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Antioxidant response to drought, cold and nutrient stress in two ploidy levels of tobacco plants: low resource requirement confers polytolerance in polyploids?

Benliang Deng · Wenchao Du · Changlai Liu · Weiwei Sun · Shan Tian · Hansong Dong

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Abstract Most polyploids can survive better under multiple stress conditions than their corresponding diploid; however, there is no established theory that can adequately explain this phenomenon at the molecular or physiological level. Here, we attempt to explain this interesting but puzzling problem from the perspectives of resource requirement and antioxidant response. In this experiment, we compared the antioxidative response and stomatal behavior of two ploidy levels of tobacco plants (tetraploid and its colchicine-induced octaploid) under drought, cold and nutrient deficit stress conditions. In comparison to tetraploid, less H_2O_2 accumulation and stronger reactive oxygen scavenging capacity (antioxidant enzyme activities and DPPH radical scavenging capacity) were observed in octaploid under stress free or stressful conditions. In accordant with these, less oxidative damage and higher redox values (ASC/DHA and GSH/GSSG) were also monitored in the octaploid than in the tetraploid under same conditions. In addition, a higher net rate of photosynthesis (Pn) and slower decline in the concentration of intercellular CO_2(Ci) were measured in the octaploid compared to the tetraploid following high concentration ABA treatment (20 mg L^{-1}), with more severe oxidative damage observed in the tetraploid than in the octaploid. On the basis of the resource acquisition theory, we consider that any environmental stress that can lower plant resource availability would favor survival in a slow-growing polyploid compared with that in a fast-growing diploid.

B. Deng · W. Du · C. Liu · W. Sun · S. Tian · H. Dong (✉)
Plant Growth and Defense Signaling Laboratory,
Department of Plant Pathology, Nanjing Agricultural University,
1 Weigang, Nanjing 210095, China
e-mail: hsdong@njau.edu.cn

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Abbreviations

ROS	Reactive oxygen species
H_2O_2	Hydrogen peroxide
SOD	Superoxide dismutase
CAT	Catalase
APX	Ascorbate peroxidase
GR	Glutathione reductase
Pn	Net photosynthesis rate
Gs	Stomatal conductance
Ci	Intercellular CO_2 concentration
ABA	Abscisic acid
TBARS	Thiobarbituric acid reactive substances
ASC	Reduced ascorbate
DHA	Oxidized ascorbate
GSH	Reduced glutathione
GSSG	Oxidized glutathione
DPPH	α,α-diphenyl-β-picrylhydrazyl
FRAP	Ferric reducing ability of plasma

Introduction

In natural environments, the availability of resources, such as water, CO_2, O_2, light, and nutrient elements, is very important for plant survival and growth. Although the drought, cold and nutrient deficiency are types of environmental stress, the mechanisms of these stresses are completely different. Drought is considered a major environmental factor that arrests plant growth and results in lower crop yields. In order to survive under conditions of

drought stress, it is necessary for plants to reduce leaf water potential, stomatal conductance, gas exchange, and normal growth (Neumann 2008; Reddy et al. 2004). Cold stress, or the low temperature exposure, can decrease plant metabolism activity and even impose severe yield penalty on crop plant (Stitt and Hurry 2002; Nord and Lynch 2009). Nutrient deficiency, in particularly the nitrogen and phosphorus deficit, is always regarded as another important limited factor for plant normal growth and metabolism (Tewari et al. 2004).

All these stresses can cause oxidative stress and lead to the formation of reactive oxygen species (ROS). These ROS can be detrimental to lipids, proteins, carbohydrates, and nucleic acids, which can accelerate plant senescence after long-term exposure (Prochazkova et al. 2001; Mittler 2002; Mittler et al. 2004). Plants have developed both enzymatic and non-enzymatic defense systems for the removal and detoxification of ROS (Mittler 2002; Mittler et al. 2004). The enzymatic system consists mainly of superoxide dismutase (SOD; EC 1.15.1.1), catalase (CAT; EC 1.11.1.6), ascorbate peroxidase (APX; EC 1.11.1.11) and glutathione reductase (GR; EC 1.6.4.2). Non-enzymatic antioxidant metabolites or low molecular weight compounds, such as ascorbates, carotenoids, tocopherols, and flavonoids, also play an essential role in ROS scavenging. The balance between ROS production and ROS removal capacity determines the rate of plant metabolism and growth (Tiwari et al. 2002; Baxter et al. 2007; Godon et al. 1998).

Polyploidization is a major driving force in plant evolution, and it is estimated that most angiosperms exist as polyploids (Comai 2005). It has long been held that polyploid species can colonize a wider range of habitats and survive better in high altitude and latitude zones that are characterized by high UV irradiation, low temperatures, nutrient-poor soils, and drought (Ehrendorfer 1980; Levin 1983). Improved stress tolerance has been reported in polyploids compared with the corresponding diploids under drought (Chandra and Dubey 2010), cold (Sugiyama 1998), UV (Niwa and Sasaki 2003), and salt (Saleh et al. 2008) stress challenges. Although these data were collected from several different plants, we have demonstrated that increases in the tolerance to more than one type of stress can be acquired to different extents by plant polyploidization (data not shown). In addition, some of these increases in stress resistance capacity are considered to be due to the enhanced SOD enzyme activity of the polyploid compared with the corresponding diploid (Chandra and Dubey 2010; Niwa and Sasaki 2003), although the conflicting results were also reported (Zhang and Kirkham 1994).

It is well known that few plants possess tolerance to more than one type of stress due to limitations in morphological and physiological trade-offs (Niinemets and Valladares 2006; Mittler 2006). Accordingly, this raises a further question: how does polyploidization confer increases in plant multi-stress tolerance to different extents compared with the corresponding diploid? The gene dosage theory can partly explain this increased tolerance (Chandra and Dubey 2010; Niwa and Sasaki 2003), and the epigenetic theory is an excellent approach for resolving this problem, although the precise mechanism has yet to be characterized (Comai 2005; Chen 2010). Hence, it is necessary to look for a simple and easily comprehensible theory that explains this polytolerance of polyploids compared with diploids at the physiological level for most stress factors. In this study, the natural allotetraploid tobacco, which is a fast-growing, moderately low nutrient/drought-tolerant, and cold-sensitive plant, and an artificially produced octaploid tobacco were compared under conditions of drought, cold and nutrient stress.

Materials and methods

Material preparation and identification

Seeds of *Nicotiana benthamiana* (allotetraploid tobacco) came from our own laboratory and were germinated in a cultivation vessel for 24 h. When the bud reached a length of 2 mm, half were transferred to another cultivation vessel and treated with 0.3% colchicine plus 2% dimethyl sulfoxide for 72 h (Nakamura et al. 1993; Zhu et al. 2006). The resulting octaploids and tetraploids were initially identified based on their special phenotypes (Paucã-Comãnescu et al. 1999; Romero-Aranda et al. 1997), and their identities were subsequently confirmed by the stomatal cell chloroplast number, a rapid and reliable method for large sample analysis (Zhu et al. 2006; Ho et al. 1990; DeMaggio and Stetler 1971).

Growth conditions and treatment

When they had reached the 7- to 8-leaf stage, plants of both ploidy levels were selected for uniformity, transplanted to plastic pots filled with vermicullite and watered with full strength Hoagland solution twice per week. After 10 days acclimative culture, the plants were divided into two groups for the following treatments.

For the cold treatment, the plants were grown under three levels of cold conditions: 25 (control), 10 and 4°C for 7 days. For the drought treatment, 0 (control), 5 and 10% PEG6000 solution were applied to each plant and their subsequent growth state was monitored. For the nutrient stress experiment, we used the 1/10 strength Hoagland solution as the control, the 1/30 and 1/100 strength as the moderate and severe deficiency treatment, respectively. For

abscisic acid (ABA) treatment, two treatments of 2 and 20 mg L^{-1} ABA (S-cis) were applied to the two groups of tobacco plant leaves with different ploidy levels for 7 days. Plants were preserved under well-watered conditions, and water-treated plants without ABA were used as the controls.

In addition, a comparison was made of the survival time of the two ploidy levels of tobacco plants under conditions of drought (5% PEG), cold (4°C), nutrient deficiency (1/100 strength of HS), shade (20 μmol s^{-1} m^{-2} irradiance) and waterlogging stress (water above the soil) exposures.

All plants were grown under controlled conditions on a 16 h light/8 h dark cycle (25/21°C) and with irradiation of 200 μmol s^{-1} m^{-2}. Seven days later, the functional leaves of the plants (mid position of the stem) were sampled for analysis. In addition, the upper-, mid- and lower-positioned leaves under favorable conditions were also collected, respectively, for carbohydrate content analysis. The data obtained for the 0 day plants (stress-free) were considered as control data.

H_2O_2 and oxidative damage determinations

H_2O_2 content was measured using the method of Gay et al. (1999). Lipid peroxidation was determined by the method of Heath and Packer (1968) with reference to the malondialdehyde content by the thiobarbituric acid reaction. Protein damage was assayed with the method of Levine (2002).

Antioxidant enzyme extraction and assay

Tobacco leaves (0.5 g) were homogenized with liquid nitrogen and then extracted with 5 mL of 50 mmol L^{-1} phosphate buffer (pH 7.0), 10 mmol L^{-1} reduced ascorbate and 4.0% (w/v) polyvinylpyrrolidone. The homogenate was centrifuged at 10,000×*g* for 30 min at 4°C, and the supernatant was collected for the enzyme assays. The SOD activity was measured with the method of Dhindsa et al. (1980); the reaction medium for APX activity was determined by the method of Nakano and Asada (1981); CAT activity was determined by following the method of Aebi (1984); the GR activity was assayed with the method of Smith et al. (1988).

Total antioxidant capacity measurement

The total antioxidant capacity reflects the non-enzymatic antioxidant capacity and was evaluated by employing two widely used methods: the Fe^{3+} reducing power (FRAP method) and α,α-diphenyl-β-picrylhydrazyl (DPPH) free radical scavenging capacity (DPPH method). The antioxidant power of foliar extracts was determined using the method of Benzie and Strain (1996). The DPPH free radical scavenging capacity of foliar extracts was determined using the method of Brand-williams et al. (1995).

Ascorbate and glutathione content determination

The ascorbate pool (ASC + DHA) and ASC/DHA were assayed according to the protocol of Fryer et al. (1998). The total glutathione pool (GSH + GSSG) and GSH/GSSG were assayed according to the protocol of Nagalakshmi and Prasad (2001).

Survival time and nonstructural carbohydrate determination

Survival time was recorded as the day on which half the plant was considered to be dead. The soluble sugar and starch content were measured with the method of Chow and Landhäusser (2004).

Analysis of gas exchange

Gas exchange was determined using a portable photosynthetic apparatus (LI-6400XT, LI-COR, USA). Photon flux density was measured at 800 μmol m^{-2} s^{-1} (outside room) and the temperature, relative humidity, and CO_2 concentration were noted to be dependent on ambient conditions. Pn, Gs, and Ci were also monitored and recorded simultaneously.

Statistical analysis

All data were analyzed using SPS 13.0 software. Means followed by the same letter are not significantly different (Duncan's multiple range test $P < 0.05$). For each stress treatment, three replicates were analyzed.

Results

Ploidy level identification and phenotype description

Nicotiana benthinana is a typical allotetraploid tobacco plant. The artificial octaploid was obtained following treatment with colchicine (Nakamura et al. 1993). The octaploid exhibited typical phenotypes of autopolyploids, such as delayed ontogenesis, slow growth, thickened and rippled leaves, a shortened stem and enhanced leaf color (Fig. 1a, b). Additionally, enhanced epidermal and stomatal cell size and decreased stomatal density were observed in contrast to their tetraploid counterparts (Fig. 1c, d). Chloroplast number was also found to be twice as large in the octaploid stomatal guard cell when compared to the tetraploid (Fig. 1c, d).

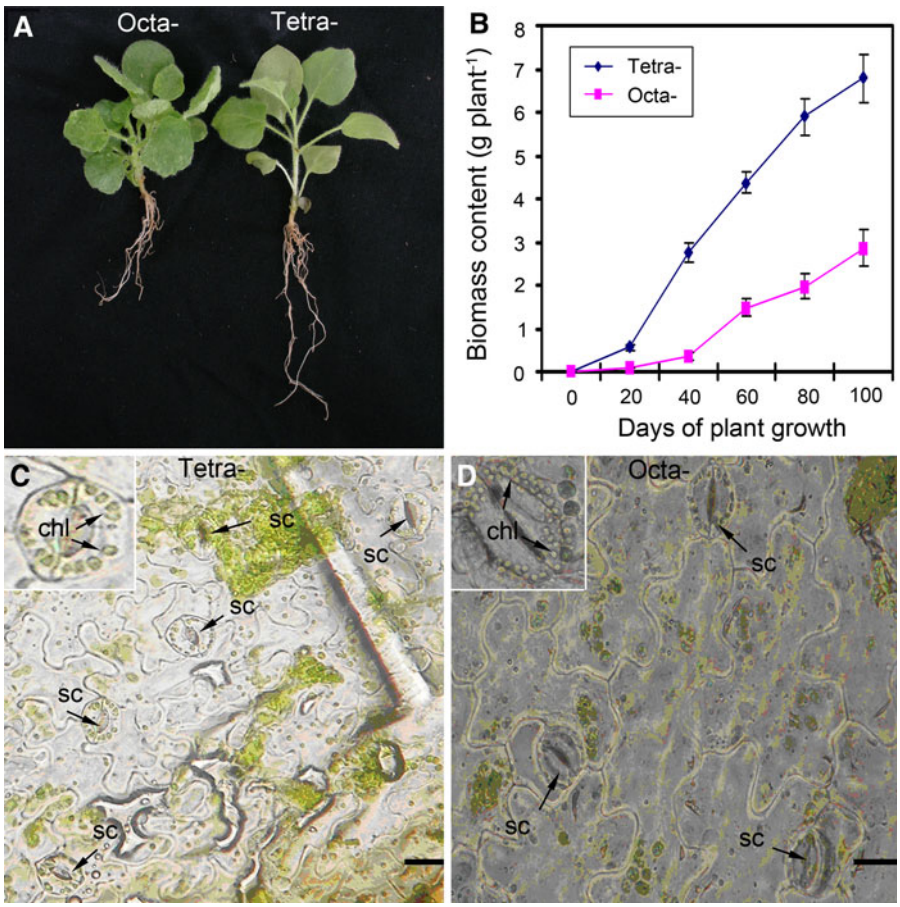

Fig. 1 Comparison of the tetraploid and octaploid tobacco plants. **a** Morphological differences between the octaploid and tetraploid; **b** biomass accumulation during the same time period; **c**, **d** contrasting sizes and densities of the stomata cells in the octaploid and tetraploid tobacco leaves, respectively. *Scale bar* = 30 μm; *sc* stomatal cell, *chl* chloroplast

H_2O_2 accumulation and oxidative damage

When compared to the octaploid, H_2O_2 accumulation and oxidative damage parameters, such as TBARS and carbonyl content, were found to increase more significantly in the tetraploid as nutrient stress increased (Table 1). This finding indicates that under nutrient deprivation, tetraploids are less tolerant to stress. Similar patterns were also observed under drought (Table 2) and cold stress (Table 3) conditions. Among these three stress conditions, the octaploid exhibited greater tolerance during drought (2.5-fold increase) and nutrient (3-fold increase), than under cold stress (1.5-fold increase) when compared to the protein damage seen in the tetraploid (Tables 1, 2, 3). This suggests the tetraploid suffered greater oxidative damage than the octaploid during both stressful and stress-free conditions.

Enzymatic and non-enzymatic antioxidant activities

Greater activity was observed in antioxidant enzymes, including SOD, CAT, APX and GR, in the octaploid plant leaves compared to the tetraploid; this difference was observed to increase when greater nutrient stresses were applied (Table 1). With regards to the non-enzymatic antioxidant capacities, such as the DPPH free radical scavenging capacity and Fe^{3+} reducing power, both lower IC_{50} values that indicate stronger DPPH scavenging capacities and higher FRAP values that indicate stronger Fe^{3+} reducing powers were measured in the octaploids compared to the tetraploids. Similar trends were also observed for stress increments and enzyme activities (Table 1) and for the other two stress conditions (Tables 2, 3). Additionally, a greater difference in non-enzymatic antioxidant capacity between the octaploid and tetraploid leaves was observed, but this was not found for antioxidant activities as the level of stress increased.

Antioxidant metabolite and redox values

Ascorbate and glutathione are two major antioxidant metabolites. The ratio of reduced to oxidized metabolites is commonly regarded as a sensitive indicator to evaluate the redox state of plant tissues (Tausz et al. 2004; Mittler 2002). Although total ascorbate and glutathione were both

Table 1 ROS metabolism related parameters were compared between the tetraploid and octaploid tobacco leaves with different concentration of HS treatment

	Control		1/30 HS		1/100 HS	
	Tetra-	Octa-	Tetra-	Octa-	Tetra-	Octa-
SOD activity	87.2 ± 4.2^{e}	98.2 ± 3.3^{d}	110.5 ± 3.8^{c}	121.3±4.9^{b}	103.1 ± 6.4^{c,d}	153.3 ± 5.4^{a}
CAT activity	76.1 ± 3.9^{b}	85.4 ± 2.2^{a}	63.2 ± 5.9^{c}	89.5 ± 4.6^{a}	36.2 ± 8.9^{d}	67.3 ± 6.8^{b,c}
APX activity	320 ± 21^{b}	367 ± 16^{a}	247 ± 36^{c}	348 ± 21^{a,b}	157 ± 32^{d}	245 ± 34^{c}
GR activity	56 ± 3.1^{e}	75.1 ± 3.8^{c}	72.2 ± 2.8^{c,d}	83.7 ± 4.1^{b}	65.7 ± 5.2^{d}	103.8 ± 6.6^{a}
DPPH scavenging	43.5 ± 2.9^{b}	25.0 ± 3.4^{d}	35.1 ± 3.2^{c}	22.2 ± 2.1^{d}	78.9 ± 6.8^{a}	17.3 ± 2.8^{e}
FRAP value	2.89 ± 0.45^{c,d}	7.86 ± 0.28^{b}	3.67 ± 0.62^{c}	8.11 ± 0.37^{b}	2.24 ± 0.34^{d}	11.67 ± 0.65^{a}
Ascorbate content	587 ± 32^{d}	669 ± 28^{c}	692 ± 24^{c}	745 ± 27^{b}	756 ± 28^{b}	912 ± 41^{a}
Glutathione content	296 ± 21^{e}	378 ± 17^{d}	369 ± 19^{d}	443 ± 26^{c}	513 ± 25^{b}	645 ± 43^{a}
ASC/DHA ratio	6.80 ± 0.18^{b}	7.53 ± 0.21^{a}	5.49 ± 0.31^{c}	6.78 ± 0.19^{b}	2.69 ± 0.86^{d}	5.55 ± 0.35^{c}
GSH/GSSG ratio	12.19 ± 0.53^{b}	13.32 ± 0.17^{a}	6.72 ± 0.57^{d}	11.81 ± 0.68^{b}	2.27 ± 0.87^{e}	8.73 ± 0.47^{c}
H_2O_2 accumulation	678 ± 45^{cd}	612 ± 28^{d}	869 ± 65^{b}	625 ± 54^{c,d}	1,209 ± 89^{a}	713 ± 67^{c}
TBARS content	340 ± 23^{d}	312 ± 29^{d}	497 ± 35^{b}	309 ± 19^{d}	978 ± 78^{a}	418 ± 38^{c}
Carbonyl content	79.1 ± 3.6^{d}	69.3 ± 2.9^{e}	131.9 ± 5.3^{b}	77.6 ± 4.5^{d}	297.5 ± 16.7^{a}	108.0 ± 7.4^{c}

Samples of leaves of tetraploid and octaploid tobacco plants in the greenhouse were harvested at a set stage of growth, which was observed when each plant was found to contain 7–8 leaves. For each plant, this appeared at separate time points: 4 weeks post-sowing for the tetraploid, and 12 weeks post-sowing for the octaploid. The data shows the mean of at least three replicates ± SE. Means followed by the same letter were not found to be significantly different (Duncan's multiple range test $P < 0.05$)

seen to increase with increased stress, the redox value showed a significantly greater decrease in the tetraploid, in comparison to the octaploid, irrespective of stress types (Tables 1, 2, 3). This suggests that under stress, antioxidant metabolites become more oxidized in the tetraploid than in the octaploid leaves.

Survival times and nonstructural carbohydrate content

Figure 2a shows a significant increase in survival time in octaploid plants than in tetraploid plants under exposure to drought conditions (survival increased by approximately 120%), nutrient deficit (increased by approximately 180%),

Table 2 ROS metabolism related parameters were compared between the tetraploid and octaploid tobacco leaves with different concentration of PEG treatment

	Control		5% PEG		10% PEG	
	Tetra-	Octa-	Tetra-	Octa-	Tetra-	Octa-
SOD activity	83.1 ± 3.9^{f}	92.5 ± 4.4^{e}	126.0 ± 5.3^{c}	137.4 ± 3.8^{b}	106.3 ± 5.9^{d}	189.5 ± 4.8^{a}
CAT activity	84.2 ± 2.8^{d}	94.8 ± 3.9^{c}	178.1 ± 7.3^{a}	136.4 ± 5.7^{b}	35.7 ± 8.5^{e}	79.5 ± 6.4^{d}
APX activity	332 ± 19^{d}	376 ± 16^{c}	548 ± 23^{a}	479 ± 29^{b}	211 ± 45^{e}	407 ± 34^{c}
GR activity	54.2 ± 3.6^{d}	72.0 ± 4.8^{c}	86.5 ± 4.6^{b}	91.1 ± 3.2^{b}	72.4 ± 5.8^{c}	112.3 ± 6.4^{a}
DPPH scavenging	41.1 ± 3.8^{b}	21.3 ± 2.2^{c}	34.7 ± 5.8^{b}	18.4 ± 3.2^{c,d}	98.5 ± 8.9^{a}	14.5 ± 2.5^{d}
FRAP value	4.55 ± 0.74^{e}	8.53 ± 0.53^{c}	6.78 ± 0.34^{d}	11.21 ± 0.48^{b}	2.23 ± 0.47^{f}	16.8 ± 0.76^{a}
Ascorbate content	576 ± 32^{e}	657 ± 28^{d}	734 ± 21^{c}	819 ± 35^{b}	853 ± 39^{b}	987 ± 45^{a}
Glutathione content	291 ± 21^{e}	369 ± 17^{d}	412 ± 28^{d}	478 ± 22^{c}	567 ± 34^{b}	649 ± 28^{a}
ASC/DHA ratio	6.95 ± 0.16^{b}	7.79 ± 0.23^{a}	5.10 ± 0.26^{c}	6.67 ± 0.18^{b}	1.87 ± 0.48^{d}	4.75 ± 0.32^{c}
GSH/GSSG ratio	12.31 ± 0.21^{b}	13.52 ± 0.17^{a}	7.80 ± 0.54^{c}	11.84 ± 0.37^{b}	1.79 ± 0.78^{d}	7.34 ± 0.64^{c}
H_2O_2 accumulation	664 ± 45^{c}	576 ± 37^{d}	956 ± 56^{b}	723 ± 46^{c}	1,478 ± 89^{a}	987 ± 65^{b}
TBARS content	302 ± 24^{d}	279 ± 27^{d}	563 ± 65^{b}	376 ± 35^{c}	1,208 ± 78^{a}	654 ± 61^{b}
Carbonyl content	84.0 ± 7.7^{d,e}	72.4 ± 4.5^{e}	167.4 ± 5.8^{b}	93.5 ± 3.8^{d}	389.2 ± 19.8^{a}	136.3 ± 8.7^{c}

Samples of leaves of tetraploid and octaploid tobacco plants in the greenhouse were harvested at a set stage of growth, which was observed when each plant was found to contain 7–8 leaves. For each plant, this appeared at separate time points: 4 weeks post-sowing for the tetraploid, and 12 weeks post-sowing for the octaploid. The data shows the mean of at least three replicates ± SE. Means followed by the same letter were not found to be significantly different (Duncan's multiple range test $P < 0.05$)

Table 3 ROS metabolism related parameters were compared between the tetraploid and octaploid tobacco leaves under low temperature exposure

	Control		10°C		4°C	
	Tetra-	Octa-	Tetra-	Octa-	Tetra-	Octa-
SOD activity	85.1 ± 4.8^{c}	96.6 ± 3.1^{b}	74.8 ± 3.2^{d}	89.0 ± 2.7^{c}	42.7 ± 5.9^{e}	109.3 ± 4.3^{a}
CAT activity	81.4 ± 3.9^{c}	89.7 ± 2.4^{b}	78.8 ± 4.2^{c}	88.1 ± 2.7^{b}	47.5 ± 6.8^{d}	112.8 ± 5.2^{a}
APX activity	346 ± 15^{c}	397 ± 19^{b}	289 ± 32^{d}	365 ± 23^{bc}	127 ± 42^{e}	498 ± 34^{a}
GR activity	58.0 ± 3.5^{c}	77.6 ± 2.4^{b}	48.8 ± 3.1^{d}	65.1 ± 4.6^{c}	25.7 ± 7.6^{e}	89.5 ± 5.2^{a}
DPPH scavenging	39.0 ± 2.7^{b}	18.1 ± 2.3^{c}	38.7 ± 2.5^{b}	15.9 ± 2.8^{c,d}	57.4 ± 3.2^{a}	11.0 ± 2.3^{d}
FRAP value	4.89 ± 0.34^{d}	9.74 ± 0.25^{c}	5.08 ± 0.19^{d}	11.22 ± 0.29^{b}	2.44 ± 0.24^{e}	15.78 ± 0.32^{a}
Ascorbate content	583 ± 21^{c}	674 ± 18^{b}	563 ± 18^{c}	687 ± 27^{b}	478 ± 42^{d}	856 ± 35^{a}
Glutathione content	301 ± 23^{c}	387 ± 27^{b}	265 ± 17^{c}	354 ± 29^{b}	198 ± 31^{d}	453 ± 24^{a}
ASC/DHA ratio	6.71 ± 0.21^{c}	7.38 ± 0.14^{ab}	7.19 ± 0.17^{b}	7.65 ± 0.22^{a}	2.17 ± 0.76^{d}	6.32 ± 0.32^{c}
GSH/GSSG ratio	12.11 ± 0.22^{b}	13.26 ± 0.39^{a}	12.94 ± 0.43^{a}	13.52 ± 0.31^{a}	4.54 ± 0.97^{c}	11.72 ± 0.52^{b}
H_2O_2 accumulation	625 ± 26^{b}	547 ± 19^{c}	549 ± 29^{c}	502 ± 31^{c}	897 ± 67^{a}	654 ± 45^{b}
TBARS content	368 ± 21^{c}	327 ± 32^{c,d}	321 ± 24^{d}	289 ± 29^{d}	756 ± 54^{a}	453 ± 42^{b}
Carbonyl content	88.1 ± 2.4^{c}	78.9 ± 4.2^{d}	77.2 ± 5.4^{d}	72.8 ± 4.1^{d}	154.6 ± 14.5^{a}	98.5 ± 6.9^{b}

Samples of leaves of tetraploid and octaploid tobacco plants in the greenhouse were harvested at a set stage of growth, which was observed when each plant was found to contain 7–8 leaves. For each plant, this appeared at separate time points: 4 weeks post-sowing for the tetraploid, and 12 weeks post-sowing for the octaploid. The data shows the mean of at least three replicates ± SE. Means followed by the same letter were not found to be significantly different (Duncan's multiple range test $P < 0.05$)

cold (increased by 70%), shade (increased by 15%) and waterlogging stress (increased by 10%). Starch and soluble sugar content were also observed in the two ploidy levels of plant to evaluate the 'deposite' resource for antioxidant synthesis. The functional leaves (mid-positioned leaves) displayed higher soluble sugar and starch content than the fresh (upper position) or old (lower position) leaves (Fig. 2b). Higher amount of starch and sugar was observed in the octaploid than in the tetraploid, irrespective of growth stage.

Gas exchange and oxidative damage with ABA treatment

Compared to the controls, low levels of application of ABA were shown to slightly close the stomata and lower the Ci values, whilst promoting the Pn. Low-dose ABA also significantly protected both plants with different ploidy levels from oxidative damage. However, during exposure to high concentrations of ABA, severe decline of Pn, Ci and Gs, and a greater increase in oxidative damage was observed in the tetraploid than in the octaploid plants (Fig. 3). This suggests that the octaploid plant can perform photosynthesis better, under limited CO_2 availability, and within a shorter duration of time where high-dose ABA can almost fully close the stomata.

Discussion

Plant polyploidization is an interesting and widespread phenomenon in nature. The process has received increased attention recently due to the finding that polyploidization results in phenotype alteration at both physiological and morphological levels (Paucã-Comãnescu et al. 1999; Romero-Aranda et al. 1997; DeMaggio and Stetler 1971). It has been well-established that enhanced environmental stresses, such as cold, drought, UV and pathogen resistance, are found in polyploids in comparison to their diploid counterparts (Sugiyama 1998; Chandra and Dubey 2010; Niwa and Sasaki 2003).

In this experiment, tolerance to different extents of enhanced drought, nutrient and cold stress was also monitored in octaploid and tetraploid tobacco plants from the perspective of oxidative damage (Tables 1, 2, 3). In accordance, lower levels of oxidative damage and H_2O_2 accumulation were measured in the octaploids compared with the tetraploid (Tables 1, 2, 3). This low level of H_2O_2 accumulation may be due to the higher activity levels of antioxidant enzyme and stronger ROS scavenging capacity in octaploids than in tetraploids (Tables 1, 2, 3).

The results showed that a very high of non-enzymatic antioxidant capacity was observed in octaploid plants than tetraploids under stressful or stress-free conditions (Tables 1, 2, 3). It is easy to understand this higher

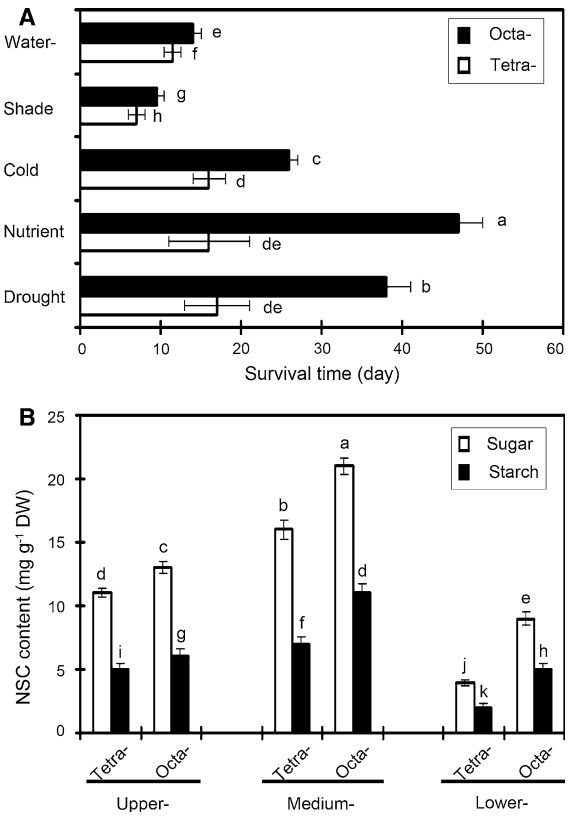

Fig. 2 Plant survival time under stress exposure and foliar NSC content under favorable condition. Survival time of two ploidy levels of tobacco plants was recorded under stress exposure (**a**); starch and soluble sugar content in the upper-, mid- and lower-positioned leaves under favorable condition (**b**). The data are the means of three independent experiments ± SE. Means followed by the *same letter* are not significantly different (Duncan's multiple range test $P < 0.05$). *NSC* nonstructural carbohydrate

accumulation of secondary metabolite in the slow-growing octaploid than in the fast-growing tetraploid, as the former has a longer growth stage (Fig. 1b). In addition, the non-enzymatic antioxidant compounds have a special function for ROS scavenging in comparison to the antioxidant enzyme because the latter becomes easily inactivated when the cell dies. Thus, the stronger non-enzymatic antioxidant may play a greater role in contributing to the enhanced stress tolerance of polyploids than in tetraploids.

However, the higher antioxidant enzyme activities were also observed in the ontogenesis-delayed octaploid in comparison to the fast-growing tetraploid counterpart under stressful or stress-free conditions (Tables 1, 2, 3). One possible explanation for this finding may be that higher accumulation of enzymatic antioxidant compound (or secondary metabolites, such as tocophenol and ascorbate) protect the antioxidant enzyme protein from oxidation by scavenging more OH^-.

It is well known that ROS play a dual role in plants: at low concentrations, they act as a signal molecule involved in promoting the normal growth and development of plants (Foreman et al. 2003; Li et al. 2009b; Kim et al. 2007; Rodríguez et al. 2002) and, at high concentrations, they can cause plant senescence or even PCD (Prochazkova et al. 2001; Tiwari et al. 2002). Figure 1b shows that the octaploid has a low growth rate and biomass accumulation capacity; its delay in the ontogenesis phenomenon compared to the tetraploid was commonly attributed to the enhanced cell size and reduction of metabolic activity (Chen et al. 2007; Comai 2005; Kondorosi et al. 2000). Considering that the ROS play a critical role in plant development and growth, including in root elongation and leaf extension, one speculative possibility is that low levels of ROS and a more reduced redox state may also contribute to the delayed ontogenesis of the octaploid compared with the tetraploid.

Some researchers consider that, in the context of stress resistance, polyploids have a gene dosage advantage over the corresponding diploids, and some possible correlation between the enhanced stress resistance and increased antioxidant capacity of polyploids compared with their diploid counterparts has been reported (Chandra and Dubey 2010; Niwa and Sasaki 2003). However, the effects of the duplicate genes of polyploids are more closely correlated with developmental programs than with the response to environmental stresses, and, indeed, the duplicate genes can eventually be lost or have the tendency to mutate (Comai 2005; Chen 2010; Allario et al. 2011). It suggested that the contribution of gene dosage effect in polyploid plant for the increased stress resistance may not so much as we thought before, at least in the autopolyploid plant. In fact, there also exists an unequal elevation of varied stress resistance for polyploid in contrast to diploid. For example, in this experiment, there is only a slight increase of shade and waterlogging (compared with the intense increase of drought or nutrient stress) tolerance in the octaploid compared with the tetraploid tobacco (Fig. 2a).

Thus, the gene dosage theory does not appear to provide a completely satisfactory explanation for the stronger stress tolerance in polyploids compare with the corresponding diploids. Although the epigenetic theory can help to explain certain stress resistance advantages of polyploidy over diploidy in several plant species at the molecular level, the conclusive resolution of this problem awaits further study (Comai 2005; Chen 2010).

The acquisition of sufficient resources, such as CO_2, light, water and nutrients, is very important for plants to survive under stressful conditions (Magyar et al. 2007; Neumann 2008; Niinemets and Valladares 2006; Chapin 1980). Gs, Pn and Ci are three parameters that can be used to evaluate plant photosynthesis performance. However, the photosynthesis and stomata conductance can both be profoundly affected by low temperature, drought or

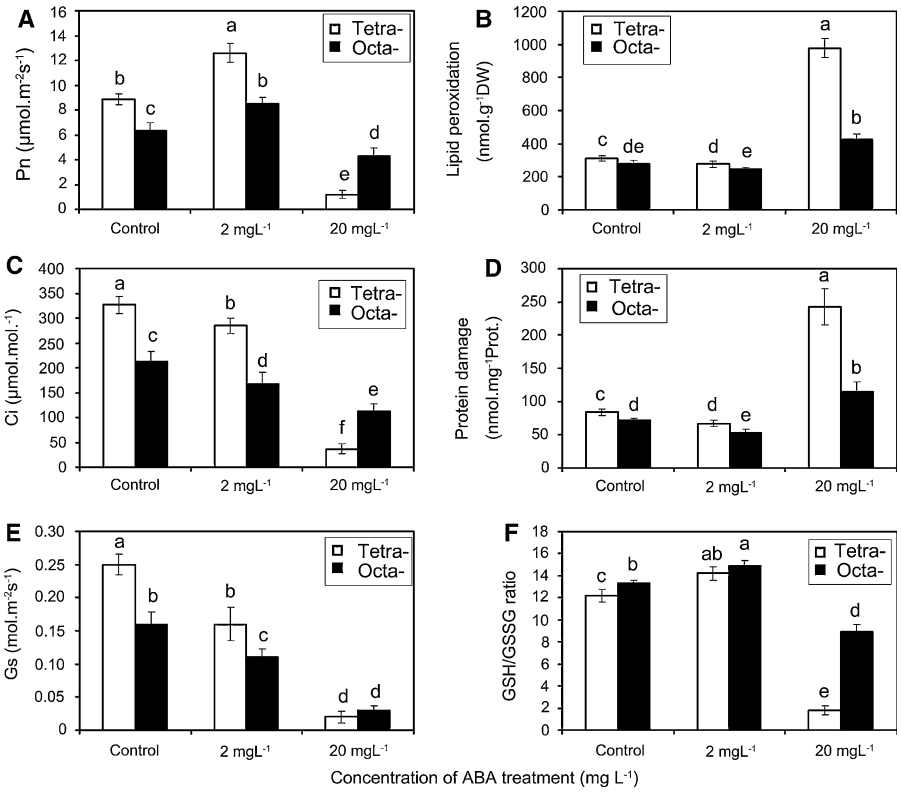

Fig. 3 Gas exchange and oxidative damage with ABA treatment. The Pn (**a**), lipid peroxidation (**b**), Ci (**c**), protein damage (**d**), Gs (**e**) and redox state, or GSH/GSSG ratio (**f**) under ABA treated conditions of the tetraploid versus the octaploid. The data are the means of three independent experiments ± SE. Means followed by the *same letter* are not significantly different (Duncan's multiple range test $P < 0.05$)

nutrient deficit stress (Nord and Lynch 2009; Stitt and Hurry 2002).

Some literature has reported that the polyploid has a low Gs, Ci and Pn under favorable conditions compared to their diploid counterparts (Xu et al. 2008; Romero-Aranda et al. 1997). It means that a stronger transpirate power is required for water and nutrient assimilation, and more CO_2 is needed to perform the normal metabolic processes in diploid than in polyploidy plants. In fact, the stomata density reduction and low light transmittance of polyploids were strong evidence to support the low CO_2 or light requirement in comparison to their diploid counterparts under stress-free conditions (Zhu et al. 2006; Ho et al. 1990; Romero-Aranda et al. 1997). However, a higher Pn and lower Ci were reported in the tetraploid compared to the diploid under stress conditions, such as drought conditions or high irradiance (Li et al. 2009a; Romero-Aranda et al. 1997). In this experiment, we also observed the Gs, Pn and Ci of the two ploidy levels of plants after treatment with ABA (Fig. 3). Compared to the control (water treatment), a low concentration of ABA (2 mg L^{-1}) can protect the tobacco plant with enhancement of the Pn coupled with a slight reduction of the Gs and Ci values (Fig. 3). This finding suggested that higher levels of CO_2 were required to maintain the higher Pn level of the tetraploid than the octaploid to satisfy its normal metabolic activity under slight stress conditions, such as a mild drought or exposure to salinity, which can trigger an increase in ABA increase. However, 20 mg L^{-1} ABA (such as severe drought or salinity exposure) can close the stomata of the two ploidy levels of tobacco plant and the Ci and Pn values were greatly decreased in the tetraploid and octaploid plants with very low Gn values (Fig. 3). This suggested that the "deposited" CO_2 was consumed more rapidly in tetraploid than in octaploid plants, while at the same time the Pn was greatly reduced in the former due to a shortage of CO_2.

We also determined the oxidative damage and redox parameter GSH/GSSG under low and high ABA exposure. Our data indicated that severe lipid peroxidation and protein damage combined with low GSH/GSSG value were observed in the tetraploid compared to the octaploid (Fig. 3). This further indicated that the tetraploid suffered greater damage under same resource availability conditions than the octaploid.

Interesting, we also can use the defense compound synthesis level to further explain the low resource requirement of octaploid than the tetraploid. Water, nutrients, CO_2, and light all are indispensable resources for normal plant growth, and any deficiency-triggered oxidative stress would divert more resources from the normal metabolic pathways to the antioxidant response pathways (Baxter et al. 2007; Godon et al. 1998). Water or nutrient deficiency or a decrease in light or O_2 availability would lead to a reduction in photosynthetic rate or changed metabolism pathway, or altered respiratory patterns respectively. Further, all these changes would result in a

decrease in available energy (Neumann 2008; Tiwari et al. 2002; Baena-González and Sheen 2008); however, the impact of such changes on octaploids would be less marked than on tetraploids due to the fact that the former has a smaller resource requirement. From an antioxidant protection perspective, these stressors (drought, cold and nutrient stress) all can trigger ROS overproduction (Tables 1, 2, 3), and hence under these conditions, plants need more antioxidant compounds to scavenge them. Fortunately, slow-growing octaploids can not only accumulate more antioxidant compounds (especially the secondary metabolite) but can also synthesize them with a low energy cost due to their slow growing feature (Scheurwater et al. 2000). Hence, slow growers would be expected to suffer less severe drought, cold or nutrient stress than fast growers (Tables 1, 2, 3).

In contrast to tetraploids, it seems that octaploids use the non-protein antioxidant compounds (longer-lived) in preference to protein antioxidant (shorter-lived) activity as 'deposite' antioxidant power under normal conditions to counteract the latent stress challenge (Tables 1, 2, 3). In addition, a further factor to which we should pay attention is the higher stability of redox state which observed in octaploid than in tetraploid (Tables 1, 2, 3). One possibility is the higher content of non-enzymatic antioxidant compound can exist longer time and contribute more to the high steady of redox state of octaploid than tetraploid over the enzymatic antioxidant.

In addition, we can even from the resource investment perspective to understand the enhanced stress tolerance of octaploid than tetraploid. Carbohydrate is not a direct antioxidant compound (Couée et al. 2006), but the storage carbon pool is always considered as an important indicator to predict stress responsiveness (Niinemets 2010). Hence, we also monitored the nonstructural carbohydrate content in two ploidy level of plants under favorable conditions. Both soluble sugar and starch were observed higher in the slow growing octaploid than in the fast growing tetraploid, and it may be reasoned that octaploids should invest more resource (such as the carbohydrate) on the antioxidant compounds synthesis than in tetraploid (Fig. 2b).

Now, we can use the low resource requirement hypothesis, combined with antioxidant defense theory, to illustrate the polytolerance and unequal elevation of stress resistance and some other interesting but puzzling problems here of polyploids compared to diploid plants.

Compared with favorable conditions, the nature of the abiotic stress challenge is also an event that includes the reduction of resource availability, such as CO_2 assimilation, nutrient element uptake, water gain and light harvest. Based on the low resource requirement hypothesis, we can not only understand the multiple tolerances that are displayed in polyploid plants in comparison to their diploid counterparts, but also understand the significant enhancement of the nutrient deficit, cold and drought stress tolerance in comparison to the slight enhancement of shade and waterlogging tolerance (Fig. 2a). As for the case of significantly decreased resources, the octaploid can be tolerant for a relative long duration of low nutrient and water/CO_2 conditions in comparison to the tetraploid due to the fact that the former has retarded metabolic activity and these resources can be re-used in the plant tissues. However, as for the shade and waterlogging stress (Causin et al. 2009; Blokhina et al. 2003), the profound reduction of light availability (from sun plant living conditions to shade plant living conditions) or ATP production (oxidative and fermentative respiration generate 36–38 and 2–3 mol ATP mol^{-1} glucose, respectively) would markedly attenuate the advantage of low resource requirements of the octaploid compared to the tetraploid plants, even for short durations of stress. This may be the reason why many reports exist with regards to enhanced cold, drought, salinity, UV and nutrient deficits (Sugiyama 1998; Chandra and Dubey 2010; Niwa and Sasaki 2003; Saleh et al. 2008), but fewer regarding shade or waterlogging stress tolerance in polyploids compared to diploid plants. It is in accordance with the fact that fewer plants would tolerant more than one of drought, shade and waterlogging stress challenges (Niinemets and Valladares 2006).

This low resource requirement hypothesis can also well explain the fast-growing cultivated crop plants with reduced stress (such as drought, low nutrient, pathogen and pest) tolerance compared with their slow-growing wild diploid counterparts (Zhang and Kirkham 1994), due to the fast growers have a high resource requirement and invest more resource on the biomass synthesis but not on defense compounds (especial the antioxidant compounds) synthesis.

In conclusion, some important and interesting findings can be summarized in here. Firstly, enhanced drought, cold and nutrient stress tolerance were observed significantly in octaploid than in tetraploid; Secondly, higher antioxidant capacity and low resource requirement both contribute to the enhanced stress tolerance of octaploid than tetraploid. In addition, low levels of H_2O_2 and more reduced redox state may contribute to the delayed ontogenesis of octaploid over tetraploid.

This work would give us a novel insight to understand the polytolerance and unequal elevation of stress tolerance of polyploid, from both resource availability and antioxidant defense perspective (Fig. 4). Some problems, such as the potential relationship of the gene dosage effect and the resource requirement capacity in stress resistance, are still required further investigation.

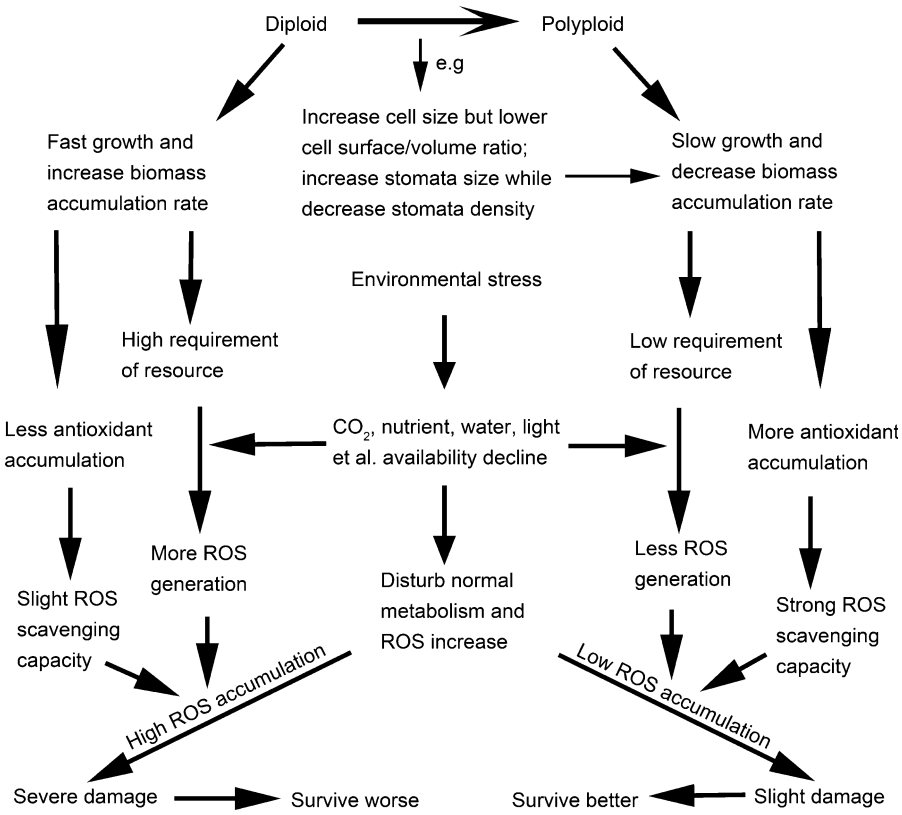

Fig. 4 A hypothetical diagram to illustrate the "low resource requirement hypothesis" described in the text. Polyploidization of the diploid slows plant growth rate and biomass accumulation under the same time interval. These traits confer the polyploids low resource requirement and high antioxidant compound accumulation. Both these properties contribute to the total ROS level decline in the polyploids compared to their diploid counterparts during exposure to stress. For further details, please refer to the text

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References

Aebi H (1984) Catalase in vitro. Methods Enzymol 105:121–126

Allario T, Brumos J, Colmenero-Flores JM, Tadeo F, Froelicher Y, Talon M, Navarro L, Ollitrault P, Morillon R (2011) Large changes in anatomy and physiology between diploid Rangpur lime (*Citrus limonia*) and its autotetraploid are not associated with large changes in leaf gene expression. J Exp Bot 62: 2507–2519

Baena-González E, Sheen J (2008) Convergent energy and stress signaling. Trends Plant Sci 13:474–482

Baxter CJ, Redestig H, Schauer N, Repsilber D, Patil KR, Nielsen J, Selbig J, Liu J, Fernie AR, Sweetlove LJ (2007) The metabolic response of heterotrophic arabidopsis cells to oxidative stress. Plant Physiol 143:312–325

Benzie IF, Strain J (1996) The ferric reducing ability of plasma (FRAP) as a measure of 'antioxidant power': the FRAP assay. Anal Biochem 239:70–76

Blokhina O, Virolainen E, Fagerstedt KV (2003) Antioxidants, oxidative damage and oxygen deprivation stress: a review. Ann Bot 91:179–194

Brand-williams W, Cuvelier ME, Berset C (1995) Use of a free radical method to evaluate antioxidant activity. Food Sci Technol 28:25–30

Causin HF, Roberts IN, Criado MV, Gallego SM, Pena LB, Ríos MC, Barneix AJ (2009) Changes in hydrogen peroxide homeostasis and cytokinin levels contribute to the regulation of shade-induced senescence in wheat leaves. Plant Sci 177:698–704

Chandra A, Dubey A (2010) Effect of ploidy levels on the activities of Δ^1-pyrroline-5-carboxylate synthetase, superoxide dismutase and peroxidase in *Cenchrus* species grown under water stress. Plant Physiol Biochem 48:27–34

Chapin FS (1980) The mineral nutrition of wild plants. Annu Rev Ecol Syst 11:233–260

Chen ZJ (2010) Molecular mechanisms of polyploidy and hybrid vigor. Trends Plant Sci 15:57–71

Chen ZJ, Ha M, Soltis D (2007) Polyploid: genome obesity and its consequences. New Phytol 174:717–720

Chow PS, Landhäusser SM (2004) A method for routine measurements of total sugar and starch content in woody plant tissues. Tree Physiol 24:1129–1136

Comai L (2005) The advantages and disadvantages of being polyploid. Nature 6:836–846

Couée I, Sulmon C, Gouesbet G, EI Amrani A (2006) Involvement of soluble sugars in reactive oxygen species balance and responses to oxidative stress in plants. J Exp Bot 57:449–459

DeMaggio AE, Stetler DA (1971) Polyploidy and gene dosage effects on chloroplasts of fern gametophytes. Exp Cell Res 67:287–294

Dhindsa RS, Dhindsa PP, Thorpe TA (1980) Leaf senescence correlated with increased levels of membrane permeability and lipid-peroxidation and decreased levels of superoxide dismutase and catalase. J Exp Bot 32:93–101

Ehrendorfer F (1980) Polyploidy and distribution. In: Lewis WH (ed) Polyploidy: biological relevance. Plenum Press, New York, pp 45–60

Foreman J, Demidchik V, Bothwell JHF, Mylona P, Miedema H, Torres MA, Linstead P, Costa S, Brownlee C, Jones JDG, Davies JM, Dolan L (2003) Reactive oxygen species produced by NADPH oxidase regulate plant cell growth. Nature 422:442–446

Fryer MJ, Andrews JR, Oxborough K, Blowers DA, Baker NR (1998) Relationship between CO_2 assimilation, photosynthetic electron transport, and active O_2 metabolism in leaves of maize in the

field during periods of low temperature. Plant Physiol 116: 571–580
Gay C, Collins J, Gebicki J (1999) Hydroperoxide assay with the ferric-xylenol orange complex. Anal Biochem 273:149–155
Godon C, Lagniel G, Lee J, Buhler J, Kieffer S, Perrot M, Boucherie H, Toledano MB, Labarre J (1998) The H_2O_2 stimulon in *Saccharomyces cerevisiae*. J Biol Chem 273:22480–22489
Heath RL, Packer L (1968) Photoperoxidation in isolated chloroplast. I. Kinetics and stoichiometry of fatty acid peroxidation. Arch Biochem Biophys 125:180–198
Ho I, Wan Y, Widholm JM, Rayburn AL (1990) The use of stomatal chloroplast number for rapid determination of ploidy level in maize. Plant Breed 105:203–210
Kim M, Kim H, Kim Y, Baek K, Oh H, Hahn K, Bae R, Lee I, Joung H, Jeon J (2007) Superoxide anion regulates plant growth and tuber development of potato. Plant Cell Rep 26:1717–1725
Kondorosi E, Roudier F, Gendreau E (2000) Plant cell-size control: growing by ploidy? Curr Opin Plant Biol 3:488–492
Levin DA (1983) Polyploidy and novelty in flowering plants. Amer Nat 122:1–24
Levine RL (2002) Carbonyl modified proteins in cellular regulation, aging and disease. Free Radic Biol Med 32:790–796
Li W, Biswas D, Xu H, Xu C, Wang X, Liu J, Jiang G (2009a) Photosynthetic responses to chromosome doubling in relation to leaf anatomy in *Lonicera japonica* subjected to water stress. Funct Plant Biol 36:783–792
Li S, Xue L, Xu S, Feng H, An L (2009b) Hydrogen peroxide acts as a signal molecule in the adventitious root formation of mung bean seedlings. Environ Exp Bot 65:63–71
Magyar G, Kun Á, Oborny B, Stuefer JF (2007) Importance of plasticity and decision-making strategies for plant resource acquisition in spatio-temporally variable environments. New Phytol 174:182–193
Mittler R (2002) Oxidative stress, antioxidants and stress tolerance. Trends Plant Sci 7:405–410
Mittler R (2006) Abiotic stress, the field environment and stress combination. Trends Plant Sci 11:15–19
Mittler R, Vanderauwera S, Gollery M, Van Breusegem F (2004) Reactive oxygen gene network in plants. Trends Plant Sci 9:490–498
Nagalakshmi N, Prasad MNV (2001) Responses of glutathione cycle enzymes and glutathione metabolism to copper stress in *Scenedesmus bijugatus*. Plant Sci 160:291–299
Nakamura A, Takashima S, Hasegawa H (1993) Simple and efficient chromosome doubling method by colchicine-treatment for haploid plantlets of tobacco. Breed Sci 43:603–612
Nakano Y, Asada K (1981) Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. Plant Cell Physiol 22:867–880
Neumann PM (2008) Coping mechanisms for crop plants in drought-prone environments. Ann Bot 101:901–907
Niinemets Ü (2010) Responses of forest trees to single and multiple environmental stresses from seedlings to mature plants: past stress history, stress interactions, tolerance and acclimation. For Ecol Manage 260:1623–1639
Niinemets Ü, Valladares F (2006) Tolerance to shade, drought, and waterlogging of temperate northern hemisphere trees and shrubs. Ecol Monogr 76:521–547
Niwa Y, Sasaki Y (2003) Plant self-defense mechanisms against oxidative injury and protection of the forest by planting trees of triploids and tetraploids. Ecotoxicol Environ Saf 55:70–81
Nord EA, Lynch JP (2009) Plant phenology: a critical controller of soil resource acquisition. J Exp Bot 60:1927–1937
Paucã-Comãnescu M, Clevering OA, Hanganu J, Gridin M (1999) Phenotypic differences among ploidy levels of *Phragmites australis* growing in Romania. Aquat Bot 64:223–234
Prochazkova D, Sairam RK, Srivastava GC, Singh DV (2001) Oxidative stress and antioxidant activity as the basis of senescence in maize leaves. Plant Sci 161:765–771
Reddy AR, Chaitanya KV, Vivekanandan M (2004) Drought-induced response of photosynthesis and antioxidant metabolism in higher plants. J Plant Physiol 161:1189–1202
Rodríguez AA, Grunberg KA, Taleisnik EL (2002) Reactive oxygen species in the elongation zone of maize leaves are necessary for leaf extension. Plant Physiol 129:1627–1632
Romero-Aranda R, Bondada BR, Syvertsen JP, Grosser JW (1997) Leaf characteristics and net gas exchange of diploid and autotetraploid citrus. Ann Bot 79:153–160
Saleh B, Allario T, Dambier D, Ollitrault P, Morillon R (2008) Tetraploid citrus rootstocks are more tolerant to salt stress than diploid. Compt Rend Biol 331:703–710
Scheurwater I, Dunnebacke M, Eising R, Lambers H (2000) Respiratory costs and rate of protein turnover in the roots of a fast-growing (*Dactylis glomerata* L.) and a slow-growing (*Festuca ovina* L.) grass species. J Exp Bot 51:1089–1097
Smith IK, Vierheller TL, Thorne CA (1988) Assay of glutathione reductase in crude tissue homogenates using 5,5′-dithiobis (2-nitobenzoic acid). Anal Biochem 175:408–413
Stitt M, Hurry V (2002) A plant for all seasons: alterations in photosynthetic carbon metabolism during cold acclimation in *Arabidopsis*. Curr Opin Plant Biol 5:199–206
Sugiyama S (1998) Differentiation in competitive ability and cold tolerance between diploid and tetraploid cultivars in *Lolium perenne*. Euphytica 103:55–59
Tausz M, Šircelj H, Grill D (2004) The glutathione system as a stress marker in plant ecophysiology is a stress-response concept valid? J Exp Bot 55:1955–1962
Tewari RK, Kumar P, Tewari N, Srivastava S, Sharma PN (2004) Macronutrient deficiencies and differential antioxidant responses—influence on the activity and expression of superoxide dismutase in maize. Plant Sci 166:687–694
Tiwari BS, Belenghi B, Levine A (2002) Oxidative stress increased respiration and generation of reactive oxygen species, resulting in ATP depletion, opening of mitochondrial permeability transition, and programmed cell death. Plant Physiol 128:1271–1281
Xu W, Zhang S, Wan S, Zhang W (2008) Photosynthetic characteristics of diploid and tetraploid radish (*Raphanus sativus* L.). Chin J Eco Agric 16:164–167 (in Chinese)
Zhang J, Kirkham MB (1994) Drought-stress-induced changes in activities of superoxide dismutase, catalase, and peroxidase in wheat species. Plant Cell Physiol 35:785–791
Zhu H, Zhang X, Xue Q (2006) Rapid determination of ploidy level of chromosome in tobacco (*Nicotiana tabacum*). J Agric Biotechnol 14:255–258 (in Chinese)