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Effect of selenium and silicon on transcription factors *NAC5* and *DREB2A* involved in drought-responsive gene expression in rice

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Abstract

Drought is one of the main environmental stresses and many investigators identified beneficial effects of both silicon and selenium on plant growth and development. To examine the effects of Si and Se on rice (*Oryza sativa* L.) responses to drought, two cultivars Giza 177 and IET 1444 pretreated with 1.5 mM Si or 0.03 mM Se were then exposed to a water stress until leaf rolling was observed. The enhanced growth of Se or Si pre-treated plants was associated with a significant increase in the content of proline and glycine betaine in both shoots and roots. Furthermore, the transcription factors (TFs), dehydration responsive element-binding protein DREB2A, and NAC5 [no apical meristem (NAM), *Arabidopsis thaliana* activating factor (ATAF), and cup-shaped cotyledon (CUC)] were over-expressed in the drought stressed rice shoots. Notably, a pretreatment with either Se or Si significantly enhanced the expression of both TFs, *DREB2A*, *NAC5*, as well as the expression of the ring domain containing *OsRDCP1* gene and some drought specific genes, such as *OsCMO* coding rice choline monooxygenase and dehydrin *OsRAB16b*. Expression of TFs and the studied genes was markedly enhanced in the Si-stressed shoots of cv. IET 1444 which favors its drought tolerance.

Additional key words: dehydration responsive element, glycine betaine, *Oryza sativa*, proline, water stress.

Introduction

Water deficit is one of the most severe environmental stresses affecting agricultural productivity worldwide (Xoconostle-Cazares *et al.* 2010). Plants regularly face the adverse effects of drought by adjustment of metabolism and gene expression for physiological and morphological adaptation. There are different transcription factors controlling the response to various abiotic and biotic stresses (Yamaguchi-Shinozaki and Shinozaki 2005). Over-expression of these genes can enhance tolerance of plants to abiotic stresses (Dubouzet *et al.* 2003). Transcriptomic analyses of barley (Talame *et al.* 2007), maize (Andjelkovic and Thompson 2006, Luo *et al.* 2010), and sorghum (Buchanan *et al.* 2005) show that thousands of genes are up- or down-regulated under abiotic stresses. Moreover, expression of thousands of genes is regulated by a variety of transcriptional cascades when plants are subjected to abiotic stresses (Qin *et al.* 2004).

Plants protect themselves against drought and salinity; one way is by over-expression of some osmoprotectants namely glycine betaine (GB) and proline (Pro) (James *et al.* 1993, Ashraf and Foolad 2007, Ashraf 2010). These compatible solutes also stabilize tertiary structure of proteins and photosynthetic apparatus (Papageorgiou and Murata 1995) and detoxify reactive oxygen species (ROS) (Ashraf and Foolad 2007, Wang *et al.* 2010).

Furthermore, a variety of genes are induced by environmental stresses in many plants (Shinozaki and Yamaguchi-Shinozaki 2000, Rabbani *et al.* 2003). The products of these genes are thought to function not only in stress tolerance but also in the regulation of gene expression and signal transduction in response to stress (Xiong *et al.* 2002, Shinozaki *et al.* 2003). Transcription factors (TFs) are among the stress induced genes and play important roles *via* transcriptional regulation of downstream genes responsible for plant tolerance to stress challenges (Gao *et al.* 2007, Lucas *et al.* 2011). These regulatory systems are achieved by specific *cis*-elements in the target gene promoter regions which are termed regulons (Nakashima *et al.* 2009, Qin *et al.* 2011). The dehydration-responsive element binding protein (*DREB2*) regulon acts in heat and osmotic stress

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Abbreviations: ABA - abscisic acid; ATAF - *Arabidopsis thaliana* activating factor; CMO - choline monooxygenase; CUC - cup-shaped cotyledon, DREB - dehydration responsive element-binding protein; GB - glycine betaine; NAC - acronym derived from the genes *NAM*/*ATAF*/*CUC*; Pro - proline; RDCP - ring domain containing protein; ROS - reactive oxygen species; TFs - transcription factors; Ub - ubiquitin.

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responses (Mizoi *et al.* 2011), whereas the binding factor *NAC* regulons [no apical meristem (NAM), *Arabidopsis thaliana* activating factor (ATAF), and cup-shaped cotyledon (CUC)] function only under an osmotic stress (Nakashima *et al.* 2009, Fujita *et al.* 2011). The well-known rice TFs, OsDREBs, which regulate downstream stress-responsive genes in an abscisic acid (ABA)-independent manner, are the major osmotic stress-inducible genes responsible for osmotolerance (Dubouzet *et al.* 2003, Hussain *et al.* 2011). Expression of *OsDREB2A* was induced by drought in rice (Chen *et al.* 2008, Wang *et al.* 2008).

NACs are plant-specific proteins with several roles in plant development and stress response (Tran *et al.* 2010). There are 140 putative *NAC* or *NAC*-like genes in the rice genome. Twenty of these genes are identified as stress-responsive genes, including *OsNAC5* (Fang *et al.* 2008). These genes encodes proteins which have a role in the production of osmolytes, detoxification and redox homeostasis, and protection of macromolecules (Hu *et al.* 2008). *OsNAC5* is induced by high salinity and drought. Overexpression of *OsNAC5* in rice plants leads to accumulation of proline and soluble sugars, and less amounts of MDA and H_2O_2. These metabolic changes protect plants from dehydration and oxidative damage under stressed conditions. Thus, OsNAC5 improves the stress tolerance of rice without causing growth defects (Takasaki *et al.* 2010, Song *et al.* 2011). *OsRAB16b* coding ring domain containing protein is one of well-characterized drought resistance-related genes (Du *et al.* 2010).

Turnover of a wide range of eukaryotic proteins is regulated by the ubiquitin (Ub)-26S proteasome pathway. Multiple Ub chains are attached to the target proteins by ubiquitin ligases E1, E2, and E3 (Kraft *et al.* 2005, Stone *et al.* 2005). It has been reported previously that RING E3 Ub ligases function in drought stress responses in rice (Bae *et al.* 2011, Ning *et al.* 2011, Park *et al.* 2011). Furthermore, the *DRIP-RING* E3 Ub ligase functions as negative regulator in the drought stress response by ubiquitinating the drought-induced *DREB2A* transcription factor (Qin *et al.* 2008). Five homologues of *Oryza sativa RING* domain containing protein family members (*OsRDCP*) identified in rice possess a single *RING* motif in their N-terminal regions. Among these rice paralogues, the *OsRDCP1* was induced by drought stress. *OsRDCP1* has a role in a subset of physiological responses to counteract dehydration stress in rice plants (Bae *et al.* 2011). Moreover, the choline monooxygenase (CMO) gene encodes the important ferredoxin-dependent enzyme choline monooxygenase which catalyzes the first step in the synthesis of glycine betaine in higher plants, (Burnet *et al.*, 1995). Recently, it has been shown that over expression of rice (*Oryza sativa*) choline monooxygenase (OsCMO), enhances glycine betaine synthesis in transgenic tobacco plants and resulted in elevated tolerance to salt stress (Luo *et al.* 2012).

Searching for suitable stress alleviators is one of the main issues for plant biologists. Many investigators have identified several beneficial effects of both silicon (Si) and selenium (Se) on plant growth and development. Both are implicated in the activation of plant defense systems. Silicon is considered to be an essential element only for some higher plants (Epstein 1999), yet its beneficial effects have been demonstrated for many plants when they are subjected to a stress (Ma and Yamaji 2006, Liang *et al.* 2007). In rice, silicon treatment decreases the transpiration rate and membrane permeability (Agarie *et al.* 1998). Although Se is not an essential element (Terry *et al.* 2000), several studies indicate that at low concentration, it may exert beneficial effects (Khattab 2004, Germ *et al.* 2007). It has been found that Se has the ability to regulate water status of plants under drought conditions (Kuznetsov *et al.* 2003).

The objective of this study was to investigate the effects of Si and Se on the regulation of transcription factors *OsNAC5* and *OsDREB2A* which modulate the expression of drought stress genes, such as *OsCMO*, *OsRDCP1*, and *OsRAB16*, thus enhancing rice seedling tolerance to drought stress.

Materials and methods

Grains of two rice (*Oryza sativa* L.) cultivars Giza 177 and IET 1444 were obtained from the Agriculture Research Center, Rice Research Institute in Giza, Egypt. The grains were surface sterilized by immersing in 1 % (m/v) sodium hypochlorite solution for 5 min and then rinsed thoroughly with distilled water. The sterilized grains were divided into three equal parts, which were soaked in water, 0.03 mM sodium selenate, or 1.5 mM potassium silicate. The experiment was conducted in a naturally lit greenhouse (day/night temperatures about 27/32 ± 2 ˚C and a 14-h photoperiod) of the Botany Department, Faculty of Science, Ain Shams University. The sterilized rice grains were sown in pots (40 × 60 cm) filled with homogenous loamy soil and sufficiently irrigated for 20 d after planting. Then a drought stress was imposed on a half of each group by withholding irrigation until leaf rolling was observed as stress indicator (about 10 d), whereas an equal amount of water was given in the well-watered treatment (flooded with water about 5 cm above soil surface). This experiment was carried out in a complete randomized design with three replicates. Both shoots and roots were used for analysis of proline and glycine betaine content, whereas only shoots were used for total RNA isolation and semi-quantitative PCR analysis.

Free proline was extracted and estimated according to Bates *et al.* (1973). Briefly, fresh tissue (0.5 g) was homogenized in 10 cm^3 of 3 % (m/v) sulphosalicylic acid, the homogenate was filtered, and the filtrate (2 cm^3) was mixed with 2 cm^3 of acid ninhydrin, 2 cm^3 of glacial acetic acid, and 4 cm^3 of toluene. Absorbance was read at 520 nm using a Spectronic 601 (Milton Roy Company, USA) spectrophotometer**.** Glycine betaine content was determined following the method of Grieve and Grattan (1983). A finely ground dry plant sample (500 mg) was mechanically shaken with 20 cm^3 of distilled water at 25 °C for 24 h. The filtrate was supplied with deionized water up to 20 cm^3 and used for GB estimation using 0.2 cm^3 of cold potassium tri-iodide solution (15.7 g of iodine and 20 g of potassium iodide dissolved in 100 cm^3 of distilled water) and 9 cm^3 of 99 % 1,2-dichloroethane. The absorbance was measured at 365 nm.

Total RNA was extracted from the shoot samples of both control and stressed plants according to the method of Chomczynski and Mackey (1995) using *TrizolBioFlux*™ reagent purchased from *BioFlux* (San Francisco, USA). The RNA was treated with RNase-free DNase I (*Invitrogen*, Carlsbad, USA) at 37 °C for 30 min to remove any contaminating genomic DNA. The extracted RNA was then reverse transcribed with an *Oligo d(T)* primer using the *Revert Aid First Strand* cDNA synthesis kit (*Fermentas*, *Thermo Scientific,* USA). The rice *RAB16b* was used as a positive control for a drought induced gene, whereas *β-actin* was used as a house-keeping gene. The resulting cDNA was subjected to PCR analysis for 35 cycles (heating at 94 °C for 45 s, annealing at 58 °C for 50 s, extension at 72 °C for 90 s, and final extension at 72 °C for 10 min) using the respective primers (Table 1) designated against the *OsNAC*, *OsCMO*, *OsDREB2A, OsRab16b*, and *OsRDCP1* genes (using the primer *Premier 5.0* software). All the primers and *Dream Taq*™*Green* PCR master mix were purchased from *Invitrogen*. Amplification products were analyzed by electrophoresis in a 1.5 % (m/v) agarose gel and visualized by ethidium bromide staining. Analysis of gel images was done using the gel analyzer *Pro v. 3.1* software.

Statistical analysis was performed using the two-way analysis of variance (*ANOVA*) test following Steel and Torrie (1980). Mean values were compared using the least significance difference test (LSD) following Snedecor and Cochran (1980).

Table 1. Sequences of the primers used in PCR.

Gene	Acc. No.	Forward	Reverse	[bp]
OsRDCP1	AK070732.1	5’-GGTTCTTTGTTCTTGTCAGTGCTG-3’	5’-GGAATGGGCACACCATTCAG-3’	404
OsDREB2	AF300971	5’-TCGATGGAGCGGGGGGAGGGGAG-3’	5’-CTACTCTAATAGGAGAAAAGGCT-3’	832
OsCMO	AJ578494.1	5'- TCCTCTCTCCGATTCCCAC- 3'	5'-AAATCACTCACCAGTCACCACT-3’	1288
OsNAC5	AB028184.1	5’-CAGCAGCTGATGGTATTGTC-3’	5’-GCACAGGTAGTACATCACCA-3’	1500
OsRab16b	NM_001074375	5’-ACAAGGGCAACAACCACCAG-3’	5’-GCTTGCAATGGCATCACAAG-3’	368
OsActin	X16280	5'-CTGGGTTCGCCGGAGATGAT-3'	5'-TGAGATCACGCCCAGCAAGG-3'	501

Results

The differences in the shoot and root lengths as well as the root/shoot ratio were recorded between seedlings of cvs. Giza 177 and IET 1444 grown under well watered and water stress conditions (Table 2). A significant reduction in the shoot length was measured in the drought-stressed rice plants, particularly cv. Giza 177. In contrast, the drought stress induced an increase in root length and root/shoot ratio of both rice cultivars compared to the well-watered control. The pretreatment with either Se or Si promoted the shoot and root elongation and also root/shoot ratio of the stressed rice cultivars.

The drought stress induced the accumulation of free proline in both the shoots and roots (Table 3). The pretreatment with either Se or Si induced further accumulation of free proline in both the shoots and roots of the drought-stressed rice seedlings compared with those of their corresponding controls. The greatest content of proline was observed in the Si-treated stressed shoots of IET 1444 followed by the roots and shoots of Giza 177.

The glycine betaine content significantly increased in the shoots and roots of the plants grown under drought and especially in cv. IET. The presoaking in either Se or Si significantly increased the accumulation of glycine betaine in the shoots and roots of both the stressed rice cultivars compared with their corresponding controls. The greatest accumulation of glycine betaine was measured in the Si-treated IET 1444 shoots (Table 3).

Roots are the primary organs that perceive abiotic stress (drought) signals and later they induce a specific signal which is transduced to shoots. A background level of expression of *OsDREB2A* and *OsRDCP1* was detected in the unstressed control shoots, whereas an over-expression was observed in the stressed shoots of both the investigated cultivars, particularly in cv. IET 1444 (Fig. 1, Table 4). Notably, the exposure of the two rice cultivars to the drought stress induced the expression of *OsNAC5*, *OsCMO*, and *OsRAB16b*, and the expression was significantly higher in cv. IET 1444 than in cv. Giza 177. The Si pretreatment induced a higher increase in the expression under drought than Se. However, no increase in the expression of the investigated genes was detected in either Se or Si treated shoots grown under the well-watered conditions (Fig. 1, Table 4).

Table 2. Effects of drought and Se or Si pretreatments on the shoot and root lengths and root/shoot ratio of the rice cultivars Giza 177 and IET 1444. Means of ten replicates ± SE.

Cultivar	Treatments	Shoot length [cm]	Root length [cm]	Root/shoot ratio
Giza 177	control	32.9 ± 0.62	5.7 ± 0.62	0.17
	control + Se	29.5 ± 0.34	8.5 ± 0.34	0.29
	control + Si	30.2 ± 0.37	9.6 ± 0.37	0.32
	drought	28.3 ± 0.56	7.4 ± 0.56	0.26
	drought + Se	29.2 ± 0.76	11.6 ± 0.76	0.40
	drought + Si	30.1 ± 0.78	11.5 ± 0.78	0.38
IET 1444	control	20.2 ± 0.71	7.3 ± 0.71	0.36
	control + Se	20.3 ± 0.52	9.5 ± 0.52	0.47
	control + Si	20.8 ± 0.45	11.3 ± 0.45	0.54
	drought	19.5 ± 0.65	7.9 ± 0.65	0.41
	drought + Se	21.6 ± 0.43	9.5 ± 0.43	0.44
	drought + Si	19.9 ± 0.56	10.8 ± 0.56	0.54
LSD at 5 %	cultivars	0.35	0.24	
	treatments	0.19	0.21	
	interaction	0.49	0.34	

Table 3. Effects of drought and Se or Si pretreatments on the content of proline and glycine betaine [μg g^{-1}(f.m.)] in rice cultivars Giza 177 and IET 1444. Means of three replicates ± SE.

Cultivar	Treatments	Shoot		Root	
		proline	glycine betaine	proline	glycine betaine
Giza 177	control	74.3 ± 2.4	76.8 ± 2.5	129.9 ± 4.4	62.2 ± 2.1
	control + Se	69.1 ± 3.4	16.9 ± 0.4	58.5 ± 1.3	19.9 ± 1.4
	control + Si	55.2 ± 1.5	19.9 ± 0.9	65.7 ± 2.3	17.7 ± 0.7
	drought	107.2 ± 3.6	90.4 ± 3.2	329.6 ± 5.7	69.5 ± 2.2
	drought + Se	209.8 ± 4.6	106.7 ± 1.8	342.7 ± 6.4	74.8 ± 3.1
	drought + Si	386.8 ± 4.7	134.1 ± 2.3	459.8 ± 4.6	82.1 ± 3.3
IET 1444	control	98.6 ± 2.6	76.1 ± 2.7	107.8 ± 1.7	25.5 ± 2.2
	control + Se	79.6 ± 4.6	20.8 ± 0.5	64.4 ± 2.3	16.9 ± 1.6
	control + Si	82.3 ± 3.2	19.5 ± 1.2	93.4 ± 2.1	17.3 ± 1.4
	drought	203.2 ± 4.2	101.6 ± 1.9	126.3 ± 1.6	38.4 ± 2.2
	drought + Se	375.0 ± 2.6	124.2 ± 2.6	192.7 ± 1.6	76.9 ± 1.2
	drought + Si	517.7 ± 5.3	196.0 ± 2.8	199.6 ± 1.8	82.8 ± 2.7
LSD at 5 %	cultivars	6.63	1.61	6.46	3.49
	treatments	5.47	3.42	17.89	7.43
	interaction	9.38	2.27	9.13	4.93

Discussion

Some genes induced during stress encode proteins/enzymes directly protecting the plant cell, whereas other are involved in stress signaling cascades (Blumwald *et al.* 2004), *e.g.*, TFs which can modulate and regulate various stress-inducible genes (Bartels and Sunkar 2005). When plants are subjected to a drought stress, the growth of shoots is rapidly reduced (Nonami and Boyer 1990, Chazen and Neumann 1994). In contrast, roots may continue to elongate under mild drought (Spollen *et al.* 1993). The increase in root length under drought stress has been attributed to their osmotic adjustment ability (Rauf and Sadaqat 2008). The drought stress increased the root/shoot ratio in both the rice cultivars and especially in cv. IET 1444. Similarly, the root/shoot ratio of tomato plants is higher in drought stressed seedlings compared to control plants (Mingo *et al.* 2004). This differential response of roots and shoots to water deficit is considered to be an adaptation of plants to dry conditions since continued root elongation facilitates water uptake from the soil (Djibril *et al.* 2005).

The tolerance mechanism is associated with an accumulation of some osmolytes, such as proline and glycine betaine, which decrease cellular osmotic potential resulting in maintenance of pressure potential (Mahajan

Table 4. Effects of drought and Se or Si pretreatments on the relative expression of OsDREB2A, OsNAC5, OsRDCP1, OsCMO and OsRab16b in the two rice cultivars Giza 177 and IET 1444. Means of three replicates ± SE.

Cultivar	Treatments	OsDREB2A	OsNAC5	OsRDCP1	OsCMO	OsRab16b
Giza 177	control	10 ± 0.9	0 ± 0	17 ± 0.7	0 ± 0	0 ± 0
	control + Se	11 ± 0.8	0 ± 0	18 ± 0.6	0 ± 0	40 ± 1.2
	control + Si	10 ± 1.0	0 ± 0	19 ± 0.5	0 ± 0	0 ± 0
	drought	20 ± 1.2	20 ± 0.5	40 ± 2.1	45 ± 1.2	71 ± 2.1
	drought + Se	52 ± 2.1	56 ± 2.6	55 ± 1.2	60 ± 2.2	0 ± 0
	drought + Si	60 ± 2.5	68 ± 2.7	62 ± 2.6	69 ± 3.5	60 ± 2.8
IET 1444	control	11 ± 2.4	0 ± 0	18 ± 0.4	0 ± 0	0 ± 0
	control + Se	10 ± 0.6	0 ± 0	18 ± 0.6	0 ± 0	58 ± 1.9
	control + Si	10 ± 0.4	0 ± 0	17 ± 0.7	0 ± 0	0 ± 0
	drought	55 ± 1.4	62 ± 2.7	55 ± 3.2	58 ± 4.3	78 ± 3.2
	drought + Se	62 ± 1.8	84 ± 3.2	70 ± 1.2	70 ± 3.5	0 ± 0
	drought + Si	82 ± 2.2	95 ± 3.9	76 ± 1.4	79 ± 3.8	75 ± 4.5
LSD at 5 %	cultivars	1.96	0.96	1.42	0.86	1.60
	treatments	1.82	1.10	2.52	0.97	0.96
	interaction	2.57	1.55	3.57	1.38	1.95

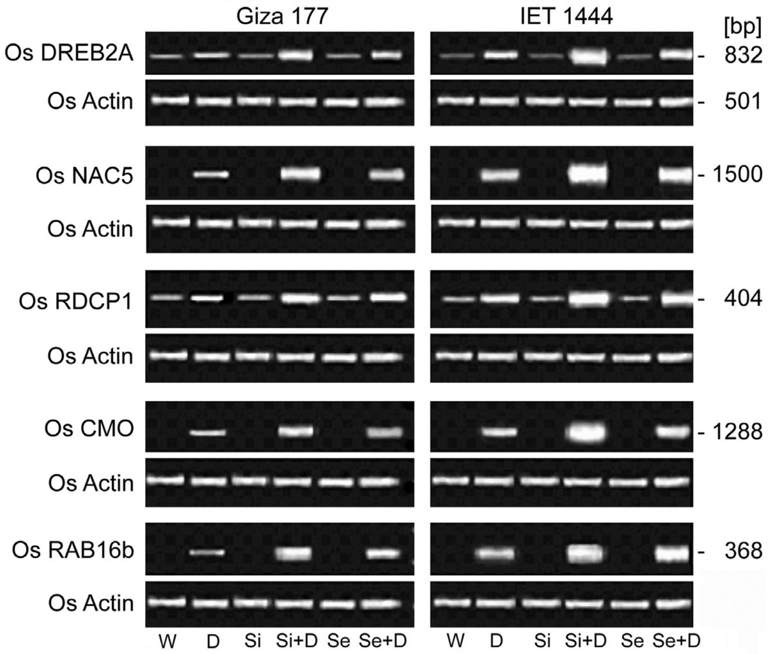

Fig. 1. PCR analyses of two TFs (OsDREB2A and OsNAC5) and two downstream genes responsible for plant tolerance to drought (OsRDCP1 and OsCMO) in shoots of two rice cultivars Giza 177 and IET 1444. OsRab16b was used as positive control for drought induced genes and actin was used as loading control. *Lanes*: W - well-watered control, D - drought, Si - control pretreated with Si, Si + D - drought stressed shoots pretreated with Si; Se - control pretreated with Se, Se + D - drought stressed shoots pretreated with Se.

and Tuteja 2005, Trovato *et al.* 2008). These compounds also contribute to detoxification of ROS, protection of membrane integrity, and stabilization of proteins (Ashraf and Foolad 2007, Liu *et al.* 2011). In some studies, the accumulation of proline under stress has been positively related to stress tolerance (Nayyar and Walia 2003) and might be taken as a parameter for selection of stress tolerance (Jaleel *et al.* 2007, Mafakheri *et al.* 2010). However, other studies reported that a proline accumulation is a symptom of stress damage rather than to be an indicator of stress tolerance (De Lacerda *et al.* 2003). Water deficit stimulated the accumulation of free proline in both the shoots and roots of the investigated rice cultivars. The proline content in the IET 1444 stressed shoot was higher than that in Giza 177. In contrast, a higher proline content was measured in the stressed root of Giza 177. The Se or Si pre-treatment stimulated a massive accumulation of proline. The accumulation of free proline is determined by its biosynthesis, catabolism, and transport between different compartments (Szabados and Savoure 2010). It has been also reported that genes involved in proline biosynthesis are highly expressed under stress conditions (Armengaud *et al.* 2004). In addition, an increase in the glycine betaine content was observed in the shoots and roots of rice plants grown under the water stress. Such increments were more pronounced in the IET 1444 cultivar compared to those of the Giza 177. Similar results have been obtained by Chaitanya *et al.* (2010) in mulberry. Glycine betaine is an inert molecule and protects the oxygen-evolving photosystem II complex and ATP synthesis in onion and rice (Mansour 1998, Rahman *et al.* 2002). The accumulation of GB in rice can be expected to be useful in efforts to improve abiotic stress tolerance and productivity (Shirasawa *et al.* 2006). The pre-treatment with either Se or Si significantly enhanced the accumulation of GB, particularly in the stressed shoots of IET 1444.

Over-expression of TFs can modulate a wide range of signaling pathways leading to stress tolerance (Chaves and Oliveira 2004, Umezawa *et al.* 2006). In the present study, the TF *OsDREB2A* was weakly expressed in the control plants and was stimulated by dehydration. The pretreatment with either Se or Si up-regulated the expression of *OsDREB2A* in the two drought-stressed rice cultivars compared to the untreated and drought stressed ones. Such results might be attributed to the enhancement of phosphorylation of the *DREB2A* protein to their active forms in the Se and Si pre-treated shoots. The over-expression of *OsDREB2A* in rice can protect cells during drought stress (Matsukura *et al.* 2010). In addition, the expression of the *OsNAC5* was not detected in all the well-watered groups, but it was detected in both the untreated and pre-treated stressed shoots. Notably, the expression was much higher in the Si and Se pre-treated and drought stressed plants. Thus, *OsNAC5* improved the stress tolerance of the two investigated rice cultivars, particularly IET 1444. These results are in accordance with Takasaki *et al.* (2010) and Song *et al.* (2011). The overexpression of *OsNAC5* resulted in an enhanced stress tolerance by up-regulating the expression of stress inducible rice genes such as *LEA3* (Takasaki *et al.* 2010).

OsRDCP1, *OsCMO*, and *OsRAB16b* are stress responsive genes which were up-regulated under the drought stress. Both Se and Si stimulated the expression of these genes in the drought-stressed shoots compared to the Se and Si untreated ones. A background expression of *OsRDCP1* was detected in the unstressed control shoots, whereas *OsCMO* was not expressed at all. The over-expression of *OsRDCP1* under drought may be involved in the inactivation or degradation of water stress-related proteins which would result in high stimulation of defense protein synthesis in rice (Park *et al.* 2011). In the present study, the expression of these genes was higher in cv. IET 1444 compared to cv. Giza 177. Their highest expression was observed in the Si-pretreated shoots. These results might be due to difference in Si and Se uptake. It was deduced that increasing the resistance of plants to various stresses is related to the amount of Si uptake and accumulation in plant organs. Plants differ widely in the amount of Si uptake (Mitani and Ma 2005). Furthermore, a large amount of Si is required for high and sustainable rice production (Savant *et al.* 1997).

In higher plants, the choline monooxygenase (CMO) gene encodes the important ferredoxin-dependent enzyme choline monooxygenase which catalyzes the first step in the synthesis of glycine betaine (Burnet *et al.* 1995). It was reported that *OsCMO* plays an important role in rice responses to abiotic stresses (Luo *et al.* 2012). It was also postulated that the *OsCMO* genes might be regulated by environmental stresses under the control of *DREB* transcription factors (Shinozaki *et al.* 2003). This is consistent with our current results showing that over-expression of *OsCMO* under the drought stress conditions was concomitant with the accumulation of glycine betaine in the rice-stressed shoots. Rice is considered a typical non glycine betaine accumulation species under normal growth conditions due to the presence of an inactive gene for *CMO* which becomes active under stress conditions (Rathinasabapathi *et al.* 1993, Shirasawa *et al.* 2006). In addition, *OsRAB16b* is one of *LEA* genes that are induced also in vegetative and reproductive tissues in response to drought, salinity, extreme temperatures, or by exogenous ABA (Tunnacliffe and Wise 2007, Bies-Ethève *et al.* 2008). *LEA* genes encode *LEA* proteins which display a role in the adaptation of plants to stresses. The expression of several groups of *LEA* genes during an environmental stress, and their role in stress tolerance has been recently reported in several species. (Lenka *et al.* 2011).

In conclusion, the present study demonstrates that the Si and Se pre-treatments of the rice cultivars could mitigate the adverse effects of the drought stress. Interestingly, both Si and Se enhanced the stress-induced expression of *OsCMO, OsRab16b*, and *OsRDCP1 via* the activation of the *DREB2A* and *NAC* TF pathways. This activation led to the production of important osmo-protectants proline and glycine betaine and consequently to the enhanced rice tolerance to the drought stress.

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