DOI: 10.1007/s10535-017-0761-4 BIOLOGIA PLANTARUM 62 (2): 307-317, 2018

Osmotic stress affects growth, content of chlorophyll, abscisic acid, Na^+, and K^+, and expression of novel *NAC* genes in contrasting rice cultivars

S. GARCÍA-MORALES[1], F.C. GÓMEZ-MERINO[1]*, L.I. TREJO-TÉLLEZ[2], L. TAVITAS-FUENTES[3], and L. HERNÁNDEZ-ARAGÓN[3]

Campus Córdoba, Colegio de Postgraduados, 94946 Amatlán de los Reyes, Veracruz, Mexico[1]
Campus Montecillo, Colegio de Postgraduados, 56230 Montecillo, Mexico[2]
Campo Agrícola Experimental Zacatepec, Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias, 62780, Zacatepec, Morelos, Mexico[3]

Abstract

Osmotic stress causes a series of morphological, physiological, biochemical, and molecular changes that alters plant growth, development, and productivity around the globe. Phytohormones, nutrients, and transcription factors may induce adaptive responses to osmotic stress in plants. We evaluated the effect of osmotic stress induced by 18.5 % polyethylene glycol (PEG) or 100 mM NaCl on growth, content of abscisic acid (ABA), chlorophyll (Chl), sodium, and potassium, and the expression of multifunctional *NAC* transcription factors in rice cultivars (the salt-tolerant Cotaxtla and salt-sensitive Tres Ríos). The PEG and NaCl decreased shoot height and increased ABA content in both cultivars, and reduced root length in cv. Tres Ríos. The PEG increased Chl content in cv. Cotaxtla leaves. NaCl reduced shoot K^+ content in cv. Tres Ríos and increased shoot and root Na^+ content in both cultivars, thus resulting in a decreased K^+/Na^+ ratio. Of the 57 *NAC* genes evaluated, two of them were repressed (*Os10g42130* and *Os07g04560*) and two other induced (*Os02g34970* and *OsNAC10*) in cv. Cotaxtla in response to PEG, whereas three of them were repressed (*Os10g42130, Os07g04560* and *Os08g10080*), and six induced (*Os02g56600, Os02g34970, Os11g08210, Os05g34830*, *OsNAC6,* and *OsNAC10*) in response to NaCl. In the cv. Tres Ríos, we found two genes repressed (*Os10g42130* and *Os07g04560*), and five induced (*Os08g33910, Os03g60080, Os06g51070*, *OsNAC6*, and *OsNAC10*) in response to PEG, while only two genes were repressed (*Os10g42130* and *Os07g04560*) but 13 induced (*Os03g21060, Os08g39110, Os03g60080, Os01g15640, Os06g51070, Os09g33490, Os04g40130, Os12g29330, Os02g36880, Os11g08210, Os05g34830, OsNAC6*, and *OsNAC10*) by NaCl. Osmotic stress affected more severely cv. Tres Ríos than cv. Cotaxtla plants. These different responses might be regulated by ABA and NAC transcription factors.

Additional key words: NaCl, *Oryza sativa*, polyethylene glycol, RT-PCR, transcription factors.

Introduction

Drought and salinity can trigger cellular dehydration in plants. In addition to osmotic stress, salinity counteracts plant growth due to the toxic effect of Na^+ within the cell (Munns and Tester 2008). Adaptations to stresses are regulated by genes that alter plant metabolism and growth (Conde *et al*. 2011). One of the most important responses to osmotic stress is the biosynthesis of the phytohormone abscisic acid (ABA). Osmotic stress triggers ABA-dependent and ABA-independent signaling pathways leading to different responses of plants to drought and salinity (Agarwal and Jha 2010).

At the molecular level, responses to osmotic stress are of multigenic character. Thanks to high throughput approaches, numerous genes that respond to osmotic stress have been reported. It has been found that some of them are involved in protection of plants from dehydration by regulating signal perception, transduction, and response networks (Pandey and Shukla 2015). Transcription factors (TF) are key proteins that regulate gene expression at the transcription level (Shiriga

Submitted 5 June 2016, *last revision* 15 May 2017, *accepted* 21 June 2017.
Abbreviations: ABA - abscisic acid; *NAC* - *NAM*, A*TAF*, and *CUC*; PEG - polyethylene glycol; RT- qPCR - reverse transcription quantitative PCR; TF - transcription factor.
Acknowledgments: SGM acknowledges the Mexico National Council of Science and Technology (CONACYT) for the Ph.D. fellowship awarded. The authors also acknowledge Prof. Ramón Marcos Soto and M. Sc. Rubén San Miguel for technical assistance in determining ABA concentration by HPLC.
* Corresponding author; e-mail: fernandg@colpos.mx

et al. 2014). A single TF can control the expression of many target genes through its specific binding to *cis*-acting elements located in the promoter of the respective target genes (Nakashima *et al*. 2007).

The *NAC* (*NAM*, *ATAF*, and *CUC*) gene family is one of the TF families found only in plants. It contains a highly conserved DNA-binding domain located in the N-terminal region and a diversified activation domain at the C-terminal region which can act as either a transcriptional activator or repressor (Shen *et al*. 2009). NAC TF are multi-functional proteins with various roles in the life cycle of plants, such as cotyledon development (Aida *et al*. 1997), lateral root development (He *et al*. 2005), flower formation (Sablowski and Meyerowitz 1998), hormone signaling (Greve *et al*. 2003), pathogen infection (Nakashima *et al*. 2007, Shan *et al*. 2016), cell division (Kim *et al*. 2006), seed development (Sperotto *et al*. 2009), senescence (Balazadeh *et al*. 2008), and response to various abiotic stresses (Hu *et al*. 2006, Nakashima *et al*. 2007, Shiriga *et al*. 2014). Bioinformatic analyses have predicted the existence of *NAC* genes in important crop and model plants, including 163 genes in poplar, 152 each in soybean, maize and tobacco, 151 in rice, 117 in *Arabidopsis thaliana*, 96 in cassava, 82 in melon, 79 in grape and mulberry, 77 in cotton, 48 in barley, 37 in maritime pine and 26 in citrus (Christiansen *et al*. 2011, Le *et al*. 2011, Nuruzzaman *et al*. 2010, 2015, Shiriga *et al*. 2014, Wei *et al*. 2016, Zhao *et al*. 2016). Importantly, an increasing number of *NAC* genes have been characterized so far. In rice, a *NAC* gene *SNAC1* has been identified in response to osmotic stress; overexpression of this gene lead to increased drought tolerance by modulating stomatal closure (Hu *et al*. 2006). Another gene, *OsNAC6/SNAC2*, is induced by abiotic stress and jasmonic acid and overexpression of this gene results in an increase in rice tolerance to cold, drought, and salinity (Hu *et al*. 2008). Further, the *OsNAC6* gene functions as a transcriptional activator in response to biotic and abiotic stresses in plants. Rice plants that constitutively overexpress the *OsNAC6* gene show increased tolerance to dehydration and salinity (Nakashima 2007). Overexpression of the *OsNAC045* gene also results in increased tolerance to drought and salinity (Zheng *et al*. 2009). Root-specific overexpression of *OsNAC10* increases root development, improves drought tolerance, and significantly increases grain yield of rice plants under field drought (Jeong *et al*. 2010). Similarly, the overexpression of *OsNAC5* results in higher tolerance to drought and increased rice grain yield under field conditions (Jeong *et al*. 2013). Importantly, recent reports confirm the pivotal role of NAC TF in osmotic stress tolerance not only in rice (Fang *et al*. 2015**,** Ghosh *et al*. 2016**,** Hong *et al*. 2016), but also in other species including *Arabidopsis* (Chen *et al*. 2016**,** Oda-Yamamizo *et al*. 2016, Sakuraba *et al*. 2016), cotton **(**Liu *et al*. 2014**)**, chickpea (Yu *et al*. 2016), and wheat (Zhang *et al*. 2016). Moreover, NAC proteins have been implicated in fruit maturation in tomato (Kou *et al*. 2016), pathogen resistance in banana (Shan *et al*. 2016), and early aging in cotton (Zhao *et al*. 2016).

In this study we aimed to evaluate the effect of osmotic stress caused either by 18.5 % PEG or 100 mM NaCl on plant growth, endogenous ABA, content of Na^+ and K^+ in leaves and roots, as well as the expression of *NAC* TF in order to determine the possible mechanisms that differentiate responses between contrasting rice cultivars. We also aimed to generate data on novel *NAC* genes as potential candidates to develop tolerance to osmotic stress in crop plants in the future.

Materials and methods

We used two rice (*Oryza sativa* L. ssp. *indica*) cultivars, Cotaxtla and Tres Ríos, obtained from the Germplasm Bank of the National Institute of Forestry, Agriculture and Livestock Research (INIFAP), Zacatepec Experimental Field, Mexico (18˚39' N.L. , 99˚12' W.L., 910 m asl). The experiment was conducted in a sawtooth-type greenhouse at the Colegio de Postgraduados Campus Montecillo Experimental Station (19˚20' N.L., 98˚53' W.L., 2250 m asl). The seeds of both cultivars were surface sterilized and germinated before sowing, and 14 d after being planted, seedlings were transferred to vessels with 9 dm^3 of Yoshida nutrient solution (Yang *et al*. 1994), according to the procedure described previously (García-Morales *et al*. 2012). We evaluated the application of 18.5 % (m/v) PEG 6000 and 100 mM NaCl; the both treatments having an osmotic potential of -0.44 MPa. The application of treatments began 20 d after transferring plants to hydroponic solution; control plants grew simultaneously in Yoshida nutrient solution with an osmotic potential of -0.035 MPa. Osmotic potential was determined by the Van´t Hoff osmotic pressure equation, using a *VAPRO*® vapor pressure osmometer (*Wescor,* Logan, UT, USA) (Bartlett *et al*. 2012).

For gene expression analysis, samples were taken 6 h after application of treatments. The second and third fully expanded leaves were sampled, and leaves of three plants were mixed and considered as a biological replicate. Total RNA was isolated using the *SV Total* RNA isolation kit (*Promega*, Madison, WI, USA), which includes treatment with DNAse I. For reverse transcription, the enzyme *SuperScript III*^{TM} *RT (Invitrogen*, Carlsbad, CA, USA) was used. We selected 57 TF primer pairs of the NAC family from a platform reported by Caldana *et al*. (2007). In addition, the *OsNAC10* gene (*Os11g03300*; *AK069257*) was included as a positive control of the expression (Jeong *et al*. 2010). Reverse transcription quantitative PCR (RT-qPCR) was performed as described by Caldana *et al*. (2007) and García-Morales *et al*. (2014) in an *ABI PRISM 7900 HT* sequence detector system (*Applied Biosystems*, Foster

City, CA, USA), using *SYBR Green PCR Master Mix* 2× (*Life Technologies*, Carlsbad, CA, USA), 250 nM of each gene-specific primer, and 10 ng of cDNA template. The gene *elongation factor 1α* (*Os03g55270*) was used as a reference gene for gene expression analysis. All analyses were performed with three technical replicates and three biological replicates. The relative expression of each gene was calculated according to the $2^{-\Delta\Delta Ct}$ method (Schmittgen and Livak 2008). In accordance with Le *et al*. (2011), and considering the biological significance of the differential expression in this study, we adopted a cut-off value of 2-fold when analyzing stress induction or repression.

The plants were harvested 6 d after application of treatments, separated into shoots and roots, and their length and fresh matter (f.m.) were determined. Root length was measured from the crown of the root to the tip of the longest root. Shoot height was measured from the crown of the plant to the apex of the flag leaf. Dry matter (d.m.) was estimated 48 h after drying the samples at 70 °C in a forced-air circulation oven.

The content of ABA was determined in the 2nd and 3rd leaves, 48 h after application of treatments. Then, 50 mg of lyophilized material was taken and 2 cm^3 of 90 % (v/v) acetone solution was added. Subsequently, the samples were shaken in an ultrasonic bath at 10 °C for 12 min following the protocol described by Olivella *et al*. (2001). The extracts were separated by thin layer chromatography (TLC, silica gel *60 F254 Multiformat* pre-scored to 5 × 5, *Merk*, Darmstadt, Germany) and then analyzed by high performance liquid chromatography (HPLC) (*Agilent Technologies*, Waldbronn, Germany) at 266 nm. In the separation, the reverse phase was used in the *SB-C8* column (250 × 4.6 mm, 5 µm, *Zorbax,* Santa Clara, CA, USA). The mobile phase was a mixture of acetonitrile (40 %, v/v) and 0.01 % (v/v) trifluoroacetic acid (40:60) at pH 2.95 under an isocratic method, and a flow rate of 1 cm^3 min^{-1}. Chlorophyll content was determined spectrophotometrically according to Arnon (1949) using 90 % (v/v) acetone solution for extraction.

To determine content of Na^+ and K^+, plants were divided into shoots and roots. The dried samples were ground, weighed, and subjected to wet digestion with a mixture of perchloric and nitric acids; extracts were read on an inductively coupled plasma-optic emission spectrometer (*725-ES* model*, Agilent ICP-OES*, Mulgrave, Victoria, Australia).

The results were analyzed using the *SAS/STAT* statistical package (*v*. 9.1; *SAS Institute Inc*., Cary, NC, USA). All data are presented as means ± standard deviations (SDs) from at least three independent replicates. Significant differences between treatments were calculated by two-way *ANOVA* following Tukey's test at $\alpha = 0.05$.

Results and discussion

The effects of osmotic stress caused by the application of 18.5 % PEG 6000 and 100 mM NaCl in hydroponics were tested in rice cultivars Cotaxtla and Tres Ríos. We previously reported that these cultivars display contrasting responses to salinity (García-Morales *et al*. 2012, 2014), being cv. Cotaxtla salt tolerant and cv. Tres Ríos salt sensitive. The plants (30-d-old) were subjected to both stresses for 6 d and shoot height, root length, as well as shoot and root dry matter were determined (Fig. 1*A-D*). Water content was reduced in both cultivars exposed to PEG to 3.15 g(H_2O) g^{-1}(d.m.) in comparison to control plants, where it was 3.69 g(H_2O) g^{-1}(d.m.), whereas plants exposed to NaCl displayed water content of 3.53 g(H_2O) g^{-1}(d.m.) similar to control plants. In both cultivars, shoot height was adversely affected (Fig. 1*A*) and a lower height was observed in plants under osmotic stress. Regarding root length, only in the cv. Tres Ríos a significant reduction in root length under osmotic stress compared to the control was found (Fig. 1*B*). In previous studies, we reported similar results in rice seedlings exposed to 100 mM NaCl, where the cv. Cotaxtla plants maintained root growth, whereas in the cv. Tres Ríos it was significantly reduced (García-Morales *et al*. 2012, 2014). Furthermore, here we found that the osmotic stress caused by 18.5 % PEG also reduced shoot height in both cultivars, whereas root length was only affected in the cv. Tres Ríos. Similarly, it was found that both 18.5 % PEG and 100 mM NaCl reduced shoot dry mass in both cultivars (Fig. 1*C*). In the cv. Cotaxtla, root dry mass was significantly reduced only by the exposure to NaCl, probably due to the toxic effect of Na^+, whereas in the cv. Tres Ríos both stress agents significantly reduced root dry mass (Fig. 1*D*). This lower biomass production has also been reported in the rice genotypes Zhenshan97B and IRAT109, which were subjected to osmotic stress for 20 d, resulting in a significant decrease in root dry mass and plant height. However, the genotype IRAT109 showed greater root growth and root depth, which could be considered as a tolerance mechanism against drought (Ji *et al*. 2012). The fact that the shoot growth was found to be more affected than the root growth is consistent with other studies. Accordingly, maintaining root growth under osmotic stress conditions is considered an important feature of adaptation to stress, due to increasing the ability to absorb water from greater depth and thus securing the survival of the whole plant (Rodrigues *et al*. 1995, Ji *et al*. 2014). Souleymane *et al*. (2016) evaluated 20 rice genotypes under salinity, showing that the most tolerant ones produced more dry matter during the vegetative growth, which is in agreement with our results (Fig. 1*C*,*D*). Thus, manipulating the growth of roots enables the development of new biotechnological strategies, which in turn can help mitigate the impact of abiotic stressors related to dehydration.

It has been found that the endogenous ABA content increases under stresses such as drought and salinity (Du

et al. 2010). Accordingly, here we found a significant increase in ABA content in both cultivars grown in PEG or NaCl as compared to the control. The highest ABA content was in the cv. Tres Ríos treated with 100 mM NaCl. Similar results have been obtained in other rice cultivars. For example, Kumar *et al*. (2013) found that in the cv. Moroberekan exposed to drought stress for 3 d, ABA content increased dramatically in rice peduncles. A similar increase in the ABA content was observed in leaves of the wheat cvs. CS and SQ1 under osmotic stress induced by PEG for 7 d (Marcinska *et al*. 2013).

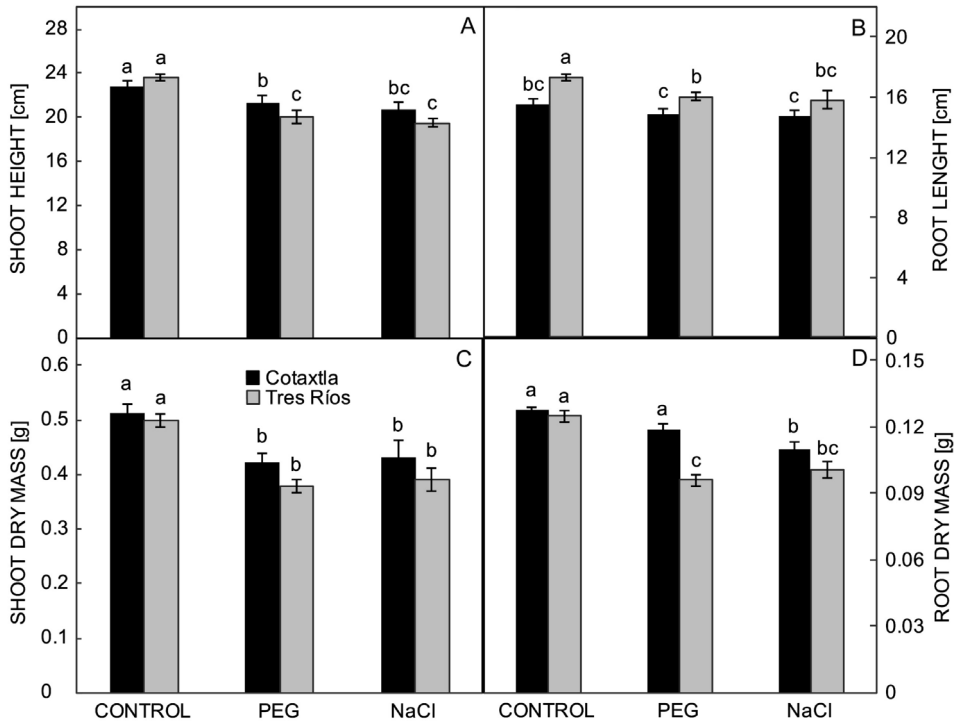

Fig. 1. Shoot height (*A*), root length (*B*), shoot dry mass (*C*), and root dry mass (*D*) in seedlings of the rice cultivars Cotaxtla and Tres Ríos. The seedlings (30-d-old) were grown hydroponically under two osmotic stress agents (18.5 % PEG or 100 mM NaCl) for 6 d. Means ± SDs, $n = 9$. Different letters indicate statistically significant differences at $\alpha \leq 0.05$ according to Tukey's test.

Total Chl content was also determined. The Chl content in cv. Cotaxtla significantly increased when it was exposed to PEG for 48 h. By contrast, the cv. Tres Ríos showed no changes in Chl content under stress conditions (Fig. 2*B*). Conversely, Shobbar *et al*. (2012) reported greater adverse effects caused by salinity (150 mM NaCl) and osmotic stress (100 and 180 mM mannitol) on Chl content in the sensitive genotype IR29 than in the tolerant genotype FL478. These differences can be attributed to the NaCl concentrations used (*i.e*., 100 mM *vs.* 150 mM), the osmotic agents used (*i.e*., mannitol *vs.* PEG), and genotypes. Paradoxically, ABA has been reported as a senescence inducer (Criado *et al*. 2007) and senescence is characterized by the breakdown of Chl and disintegration of chloroplasts (Balazadeh *et al*. 2008). However, the onset and progress of this process can be influenced by different growth regulators and stress conditions (Sperotto *et al*. 2009), among which osmotic stress caused by drought and salinity stands out. In our study, this relationship was not found, since the cv. Tres Ríos (stress sensitive) had the highest ABA content and showed no change in Chl content. However, the increase in Chl content could be also explained as a compensatory mechanism to maintain photosynthetic activity under osmotic stress (Shobbar *et al*. 2012). Although ABA may trigger complex biochemical networks in response to abiotic stresses, including chloroplast disintegration, reduced photosynthetic activity, and senescence, plant cells may counteract such damage by activating different tolerance mechanisms such as the induction of TF gene expression. In fact, the genes *ONAC022* (Hong *et al*. 2016), *ANAC046* (Oda-Yamamizo *et al*. 2016), *NAC016* (Sakuraba *et al*. 2016), *VviNAC1* (Rattanakon *et al*. 2016), and *GhNAC12* (Zhao *et al*. 2016) mediate ABA-dependent stress responses, whereas the gene *SNAC3* regulates stress responses in an ABA-independent manner (Fang *et al*. 2015).

In the cv. Cotaxtla, the shoot K^+ content remained without significant changes in PEG solution compared to the control, although an evident increase under 100 mM NaCl was observed. In the cv. Tres Ríos, the K^+ content was also unaffected by PEG, but a significant decrease was found with 100 mM NaCl (Fig. 3*A*). Similar results were observed in cv. IR64, wherein the K^+ content is unaffected by PEG, but it is significantly reduced under

NaCl treatment (Castillo *et al*. 2007). On the other hand, the results observed in the present study are consistent with the findings previously reported by our working group (García-Morales *et al*. 2012). Accordingly, under salt stress conditions, the K^+ content in shoots increased in the cv. Cotaxtla, whereas in the cv. Tres Ríos it was significantly reduced. Regarding K^+ content in roots, it decreased significantly in both cultivars after the application of NaCl or PEG (Fig. 3*B*).

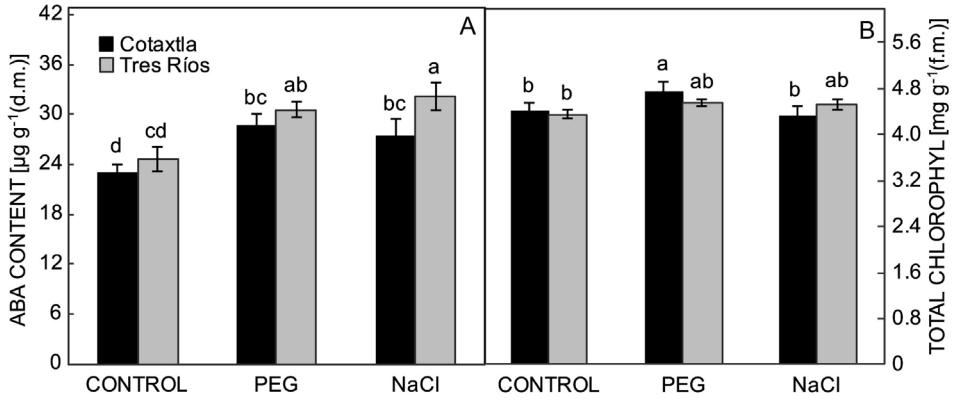

Fig. 2. ABA (*A*) and total chlorophyll (B) content in leaves of rice cultivars Cotaxtla and Tres Ríos. The seedlings (30-d-old) were grown hydroponically under two osmotic stress agents (18.5 % PEG or 100 mM NaCl) for 48 h, then the first two newly-developed leaves were taken for analysis. Means ± SDs, $n = 3$. Different letters indicate statistically significant differences at $\alpha \leq 0.05$ according to Tukey's test.

As expected, a significant increase in shoot and root Na^+ content was observed under salt stress in both cultivars (Fig. 3C,*D*). However, the root Na^+ content was higher in the cv. Cotaxtla (Fig. 3*D*) than in the cv. Tres Ríos. This result is similar to that observed in rice cultivars IR29 and FL478 exposed to 150 mM NaCl and different concentrations of mannitol (Shobbar *et al*. 2012). Furthermore, Castillo *et al*. (2007) also reported increased shoot Na^+ content in the cv. IR64 grown under salt stress, but no effect of PEG on Na^+ absorption.

Under suitable environmental conditions, plants maintain a high K^+/Na^+ ratio, and strategies to keep this ratio high include the exclusion of cytosolic Na^+ and/or Na^+ compartmentalization, mainly into the vacuole (Blumwald 2000). Plants exposed to salinity for prolonged periods tend to show symptoms of K^+ deficiency due to either decreased absorption or less retention of this nutrient in the different tissues, and at the same time plants exhibit a greater accumulation of Na^+. This leads to a decrease in the K^+/Na^+ ratio and thus a disruption of some physiological and biochemical processes, especially in actively growing plant tissues (Chakraborty *et al*. 2016). Therefore, the maintenance of a high K^+/Na^+ ratio is an important mechanism of tolerance to salinity (Wakeel 2013, Shabala *et al*. 2015). A decrease in the cytosolic K^+/Na^+ ratio under salt stress is characterized by an increase in the Na^+ influx and a K^+ efflux from plant cells (Wakeel 2013). Therefore, under salt stress conditions, higher K^+ availability favors the activity of high affinity potassium transporters (HKTs) and non-selective cation channels (NSCCs) resulting in increased K^+ uptake by minimizing the Na^+ uptake and avoiding the K^+ efflux from the cells to maintain an optimal K^+/Na^+ ratio for plant metabolism (Chakraborty *et al*. 2016). The maintenance of K^+ homeostasis is essential for enzymatic activity and represents a pivotal adaptive characteristic of plants tolerant to salinity (Hanin *et al*. 2016). In our experiments, the shoot and root K^+/Na^+ ratio was drastically reduced in both cultivars by the application of 100 mM NaCl (Fig. 3*E*,*F*). It was not possible to identify differences between the cultivars, because the NaCl concentration and the exposure time were not high enough to reach a toxic Na^+ level. Nevertheless, when considering the root Na^+ content, we observed that the cv. Cotaxtla had a higher root Na^+ content than cv. Tres Ríos (Fig. 3*D*), which is not reflected in a higher Na^+ content in the shoot (Fig. 3*C*). This response could indicate that the cv. Cotaxtla is capable of re-translocating Na^+ into the culture medium or compartmentalizing it into the root vacuoles and thus avoiding a toxic effect on the shoot, as has been reported in the cv. IR64 (Castillo *et al*. 2007). Furthermore, in order to maintain a higher K^+/Na^+ ratio, a crucial response is the activation of K^+ channels, such as AKT1. This sort of channels, as well as other transporters involved in Na^+ exclusion, tend to increase the tolerance to salinity, since they maintain an osmotic balance in plant cells (Mekawy *et al*. 2015). According to Ahmad *et al*. (2016), the overexpression of the rice *OsAKT1* potassium channel increases K^+ uptake, which is beneficial for plants growing under osmotic stress induced by PEG (5 - 10 %). Instead, when plants are exposed to NaCl (60 - 70 mM) no differences concerning K^+ or Na^+ uptake are observed. Conversely, we did find differences regarding K^+ and Na^+ content, which might be due to the higher NaCl concentration tested (100 mM NaCl), but also by the different genotypes evaluated.

For *NAC* TF gene family expression analysis,

30-d-old rice seedlings exposed to 18.5 % PEG or 100 mM NaCl for 6 h were used. Here we compare the expression of 57 TF-encoding genes of the NAC family. In the cv. Cotaxtla expressions of four genes were changed by 18.5 % PEG, two of them repressed (*Os10g42130* and *Os07g04560*) and the other two induced (*Os02g34970* and *OsNAC10*); in addition, nine genes were regulated by 100 mM NaCl, three of them repressed (*Os10g42130, Os07g04560*, and *Os08g10080*) and the other six genes induced (*Os02g56600, Os02g34970, Os11g08210, Os05g34830*, *OsNAC6,* and *OsNAC10*). In the case of the cv. Tres Ríos, we found seven genes regulated by PEG, two repressed (*Os10g42130* and *Os07g04560*) and five induced (*Os08g33910, Os03g60080, Os06g51070*, *OsNAC6*, and *OsNAC10*), as well as 15 genes regulated by NaCl, of which only two genes were repressed (*Os10g42130* and *Os07g04560*) and the rest induced (Table 1).

We found that the genes *Os10g42130* and *Os07g04560* were repressed in both cultivars and under both stresses. In the case of the *Os07g04560* gene, we had previously reported its repression in the leaves of both cultivars exposed to 100 mM NaCl for 3 and 6 h (García-Morales *et al*. 2014). Similarly, Nuruzzaman *et al*. (2012) reported the down-regulation of this gene in roots of rice plants exposed to severe drought. Furthermore, the *Os10g42130* gene has been reported to be regulated by different biotic and abiotic stresses

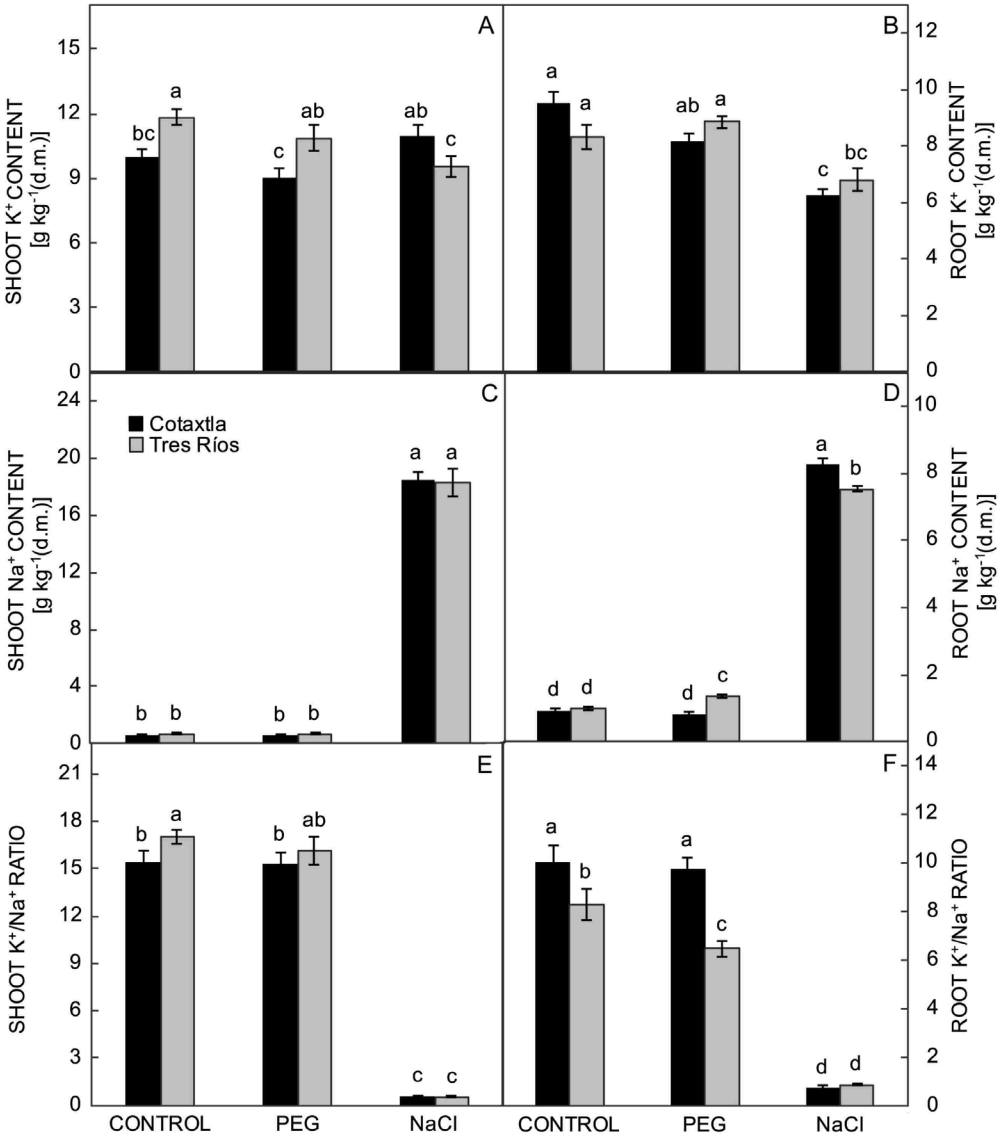

Fig. 3. Shoot K^+ (*A*) and root K^+ (*B*) content, shoot Na^+ (*C*) and root Na^+ (*D*) content, shoot K^+/Na^+ ratio (*E*), and root K^+/Na^+ ratio (*F*) in seedlings of rice cultivars Cotaxtla and Tres Ríos. The seedlings (30-d-old) were grown hydroponically under two osmotic stress agents (18.5 % PEG or 100 mM NaCl) for 6 d. Means ± SDs, $n = 3$. Different letters indicate statistically significant differences at $\alpha \leq 0.05$ according to Tukey's test.

Table 1. Relative expressions of the *NAC* family genes in leaves of rice seedlings of the cultivars Cotaxtla and Tres Ríos, using RT-qPCR and taking the *elongation factor 1α* as reference gene. The seedlings (30-d-old) were exposed to 18.5 % PEG 6000 or 100 mM NaCl for 6 h. Samples of the second and third fully expanded leaves were taken from three seedlings. Means ± SDs, $n = 3$. Genes that were induced or repressed with a fold change equal to or greater than 2 are marked *in bold*. NAM - no apical meristem.

Identifier	Annotation	cv. Cotaxtla		cv. Tres Ríos	
		18.5 % PEG	100 mM NaCl	18.5 % PEG	100 mM NaCl
Os02g56600	putative NAM protein	1.72 ± 0.31	**2.36 ± 0.71**	-1.50 ± 0.40	-1.48 ± 0.48
Os03g21060	putative NAM protein	-1.21 ± 0.12	-1.15 ± 0.18	1.35 ± 0.06	**2.40 ± 0.82**
Os08g33910	similar to NAM-like protein	1.14 ± 0.11	1.52 ± 0.36	**2.33 ± 0.82**	**2.44 ± 1.34**
Os03g60080	putative NAC-domain protein	-1.41 ± 0.52	-1.41 ± 0.53	**2.24 ± 0.12**	**2.09 ± 0.76**
Os10g42130	putative NAM protein	**-3.98 ± 1.6**	**-2.41 ± 1.61**	**-2.98 ± 0.29**	**-2.15 ± 0.37**
Os01g15640	putative NAM protein	-1.20 ± 0.15	-1.26 ± 0.09	1.62 ± 0.25	**2.31 ± 0.36**
Os07g04560	hypothetical protein	**-2.28 ± 0.48**	**-7.48 ± 1.46**	**-4.83 ± 1.06**	**-5.77 ± 1.21**
Os06g51070	NAM-like protein (imported from *Arabidopsis thaliana*)	1.82 ± 0.92	-1.56 ± 0.44	**2.55 ± 1.25**	**2.12 ± 0.25**
Os09g33490	similar to NAC domain protein NAC2	-1.21 ± 0.08	1.09 ± 0.1	1.64 ± 0.39	**2.22 ± 0.20**
Os02g34970	hypothetical protein	**2.30 ± 0.21**	**5.49 ± 0.54**	1.48 ± 0.09	1.68 ± 0.65
Os04g40130	similar to probable salt-inducible protein (imported from *Arabidopsis thaliana*)	1.35 ± 0.17	1.46 ± 0.57	1.62 ± 0.53	**2.18 ± 0.39**
Os08g10080	similar to NAC domain protein NAC1	-1.29 ± 0.44	**-2.09 ± 0.51**	-1.84 ± 0.36	1.68 ± 0.11
Os12g29330	similar to NAC domain protein NAC2	1.24 ± 0.3	1.18 ± 0.13	1.72 ± 0.73	**2.07 ± 0.36**
Os02g36880	OsNAC1 protein	-1.62 ± 0.58	-1.54 ± 0.22	-1.62 ± 0.85	**2.06 ± 0.17**
Os11g08210	OsNAC5 protein (imported from rice)	1.97 ± 0.69	**2.63 ± 0.79**	1.55 ± 0.19	**2.04 ± 0.36**
Os05g34830	OsNAC5 protein (imported from rice)	1.87 ± 0.65	**2.43 ± 0.77**	1.32 ± 0.04	**2.10 ± 0.30**
Os01g66120	OsNAC6	-1.33 ± 0.13	**2.38 ± 1.21**	**4.04 ± 1.91**	**6.52 ± 2.32**
Os11g03300	OsNAC10	**3.38 ± 0.91**	**2.72 ± 0.73**	**8.33 ± 1.86**	**5.94 ± 1.66**

(Nuruzzaman *et al*. 2010, 2015). Regarding the *Os03g21060* gene, we found no differential gene expression in the cv. Cotaxtla, but in the cv. Tres Ríos it was induced only by NaCl. Induction of this gene has been reported also in the rice genotypes NL13 and IR64, both classified as drought-sensitive, but it was not differentially expressed in the drought tolerant line NL10 (Nuruzzaman *et al*. 2012). These findings are consistent with our results since this gene did not show regulation in the cv. Cotaxtla but it did in the cv. Tres Ríos. Additionally, the expression of the *Os08g33910* gene was induced by both PEG and NaCl only in the cv. Tres Ríos. Interestingly, Nuruzzaman *et al*. (2012) reported that this gene is not regulated by water deficit or plant hormones such as ABA, auxin, gibberellic acid, cytokinin, or jasmonic acid.

The *Os03g60080* gene expression did not change in the cv. Cotaxtla, but it was induced in the cv. Tres Ríos by both PEG and NaCl. The expression of this gene has also shown induction in the leaves of NL10 and NL13 under mild and severe drought, as well as after treatment with various plant hormones (Nuruzzaman *et al*. 2012). This gene, named *SNAC1,* is involved in stomatal closure and seed growth, and belongs to the subgroup SNAC (stress-associated NAC). When the *Os03g60080/SNAC1* gene is overexpressed, stomatal closure stimulation is observed in rice flag leaves under drought (Hu *et al*. 2006). On the other hand, Lin *et al*. (2007) found that the *Os03g60080* gene is expressed primarily in roots, and its expression is relatively low in stems, pods, and leaves. However, the amount of transcripts is increased significantly in leaves after infection with phyto-pathogenic fungi and after application of methyl jasmonate, ABA, and ethylene. Likewise, this gene has been classified as a key element inducing resistance/ tolerance mechanisms in response to biotic and abiotic stresses (Nuruzzaman *et al*. 2015). The *Os03g60080* (*OsNAC1*) gene has also been found to be 4-fold more expressed at low-temperature (10 °C) in rice tolerant genotypes (M 202 and ARR 09) than in sensitive genotypes (Ghosh *et al*. 2016).

The *Os01g15640* gene was only induced by 100 mM NaCl in the cv. Tres Ríos, whereas Nuruzzaman *et al*. (2010) found that this gene is induced by both biotic and abiotic stresses. On the other hand, the *Os02g34970* gene was only induced in the cv. Cotaxtla, both by PEG and NaCl, which is consistent with the findings reported by Nuruzzaman *et al*. (2012), since under mild and severe stress this gene is specifically induced in root, leaf, and panicle of NL10 (drought tolerant), but not in NL13. It is also induced by treatment with hormones, by drought, and by submergence. This gene belongs to the subgroup ONAC6, a member of the main group A, which encompasses the least studied genes among all *NAC* genes (Nuruzzaman *et al*. 2010). Nuruzzaman *et al*. (2012) found that the *Os08g10080* gene is highly expressed in the panicle, root, and leaves of the NL10 genotype under control conditions, mild, and severe water deficit, whereas we observed a significant repression of this gene by 100 mM NaCl in the

cv. Cotaxtla. Interestingly, overexpression of the wheat *TaNAC-S* gene, an orthologue of the rice *Os08g10080* gene, delays leaf senescence and increases grain nitrogen content in wheat (Zhao *et al*. 2015), indicating a role as a potential negative regulator of senescence.

Under our experimental conditions, the expression of the *Os02g36880* gene was induced by NaCl only in the cv. Tres Ríos. Interestingly, Nuruzzaman *et al*. (2012) found that this gene is up-regulated in leaves and panicle of the NL10 and NL13 lines, both under mild and severe drought stress. Additionally, the *Os11g08210* and *Os05g34830* genes were specifically induced by salinity in both evaluated cultivars; both genes are included in the subgroup SNAC, the best characterized subgroup of the NAC TF family, and they are induced by drought, salinity, and hormones (Nuruzzaman *et al*. 2010). According to Ghosh *et al.* (2016), *OsNAC5* (*Os11g08210*) shows a higher transcript accumulation in tolerant genotypes than in sensitive genotypes after low temperature (10 °C) treatment, suggesting that stress tolerance might be due to higher expression of this stress-responsive TF. Furthermore, Nuruzzaman *et al*. (2012) reported that the *Os05g34830* gene is specifically activated in NL10 line roots under severe osmotic stress, and in leaves of both NL10 and NL13 under drought and salinity. Accordingly, we found similar results in our rice cultivars evaluated. The *Os05g34830* gene is also activated by plant hormones (Nuruzzaman *et al*. 2010) and biotic stresses (Nuruzzaman *et al*. 2015).

The *OsNAC6* (*Os01g66120*) gene was induced by 100 mM NaCl in both cvs. Cotaxtla and Tres Ríos. Nonetheless, its expression was not changed in the cv. Cotaxtla exposed to PEG. According to Ohnishi *et al*. (2005), the *OsNAC6* gene is induced by abiotic stresses and treatment with jasmonic acid, and the overexpression of this gene results in an increased rice tolerance to drought, salinity, and cold. Moreover, the overexpression of the *OsNAC6* gene induces higher resistance to fungus *Magnaporthe grisea* (Nakashima *et al*. 2007), stimulates a better germination and growth rate under high salinity, increases tolerance to PEG and results in greater sensitivity to ABA (Hu *et al*. 2008). In addition, Moumeni *et al*. (2015) reported the induction of the *OsNAC6* gene in a transcriptional profiling of the leaves of near-isogenic rice lines with contrasting drought tolerance at the reproductive stage in response to water deficit.

We selected the *OsNAC10* gene as a positive control for this study, since it has been found to be induced by drought, salinity, and ABA (Jeong *et al*. 2010). Indeed, its expression was induced in response to salt stress in both cultivars tested. Interestingly, this gene has recently been reported to be induced upon viral infection (Nuruzzaman *et al*. 2015) and is therefore associated with a wide range of stress responses and growth regulation. The wheat *TaNAC29* gene exhibits relatively high homology with *OsNAC10*, and plays important role in the senescence and in response to salt and drought stresses. Furthermore, ABA signal pathway and antioxidant enzyme systems are involved in TaNAC29-mediated stress tolerance mechanisms (Huang *et al*. 2015). Just recently, Zhou *et al*. (2016) reported induction of the genes *SNAC1* (*Os03g60080*), *OsNAC5* (*Os11g08210*) and *OsNAC6* (*Os01g66120*) in roots and leaves of the wild rice (*Oryza rufipogon* Griff.) genotype Dongxiang under salt stress (200 mM NaCl). These results are in full agreement with our findings, since in the cv. Tres Ríos all these genes were induced in leaves when plants were exposed to 100 mM NaCl, whereas in the cv. Cotaxtla leaves only *OsNAC5* and *OsNAC6* were induced. Interestingly, the expression of the *SNAC1* gene was evidently contrasting, as it was induced in the cv. Tres Ríos but repressed in the cv. Cotaxtla. Although the NAC family comprises one of the largest plant-specific TFs, only a few genes have been characterized so far. Therefore, further studies aimed at elucidating the functional roles of more *NAC* genes and proteins need to be conducted. Nuruzzaman *et al*. (2010) reported that the *NAC* gene family in rice is grouped into two large clusters, A and B, and these in turn are divided into subgroups, depending on their NAC domain. Within these two main groups, cluster A members have less homology with the *NAC* genes of *A. thaliana* and show greater diversity in the NAC domain structure, whereas cluster B members show high homology with the genes of *A. thaliana* and those of other species. In this research we found genes previously reported to be regulated by osmotic stress such as *Os10g42130, Os11g08210*, *Os05g34830, OsNAC6*, and *OsNAC10*, which belong to cluster B and which have been more widely studied. However, we also found the *Os02g34970* gene (from cluster A), which may be a gene with the potential to regulate responses to osmotic and other types of stress. It has been shown that a single NAC TF can function as a regulator of different processes and can also mediate the interconnection between different signaling pathways (Nuruzzaman *et al*. 2012). Additionally, the *Os08g10080* gene has not yet been characterized in rice. This gene may be involved in the control of senescence, since its ortholog *TaNAC-S* found in wheat is involved in the regulation of leaf senescence and nitrogen content in wheat leaves (Zhao *et al*. 2015).

In conclusion, our experiments demonstrated that osmotic stress induced by PEG and NaCl reduced plant height, root length, shoot and root dry matter, as well as the content of K^+ in roots and the corresponding K^+/Na^+ ratio, and increased the ABA content in leaves. From the 57 *NAC* genes evaluated, PEG regulated a lower number of them (8 genes) in comparison to NaCl (18 genes). Plant growth in the cv. Tres Ríos was more affected by the osmotic stress, which confirms that this cultivar is more stress-sensitive than the cv. Cotaxtla. The cv. Cotaxtla maintained growth and displayed a higher content of K^+ in shoots in response to NaCl, though they contained more Na^+ in roots than the cv. Tres Ríos. The cv. Cotaxtla plants also increased the total Chl content and maintained a higher K^+/Na^+ ratio when treated with PEG, which indicates a better capacity to tolerate osmotic

stress. Such contrasting responses might be regulated by ABA and the differential expression of *NAC* genes. Furthermore, we were able to confirm that the *NAC* genes *Os10g42130, Os11g08210*, *Os05g34830, OsNAC6*, and *OsNAC10* are induced by PEG and NaCl, while reporting for the first time the discovery of novel *NAC* genes, namely *Os03g60080, Os02g34970*, and *Os08g10080*, responding to osmotic stress, which may represent potential candidates for further rice breeding programs. Nevertheless, identifying the function of *NAC* genes under different stresses remains a major challenge. Importantly, understanding the mechanisms that are controlled by NAC proteins is crucial for the development of crops with increased tolerance to environmental stresses. It is also important to analyze the accompanying elements that interact with the NAC proteins and which together regulate the transcription of target genes.

References

Aida, M., Ishida, T., Fukaki, H., Fujisawa, H., Tasaka, M.: Genes involved in organ separation in *Arabidopsis*: an analysis of the cup-shaped cotyledon mutant. - Plant Cell **9**: 841-857, 1997.

Agarwal, P.K., Jha, B.: Transcription factors in plants and ABA dependent and independent abiotic stress signalling. - Biol. Plant. **54**: 201-212, 2010.

Ahmad, I., Mian, A., Maathuis, F.J.: Overexpression of the rice *AKT1* potassium channel affects potassium nutrition and rice drought tolerance. - J. exp. Bot. **67**: 2689-2698, 2016.

Arnon, D.I.: Copper enzymes in isolated chloroplasts - polyphenoloxidase in *Beta vulgaris*. - Plant Physiol. **24**: 1-15, 1949.

Balazadeh, S., Riaño-Pachón, D.M., Mueller-Roeber, B.: Transcription factors regulating leaf senescence in *Arabidopsis thaliana*. - Plant Biol. **1** (Suppl.): 63-75, 2008.

Bartlett, M.K., Scoffoni, C., Ardy, R., Zhang, Y., Sun, S., Cao, K., Sack, L.: Rapid determination of comparative drought tolerance traits: using an osmometer to predict turgor loss point. - Methods Ecol. Evol. **3**: 880-888, 2012.

Blumwald, E.: Sodium transport and salt tolerance in plants. - Curr. Opin. Cell Biol. **12**: 431-434, 2000.

Caldana, C., Scheible, W.R., Mueller-Roeber, B., Ruzicic, S.: A quantitative RT-PCR platform for high-throughput expression profiling of 2500 rice transcription factors. - Plant Methods **3**: 7, 2007.

Castillo, E.G., Tuong, T.P., Ismail, A.M., Inubushi, K.: Response to salinity in rice: comparative effects of osmotic and ionic stresses. - Plant Prod. Sci. **10**: 159-170, 2007.

Chakraborty, K., Bhaduri, D., Meena, H.N., Kalariya, K.: External potassium (K^+) application improves salinity tolerance by promoting Na^+-exclusion, K^+-accumulation and osmotic adjustment in contrasting peanut cultivars. - Plant Physiol. Biochem. **103**: 143-53, 2016.

Chen, X., Cheng, J., Chen, L., Zhang, G., Huang, H., Zhang, Y., Xu, L.: Auxin-independent *NAC* pathway acts in response to explant-specific wounding and promotes root tip emergence during *de novo* root organogenesis in *Arabidopsis*. - Plant Physiol. **170**: 2136-2145, 2016.

Christiansen, M.W., Holm, P.B., Gregersen, P.L.: Characterization of barley (*Hordeum vulgare* L.) NAC transcription factors suggests conserved functions compared to both monocots and dicots. - BMC Res. Notes **4**: 302, 2011.

Conde, A., Chaves, M.M., Gerós, H.: Membrane transport, sensing and signaling in plant adaptation to environmental stress. - Plant Cell Physiol. **52**: 1583-1602, 2011.

Criado, M.V., Roberts, I.N., Echeverria, M., Barneix, A.J.: Plant growth regulators and induction of leaf senescence in nitrogen-deprived wheat plants. - J. Plant Growth Regul. **26**: 301-307, 2007.

Du, H., Wang, N., Cui F., Li, X., Xiao, J., Xiong, L.: Characterization of the beta-carotene hydroxylase gene *DSM2* conferring drought and oxidative stress resistance by increasing xanthophylls and abscisic acid synthesis in rice. - Plant Physiol. **154**: 1304-1318, 2010.

Fang, Y., Liao, K., Du, H., Xu, Y., Song, H., Li, X., Xiong, L.: A stress-responsive NAC transcription factor SNAC3 confers heat and drought tolerance through modulation of reactive oxygen species in rice. - J. exp. Bot. **66**: 6803-6817, 2015.

García-Morales, S., Trejo-Téllez, L.I., Gómez-Merino, F.C., Caldana, C., Espinosa-Victoria, D., Herrera-Cabrera, E.: Growth, photosynthetic activity and potassium and sodium concentration in rice plants under salt stress. - Acta Sci. **34**: 317-324, 2012.

García-Morales, S., Gómez-Merino, F.C., Trejo-Téllez, L.I.: *NAC* transcription factor expression, amino acid concentration and growth of elite rice cultivars upon salt stress. - Acta Physiol. Plant. **36**: 1927-1936, 2014.

Ghosh, T., Rai, M., Tyagi, W., Challam, C.: Seedling stage low temperature response in tolerant and susceptible rice genotypes suggests role of relative water content and members of *OsSNAC* gene family. - Plant Signal Behav. **11**: e1138192, 2016.

Greve, K., La Cour, T., Jensen, M.K., Poulsen, F.M., Skriver, K.: Interactions between plant RING-H2 and plant-specific NAC (NAM/ATAF1/2/CUC2) proteins: RING-H2 molecular specificity and cellular localization. - Biochem. J. **371**: 97-108, 2003.

Hanin, M., Ebel, C., Ngom, M., Laplaze, L., Masmoudi, K.: New insights on plant salt tolerance mechanisms and their potential use for breeding. - Front. Plant Sci. **7**: 1787, 2016.

He, X.J., Mu, R.L., Cao, W.H., Zhang, Z.G., Zhang, J.S., Chen, S.Y.: AtNAC2, a transcription factor downstream of ethylene and auxin signaling pathways, is involved in salt stress response and lateral root development. - Plant J. **44**: 903-916, 2005.

Hong, Y., Zhang, H., Huang, L., Li, D., Song, F.: Overexpression of a stress-responsive *NAC* transcription factor gene *ONAC022* improves drought and salt tolerance in rice. - Front. Plant Sci. **7**: 4, 2016.

Hu, H., Dai, M., Yao, J., Xiao, B., Li, X., Zhang, Q., Xiong, L.: Overexpressing a *NAM*, *ATAF*, and *CUC* (*NAC*) transcription factor enhances drought resistance and salt tolerance in rice. - Proc. nat. Acad. Sci. USA **103**: 12987-12992, 2006.

Hu, H.H., You, J. Fang, Y.J., Zhu, X., Qi, Z.Y., Xiong, L.Z.: Characterization of transcription factor gene *SNAC2* conferring cold and salt tolerance in rice. - Plant mol. Biol. **67**: 169-181, 2008.

Huang, Q., Wang, Y., L.I., B., Chang, J., Chen, M., Li, K.,

Yang, G., He, G.: TaNAC29, a NAC transcription factor from wheat, enhances salt and drought tolerance in transgenic *Arabidopsis*. - BMC Plant Biol. **15**: 268, 2015.

Jeong, J.S., Kim, Y.S., Baek, K.H., Jung, H., Ha, S.H., Choi, Y.D., Kim, M., Reuzeau, C., Kim, J.K.: Root-specific expression of *OsNAC10* improves drought tolerance and grain yield in rice under field drought conditions. - Plant Physiol. **153**: 185-197, 2010.

Jeong, J.S., Kim, Y.S., Redills, M.C., Jang, G., Jung, H., Bang, S.W., Choi, Y.D., Ha, S.H. Reuzeau, C., Kim, J.K.: *OsNAC5* overexpression enlarges root diameter in rice plants leading to enhanced drought tolerance and increased grain yield in the field. - Plant Biotechnol. J. **11**: 101-114, 2013.

Ji, H., Liu, L., Li, K., Xie, Q., Wang, Z., Zhao, X., Li, X.: PEG-mediated osmotic stress induces premature differentiation of the root apical meristem and outgrowth of lateral roots in wheat. - J. exp. Bot. **65**: 4863-4872, 2014.

Ji, K.X., Wang, Y.Y., Sun, W.N., Lou, Q.J., Mei, H.W., Shen, S.H., Chen, H.: Drought-responsive mechanisms in rice genotypes with contrasting drought tolerance during reproductive stage. - J. Plant Physiol. **169**: 336-344, 2012.

Kim, Y.S., Kim, S.G., Park, J.E., Park, H.Y., Lim, M.H., Chu, N.H., Park, C.M.: A membrane-bound NAC transcription factor regulates cell division in *Arabidopsis*. - Plant Cell **18**: 3132-3144, 2006.

Kou, X., Liu, C., Han, L., Wang, S., Xue, Z.: NAC transcription factors play an important role in ethylene biosynthesis, reception and signaling of tomato fruit ripening. - Mol. Genet. Genomics **291**: 1205-1217, 2016.

Kumar, K.A., Kumar, M.S, Sudha, M., Vijayalakshmi, D., Vellaikkumar, S., Senthil, N., Raveendran, M.: Identification of genes controlling ABA accumulation in rice during drought stress and seed maturation. - Int. J. adv. biotechnol. Res. **4**: 481-487, 2013.

Le, D.T., Nishiyama, R., Watanabe, Y., Mochida, K., Yamaguchi-Shinozaki, K., Shinozaki, K., Tran, L.S.P.: Genome-wide survey and expression analysis of the plant-specific NAC transcription factor family in soybean during development and dehydration stress. - DNA Res. **18**: 263-276, 2011.

Lin, R., Zhao, W., Meng, X., Wang, M., Peng, Y.: Rice gene *OsNAC19* encodes a novel NAC-domain transcription factor and responds to infection by *Magnaporthe grisea*. - Plant Sci. **172**: 120-130, 2007.

Liu, G., Li, X., Jin, S., Liu, X., Zhu, L., Nie, Y., Zhang, X.: Overexpression of rice *NAC* gene *SNAC1* improves drought and salt tolerance by enhancing root development and reducing transpiration rate in transgenic cotton. - PLoS ONE **9**: e86895, 2014.

Marcinska, I., Czyczylo-Mysza, I., Skrzypek, E., Grzesiak, M.T., Janowiak, F., Filek, M., Dziurka, M., Dziurka, K., Waligórski, P., Juzon, K., Cyganek K., Grzesiak, S.: Alleviation of osmotic stress effects by exogenous application of salicylic or abscisic acid on wheat seedlings. - Int. J. mol. Sci. **14**: 13171-13193, 2013.

Mekawy, A.M., Assaha, D.V., Yahagi, H., Tada, Y., Ueda, A., Saneoka, H.: Growth, physiological adaptation, and gene expression analysis of two Egyptian rice cultivars under salt stress. - Plant Physiol. Biochem. **87**: 17-25, 2015.

Moumeni, A., Satoh, K., Venuprasad, R., Serraj, R., Kumar, A., Leung, H., Kikuchi, S.: Transcriptional profiling of the leaves of near-isogenic rice lines with contrasting drought tolerance at the reproductive stage in response to water deficit. - BMC Genomics **16**: 1110, 2015.

Munns, R., Tester, M.: Mechanisms of salinity tolerance. - Annu. Rev. Plant Biol. **59**: 651-681, 2008.

Nakashima, K., Tran, L.S., Van Nguyen, D., Fujita, M., Maruyama, K., Todaka, D., Ito, Y., Hayashi, N., Shinozaki, K., Yamaguchi-Shinozaki, K.: Functional analysis of a NAC-type transcription factor OsNAC6 involved in abiotic and biotic stress-responsive gene expression in rice. - Plant J. **51**: 617-630, 2007.

Nuruzzaman, M., Manimekalai, R., Sharoni, A.M. Satoh, K., Kondoh, H., Ooka, H., Kikuchi, S.: Genome-wide analysis of *NAC* transcription factor family in rice. - Gene **465**: 30-44, 2010.

Nuruzzaman, M., Sharoni, A.M., Satoh, K., Moumeni, A., Venuprasad, R., Serraj, R., Kumar, A., Leung, H., Attia, K., Kikuchi, S.: Comprehensive gene expression analysis of the *NAC* gene family under normal growth conditions, hormone treatment, and drought stress conditions in rice using near-isogenic lines (NILs) generated from crossing Aday Selection (drought tolerant) and IR64. - Mol. Genet. Genomics **287**: 389-410, 2012.

Nuruzzaman, M., Sharoni, A.M., Satoh, K., Karim, M.R., Harikrishna, J.A., Shimizu, T., Sasaya, T., Omura, T., Haque, M.A., Hasan, S.M., Ahmad, A., Kikuchi, S.: NAC transcription factor family genes are differentially expressed in rice during infections with Rice dwarf virus, Rice black-streaked dwarf virus, Rice grassy stunt virus, Rice ragged stunt virus, and Rice transitory yellowing virus. - Front. Plant Sci. **6**: 676, 2015.

Oda-Yamamizo, C., Mitsuda, N., Sakamoto, S., Ogawa, D., Ohme-Takagi, M., Ohmiya, A.: The NAC transcription factor ANAC046 is a positive regulator of chlorophyll degradation and senescence in Arabidopsis leaves. - Sci. Rep. **6**: 23609, 2016.

Ohnishi, T., Sugahara, S., Yamada, T., Kikuchi, K., Yoshiba, Y., Hirano, H.Y., Tsutsumi, N.: *OsNAC6*, a member of the NAC gene family, is induced by various stresses in rice. - Genes Genet. Syst. **80**: 135-139, 2005.

Olivella, C., Vendrell, M., Save R.: [Determinacion de ácido abscisico, ácido indolacetico, zeatina y ribosido de zeatina en hojas desarrolladas de *Gerbera jamesonii* cv Bolus y su variacion con la edad]. - Invest. Agr. Prod. Prot. Veg. **16**: 333-342, 2001. [In Spanish]

Pandey, V., Shukla, A.: Acclimation and tolerance strategies of rice under drought stress. - Rice Sci. **22**: 147-161, 2015.

Rattanakon, S., Ghan, R., Gambetta, G.A., Deluc, L.G., Schlauch, K.A., Cramer, G. R.: Abscisic acid transcriptomic signaling varies with grapevine organ. - BMC Plant Biol. **16**: 72, 2016.

Rodrigues, M.L., Pacheco, C.M., Chaves, M.M.: Soil-plant water relations, root distribution and biomass partitioning in *Lupinus albus* L. under drought conditions. - J. exp. Bot. **46**: 947-956, 1995.

Sablowski, R.W., Meyerowitz, E.M.: A homolog of *NO APICAL MERISTEM* is an immediate target of the floral homeotic genes *APETALA3/PISTILLATA*. - Cell **92**: 93-103, 1998.

Sakuraba, Y., Han, S.H., Lee, S.H., Hörtensteiner, S., Paek, N.C.: *Arabidopsis* NAC016 promotes chlorophyll breakdown by directly upregulating *STAYGREEN1* transcription. - Plant Cell Rep. **35**: 155-166, 2016.

Schmittgen, T.D., Livak, K.J.: Analyzing real-time PCR data by the comparative C_T method. - Nat. Protocols **3**: 1101-1108, 2008.

Shabala, S., Wu, H., Bose, J.: Salt stress sensing and early signaling events in plant roots: current knowledge and

hypothesis. - Plant Sci. **241**: 109-119, 2015.

Shan, W., Chen, J.-Y., Kuang, J.-F., Lu, W.-J.: Banana fruit NAC transcription factor MaNAC5 cooperates with MaWRKYs to enhance the expression of pathogenesis-related genes against *Colletotrichum musae*. - Mol. Plant Pathol. **17**: 330-338, 2016.

Shen, H., Chen, F., Dixon, A.: A bioinformatic analysis of *NAC* genes for plant cell wall development in relation to lignocellulosic bioenergy production. - Bioenerg. Res. **2**: 217-232, 2009.

Shiriga, K., Sharma, R., Kumar, K., Yadav, S.K., Hossain, F., Thirunavukkarasu, N.: Genome-wide identification and expression pattern of drought-responsive members of the NAC family in maize. - Meta Gene **2**: 407-417, 2014.

Shobbar, M.-S., Azhari, O., Shobbar, Z.-S., Niknam, V., Askari, H., Pessarakli, M., Ebrahimzadeh, H.: Comparative analysis of some physiological responses of rice seedlings to cold, salt, and drought stresses. - J. Plant Nutr. **35**: 1037-1052, 2012.

Souleymane, O., Nartey, E., Manneh, B., Danquah, E., Ofori, K.: Phenotypic variability of 20 rice genotypes under salt stress. - Int. J. Plant Breed. Genet. **10**: 45-51, 2016.

Sperotto, R.A., Ricachenevsky, F.K., Duarte, G.L., Boff, T., Lopes, K.L., Sperb, E.R., Grusak, M.A., Fett, J.P: Identification of up-regulated genes in flag leaves during rice grain filling and characterization of *Os*NAC5, a new ABA-dependent transcription factor. - Planta **230**: 985-1002, 2009.

Wakeel, A.: Potassium-sodium interactions in soil and plant under saline-sodic conditions. - J. Plant Nutr. Soil Sci. **176**: 344-354, 2013.

Wei, S., Gao, L., Zhang, Y., Zhang, F., Yang, X., Huang, D: Genome-wide investigation of the NAC transcription factor family in melon (*Cucumis melo* L.) and their expression analysis under salt stress. - Plant Cell Rep. **35**: 1827-1839. 2016.

Yang, X., Römheld, V., Marschner, H.: Effect of bicarbonate on root growth and accumulation of organic acids in Zn-inefficient and Zn-efficient rice cultivars (*Oryza sativa* L.). - Plant Soil **164**: 1-7, 1994.

Yu, X., Liu, Y., Wang, S., Tao, Y., Wang, Z., Shu, Y., Peng, H., Mijti, A., Wang, Z., Zhang, H., Ma, H.: CarNAC4, a NAC-type chickpea transcription factor conferring enhanced drought and salt stress tolerances in *Arabidopsis*. - Plant Cell Rep. **35**: 613-627, 2016.

Zhang, L., Zhang, L., Xia, C., Zhao, G., Jia, J., Kong, X.: The novel wheat transcription factor TaNAC47 enhances multiple abiotic stress tolerances in transgenic plants. - Front. Plant Sci. **6**: 1174, 2016.

Zhao, D., Derkx, A.P., Liu, D.C., Buchner, P., Hawkesford, M.J.: Overexpression of a NAC transcription factor delays leaf senescence and increases grain nitrogen concentration in wheat. - Plant Biol. **17**: 904-913, 2015.

Zhao, F., Ma, J., Li, L., Fan, S., Guo, Y., Song, M., Wei, H., Pang, C., Yu, S.: *GhNAC12*, a neutral candidate gene, leads to early aging in cotton (*Gossypium hirsutum* L). - Gene **576**: 268-274, 2016.

Zheng, X., Chen, B., Lu, G., Han, B.: Overexpression of a *NAC* transcription factor enhances rice drought and salt tolerance. - Biochem. biophys. Res. Commun. **379**: 985-989, 2009.

Zhou, Y., Yang, P., Cui, F., Zhang, F., Luo, X., Xie, J.: Transcriptome analysis of salt stress responsiveness in the seedlings of Dongxiang wild rice (*Oryza rufipogon* Griff.). - PLoS ONE **11**: e0146242, 2016.