



Detection of genes providing salinity-tolerance in rice

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ABSTRACT. The present study aimed to identify salinity-tolerant genes in three cultivars (BRS-7 Taim, BRS Querência and BRS Atalanta) of *Oryza sativa* L. ssp. *indica* S. Kato and in three cultivars (BRS Bojuru, IAS 12-9 Formosa and Goyakuman) of *Oryza sativa* L. ssp. *japonica* S. Kato. Ten days after emergence seedlings were transferred to a greenhouse and placed in a 15L vessel with half strength Hoagland nutrient solution, which was changed every four days, under controlled temperature and humidity. Plants were harvested 56 days after transfer. DNA extraction was carried out by CTAB method and salinity-tolerant genes SOS and CK1 were identified by *in silico* research. Amplification of gene sequence was performed with *in silico* primers. Bands were detected by agar gel electrophoresis and visualized under ultraviolet light after staining with ethidium bromide. Gene SOS1 fragments were present in all cultivars, except in BRS Atalanta, whereas CK1 gene was present in all evaluated cultivars. Results show that salinity-tolerant genes under analysis were identified in the two sub-species.

Keywords: *Oryza sativa* L., cultivars, salinity-tolerance, genes, *in silico*.

Detecção de genes que conferem tolerância à salinidade em planta de arroz

RESUMO. O estudo teve como objetivo identificar genes envolvidos na tolerância à salinidade em três cultivares (BRS-7 Taim, BRS Querência e BRS Atalanta) de *Oryza sativa* L. ssp. *indica* S. Kato e em três cultivares (BRS Bojuru, IAS 12-9 Formosa e Goyakuman) de *Oryza sativa* L. ssp. *japonica* S. Kato. As plântulas foram transferidas para casa de vegetação, sob temperatura e umidade relativa controladas, dez dias após a emergência e colocadas em bacia com capacidade para 15 litros, contendo solução nutritiva de Hoagland, meia força, a qual foi mudada em intervalos de quatro dias. A coleta foi aos 56 dias após a transferência. A extração de DNA foi realizada pelo método CTAB. Os genes SOS e CK1, envolvidos na tolerância à salinidade, foram identificados por meio de pesquisa *in silico*. A amplificação das sequências do gene foi realizada utilizando-se *primers* também desenhados *in silico*. As bandas foram detectadas por eletroforese em gel de agarose e visualizadas em luz ultravioleta após coloração com brometo de etídio. Fragmentos do gene SOS1 foram encontrados em todas as cultivares, exceto para BRS Atalanta. O gene CK1 esteve presente em todas as cultivares avaliadas. Os resultados mostram que os genes estudados estão presentes em ambas as subespécies.

Palavras-chave: *Oryza sativa* L., cultivares, tolerância à salinidade, genes, *in silico*.

Introduction

Rice (*Oryza sativa* L.), a basic diet for approximately 2.5 billion people, is grown and consumed in all the five continents. It has a strategic role in the economic and social development of the most populous nations of Asia, Africa and Latin America. It is the second cereal most cultivated in the planet and occupies approximately 158 million hectares of land with a production of almost 662 million tons of grains. Brazil produces between 11 and 13 million tons of rice and is the ninth largest producer with nearly 82% of production within the Mercosur area (EMBRAPA, 2011).

The genus *Oryza* comprises 22 species, among which *O. sativa* L. is the most relevant due to its participation in human diet. The species is subdivided into several subspecies, among which the *indica* and the *japonica* are the most important. Rice features two ploidy levels, diploid and triploid, and the genome of the subspecies *indica* has 466 million bp (base pairs) and between 45000 and 56000 genes, whereas the *japonica* has 420 million bp and between 32000 and 50000 genes. Although rice is moderately sensitive to salinity (FAGERIA et al., 2010), there are variations in salinity tolerance among the genotypes of the same species. *O. sativa* ssp. *japonica* cultivars are

moderately tolerant, whereas *O. sativa* ssp. *indica* cultivars are sensitive to salinity (LIMA et al., 2004).

Floodplains and highlands are the two most important topology systems in Brazilian rice culture (EMBRAPA, 2011). The floodplain soils of the Brazilian southern state of Rio Grande do Sul comprise common characteristics of hydromorphism, albeit with great variations. Incorrect natural drainage of hydromorphic soils is generally caused by a predominantly plain topography associated with a profile with a practically impervious surface and sub-surface layers. The irrigation system by flooding is widely used in the Brazilian southern regions and requires characteristic soils to avoid degradation and to achieve a good development. However, the best conditions are rarely met within the same culture. Even though water is of excellent quality, irrigation practices may be an important factor for soil salinization. This is true if the soil is not treated adequately, especially in a system that does not provide drainage. In fact, it is the main negative impact of irrigated agriculture (MANTOVANI et al., 2006). Area affected by saline stress in Brazil amounts to 2% of the total area. Since flooding is the main irrigation system for rice grown in Rio Grande do Sul, it may lead to salinity when soil drainage is inadequate. Further, the farmlands of the coastal regions use the water from the Laguna dos Patos, which are prone to salinization owing to the addition of sea water when the Laguna's water level is low. This fact occurs mainly in January and February under high rainfall and low evaporation-transpiration, coinciding with the reproduction phase of the rice culture (MACLEAN et al., 2002). Several studies have shown that rice cultivars with high yield potential in the states of Rio Grande do Sul and Santa Catarina are non-tolerant to irrigation with NaCl rates equal to or higher than 0.25% in the water.

Salinity is one of the most impacting environmental stresses for rice culture due to its restrictions in production, a counter-event in the current increasing food demand. Plants tolerate salinity through mechanisms as (i) vacuolar compartmentalization for low salt levels in the cytoplasm; (ii) minimization of cell's exposure time to ionic imbalance (TESTER; DAVENPORT, 2003; ASHRAF, 2004) to avoid sodium deleterious effects. Ion storing is another mechanism for osmotic adjustment (MUNNS, 2005; MARENCO; LOPES, 2007). Ions are transported by Na^+/H^+ antiport transporters on the tonoplast or in the plasmatic membrane (APSE; BLUMWALD, 2007). Their

function is to limit entry and exit of Na^+ in the cells to regulate the compartmentalization of Na^+ in the vacuole and the selective importation of potassium instead of sodium (EPSTEIN; BLOOM, 2006).

Na^+ accumulation causes a higher tolerance to salinity (APSE; BLUMWALD, 2007). Sodium exclusion and vacuolar compartmentalization are studied through SOS signaling which regulates gene expression involved in ionic homeostasis. SOS1 restricts Na^+ absorption in the root, removes Na^+ from the cytosol and promotes its export to the apoplast so that the rapid accumulation of Na^+ in the aerial part could be limited and prevented (QI; SPALDING, 2004). SOS triggering is done by Ca^{2+} cytoplasm signal (ZHU, 2002). Besides being associated with the maintenance of stability of the membrane and the cell wall (EPSTEIN; BLOOM, 2006), Ca^{2+} has also the role of secondary messenger which involves environmental signals for growth and development (MARENCO; LOPES, 2007). Kinases and/or phosphatases are, as a rule, messenger receivers. Protein kinase regulated by a more abundant Ca^{2+} is the Ca^{2+} -dependent kinase protein, also known as CDPK or CPK (TEIGE et al., 2004). CDPKs are a class of calcium-activated proteins, albeit insensitive to calmodulin, and are commonly found in plants such as *Nicotiana tabacum* L. (ZHANG et al., 2005), *Oryza sativa* L. (YANG et al., 2003) and *Cucumis sativus* L. (KUMAR et al., 2004). They have a key role in the response to stimuli, by receiving signals from the receptors which are sensitive to environmental conditions and convert them into responses, such as changes in metabolism, gene expression and cell growth (PRISCO; GOMES FILHO, 2010). The calcium-dependent and calmodulin-independent gene *CK1* in rice recognizes such signals as a response to environmental stress.

Agricultural and environmental managements must understand the physiological processes and the mechanisms by which plants identify environmental signals and trigger the cell mechanism (XIONG et al., 2002) for adaptation and acclimation, which provide tolerance and ensure plants survival (SHINOZAKI; DENNIS, 2003). The discovery of new genes, the determination of expression patterns to abiotic stress and a better comprehension of their role in stress adaptation provide the basis for new strategies to improve the tolerance of cultures to stress (CUSHMAN; BOHNERT, 2000; CHAVES et al., 2003; GAO et al., 2013).

The present study analyzes the presence of genes *SOS1* and *CK1* involved in salinity tolerance in *O. sativa* ssp. *japonica* and *O. sativa* ssp. *indica* cultivars.

Material and methods

Plant material consisted of genotypes *O. sativa* ssp. *japonica* tolerant to salinity and *O. sativa* ssp. *indica* sensitive to saline stress (LIMA et al., 2004, 2005). Cultivars of *Oryza sativa* L. ssp. *japonica* S. Kato were BRS Bojurú, IAS 12-9 Formosa and Goyakuman, whereas cultivars of *O. sativa* L. ssp. *indica* S. Kato were BRS-7 Taim, BRS Querência and BRS Atalanta.

Sowing occurred in plastic trays with washed sand. Seedlings were transferred to a greenhouse at 25°C and 80% humidity on the 10th day after emergence. Seedlings were placed in 15 L vessel with half strength Hoagland nutrition solution (HOAGLAND; ARNON, 1950), which was changed at 4-day intervals. Plants were harvested 56 days after transference and immediately stored in a freezer. One plant from each cultivar was ground with dry ice and stored in plastic bags, labeled and immediately placed in a freezer for later DNA extraction.

Plant genomic DNA from each cultivar was extracted using 2% CTAB extraction buffer (SAGHAI-MAROOF et al., 1984) with 200 mg of the plant material. DNA quantification and integrity were processed with 4 µL DNA and 4 µL buffer in 1 % agar gel, in a buffer TBE 1X, stained with 0.2 µg mL⁻¹ ethidium bromide (SAMBROOK et al., 1989), at 100 V for 50 minutes. After electrophoresis, gels were analyzed in a transilluminator under ultraviolet light (254 nm) and images were digitized. Marker λ Hind III (Invitrogen) was the reference for molecular mass. Gene detection comprised genomic DNA extraction and PCR reactions with primers for the amplification of gene fragments involved in salinity tolerance.

In silico analysis undertaken at the National Center for Biotechnology Information (NCBI) identified genes involved in salinity tolerance, or rather, genes AY785147 – SOS with 3660 bp and AF319481 – CK1 with 4206 bp, described as: AY785147: SOS1-1-F, 5'-GAAGAACTTTTCGATGCAGGA-3'; SOS1-1-R, 5'-ATTTCCCAGAAATGATGCAA-3'; SOS1-2-F, 5'-TGGACAGATTAGCAGCAACA-3'; SOS1-2-R, 5'-TTGGGTAGGAACAAGATCCA-3' and SOS1-3-F, 5'-GCTCTAATAAGCGCACAGC-3'; SOS1-3-R, 5'-CCAACAATTACTTGGGTTGCGGGCTTCAGAACTGACAGA-3'. AF319481: CK1-1-F, 5'-

GGTTTTTCCAACCAACTCCT-3'; CK1-1-R, 5'-GGGCTTCAGAACTGACAGA-3'; CK1-2-F, 5'-GAGAACTTCCTGCTGCTGAG-3'; CK1-2-R, 5'-ATCTTCCTGACGAGGTCCTT-3' and CK1-3-F, 5'-TACAGCATTCCAAGTGCTC A-3'; CK1-3-R, 5'-TGCTCAATTTCGTTGTCT GA.

Three pairs of primers were designated for each gene, one for each 1200 bp for gene *SOS1* and 1400 bp for gene *CK1*, to amplify different regions within the gene by Primer3 (MIT, 2006).

Each 20 µL of the reaction contained 2 µL DNA; 1.0 Taq DNA polymerase (Invitrogen), and the following final concentrations: 0.5 µM of forward and reverse primer; 0.2 mM of dNTPs; buffer PCR 1X and 2.5 mM of MgCl₂.

Amplification reactions were undertaken in a PTC-100 MJ Research, thermocycler with the following amplification program: initial denaturation at 95°C for 3 minutes, followed by 35 cycles of denaturation at 95°C for 30 seconds, annealing at 58°C for 30 seconds, an extension at 72°C for 30 seconds; a final extension for 10 minutes at 72°C. Further, 4 µL of buffer was added to amplified DNA and amplification products were detected by 1.5% agar gel electrophoresis in buffer TBE 1X, at 120 V for 60 minutes. DNA agarose gels stained by ethidium bromide was photographed under ultraviolet light (254 nm) and images were then digitized. Results were evaluated by visually comparing the band patterns after amplification by PCR. Molecular mass marker ladder (100 bp, Invitrogen) was used to estimate molecular mass.

Results and discussion

Since cultivar BRS Atalanta failed to produce amplification of *SOS* gene in any primer, it allows inferring that it did not contain the salinity tolerance gene. In other cultivars, amplification occurred only in primer *SOS1-3*, with 600 bp bands. This fact suggested that these cultivars had the *SOS1* gene (Figure 1).

DNA was amplified for all tolerant and sensitive varieties of gene *CK1* by the three primers (Figure 2). However, for primer *CK1-1*, cultivars BRS-7 Taim and BRS Atalanta revealed bands with low intensity. In the case of primer *CK1-2*, bands had a similar intensity pattern, with the exception of cultivar IAS 12-9 Formosa, whose band was more intense. Primers *CK1-1* and *CK1-2* produced 600 bp bands. Bands of primer *CK1-3* (400 bp) presented the lowest intensity compared to that of

primers CK1-1 and CK1-2. Further, cultivars BRS-7 Taim, BRS Querência and Goyakuman failed to amplify the primer CK1-3.

Considering that primers were designed from a single gene and only the region of primer SOS1-3 showed amplification, it is possible that this region of the gene may have been preserved during evolution. The gene may have undergone some

alterations in the region comprised by the other two primers. The results suggest the presence of the gene in cultivars BRS-7 Taim, IAS 12-9 Formosa, BRS Querência, Goyakuman and BRS Bojurú. Bands in the later were less intense. SOS1 transcript is present in *Arabidopsis* plants without any saline stress, although their levels are increased by treatment NaCl (SHI et al., 2003).

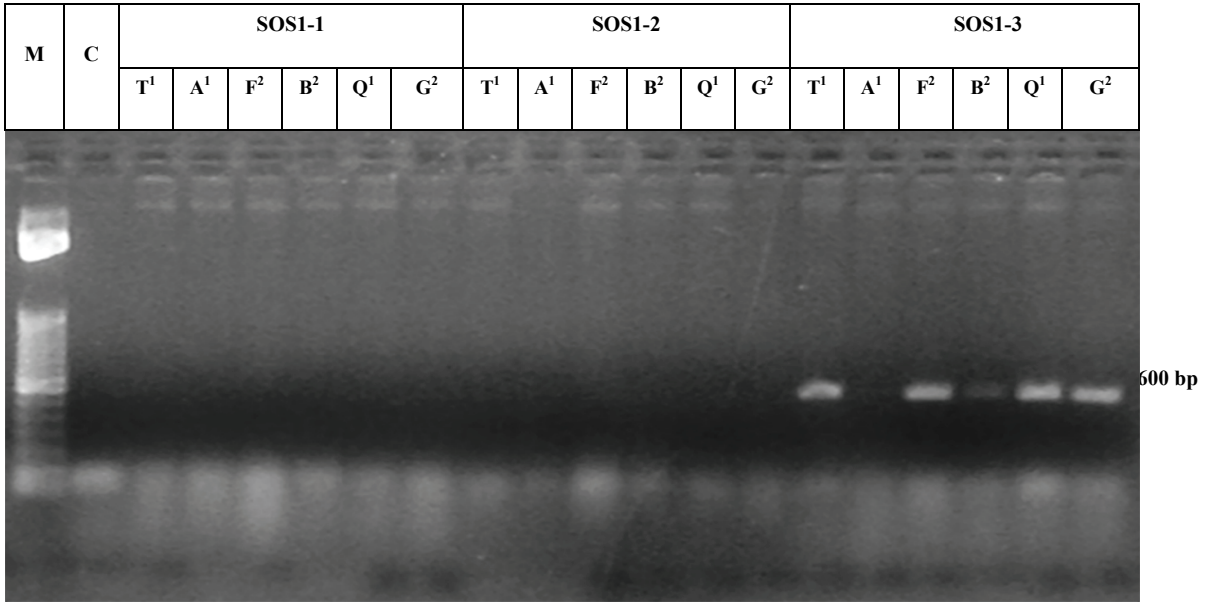


Figure 1. Electrophoresis gel for detection of the gene SOS1 of rice cultivars Taim (T), Atalanta (A), Formosa (F); Bojurú (B); Querência (Q) and GoyaKuman (G), using the *primers* SOS1-1; SOS1-2 and SOS1-3. Ladder 100 bp molecular weight (M) and control (C), without DNA only with 2 μ L H₂O. Based on previous research, ¹ and ² indicate cultivars sensitive and tolerant to NaCl, respectively.

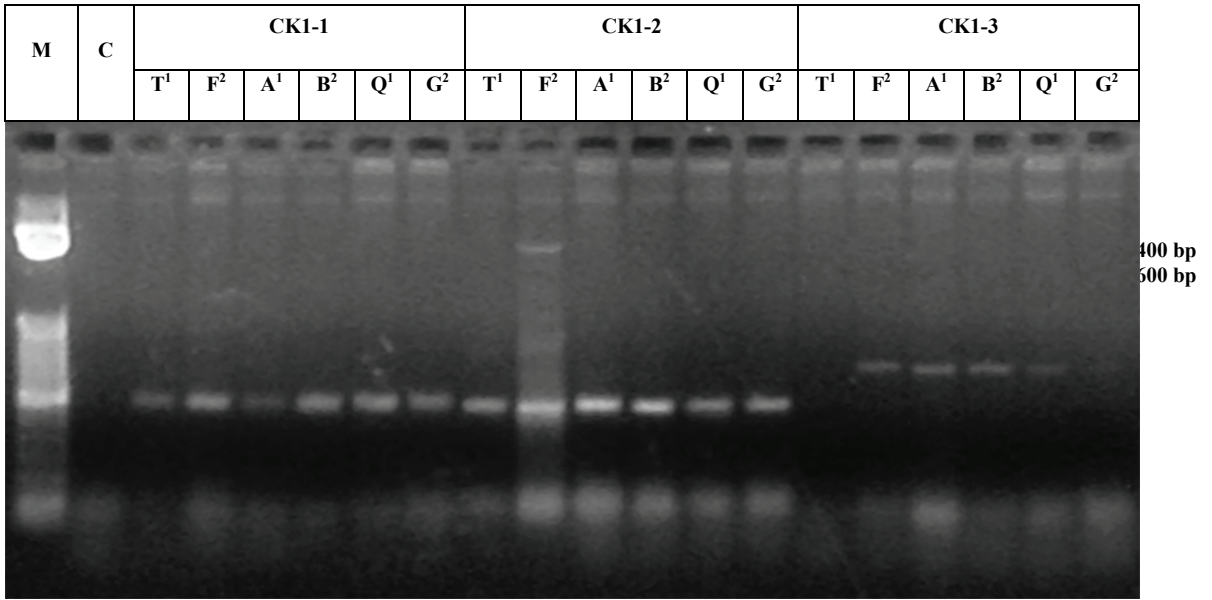


Figure 2. Electrophoresis gel for detection of the gene CK1 of rice cultivars Taim (T), Atalanta (A), Formosa (F); Bojurú (B); Querência (Q) and GoyaKuman (G), using the *primers* CK1-1; CK1-2 and CK1-3. Ladder 100 bp molecular weight (M) and control (C) without DNA only with 2 μ L H₂O. Based on previous research, ¹ and ² indicate cultivars sensitive and tolerant to NaCl, respectively.

Canola transgenic plants (*Brassica napus* L.) in which *Arabidopsis* SOS1 genes were inserted, grew, flowered and produced seeds in the presence of 200 mM NaCl, whereas the wild ones were severely affected (ZHANG et al., 2001). Similar results were obtained by Borsani et al. (2001) with tomato (*Lycopersicon esculentum* L.).

Analyses of *Arabidopsis* SOS mutants revealed a signaling pathway for the regulation of ionic homeostasis and for salinity tolerance (ZHU, 2001). SOS1 may be active as an antiport transporter and as ionic channel in the loading of xylem or even as a Na⁺/H⁺ symport transporter in the unloading of xylem. An antiport transporter of the Na⁺/H⁺ membrane was identified in *Arabidopsis*, which was codified by gene SOS1, whose expression was increased in plants with 25 mM of NaCl (SHI et al., 2003). Identification and comparison of genes induced by saline stress in rice and *Arabidopsis* indicate that molecular mechanisms, similar in the two species, exist as a response to stress (RABBANI et al., 2003). The two cultivars, *O. sativa* ssp. *japonica* and *O. sativa* ssp. *indica* contain gene CK1 which recognizes the signal as a response to environmental stress.

Ca²⁺ signaling mechanism originally found in *Arabidopsis* is also present in rice and gene *OsCK1* has an important role in its response to different stress types (KIM et al., 2003). There are approximately 34 CDPK genes in the *Arabidopsis* genome, which are involved in the response to environmental stress and hormone signaling. Survival and growth are dependent on the restoration of ionic homeostasis (APSE; BLUMWALD, 2007) which is undertaken by transporters. The relevance of transporters Na⁺/H⁺ in salinity tolerance is proved by the detection of *AtNHX1*, a super-expression of one of them, which increases tolerance to saline stress (APSE; BLUMWALD, 2007).

Studies illustrates the combined use of genetic, physiological and biochemical researches to the elucidation of the molecular determinants of ion homeostasis and its regulatory signal in plants, contributing to the development of genotypes tolerant to salinity (MANSOUR; SALAMA, 2004). Moreover, the use of cultivars tolerant to salinity is a viable alternative for the reuse of degraded areas and the use of low quality water (WILLADINO et al., 1999). Tolerance mechanisms to salinity has proved efficient in transgenic *Arabidopsis* (APSE; BLUMWALD, 2007) tobacco (WU et al., 2004) and cotton (HE et al., 2005), showing increased expression of genes that encode protein synthesis.

However, it is essential to find out how these genes can interfere with the molecular mechanisms of plant resistance.

In the future, tests should be performed to check the expression of the genes used in this work, with different subspecies and under different concentrations of salinity through the various existing techniques for measuring gene expression in plants subjected to stress. Currently, the most accurate quantitative analysis for the expression of a particular gene is the real time quantitative PCR (RT-qPCR), providing rapid and reproducible data (GINZINGER, 2002). After a clear understanding of genes and their products (proteins and other biomolecules), it would be possible to identify the mechanisms and molecular markers to be used effectively in breeding programs to develop cultivars resistant to salinity (SILVEIRA et al., 2010).

Conclusion

The genes analyzed are present in the two rice sub-species.

Acknowledgements

The authors would like to thank the Coordination of Improvement of Higher Education Personnel (CAPES) for the scholarship to the first author and for funding by PROAP/ CAPES.

References

- APSE, M. P.; BLUMWALD, E. Na⁺ transport in plants. **FEBS Letters**, v. 581, n. 7, p. 2247-2254, 2007.
- ASHRAF, M. Some important physiological selection criteria for salt tolerance in plants. **Flora**, v. 199, n. 5, p. 361-376, 2004.
- BORSANI, O.; CUARTERO, J.; FERNÁNDEZ, J. A. C.; VALPUESTA, V.; BOTELLA, M. A. Identification of two loci in tomato reveals distinct mechanisms for salt tolerance. **The Plant Cell**, v. 13, n. 4, p. 873-887, 2001.
- CHAVES, M. M.; MAROCO, J. P.; PEREIRA, J. S. Review: Understanding plant responses to drought – from genes to the whole plants. **Functional Plant Biology**, v. 30, n. 3, p. 239-264, 2003.
- CUSHMAN, J. C.; BOHNERT, H. J. Genomic approaches to plant stress tolerance. **Current Opinion Plant Biology**, v. 3, n. 2, p. 117-124, 2000.
- EMBRAPA-Empresa Brasileira de Pesquisa Agropecuária. Agência de informação da Empresa Brasileira de Pesquisa Agropecuária – Arroz. EMBRAPA Arroz e feijão. Available from: <<http://www.cnpaf.embrapa.br>>. Access on: June 3, 2011.
- EPSTEIN, E.; BLOOM, A. J. **Nutrição mineral de plantas: princípios e perspectivas**. 2. ed. Londrina: Editora Planta, 2006.

- FAGERIA, N. K.; SOARES FILHO, N. S.; GHEYI, H. R. Melhoramento genético vegetal e seleção de cultivares tolerantes à salinidade. In: GHEYI, H. R.; DIAS, N. S.; LACERDA, C. F. (Ed.). **Manejo da salinidade na agricultura: estudo básico e aplicado**. Fortaleza: Instituto Nacional de Ciência e Tecnologia em Salinidade, 2010. p. 212-225.
- GAO, R.; DUAN, K.; GUO, G.; DU, Z.; CHEN, Z.; LI, L.; HE, T.; LU, R.; HUANG, J. Comparative transcriptional profiling of two contrasting barley genotypes under salinity stress during the seedling stage. **International Journal of Genomics**, v. 2013, p. 1-19, 2013.
- GINZINGER, D. G. Gene quantification using real-time quantitative PCR: An emerging technology hits the mainstream. **Experimental Hematology**, v. 30, n. 6, p. 503-512, 2002.
- HE, C.; YAN, J.; SHEN, G.; FU, L.; HOLADAY, A. S.; AULD, D.; BLUMWALD, E.; ZHANG, H. Expression of an *Arabidopsis* vacuolar sodium/proton antiporter gene in cotton improves photosynthetic performance under salt conditions and increases fiber yield in the field. **Plant Cell Physiology**, v. 46, n. 11, p. 1848-1854, 2005.
- HOAGLAND, D. R.; ARNON, D. I. **The water-culture method for growing plants without soil**. Berkely: University of California Agricultural Experiment Station, 1950. (Circular n. 347, p. 34).
- KIM, K.-N.; LEE, J.-S.; HAN, H.; CHOI, S. A.; GO, S. J.; YOON, I. S. Isolation and characterization of a novel rice Ca^{2+} -regulated protein kinase gene involved in responses to diverse signals including cold, light, cytokinins, sugars and salts. **Plant Molecular Biology**, v. 52, n. 6, p. 1191-1202, 2003.
- KUMAR, K. G. S.; ULLANAT, R.; JAYABASKARAN, C. Molecular cloning, characterization, tissue-specific and phytohormone-induced expression of calcium-dependent protein kinase gene in cucumber (*Cucumis sativus* L.). **Journal of Plant Physiology**, v. 161, n. 9, p. 1061-1071, 2004.
- LIMA, M. G. S.; LOPES, N. F.; BACARIN, M. A.; MENDES, C. R. Efeito do estresse salino sobre a concentração de pigmentos e prolina em folhas de arroz. **Bragantia**, v. 63, n. 3, p. 335-340, 2004.
- LIMA, M. G. S.; LOPES, N. F.; MORAES, D. M.; ABREU, C. M. Qualidade fisiológica de sementes de arroz ao estresse salino. **Revista Brasileira de Sementes**, v. 27, n. 1, p. 54-61, 2005.
- MACLEAN, J. L.; DAWE, D. C.; HARDY, B.; HETTEL, G. P. **Rice almanac: Source book for the most important economic activity on earth**. 3rd ed. Wallingford: CABI Publishing, 2002.
- MANSOUR, M. M. F.; SALAMA, K. H. A. Cellular basis of salinity tolerance in plants. **Environmental and Experimental Botany**, v. 52, n. 2, p. 113-122, 2004.
- MARENCO, R. A.; LOPES, N. F. **Fisiologia vegetal: fotossíntese, respiração, relações hídricas e nutrição mineral**. 2. ed. Viçosa: UFV, 2007.
- MANTOVANI, E. C.; BERNARDO, S.; PALARTTI, L. F. **Irrigação: princípios e métodos**. Viçosa: UFV, 2006.
- MIT-Massachusetts Institute of Technology. Available from: <<http://www.frodo.wi.mit.edu/cgi-bin/primer3/>>. Access on: Aug. 5, 2006.
- MUNNS, R. Genes and salt tolerance: bringing them together. **New Phytologist**, v. 167, n. 3, p. 645-663, 2005.
- PRISCO, J. T.; GOMES FILHO, E. Fisiologia e bioquímica do estresse salino em plantas. In: GHEYI, H. R.; DIAS, N. S.; LACERDA, C. F. (Ed.). **Manejo da salinidade na agricultura: estudo básico e aplicado**. Fortaleza: Instituto Nacional de Ciência e Tecnologia em Salinidade, 2010. p. 148-164.
- QI, Z.; SPALDING, E. P. Protection of plasma membrane K^+ transport by the Salt Overly Sensitive Na^+ - H^+ antiporter during salinity stress. **Plant Physiology**, v. 136, n. 1, p. 2548-2555, 2004.
- RABBANI, M. A.; MARUYAMA, K.; ABE, H.; KHAN, M. A.; KATSURA, K.; ITO, Y.; YOSHIWARA, K.; SEKI, M.; SHINOZAKI, K.; YAMAGUCHI-SHINOZAKI, K. Monitoring expression profiles of rice genes under cold, drought, and high-salinity stresses and abscisic acid application using cDNA microarray and RNA gel-blot analyses. **Plant Physiology**, v. 133, n. 4, p. 1755-1767, 2003.
- SAGHAI-MAROOF, M. A.; SOLIMAN, K. M.; JORGENSEN, R. A.; ALLARD, R. W. Ribosomal DNA spacer length polymorphism in barley: Mendelian inheritance, chromosome location and population dynamics. **Proceedings of the National Academy of Science of the United States of America**, v. 81, n. 24, p. 8014-8018, 1984.
- SAMBROOK, J.; FRITSCH, F.; MANIATS, T. **Molecular cloning laboratory manual**. 2nd ed. Cold Spring: Harbor Laboratory Press, 1989.
- SHI, H.; LEE, B. H.; WU, S. J.; ZHU, J. K. Overexpression of a plasma membrane Na^+ / H^+ antiporter gene improves salt tolerance in *Arabidopsis thaliana*. **Nature Biotechnology**, v. 21, n. 1, p. 81-85, 2003.
- SHINOZAKI, K.; DENNIS, E. S. Cell signalling and gene regulation global analyses of signal transduction and gene expression profiles. **Current Opinion in Plant Biology**, v. 6, n. 5, p. 405-409, 2003.
- SILVEIRA, J. A. G.; SILVA, S. L. F.; SILVA, E. N.; VIÉGAS, R. A. Mecanismos biomoleculares envolvidos com a resistência ao estresse salino em plantas. In: GHEYI, H. R.; DIAS, N. S.; LACERDA, C. F. (Ed.). **Manejo da salinidade na agricultura: Estudo básico e aplicado**. Fortaleza: Instituto Nacional de Ciência e Tecnologia em Salinidade, 2010. p. 161-180.
- TEIGE, M.; SCHEIKL, E.; EULGEM, T.; DOCZI, R.; ICHIMURA, K.; SHINOZAKI, K.; DANGL, J. L.; HIRT, H. The MKK2 pathway mediates cold and salt stress signaling in *Arabidopsis*. **Molecular Cell**, v. 15, n. 1, p. 141-152, 2004.
- TESTER, M.; DAVENPORT, R. Na^+ tolerance and Na^+ transport in higher plants. **Annals of Botany**, v. 91, n. 5, p. 503-527, 2003.

- WILLADINO, L.; MARTINS, M. H. V.; CAMARA, T. R.; ANDRADE, A. G.; ALVES, G. D. Resposta de genótipos de milho ao estresse salino em condições hidropônicas. **Scientia Agricola**, v. 56, n. 4, p. 1209-1213, 1999.
- WU, C. A.; YANG, G. D.; MENG, Q. W.; ZHENG, C. C. The cotton GhNHX1 gene encoding a novel putative tonoplast Na^+/H^+ antiporter plays an important role in salt stress. **Plant Cell Physiology**, v. 45, n. 5, p. 600-607, 2004.
- XIONG, L.; SCHUMAKER, K. S.; ZHU, J.-K. Cell signaling during cold, drought, and salt stress. **The Plant Cell**, v. 14, suppl. 1, p. 165-183, 2002.
- YANG, G.; SHEN, S.; YANG, S.; KOMATSU, S. *OsCDPK13*, a calcium-dependent protein kinase gene from rice, is induced in response to cold and gibberellin. **Plant Physiology and Biochemistry**, v. 41, n. 4, p. 369-374, 2003.
- ZHANG, H.-X.; HODSON, J. N.; WILLIAMS, J. P.; BLUMWALD, E. Engineering salt-tolerant Brassica plants: Characterization of yield and seed oil quality in transgenic plants with increased vacuolar sodium accumulation. **Proceedings of the National Academy of Science of the United States of America**, v. 98, n. 22, p. 12832-12836, 2001.
- ZHANG, M.; LIANG, S.; LU, Y.-T. Cloning and functional characterization of NtCPK4, a new tobacco calcium-dependent protein kinase. **Biochemical et Biophysical Acta**, v. 1729, n. 3, p. 174-185, 2005.
- ZHU, J.-K. Cell signaling under salt, water and cold stresses. **Current Opinion In Plant Biology**, v. 4, n. 5, p. 401-406, 2001.
- ZHU, J.-K. Salt and drought stress signal transduction in plants. **Annual Review Plant Biology**, v. 53, n. 1, p. 247-273, 2002.
- Received on December 5, 2011.*
Accepted on August 2, 2013.
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