Plant Omics Journal POJ

POJ 9(1):19-25 (2016) ISSN:1836-3644

Evolution of aerenchyma formation in a maize breeding program

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Abstract

Approximately 28 million hectares of intermittently flooded land with agricultural potential in Brazil could be used for rice crop rotation to increase production. Understanding the flood tolerance mechanisms in 'Saracura' is of paramount importance for maize and other crops. Thus, we evaluated the expression levels of the superoxide dismutase, ascorbate peroxidase, guaiacol peroxidase, and catalase antioxidant genes and cell wall loosening (XET) enzyme in maize roots under flooding as well as associated expression with the initiation of aerenchyma formation, which is the most important plant adaptation to flooding. We collected data on 'Saracura' at the beginning of the breeding program (cycle 1), a time considered more sensitive to flooding, and during the last selection cycle (cycle 18), a time considered more resistant to flooding. Maize plants were flooded for 12 and 24h, and roots were collected for anatomic (aerenchyma density in the root cortex) and gene expression analyses by qRT-PCR. Our results showed that there was a proportional graded increase in aerenchyma formed in the root cortex with increased flooding time that was most pronounced during selection cycle 18. Antioxidant enzyme gene expression levels at both time points were similar: high expression just after germination, decreased expression after 12h, and increased expression after 24h of flooding. Aerenchyma formation was detectable after 24h of flooding, which coincided with the highest antioxidant enzyme gene expression levels. These results indicate that the correlation between antioxidant enzyme activities and flooding can act as a molecular flag to improve flood tolerance in 'Saracura'. Our results are important to understand the induction of aerenchyma formation and flooding tolerance in 'Saracura'.

Keywords: Flooding; gene expression; antioxidant system enzymes; *Zea mays* L.; aerenchyma formation.
Abbreviations: CO_2_carbon dioxide; O_2_oxygen; ROS_reactive oxygen species; H_2O_2_hydrogen peroxide; CAT_catalase; GPX_guaiacol peroxidase; POD_ascorbate peroxidase; XET_xyloglucan endotransglucosylase; SOD_superoxide dismutase; CRD_ completely randomized; LPC_lysophosphatidylcloline; PCR_polymerase chain reaction; CT_threshold cycle; PA_proportional area.

Introduction

Approximately 28 million intermittently flooded hectares in Brazil have agricultural potential (Kavalco et al., 2014). This environmental condition leads to restricted diffusion of atmospheric air, increased accumulation of carbon dioxide (CO_2), and decreased oxygen (O_2) in soil, known as hypoxia ($[O_2]$ < 4 %) or anoxia, in extreme cases ($[O_2]$ ~0%) (Muhlenbock et al., 2007; Pezeshki and DeLaune, 2012). Under oxygen-deficient conditions plant growth is drastically inhibited, photosynthetic rate is gradually decreased, and mitochondrial membrane integrity is lost, thereby blocking aerobic respiration. Thus, energy production is restricted to fermentation, with much lower energy yields (Fukao and Bailey-Serres, 2004; Sairam et al., 2008; Banti et al., 2013; Sayed and Gadallah, 2014). Moreover, roots grow only at the soil surface and cannot fully exploit soil volume as under normal oxygenation conditions. In addition, plants produce more reactive oxygen species (ROS), such as hydrogen peroxide (H_2O_2) and other free radicals (Sairam et al., 2008; Guo et al., 2011; Porto et al., 2013).

Plants subjected to hypoxia undergo various morphoanatomical changes to withstand the low oxygen concentrations (Gibbs et al., 2011). Two of the most well-known adaptations are aerenchyma formation and adventitious root development (Bailey-Serres and Voesenek, 2008; Yu et al., 2015). These two adaptations allow plants to keep air inside their cells and survive under hypoxic conditions.

There is a shortage of commercial species adapted to flooded conditions due to low survival, except flooded rice (Silva, 2007). According to FAO (2013), maize (*Zea mays*) is the most produced cereal in Brazil and contributes to about 80% of the country's grain production along with soybeans. Maize can adapt to most climatic conditions due to its allogamous nature (Allard, 1960; Rowan and Barret, 2008). Researchers from the Brazilian Agricultural Research Corporation, EMBRAPA Maize and Sorghum, developed the maize BRS-4154 variety 'Saracura' after considering these genetic characteristics and planting crops in flooded areas immediately after rice is planted to better use the flooded land in Brazil (Parentoni et al., 1997). After several mass selection cycles under intermittent flooding conditions, this variety has proved to be one of the most suitable for cultivation in flooded areas (Alves et al., 2002). Further studies have shown significant genetic gains during selection

cycles in different experiments related to flooding (Souza et al., 2010; Souza et al., 2012). Increased catalase (CAT), guaicol peroxidase (GPX), and ascorbate peroxidase (POD1) antioxidant enzyme activities have been observed in roots throughout the selection cycles for 'Saracura' (Pereira et al., 2010). These antioxidant enzymes specifically removed cellular H_2O_2 (Moller et al., 2007). Pereira et al. (2010) reported increased root density of aerenchyma in 'Saracura', suggesting a relationship between antioxidant enzyme activities and increased area of aerenchyma, which is an essential feature of flood tolerance. Thus, the aim of this study was to evaluate the gene expression levels of three antioxidant enzymes [ascorbate peroxidase (POD), catalase (CAT) and superoxide dismutase (SOD)] and cell wall loosening enzyme [xyloglucan endotransglucosylase (XET)], of 'Saracura' seedlings roots during cycles 1 and 18 and correlate these changes to the induction of aerenchyma formation.

Results

Anatomical analysis

The anatomical results revealed significant differences in the proportion of root cortex area occupied by aerenchyma. About 4% of the root cortex was occupied by aerenchyma on cycle 1, without flooding, whereas it increased to almost 7% on cycle 18, demonstrating good breeding program efficiency. The proportion of area occupied by aerenchyma increased rapidly to about 12% on the cycles 1 and 18 after 12h of flooding (Table 1). The proportion of cortex occupied by aerenchyma reached 15% on the cycle 1 and about 20% in cycle 18 after 24h of flooding. The differences between flooding time and between cycles were significant.

Roots in cycle 1 presented a smaller proportion of cortex area occupied by aerenchyma than those in cycle 18 regardless of flooding time. These results indicate a gradual increase in the proportion of aerenchyma area during flooding (Table 1 and Fig. 1), and the cortex area occupied by aerenchyma was larger during cycle 18 even in unflooded plants. These results demonstrate that the flood tolerance trait is incorporated in the genome.

Expression of antioxidant genes and cell wall enzymes

XET and POD1 were highly expressed, whereas CAT and SOD were expressed at low levels just after germination of Saracura' roots during selection cycle 1 before flooding (Fig. 2). The expression levels of all genes decreased after 12h of hypoxia, but POD1 remained the highest expressed gene, followed by XET, and CAT. SOD had practically no expression during selection cycles 1 and 18 and after 3h of flooding (Fig. 2). The expression levels of all of these genes increased again after 24h of flooding and maintained the levels observed after germination (Fig. 2).

The gene expression results for 'Saracura' roots during selection cycle 18 followed the same pattern observed during cycle 1; high expression of POD1 and XET and lower expression of CAT and SOD. In general, the expression level of all genes increased, (Fig. 2), decreased after 12h of flooding (Fig. 2), and then increased again after 24h of flooding (Fig. 2), but did not reach the initial level. Although similar gene expression patterns were observed during cycles 1 and 18, the levels were always higher during cycle 18 than those during cycle 1, which may be related to the better adaptability of this maize cultivar to flooding and formation of aerenchyma.

Discussion

The results of this study agree with those reported by Pereira et al. (2008), who observed a significant increase in the proportion of cortex area occupied by aerenchyma in 'Saracura' roots subjected to 2 months of hypoxic stress. Aerenchyma begins forming after 24h of hypoxia, and the proportion of cortex occupied by aerenchyma reaches 12% and then gradually increases to 50% after 4 days of flooding (Dantas et al., 2001; Lopes et al, 2005). According to these studies, aerenchyma forms in 'Saracura' seedlings during the first hours of hypoxia, reaching 12% of the cortex area 24h after the stress starts. Thereafter, aerenchyma forms progressively to include 50% of the total cortex area after 4 days and 98% after 7 days of flooding (Souza et al., 2012). Pereira et al. (2008) reported a progressively increasing proportion of aerenchyma in the root cortex after the 'Saracura' maize genetic selection cycle 18. Aerenchyma allows oxygen to reach internal root tissues (Bouranis et al., 2006; Voesenek and Bailey-Serres, 2015), which is crucial to maintain aerobic metabolism in roots in hypoxic or anoxic environments (Insausti et al., 2001; Gunawardena, 2008). This may be the most important strategy adopted by the 'Saracura', as it enables the plant to grow and survive for much longer periods during flooding, compared to other cultivars from the Brazilian germplasm. Previous studies (Dantas et al., 2001; Alves et al., 2002; Lopes et al., 2005; Pereira et al., 2008; Souza et al., 2009; Pereira et al., 2010; Souza et al., 2012) confirmed the superior flood tolerance of the 'Saracura' cultivar compared to that of the BR 107 cultivar, which is consistent with the increased proportion of cortex occupied by aerenchyma found in this study.

In another study conducted by Pereira et al. (2010), CAT and GPX activities decreased during the last few selection cycles, whereas POD1 activity increased. This reduction in antioxidant activity is related to an imbalance in H_2O_2 degradation, which promoted the increase in the amount of aerenchyma formed during the final cycles. CAT gene expression was upregulated during cycle 1 after 12h of flooding and POD1 was upregulated after 24 h of flooding. SOD was not expressed during either cycle after 12 and 24h of flooding, whereas XET gene expression increased during cycle 18 after 12 and 24h of flooding (Fig. 2).

The enhanced gene expression observed during cycle 18 suggests that 'Saracura' roots are more responsive to the oxygen deficit and that more genes related to oxidative stress are expressed. In fact, these two stressors are closely related, as the second one is a consequence of the first (Gunawardena et al., 2001; Evans, 2004; Deuner et al., 2008; Zanandrea, 2010; Steffens, 2014; Kamal and Komatsu, 2015b). Many researchers have associated XET, POD1, CAT, and SOD enzyme activities with increased flood tolerance in 'Saracura' (Dantas et al., 2001; Lopes et al., 2005; Pereira et al., 2010; Porto et al, 2013). Tolerance to anaerobic stress can be improved by increasing antioxidant enzyme activities (Mehlhorn et al., 1996; Biermelt et al., 1998; Jiang and Huang, 2001, Lin et al., 2007; Chiang et al., 2014). In the present study, the increased flood tolerance of the 'Saracura' occurred along with increased expression of antioxidant enzyme genes during the two selection cycles. Hypoxia induces cellulase and XET activities, leading to disorders of cell wall components and formation of aerenchyma (Brett

Table 1. Proportion of aerenchyma in the total cortex area (PA) of 'Saracura' maize roots and growth during selection cycles 1 and 18 at different flooding times.

Cycle 1	PA (%) C1	Cycle 18	PA (%) C18
0 h	4. 36 A a	0 h	6.9 B a
12 h	11.57 A b	12 h	12.03 B b
24 h	15. 75 A b	24 h	19.55 B c

Uppercase letters represent differences between cycles and lowercase letters represent differences between different flooding times within the same cycle.

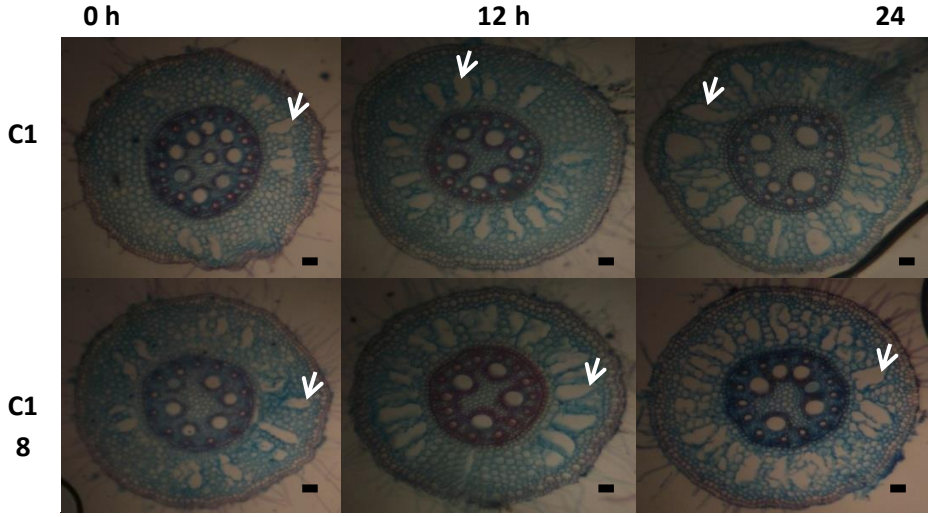

Fig 1. Aerenchyma formation in 'Saracura' root during selection cycles 1 and 18 after flooding the substratum. Bars = 100 µm. Arrows show aerenchyma.

And Waldron, 1990; Vitorino et al., 2001; Manzur et al., 2014). Porto et al. (2013) demonstrated that expression of the poligalacturonase gene, which is a cell wall loosening enzyme in *Saracura coleoptile* that appears just after germination, is high, decreases after 4.5 days of hypoxia, and then increases on day 6. The XET gene allows for the growth and development of seedlings immediately after seed germination through a cell wall loosening effect (Asada, 1999; Fries et al., 2007; Porto et al., 2013). This may explain the high XET expression after germination and the subsequent decrease after 12h of flooding in this study.

The aerenchyma that forms in maize roots under hypoxic conditions has a lysigenous origin, high cellulase activity, and is induced by apoptosis (Dantas et al., 2001; Gunawardena et al., 2001a; Gunawardena et al., 2001b; Gadjev et al., 2008; Petrov et al., 2015). The activity of the cell wall loosening enzyme is closely linked to formation of aerenchyma (Saab and Sachs, 1996), which is usually found during the early stages of flooding in the maize coleoptile (Dantas et al., 2001). The high antioxidant enzyme activities that are observed immediately after germination are due to neutralization of the free radicals formed during germination and plant growth (Asada, 1999; Fries et al., 2007; Porto et al., 2013; Balakhnina et al., 2015). Dismutation of O_2^- is catalyzed by SOD. CAT, POD1, and other peroxidases reduce H_2O_2 to H_2O. Thus, these enzymes constitute the frontline defense against ROS (Léon et al., 2002; Xi et al., 2010; Chiang et al., 2014).

Kamal (2015a) found that the POD1 protein was downregulated in soybean cotyledons under flooding stress, which is the opposite of what was found in this study. This contrary result can be explained since the upregulation of POD1 was detoxifying and may explain the ability of 'Saracura' to survive longer under intermittent flooding. Another study that supported these results was reported by Chiang (2014a), who created flood-tolerant rice plants by transforming them with the eggplant POD1 gene fused with a constitutive promoter.

The gene expression patterns observed in this study during cycles 1 and 18 can be explained based on these relationships. SOD was active during germination, as it generates H_2O_2 that needs to be eliminated by peroxidases and catalases, justifying the high expression of their genes, particularly POD1, soon thereafter. SOD enzyme expression does not change during flooding (Fries et al., 2007; Chiang et al., 2014b). Several authors have shown that POD1 activity increases at the detriment of CAT (Dantas et al., 2001; Lopes et al., 2005; Pereira et al., 2010) during flooding. CAT is specialized to remove high concentrations of toxic cellular peroxides (Mittler, 2002), suggesting that there some ROS may not be removed by POD1 (Madhusudhan et al., 2003).
Greater formation of aerenchyma in maize roots during cycle 18 was detected after 12 h of flooding (Table 2), when the levels of antioxidant enzyme gene expression were lower, compared to the initial period (Fig. 2). This low expression may have led to a decrease in enzyme synthesis and an accumulation of cellular ROS. Accumulating ROS lead to lipid peroxidation and cell death (Cakmak and Horst, 1991; Mittler et al., 2004; Zanandrea et al., 2010; Baxter et al., 2013), which can generate lysigenous aerenchyma. The accumulation of ROS is a molecular flag in response to various stimuli, including hypoxia after 12–24 h of flooding (Neill et al., 2002). Antioxidant gene expression is activated, and there is a direct relationship between enzyme synthesis and expression level. The increased antioxidant and cell wall loosening enzyme activities we observed (Fig. 2) prevented cell collapse by scavenging free radicals. Nevertheless, these enzymes increased flooding tolerance and subsequent survival of 'Saracura' during hypoxia. The significant increase in the proportion of aerenchyma in the root cortex

Table 2. Polymerase chain reaction primers with NCBI and GenBank identification.

Gene	Orientation	Sequence 5′ --------3′	NCBI/GenBank	Amplicon (bp)
Ascorbate Peroxidase (POD1)	*Forward*	GCTGAGTGACCCTGTCTTCC	FJ797426.1	102
	Reverse	AGTTCGGAGAGCCTTGAGGTG		102
Catalase (CAT1)	*Forward*	CCAAACTATCTGATGCTTCC	X60135.1	63
	Reverse	ATCATGGTGGTTATTGTGGT		63
Xyloglucan Endotransglycosylase (XET)	*Forward*	TCTACCTGTCGTCGCAGAACTC	NM_001155512.1	98
	Reverse	CCGGTTGCCCAGGAACT		98
Superoxide Dismutase (SOD1)	*Forward*	GCATAGCCGAGGCAACCAT	AB093581.1	106
	Reverse	AACTGAATTTGCGCCAGTCAA		106
Ubiquitin	*Forward*	AAGGCCAAGATCCAGGACAA	XM_008657649.1	69
	Reverse	TTGCTTTCCAGCGAAGATGA		69
Alcohol Dehydrogenase (ADH)	*Forward*	AGGACGCTGAGTTAAGACC	XM_008650471	104
	Reverse	CACATTTGGCAGATCAGTGC		104

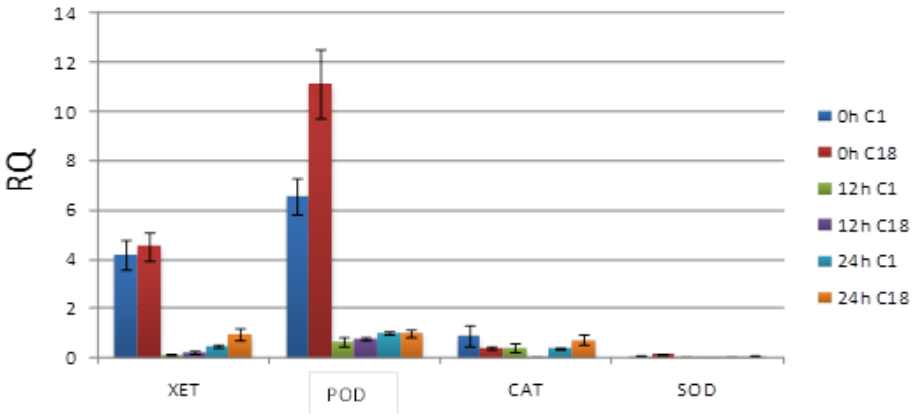

Fig 2. Ascorbate peroxidase (POD1), xyloglucan endotransglycosylase (XET), catalase (CAT), guaiacol peroxidase (GPX), and superoxide dismutase (SOD) gene expression in 'Saracura' roots during selection cycles 1 and 18. Time: 0, 12, and 24 h of hypoxia. RQ, relative quantity

during cycle 18 was absent during cycle 1 but started to increase after 12 h of flooding. This behavior was attributed to the increased formation of ROS in the region.

Materials and Methods

Plants and experimental design

We used the caryopsis of the BRS 4154 maize cultivar, which is also called 'Saracura'. The selection program has been conducted by Embrapa maize and sorghum since 1986. Selection was carried out in trays in poorly drained alluvial soil. Flooding was initiated about 30 days after planting by intermittently filling the pan every 2 days to a 20 cm water depth and allowing it to drain naturally. Mass selection was conducted after the stress was applied and only the best plants were used for cycles 1–18 (Parentoni et al. 1998)
Caryopses were grown for these two cycles in plastic trays filled with vermiculite and were divided horizontally. The caryopses were arranged on each side to avoid possible environmental interference. The trays were kept in a growth chamber at 26 ± 2°C and a 12h photoperiod for 4 days. To avoid possible hypoxia during germination, perforations were made in the tray bottoms, and the holes were sealed with Durepox© to induce hypoxia. Water was added above the substrate level until a 1 cm blade formed and remained for different periods (12 and 24h). Plants that were not watered were used as a control.

Detecting the formation of aerenchyma

Primary roots were collected from three seedlings per replicate for the anatomical analysis and were fixed in formalin, acetic acid, and 70% ethanol for 72h and then stored in 70% ethanol until data collection (Kraus and Arduim, 1997). Two cm cuts were made into the pilifera root zone for the evaluation. The pilifera root fragments were cut into cross sections using a microtome, clarified with 5% sodium hypochlorite for 10 min, rehydrated for 10 min, stained with "astrablau" (7.5:2.5, safranin solution: astra blue), and assembled on slides with 50% glycerin (Kraus and Arduim, 1997). Photographs of the sections were obtained with an optical microscope coupled to a digital camera, and total cortex area, area of the aerenchyma, and the proportion of the cortex area occupied by aerenchyma were measured with the UTHSCSA ImageTool analysis software. Three replciates were taken to calculate the mean for each anatomical characteristic. The proportion of area in the cortex occupied by the aerenchyma was calculated by dividing total aerenchyma area by total cortex area.

RNA extraction

RNA was extracted from roots using TriReagent (Sigma, St. Louis, MO, USA), according to the manufacturer's instructions with some modifications.

The samples were stored at −20°C. RNA integrity was assessed spectroscopically, and quality was evaluated by agarose gel electrophoresis. DNAse Free was applied from the DNAse Turbo Free kit (Ambiom, Austin, TX, USA). RNA was quantified using Nanodrop® Spectrophotometer ND-1000 (NanoDrop Technologies, Wilmington, DE, USA), and cDNA was synthesized using the High Capacity cDNA Reverse Transcription cDNA Kit (Applied Biosystems, Foster City, CA, USA).

The primers were designed using the Primer Express 3 program (Applied Biosystems) (Table 2), and cDNA quality by PCR amplification using oligonucleotides corresponding to endogenous ubiquitin and alcohol dehydrogenase (Livak and Scmittgen, 2001; Scgikdberg et al., 2009). Gene expression was quantified using the ABI PRISM 7500 Real-Time PCR (Applied Biosystems) and the SYBR Green detection system. Relative gene expression was determined by the comparative CT method after diluting the samples 1:10. The expression analysis was processed in triplicates. After amplification, data were analyzed using 7500 Fast Software ver. 2.1 (Applied Biosystems). Each sample was normalized with the mean expression value of the endogenous genes and the equation ΔCT = CT (target gene) − CT (endogenous). Relative quantification values were obtained using $-2^{\Delta\Delta CT}$ and calibrated with the equation ΔΔCT = ΔCT (sample) − ΔCT (calibrated sample). Expression of all genes in a 12 h flood treatment sample was analyzed as a calibrator. All qPCR analyses followed the MIQE guidelines for qPCR analysis (Bustin et al., 2009).

Statistical analysis

The experimental design was completely randomized and each tray was considered a repeat. Three replicate measurements were taken for each anatomical characteristic. Tukey's test was used to make comparisons. A *p*-value < 0.05 was considered significant. Sisvar software (Ferreira, 2011) was used to conduct the analysis.

Conclusion

We conclude that antioxidant enzyme gene expression was correlated with formation of aerenchyma and acted as a molecular flag to activate the flood-tolerance mechanism in 'Saracura'. These results are for understanding the mechanisms of flood tolerance in maize. t These results could be extrapolated to other cultivars adapted to micro-environments or other flood-tolerant crop species.

Acknowledgements

The authors are grateful to the Brazilian Agricultural Research Corporation, EMBRAPA, to the researcher Dr. Paulo César Magalhães for providing seeds, to Professor Dr. Evaristo Mauro Castro for the facilities to conduct the anatomical analyzes and to CNPq, CAPES, and FAPEMIG for grants and a scholarship.

References

Allard RW (1960) Bases genéticas do melhoramento em plantas alógamas. In Allard RW (Ed.), Princípios do melhoramento genético das plantas. Translators: A. Blumenschein, E. Paterniani, J. T. Gurgel, and R. Vencovsk.. New York, NY, USA: John Wiley and Sons, Inc. 135-189.

Alves JD, Magalhães MM, Goulart PF, Dantas BF, Gouvêa JA, Purcino RP (2002) Mecanismos de tolerância da variedade de milho "saracura" (BRS 4154) ao alagamento. Rev Bras de Milho e Sorgo. 1: 33-40.

Asada K (1999) The water-watercycle in chloroplasts: scavening of active oxygens and dissipation of excess photons. Annu Rev Plant Physiol Plant Mol Biol. 50: 601-639.

Bailey-Serres J, Voesenek LA (2008) Flooding stress: acclimations and genetic diversity. Annu Rev Plant Biol. 59: 313-339.

Balakhnina T, Bulak P, Nosalewicz M, Pietruszewski S, Wlodarczyk T (2015) The influence of wheat *Triticum aestivum* L. seed pre-sowing treatment with magnetic fields on germination, seedling growth, and antioxiant potential under optimal soil watering and flooding. Acta Physiol Plant. 37: 1-10.

Banti V, Giuntoli B, Gonzali S, Loreti E, Magneschi L, Novi G (2013) Low oxygen response mechanisms in green organisms. Int J Mol Sci. 14: 4734-4761.

Baxter A, Mittler R, Suzuki N (2013) ROS as key players in plant stress signalling. J Exp Bot. 1-12.

Biermelt S, Keetman U, Albrecht G (1998) Re-aeration following hypoxia or anoxia leads to activation of the antioxidative defense system in roots of wheat seedlings. Plant Physiol. 116: 651-658.

Bouranis DL, Chorianopoulou SN, Kollias C, Maniou P, Protonotarios VE, Siyiannis VF (2006) Dynamics of aerenchyma distribution in the cortex of sulfate-deprived adventitious roots of maize. Ann Bot-London. 97: 695-704.

Brett C, Waldron K (1990) Physiology and biochemistry of plant cell walls. In: Topics in Plant Physiology. Netherlands, Springer. 20-256.

Bustin SA, Benes V, Garson JA, Hellemans J, Huggett J, Kubista M, Mueller R, Nolan T, Pfaffl MW, Shipley GL, Vandesompele J, Wittwer CT (2009) The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. Clin Chem. 55: 4 611-622.

Cakmak I, Horst WJ (1991) Effect of aluminium on lipid peroxidation, superoxide dismutase, catalase and peroxidase activities in root tips of soybean (Glycine max). Physiol Plantarum. 83: 463-468.

Chiang CM, Chen LF, Shih SW, Lin KH (2014a) Expression of eggplant ascorbate peroxidase increases the tolerance of transgenic rice plants to flooding stress. J Plant Biochem Biot. 1-11.

Chiang CM, Kuo WS, Lin KH (2014b) Cloning and gene expression analysis of sponge gourd ascorbate peroxidase gene and winter squash superoxide dismutase gene under respective flooding and chilling stresses. Hort Environ Biote. 55: 129-137.

Dantas BF, Aragão CA, Alves JD (2001) Cálcio e desenvolvimento de aerênciquimas e atividade de celulase em plântulas de milho submetidas a hipoxia. Sci Agr. 58: 251-257.

Deuner S, Alves AJ, Deitos DF, Zanandrea I, Almeida AL, Castro PH (2008) Peróxido de hidrogênio e ácido ascórbico influenciando a atividade de enzimas antioxidantes de mudas de cafeeiro. Ceres. 55: 135-140.

Evans D (2004) Aerenchyma formation. New Phytol. 161: 35-39.

FAO (2013) FAOSTAT. Retrieved march 24, 2015, from faostat.fao.org

Ferreira DF (2011) Sisvar: a computer statistical analysis system. Cienc Agrotec. 35: 1039-1042.

Fries DD, Alves JD, Delú Filho N, Magalhães PC, Goulart PF, Magalhães MM (2007) Crescimento de plântulas de milho "saracura" e atividade de alfa amilase e invertases associados ao aumento da tolerância ao alagamento exercido pelo cálcio exógeno. Bragantia. 66: 1-9.

Fukao T, Bailey-Serres J (2004) Plant responses to hypoxia is survival a balancing act? Trends Plant Sci. 9: 449-456.

Gadjev I, Stone JM, Gechev TS (2008) Programmed cell death in plants: new insights into redox regulation and the role of hydrogen peroxide. Int Rev Cel Mol Bio. 270: 87-144.

Gibbs DJ, Lee SC, Isa NM, Gramuglia S, Fukao T, Bassel GW (2011) Homeostatic response to hypoxia is regulated by the N-end rule pathway in plants. Nature. 479: 415-419.

Gunawardena A H (2008) Programmed cell death and tissue remodelling in plants. J Exp Bot. 59: 445-451.

Gunawardena AH, Pearce DM, Jackson MB, Hawes CR, Evans DE (2001a) Characterisation of programmed cell death during aerenchyma formation induced by ethylene or hypoxia in roots of maize (Zea mays L.). Planta. 212: 2005-2014.

Gunawardena AH, Pearce DM, Jackson MB, Hawes CR, Evans DE (2001b) Rapid changes in cell wall pectic polysaccharides are closely associated with early stages of aerenchyma formation, a spatially localized form of programmed cell death in roots of maize (*Zea mays* L.) promoted by ethylene. Plant Cell Environ. 24: 1369-1375.

Guo XY, Huang ZY, XU A, Zhang ZS (2011) A comparison of physiological, morphological and growth responses of 13 hybrid poplar clones to flooding. Forestry. 84: 1-12.

Insausti P, Grimoldi AA, Chaneton EJ, Vasellati V (2001) Flooding induces a suite of adaptive plastic responses in the grass *Paspalum dilatatum*. New Phytol. 152: 291-299.

Jiang Y, Huang B (2001) Effects of calcium on antioxidant activities and water relations associated with heat tolerance in two cool-season grasses. J Exp Bot. 52: 341-349.

Kamal AH, Rashid H, Sakata K, Komatsu S (2015a) Gel-free quantitative proteomic approach to identify cotyledon proteins in soybean under flooding stress. J Proteomics. 112: 1-13.

Kamal AH, Komatsu S (2015b) Involvement of reactive oxygen species and mitochondrial proteins in biotphoton emission in roots of soybean plants under flooding stress. J Proteome Res. 1: 2219-2236.

Kavalco SAF, Figueiredo R, Groli EL, Zimmer CM, Baretta D, Tessmann EW (2014) Análise de trilha em genótipos de trigo submetidos ao estresse por encharcamento. Semina: Ci Agrárias. 35: 1683-1695.

Kraus JE, Arduim M (1997) Manual básico de métodos em morfologia vegetal. Rio de Janeiro, RJ, Brazil: EDUR. 198p.

Léon AM, Palma JM, Corpas FJ, Gómez M, Romero-Puertas MC, Chatterjee D (2002) Antioxidative enzymes in cultivars of pepper plants with different sensitivity to cadmium. Plant Physiol Bioch. 40: 813-820.

Lin KH, Lo HF, Lin CH, Chan MT (2007) Cloning and expression analysis of ascorbate peroxidase gene from eggplant under flooding stress. Bot Stud. 48: 25-34.

Livak KJ, Scmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2(-DeltaDeltaCT) method. Methods. 25: 402-408.

Lopes MJ, Souza IR, Magalhães PC, Gama EE, Alves JD, Magalhães MM (2005) Oxidação protéica e peroxidação lipídica em plantas de seleção do milho "Saracura", sob encharcamento contínuo. *Rev Bras de Milho e Sorgo*. 4: 362-373.

Madhusudhan R, Ishikawa T, Sawa Y, Shigeoka S, Shibata H (2003) Characterization of an ascorbate peroxidase in plastids of tobacco BY-2 cells. Physiol Plant. 117: 550-557.

Manzur ME, Grimoldi AA, Insausti P, Striker GG (2014) Radial oxygen loss and physical barriers in relation to root tissue age in species with different types of aerenchyma. Funct Plant Biol. 42: 9-17.

Mehlhorn H, Lelandais M, Korth HG, Foyer CH (1996) Ascorbate is the natural substrate for plant peroxidases. FEBS Lett. 378: 203-206.

Mittler R (2002). Oxidative stress, antioxidants and stress tolerance. Trends Plant Sci. 7: 405-410.

Mittler R, Vanderauwera S, Gollery M, Breusegem FV (2004) Reactive oxygen gene network of plants. Trends Plant Sci. 9: 490-498.

Moller IM, Jensen PE, Hansson A (2007) Oxidative modifications to cellular components in plants. Annu Rev Plant Biol. 58: 459-481.

Muhlenbock PM, Plaszczyca M, Plaszczyca M, Mellerowicz E, Karpinski S (2007) Lysigenous aerenchyma formation in *Arabidopsis* is controlled by lesion simulating disease. Plant Cell. 19: 3819-3830.

Neill S, Desikan R, Hancock J (2002) Hydrogen peroxide signaling. Curr Opin Plant Biol. 5: 388-395.

Parentoni SN, Gama EE, López MA, Santos MX, Guimarães PE, Pacheco CA (1997) Seleção para a tolerância ao encharcamento na variedade de milho CMS54-saracura. Reunion Latinoamericana: 4 Reunion de la zona andina de investigadores en maiz. 17: 368-373. Cartagena de Indias: 4 Reunion Latinoamericana.

Parentoni SN, Gama EE, López MA, Santos MX, Guimarães PE, Pacheco CA, Meirelles WF, Souza IRP, Correa LA (1998) Nove ciclos de seleção para tolerância ao encharcamento na variedade de milho CMS54-saracura. CNMS. 22: 1-5.

Pereira FJ, Castro EM, Souza TC, Magalhães PC (2008) Evolução da anatomia radicular do milho "saracura" em ciclos de seleção sucessivos. Pesq Agropec Bras. 43: 1649-1656.

Pereira FJ, Magalhães PC, Souza TC, Castro EM, Alves JD (2010) Atividade do sistema antioxidante e desenvolvimento de aerênquima em raízes de milho "saracura". Pesq Agropec Bras. 45: 16-24.

Petrov V, Hille J, Mueller-Roeber B, Gechev TS (2015) ROS-mediated abiotic stress-induced programmed cell death in plants. Front Plant Sci. 6: 1-16.

Pezeshki SR, DeLaune RD (2012) Soil oxidation-reduction in wetlands and its impact on plant functioning. Biology. 1: 196-221.

Porto BN, Alves JD, Magalhães PC, Castro EM, Campos NA, Souza KR (2013) Calcium-dependent tolerant response of cell wall in maize mesocotyl uner flooding stress. J Agron Crop Sci. 199 :134-143.

Rowan DH, Barret DS (2008) Adaptation from standing genetic variation. Trends Ecol Evol. 23: 38-44.

Saab IN, Sachs MM (1996) A flooding-induced xyloglucan endo-transglycosylase homolog in maize is responsive to ethylene and associated with aerechyma. Plant Physiol. 112: 385-391.

Sairam RK, Kumutha D, Ezhilmathi K, Deshmukh PS, Srivastava GC (2008) Physiology and biochemistry of waterlogging tolerance in plants. Biol Plantarum. 52: 401-412.

Sayed SA, Gadallah MA (2014) Effects of silicon on *zea mays* plant exposed to water and oxygen deficiency. Russ J Plant Physiol. 61: 460-466.

Scgikdberg TA, Norden TD, Nelson DD, Jenkins GR (2009) Evaluating precision and accuracy when quantifying different endogenou control reference genes in maize using real-time PCR. J Agr Food Chem. 57: 2903-2911.

Silva SD, Sereno MJ, Silva CF, Oliveira AC, Neto JB (2007) Inheritance of tolerance to flooded soils in maize. Crop Breed Appl Biot. 7: 165-172.

Souza TC, Castro EM, Magalhães PC, Alves ET, Pereira FJ (2012) Early characterization of maize plants in selection cycles under soil flooding. Plant Breeding. 131: 493-501.

Souza TC, Castro EM, Pereira FJ, Parentoni SN, Magalhães PC (2009) Morpho-anatomical characterization of root in recurrent selection cycles for flood tolerance of maize (*Zea mays* L.). Plant Soli Environ. 55: 504-510.

Souza TC, Magalhães PC, Pereira FJ, Castro EM, Junior JMS, Parentoni SN (2010) Leaf plasticity in successive selection cycles of "saracura" maize in response to periodic soil flooding. Pesq Agropec Bras. 45: 16-24.

Steffens B (2014) The role of ethylene and ROS in salinity, heavy metal, and flooding responses in rice. Front Plant Sci. 5: 1-5.

Vitorino PG, Alves JD, Magalhães PC, Magalhães MM, Lima LC, Oliveira LE (2001) Flooding tolerance and cell wall alterations in maize mesocotyl during hypoxia. Pesq Agropec Bras. 36: 1027-1035.

Voesenek LA, Bailey-Serres J (2015) Flood adaptive traits and processes: an overview. New Phytol. 206: 57-73.

Xi D M, Liu, W S, Yang G D, Wu C A, Zheng C C (2010) Seed-specific overexpression of antioxidant genes in *Arabidopsis* enhances oxidative stress tolerance during germination and early seedling growth. Plant Biotechnol J. 8: 796-806.

Yu B, Zhao CY, Li J, Li JY, Peng G (2015) Morphological, physiological, and biochemical responses of *Populus euphratica* to soil flooding. Photosynthetica. 53: 110-117.

Zanandrea I, Alves JD, Deuner S, Goulart PF, Henrique PC, Silveira NM (2010) Tolerance of *Sesbania virgata* plants to flooding. Aust J Bot. 57: 661-669.