


# Aerenchyma formation in the initial development of maize roots under waterlogging

Kamila Rezende Dázio de Souza  · Meline de Oliveira Santos · Cíntia Aparecida Andrade · Dayane Meireles da Silva · Nádia Alves Campos · Jose Donizeti Alves

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**Abstract** We investigated the role of antioxidant and cell wall loosening enzymes in aerenchyma formation in roots of Saracura maize (*Zea mays*) from two different selection cycles with contrasting tolerance to waterlogging. Plantlets of Saracura maize from the 1st cycle (C1—sensitive) and 18th cycle (C18—tolerant) of selection were subjected to waterlogging for 0, 24, 48, 72, 96, and 120 h, and roots were collected for biochemical and anatomical analyses. Plantlets of cycle 18 exhibited higher aerenchyma formation, exodermis thickening, and higher activity of alcohol dehydrogenase, polygalacturonase, cellulase, and antioxidant enzymes than plantlets of cycle 1. Although these processes also occurred in C1 plantlets, they were independent of the waterlogging and

therefore were initiated later. C18 plantlets exhibited biochemical and physiological characteristics that may have contributed to the higher water excess tolerance of Saracura maize plantlets from the later selection cycle. Higher aerenchyma formation in C18 plantlets is related to higher and early activity of cell wall loosening enzymes and higher activity of antioxidant enzymes than in C1 plantlets.

**Keywords** Antioxidants · Cell wall · Anatomy · Tolerance

## 1 Introduction

One of the potential impacts of global climate changes is the intensification of flooding in different crop areas around the world (Durack et al. 2012). In this scenario, plant growers should change land use or, grow more tolerant crops to water excess. Indeed, scientists are concerned about these issues and have been working on tools to identify or develop cultivars that are tolerant to waterlogging (Voeselek and Bailey-Serres 2013). In maize (*Zea mays* L.), considerable effort has been made to identify traits associated to tolerance to hypoxia (Chugh et al. 2012; Mano and Omori 2013).

Plant survival in root environments with low oxygen availability depends on the development of morpho-anatomical and physiological responses (Voeselek and Bailey-Serres 2013; Yu et al. 2015),

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K. R. D. de Souza (✉) · C. A. Andrade ·  
D. M. da Silva · J. D. Alves  
Programa de Pós-Graduação em Fisiologia Vegetal, Setor  
de Fisiologia Vegetal, Departamento de Biologia,  
Universidade Federal de Lavras, Campus Universitário,  
Caixa Postal 3037, Lavras, MG, Brazil  
e-mail: krdazio@hotmail.com

M. de Oliveira Santos  
Empresa de Pesquisa Agropecuária de Minas Gerais -  
EPAMIG Sul, Campus da Universidade Federal de Lavras  
- UFLA, Rodovia Lavras/Ijaci km 02,  
Caixa Postal 176, Lavras, MG CEP: 37200-000, Brazil

N. A. Campos  
Katholieke Universiteit Leuven, KU Leuven, Willem de  
Croylaan 42, PO Box 3000, Heverlee, Leuven, Belgium

such as aerenchyma formation, endodermis and exodermis thickening, activation of anaerobic enzymes and antioxidant metabolism, associated to induction of adventitious root growth and lenticels development (Colmer and Voesenek 2009; Imaz et al. 2013). Plants that maintain cellular homeostasis through these processes can survive for longer periods under waterlogging (Gechev et al. 2006). This tolerance is expressed by the maintenance or increasing in plant biomass under suboptimal conditions (Mendiondo et al. 2016).

The formation of aerenchyma responding to oxygen deprivation is a key trait to survive longer under hypoxia. These structures typically provide a channel that connects shoots and roots, allowing oxygen flux and thereby facilitating aerobic respiration in submerged organs (Mano and Omori 2013). Aerenchyma formation in roots is a process mediated by ethylene and related to the generation of reactive oxygen species (ROS), culminating in programmed cell death (PCD) and cell wall degradation (Gunawardena et al. 2001; Porto et al. 2013; Pires et al. 2015; Yamauchi et al. 2013).

A relationship has been observed between PCD and oxidative stress in plants under stress conditions (Gunawardena et al. 2001; Yamauchi et al. 2011). Specifically, just after the transition from normoxia to hypoxia it is observed an increase in ROS production, which is related to damages to cellular components, being part of the PCD process. However, the propagation of oxidative cycles in cells is avoided by the activation of enzymatic and non-enzymatic antioxidant system (Gill and Tuteja 2010). Therefore, the balance between ROS generation and antioxidant enzymes will determine the occurrence of PCD or cell homeostasis.

The Saracura maize (BRS-4154) is a variety tolerant to flooding. This trait was improved by the breeding program (Maize and Sorghum National Research Center) through recurrent selection. It is known that in these plants the aerenchyma formation represents a crucial mechanism to waterlogging tolerance. Therefore, plants from the first selection cycle (Cycle 1—C1) are more sensitive to waterlogging than plants from the last selection cycle (Cycle 18—C18) (Ferreira et al. 2007). Besides that, several studies have demonstrated that aerenchyma formation increases in this maize cultivar over the selection cycles, so the plants from Cycle 1 show

few aerenchymas than plants from Cycle 18 (Dantas et al. 2001; Vitorino et al. 2001; Fries et al. 2007; Pereira et al. 2008, 2010; Souza et al. 2009, 2010, 2012; Porto et al. 2013; Pires et al. 2015; Campos et al. 2016).

Despite the extensive studies about the tolerance of Saracura maize to waterlogging, most of them have been developed under greenhouse or field conditions using plants subjected to intermittent waterlogging from the stages V6 to R6 (Pereira et al. 2008, 2010; Souza et al. 2009, 2010, 2011; Pires et al. 2015), which may be influenced by different conditions of biological and climate factors. Those valuable studies showed a variety of root and leaf morpho-anatomical and physiological modifications of Saracura maize that successfully resulted in higher tolerance to waterlogging, culminating in higher productivity under water excess. On the other hand, studies with plantlets were conducted with plantlets from first selection cycles under completely submerged conditions and using calcium to increase the tolerance to waterlogging (Dantas et al. 2001; Vitorino et al. 2001; Fries et al. 2007). These studies were basically focused on the mesocotyl region of the plantlets only. Later, differential expression of superoxide dismutase (SOD), xyloglucan endo-transglycosylase (XET), alcohol dehydrogenase (ADH) and polygalacturonase (PG) at the mesocotyl region of completely submerged maize plantlets from C1 and C18 were observed (Porto et al. 2013).

Still, the gene expression of SOD, CAT, peroxidase (POD) and XET in roots of C1 and C18 plantlets at the first hours of waterlogging has been also investigated (Campos et al. 2016). It was evident the change in gene expression at the first 24 h of waterlogging, and the increased expression of antioxidant enzymes in roots of plantlets from C18 in relation to those from C1. To confirm the occurrence of the physiological events related to the genes studied by Campos et al. (2016), we conducted an experiment with plantlets of C1 and C18 for 120 h of waterlogging to test the hypothesis that the changes in the first hours of waterlogging are due to the early physiological responses of Saracura maize to initiate the morpho-anatomical modifications related to the tolerance to waterlogging. Here, we investigated the role of antioxidant and cell wall loosening enzymes in aerenchyma formation in roots of Saracura maize from two different selection cycles with contrasting tolerance to waterlogging.

## 2 Material and methods

### 2.1 Plant material and waterlogging conditions

Caryopses of Saracura maize cv. 'BRS-4154' from cycles 1 and 18 were sown in polypropylene trays (34 × 23 × 7 cm) containing moist vermiculite. On the 4th day after sowing, plantlets from cycles 1 and 18 (two to three cm in height) were submitted to two treatments: control and waterlogging. Control plantlets were cultivated with substrate humidity maintained close to the field capacity of soil water retention, and waterlogging was performed by maintaining a layer of water of approximately 1 cm above the substrate. The experiment was carried out in a greenhouse with  $28 \pm 4$  °C and a 12 h photoperiod. After different times of waterlogging (0, 24, 48, 72, 96, and 120 h) the roots of the plantlets were collected, properly washed under tap water and stored for biochemical and anatomical analyses. For the biochemical analyses the samples were collected and immediately immersed in liquid nitrogen, then stored at  $-78$  °C. For the anatomical analyses, the roots were fixed in a solution of formaldehyde, acetic acid and ethanol 70% (FAA 70), for 72 h and, then, were stored in 70% ethanol.

### 2.2 Anatomical evaluation

Cross sections were taken from the second to third centimeters of the piliferous zone of the primary root using a table microtome. Sections were then clarified in 5% (v/v) sodium hypochlorite, washed in distilled water, stained with safrablau (safranin 7.5: astra blue 2.5), and placed in semi-permanent slides fixed with 50% (v/v) glycerin (Kraus and Arduin 1997). The photomicrographs were obtained from an optical microscope (Olympus BX-60) coupled to a digital camera (Canon Poxer Shot A620). The photomicrographs were used for measurements of total aerenchyma area in root cortex, total root cortex area and thickness of endodermis and exodermis. The proportion of aerenchyma in relation to cortex area was obtained by dividing total aerenchyma area in root cortex by total root cortex area. The measurements were performed using the software UTHSCSA Image Tool (University of Texas, San Antonio, TX, USA). Finally, the average of each treatment was the result of

18 observations per replicate, comprising 54 observations per treatment.

### 2.3 Enzymatic activity

Alcohol dehydrogenase (ADH) activity was determined following the methods proposed by Yamano-shita et al. (2005). For this, 0.2 g of plant tissue were ground in liquid nitrogen and added to 50 mM Tris–HCl pH 6.8, 15% glycerol and 10 mM 2-mercaptoethanol. The extracts were centrifuged at 13.000g for 15 min at 4 °C. Aliquots of the enzymatic extract were added to 150 mM Tris–HCl pH 8.0 and 0.3 mg mL<sup>-1</sup> nicotinamide adenine dinucleotide (NAD<sup>+</sup>), previously incubated at 30 °C for 15 min. Then, ethanol was added to start the reactions and the absorbances were detected at 340 nm, for 3 min every 15 s using a spectrophotometer GBC Cintra 10 (Gbc Scientific Equipment).

Cellulase activity was determined according Walker et al. (2006) with modifications. The enzyme extract for cellulase activity determination was obtained by grinding 0.3 g of plant material in liquid nitrogen, followed by adding 1 M NaCl. The samples were centrifuged at 12.000g for 30 min at 4 °C. Then, the mixture of 400 µL of the supernatant and 400 µL of 1% caboxymethyl-cellulose was incubated into water bath for one hour at 30 °C. After that, the extracts were deproteinated by adding 0.3 N barium hydroxide and 5% zinc sulfate (Markovic et al. 1975), followed by centrifugation at 8.000g for 15 min. Finally, aliquots of 400 µL of the deproteinated extract were used to determine cellulase activity by dinitrosalicylic acid method—DNS (Miller 1959). One unit of cellulase was considered as the amount of enzyme required to break 1 mol of reducing sugars per hour.

The extraction of polygalacturonase (PG) was performed according to a modified method proposed by Buescher and Furmanski (1978), by grinding 1.5 g of plant tissue in 5 mL of 1 M NaCl. Then, PG activity was assayed by adding 0.25% citric pectin to the enzyme extract, and incubation in water bath for 120 min at 30 °C. The reaction was stopped by boiling the samples for 5 min. After that, the extracts were deproteinated by adding 0.3 N barium hydroxide and 5% zinc sulfate. Finally, samples were filtered in filter paper, and aliquots of the deproteinated solution were

used to determine the enzyme activity by Somoghi-Nelson (Nelson 1944).

Antioxidant enzymes were obtained by grinding 0.3 g of roots in liquid nitrogen, following a modified protocol proposed by Biemelt et al. (1998). Plant material was ground in liquid nitrogen and suspended in 1500  $\mu\text{L}$  of a buffer composed by 100 mM potassium phosphate 7.8 pH, 0.1 mM ethylenediaminetetraacetic acid (EDTA), 200 mM ascorbic acid. After centrifugation at 13,000g for 10 min at 4 °C, the supernatants were collected and used in the quantification of the activity of superoxide dismutase (SOD) (Giannopolitis and Ries 1977), catalase (CAT) (Havir and McHale 1987) and ascorbate peroxidase (APX) (Nakano and Asada 1981). Specific activity was determined through the quantification of proteins (Bradford 1976).

For SOD activity determination, aliquots of the supernatant were added to the incubation medium containing 50 mM potassium phosphate 7.8 pH, 14 mM methionine, 0.1  $\mu\text{M}$  (EDTA), 75  $\mu\text{M}$  nitroblue tetrazolium chloride (NBT), and 2  $\mu\text{M}$  riboflavin. Then, the tubes were illuminated with a 20 W lamp for 7 min and the absorbances were determined at 560 nm. One unit of SOD was considered by the ability of the enzyme to inhibit 50% of the NBT photoreduction.

APX activity was determined by adding aliquots of the supernatant to an incubation medium composed by 100 mM potassium phosphate 7.0 pH, 0.5 mM ascorbic acid, previously incubated at 28 °C. Then, 0.1 mM hydrogen peroxide was added to the medium and the absorbances were performed at 290 nm, for 3 min in intervals of 15 s. The coefficient of molar extinction was 2.8  $\text{mM}^{-1} \text{cm}^{-1}$ .

The determination of CAT activity was performed by adding aliquots of the supernatant to a medium containing 100 mM potassium phosphate 7.0 pH, previously incubated at 28 °C. Then, 12.5 mM hydrogen peroxide were added to the medium and the absorbances were performed at 240 nm, for 3 min in intervals of 15 s. The coefficient of molar extinction was 36  $\text{mM}^{-1} \text{cm}^{-1}$ .

## 2.4 Biometric analyses

Biomass accumulation was determined by the dry mass of shoots and roots. For this, shoots and roots were dried in a forced-air oven at 65 °C until

constant weight. Dry mass was measured using a scale.

## 2.5 Experimental design and statistics

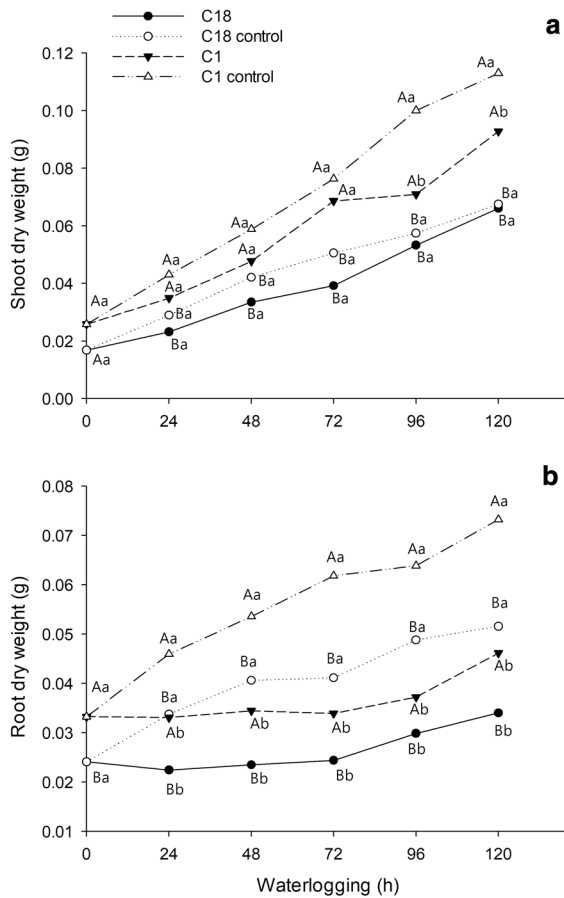
The experiment was performed using a completely randomized design, with two cycles of selection (C1 and C18), six waterlogging times (0, 24, 48, 72, 96, and 120 h), and two treatments (control and waterlogged plants). There were three biological and three experimental replicates. Each biological replicate was composed of one plantlet. Data were subjected to a two-way ANOVA with two cycles of selection and two treatments as independent variables at each time of analysis. Differences between variables were assessed with an F test, and the averages were compared by the Scott–Knott's test ( $p \leq 0.05$ ). Statistical analyses were performed in the statistical software SISVAR.

## 3 Results

Waterlogging caused a decrease in dry weight of C18 and C1 plantlets of Saracura maize throughout the experiment (Fig. 1). Greater reductions in growth were observed for roots in relation to shoots. In fact, the biomass accumulation in roots of both C1 and C18 plantlets ceased until 72 h. Also, this reduction was more pronounced in C1 plantlets than in C18 plantlets. After 120 h of waterlogging, it was observed a reduction of 19% in C1 and 2% in C18 shoot dry mass when compared to control plantlets (Fig. 1a). For root dry mass, these reduction was 37% in C1 and 33% in C18 plantlets (Fig. 1b).

Roots of C18 plantlets under waterlogging had a higher proportion of aerenchyma in their cortex when compared to C1 plantlets, throughout the study (Table 1). There was a progressive increase in aerenchyma formation for plantlets from both selection cycles (Fig. 2). However, after 120 h of waterlogging, C1 plantlets had 27% of the cortex area covered by aerenchyma compared to 46% in C18 plantlets. C1 plantlets showed a higher level of endodermis thickening until 96 h of waterlogging, while C18 plantlets were characterized by higher thickening of the exodermis only at 120 h.

The plantlets of C1 and C18 showed higher cellulase and PG activity under waterlogging than



**Fig. 1** Shoot and root dry weight of plantlets of Saracura maize from selection cycles 1 and 18 after 120 h under waterlogging. Capital letters compare cycles within each treatment (control and waterlogging) and lower-case letters compare the effects of the treatments within each cycle, based on the Scott–Knott test ( $p \leq 0.05$ )

under control conditions (Fig. 3). Until 48 h, C18 plantlets showed higher enzyme activity than C1 plantlets. After 120 h of waterlogging, C1 and C18 plantlets had similar levels of cellulase activity. In general, there was a decrease in cellulase activity throughout the experiment for plantlets from both selection cycles (Fig. 2a). PG activity in C18 plantlets increased during the first 24 h of waterlogging, then remained stable until 72 h and started to decrease after 96 h of waterlogging (Fig. 2b). In contrast, in C1 plantlets, PG activity increased until 72 h of waterlogging, then declined until 120 h.

The plantlets of C18 showed higher ADH activity than C1 plantlets after 24 h of waterlogging (Fig. 4). However, after 72 h of waterlogging this activity was similar for plantlets from both selection cycles. In general, there was an increase in ADH activity until 72 h of water excess, after which activity decreased until 120 h for plantlets from both cycles.

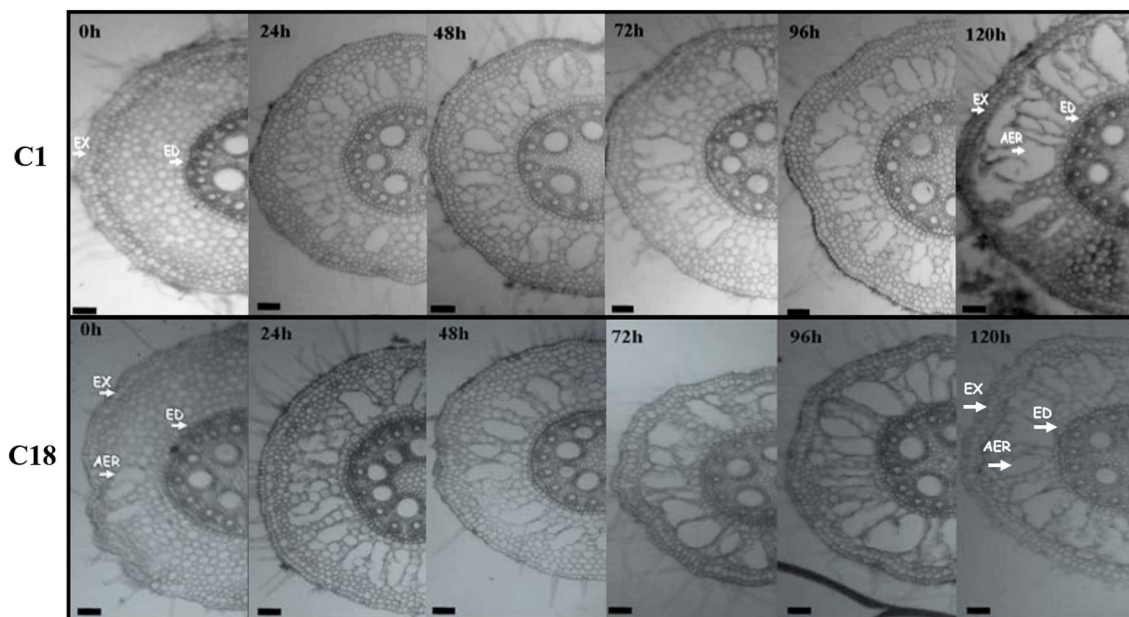
We observed a great influence on the activity of antioxidant enzymes by waterlogging (Fig. 5). SOD activity was higher in waterlogged plantlets than in control plantlets from both cycles after 24 h of waterlogging (Fig. 5a). C18 plantlets had higher SOD activity than C1 plantlets from 72 h to 120 h of waterlogging. APX activity was higher in C18 plantlets than C1 plantlets after 72 h of waterlogging (Fig. 5b). C18 plantlets showed a constant level of APX activity until 24 h of water excess, increasing until 72 h of waterlogging. In C1 plantlets, APX activity increased until 24 h and then remained constant until the end of the evaluation. CAT activity was also influenced by waterlogging, but only for C18

**Table 1** Proportion of aerenchyma in relation to the cortex area (%) and thickening of endodermis and exodermis in roots of Saracura maize from Cycles 1 (C1) and 18 (C18) after exposure to waterlogging for 120 h

Waterlogging	Aerenchyma (%)		Endodermis ( $\mu\text{m}$ )		Exodermis ( $\mu\text{m}$ )	
	C1	C18	C1	C18	C1	C18
0 h	0.99 Bd	4.74 Ae	28.42 Ab	21.99 Bd	36.25 Ad	36.35 Ad
24 h	8.60 Bc	18.09 Ad	28.12 Ab	25.53 Bc	46.26 Ac	50.31 Ac
48 h	19.10 Bb	27.85 Ac	31.26 Aa	27.39 Bb	47.50 Ac	49.51 Ac
72 h	19.28 Bb	36.88 Ab	31.10 Aa	27.35 Bb	52.46 Ab	55.28 Ab
96 h	28.54 Ba	40.37 Ab	31.26 Aa	27.92 Bb	56.05 Ab	57.82 Ab
120 h	27.09 Ba	46.20 Aa	30.47 Aa	31.26 Aa	60.07 Ba	68.62 Aa

For each waterlogging time, different capital letters indicate significant differences ( $p < 0.05$ ) between C1 and C18. For each cycle (C1 or C18) different lower-case letters indicate significant differences ( $p < 0.05$ ) between waterlogging times





**Fig. 2** Cross sections of roots of Saracura maize from Cycles 1 (C1) and 18 (C18) exposed to different times of waterlogging. Arrows indicate the progression of aerenchyma formed in root cortex (AER), endodermis (END) and exodermis (EX). Bars = 100  $\mu$ m

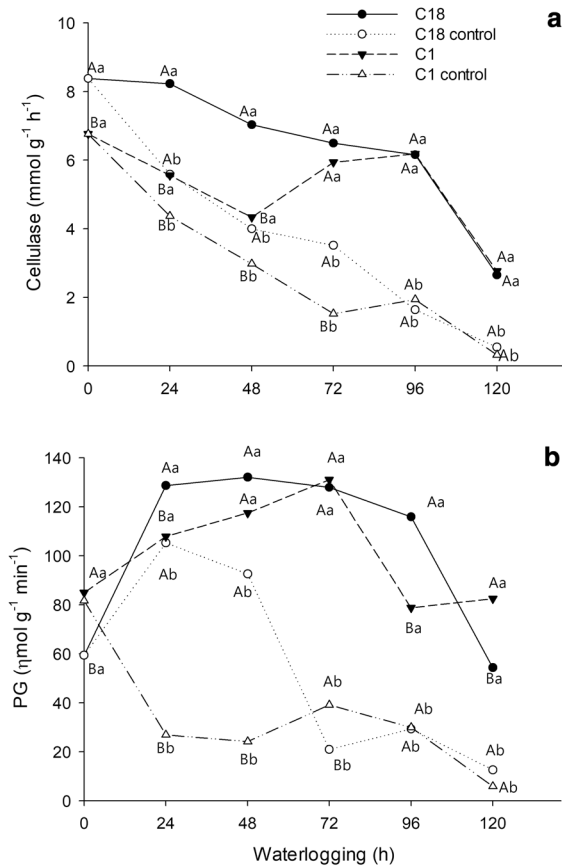
plantlets (Fig. 5c). After 24 h of waterlogging, C18 plantlets had higher CAT activity than the control, and higher CAT activity than C1 plantlets throughout the experiment. The constitutive antioxidant enzymes activities were more pronounced in C18 plantlets than C1 plantlets. Also, the responses of these antioxidant systems was higher when induced by waterlogging. The activation of antioxidant enzymes started with the activity of CAT after 24 h, followed by SOD and APX after 72 h of waterlogging.

#### 4 Discussion

This study first reports the roots aerenchyma formation in plantlets just after the germination, which should be associated to the already reported better performance of the Saracura maize from C18 than plants from C1 cycle (Pereira et al. 2008, 2010; Pires et al. 2015; Souza et al. 2009, 2010, 2011, 2012). Therefore, our results show that the higher hypoxia tolerance of C18 plantlets may be associated to a faster response when root hypoxia occurs. The C18 plantlets improve the activity of enzymes involved in cell wall loosening, associated to a more efficient antioxidant defense. This association is important to perform the lysigeny in a

controlled manner in the root tissues. The better performance of C18 plantlets was also associated to a faster response of respiratory metabolism, once C18 plants show higher activity of alcohol dehydrogenase just after being subjected to waterlogging. Despite the lack of information about the oxygen concentration in water during the waterlogging time, the hypoxia condition was strongly suggested by the induction of ADH activity in plants exposed to waterlogging conditions. Also, the lower biomass accumulation in both maize plants reinforces the hypoxia conditions.

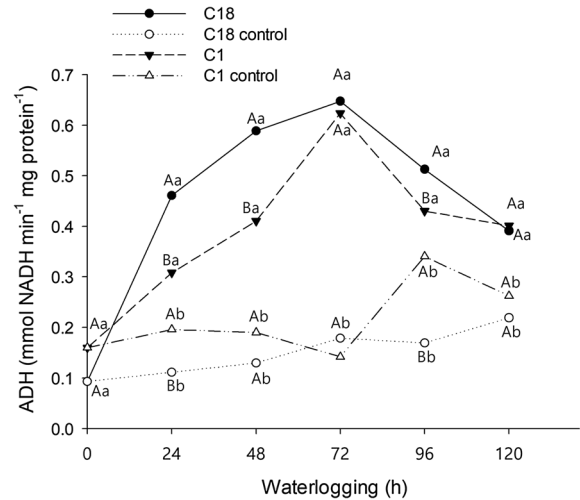
C18 plantlets showed constitutive aerenchyma in their roots, and it is known these structures are associated with higher tolerance to water excess (Imaz et al. 2013; Mano and Omori, 2013; Voesenek and Sasidharan 2013). Aerenchyma are spaces for oxygen storage that facilitate gas movement in the root cortex and therefore facilitate aerobic respiration in submerged organs (Mano and Omori 2013). Together with aerenchyma formation, the thickening of endodermis and exodermis improves gas diffusion and reduces radial oxygen loss from the roots (Abiko et al. 2012; Watanabe et al. 2013). Endodermal and exodermal thickening also provides support for lateral roots, especially in young plants, which is necessary when the aerenchyma appearance is associated to cell



**Fig. 3** Cellulase and Polygalacturonase (PG) activity of plantlets of Saracura maize from selection cycles 1 and 18 under waterlogging. For each treatment (control or waterlogging) different capital letters indicate differences ( $p < 0.05$ ) between C1 and C18. For each cycle (C1 or C18) different lower-case letters indicate significant differences ( $p < 0.05$ ) between plants submitted to waterlogging and those not submitted to it

wall structures degradation. However, while C1 plantlets showed higher endodermal thickening, in C18 this occurred only at 120 h of waterlogging (Table 1). Pereira et al. (2008) and Souza et al. (2009) observed a maintenance or a reduction in exodermal and endodermal thickening along the selection cycles of young plants of Saracura maize. Those findings suggest that the thickening of endodermis and exodermis does not seem to be the most responsible for the higher tolerance of C18 plantlets to waterlogging.

The profiles of PG and cellulase activities were different between plantlets from the two selection cycles. C18 plantlets had higher cellulase activity than

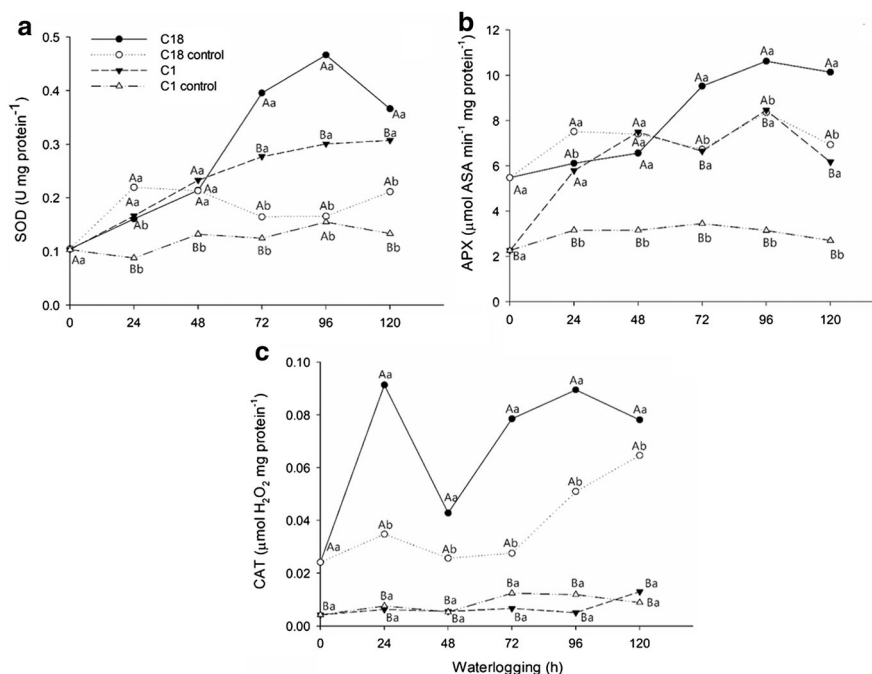


**Fig. 4** Activity of alcohol dehydrogenase (ADH) of plantlets of Saracura maize from selection cycles 1 and 18 under waterlogging. For each treatment (control or waterlogging) different capital letters indicate differences ( $p < 0.05$ ) between C1 and C18. For each cycle (C1 or C18) different lower-case letters indicate significant differences ( $p < 0.05$ ) between plants submitted to waterlogging and those not submitted to it

C1 plantlets in the first 48 h of waterlogging, overlapping with higher levels of aerenchyma formation. In C18 plantlets, cellulase activity was constant until 24 h of waterlogging, during which there was an increase in PG. This observation supports the activity of the cell wall loosening enzyme by the PG enzyme preceding the cellulase activity, which is a cell wall degrading enzyme (Zhou et al. 2011), promoting the aerenchyma formation. After 120 h of waterlogging, PG and cellulase activities decreased, probably due to the low availability of substrate for those enzymes given the high frequency of aerenchyma formation in the root cortex, showing the coordination between cell wall loosening and degradation enzymes activities; this is also associated with antioxidant metabolism and aerenchyma formation in root tissues.

Aerenchyma appearance under waterlogging is mediated by ethylene, whose production is stimulated under conditions of low oxygen availability. Pires et al. (2015) observed increases in ethylene production in roots of C18 plantlets under waterlogging, which is associated to the increase in the activities of the enzymes related to cell wall metabolism in cortical cells, leading to programmed cell death and the formation of lysigenous aerenchyma (Evans 2004). The process begins with the action of a pectinase

**Fig. 5** Activity of superoxide dismutase (a), ascorbate peroxidase (b), and catalase (c) of plantlets of *Saracura* maize from selection cycles 1 and 18 under waterlogging. For each treatment (control or waterlogging), different capital letters indicate significant differences ( $p < 0.05$ ) between C1 and C18. For each cycle (C1 or C18), different lower-case letters indicate significant differences ( $p < 0.05$ ) between plantlets submitted to waterlogging and those not submitted to it



enzyme (PG) that opens the access for the enzymes that cause cell wall degradation (cellulases), leading to cellular lysis (Bragina et al. 2003).

This study also showed for the first time that aerenchyma formation in C18 plantlets is induced just after a short-term hypoxia conditions, showing a marked shift in respiratory metabolism to anaerobic route during the first 24 h of waterlogging (Fig. 3). This ability to biophysically perceive the environment and shift their metabolism to anaerobic respiration are considered an important trait to tolerate hypoxia, once these plants can maintain a minimum level of energy (ATP and NADH) production for basal metabolism (Kumutha et al. 2008). In this context, we can infer that *Saracura* maize acquired mechanisms of perception of low oxygen availability over the selection cycles, and these plants were able to rapidly express ADH leading to efficient use of carbohydrates and maximizing survival under waterlogging. As a consequence of the high proportion of aerenchyma (Fig. 2) in the root cortex of C18 plantlets, higher oxygen diffusion may contribute to the reduction in ADH activity after 72 h of hypoxia (Benz et al. 2007). Thus, we showed that C18 plantlets have a rapid mechanism of deviation to anaerobic respiration at the beginning of waterlogging, and when aerenchyma

formation provides enough oxygen there is a decrease in ADH activity.

As a consequence of the increase in ethylene concentration (Voesenek and Bailey-Serres 2015) there is an increase in ROS generation and high activity of the enzymes controlling cell wall loosening and degradation, culminating in aerenchyma formation (Gunawardena et al. 2001). Besides ethylene production, higher ROS levels were correlated to lysigenous aerenchyma in *Saracura* maize (Pires et al. 2015). Programmed cell death (PCD), a highly regulated event, is a natural process to eliminate cells that are disturbing homeostasis (Pennell and Lamb 1997) and it can be stimulated by biotic and abiotic stresses (Van Breusegem and Dat 2006). In this way, antioxidant system activity in C18 *Saracura* maize was enough to prevent uncontrolled oxidative damages, but probably sustained a basal response of ROS necessary for aerenchyma formation.

Aerenchyma formation is a very important anatomical modification under waterlogging, once these tubes formed between roots and shoots allow the oxygenation and maintenance of cell metabolism. In roots, aerenchyma are related to the root hydraulic conductivity, where water and nutrients absorption can occur even under lower rates (Irfan et al. 2010). Despite the increase in aerenchyma formation at the first hours of



waterlogging, it was observed a maintenance of root biomass until 72 h in C1 and C18 plantlets under waterlogging, followed by a slight increase in root biomass. This response gives some evidence for the investment of roots in morpho-anatomical and physiological modifications aiming to survive under waterlogging. Nevertheless, when compared to C1 plantlets, it was observed lower growth of C18 plantlets under waterlogging. However, comparing the plantlets under control and waterlogged conditions, C18 plantlets suffered less with waterlogging, culminating in lower biomass loss in plantlets under waterlogging.

The increased capacity for maintaining biomass in C18 plantlets after 120 h of waterlogging (Fig. 1) was related to higher aerenchyma formation and antioxidant enzymes response. These morphological responses were performed due to a faster response of the respiratory metabolism associated with higher activities of the enzymes involved in lysigeny in the cortex of the root cells. Taken together, these responses conferred higher waterlogging tolerance for C18 when compared with C1, especially during the initial growth stages, during the first hours of waterlogging.

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