Flooding is one of the abiotic stresses and has a significant impact on plant survival in many regions of the world. The problem is becoming more aggravating with the global warming, which will result in more irregular weather in the future (Qiu et al. 2007). Flooding is characterized by low availability of oxygen (hypoxia), because the soil pores are filled up with water, which leads to a decrease in the concentration of oxygen in the soil as well as in the plant parts (Bailey-Serres and Voesenek 2008). Plant metabolism is switched from aerobic to anaerobic route, resulting in a severe impairment in the growth of plants (Sairam et al. 2008).

Maize is a crop sensitive to flooding and its inability to tolerate waterlogged soil results in substantial yield loss (Zaidi et al. 2007). However, a wide genetic variability in the species of *Zea mays* L., can be an invaluable tool for the breeders for development of flooding-tolerant maize plants (Zaidi et al. 2004). Tolerant plants are able to modulate their metabolism, morphology, and anatomy to survive and reproduce in flooded condition (Fernández 2006). Maize plants develop lysigenous aerenchyma as an adaptation mechanism to avoid the lack of oxygen in flooded regions (Gunawardena et al. 2001). These are parenchyma tissues with big intercellular spaces (originated from cell death) filled up with gases that allow the transportation of oxygen from the stem to the roots that are under hypoxia condition (Evans 2004).

In Brazil, flood plains or wetlands (alluvial and hydromorphic soils) with temporary floods restrict the diversity of agriculture to rice cultivation only (Silva et al. 2007). With this concern and the possibility of crop rotation with maize on flood plains and wetlands, the Maize and Sorghum National Research Center developed, through a stratified phenotypic recurrent selection, a variety of maize known as Saracura-BRS-4154, which is able to survive, produce, and tolerate periodic soil flooding (Ferreira et al. 2008). The name ‘Saracura’ was given after a bird frequently found in Brazilian wetlands.

Morpho-anatomical characterization of root in recurrent selection cycles for flood tolerance of maize (*Zea mays* L.)

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ABSTRACT

Changes in root anatomical structures at successive cycles of selection (cycle 1 to cycle 18, alternating) were observed in the study of maize (*Zea mays* L. cv. Saracura-BRS 4154) capable to survive and produce in temporarily flooded soils; this cultivars was developed by the Maize and Sorghum National Research Center through stratified phenotypic recurrent selection for cultivation wetland soils. Field trial was carried out and flooding of the soil was initiated at the six-leaf stage; the soil was flooded with water (20-cm deep) three times per week. Root sample was collected, fixed, and selected for observation in photon microscope. A gradual increase in the number of aerenchyma, the proportion of vascular cylinder, smaller metaxylem, and phloem and epidermis width, and a decrease in exodermis and cortex were observed in successive selection cycles. Such phenotypic changes impart the flood tolerance ability to this maize cultivar.

Keywords: soil flooding; *Zea mays* L.; hypoxia; root anatomy
Currently, this variety is at the 18th annual cycle of selection. Its capacity to tolerate intermittent periods of flooding is due to the presence of different biochemical and physiological mechanisms and also due to the morpho-anatomical changes in the roots, such as the formation of adventitious roots, aerenchyma, and changes in the vascular cylinder (Pereira et al. 2008).

There are few quantitative studies on the classical selection by recombination of genotypes and their morpho-anatomical gains. Although some basic studies with cv. Saracura were already undertaken (Vitorino et al. 2001, Pereira et al. 2008), there is no anatomical study comparing all of the cycles of selection in the field conditions, where all of the biotic and abiotic limiting factors are present. In this context, this work was developed with the objective of characterizing the modifications in the root anatomical structures at successive cycles of selection in the maize cv. Saracura-BRS 4154 under flooded conditions.

MATERIAL AND METHODS

The experiment was carried out from October 2007 to January 2008 under field conditions at the Maize and Sorghum National Research Center, Sete Lagoas, Minas Gerais state, Brazil (climate AW of savanna with dry winter, altitude 732 m, south latitude 19°28’, and west longitude 44°15’). Maximum and minimum temperatures registered during the evaluation period were 30°C and 18°C, respectively. Average relative humidity was 64.3% and the accumulated precipitation was 852 mm.

The area was leveled and divided into plots of four 5-m-long rows spaced 0.9 m from each other in order to facilitate the flooding. We used the randomized block design with three replications. The distance between the plants in the plot line was 0.2 m and the useful area corresponded to two central rows. Water from a nearby reservoir was used for flooding the lots, conducted by pumps to the experiment location. The fertilization of the soil, classified as Tb Fluvic Neosol, Typical Eutrophic, clay texture, phase plain relief wetland fields, was performed according to the recommendations from the chemical analysis. Basic 5-20-20 + Zn fertilizer in doses of 400 kg/ha was applied at sowing and, after the germination of the caryopses, two top dressings with ammonium sulphate were carried out in doses of linear 20 g/m, first at the stage of six leaves and the second at the stage of eight leaves. All of the phytosanitary treatments required by the culture were applied.

The flooding was initiated at the stage of six leaves (when the growth meristem of the plants is already above the ground surface) (Magalhães et al. 2007), with addition of a 20-cm-deep water three times a week, and ended at the physiological maturity of the plants. The genetic material used constituted the alternating cycles of selection of the maize cv. Saracura-BRS 4154: C1, C3, C5, C7, C9, C11, C13, C15, C17, and C18 and one variety BR 107 as control, known for its susceptibility to flooding.

At flowering, phase the most susceptible to stress caused by excess water (Zaidi et al. 2004), the roots were dug out, washed under running water and cut at the crown region. Complete roots (with apical, elongation, maturation, and differentiation zones) from two plants of each replication were randomly collected and fixed in formaldehyde, acetic acid, and 70% ethanol solution (FAA 70) for 48 h and then preserved in 70% ethanol. The transverse sections were cut at 4 cm from the root apex with a table microtome. They were clarified with 5% sodium hypochlorite for 10 min, rehydrated for 10 min, stained with Astrablau (7.5:2.5 safranine and Astra blue solution) and mounted on slides with 50% glycerin. The sections were photographed in Olympus BX-60 photon microscope coupled to a digital camera. The photomicroographies were used to measure the following parameters: aerenchyma total area (ATA), proportion occupied by the aerenchyma in the cortex (PA), proportion of the vascular cylinder area in relation to the root total area (PVC), proportion of the cortex area in relation to the root total area (PC), cortex width (CW), width of the suberized cell layer, present in the hypodermis region (exodermis) (SC), cortex cell diameter (CCD), epidermis width (EPW), endodermis width (EW), metaxylem cell diameter (XD), metaxylem number (XN), and phloem width in the vascular cylinder (PL). The proportion of the area occupied by the aerenchyma in the cortex was calculated by dividing the aerenchyma total area by the cortex total area.

The measurements were taken with the image analysis program UTHSCSA ImageTool, using calibrations undertaken with microscope rule photographed in the same scale as the photomicroographies. The final data were determined by taking the average measure of roots from two plants of each replication. In each root, five measurements of each anatomical trait were taken. For the statistical analysis of the results, we applied the analysis of variance and the Scott-Knott test for the mean comparison at 0.05 significance, using the Sisvar program, version 4.3.
RESULTS AND DISCUSSION

The results showed anatomical differences in the cortex throughout the cycles of selection. Aerenchyma (ATA) started increasing significantly from 11th cycle onwards (Table 1). The 18th cycle, followed by the 17th, presented the highest ATA 98% of control. Throughout the cycles we could observe a progressive increase in the aerenchyma tissue area and, consequently, an increase in the proportion of aerenchyma in the cortex (PA) (Figure 1). The control showed 1.6% of PA in the cortex, while in C18, the value reached 42.4% (Table 1).

The increase of root porosity through the formation of a great amount of aerenchyma in the cortex of the 18th cycle under hypoxia helps to keep the aerobic respiration, because these structures increase the longitudinal oxygen flow from the aboveground part to the root (Colmer 2003b). Besides, they are responsible for the ventilation (root-atmosphere) of excess gas such as ethylene, methane, and carbon dioxide, which can have growth retarding effect at higher concentrations. With the presence of aerichyma, the metabolic cost also decreases due to a decrease in cell respiration (Lynch and Ho 2005). All of these characteristics attributed to the presence of aerichyma make the maize 'Saracura', represented by the 18th cycle, the most successful in flooded soils.

The presence of aerichyma in these conditions was also reported for other crops, such as soybean (Thomas et al. 2005), wheat, barley, and oat (Setter and Waters 2003). An intensive production of aerichyma was observed in other maize cultivars, such as cv. Seneca Horizon (Enstone and Peterson 2005) and cv. Single Cross 704 (Pourabd al et al. 2008). Lizaso et al. (2001) observed 30% or higher aerichyma production in a Venezuelan maize variety tolerant to flooding, compared to a sensitive variety. This structure is, therefore, an adaptive response common to plants in flooded environments. The present data on a progressive increase in the formation of aerichyma through the selection cycles in the cv. Saracura maize in field conditions (Table 1) corroborate with the results of Pereira et al. (2008) in a greenhouse.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>ATA (mm²)</th>
<th>PA (%)</th>
<th>PVC (%)</th>
<th>PC (%)</th>
<th>CW (µm)</th>
<th>SC (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BR107</td>
<td>0.03ds</td>
<td>1.63f</td>
<td>0.20b</td>
<td>0.80a</td>
<td>647.39a</td>
<td>116.04a</td>
</tr>
<tr>
<td>C1</td>
<td>0.38c</td>
<td>11.74e</td>
<td>0.21b</td>
<td>0.77b</td>
<td>604.48a</td>
<td>94.89b</td>
</tr>
<tr>
<td>C3</td>
<td>0.33c</td>
<td>19.28d</td>
<td>0.20b</td>
<td>0.80a</td>
<td>565.29a</td>
<td>90.36b</td>
</tr>
<tr>
<td>C5</td>
<td>0.43c</td>
<td>13.53e</td>
<td>0.15b</td>
<td>0.85a</td>
<td>617.20a</td>
<td>84.70b</td>
</tr>
<tr>
<td>C7</td>
<td>0.51c</td>
<td>25.16c</td>
<td>0.19b</td>
<td>0.81a</td>
<td>499.93b</td>
<td>87.45b</td>
</tr>
<tr>
<td>C9</td>
<td>0.53c</td>
<td>20.12d</td>
<td>0.25a</td>
<td>0.75b</td>
<td>514.44b</td>
<td>85.96b</td>
</tr>
<tr>
<td>C11</td>
<td>0.81b</td>
<td>30.40c</td>
<td>0.27a</td>
<td>0.73b</td>
<td>469.10b</td>
<td>77.96b</td>
</tr>
<tr>
<td>C13</td>
<td>0.95b</td>
<td>35.09b</td>
<td>0.25a</td>
<td>0.75b</td>
<td>438.04b</td>
<td>60.99c</td>
</tr>
<tr>
<td>C15</td>
<td>0.99b</td>
<td>29.34c</td>
<td>0.23a</td>
<td>0.77b</td>
<td>457.87b</td>
<td>55.34c</td>
</tr>
<tr>
<td>C17</td>
<td>1.58a</td>
<td>42.17a</td>
<td>0.26a</td>
<td>0.74b</td>
<td>482.89b</td>
<td>68.21c</td>
</tr>
<tr>
<td>C18</td>
<td>1.74a</td>
<td>42.41a</td>
<td>0.25a</td>
<td>0.75b</td>
<td>422.51b</td>
<td>54.94c</td>
</tr>
<tr>
<td>CV (%)</td>
<td>15.98</td>
<td>15.95</td>
<td>13.42a</td>
<td>4.01</td>
<td>9.81</td>
<td>16.55</td>
</tr>
</tbody>
</table>

* means followed by the same letters within columns are not significantly different by the Scott-Knott test at the 0.05 probability level; ATA – aerichyma total area found in the cortex; PA – proportion occupied by the aerichyma in the cortex; PVC – proportion of the vascular cylinder area in relation to the root total area; PC – proportion of the cortex area in relation to the root total area; CW – cortex width; SC – width of the suberized cell layer, present in the hypodermis region (exodermis)
mately by 7% smaller compared to the control and C1. It is possible that under field conditions, limiting factors (such as humidity and temperature), different from those observed at greenhouse, could be altering the expression of cv. Saracura maize.

A decrease in water conductivity and smaller CW were observed from C7 (Table 1). This can lead to a higher conductivity because the distance necessary for the water to reach the vascular cylinder is going to be shorter, which increases the plant capacity to tolerate the stress.

Many studies described changes in the vascular cylinder in response to environmental stresses. Models reported the importance of smaller cylinders in avoiding damages in roots with low porosity and oxygen diffusion (Colmer 2003b). A smaller vascular cylinder would have more chances of transporting oxygen, water, and photoassimilates, in plants with roots under hypoxia conditions. The first cycles and the control showed smaller cylinders but in C9 onwards there was a significant increase in the cylinder size (Table 1), which could lead to a higher conduction in flooding conditions. A higher investment in vascular cylinder is possible in these cycles, as the high porosity and high oxygen diffusion are found in the respective cortex.

The layer of suberized cells (SC) present in the hypodermis regions (exodermis) decreased only after C13 (29.01% of control) onwards to C18 (53.2% control) (Table 1 and Figure 1). Similar barriers were also reported in rice (Colmer 2003a) and grasses (Glyceria maxima and Phragmites australis) (Soukup et al. 2007).
The presence of a thick barrier in the hypodermis cells of the BR 107 variety as well as of the first selection cycles of cv. Saracura maize helps avoid the radial loss of oxygen to the rhizosphere and increases the longitudinal diffusion in the aerenchyma (Colmer 2003b), since this tissue is not highly specialized in these genotypes. Furthermore, these suberized layers help avoid partial absorption of phytotoxins (Soukup et al. 2002). However, there are negative consequences regarding the presence of this barrier, as it can diminish the release of ethylene and methane present in excess and decrease the intake of water and nutrients.

A thin exodermis layer allows free radial movement of oxygen and creates aerated conditions in the rhizosphere, protecting the roots from the presence of phytotoxins (Soukup et al. 2002). However, there are negative consequences regarding the presence of this barrier, as it can diminish the release of ethylene and methane present in excess and decrease the intake of water and nutrients.

Table 2. Dimensions of cells and tissues in root system of cv. Saracura maize, throughout the successive cycles of selection (alternating) and of variety BR107, under intermittent flooding conditions

<table>
<thead>
<tr>
<th>Treatments</th>
<th>CCD (µm)</th>
<th>EPW (µm)</th>
<th>EW (µm)</th>
<th>XD (µm)</th>
<th>XN</th>
<th>PL (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BR107</td>
<td>33.88e</td>
<td>21.95c</td>
<td>7.98a</td>
<td>131.45a</td>
<td>9.00b</td>
<td>63.01f</td>
</tr>
<tr>
<td>C1</td>
<td>35.21e</td>
<td>23.87c</td>
<td>6.80a</td>
<td>119.40b</td>
<td>12.00b</td>
<td>65.44f</td>
</tr>
<tr>
<td>C3</td>
<td>37.67d</td>
<td>24.45c</td>
<td>7.78a</td>
<td>108.55c</td>
<td>12.83b</td>
<td>83.14a</td>
</tr>
<tr>
<td>C5</td>
<td>35.08e</td>
<td>23.02c</td>
<td>8.24a</td>
<td>098.62d</td>
<td>13.83b</td>
<td>82.45c</td>
</tr>
<tr>
<td>C7</td>
<td>39.27d</td>
<td>27.86c</td>
<td>8.10a</td>
<td>099.49d</td>
<td>12.33b</td>
<td>86.77d</td>
</tr>
<tr>
<td>C9</td>
<td>40.84d</td>
<td>28.84b</td>
<td>7.88a</td>
<td>101.34d</td>
<td>16.33b</td>
<td>96.74c</td>
</tr>
<tr>
<td>C11</td>
<td>48.75b</td>
<td>28.26b</td>
<td>8.22a</td>
<td>100.97d</td>
<td>14.50b</td>
<td>87.35d</td>
</tr>
<tr>
<td>C13</td>
<td>45.25c</td>
<td>28.39b</td>
<td>8.65a</td>
<td>96.59d</td>
<td>15.66b</td>
<td>98.77c</td>
</tr>
<tr>
<td>C15</td>
<td>49.83b</td>
<td>29.01b</td>
<td>8.66a</td>
<td>96.04d</td>
<td>19.83a</td>
<td>104.52b</td>
</tr>
<tr>
<td>C17</td>
<td>50.29b</td>
<td>32.19a</td>
<td>8.89a</td>
<td>87.21e</td>
<td>20.00a</td>
<td>119.94d</td>
</tr>
<tr>
<td>C18</td>
<td>57.31a</td>
<td>33.75a</td>
<td>9.16a</td>
<td>69.08f</td>
<td>22.33a</td>
<td>121.67a</td>
</tr>
<tr>
<td>CV(%)</td>
<td>5.190</td>
<td>7.250</td>
<td>6.750</td>
<td>2.740</td>
<td>15.69</td>
<td>2.850</td>
</tr>
</tbody>
</table>

*means followed by the same letters within columns are not significantly different by Scott-Knott test at the 0.05 probability level; CCD – cortex cell diameter; EPW – epidermis width; EW – endodermis width; XD – metaxylem cell diameter; XN – metaxylem number; PL – phloem width in the vascular cylinder

The presence of a thick barrier in the hypodermis cells of the BR 107 variety as well as of the first selection cycles of cv. Saracura maize helps avoid the radial loss of oxygen to the rhizosphere and increases the longitudinal diffusion in the aerenchyma (Colmer 2003b), since this tissue is not highly specialized in these genotypes. Furthermore, these suberized layers help avoid partial absorption of phytotoxins (Soukup et al. 2002). However, there are negative consequences regarding the presence of this barrier, as it can diminish the release of ethylene and methane present in excess and decrease the intake of water and nutrients.

A thin exodermis layer allows free radial movement of oxygen and creates aerated conditions in the rhizosphere, protecting the roots from the presence of phytotoxins and offering bigger superficial area (Armstrong et al. 2000). The cycle 18 of cv. Saracura maize was more adapted to the flooding as a result of a thin suberized layer in the hypodermis (SC). This characteristic allows high radial diffusion of oxygen, creating an aerobic rhizosphere without harmful losses to the cortex due to an elevated proportion of aerenchyma, which is, in turn, responsible for a higher oxygen supply. Pi et al. (2008), studying eight species from flooded regions also observed higher adaptation of Acanthus ilicifolius and Avicennia marina due to the presence of high proportions of aerenchyma and thin exodermis.

The cortex cell diameter (CCD) showed a gradual increase with the selection cycle (Table 2 and Figure 1); in C18 it was 40.9% of the control and in C1 it was 38.6%. The increase in diameter of the cortex cells is related to the decrease in the number of cortex cell layers and increase in the intercellular spaces. Bigger cortex cells can diminish the energy expense by diminishing the cell pool undergoing respiration in the cortex, in the same way it is known for aerenchyma.

The epidermis width (EPW) was bigger in the cycles 17 and 18 and smaller in the control and initial cycles. EPW increased by 36.25% compared to control (variety BR 107) in C18 and by 29.2% compared to C1 (Table 2). An increase in EPW in hypoxic conditions due to flooding was reported in Carex acuta and Juncus effusus (Visser et al. 2000). This characteristic presented by the last cycles of cv. Saracura maize can make the entrance of pathogens and toxic compounds through the root more difficult.

There was no significant difference in endodermis width (EW) (Table 2). This result is in sharp contrast to a gradual and significant increase of endodermis throughout the cycles of selection in cv. Saracura maize in greenhouse condition (Pereira et al. 2008). However, Enstone and Peterson (2005) reported a decrease in the EW of maize cv. Seneca Horizon and concluded that this could facilitate oxygen diffusion in the vascular cylinder.
A progressive decrease in metaxylem cell diameter (XD) and an increase in vessel number (XN) were observed throughout the cycles of selection (Table 2 and Figure 2). In C18 the XD decreased by 47.4% and XD increased by 59.7% compared to control. Compared to C1, C18 showed a decrease of 42.1% in XD and an increase of 46.3% in XN. The higher number of xylem vessels with smaller diameter found in the last cycles of selection in cv. Saracura maize can be related to a guarantee for water flow. However, the ability of roots in absorbing water is affected by the lack of oxygen in flooded environments (Dell’Amico et al. 2001, Tournaire-Roux et al. 2003). Similar results on the characteristics of xylem vessels were reported in grasses (Wahl et al. 2000).

The highest values of phloem width in the vascular cylinder (PL) were found in the C17 and C18 and the lowest values in the variety BR 107 and in C1 (Table 2). C18 showed an increase in the phloem width of 48.2% compared to the variety BR 107 and of 46.2% compared to C1. There is inhibition of photoassimilate transport to the root in plants under flooding conditions (Chen et al. 2005). A thicker phloem tissue in the root might be favorable for an increase in the phloem transport from the aboveground parts to the roots. An increase in the phloem width and in the metaxylem diameter was also observed throughout cv. Saracura cycles of selection developed in a greenhouse (Pereira et al. 2008).

The progressive change in root anatomical features that facilitate flood tolerance in plants shows the efficacy of the selection cycles for maize breeding.

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