# Leaf plasticity in successive selection cycles of 'Saracura' maize in response to periodic soil flooding

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Abstract – The objective of this work was to assess the effect of successive selection cycles on leaf plasticity of 'Saracura' maize BRS-4154 under periodical flooding in field conditions. Soil flooding started at the six-leaf stage with the application of a 20-cm depth water layer three times a week. At flowering, samples of leaves were collected and fixed. Paradermic and transverse sections were observed under photonic microscope. Several changes were observed throughout the selection cycles, such as modifications in the number and size of the stomata, higher amount of vascular bundles and the resulting decrease of the distance between them, smaller diameter of the metaxylem, decrease of cuticle and epidermis thickness, decrease of number and size of bulliform cells, increase of phloem thickness, smaller sclerenchyma area. Therefore, the successive selection cycles of 'Saracura' maize resulted in changes in the leaf anatomy, which might be favorable to the plant's tolerance to the intermittent flooding of the soil.

Index terms: *Zea mays*, hypoxia, leaf anatomy, tolerance to flooding.

## Plasticidade foliar nos sucessivos ciclos de seleção do milho 'Saracura' em resposta ao alagamento intermitente do solo

Resumo – O objetivo deste trabalho foi avaliar o efeito de sucessivos ciclos de seleção na plasticidade foliar do milho 'Saracura' BRS-4154 sob alagamento intermitente do solo em condições de campo. O alagamento do solo iniciou-se no estádio de seis folhas com a aplicação de uma lâmina de 20 cm de água três vezes por semana. No florescimento, foram coletadas e fixadas amostras das folhas. Secções paradérmicas e transversais de amostras foram observadas em microscópio fotônico. Várias modificações foram observadas durante os ciclos de seleção, como o número e o tamanho dos estômatos, a maior quantidade de feixes vasculares e a consequente diminuição da distância entre eles, o metaxilema com diâmetros menores, a diminuição da espessura da cutícula e da epiderme, a diminuição no número e tamanho das células buliformes, o aumento da espessura do floema e a menor área de esclerênquima. Os sucessivos ciclos de seleção do milho 'Saracura', portanto, levaram a mudanças na anatomia foliar, as quais podem ser favoráveis à tolerância da planta ao alagamento intermitente do solo.

Termos para indexação: *Zea may*s, hipoxia, anatomia foliar, tolerância ao alagamento.

## Introduction

The excess of water in the soil can lead to dramatic consequences in the process of gas diffusion. Since gases diffuse 10,000 times faster in the air than in the water, an impoverishment of oxygen availability (hypoxia) results both in the soil and in the plant parts (Dat et al., 2004). Under oxygen restriction, the tricarboxylic acid cycle is interrupted due to the absence of reducing power, and the anaerobic metabolism is activated (Sairam et al., 2008).

This situation can be found in flooded soils. However, more attention has been directed to the adaptative mechanisms of roots rather than to that of leaves (Bragina et al., 2004). Up to this moment, few studies have shown leaf modifications due to root flooding. Nonetheless, some morphological (Huber et al., 2009) and anatomical (Stoyanova et al., 2002; Yordanova et al., 2005) alterations have been found. Comparatively, evidences of leaf plasticity are more known in completely flooded plants. Physiological

as well as anatomical alterations have been observed in those plants, which are also known as amphibious plants (Voesenek et al., 2006; Mommer et al., 2007).

Plasticity can be defined as the ability of plants to adjust their performance through morphological or physiological modification in response to environment variation (Navas & Garnier, 2002). Although maize (*Zea mays* L.) can be sensitive to flooding, it possesses considerable genetic variability to tolerance (Zaidi et al., 2007), and can expressively respond to these conditions with the plasticity of its organs.

The inability of the maize crop to respond well to the low availability of oxygen in the rhizosphere caused by root flooding results in substantial losses in productivity (Zaidi et al., 2007). In Brazil, large agricultural limitations are found in the flooded plains or wetlands (alluvial and hydromorphic soils), where the periodical floodings restrict the agricultural practice, except for rice crops, which are more adapted (Silva et al., 2007). Concerned with this fact and thinking of the possibility of maize being a good crop option for Brazilian wetlands, Embrapa Milho e Sorgo has developed, by means of a stratified phenotypic recurrent selection, a maize variety known as 'Saracura BRS-4154', which is capable of surviving, yielding and tolerating intermittent periods of soil flooding (Ferreira et al., 2007). This variety is currently in its 18th annual selection cycle. Its ability to tolerate intermittent periods of flooding is due to the presence of different biochemical and physiological mechanisms, and also to root morphoanatomical modifications, such as the formation of aerenchyma and the modification of the vascular tissues (Ferreira et al., 2008; Pereira et al., 2008). Leaves of the 'Saracura' maize (BRS-4154) cultivated in a greenhouse with root flooding presented variations in the diameter of the vascular bundles as well as in the size and amount of stomata (Melo et al., 2004).

The objective of this work was to evaluate the effect of successive selection cycles on leaf plasticity of 'Saracura' maize under periodical flooding in field conditions.

## Materials and Methods

The experiment was carried out from October 2007 to January 2008, under field conditions, at Embrapa Milho e Sorgo, Sete Lagoas, Minas Gerais state, Brazil

(Köppen climate classification Aw, savanna with dry winter, 732-m altitude, 19°28'S, 44°15'W). The maximum and minimum temperatures registered during the evaluation period were 30 and 18°C respectively. The average relative humidity was of 64.3%, and the accumulated precipitation was of 852 mm.

With the purpose of facilitating the flooding, the area was leveled and divided into lots. A randomized block design with three replicates was used. The experimental plots were composed of four 5-m-long rows with 0.9-m spacing from each other. The distance between the plants in the plot line was of 0.2 m, and the useful area corresponded to two central rows. Water from a nearby reservoir was used for flooding the lots and conducted by pumps to the experiment's location. The fertilization of the soil, classified as wetland fields' plain-relief phase Entisol with clayey texture ("Neossolo Flúvico Tb eutrófico típico, textura argilosa") (Santos et al., 2006), was performed according to the chemical analysis. Basic 5–20–20 + Zn fertilizer in doses of 400 kg ha^{-1} were applied at sowing and after the germination of the caryopses, two top dressings with ammonium sulphate were carried out in doses of 20 grams per linear meter, the first at the stage of six leaves and the second at the stage of eight leaves. All phytosanitary treatments required by the crop were applied.

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

At flowering, the phase most susceptible to the stress caused by water excess (Zaidi et al., 2007), a sample of the medium third of a totally expanded leaf below the flag leaf from two plants of each replicate was randomly collected and fixed in formaldehyde, acetic acid, and 70% ethanol solution (FAA 70) for 48 hours and then preserved in 70% ethanol. Transversal sections of the mesophyll and midrib were performed with Table microtome, and the paradermical sections were manually acquired from the adaxial and abaxial surfaces at the Laboratório de Anatomia Vegetal of Universidade Federal de Lavras. The transversal

sections 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 paradermical sections were also clarified with 5% sodium hypochlorite for 10 min, rehydrated for 10 min, stained with 2% safranine and mounted on slides with 50% glycerin.

The sections were photographed in an Olympus BX-60 photon microscope coupled to a digital camera. The parameters measured in the paradermic section of the adaxial and abaxial surfaces were: stomatal density; number of stomata per square millimeter; stomatal index, 100(number of stomata per square millimeter/number of epidermis cells); polar diameter of the stoma; and stomatal functionality, polar diameter/equatorial diameter. The parameters evaluated for the midrib were: number of vascular bundles (NV); total number of metaxylem vessels in the vascular bundles; metaxylem vessel diameter in the midrib (VD); Carlquist's vulnerability index, VD/NV (Carlquist, 1975); phloem thickness in the midrib vascular bundles; medium diameter of the ten parenchyma cells; and sclerenchyma area. For the leaf blade, the following were measured: adaxial epidermis thickness, abaxial epidermis thickness, mesophyll thickness, adaxial cuticle thickness, phloem thickness of the vascular bundles, diameter of metaxylem vessels, distance between the vascular bundles, number of bulliform cells at every 2 mm, and polar diameter of the bulliform cells. The anatomical evaluations of the leaf blades were performed in the region of the fourth vascular bundle, counting from the midrib toward the leaf border, where a higher uniformity in the thickness of the leaf blade was observed. Vascular bundles present in the largest midrib and in the fourth biggest vascular bundle after the central vein were used as standard for the vascular bundle evaluation.

The measurements were made using the UTHSCSA ImageTool (University of Texas, San Antonio, USA) image analysis program, and the calibrations were undertaken with a microscope ruler photographed in the same scale as the photomicrographies. The final data were determined by taking the average measurement of leaves from two plants of each replicate. In each leaf, five measurements of each anatomical trait were taken. For the statistical analysis of the results, analysis of variance (ANOVA) and the Scott-Knott test for the

means comparison at 5% probability were applied using Sisvar version 4.0 (Ferreira, 2000).

## Results and Discussion

Modifications in the paradermic sections were observed both on the adaxial and abaxial surfaces of the leaf (Figure 1). The stomatal density on the adaxial surface was statistically higher in the C17 and C18 in comparison to the other cycles and the control (Table 1). The stomatal density of the C18 presented an increase of 45.2 and 41.9% on the adaxial surface compared to the BR 107 and the C1 respectively. The stomatal index on the adaxial surface was also higher in the last two cycles and smaller in the control. C18 presented an increase of 42.76% in comparison to the control, and of 30.4% in comparison to the C1. The adaxial stomatal functionality was higher in C15, C17, and C18 in comparison to the other cycles and
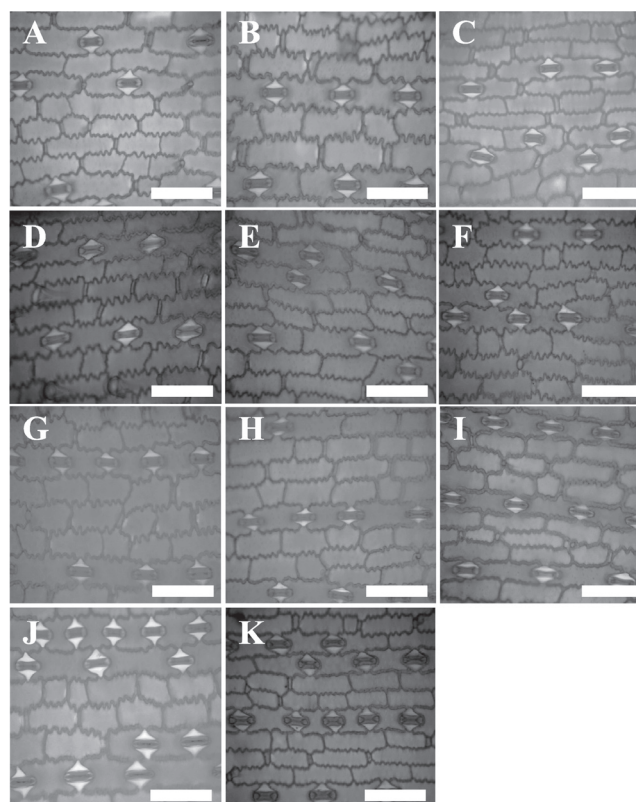


**Figure 1.** Presence of stomata on the adaxial surface throughout the selection cycles of 'Saracura' maize submitted to periodical flooding. A, BR 107 (control); B, C, D, E, F, G, H, I, J and K, cycles 1, 3, 5, 7, 9, 11, 13, 15, 17 and 18 respectively. Bar = 100 μm.

the control, which were not different from each other. C18 presented 29.4% more functionality than the control and 25.4% more than C1. The polar diameter of the stoma from the adaxial surface was higher in C13, C15, C17 and C18 in comparison to the initial cycles and the control. On the abaxial surface, despite the fact that stomatal density had higher values than on the adaxial surface, no significant differences were detected among the treatments. The stomatal index of the abaxial surface presented difference and higher values in C9, C11, C15, C17 and C18. The cycle with the highest average (C9) presented a stomatal index 59.9% higher than that of the control and 26.4% higher than that of C1. There was no significant difference in the stomatal functionality of the abaxial surface. However, for stomatal polar diameter, the control together with C1 presented significantly lower values than the other selection cycles.

Stomata density can also vary in other types of abiotic stresses, such as drought, when a decrease in the stomata density occurs (Bergmann, 2004). The closing of the stomata is one of the most prominent responses to flooding, leading to a decrease in gas exchange (Nicolás et al., 2005) and consequently to a decrease in photosynthesis and in stoma conductance. However, an optimization promoted by an increase in the number of stomata can favor a greater intake of carbon dioxide gas, which diminishes the stomata resistance and facilitates the carbon assimilation process (Henrique et al., 2010). This probably occurred in the 'Saracura' maize, because there was an increase in the stomata number, both by area and by number of epidermis cells

in the last selection cycles, which might benefit the gas exchange. Changes in the size of barley stomata were observed in studies of flooding (Yordanova et al., 2005). However, other authors did not observe changes in the amount and size of stomata in leguminous from flooded regions (*Lotus corniculatus* L. and *Lotus glaber* Mill.) (Striker et al., 2005), which shows that the stomata changes are not very clear in all of the species exposed to this kind of stress. Many studies have described that 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). Higher functionality in the last cycles can favor higher water use efficiency, because they can get carbon dioxide gas with a smaller area of stoma opening, thus avoiding greater loss of water. The same can be observed by the increase in the polar diameter in the last cycles, which is directly related to the stoma functionality. All these characteristics can favor a higher $CO_2$ absorption, an important factor that can increase the quantity of internal carbon, which predisposes a higher energy production for the survival and growth of the crop in flooded areas. However, ecophysiological and biochemical studies should be carried out to support these suggestions, because in some species, including maize, an inhibition in the carboxylation can occur in flooded areas (Yordanova et al., 2005).

The number of vascular bundles increased throughout the selection cycles (Table 2). C3 and the control showed the lowest average, while C15 and C18 presented the highest average. There was an increase of 31.5% in C18 in comparison to the control,

**Table 1.** Parameters measured on the paradermical sections of the adaxial and abaxial surfaces of leaves of 'Saracura' maize of successive selection cycles (C1 to C18) submitted to intermittent flooding[1].

| Treatment | Adaxial surface | | | | Abaxial surface | | | |
|---|---|---|---|---|---|---|---|---|
| | SD | SI | SF | SPD (μm) | SD | SI | SF | SPD (μm) |
| BR 107 | 59.81b | 9.72c | 1.25b | 43.18b | 77.41a | 10.61b | 1.18a | 41.83b |
| C1 | 63.33b | 11.81c | 1.32b | 42.94b | 84.44a | 13.04b | 1.45a | 42.32b |
| C3 | 63.30b | 11.53c | 1.30b | 44.28b | 91.48a | 13.70b | 1.53a | 44.56a |
| C5 | 63.33b | 11.81c | 1.42b | 44.57b | 84.44a | 13.04b | 1.51a | 45.83a |
| C7 | 70.37b | 13.80b | 1.38b | 45.01b | 87.96a | 13.62b | 1.60a | 45.76a |
| C9 | 63.33b | 12.13c | 1.42b | 44.22b | 105.55a | 17.73a | 1.42a | 46.07a |
| C11 | 66.85b | 12.71c | 1.50b | 45.78b | 109.07a | 16.13a | 1.55a | 46.44a |
| C13 | 77.41b | 14.58b | 1.45b | 46.66a | 95.00a | 12.68b | 1.49a | 46.63a |
| C15 | 77.41b | 13.95b | 1.74a | 48.02a | 95.00a | 14.87a | 1.58a | 46.52a |
| C17 | 98.52a | 17.51a | 1.74a | 48.55a | 98.54a | 17.18a | 1.56a | 46.12a |
| C18 | 109.07a | 16.98a | 1.77a | 48.26a | 110.55a | 15.96a | 1.65a | 47.57a |
| CV (%) | 18.15 | 12.69 | 14.91 | 4.18 | 15.81 | 13.67 | 9.78 | 3.96 |

[1]Means followed by equal letters within columns do not differ significantly by the Scott-Knott test, at 5% probability. SD, stomatal density; SI, stomatal index; SF, stomatal functionality; SPD, stomatal polar diameter.

and of 37% in comparison to C3. The total number of metaxylem vessels in the vascular bundles also increased throughout the cycles: C1, C3 and the control were statistically similar, with the lowest averages, and C13, C15, C17 and C18 presented the highest total number. C1 corresponded to 42% of the total number of metaxylem vessels in the vascular bundles of C18. Regarding the diameter of metaxylem vessels, it was observed that C1, C3, C5 and the control presented the highest average, and C13, C15, C17 and C18, the lowest average. The diameter of the metaxylem vessels of C18 decreased 37.9% in comparison to C1 (Table 2, Figure 2 A to C). The highest value of Carlquist's vulnerability index was found in the control, and C13, C15, C17 and C18 corresponded to the lowest index, being statistically similar to each other. Still regarding the midrib vascular bundles, an increase in the phloem thickness was observed starting on the fifth cycle. C18 showed an increase of 26.1% in comparison to the control and of 24.3% in comparison to C1 (Table 2, Figure 2 A to C). There was no difference among the diameter of the parenchyma cells. For the sclerenchyma area in the midrib, a decrease was observed throughout the cycles. The greatest area was found in the control and in C1. From C7 to C18, no statistical difference was detected. The sclerenchyma area of C18 corresponded to 68% of the control's area and to 70.1% of cycle 1's area.

The lack of oxygen in flooded areas can cause a decrease in water conductivity in plants (Dell'Amico et al., 2001). The low conductivity leads to the cavitation process, which can diminish the plant capacity in transporting water and fixing carbon (Mostajeran & Rahimi-Eichi, 2008). When the species have higher quantity of vessels, there is a higher water flow and higher chances to avoid cavitation (Zimmermann, 1983). The diameter of metaxylem vessels is also an important factor in the water flow, because vessels with greater caliber are more prone to cavitation than vessels with smaller caliber (Vasellati et al., 2001). Therefore, the increase in the number of bundles and metaxylem vessels in the last selection cycles of 'Saracura' maize could be protecting the water system and possibly favoring the water absorption in the root, the flow and the distribution of water in the leaves. Such protection in the conducting system is shown by the smaller value of Carlquist's vulnerability index in C13, C15, C17 and C18 (Table 2). A smaller metaxylem vessel diameter in the last selection cycles can also avoid cavitation and embolism due to the increase in the number of water molecules in contact to xylem cell walls per water volume (Hacke & Sperry, 2001).

An increase in the thickness of the phloem can be an important adaptation for the plants submitted to flooding. With the hypoxia, the transportation of photosynthates to the root is inhibited (Liao & Lin, 2001), and, at the same time, lower energy production occurs due to the blockage of oxidative phosphorylation. Furthermore, the decrease of photosynthate flow to the root can cause accumulation of carbohydrates in the chloroplasts, which leads to inhibition of the photosynthesis and decrease of sugars (Liao & Lin, 2001). The roots, as a response to the absence of sugar, deplete the carbohydrate reserves, which makes a

**Table 2.** Parameters measured midrib anatomical parameters from successive selection cycles (C1 to C18) of 'Saracura' maize submitted to intermittent flooding[1].

| Treatment | NV | TV | VD (μm) | VI | PL (μm) | PC (μm) | SC (μm$^2$) |
|---|---|---|---|---|---|---|---|
| BR 107 | 12.33d | 7.17c | 74.55a | 10.67a | 53.62b | 75.90a | 35,027.67a |
| C1 | 13.50c | 7.50c | 66.85a | 9.00b | 54.91b | 95.03a | 33,977.50a |
| C3 | 11.33d | 7.67c | 68.22a | 9.00b | 60.78b | 75.72a | 29,179.10b |
| C5 | 13.50c | 9.33b | 65.83a | 7.33c | 65.09a | 90.47a | 29,988.61b |
| C7 | 13.17c | 10.33b | 56.72b | 5.67d | 64.60a | 93.99a | 27,496.26c |
| C9 | 14.50b | 11.00b | 53.88b | 5.00d | 65.67a | 99.10a | 26,759.92c |
| C11 | 15.17b | 11.17b | 52.82b | 4.67d | 67.65a | 104.03a | 25,268.84c |
| C13 | 15.33b | 14.00a | 47.24c | 3.33e | 66.75a | 102.91a | 26,113.09c |
| C15 | 16.67a | 15.17a | 44.00c | 2.67e | 65.81a | 94.02a | 24,380.54c |
| C17 | 16.00b | 15.17a | 46.24c | 3.00e | 67.00a | 93.80a | 24,253.33c |
| C18 | 18.00a | 17.83a | 41.52c | 2.33e | 72.57a | 95.71a | 23,845.13c |
| CV (%) | 6.48 | 16.16 | 7.79 | 16.93 | 7.33 | 18.49 | 8.43 |

[1]Means followed by equal letters within columns do not differ significantly by the Scott-Knott test at 5% probability level. NV, number of vascular bundles; TV, total number of metaxylem vessels in the vascular bundles; VD, metaxylem vessel diameter; VI, Carlquist's vulnerability index; PL, phloem thickness in the midrib vascular bundles; PC, diameter of the parenchyma cells; SC, sclerenchyma area.

higher intake of photosynthates necessary (Liao & Lin, 2001). The increase of the number of vascular bundles and the phloem thickness in the last selection cycles of 'Saracura' maize could increase the photosynthates transportation from the leaf to other parts of the plant, as well as avoid photosynthesis inhibition.

The diameter of the parenchyma cells can influence the intercellular spaces, increasing the area of gases, such as oxygen (Souza et al., 2009). Furthermore, the death of the biggest cells in the aerenchyma formation could originate larger spaces in the leaf. However, no increase in the diameter of such cells or formation of aerenchyma throughout the selection cycles was verified.

Several studies had shown the increase of sclerenchyma in response to low water availability (Bacelar et al., 2004), an alteration that is important for the plants to avoid water loss. The first cycles produced



**Figure 2.** Anatomical characteristics of transversal sections of midrib and leaf blade. Control BR 107 (A, D, G, J); selection cycle 1 (B, E, H, K); selection cycle 18 (C, F, I, L). Midrib (A, B, C) and leaf blade (D, E, F) with vascular bundle detail, leaf blade evidencing epidermis (G, H, I), showing the bulliform cells (J, K, L). Bar = 100 μm.

higher quantity of this tissue, which shows a possible attempt to save water, since these cycles showed a more vulnerable vascular system than the last cycles. Lower investment in the sclerenchyma area occurred after C7. The reduction of sclerenchyma can lead to the production of other more important tissues, such as phloem, which facilitates the survival of the cycles of 'Saracura' maize in flooded environments.

Adaxial epidermis thickness and abaxial epidermis thickness significantly decreased throughout the selection cycles (Table 3). There was a decrease of 28.7% in the adaxial epidermis of C18 when compared to the control, and of 20.1% in comparison to C1 (Table 3, Figure 2 G to I). The abaxial epidermis of C18, on the other hand, decreased 24.4% in comparison to the control and 15.9% in comparison to C1. No statistically significant differences were observed for the mesophyll thickness. Regarding the cuticle, a significant decrease started to show in the fifth cycle, and C5, C7, C9, C11, C13, C15, C17 and C18 were all similar. C18 cuticle decreased approximately 34.5% in comparison to the control and to C1. Conversely, phloem thickness of the vascular bundles in the leaf blade presented statistically higher values from C11 to C18. The last cycle resulted in an increase of 25.72% in comparison to the control, and of 19.67% in comparison to C1 (Table 3, Figure 2 D to F). A decrease in diameter of metaxylem vessels was observed throughout the selection cycles (Figure 2 D, E and F). The greatest metaxylem diameter was found in the control (66.50 μm), which was statistically similar to that of C1, C3, C5 and C7, C18, together with C9, C11, C13, C15 and C17, resulted in the smallest diameter. C1 and C3 as well as the sensitive variety BR 107 presented the biggest distances between the vascular bundles, and were similar to each other. From C5 to C18 there was a decrease in this distance; however, the cycles did not differ from each other. C18 in comparison to BR 107 and C1 presented a decrease of 15.49 and 12.14%, respectively. The number of bulliform cells of the control was statistically similar to that of C1, C3 and C5. Starting in C7 to C18, the averages were equal and smaller. C18 had a decrease of 31.4% in comparison to that of the control, and of 30% in comparison to C1. Diameter of the bulliform cells also decreased throughout the selection cycles (Table 3, Figure 2 J to L). Control and C1, C3, C7 and C9 were statistically similar to each other, and corresponded to the highest averages.
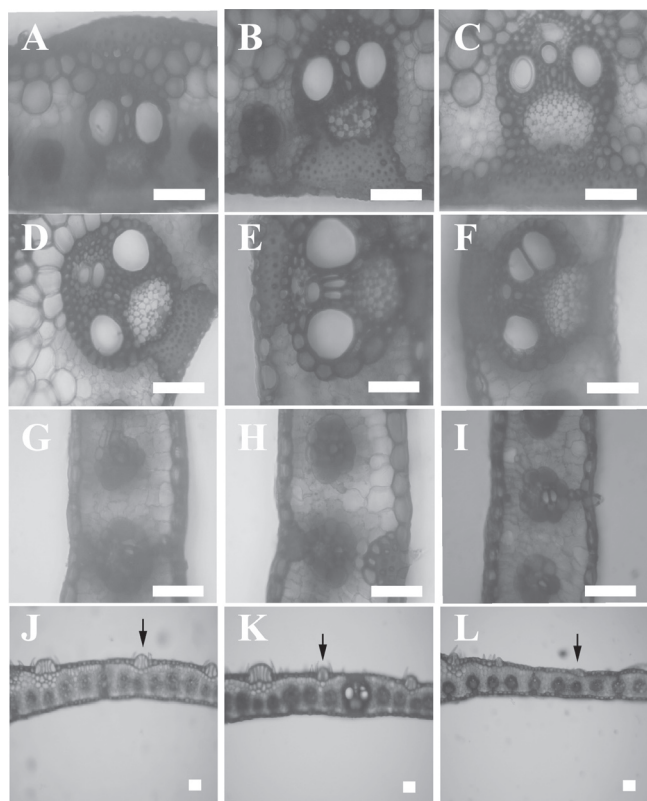
**Table 3.** Leaf blade anatomical parameters (µm) from successive selection cycles (C1 to C18) of 'Saracura' maize submitted to intermittent flooding[1].

| Treatment | DET | BET | MPT | DCT | PHT | DMV | DVB | NBC | PDB |
|---|---|---|---|---|---|---|---|---|---|
| BR 107 | 26.96a | 24.15a | 181.45a | 14.57a | 48.94b | 66.50a | 180.29a | 8.50a | 68.95a |
| C1 | 24.57a | 22.86a | 182.28a | 14.55a | 52.93b | 65.95a | 173.41a | 8.33a | 73.35a |
| C3 | 27.28a | 22.55a | 177.73a | 14.12a | 43.74b | 64.83a | 172.86a | 8.50a | 66.90a |
| C5 | 27.02a | 24.30a | 178.29a | 11.44b | 50.79b | 66.28a | 162.35b | 8.33a | 55.85b |
| C7 | 27.26a | 22.00a | 164.77a | 11.64b | 49.36b | 70.11a | 153.30b | 6.67b | 61.70a |
| C9 | 23.82a | 22.84a | 174.15a | 11.95b | 49.73b | 61.85b | 156.20b | 7.33b | 61.47a |
| C11 | 23.61a | 20.76b | 167.66a | 10.78b | 55.92a | 59.67b | 152.38b | 7.33b | 53.36b |
| C13 | 20.69b | 20.28b | 170.18a | 10.75b | 57.35a | 62.43b | 155.22b | 5.67b | 52.41b |
| C15 | 20.82b | 21.42b | 171.37a | 10.66b | 61.65a | 59.89b | 157.33b | 6.33b | 49.72b |
| C17 | 20.77b | 19.69b | 175.26a | 10.24b | 66.20a | 56.80b | 159.94b | 6.17b | 57.66b |
| C18 | 19.62b | 19.22b | 156.61a | 9.53b | 65.89a | 54.05b | 152.36b | 5.83b | 49.68b |
| CV (%) | 9.18 | 7.96 | 4.52 | 9.69 | 12.27 | 06.38 | 3.01 | 14.38 | 10.31 |

[1]Means followed by equal letters within columns do not differ significantly by the Scott-Knott test at 5% probability level. DET, adaxial epidermis thickness; BET, abaxial epidermis thickness; MPT, mesophyllum thickness; DCT, adaxial cuticle thickness; PHT, phloem thickness in the vascular bundles; DMV, diameter of metaxylem vessels; DVB, distance between the vascular bundles; NBC, number of bulliform cells at every 2 mm; PDB, polar diameter of the bulliform cells.

Although flooding may lead to water deficit (Dell'Amico et al., 2001; Tournaire-Roux et al., 2003), the last selection cycles showed thinner epidermis and cuticle. This modification may be due to low requirement of those selection cycles in retaining water, since they are probably more adapted and therefore establish a greater water conductivity in the flooded environment.

With the leaves completely immersed in water, the mesophyll can modify itself and become thinner, therefore diminishing loss of water and increasing gas diffusion (Mommer et al., 2007). Stoyanova et al. (2005) did not find differences in the mesophyll of the maize cultivar Knezha 6112L submitted to flooding. Even though no significant differences in the mesophyll of the selection cycles of 'Saracura' maize were observed in the present work, Melo et al. (2004) found significant differences in C14 of the same variety when submitted to flooding in a greenhouse.

A greater number of bulliform cells with bigger polar diameter in the initial cycles is possibly related to leaf curling, which leads to a decrease in the transpiration surface area (Alvarez et al., 2008). This fact is important at the initial cycles, because they are more sensitive to flooding. The bulliform cells are motor cells that promote folding and curling of the leaves. The last selection cycles are probably more physiologically adapted to the periodical flooding, allowing the leaf to uncurl with increase in the photosynthetic surface without increase in transpiration.

The selection cycles of 'Saracura' maize under flooding presented characteristics that are typical of C4 plants, but with more intensified stomatal characters to increase carbon dioxide gas concentration and to use water more efficiently, as well as higher capacity of the conducting tissues (both phloem and xylem). It was observed that from C5 to C18 there was a smaller distance between the vascular bundles and, as a consequence, smaller internerval space and higher venal density (Roth-Nebelsick et al., 2001). The distance between vascular bundles can be related to the more efficient transportation and distribution of carbohydrates and water to the mesophyll cells. Studies on parallel vascular bundles in grasses showed that C4-type species present smaller distance between the vascular bundles than C3 type (Ueno et al., 2006). Such characteristic, present in C4-type plants, conveys higher ability in photosynthate translocation and higher water distribution in environments with water restriction and high temperatures (Sage, 2004). Just as a greater phloem thickness could increase the photosynthetic flow, a reduced space between the vascular bundles in the selection cycles of 'Saracura' maize could also increase this flow, because it would promote higher photosynthate distribution (Roth-Nebelsick et al., 2001) in addition to a higher water distribution.

## Conclusion

The successive selection cycles of 'Saracura' maize result in modifications in leaf anatomy that are potentially favorable to its tolerance to the periodical soil flooding.

## Acknowledgements

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