ANATOMICAL CHANGES IN *Urochloa plantaginea* AND *Urochloa platyphylla* UNDER DIFFERENT SOIL MOISTURE CONDITIONS

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Abstract

Urochloa plantaginea and Urochloa platyphylla are common weeds in the highland area. However, in recent years, they have been found in wetlands and poorly drained soils, but the biology and behavior of the species in these conditions are not known. Thus, the objective was to assess anatomical changes in plants of Urochloa plantaginea and Urochloa platyphylla grown under different soil moisture conditions, as well as the adaptive structures generated as a result of each environment. A completely randomized experimental design in the form of a 2x2 factorial design was used, with factor A being two species of Urochloa (U. plantaginea and U. platyphylla), and species B being three soil moisture conditions (50 and 100% FC and 5 cm water depth), with four repetitions. The assessments were performed by means of anatomical cuts, observing the number and diameter (micrometers - µm) of aerenchymas in stems, roots and leaves; total diameter and the central root cylinder (µm); diameter of the fistula medulla and cortex (µm) in stems; mesophyll thickness and leaf midrib (µm). It was found that, for the two species of Urochloa, the water depth condition induced an increase in the number and diameter of aerenchymas in roots and leaves and provided a larger diameter of the fistulous pith in stems. The diameter of the central cylinder and the thickness of the leaf mesophyll midrib were more compact at 50% FC, also, for both species. Therefore, the adaptive structures generated vary as a result of the field capacity of the soil.

Keywords: Anatomy; Brachiaria; Hypoxia;

1. Introduction

Throughout their life cycle, plants are subjected to different environmental conditions, oftentimes unfavorable to their development. Water stress can occur due to both a lack or excess of water supplied (Andrade et al., 2020), causing morphophysiological changes in plants, such as increased stomatal resistance, reduced photosynthesis (Bonfim-Silva et al., 2011), changes in transpiration rates, growth inhibition, leaf wilting and/or abscission, reduced yielding, etc. (Rodrigues et al., 2019).

In irrigated-rice crops, soil flooding causes a deficit of oxygen for the root system of plants, favoring weed control, due to physiological disturbances in some species, such as reduced root oxygenation and production of reactive oxygen species (ROS) (Ismail et al., 2012; Gealy et al., 2014). Under this stressful condition, plants develop morphological and physiological responses, which give them an ability to tolerate or not flooded environments.

The mechanisms through which plants tolerate excessive moisture in the soil vary from species to species, and so does their ability to react by adapting morphologically and physiologically to this environmental stress (Taiz & Zeiger, 2013; Liu, 2014). In irrigated-rice crops, aerenchymas are formed in roots, stems and leaves (Joshi & Kumar, 2012), and there is an increase in adventitious roots (Kroth, 2013; Yin et al., 2013; Dalmolin et al., 2018;) and/or formation of larger intercellular spaces that allow greater oxygen diffusion from the aerial part to the root system, temporarily maintaining the aerobic respiration process (Pareek et al., 2011; Pegg et al., 2020).

Plants tend to modify important tissues such as chlorophyll parenchymas in the leaf mesophyll, and cortical or medullary parenchyma in stems and roots, in order to adapt. The formation of lysigenous or schizogenous spaces, including aerenchymas, is intensified (Hossain & Udin, 2011) by the disruption of the cell wall, mainly by the higher concentration of ethylene, which stimulates their formation, with an increase in the cell concentration of ethanol and acetaldehyde, and acidification of the cytoplasm due to the presence of lactate. These compounds trigger the formation of these anaerobiosis-adaptive tissues by promoting cell disruptions (cell wall), increasing the occurrence of these aeriferous spaces (Alves et al., 2002).

Plants of the genus *Urochloa* P. Beauv., popularly known as brachiaria, or marmalade grass, have 18 species registered in Brazil (Shirasuna, 2015), inhabiting the South, Southeast and Midwest, mainly in soybean and corn fields. In recent years, infestation levels in irrigated-rice areas in Rio Grande do Sul have risen, which may cause high losses in grass yields due to a highly competitive ability (Galon et al., 2015). According to Velho et al. (2012), the presence of 25 plants/m² of this species in competition with irrigated rice caused a 96% negative impact on yielding.

Irrigated-rice areas are characterized by a flooding period; however, the high cost of irrigation has decreased the water depth over the years, thus favoring the adaptation of weeds such as *Urochloa plantaginea* (Link) R.D. Webster (Marmalade grass) and *Urochloa platyphylla* (Munro ex C. Wright) R.D. Webster (Dwarf bamboo) (Kissmann, 1997; SOSBAI, 2014). The *U. platyphylla* species has been found in flooded environments (SOSBAI, 2014), whereas the *U. plantaginea* species is a more recent weed, typically inhabiting highlands (Kissmann, 1997), adapting to the anaerobic environment of southern lowlands (SOSBAI, 2014).

Plants can reduce the damage caused by excess water through morphological adjustments, with a decrease in the below/above ground biomass ratio, development of hypertrophied lenticels, of adventitious roots, and aerenchyma formation, which will increase the transport of oxygen to the roots or reduce root loss of oxygen to the soil (Gao et al., 2016). In this context, the objective of this study was to assess anatomical changes in plants of *Urochloa plantaginea* and *Urochloa platyphylla* subjected to different soil moisture conditions, in order to identify adaptive structures generated as a result of each environment.

2. Material and methods

The experiment was run in a greenhouse at the Biology Department of the Federal University of Santa Maria [Universidade Federal de Santa Maria] – UFSM, Santa Maria, RS, with 6 m x 20 m and 5 m in height. A completely randomized experimental design was used in the form of a 2 x 2 factorial design, with factor A being two species of *Urochloa* (*U. plantaginea* and *U. platyphylla*), and factor B being three soil moisture conditions (50% and 100% system field capacity (FC) and 5 cm water depth), with four repetitions. The samples of *U. plantaginea* and *U. platyphylla* were collected from a commercial irrigated-rice field in the city of Itaqui, located on the west border of the state of Rio Grande do Sul (LAT: 29° 14' 09,03" S LONG: 56°20' 07,80" W). Inflorescences of random plants belonging to the target populations were collected and stored in porous paper bags until the moment of sowing.

In the greenhouse, the seeds were sown into pots with capacity for 7.5 L, filled with 2.5 kg of organic substrate and 4.0 kg of medium-texture, sterilized sand. After emergence, only one plant per experimental unit remained. For said amounts of water to be supplied, the gravimetric moisture content of the sand-substrate system was calculated in accordance with the manual of soil methods and analyses (Embrapa, 1997), and from the moisture-column methodology proposed by Forsythe (1975), the amount of water required for each experimental unit to reach 100% FC was determined through the total saturation of the system. When total saturation was reached, the total mass of the experimental unit was checked and maintained until the end of the experiment. Irrigation was performed regularly by means of the weighing method, using an ACS System electronic scale with a 5 g precision, with water being added until the pre-determined total mass was reached (pot + sand-dry substrate system + 100% field capacity for floodplain environments, and 50% for hilly grassland environments).

For a proper plant establishment, the hydric conditions of the soil were established when the plants had an average of 2-3 leaves – stage 13 on the BBCH-scale (Meier, 2001). The material for making the glass slides was collected when the plants were in the reproductive stage of full flowering – stage 65 on the BBCH-scale (Meier, 2001). From each experimental unit, cuts were made in the middle third of the flag leaf, stem and main root, using a scalpel. The making of the slides followed the Jung historesin technique, modified by Mariath & Santos (1996). The material was embedded in hydroxyethyl methacrylate, in accordance with the protocol by Gerrits & Smid (1983). The cross sections (anatomical cuts) were obtained by sectioning the material embedded in historesin, using a rotary microtome, with the sections being processed and fixed on a glass slide and later stained with 0.05% toluidine blue (Feder & O'Brien, 1968).

The anatomical assessments of the sections were performed with the aid of an Axio Scope.A1 optical microscope equipped with a high-resolution Axiocam digital camera. Using a 5x objective lens and a 10x ocular lens, images with 2560x1920 pixels and 96 dpi resolution were obtained and stored in JPEG format, then analyzed using the Zenn 2012 image processing software. The anatomical parameters assessed included: root aerenchyma diameter (micrometers – µm), root diameter (µm), number of root aerenchyma, central root cylinder diameter (µm), root cortex thickness (µm), stalk aerenchyma diameter (µm), number of stalk aerenchyma, stalk fistula thickness (µm), stem cortex thickness(µm), leaf mesophyll thickness (µm), leaf midrib thickness (µm), and leaf aerenchyma diameter (µm). The measures for aerenchyma diameter, stalk fistula thickness, central cylinder diameter and root diameter were taken in two directions due to the ellipsoidal shape of these structures, using the average value.

The experimental errors were tested for normal distribution through the Shapiro-Wilk test, and the homogeneity of the variances was checked by means of Bartlett's test, with the aid of the Action program (Estatcamp, 2014). Subsequently, the analysis of variance (ANOVA) and the Scott-Knott test for grouping the means were carried out, at a 5% probability of error ($p<0.05$), using the Sisvar® 5.3 statistical program (Ferreira , 2011).

3. Results and discussions

A statistically significant difference was found in stem, root and leaf morphologies by the Scott-Knott test ($p \leqslant 0.05$) for all variables analyzed, with the exception of only the "leaf mesophyll midrib thickness" variable. For number of aerenchyma in stems of *U. plantaginea* (Table 1), less aerenchyma formation was observed in the 50% FC condition (0.25). When the amount of water in the soil doubled to 100% FC, 149 times more aerenchyma units were obtained per section and with a water depth 94 times greater than at 50% FC. These results show that the collected access is of recent introduction in the flooded environment where it was collected, and still has anatomical characteristics of a plant that develops in a well-drained highland environment, when not subjected to soil anaerobiosis (SOSBAI, 2014).

Table 1. Anatomical parameters assessed in the cross sections of stems of *Urochloa plantaginea* and *Urochloa platyphylla*. UFSM, 2014.

Treatment[3]	Aerenchyma diameter (μm)[2]	Fistula diameter (μm)	Cortex thickness (μm)	Number of aerenchymas
		Urochloa plantaginea		
Depth	196.418 a	774.870000 a	711.242 a	23.500000 b
100%	115.257 b	690.215000 b	516.391 b	37.250000 a
50%	4.840 c	314.627500 c	472.390 c	0.250000 c
C.V.(%)	48.19	1.93	13.58	18.75
		Urochloa platyphylla		
Depth	170.493 a[1]	787.425 a	401.873 a	43.750 b
100%	163.640 a	635.415 b	380.205 b	77.500 a
50%	120.638 b	538.705 c	216.434 c	67.500 a
C.V.(%)	48.19	30.70	18.45	8.99

[1]Means followed by the same letter in the column do not differ significantly from each other by the Scott Knott test, at a 5% probability of error.

[2]Unit (μm) = micrometers.

[3]Treatments: 5 cm water depth; 100% field capacity; 50% field capacity.

In *U. platyphylla* (Table 1), the water depth condition was the one with the smallest number of stem aerenchymas formed. As for the 50% FC, it formed 1.54 times fewer aerenchymas, and for 100% FC this ratio was 1.77 times lower. This species, being acclimated to flooded areas, showed little change in aerenchyma formation comparing the three soil moisture conditions, assuming that this characteristic is already incorporated into the genotype of the species. The latter presents an amount of aerenchymas, even in conditions of low soil moisture, so a 50% FC was enough to develop the aeriferous tissues, differently from what happened with *U. plantaginea*, which formed virtually no aerenchymas under this condition.

Figure 1 shows that, in the water depth condition (A), *U. plantaginea* presented a smaller number of areas with stem aerenchyma (Ae), but a greater average diameter compared to the 100% FC condition (B). For the average diameter of the fistulous pith (Fp) and cortex thickness (Co), the treatments with water depths resulted in a larger fistula diameter and greater cortex thickness compared to 100% (B) 50% FC (C). This fact evidences that, as the amount of water in the soil increased, until the hypoxia condition, there was an increase in stem thickening, due to the presence of a thicker subepidermal sclerenchymatic layer, that is, there was a greater compensatory reinforcement to the increase in the cavity of the fistulous pith to provide resistance to the growth of the stem.

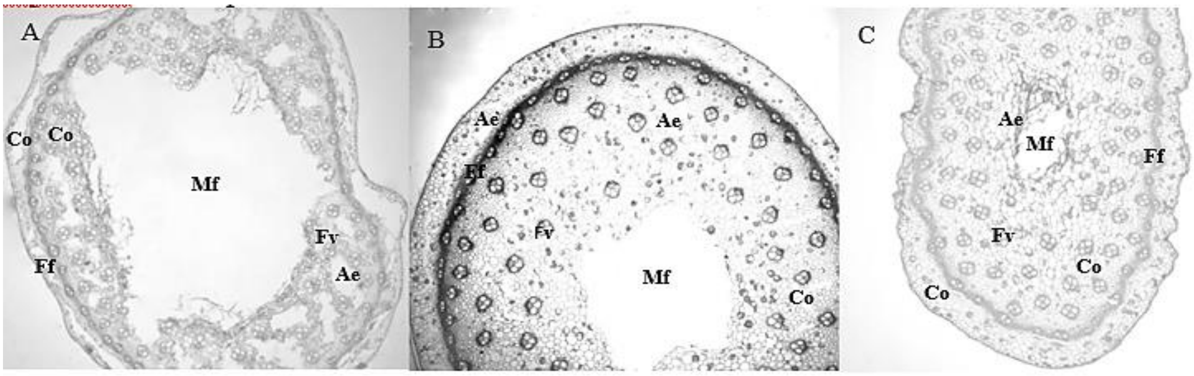

Figure 1. Anatomical changes in stems of *Urochloa plantaginea* under three soil moisture conditions: (A) Water depth, (B) 100% field capacity and (C) 50% field capacity. Image: 300 µm.
Fistulous pith (Fp); Cortex (Co); Aerenchymas (Ae), Fiber bundle (Fb); Vascular bundles (Vb).

The stem anatomy of *Urochloa platyphylla* (Figure 2) showed an increase in the thickness of the fistulous pith (Fp) as the amount of water in the soil increased, with a greater value for fistula diameter in the water depth condition, followed by 100% and 50 % FC, which showed the smallest diameter. Regarding stem cortex thickness, it followed the same trend as the fistulous pith, with greater cortical thickness in the water depth condition and the lowest thickness at 50% FC.

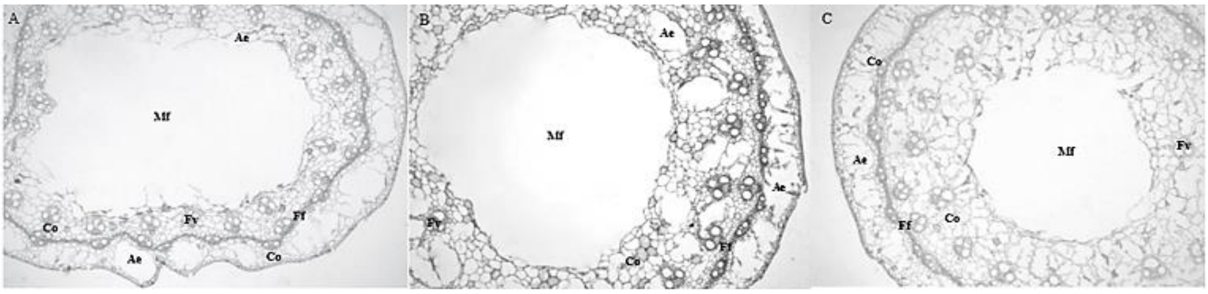

Figure 2. Anatomical changes in stems of *Urochloa platyphylla* under three soil moisture conditions: (A) Water depth, (B) 100% field capacity and (C) 50% field capacity. Image: 300 µm.
Fistulous pith (Fp); Cortex (Co); Aerenchymas (Ae), Fiber bundle (Fb); Vascular bundles (Vb)

The anatomical analysis of the stem sections of *U. plantaginea* (Figure 1) and *U. platyphylla* (Figure 2) species allows observing that, initially, the stem cortical aerenchyma seems to be formed in a lysigenous manner, stimulated by the hypoxia condition. Subsequently, the aerenchymas located at the edges of the fistulous pith have their wall broken and join the pith, expanding its space, which was made evident when its diameter was measured, which proved to be larger in the water depth condition, thus explaining why there is a smaller number of cortical aerenchymas in the count in said condition. In this sense, it can be inferred that the fistula must function as a large aeriferous stem channel. For Joshi & Kumar (2012), lysigenous and schizogenous aerenchymas are important tissue systems developed by plants that produce the same final result, forming aerenchymas for survival in flooded soils.

U. plantaginea and *U. platyphylla* presented a larger aerenchyma diameter, fistula diameter, and thicker cortex in the water depth condition. Studying wheat (*Triticum aestivum*), Hossain & Uddin (2011) found that the plants went through anatomical adaptations, such as formation of lysigenous aerenchyma in stems and roots to survive in an anaerobic environment caused by excess water in the soil. *Lysigenous aerenchymas* are formed through the disruption of the cells (Hossain & Uddin cells, 2011); the formation of this type of aerenchyma in irrigated-rice stems and roots is common (Joshi & Kumar, 2012) and involves an accumulation of ethylene in plants due to the formation of its precursor ACC (1-aminocyclopropane 1-carboxylic acid). A lack of oxygenation blocks the conversion of ACC to ethylene, causing an accumulation of by-products of anaerobic fermentation such as ethanol and acetaldehyde (Both et al., 2014), as well as of reactive compounds such as H_2O_2, which is induced in hypoxia conditions and can lead to cell death (Yamauchi et al., 2013).

Analyzing the data obtained with the anatomical cuts in the leaves of the two species assessed (Table 2), both maintained the number of aerenchymas higher in the water depth condition because, according to Voesenek & Sasidharan (2013), aerenchyma formation is one of the metabolic changes that may occur during the soil flooding period. However, the diameter of *U. plantaginea* aerenchymas is greater in the 100% condition than in the flooded environment, whereas for *U. platyphylla* it was larger with water depth. The thickness of the leaf mesophyll was affected by the water depth condition, showing that, under excess water condition, this species has its homogenous chlorophyll parenchyma shortened, more than under the other soil moisture conditions, making this mesophyll more compact, but without effect on the midrib, which did not differ statistically in the 50 and 100% FC conditions.

Table 2. Anatomical parameters assessed in the cross sections of leaves of *Urochloa plantaginea* and *Urochloa platyphylla*. UFSM, 2014.

Treatment[3]	Aerenchyma diameter (µm)[2]	Leaf mesophyll thickness (µm)	Leaf mesophyll midrib thickness (µm)	Number of aerenchymas
		Urochloa plantaginea		
Depth	36.146 b	123.391 b	189.910 a	9.45 a
100%	57.293 a	142.027 a	193.500 a	7.26 b
50%	33.050 b	142.346 a	172.060 a	0.42 c
C.V.(%)	29.61	5.50	10.24	7.32
		Urochloa platyphylla		
Depth	104.128 a[1]	133.473 b	181.390 a	12.34 a
100%	71.522 b	159.830 a	184.740 a	8.35 b
50%	55.296 b	155.167 a	169.115 a	4.58 c
C.V.(%)	29.61	28.96	6.15	17.36

[1]Means followed by the same letter in the column do not differ significantly from each other by the Scott Knott test, at a 5% probability of error.

[2]Unit (µm) = micrometers.

[3]Treatments: 5 cm water depth; 100% field capacity; 50% field capacity.

In the leaves of *U. plantaginea* (Figure 3), the highest average number of areas with aerenchymas (Ae) was found in the water depth condition (A). In addition, the increase in the amount of water in the soil promoted the formation of a more compact homogeneous chlorophyll tissue, with a smaller leaf mesophyll diameter in water depth conditions compared to 50% and 100% FC, which presented statistically equal measures among themselves, a similar behavior as to leaf morphology between both species analyzed.

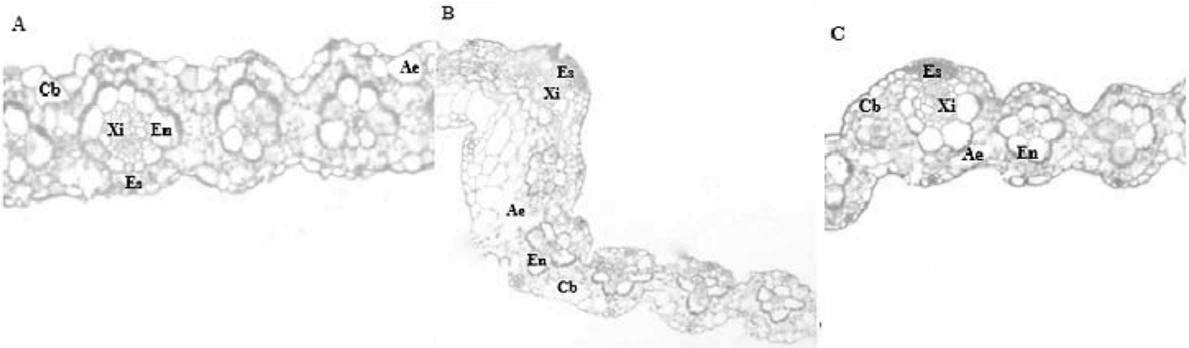

Figure 3. Anatomical changes in leaves of *Urochloa plantaginea* under three soil moisture conditions: (A) Water depth, (B) 100% field capacity and (C) 50% field capacity. Image: 50 µm.
Bulliform cells (Bc), Bundle sheath endoderm (En); Aerenchyma (Ae); Xylem (Xi); Sclerenchyma bundles (Sc).

As shown in Figure 4, the largest number of areas with aerenchymas (Ae) and their largest diameter were found when the plants were subjected to the water depth, because the hypoxia condition induced the leaves of *U. platyphylla* to present smaller and more compact cells in the homogeneous chlorophyll parenchyma when the leaf mesophyll was assessed. In the 50% and 100% FC conditions, they were similar, presenting a thicker leaf mesophyll compared to the water depth.

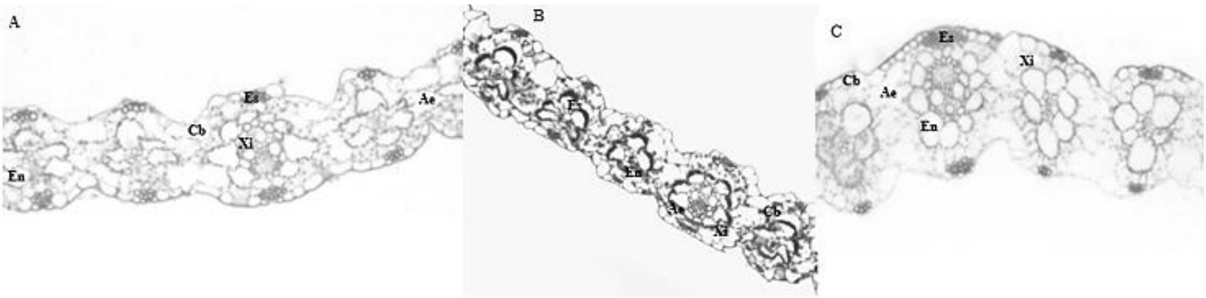

Figure 4. Anatomical changes in leaves of *Urochloa platyphylla* under three soil moisture conditions: (A) Water depth, (B) 100% field capacity and (C) 50% field capacity. Image: 50 µm.
Bulliform cells (Bc), Bundle sheath endoderm (En); Aerenchyma (Ae); Xylem (Xi); Sclerenchyma bundles (Sc).

Significant changes were observed in the conducting tissues of the leaves of the assessed species (Figures

3 and 4). The midrib diameter did not change due to the flooding, but the conducting vessels of the secondary ribs showed differences between the treatments with more or less water in the soil. Anatomical differences were found as to the thickening and lignification of the xylem walls, phloem walls or vascular bundles, which became less thick in the water depth condition, loosening the wall, which is deformed and less thick. Medri et al. (2011), while studying the morphoanatomy of vegetative organs of *Aegiphila sellowina* Cham. (Lamiaceae), reported that flooding reduced the midrib diameter and the xylem thickness in leaves. The phloem showed no significant anatomical changes between the flooding and control treatments.

Concerning the roots (Table 3), the conducting tissues proved to be affected by the treatments. The diameter of the central cylinder reduced as the amount of water in the soil increased, with the smallest diameter in the water depth condition, and the largest diameter at 50% FC. In this sense, the cortex/central cylinder ratio in *U. platyphylla* is higher (1.70) for the water depth condition compared to 100% FC (1.40) and 50% FC (1.04), with a reduction of 41.27% in the diameter of the central cylinder compared to the cortex in this flooding condition. For *U. plantaginea*, despite a smaller central cylinder diameter being found in water depth compared to the other treatments, there is a balanced cortex/central cylinder ratio (1.02) in water depth, which is also observed for 100% FC (1.06). For 50% FC, the ratio is 0.39; under this condition of less water, for this species the cortex was 39.49% smaller than the central cylinder, which is the opposite of what happened with the *U. platyphylla* species.

Table 3. Anatomical parameters assessed in the cross sections of roots of *Urochloa plantaginea* and *Urochloa platyphylla*. UFSM, 2014.

Treatment[3]	Aerenchyma diameter (µm)[2]	Central cylinder diameter (µm)	Root diameter (µm)	Number of aerenchymas
		Urochloa plantaginea		
Depth	113.967 b	314.600 c	638.585 c	16.000 b
100%	277.220 a	469.795 b	970.535 a	24.750 a
50%	43.333 c	510.500 a	712.145 b	4.750 c
C.V.(%)	72.95	13.59	5.68	23.0
		Urochloa platyphylla		
Depth	208.131 a[1]	278.330 c	752.230 b	28.000 a
100%	133.476 b	309.085 b	751.880 b	29.250 a
50%	106.878 b	474.250 a	970.670 a	16.250 b
C.V.(%)	72.95	39.54	5.23	3.85

[1]Means followed by the same letter in the column do not differ significantly from each other by the Scott Knott test, at a 5% probability of error.

[2]Unit (µm) = micrometers.

[3]Treatments: 5 cm water depth; 100% field capacity; 50% field capacity.

Regarding root diameter and root central cylinder diameter for *U. plantaginea*, the water depth showed the lowest values for these parameters, which were statistically inferior to those of the other soil moisture conditions (Table 3). The 50% field capacity was the treatment that provided the largest central cylinder diameter, and at 100% FC there was a larger root diameter, a larger number of areas with aerenchymas and a higher average aerenchyma diameter for *U. plantaginea*. In addition, it was possible to observe that, as the amount of water in the soil increased, the number of root aerenchymas increased as well for both species, being statistically greater in water depth, followed by 100% and 50% FC. The formation of aeriferous parenchymas (aerenchymas) is a common and important anatomical change for survival in a soil anaerobiosis condition (Medri et al., 2011) and was found in the leaves, roots and stems assessed in this study.

The roots of *U. plantaginea* (Figure 5) showed a greater number of areas with aerenchymas and a larger aerenchyma diameter in the 100% FC condition, followed by water depth. Both differed statistically from the 50% FC condition, which showed lower values for these parameters. The conducting vessels have less thick cell walls and less lignification, which is also seen in the endoderm, where the reinforcement of cellulose and lignin in "U" is less developed with the increase in the amount of water in the soil. Cell loosening deforms the sections, due to the less structured cell walls under anoxia.

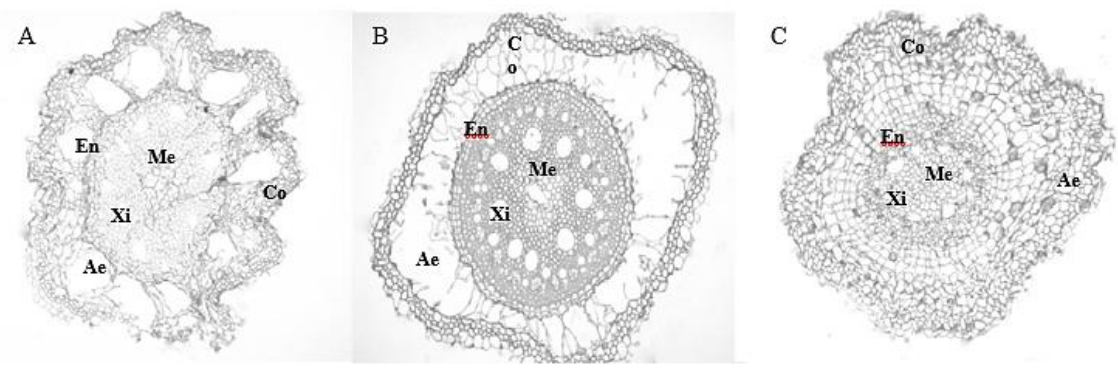

Figure 5. Anatomical changes in roots of *Urochloa plantaginea* under three soil moisture conditions: (A) Water depth, (B) 100% field capacity and (C) 50% field capacity. Image: 300 µm.
Cortex (Co); Central cylinder medulla (Me); Endoderm (En); Xylem (Xi); Aerenchyma (Ae).

The diameter of the central cylinder of *U. platyphylla* roots reduces as the amount of water in the soil increases, reaching the highest average value in the 50% FC condition, differing from 100% FC and water depth, which presented lower values for this parameter (Figure 6). When the root diameter was assessed, including cortex and central cylinder, no significant difference was found between 100% FC and water depth, whose values were statistically lower than those for 50% FC, the soil moisture condition with the largest average root diameter.

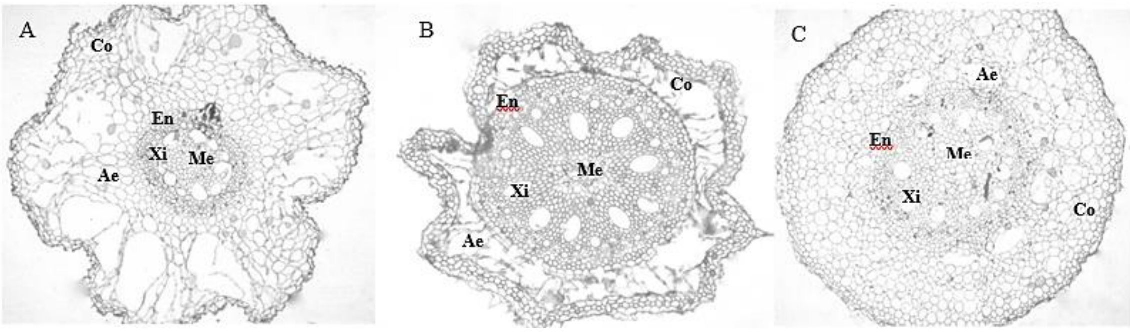

Figure 6. Anatomical changes in *Urochloa platyphylla* roots under three soil moisture conditions: (A) Water depth, (B) 100% field capacity and (C) 50% field capacity. Image: 300 µm. Cortex (Co); Central cylinder medulla (Me); Endoderm (En); Xylem (Xi); Aerenchyma (Ae).

The results found in this study show that both the *Urochloa plantaginea* and *Urochloa platyphylla* species developed under the three different soil moisture conditions, including water depth. It can be inferred that, despite being plants typical of drier environments, the two have adaptive mechanisms that allowed their development and growth in a water depth environment, different from their natural habitat. The development of tissues specialized in transporting oxygen from the aerial part to the roots (aerenchymas) was the main adaptive event found for *U. plantaginea* and *U. platyphylla* grown in water depth.

5. Conclusion

The results presented in this research allow concluding that, in the water depth condition, significant changes occurred in the morphoanatomy of the plants of *Urochloa plantaginea* and *Urochloa platyphylla*, which vary between species according to the greater or lesser ability of the plant to adapt to environments with low root oxygenation.

The increase in the amount of water in the soil led to the formation of a more compact homogeneous chlorophyll tissue, with a less thick leaf mesophyll under water depth conditions for the two species studied.

The flooding of the soil induced an increase in the number and diameter of aerenchymas in roots and leaves, while for the stem a larger fistulous pith diameter was found. The diameters of the central root cylinder and of the leaf mesophyll were smaller (more compact tissues) in water depth than at 50% and 100% field capacity.

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