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Physiological and anatomical differences between subtropical forage plants grown in waterlogged alkaline-sodic soil






Abstract – The objective of this work was to evaluate the effects of 26 days of waterlogging, compared with field capacity, on different traits of the forage plants 'Finecut' *Chloris gayana*, 'Klein Verde' *Panicum coloratum*, and 'Shawnee' *Panicum virgatum*, grown in alkaline-sodic soil. Shoot and root dry mass, leaf greenness index, electrolyte leakage, and root histology were evaluated. The histological analysis was performed along the mid-portion of adventitious roots with a 2.0 mm diameter. Waterlogging inhibited the growth of *P. coloratum*, in addition to decreasing the leaf greenness index and causing injuries in the cell membrane of *C. gayana* and *P. coloratum*. At field capacity, only *C. gayana* and *P. coloratum* had aerenchyma; however, waterlogging induced the development and increased the area of the aerenchyma in *P. virgatum* and *C. gayana*, respectively. Waterlogging also thickened the exodermis and endodermis of all three genotypes, inducing a greater number of exodermis cell rows and a thicker internal tangential cell wall of the endodermis in *C. gayana* and *P. virgatum*. Although *P. coloratum* growth is more sensitive to waterlogging, there are radial oxygen loss barriers in the roots of the three evaluated genotypes.

Index terms: *Chloris gayana*, *Panicum coloratum*, *Panicum virgatum*, alkalinity, flooding, plant anatomy.

Diferenças fisiológicas e anatômicas entre gramíneas subtropicais crescidas em solo alcalino-sódico alagado

Resumo – O objetivo deste trabalho foi avaliar os efeitos de 26 dias de alagamento, em comparação à capacidade de campo, sobre diferentes atributos das gramíneas *Chloris gayana* 'Finecut', *Panicum coloratum* 'Klein Verde' e *Panicum virgatum* 'Shawnee', cultivadas em solo alcalino-sódico. Foram avaliados massa seca da parte aérea e da raiz, índice de verdor da folha, extravasamento de eletrólitos e histologia da raiz. A análise histológica foi realizada na porção média de raízes adventícias com 2,0 mm de diâmetro. O alagamento inibiu o crescimento de *P. coloratum*, além de ter diminuído o índice de verdor foliar e causado lesão da membrana celular em *C. gayana* e *P. coloratum*. Em capacidade de campo, apenas *C. gayana* e *P. coloratum* apresentaram aerênquima; no entanto, o alagamento induziu o desenvolvimento e o aumento da área do aerênquima em *P. coloratum* e *C. gayana*, respectivamente. O alagamento também engrossou a exoderme e a endoderme dos três genótipos, tendo induzido maior número de células da exoderme e maior espessura da parede celular tangencial interna da endoderme em *C. gayana* e *P. virgatum*. Embora o crescimento de *P. coloratum* seja mais sensível ao alagamento, há barreiras radiais de perda de oxigênio nas raízes dos três genótipos avaliados.

Termos para indexação: *Chloris gayana*, *Panicum coloratum*, *Panicum virgatum*, alcalinidade, inundações, anatomia vegetal.

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Introduction

Alkalinity, sodicity, and waterlogging restrict plant growth and development (Ashraf, 2012; Zhu, 2016). The simultaneous occurrence of these stresses strongly limits forage production in several areas of the Flooding Pampa region in Argentina (Cicore et al., 2015). Historically, in this area, soil waterlogging occurs frequently in winter and early spring, but this event has been increasing over the last decades, partially due to worldwide climate change, mainly to more intense and unpredictable rainfalls (Hirabayashi et al., 2013).

Waterlogging decreases oxygen availability in soils, inhibiting the production of adenosine triphosphate in plant roots, whose hydraulic conductivity is consequently reduced, in addition to causing water stress to plants, whose first responses are a reduced leaf growth and stomata closure (Pezeshki & DeLaune, 2012). Herzog et al. (2016) found that a decreased CO₂ concentration in intercellular spaces reduces net photosynthesis, leading to an excessive production of reactive oxygen species, with a decreased photosynthetic rate due to non-stomatal causes.

In this scenario, perennial C₄ Poaceae are tropical forages known for their high productivity (Gherbin et al., 2007; Siri Prieto et al., 2017), such as switchgrass (*Panicum virgatum* L.), and for their ability to cope with different abiotic stresses, including waterlogging (Imaz et al., 2015; Striker et al., 2017) and soil alkalinity (García et al., 2018; Pesqueira et al., 2017), as Rhodes grass (*Chloris gayana* Kunth) and Kleingrass (*Panicum coloratum* L.), even in soils with restrictions to plant growth (Lowry et al., 2014; Hu et al., 2015, 2022).

Stress avoidance and tolerance are two main strategies used by plants to survive or thrive in waterlogged soils (Bailey-Serres et al., 2012; Gao et al., 2015). To mitigate or prevent the stress caused by hypoxia, for example, some species undergo anatomical and morphological changes in their roots to increase the internal availability of O₂, required to maintain their energetic status, function, and growth (Pedersen et al., 2021). According to the same authors, among these changes are the development or expansion of the aerenchyma tissue, increasing the number of newly emerged adventitious roots, as well as the deposition of suberin or lignin in root tissues.

Aerenchyma development can be a constitutive or a waterlogged-induced characteristic (Jackson &

Colmer, 2005). *Chloris gayana* and *P. coloratum*, for example, have a constitutive aerenchyma, which allows the plant to avoid the effects of waterlogging (Imaz et al., 2012, 2015; Striker et al., 2017). Both species also have the ability to fully recover after different periods of waterlogging throughout the year (Imaz et al., 2015). In the case of *P. virgatum* plants, there is evidence of the presence of the aerenchyma in different cultivars exposed to flooding conditions (Skinner et al., 2009), but not that it is constitutive.

Barriers to radial oxygen loss, mainly composed of suberin deposits in the endodermis, are important for the transport of oxygen over long distances, enabling cell respiration at the root tip, while suberin deposits may block the entry of potentially toxic compounds normally present in highly reduced soils (Soukup et al., 2007; Ejiri et al., 2021). In other *Poaceae*, suberin depositions were detected and quantified under anaerobic conditions (Soukup et al., 2007; Manzur et al., 2015), but there are no known reports about the presence of a radial oxygen loss barrier in *C. gayana*, *P. coloratum*, and *P. virgatum*.

The objective of this work was to evaluate the effects of 26 days of waterlogging, compared with field capacity, on different traits of the forage plants 'Finecut' *C. gayana*, 'Klein Verde' *P. coloratum*, and 'Shawnee' *P. virgatum*, grown in alkaline-sodic soil.

Materials and Methods

The experiment was carried out in the greenhouse and laboratories of Facultad de Ciencias Agrarias of Universidad Nacional de Lomas de Zamora, located in Buenos Aires, Argentina. The experimental design was completely randomized, in a 2×3 factorial design, corresponding to two soil water conditions (field capacity and waterlogged soils) and three genotypes ('Finecut' *C. gayana*, 'Shawnee' *P. virgatum*, and 'Klein Verde' *P. coloratum*), with three replicates. The experimental units were pots with one plant each, managed separately during the experiment for independent observations.

Seeds of *C. gayana* and *P. coloratum*, provided by Oscar Peman S.A. (Sinsacate, Córdoba, Argentina), and of *P. virgatum*, by Instituto Nacional de Tecnología Agropecuaria (Ciudad Autónoma de Buenos Aires, Argentina), were sown in 18 black polyethylene pots, with a 4.0 L capacity, filled with alkaline-sodic soil. The soil presented pH 8.3, 0.98 dS m⁻¹ electrical

conductivity of the saturated paste extract (EC_s), 26.2% exchange sodium percentage (ESP), and 3.6% organic matter. The soil was collected in the municipality of Chascomús, in the province of Buenos Aires, Argentina (35°34'42.865"S, 58°0'49.865"W).

To simulate waterlogging, 42 days after sowing, half of the pots from each genotype were randomly selected and immersed, up to 3.0 cm above soil level, for 26 days in plastic vessels filled with tap water. The water was changed every four days using a siphon system to prevent algae overgrowth. Control pots remained at field capacity. The averages of the maximum and minimum temperatures in the greenhouse were 25.3±2.78 and 12.5±3.16°C, respectively. The photoperiod of 16 hours was achieved using natural and artificial light (fluorescent lamps).

The dissolved oxygen content of the water in the plastic vessels was periodically monitored throughout the experiment by subtly agitating the water with the DO-5510 oxygen-sensor probe (Lutron Electronic Enterprise Co., Ltd., Taipei, Taiwan) and taking measurements at a 10 cm depth. The dissolved oxygen was 7.3 and 5.7 mg L⁻¹ when water was changed and four days later, respectively.

For fertilization, 200 mL urea solution (5.0 g L⁻¹) and 200 mL diammonium phosphate solution (0.52 g L⁻¹) were applied twice per pot, once on the fifth and once on the tenth day after the beginning of the treatment.

The plant variables measured at the end of the experiment were: leaf greenness index, electrolyte leakage, histological root traits, and shoot and root dry mass. The leaf greenness index was used to estimate chlorophyll content through three readings in the third leaf blade of each plant with the CL01 chlorophyll meter (Hansatech Instruments Ltd, Norfolk, United Kingdom).

Electrolyte leakage was used to estimate cell membrane damage (Hossain & Uddin, 2011). For this, test tubes were filled with 10 mL distilled water, whose electrical conductivity (EC_{dw}) was measured using the 850038 equipment (Sper Scientific Direct: Environmental Measurement Instruments, Scottsdale, AZ, USA). On harvest day, three subsamples of 1.0 cm² area were randomly selected from the last fully-expanded leaf and washed in distilled water to remove any solutes or lysed cells. After washing, the subsamples were dried superficially and put in test tubes, kept immersed in distilled water. The capped test tubes were placed in the BM021 shaker (Biomint,

Buenos Aires, Argentina) at room temperature and under a low light intensity of 80 μmol m⁻² s⁻¹ to reach compensation point; light intensity was measured using the MQ-301 radiometer (Apogee Instruments, Inc., Logan, UT, USA). After 6 hours, the electrical conductivity of the solution in the test tubes ($EC_{initial}$) was read. Then, the test tubes were autoclaved in the VZ300 equipment (Villar y Zaurdo S.R.L., Buenos Aires, Argentina) at 1.0 atm, for 15 min, to kill leaf tissues. After being autoclaved, the tubes were left at room temperature (25°C) to cool down and, then, another electrical conductivity reading (EC_{final}) was taken. The estimative of electrolyte leakage (EL) was calculated as: $EL (\%) = [(EC_{initial} - EC_{dw}) / (EC_{final} - EC_{dw})] \times 100$.

For the histological analysis at the end of the experiment, five subsamples from each plant were taken from the mid-portion of adventitious roots with a 2.0 mm diameter. The subsamples were fixed in FAA (50% ethanol 96°, 10% formaldehyde, and 5% glacial acetic acid), after which they were immersed in a sequence of ascending concentrations of ethanol for tissue dehydration. The roots were clarified using xylene and embedded in paraffin. Sections of 13 μm of the paraffin-embedded subsamples were cut using the KD-1508A vertical rotary microtome (Zhejiang Jinhua Kedi Instrumental Equipment CO., LTD., Zhejiang, China). The obtained sections were stained with safranin and fast green (D'Ambrogio de Argüeso, 1986). The subsamples were, then, examined in the bright NLCD-307B LCD digital binocular optical microscope (Serico, Shanghai, China) with a built-in digital camera. The percentage of cortical aerenchyma area and the stele:root ratio were determined using the ImageJ software (Schneider et al., 2012). The cell wall thickness of the endodermis and exodermis was measured using a 10×/18 mm micrometer eyepiece.

After the evaluation of chlorophyll content and cell membrane damage, shoot and root dry mass was determined. For this, plants were harvested individually, and the shoots were separated from the roots and dried, at 70°C, in the SL60S oven (San Jor, Buenos Aires, Argentina) until reaching a constant mass.

The degree of root hypoxia (low content of dissolved oxygen) was indirectly measured using the redox potential of the soil at the end of the experiment, which was obtained with the TPX-I digital thermometer-pH-meter, combining a platinum electrode and a platinum ring (Altronix, Buenos Aires, Argentina). This was

possible because the soil redox potential is related to the dissolved oxygen in waterlogged soils (Fiedler et al., 2007).

Since the experimental units were kept independent, the residuals were also considered independent. Data was checked for normality and homogeneity of variances using Shapiro-Wilk's and Levene's tests, respectively. A statistical analysis was carried out using generalized linear models for a completely randomized design, with a factorial arrangement of two factors with interaction. When heteroscedasticity was detected, mixed model algorithms were used to incorporate it, by selecting the most appropriate matrix of variances and covariances for residuals using the Akaike information criterion. The F-test was used in the mixed models.

Means were compared by the test of Di Rienzo-Guzmán-Casanoves (Di Rienzo et al., 2002), chosen because of its simplicity and because there are no overlaps as in Tukey's test; although this method is more suitable to a large number of groups, six treatments (combination of the two studied factors) are considered sufficient. When an interaction was detected, the means of the six treatments were compared; however, when the interaction was not significant, the means of the significant factors were compared.

All statistical analyses were conducted using the Infostat software (Di Rienzo et al., 2020), and $\alpha=0.05$ was used in all hypotheses tests.

Results and Discussion

The assumption of normality was met in all studied variables. The heterogeneity of variances between species was observed for shoot dry mass ($p=0.0017$), root dry mass ($p=0.0088$), endodermis thickness ($p=0.0005$), and thickness of the internal tangential cell wall of the endodermis ($p<0.001$).

Shoot dry mass ($p=0.0249$) was significantly affected by the interaction between factors, i.e., soil water condition and genotype (Figure 1 A). Specifically, the values of shoot dry mass were not affected by water condition in *Chloris gayana* and *P. virgatum*, but were lower in waterlogged plants of *P. coloratum*, compared with the control. Among the studied genotypes, *C. gayana* stood out for its highest shoot dry mass per plant, both under field capacity and waterlogged conditions, producing 1.78 and 2.32 times

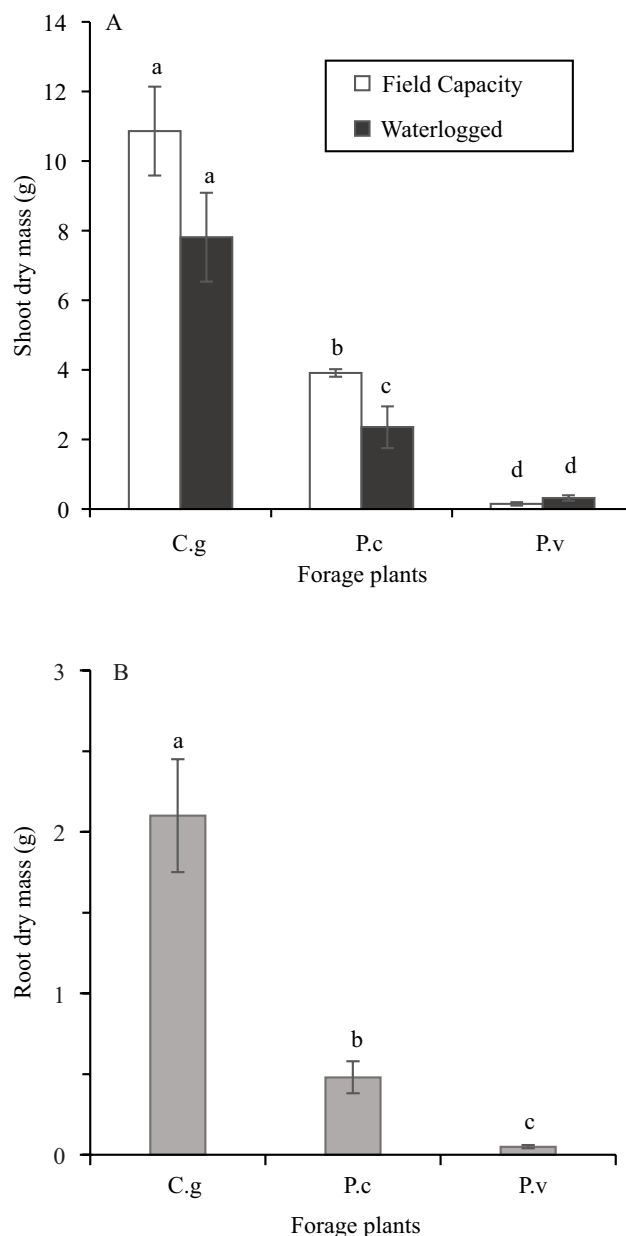


Figure 1. Means of the dry mass of: A, shoots of 'Finecut' *Chloris gayana* (C.g), 'Klein Verde' *Panicum coloratum* (P.c), and 'Shawnee' *Panicum virgatum* (P.v) plants grown 42 days in pots with alkaline-sodic soil at field capacity and 26 days under two water conditions (field capacity and waterlogging, $n = 3$); and B, roots of C.g, P.c, and P.v plants grown 68 days in pots with alkaline-sodic soil, regardless of the water condition ($n = 6$). The alkaline-sodic soil presented: pH 8.3, 0.98 dS m^{-1} electrical conductivity, and 26.2% exchange sodium percentage. Different letters represent significant differences between means by the test of Di Rienzo-Guzmán-Casanoves (Di Rienzo et al., 2002), at 0.5% probability.

more than *P. coloratum* and 71.4 and 24.19 times more than *P. virgatum*, respectively.

No significant interaction was observed between factors for root dry mass ($p=0.4098$). Although there were no differences between water conditions ($p=0.3608$), genotypes ($p<0.0001$) differed between themselves. The highest root dry mass of 2.1 ± 0.35 g was obtained for *C. gayana*, compared with those of 0.48 ± 0.1 and 0.05 ± 0.01 g, respectively, for *P. coloratum* and *P. virgatum*, regardless of the water condition (Figure 1 B). However, after four growth cycles in the field in the alkaline-sodic soil ($\text{pH} = 9.8$, $\text{ECs} = 0.69$ dS m^{-1} , and $\text{ESP} = 26.2\%$), the mean dry mass of *P. coloratum* did not differ from that of *C. gayana* (Pesqueira et al., 2017). In a previous study conducted in a greenhouse with potted plants, Makar (2019) found that alkaline-sodic soil conditions ($\text{pH} = 8.3$ and $\text{ESP} = 26.2\%$) greatly restricted the growth of six-week-old plants of 'Shawnee' *P. virgatum*, compared with those grown in neutral soil ($\text{pH} = 6.3$ and $\text{ESP} = 5.02\%$), which was attributed to a severe reduction of 70.6% in shoot dry mass. Clearly, alkalinity represents a great restriction for the growth of 'Shawnee' *P. virgatum* plants.

The leaf greenness index is a good parameter to estimate photosynthetic activity and chlorophyll content (Table 1), showing a significant interaction between the studied factors ($p=0.033$). The values obtained for this index decreased after 26 days of waterlogging in *C. gayana* and *P. coloratum* plants, but did not differ in *P. virgatum* leaves. Xiong et al. (2015) found a close relationship between leaf greenness values and chlorophyll content per leaf area ($r=0.84$) and nitrogen content ($r=0.80$) in monocots, such as rice (*Oryza sativa* L.) and maize (*Zea mays* L.).

Table 1. Mean and standard error of the leaf greenness index of *Chloris gayana*, *Panicum virgatum*, and *Panicum coloratum* plants under field capacity and waterlogged conditions.

Genotype	Leaf greenness index ⁽¹⁾	
	Field capacity	Waterlogging
'Finecut' <i>C. gayana</i>	9.16±0.80c	6.37±0.06b
'Shawnee' <i>P. virgatum</i>	3.29±0.38a	3.76±0.31a
'Klein Verde' <i>P. coloratum</i>	10.93±0.87d	8.44±0.69c

⁽¹⁾Different letters indicate significant differences by the test of Di Rienzo-Guzmán-Casanoves (Di Rienzo et al., 2002), at 0.5% probability.

As greenness index values are correlated with nitrogen content within a genotype, the decreased foliar nitrogen content observed in waterlogged plants could be caused by the inhibition of nitrogen absorption (Wu et al., 2014) or by an accelerated senescence (Ploschuk et al., 2018) in comparison with plants grown in field-capacity soils.

Waterlogging also induced cell-membrane damage in *C. gayana* and *P. coloratum* plants as electrolyte-leakage percentage increased. Although water conditions and genotypes affected the percentage of electrolyte leakage, no significant interactions were found between them. Under waterlogging, electrolyte leakage was $10.31\pm 1.87\%$, double that of $5.54\pm 1.09\%$ of the control ($p=0.0119$). The values obtained for the electrolyte leakage of *C. gayana* were $11.55\pm 1.81\%$, higher than that of $5.30\pm 0.77\%$ of *P. coloratum* ($p=0.001$), regardless of the water condition. Despite this, the biomass production of the flooded plants of *C. gayana* did not significantly decrease compared with that of the control (Figure 1).

The mean values of the redox potential of the soil samples were 268.67 ± 11.95 and 231.39 ± 12.09 mV at field capacity and flooding, respectively. The roots of the waterlogged plants show a decreased redox potential since the reductive state of the soil leads to an increase in oxygen demand and in phytotoxin production, which can cause severe stress to plant roots (Pezeshki & DeLaune, 2012).

Considering the anatomical/morphological changes in plants as a response to reduced soil conditions, the roots of the three genotypes showed aerenchyma when subjected to waterlogging. A significant interaction between factors was found for cortical aerenchyma percentage ($p=0.0182$) and the stele:root ratio ($p=0.0016$). At field capacity, the roots of *C. gayana* and *P. coloratum* showed similar proportions of 36.4 and 36.17% constitutive cortical aerenchyma, respectively; however, there was no visible aerenchyma in the roots of *P. virgatum*. Under waterlogging, only *C. gayana* roots showed an increased proportion of aerenchyma, occupying 33.5% of the root cortical area, compared with the plants at field capacity, whose values were 56.83 and 34.63% (Table 2 and Figure 2).

Internal aeration is crucial for root growth under waterlogged conditions. Along with the aerenchyma, the development of newly adventitious roots is another strategy to cope with hypoxia (Ashraf, 2012). The

lowest stele:root ratio was detected in *C. gayana* plants grown under waterlogging due to the increase in the aerenchyma area in the roots, when compared with plants at field capacity (Figure 2 and Table 2). Similarly, Imaz et al. (2012) also detected an increase of 50% in the root aerenchyma area in *C. gayana*. These authors, however, found that waterlogging induced a 40% increase in the aerenchyma area in *P. coloratum*, which was not observed in the present study. This difference could be attributed to the fact that Imaz et al. (2012) used a mix of sand and topsoil from a low land area of the Floody Pampa region (1:1) as a plant substrate, possibly preventing the effect of soil compaction on the roots, a factor that also affects soil aeration at field capacity (Mentges et al., 2016).

Although 'Shawnee' *P. virgatum* showed no aerenchyma in its roots under control conditions, it developed aerenchyma tissue, representing 33% of the root cortical area, in its adventitious roots when subjected to waterlogging (Figure 2 and Table 2), a finding reported for the first time in the present work. This octoploid cultivar belongs to the upland ecotypes and had not yet been studied under these stress conditions. Even if it typically occurred in upland areas that are not subjected to flooding, no growth differences were found between soil water conditions (Figure 1). Under flooding conditions in a greenhouse, Barney et al. (2009) evaluated four different ecotypes, 2 upland (Cave-in-Rock and Blackwell) and two lowland (Alamo and Kanlow), observing that the plants germinated, established, and flowered in both of them. This led the authors to suggest that *P. virgatum* is a facultative wetland species since its different cultivars performed well under flooded and stress-free conditions; only slight reductions in yield occurred in the upland ecotype. Skinner et al. (2009) concluded that some *P. virgatum* cultivars tested under

semi-controlled conditions, in a saturated soil with 17% moisture content, showed a high variability in aerenchyma development (Skinner et al., 2009).

Another histological change to maintain an adequate level of oxygen within plant roots is the development of an oxygen-impermeable barrier in the endodermis and exodermis cell walls, measured by the thickness of these tissues. For these parameters, there were no interactions between water condition and genotype (Figure 3). However, differences were observed in the thickness of the endodermis ($p < 0.0001$) and exodermis ($p < 0.0001$) of *C. gayana*, *P. coloratum*, and *P. virgatum* (Table 3). For plants grown in waterlogged soils, the mean thickness of the endodermis and exodermis increased from 19.8 ± 0.3 to 21.99 ± 0.41 μm and from 48.19 ± 1.78 to 60.86 ± 2.44 μm , respectively, i.e., 11 and 26% in relation to that of the control.

The increase in the thickness of the exodermis of *C. gayana* and *P. virgatum* can be explained by the significant difference in the number of exodermal cell rows and by the increase in the thickness of the internal tangential cell walls, both significantly affected by the interaction between genotype and soil water condition ($p < 0.0001$) (Table 4). Waterlogging increased the number of cell rows of the exodermis and the thickness of the internal tangential cell walls of the endodermis of *C. gayana* in 22 and 32%, respectively, and of *P. virgatum*, in 55 and 66%, respectively, but not of *P. coloratum*.

Although there are no known reports on the development of radial oxygen-loss barriers in the perennial species under study, the roots of many wetland plants contain a complete or partial barrier to radial oxygen loss in their epidermis, exodermis, or subepidermal layers (Ejiri et al., 2021). Partial barriers can be constitutive, i.e., formed even in the absence of the stress signal, which is detected in most annual

Table 2. Mean and standard error of the stele:root ratio and of the proportion of cortical aerenchyma in roots of *Chloris gayana*, *Panicum coloratum*, and *Panicum virgatum*, plants under field capacity and waterlogged conditions⁽¹⁾.

Genotype	Stele:root ratio		Cortical aerenchyma (%)	
	Field capacity	Waterlogging	Field capacity	Waterlogging
'Finecut' <i>C. gayana</i>	0.41±0.010A	0.30±0.03B	36.40±6.14a	56.83±2.17b
'Klein Verde' <i>P. coloratum</i>	0.39±0.004A	0.42±0.03A	36.17±3.19a	34.63±5.59a
'Shawnee' <i>P. virgatum</i>	0.39±0.010A	0.40±0.02A	0.0c	33.45±5.68a

⁽¹⁾Different capital and lowercase letters indicate significant differences within the stele:root ratio and cortical aerenchyma percentage, respectively, by the test of Di Rienzo-Guzmán-Casanoves (Di Rienzo et al., 2002), at 0.5% probability.

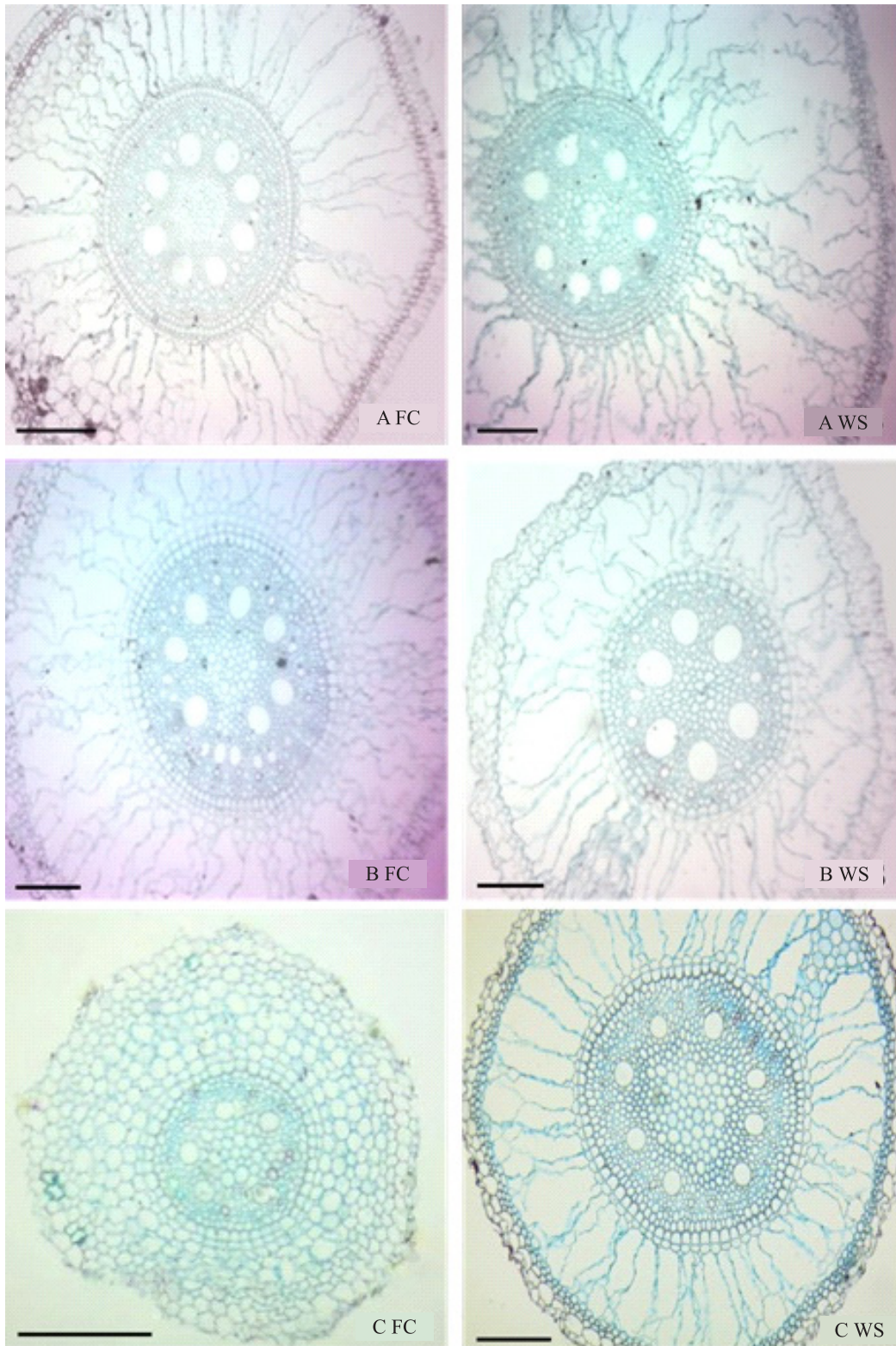


Figure 2. Double successive safranin-fast green combined staining of root cross-sections of 'Finecut' *Chloris gayana* (A), 'Klein Verde' *Panicum coloratum* (B), and 'Shawnee' *Panicum virgatum* (C) plants under field capacity (FC) and waterlogging (WS) conditions (bar = 1,000 μm).

Table 3. Mean and standard error of the endodermis and exodermis thickness of roots of *Chloris gayana*, *Panicum coloratum*, and *Panicum virgatum* plants⁽¹⁾.

Genotype	Endodermis (µm)	Exodermis (µm)
'Finecut' <i>C. gayana</i>	184.9±2.9c	378.0±13.1c
'Klein Verde' <i>P. coloratum</i>	231.0±3.7a	668.2±15.7a
'Shawnee' <i>P. virgatum</i>	210.9±4.7b	589.6±17.3b

⁽¹⁾Different letters indicate significant differences by the test of Di Rienzo-Guzmán-Casanoves (Di Rienzo et al., 2002), at 0.5% probability.

Table 4. Mean and standard error of number of cell rows in the exodermis and of thickness of internal tangential cell walls of the endodermis of roots of *Chloris gayana*, *Panicum coloratum*, and *Panicum virgatum* plants grown under field capacity (FC) and waterlogged (W) conditions⁽¹⁾.

Genotype	Soil water condition	Number of cell rows in the exodermis	Endodermis internal tangential cell wall (µm)
'Finecut' <i>C. gayana</i>	FC	2.3±0.04b	1.9±0.10a
	W	2.8±0.05c	2.5±0.09b
'Klein Verde' <i>P. coloratum</i>	FC	3.0±0.02d	2.8±0.11b
	W	3.0±0.03d	2.8±0.11b
'Shawnee' <i>P. virgatum</i>	FC	2.0±0.02a	2.4±0.09b
	W	3.1±0.03d	4.0±0.22c

⁽¹⁾Different letters indicate significant differences within each column by the test of Di Rienzo-Guzmán-Casanoves (Di Rienzo et al., 2002), at 0.5% probability.

wild *Echinochloa* spp., and are closely associated with exodermal suberization (Ejiri & Shiono, 2019).

Soukup et al. (2007) observed longitudinal profiles of radial oxygen loss measurements along the roots of *Glyceria maxima* (Hartm.) Holmb. in a stagnant solution, with minimum values at 30 mm from the root tip. Manzur et al. (2015) concluded that suberin deposition begins to increase from 2 cm away/on from the root apex, with greater losses towards the base of the root of *Paspalidium geminatum* (Forssk.) Stapf in Prain. This distance from the root apex and the root section chosen for the present study are coincident.

Conclusions

1. 'Shawnee' *Panicum virgatum* roots undergo anatomical changes to cope with waterlogging even

when plant growth is inhibited due to alkaline-sodic conditions.

2. There are radial oxygen loss barriers in the roots of 'Finecut' *Chloris gayana*, 'Klein Verde' *Panicum coloratum*, and 'Shawnee' *P. virgatum*.

3. The growth of 'Klein Verde' *P. coloratum* plants is more sensitive to waterlogging than that of 'Finecut' *C. gayana* and 'Shawnee' *P. virgatum*.

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