Root length is proxy for high-throughput screening of waterlogging tolerance in Urochloa spp. grasses

Citation:

de la Cruz Jiménez, J.; Cardoso, J.A.; Kotula, L.; Veneklaas, E.J.; Pedersen, O.; Colmer, T.D. (2020) Root length is proxy for high-throughput screening of waterlogging tolerance in Urochloa spp. grasses. Functional Plant Biology, Online first paper (8 December 2020). ISSN: 1445-4416

Publisher's DOI:

https://doi.org/10.1071/FP20200

Access through CIAT Research Online:

https://hdl.handle.net/10568/111275

Root length is proxy for high-throughput screening of waterlogging tolerance in *Urochloa* grasses

Juan de la Cruz Jiménez[1*], Juan A. Cardoso[2], Lukasz Kotula[1], Erik J. Veneklaas[1,3,4], Ole Pedersen[1,5] and Timothy D. Colmer[1,4]

[1] UWA School of Agriculture and Environment, Faculty of Science, The University of Western Australia, 35 Stirling Highway, Crawley, WA 6009, Australia.

[2] International Center for Tropical Agriculture (CIAT), Km 17 Recta Cali – Palmira, Colombia.

[3] UWA School of Biological Sciences, Faculty of Science, The University of Western Australia, 35 Stirling Highway, Crawley, WA 6009, Australia.

[4] The Institute of Agriculture, The University of Western Australia, 35 Stirling Highway, Crawley, WA 6009, Australia.

[5] Department of Biology, University of Copenhagen, Universitetsparken 4, 3rd floor, 2100 Copenhagen, Denmark

*Corresponding author: juan.jimenezserna@research.uwa.edu.au

Running Head: Root traits for waterlogging tolerance in Urochloa

Abstract

C4 perennial *Urochloa* grasses are widely planted in extensive areas in the tropics. These areas are continuously facing waterlogging events, which limits plant growth and production. However, no commercial cultivar combining excellent waterlogging tolerance with superior biomass production and nutritional quality is available. The objective of this study was to identify root traits that can be used for selecting waterlogging tolerant *Urochloa* species. Root respiration, root morphological, architectural and anatomical traits were evaluated in eight contrasting *Urochloa* genotypes grown under aerated or deoxygenated stagnant solutions. Moreover, modelling of internal aeration was used to relate differences in root traits and root growth in waterlogged soils. Increased aerenchyma formation in roots, reduced stele area and development of a fully suberized exodermis are characteristics improving internal aeration of roots and therefore determining waterlogging tolerance in these C4 forage grasses. Waterlogging-tolerant genotypes had steeper root angles and greater root lengths than the waterlogging-sensitive genotypes. In stagnant conditions, waterlogging-tolerant genotypes which had a greater proportion of aerenchyma and reduced stele area in root cross-sections also had deeper roots, steeper root angle and larger root biomass, which in turn, allowed for greater shoot biomass. Total root length had the strongest positive influence on shoot dry mass and can therefore be used as proxy for selecting waterlogging tolerant *Urochloa* genotypes.

Keywords: Root anatomy, tropical grasses, abiotic stress, root angle, root respiration, suberin, lignin, radial oxygen loss, root internal aeration, root length modelling.

Introduction

Waterlogged soils affecting forage production are considered as one of the major constraints impeding livestock intensification in the American tropics (Rao et al. 2011). During waterlogging, the porous space in soil is filled with water and O_2 diffusion is highly restricted (Armstrong 1979). Due to a lack of O_2, root respiration is impeded and nutrient uptake is restricted (Colmer and Greenway 2011), resulting in reduced growth and sometimes mortality. The frequency of heavy rainfall events is likely to increase in several regions of the tropics (Hirabayashi et al. 2013),

therefore, the development of tropical forages adapted to soil waterlogging conditions should be a priority for the productivity of these areas.

C4 grasses from the genus *Urochloa* have been extensively planted in the tropics to sustain livestock production (Miles et al. 2004). Among the *Urochloa* grasses, *U. humidicola* genotypes have been identified as superior to other varieties when challenged by soil waterlogging (Dias-Filho and Carvalho 2000; Cardoso et al. 2013; 2014; Jiménez et al. 2015*a*; Supplementary Table 1). The tolerance of *U. humidicola* to waterlogging (based on the evaluation of several intraspecific genotypes) is associated with traits improving internal aeration of roots, including greater aerenchyma development, reduced stele proportion to the cortex, and a barrier to impede radial O_2 loss (ROL) to the rhizosphere via deposition of suberin and lignin in the outer part of the roots (Cardoso et al. 2013; 2014; Jiménez et al. 2015*b*; 2019). The enhanced O_2 transport from shoots to roots in plants under waterlogging conditions allows a greater root growth and higher uptake and translocation of nutrients (Armstrong 1979; Armstrong et al. 1983; Gibbs et al. 1988; Armstrong and Drew 2002; Colmer and Greenway 2011).

The root growth into a waterlogged substrate is constrained by the internal capacity to transport O_2 from shoots to the root tip (Armstrong 1979). The effectiveness of the internal longitudinal O_2 transport into and along the roots depends on the longitudinal O_2 diffusion path-length, the amount and distribution of pore space resistance, the formation of barriers to impede ROL to the rhizosphere and the O_2 demand by tissues along the path (Armstrong et al. 1983). Waterlogging tolerant genotypes (with greater capacity for internal O_2 movement) usually have longer roots and faster root growth under anoxic soil conditions than sensitive genotypes. Mathematical models computing root anatomical characteristics have provided the theoretical basis of internal aeration processes and its relation to the maximum root length attained in waterlogged soils (Armstrong 1979). These models have recently been modified to compute variations in the proportion and respiratory activities of the stele and cortex, which substantially impacts the internal capacity to transport O_2 and therefore the accuracy of models (Pedersen et al. 2020). The direction of the root elongation into the soil is determined by the basal root angle and thus, steeper root angles allow greater rooting depth. This has been shown in non-waterlogged conditions (e.g., wheat, Oyanagi 1994; Wasson et al. 2012; Anzooman et al. 2019; rice, Uga et al. 2013; Kato et al. 2016;

Ramalingam et al. 2017; maize, Liakat et al. 2015), but variation in root angle and its influence on root length in plants grown under low-O_2 conditions remain largely unexplored.

The evaluation of root anatomical traits improving internal aeration is a time-consuming and technically specialized activity, thus, limiting their use for phenotyping large breeding populations of plants. Therefore, the objective of this study was to identify root traits that can be used as proxies for waterlogging tolerance in *Urochloa* grasses, one of the most important tropical forage grasses. Root and shoot dry mass, root morphological and architectural traits (root length, root cross-sectional area, root angle) and root anatomical traits (stele area and aerenchyma percentage, suberin and lignin deposition) were evaluated in eight contrasting *Urochloa* genotypes grown for two weeks under aerated or stagnant solutions. In addition, the maximum root length achieved in waterlogged conditions was modelled using the root respiration rates measured in two contrasting *Urochloa* genotypes. We hypothesized that longer roots is a trait that can be used as proxy for waterlogging tolerance. This knowledge will extend our understanding of plant responses to low-O_2 conditions and will serve as a basis for improving phenotyping in tropical grass breeding programs.

Materials and Methods

Plant material and growing conditions

Seeds for different accessions (Supplementary Table 1) of the waterlogging-sensitive species *U. brizantha* (cv. Marandú "CIAT6294", cv. Toledo "CIAT26110", CIAT26124), waterlogging-sensitive hybrid Mulato II (*U. ruzisiensis* x *U. decumbens* x *U. brizantha*, "CIAT36087") and waterlogging-tolerant species *U. humidicola* (cv. Tully "CIAT679", cv. Llanero "CIAT6133", CIAT16888, CIAT26570), were sown in sterilized river sand. Seeds were irrigated daily with DI water. Seeds of *U. humidicola* genotypes (slower germination) were sown three weeks before being transplanted into 3.7 L pots filled with nutrient solution, and seeds of the other four genotypes 2 weeks before transplanting. Four plants were transplanted into individual pots and were held by foam in holes in the lid of each pot. Plantlets were grown in a nutrient solution known to maximize the phenotypic differences in growth of *Urochloa* plants under stagnant compared to aerated solutions (Jiménez et al. 2019). The nutrient solution contained (in µM): 5000 NO_3^-, 500

114 NH_4^+, 3000 K^+, 3000 Ca^{2+}, 1500 Mg^{2+}, 1600 Na^+, 50 $H_2PO_4^-$, 2860 SO_4^{2-}, 50 Fe-EDTA, 10 Mn^{2+},
115 10 Zn^{2+}, 2 Cu^{2+}, 60 H_3BO_3, 50 SiO_3^{2-}, 0.010 MoO_4^{2-} and 3324 Cl^-, 0.010 μM Ni^{2+}; HCl was used
116 to adjust the pH to 4.2 to mimic the acidic conditions of tropical soils (Wenzl et al. 2003).

117 Plantlets were grown with the roots in aerated nutrient solution for one week to allow recovery
118 after transplanting. Pots were covered with Al foil to reflect sunlight and minimize changes in the
119 nutrient solution temperature. After one week of regrowth, plants were exposed to either aerated
120 or stagnant root-zone treatments during 2 weeks. Aerated solutions were continuously bubbled
121 with air. Stagnant solutions were made by dissolving agar at 0.1% (w/v) in the nutrient solution
122 and pre-flushing it with high purity N_2 gas to purge out O_2. This stagnant solution was continuously
123 flushed with N_2 when syphoning stagnant solutions from preparation tanks into pots so as to avoid
124 any O_2 mixing into the solution. The higher viscosity of the 0.1% agar solution prevents
125 convection, so that it mimics the changes in gas composition (low O_2, but increased ethylene in
126 roots) which occur in waterlogged soils (Wiengweera et al. 1997) since any O_2 entry is greatly
127 impeded. Oxygen concentrations in deoxygenated stagnant solutions remain very low (c. 0 – 0.2
128 mg L; Kotula et al., 2009; 2015). Nutrient solutions were renewed every week. The experiment
129 was run in a completely randomized design with two treatments (aerated and stagnant), eight
130 genotypes and four replications. Plants were grown in a greenhouse at 30/19 °C day/night air
131 temperatures; 12 hours daylight; located at CIAT, Cali, Colombia and during the months of March
132 to and April, 2018.

133 *Harvest*

134 The harvest was conducted after 2 weeks of treatments. Two out of four plants per pot were
135 separated into shoots (stems and leaves) and roots (the remaining two plants were used for root
136 architecture and root anatomy determination, see below). The number of main roots ('nodal' or
137 'adventitious' roots) per plant were counted for these two plants. Dry weights were measured after
138 oven drying tissues at 60 °C for three days. Data for two plants from each pot were pooled and the
139 mean, expressed on a per plant basis, was used to provide one replicate. There were 4 replicate
140 pots for each treatment x genotype combination.

141 *Root architecture*

142 The *Urochloa* root system consists of one seminal root and several adventitious (nodal) roots, each
143 having several lateral roots. The seminal root is small and typically dies back a few days after the

adventitious root system is established (4 to 7 adventitious roots) and therefore it was not used for analysis. Root extension was determined by measuring the length of an individual adventitious root (initial length of 6-10 cm, one plant per pot previously marked with a cotton thread) using a ruler at 0 and 14 days after treatments commencement, and is expressed in cm per day. One of the other two remaining plants per pot was removed, laid down on a protractor and photographed from a nadir view at 20 cm height using a 13 megapixels digital camera (Nikon, Coolpix, P6000, Japan). The root angle was determined by measuring the angle of the basal 5 cm formed relative to the vertical axis (Supplementary Fig 1), thus, small angles indicate roots growing downwards. After photographing, all roots from each plant were separated and scanned at a resolution of 300 dpi in a flatbed scanner (EPSON Expression 1680, Japan). The total length of main and laterals roots were measured using the scanned images and the WinRhizo software (Regent Instruments, Canada). Main axes and lateral roots were differentiated based on their diameter ranges that were determined both microscopically (see details below) and using the WinRhizo software in random samples.

Root anatomy

One root (~100 mm in length) was excised from the remaining plant in each pot (same plant used for root extension rate). Roots were fixed in 1.6% (v/v) paraformaldehyde in phosphate-buffered saline, pH 7.4 and stored at 4 °C until required. Segments of 10 mm length were excised at distances of c. 50 mm behind the root tip and were embedded in 5% (w/v) warm agar. Cross sections were obtained by cutting solid agar blocks using a vibrating blade microtome (VT 1000S Leica, Wetzlar, Germany). Adhered agar was removed by clearing root sections with 85% lactic acid saturated with chloride hydrate for 1 h at 70 °C (Lux et al. 2005) and washing several times with DI water. Suberin was visualized by green-yellow colour after staining cross-sections with 0.01% (w/v) Fluorol Yellow 088 in polyethylene glycol glycerol for 1 h (Brundrett et al. 1991) and viewed under UV light (Axioscope2 plus, Zeiss, Oberkochen, Germany; Excitation G365, Emission LP397). Root sections containing suberin were photographed with a Zeiss AxioCam Digital Camera. Lignin was visualized by brown colour after treating root cross-sections successively with 1% (w/v) $KMnO_4$, 12% HCl and a concentrated solution of ammonia for the Mäule reaction (Kutscha and Gray 1972). These cross-sections were viewed and photographed under white light microscope (AxioCam ERc5s, Zeiss, Oberkochen, Germany; software ZEN 2012). The root cross-sectional area, aerenchyma percentage (% gas-filled large spaces in the root

cortex) and the stele area at c. 50 mm behind the root apex were determined using white light images and the ImageJ software (National Institutes of Health, Bethesda, USA). The ratio (area) of each root tissue was calculated using the cross-sectional areas.

O_2 consumption of root segments

The purpose of these measurements was to obtain data on root respiration for use in modelling of possible maximum root lengths based on internal O_2 movement to the apex (next section). Seeds of waterlogging-sensitive *Urochloa* hybrid cv. "Mulato II" (CIAT 36087) and waterlogging-tolerant *Urochloa humidicola* cv. "Tully" (CIAT 679) were sown in sterilized sand and then transferred to deoxygenated stagnant solutions as explained above but in a constant temperature room at 30 °C, with PAR at shoot height of 150 µmol m^{-2} s^{-1}, 12 h light/dark at the University of Copenhagen. After two weeks of growth in stagnant conditions, rates of O_2 consumption by excised root segments were measured following the procedure described in Pedersen et al. (2013) using a MicroResp system (Unisense A/S, Aarhus, Denmark).

Root segments of 10 mm length (45-55 mm behind the root tip) were excised and inserted in 4-ml glass vials containing nutrient solution (same as used to grow plants, see above, but lacking agar) at O_2 in equilibrium with air. Each vial contained a glass-coated magnetic stir bar and the stirring rate was set to 600 rpm using the stirrer controller unit (MR2-St-Co, Unisense A/S). The vials were placed in a rack and submerged into a constant temperature bath (30 °C) and left to stabilize for c. 15 min. Oxygen consumption by the root segment was measured in each vial using an O_2 optode (OP-MR, Unisense A/S); O_2 in the medium declined from air equilibrium (20.6 kPa) to no less than 16 kPa as O_2 was consumed by the root segments. Vials without tissue served as blanks. The volume of each vial and the fresh mass of the root segment were determined immediately after finishing the O_2 measurements. Experiments were run for 1.5 – 2.5 h, depending on how quickly O_2 was depleted from the vials.

Oxygen consumption (root respiration; '*Resp*', nmol O_2 g^{-1} FM s^{-1}) rates were calculated using Rate (Sensortrace Suite version 2.3.100, Unisense A/S) as follows:

$$Resp = \frac{(C_1 - C_2) \times Vol}{(t_2 - t_1) \times FM} \qquad \text{Eqn (1)}$$

Where C_2-C_1 (µmol O_2 L^{-1}) is the difference in O_2 concentration in the solution within the vial at two time points, t_1 and t_2 (s) is the time between time points, *Vol* (L) is the volume of the vial and

FM is the fresh mass (g) of the root tissue. Respiration of root segments for both genotypes was measured several times (n = 3 to 6) on different roots. In addition, for root segments of waterlogging tolerant *U. humidicola* cv. Tully that constitutively form a barrier to radial O_2 loss at 50 mm behind the root (i.e., also restricting O_2 consumption from the external medium, cf. Jiménez et al. 2019), the root segments were sliced opened (to allow O_2 consumption by root tissue) and the O_2 consumption rate was measured as explained above. Small differences in initial respiration rates possibly due to wounding (cf. Gronewald and Hanson 1982) were not included in the calculations; as indicated by linear regressions of the slope among sequential O_2 consumption measurements had stabilized during the measurements which lasted 1.5-2.5 h and a period of 30-45 mins within this was used to calculate the respiration rate.

Modelled maximum root lengths

The maximum length of adventitious roots when growth is supported by internal O_2 diffusion from the shoot into and along the roots as the only O_2 source to the growing apex, was calculated using the model proposed by Armstrong (1979). Based on the assumption that there is no lateral diffusion of O_2 to the waterlogged rhizosphere (i.e. no ROL) and that respiration is homogenous across the root cross-section, the maximum aerated path length equals the maximum length of adventitious roots in waterlogged soils and can be calculated from the following equation (Armstrong 1979):

$$L = \sqrt{\frac{2\,Co\,D\,\varepsilon\,\tau}{Mt}} \qquad \text{Eqn (2)}$$

where L is the maximum aerated path length, *Co* is the cortex O_2 status at the root-shoot junction, *D* is the diffusion coefficient of O_2 in gas phase, ε is the fractional root porosity (assuming uniformity along the entire length of the root), τ is the tortuosity factor (assumed to be 1.0) and *Mt* is total root tissue respiration (assuming constant respiration along the root).

In addition, the maximum length of adventitious roots was calculated using a modified version of Armstrong's model which includes also the variation in the respiratory activities of the stele and cortex tissues, as well as the proportions of these tissues in roots (Pedersen et al. 2020). This modified model incorporates the fractions of cortex respiration (*Mc*; respiration corrected for volume-based as tissue porosity changes) and stelar respiration (*Ms*) as components for the

respiration of the root as a whole, which enables assessment of the influence of the stele size as a root trait influencing internal aeration (Pedersen et al. 2020).

Maximum root lengths were calculated for all eight genotypes using the root respiration rates measured for waterlogging-sensitive *U.* hybrid cv. Mulato II and waterlogging-tolerant *U. humidicola* cv. Tully as follows: 1) averaging the respiration rates of both sensitive and tolerant genotypes and using the mean respiration for all genotypes and; 2) respiration rates from sensitive and tolerant genotypes were used for the modelling of maximum root length of all sensitive and tolerant genotypes, respectively.

Statistical analyses of data

Statistical differences between data values for treatments and genotypes were evaluated through two-way ANOVA. The multiple comparison Tukey test was run further to separate differences between means for each variable analyzed. Pearson's correlation coefficient between different traits were calculated. Statistical analyses were run in the software R (R core team 2012), using the library Agricolae (Mendiburu 2014).

Results

Growth of Urochloa *genotypes in aerated or stagnant conditions*

In aerated conditions, shoot dry mass was similar between the eight different genotypes evaluated, averaging 1.77 and 2.14 g per plant for sensitive (Marandú, Toledo, CIAT26124, Mulato II) and tolerant (Tully, Llanero, CIAT16888, CIAT26570) genotypes, respectively. Average shoot dry mass under stagnant conditions, expressed as a percentage of controls (aerated treatments), decreased 41% in sensitive and 26% in tolerant genotypes (Supplementary Table 2). Sensitive genotypes all showed similar root dry mass in aerated conditions, and had on average 53% lower root dry mass in stagnant compared to aerated conditions (Supplementary Table 2). In contrast, the root dry mass of the tolerant genotypes was not significantly reduced under stagnant conditions, except for the cv. Tully. Under stagnant conditions, the average root dry mass of the tolerant genotypes was 1.6-fold higher than that of the sensitive genotypes (Supplementary Table 2).

Root architecture of Urochloa *genotypes in aerated or stagnant conditions*

The average number of main roots in plants grown in stagnant solutions (for all genotypes) was 1.2-fold higher than that of aerated controls. However, the average number of main roots was only significantly higher in stagnant in comparison to aerated treatments for the tolerant genotypes, except for cv. Llanero (Table 1). The stagnant treatment resulted in slower root extension rate for all genotypes. The average root extension rate in stagnant treatments as percentage of controls was 12% for sensitive and 35% for tolerant genotypes (Table 1). In aerated conditions, the average root extension rate of the sensitive genotypes was 1.5-fold higher than that of tolerant genotypes. On the contrary, in stagnant conditions, the average root extension rate of the tolerant genotypes was 1.9-fold higher than that of sensitive genotypes (Table 1). The root angle relative to the vertical axis was steeper in the tolerant genotypes (ranging from 17 to 27°) than in the sensitive genotypes (ranging from 31 to 48°). This trait was not influenced by the aeration treatment, except for Toledo where the root angle was shallower in the stagnant treatments (Table 1).

The total length of both main axes of adventitious roots and their lateral roots was significantly lower in stagnant in comparison to aerated treatments for all genotypes, except for Llanero and CIAT 16888 (Table 1). In stagnant conditions, the average total length of main axes of roots as percentage of controls was 31% for sensitive and 66% for tolerant genotypes (Table 1). The average total length of lateral roots in stagnant treatments as percentage of controls was 38% for sensitive and 72% for tolerant genotypes (Table 1).

Root anatomy

The root cross-sectional area at 50 mm behind the root tip significantly increased under stagnant conditions in comparison to aerated treatments in three of the eight genotypes, being on average (for all genotypes) 1.4-fold greater in stagnant than in aerated conditions (Table 2). This increase in the root cross-sectional area is driven by a larger cortex which contains root aerenchyma (Table 2) and had greater elongation of the cortical cells which remained intact under stagnant conditions (see Supplementary Fig 2). The average percentage of root aerenchyma significantly increased from 3.2 to 13.3% and from 9.8 to 23.1% from aerated to stagnant treatments for sensitive and tolerant genotypes, respectively (Table 2). The aerenchyma formation is a constitutive trait for the tolerant genotypes but it is not or barely present in sensitive genotypes in aerated conditions. The percentage of aerenchyma in the root cross section of the tolerant genotypes was 3.7- or 1.8-fold greater than in sensitive genotypes for aerated or stagnant conditions, respectively. Under stagnant

conditions, the stele area at 50 mm behind the root tip was reduced for cv. Marandú and increased for cv. Mulato II. No significant changes in stele area were found for the waterlogging tolerant genotypes (Table 2). The average (for all genotypes) cortex to stele ratio (CSR) in stagnant was 1.5-fold higher than in aerated controls. However, the CSR was only significantly higher in stagnant in comparison to aerated treatments for the tolerant genotypes (Table 2).

The outer part of the root of all genotypes was characterized by a multi-seriate layer composed by one epidermal cell, one or two exodermal cells and one or two cells of sclerenchyma. Tolerant genotypes exhibited one layer of bigger exodermal cells while sensitive genotypes exhibited two but smaller exodermal layers (Fig 1). Deposition of lignin, was evident in the sclerenchyma of all genotypes evaluated under both treatments, except for the hybrid Mulato II (Fig 1). All of the tolerant genotypes exhibited two layers of lignified sclerenchyma, except for the genotype CIAT16888 which only exhibited one. In contrast, sensitive genotypes only exhibited one layer of lignified sclerenchyma, except for Mulato II which did not exhibit sclerenchyma at all.

At a distance of 50 mm behind the root tip, deposition of suberin was exhibited in some cells of the exodermis in the sensitive genotypes, indicating a patchy suberin development (Fig 2). In contrast, suberin lamella was exhibited in all exodermal cells in tolerant genotypes (Fig 2). The suberin deposition was not affected by the aeration versus stagnant treatments.

The correlation between total root length and dry mass of shoots was positive and significant for aerated (0.44, $P \leq 0.1$) and stagnant treatments (0.94, $P \leq 0.001$). In aerated conditions, the root angle measured relative to the vertical axis was neither related to dry mass of roots (0.22, $P=0.23$) nor related to the dry mass of shoots (0.16, $P=0.39$). However, in stagnant conditions, plants with steeper root angle had higher dry mass of roots (-0.53, $P \leq 0.01$) and dry mass of shoots (-0.53, $P \leq 0.01$; Fig. 3). The relationship between the percentage of root aerenchyma and the dry mass of shoots was strong for stagnant (0.66, $P \leq 0.001$) but not for aerated treatments (0.22, $P= 0.24$). Similarly, in stagnant conditions, the relationship between root extension rate and both dry mass of roots (0.73, $P \leq 0.001$) and dry mass of shoots (0.59, $P \leq 0.001$) was stronger than in aerated conditions with a correlation of 0.35 ($P \leq 0.5$) for dry mass of roots and no correlation of 0.02 ($P=0.91$) for dry mass of shoots (Fig 3). In stagnant conditions, total root length had the strongest positive influence on shoot dry mass (Supplementary Table 3).

O_2 consumption of root segments

The average O_2 consumption (respiration rate) of roots from waterlogging-sensitive cv. Mulato II grown for two weeks in deoxygenated stagnant solutions was 2.94 nmol O_2 g FW sec^{-1} (SE= 0.6, n=4). The average O_2 consumption rate of roots of waterlogging-tolerant *U. humidicola* cv. Tully was 0.91 and 2.22 nmol O_2 g FW sec^{-1} for intact (SE= 0.3, n=6) and sliced opened (SE= 0.2, n=4) root segments, respectively. There were not significant differences between the respiration rates of intact segments of the waterlogging-sensitive and sliced opened segments from the waterlogging-tolerant genotypes (P=0.29). In the waterlogging-tolerant cv. Tully, the average O_2 consumption of sliced opened root segments was 2.4-fold higher than that of intact root segments, highlighting the strength of a barrier to ROL to impede radial O_2 diffusion across the outer tissues (inwards as well as outwards) and thus also impeding O_2 consumption from the medium. The rate from the sliced opened root segments (and not the rate from the intact root segment) was used for modelling maximum root lengths in waterlogging-tolerant cv. Tully, as this rate would better represent O_2 consumption by tissues when O_2 is available internally via aerenchyma. For the waterlogging-sensitive cv. Mulato II, the average O_2 consumption rate of intact root segments was used, as this genotype does not form a barrier to ROL (Supplementary Fig 3) and so O_2 could enter the tissues and was assumed to represent the rate likely also with O_2 available internally via aerenchyma.

Modelled maximum root lengths

The predicted maximum length of adventitious roots when using the original model (Armstrong 1979) and assuming similar respiration rates among genotypes (i.e., the average respiration rate assessed for all genotypes) averaged (for four genotypes) 144 mm for waterlogging-sensitive and 208 mm for waterlogging-tolerant genotypes (Table 3). The predicted maximum length of adventitious roots, however, decreased 8 mm for sensitive (average of 136 mm) and increased 14 mm for tolerant genotypes (average of 221 mm) when assuming different respiration rates (i.e., the respiration of sensitive cv. Mulato II assumed equal to all sensitive genotypes and the respiration rate of tolerant cv. Tully assumed equal to all tolerant genotypes). Moreover, these predicted values of maximum root length attained in waterlogged soils, decreased for the sensitive (average 82 mm) and increased (average 360 mm) for the tolerant genotypes when including differences in tissue respiration based on the differences in the proportion of the stele in these roots (smaller stele in tolerant genotypes, Pedersen et al. 2020; Table 3). The correlations between modelled and observed maximum root lengths were all positive ($P<0.05$) and increased as based

on the Pearson correlation coefficient from an average of 0.63 from calculations using the original model (Armstrong 1979) to 0.70 from calculations using the model including variation in respiratory activity of stele and cortex (Pedersen et al. 2020; Supplementary Fig 4).

Discussion

This study documents the responses of eight *Urochloa* genotypes (four considered to be waterlogging-sensitive and four waterlogging-tolerant, see Supplementary Table 1) to low O_2 in the root-zone. Root anatomical and architectural characteristics improving internal aeration represent major changes determining waterlogging tolerance in these C4 forage grasses. Likewise, results indicated that genotypes with longer roots produce greater biomass under stagnant conditions. This trait can therefore be used as a proxy for selecting waterlogging tolerant *Urochloa* genotypes.

All genotypes evaluated in this study increased the percentage of root aerenchyma under stagnant conditions (Table 2). However, the percentage of aerenchyma was higher for the waterlogging-tolerant *U. humidicola* genotypes. Aerenchyma is a constitutive trait found in *U. humidicola* genotypes under aerated conditions that can be further increased under low-O_2 conditions (Cardoso et al. 2013, 2014; Jiménez et al. 2015*b*, 2019; this study). Many wetland species have roots with constitutive aerenchyma (Justin and Armstrong 1987). The presence of constitutive aerenchyma in roots is advantageous to plants when a soil is initially flooded, as O_2 can diffuse from shoots into and along the roots to allow root respiration and growth in anoxic substrates (Colmer and Voesenek 2009; Yamauchi et al. 2019b). Moreover, the amount of aerenchyma increases further during waterlogging, so-called 'inducible' aerenchyma, both in wetland species and in many non-wetland species (Justin and Armstrong 1987; Colmer and Voesenek 2009). In addition to a higher root aerenchyma percentage, tolerant genotypes in stagnant conditions had also higher cortex to stele ratio (CSR) than sensitive genotypes (Table 2). The stele is a low porosity tissue of high diffusive resistance to O_2, with a higher O_2 consumption rate than the cortical tissue (Armstrong and Beckett 1987; Armstrong et al. 1991; Aguilar et al. 2003). Therefore, higher percentage of aerenchyma and higher CSR are traits acting together to improve root aeration of waterlogging-tolerant genotypes (cf. Yamauchi et al. 2019a).

382 Under stagnant conditions, major differences in root growth were apparent between sensitive and
383 tolerant genotypes. In stagnant conditions, the total length of main axes of adventitious roots was
384 1.8-fold greater in tolerant genotypes than in sensitive genotypes (Table 1). This greater total root
385 length (main axes) in tolerant genotypes was influenced by a higher number of longer main roots
386 developed in stagnant conditions, than for the sensitive genotypes (Table 1). In most, if not all
387 species, the length of roots is severely reduced under waterlogging conditions (clover, Gibberd et
388 al. 2001; wheat, Wiengweera and Greenway 2004; Haque et al. 2012; barley, Kotula et al. 2015).
389 The reduction of root growth in stagnant conditions is attributed to O_2 concentrations approaching
390 zero in the root apical zone since this tissue becomes more distant from the O_2 source as roots
391 grow further into an anoxic medium (Armstrong 1979; Kotula et al. 2015); root extension is
392 reduced below a threshold level of O_2 (the critical O_2 partial pressure for root extension, Armstrong
393 and Webb 1985). The apical O_2 concentration in the root declines as roots grow and the distance
394 from the root-shoot junction to the root tip increases (Armstrong 1979), when the only source of
395 O_2 is supplied via internal gas-phase diffusion for roots in an anoxic medium (Armstrong et al.
396 1983). Therefore, an improved internal O_2 movement driven by an increased aerenchyma and
397 higher CSR in roots of tolerant genotypes (Table 2) allows greater root growth than that of sensitive
398 genotypes. This conclusion was supported by mathematical modelling that predicted longer roots
399 in tolerant in comparison with sensitive genotypes (even when using similar respiratory O_2
400 consumption for calculations, Table 3). The correlation between modelled and observed maximum
401 root lengths increased when using the mathematical model that includes the variation in respiratory
402 consumption in cortex and stele tissues (Pedersen et al. 2020) in comparison to the original model
403 that does not (Armstrong 1979; Supplementary Fig 4); highlighting the importance of an improved
404 internal aeration to sustain root growth into anoxic substrates.

405 The total length of lateral roots under stagnant treatments was on average 1.7-fold higher in tolerant
406 than in sensitive genotypes (Table 1). Greater lateral root formation has previously been associated
407 to waterlogging-tolerant but not -sensitive genotypes of pasture legumes (Gibberd et al. 2001). The
408 production of lateral roots can have a significant influence on the O_2 regime of the primary root.
409 Lateral roots are reliant on the O_2 available from the aerenchyma within the main root, thus, an
410 increase in the number of laterals reduces the apical O_2 concentration in main roots, as
411 demonstrated experimentally by an increase of main root O_2 upon excision of the lateral roots
412 (Armstrong et al. 1983; Sorrell et al. 2000). Greater aeration capacity of the main axes of

adventitious roots of tolerant genotypes allows greater lateral root formation than in sensitive genotypes with poorer aeration capacity (cf. Sorrell et al. 2000). This root architecture in waterlogging-tolerant *U. humidicola* genotypes, resembles the basic architecture of wetland rice root systems which consist of aerenchymatous primary roots with barriers to impede ROL, conducting O_2 down to short, fine and gas-permeable laterals. This system provides a compromise between the need for internal aeration and the need for a large nutrient absorbing surface per unit root mass (Kirk 2003).

The outer part of the main axes of adventitious roots of all *Urochloa* genotypes evaluated here, were characterized by a multiseriate band of cells. This characteristic has been suggested to increase mechanical strength of roots with higher porosity under low-O_2 conditions (cf. Striker et al. 2007). Tolerant genotypes had one layer of bigger exodermal cells whereas sensitive genotypes exhibited two smaller exodermal layers. These cell layers would determine the 'strength' (i.e. degree of impedance) of the barrier to ROL in these roots. A stronger O_2 diffusion impedance is determined by the cell wall composition (see next paragraph), as well as the path-length across this tissue and O_2 consumption rates of these cells. Further studies are needed to clarify the relation between cell size and number and the respiratory consumption of O_2 by epidermal/hypodermal cells in roots of plants growing in low-O_2 conditions.

Lignified sclerenchyma and suberized hypodermis/exodermis in roots are both found in roots with barriers to ROL (Armstrong 1979; Kotula et al. 2009; Abiko et al. 2012; Jiménez et al. 2019). However, detailed studies comparing both suberin and lignin deposition in the outer part of the root and O_2 profiles across the tissues of roots have indicated that suberisation rather than lignification is responsible for restricting ROL (De Simone et al. 2003; Shiono et al. 2014; Kotula et al. 2017). Therefore, complete suberin lamellae formation in waterlogging-tolerant but not -sensitive *Urochloa* genotypes (Fig 2) is very likely contributing to restrict ROL and thus improve root longitudinal O_2 transport under low-O_2 conditions in the root zone.

Urochloa humidicola waterlogging-tolerant genotypes had steeper root angles and greater root lengths than the waterlogging-sensitive genotypes (Table 1). Plants with steeper root angles had a greater number of roots, greater dry mass of roots and greater dry mass of shoots in stagnant treatments but not in aerated conditions (Fig 3). Moreover, in stagnant conditions, genotypes with higher root extension rate had more dry mass of roots and more dry mass of shoots; this trend was

not similar in aerated conditions in which there was a weak relation between root extension rate and dry mass of roots (0.35, $P \leq 0.5$) and no relationship between root extension rate and dry mass of shoots (0.02, $P=0.91$). The root extension into a waterlogged soil is largely determined by the internal movement of O_2 from the atmosphere into and along the root axis and to the root apex (Armstrong 1979). Therefore, the improved aeration efficiency (higher aerenchyma, higher CSR and a full suberin lamella in the hypodermis/exodermis) in tolerant genotypes allows greater root growth to explore for resources and therefore greater shoot biomass.

In conclusion, in stagnant conditions, waterlogging-tolerant *U. humidicola* genotypes which had a greater proportion of aerenchyma and higher CSR also had deeper roots, steeper root angle and larger root biomass, which in turn, presumably provided for a greater leaf biomass (Tables 1 and 2, Fig 3). These findings suggest that rooting depth may be used as a proxy for aerenchyma formation and root internal aeration efficiency in *Urochloa* grasses grown under waterlogged soil conditions. Deeper roots (longer roots with steeper angles) are particularly important for transient waterlogging after the water level recedes, as these roots growing downwards should be able to take up more water and resources to support recovery while the upper soil layers dry out. Substantial variation in root anatomy and root morphology traits were found among the 8 genotypes evaluated in this study, so characterization of more *Urochloa* genotypes under waterlogged conditions could reveal additional genotypes showing desirable characteristics upon which, together with other agronomic characteristics, a breeding program could be designed.

Conflicts of interest

The authors have no conflicts of interest to declare.

Acknowledgements

We thank Dr. Luis Guillermo Santos from the CIAT's Genetic Resources Unit for providing seeds and Valheria Castiblanco and Luis Miguel Hernandez for allowing access to greenhouse facilities. We are grateful to Alfer Valencia for assistance during the experiment set-up and harvest process. JCJ is grateful to COLCIENCIAS (Colombian government) for a PhD scholarship grant and to The University of Western Australia for a reduced-fees scholarship. We thank ICESI University for the use of the vibrating microtome. The authors acknowledge use of the Microscopy Australia

facilities at the Centre for Microscopy, Characterisation & Analysis, The University of Western Australia, a facility funded by the University, State and Commonwealth Governments. JCJ thanks the R.N. Robertson Travelling Fellowship, the Australian Society of Plant Scientists and the University of Western Australia for a travel award to visit the University of Copenhagen. This work was partially undertaken as part of the CGIAR Research Program on Livestock. We thank all donors that globally support work through their contributions to the CGIAR system.

List of Figures

Figure 1. Development of lignified sclerenchyma of genotypes Marandú (a, i), CIAT26124 (b, j), Toledo (c, k), Mulato II (d, l), Llanero (e, m), Tully (f, n), CIAT 16888 (g, o) and CIAT 26570 (h, p) grown in aerated (left column: a – h) or deoxygenated stagnant solutions (right column: i – p) for 2 weeks. Cross-sections were made at 50 mm behind the apex and syringyl groups of lignin were stained orange/brown with $KMnO_4$ and HCl, see black arrowheads pointing to examples. Abbreviations: Ep, epidermis; Ex, exodermis; Sc, Sclerenchyma Co, cortical cells. Scale Bar = 200 µm.

Figure 2. Development of suberized exodermis of genotypes Marandú (a, i), CIAT26124 (b, j), Toledo (c, k), Mulato II (d, l), Llanero (e, m), Tully (f, n), CIAT 16888 (g, o) and CIAT 26570 (h, p) grown in aerated (left column: a – h) or deoxygenated stagnant solutions (right column: i – p) for 2 weeks. Cross-sections were made at 50 mm behind the apex and suberin deposition was stained yellow-greenish with Fluorol Yellow 088 and viewed under UV illumination. See white arrows pointing to examples of suberin and arrowheads pointing to 'passage cells' without suberin deposition. Abbreviations: Ep, epidermis; Ex, exodermis; Sc, Sclerenchyma; Co, cortical cells. Scale Bar = 200 µm.

Figure 3. Binary relationships and Pearson's correlation coefficients between root architectural and anatomical traits and growth of eight waterlogging contrasting *Urochloa* genotypes grown in aerated conditions (upper right, empty black dots) or deoxygenated stagnant solutions (lower left, gray dots). RAer= root aerenchyma (%), RExt= root extension rate (cm per day), TRL= Total root length including both main and lateral roots (cm), RAngle= root angle measured to the Y-axis,

NoRoots= number of main roots, DMR= dry mass of roots (g per plant), DMS= dry mass of shoots (g per plant). n= 32 for each biplot. Pearson's correlation coefficients are indicated with their statistical significance as follows: *·P≤0.5, *P≤0.1, **P≤0.01, ***P≤0.001.*

References

Abiko T, Kotula L, Shiono K, Malik AI, Colmer TD, Nakazono M (2012). Enhanced formation of aerenchyma and induction of a barrier to radial oxygen loss in adventitious roots of *Zea nicaraguensis* contribute to its waterlogging tolerance as compared with maize (*Zea mays* ssp. *mays*). *Plant, Cell and Environment* **35**, 1618-1630.

Aguilar EA, Turner DW, Gibbs DJ, Armstrong W, Sivasithamparam K (2003). Oxygen distribution and movement, respiration and nutrient loading in banana roots (*Musa* spp. L.) subjected to aerated and oxygen-depleted environments. *Plant and Soil* **253**, 91-102.

Anzooman M, Christopher J, Dang YP, Taylor J, Menzies NW, Kopittke PM (2019). Chemical and physical influence of sodic soils on the coleoptile length and root growth angle of wheat genotypes. *Annals of Botany* **124**, 1043-1052.

Armstrong W (1979). Aeration in higher plants. In: Woolhouse HW, ed. *Advances in Botanical Research*. London: Academic Press, 225-332.

Armstrong W, Healy MT, Lythe S (1983). Oxygen diffusion in pea II. Oxygen concentrations in the primary pea root apex as affected by growth, the production of laterals and radial oxygen loss. *New Phytologist* **94**, 549-559.

Armstrong W, Webb T (1985). A critical oxygen pressure for root extension in rice. *Journal of Experimental Botany* **36**, 1573-1582.

Armstrong W, Beckett PM (1987). Internal aeration and the development of stelar anoxia in submerged roots: multi-shelled mathematical model combining axial diffusion of oxygen in the

cortex with radial losses to the stele, the wall layers and the rhizosphere. *New Phytologist* **105**, 221-245.

Armstrong W, Justin SHFW, Beckett PM, Lythe S (1991). Root adaptation to soil waterlogging. *Aquatic Botany* **39**, 57-73.

Armstrong W, Drew MC (2002). Root growth and metabolism under oxygen deficiency. In: Waisel Y, Eshel A, Kafkafi U, eds. *Plant roots: the hidden half,* 3rd ed. New York: Marcel Dekker, 729-761.

Brundrett MC, Kendrick B, Peterson CA (1991). Efficient lipid staining in plant material with sudan red 7B or fluorol yellow 088 in polyethylene glycol-glycerol. *Biotechnic & Histochemistry* **66**, 111-116.

Caetano LPdS, Dias-Filho MB (2008). Responses of six *Brachiaria* spp. accessions to root zone flooding. *Revista Brasileira de Zootecnia* **37**, 795-801.

Cardoso JA, Rincón J, Jiménez JC, Noguera D, Rao IM (2013). Morpho-anatomical adaptations to waterlogging by germplasm accessions in a tropical forage grass. *Annals of Botany Plants*, plt047. doi: 10.1093/aobpla/plt047

Cardoso, JA, Jiménez JC, and Rao IM (2014). Waterlogging induced changes in root architecture of germplasm accessions of the tropical forage grass *Brachiaria humidicola*. *Annals of Botany Plants*, plu017. doi: 10.1093/aobpla/plu017

Colmer TD, Voesenek LACJ (2009). Flooding tolerance: suites of plant traits in variable environments. *Functional Plant Biology* **36**, 665-681.

Colmer TD, Greenway H (2011). Ion transport in seminal and adventitious roots of cereals during O_2 deficiency. *Journal of Experimental Botany* **62**, 39-57.

564 De Simone O, Haase K, Müller E, Junk WJ, Hartmann K, Schreiber L, Schmidt W (2003).
565 Apoplasmic barriers and oxygen transport properties of hypodermal cell walls in roots from four
566 Amazonian tree species. *Plant Physiology* **132**, 206-217.
567
568 Dias-Filho MB, Carvalho CJR (2000). Physiological and morphological responses of *Brachiaria*
569 spp. to flooding. *Pesquisa Agropecuari Brasileira* **10**, 1959-1966.
570
571 Gibbs J, Turner DW, Armstrong W, Darwent MJ, Greenway H (1998). Response to oxygen
572 deficiency in primary maize roots. I. Development of oxygen deficiency in the stele reduces radial
573 solute transport to the xylem. *Australian Journal of Plant Physiology* **25**, 745-758.
574
575 Gibberd MR, Gray JD, Cocks PS, Colmer TD (2001). Waterlogging tolerance among a diverse
576 range of *Trifolium* accessions is related to root porosity, lateral root formation and `aerotropic
577 rooting'. *Annals of Botany* **88**, 579-589.
578
579 Gronewald JW, Hanson JB (1982). Adenine nucleotide content of corn roots as affected by injury and
580 subsequent washing. *Plant Physiology* **69**, 1252-1256
581
582 Haque ME, Oyanagi A, Kawaguchi K (2012). Aerenchyma formation in the seminal roots of
583 Japanese wheat cultivars in relation to growth under waterlogged conditions. *Plant Production*
584 *Science* **15**, 164-173.
585
586 Hirabayashi Y, Mahendran R, Koirala S, Konoshima L, Yamazaki D, Watanabe S et al. (2013).
587 Global flood risk under climate change. *Nature Climate Change* **3**, 816-821.
588
589 Jiménez JdlC, Cardoso JA, Arango-Londoño D, Fischer G, Rao I (2015a). Influence of soil fertility
590 on waterlogging tolerance of two *Brachiaria* grasses. *Agronomía Colombiana* **33**, 20-28.
591
592 Jiménez JdlC, Cardoso JA, Dominguez M, Fischer G, Rao I (2015b). Morpho-anatomical traits of
593 root and non-enzymatic antioxidant system of leaf tissue contribute to waterlogging tolerance in
594 *Brachiaria* grasses. *Grassland Science* **61**, 243-252.

595
596 Jiménez JdlC, Kotula L, Veneklaas EJ, Colmer TD (2019). Root-zone hypoxia reduces growth of
597 the tropical forage grass *Urochloa humidicola* in high-nutrient but not low-nutrient conditions.
598 *Annals of Botany* **124**, 1019-1032.
599
600 Justin SHFW, Armstrong W (1987). The anatomical characteristics of roots and plant response to
601 soil flooding. *New Phytologist* **106**, 465-495.
602
603 Kato Y, Abe J, Kamoshita A, Yamagishi J (2006). Genotypic variation in root growth angle in rice
604 (*Oryza sativa* L.) and its association with deep root development in upland fields with different
605 water regimes. *Plant and Soil* **287**, 117-129.
606
607 Kirk GJD (2003). Rice root properties for internal aeration and efficient nutrient acquisition in
608 submerged soil. *New Phytologist* **159**, 185-194.
609
610 Kotula L, Ranathunge K, Schreiber L, Steudle E (2009). Functional and chemical comparison of
611 apoplastic barriers to radial oxygen loss in roots of rice (*Oriza sativa* L.) grown in aerated or
612 deoxygenated solution. *Journal of Experimental Botany* **60**, 2155-2167.
613
614 Kotula L, Clode PL, Striker GG, et al. (2015). Oxygen deficiency and salinity affect cell-specific
615 ion concentrations in adventitious roots of barley (*Hordeum vulgare*). *New Phytologist* **208**, 1114-
616 1125.
617
618 Kotula L, Schreiber L, Colmer TD, Nakazono M (2017). Anatomical and biochemical
619 characterisation of a barrier to radial O_2 loss in adventitious roots of two contrasting *Hordeum*
620 *marinum* accessions. *Functional Plant Biology* **44**, 845-857.
621
622 Kutscha NP, Gray JR (1972). The suitability of certain stains for studying lignification in balsam
623 fir *Abies balsamea* L. Mill. *Technical Bulletin* 53, 1-70. Life Sciences and Agriculture Experiment
624 Station, University of Maine, Orona.
625

626 Mendiburu F (2014)**.** Agricolae: statistical procedures for agricultural research. R package version
627 1.1-8. In: http://CRAN.R-project.org/package=agricolae; consulted: October, 2017.
628
629 Miles JW, Do Valle CB, Rao IM, Euclides VPB (2004). Brachiariagrasses. *Warm Season (C4)*
630 *Grasses*, Agronomy Monograph no. 45. American Society of Agronomy, Crop Science Society of
631 America, Soil Science Society of America, 677 S. Segoe Rd, Madison, WI 53711, USA.
632
633 Liakat M, Luetchens J, Nascimento J, Shaver TM, Kruger GR, Lorenz AJ (2015). Genetic
634 variation in seminal and nodal root association with grain yield of maize under conditions. *Plant*
635 *and Soil* **397**, 213-225.
636
637 Lux A, Morita S, Abe J, Ito K (2005). An improved method for clearing and staining free-hand
638 sections and whole-mount samples. *Annals of Botany* **96**, 989-996.
639
640 Oyanagi A (1994). Gravitropic response growth angle and vertical distribution of roots of wheat
641 *(Triticum aestivum* L.). *Plant and Soil* **165**, 323-326.
642
643 Pedersen O, Colmer TD, Sand-Jensen K (2013). Underwater photosynthesis of submerged plants
644 – recent advances and methods. *Frontiers in Plant Science* **4**, doi: 10.3389/fpls.2013.00140.
645
646 Pedersen O, Sauter M, Colmer TD, Nakazono M (2020). Regulation of root adaptive anatomical
647 and morphological traits during low soil oxygen. *New Phytologist*, in press. doi:
648 10.1111/nph.16375
649
650 Ramalingam P, Kamoshita A, Deshmukh V, Yaginuma S, Uga Y (2017). Association between
651 root growth angle and root length density of a near-isogenic line of IR64 rice with *DEEPER*
652 *ROOTING 1* under different levels of soil compaction. *Plant Production Science* **20**, 162-175.
653
654 Rao I, Miles J, Wenzl P, Louw-Gaume A, Cardoso JA, Ricaurte J, Polania J, Rincón J, Hoyos V,
655 Frossard E, Wagatsuma T, Horst W (2011). Mechanisms of adaptation of brachiariagrasses to

656 abiotic stress factors in the tropics. Proceedings of the III International Symposium on Forage
657 Breeding November 7th to 11th. Bonito, Brazil.
658
659 Shiono K, Yamauchi T, Yamazaki S, Mohanty B, Malik AI, Nagamura Y, Nishizawa NK,
660 Tsutsumi N, Colmer TD, Nakazono M (2014). Microarray analysis of laser-microdissected tissues
661 indicates the biosynthesis of suberin in the outer part of roots during formation of a barrier to radial
662 oxygen loss in rice (*Oryza sativa*). *Journal of Experimental Botany* **65**, 4795-4806.
663
664 Sorrell BK, Mendelssohn IA, Mckee KL, Woods RA (2000). Ecophysiology of wetland plant
665 roots: a modelling comparison of aeration in relation to species distribution. *Annals of Botany* **8**,
666 675-685.
667
668 Striker GG, Insausti P, Grimoldi AA, Vega AS (2007). Trade-off between root porosity and
669 mechanical strength in species with different types of aerenchyma. *Plant, Cell and Environment*
670 **30**, 580-589.
671
672 Uga Y, Sugimoto K, Ogawa S et al. (2013). Control of root system architecture by *DEEPER*
673 *ROOTING 1* increases rice yield under drought conditions. *Nature Genetics* **45**,
674 doi:10.1038/ng.2725
675
676 Wasson AP, Richards RA, Chatrath R, Misra SC, Prasad SV, Rebetzke GJ, Kirkegaard JA,
677 Christopher J, Watt M (2012). Traits and selection strategies to improve root systems and water
678 uptake in water-limited wheat crops. *Journal of Experimental Botany* **63**, 3485-3498.
679
680 Wenzl P, Mancilla LI, Mayer JE, Albert R, Rao IM (2003). Simulating infertile acid soils with
681 nutrient solutions: the effects on *Brachiaria* species. *Soil Science Society of America Journal* **67**,
682 1457-1469.
683
684 Wiengweera A, Greenway H (2004). Performance of seminal and nodal roots of wheat in stagnant
685 solution: K^+ and P uptake and effects of increasing O_2 partial pressures around the shoot on nodal
686 root elongation. *Journal of Experimental Botany* **55**, 2121-2129.

Wiengweera A, Greenway H, Thomson CJ (1997). The use of agar nutrient solution to simulate lack of convection in waterlogged soils. *Annals of Botany* **80**, 115-123.

Yamauchi T, Abe F, Tsutsumi N, Nakazono M (2019a). Root Cortex Provides a Venue for Gas-Space Formation and Is Essential for Plant Adaptation to Waterlogging. *Frontiers in Plant Science* **10**, 259. doi: 10.3389/fpls.2019.00259

Yamauchi T, Tanaka A, Inahashi H, Nishizawa NK, Tsutsumi N, Inukai Y, Nakazono M (2019b). Fine control of aerenchyma and lateral root development through AUX/IAA- and ARF-dependent auxin signalling. *Proceedings of the National Academy of Science* **116**, 20770-20775.

717 **Table 1**. Number of main (adventitious) roots, root extension rate, root angle, length of the main axes of adventitious roots and length
718 of the lateral roots of eight *Urochloa* genotypes after 2 weeks of growth in aerated or stagnant deoxygenated nutrient solutions.
719 Different letters indicate statistically significant differences (P<0.05, Tukey test). The root angle was determined by measuring the
720 root angle of the basal 5 cm of the first adventitious root formed relative to the vertical axis. Initial lengths of roots used for extension
721 rate measurements were 6 to 10 cm.

722

Genotypes	Tolerance to waterlogging	Number of roots		Root extension rate (cm day^{-1})		Root angle (°)		Main roots length (cm)		Lateral roots length (cm)	
		Aerated	Stagnant	Aerated	Stagnant	Aerated	Stagnant	Aerated	Stagnant	Aerated	Stagnant
Marandú	Sensitive	12 def	9 f	2.4 ab	0.3 g	31 bcde	36 bc	8362 a	2522 ef	1429 ab	470 ef
CIAT26124	Sensitive	11 ef	11 ef	2.4 ab	0.2 g	32 bcd	39 ab	9149 ab	2232 f	1223 a	385 f
Toledo	Sensitive	9 f	11 ef	2.7 a	0.3 g	36 bc	46 a	8501 ab	2658 ef	1322 ab	533 ef
Mulato II	Sensitive	16 bcd	14 cde	2.1 bc	0.4 g	40 ab	38 ab	7587 ab	2917 def	1262 abc	586 ef
Llanero	Tolerant	13 cdef	16 bc	1.4 de	0.4 fg	20 f	22 def	5315 cd	4231 cde	885 cd	767 def
Tully	Tolerant	15 cde	19 ab	1.5 cd	1.0 ef	18 f	17 f	8585 a	4621 cd	1430 ab	859 def
CIAT16888	Tolerant	12 def	20 a	1.9 bcd	0.3 g	21 ef	25 def	6188 bc	4698 cde	1014 bcd	775 de
CIAT25670	Tolerant	17 bc	21 a	1.5 cde	0.6 fg	27 cdef	20 f	8956 a	5495 bc	1400 a	1026 cd

723

724

725

726

727 **Table 2**. Root cross-sectional area, aerenchyma percentage of root cross-section, stele area of adventitious roots and cortex to stele ratio
728 at 50 mm behind the root tip of eight *Urochloa* genotypes grown in aerated or stagnant deoxygenated solutions for 2 weeks. Different
729 letters indicate statistically significant differences ($P< 0.05$, Tukey test).
730

Genotypes	Tolerance to waterlogging	Root cross-sectional area (mm^2)				Aerenchyma (%)				Stele area (mm^2)				Cortex/Stele (mm^2)			
		Aerated		Stagnant		Aerated		Stagnant		Aerated		Stagnant		Aerated		Stagnant	
Marandu	Sensitive	4.04	ab	3.59	abc	3.2	de	13.3	c	0.79	a	0.59	bc	2.69	g	3.75	fg
CIAT26124	Sensitive	2.19	de	2.61	bcde	1.5	e	13.0	c	0.39	def	0.38	def	3.25	g	4.36	defg
Toledo	Sensitive	2.06	de	4.52	a	1.5	e	5.1	d	0.48	cde	0.59	bc	3.05	g	4.99	cdefg
Mulato II	Sensitive	2.70	bcd	4.07	a	6.4	d	10.3	c	0.50	cd	0.67	ab	3.07	g	3.95	efg
Llanero	Tolerant	1.29	e	2.43	cde	11.2	c	23.1	a	0.16	h	0.22	gh	4.34	defg	6.81	bc
Tully	Tolerant	2.52	cde	3.76	abc	12.5	c	17.3	b	0.28	fgh	0.29	fgh	5.62	cde	9.31	a
CIAT16888	Tolerant	1.81	de	2.46	cde	9.8	c	19.0	b	0.23	gh	0.28	fgh	4.94	cdefg	6.08	cd
CIAT25670	Tolerant	2.49	cde	3.92	ab	12.9	c	17.4	b	0.28	fgh	0.34	efg	5.56	cdef	8.09	ab

Table 3. Modelled maximum lengths of adventitious roots attained in waterlogging conditions of eight *Urochloa* genotypes (four sensitive and four tolerant).

Genotype	Tolerance to waterlogging	Original model*		Model including stele proportion**	
		Maximum root length (mm)		Maximum root length (mm)	
		Averaged resp. rates¥	Sensitive vs tolerant resp.Φ	Averaged resp. rates¥	Sensitive vs tolerant resp.Φ
Marandú	Sensitive	167	158	94	89
La Libertad	Sensitive	165	156	98	93
Toledo	Sensitive	99	93	62	59
Mulato II	Sensitive	144	136	83	78
Llanero	Tolerant	234	249	377	401
Tully	Tolerant	195	208	361	385
CIAT16888	Tolerant	206	220	317	338
CIAT26570	Tolerant	196	209	340	363

* This model was calculated using equation (2) and assuming constant respiration.

** This model also uses equation (2) but differences in respiration rates among cortex and stele tissues are computed.

¥ The respiration rates were averaged between both sensitive and tolerant genotypes and the mean was used for calculations.

Φ Respiration rates from waterlogging-sensitive cv. Mulato II and waterlogging-tolerant cv. Tully were used for the modelling of maximum root length of all sensitive and tolerant genotypes, respectively.

The presence of a tight barrier was computed for tolerant genotypes while no barrier was assumed for sensitive genotypes, based in suberin depositions in exodermal layers. The numerical values used in the models are as follows: Cortex O_2 status at the root-shoot junction (Co): 2.7 10^{-4} g cm^{-3}; diffusion coefficient of oxygen in air (D): 0.258 cm^2 s^{-1} (at 30 °C); fractional root porosity (ε): different values summarised in Table 2 but expressed as proportion (i.e., 0.13 instead of 13%); Tortuosity (τ): 1.0; tissue respiration (M): different values of root respiration were used for comparison: 1) the average values between the two genotypes evaluated (i.e., 2.63 nmol O_2 g FW sec^{-1}) and 2) the respiration rate of waterlogging-sensitive cv. Mulato II (i.e., 2.94 nmol O_2 g FW sec^{-1}) assumed equal for all sensitive genotypes and the respiration rate of waterlogging-tolerant cv. Tully (i.e., 2.31 nmol O_2 g FW sec^{-1}) assumed equal for all tolerant genotypes. For the model calculations root respiration values were expressed based in volume (according to porosities in Table 2 and expressed as ng O_2 cm^{-3} s^{-1}). Respiration values for stelar tissues are assumed to be 3-fold higher than those of cortical tissues (Pedersen et al. 2020).