

Supplementary Material

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

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Table S1. Waterlogging tolerance, shoot dry mass, root dry mass and root aerenchyma (% of air spaces in the root cross-section) of eight contrasting *Urochloa* genotypes evaluated in this study

Reference values in the table represent waterlogged treatments from experiments where plants were grown during two to four weeks in waterlogged soils.

Genotype	Tolerance to waterlogging	Shoot dry mass (g per plant)	Root dry mass (g per plant)	Root aerenchyma (%)	References
<i>U. brizantha</i> cv. Marandú	Sensitive	0.8 - 1.9	0.19 - 0.34	9 - 19	Caetano and Dias-filho 2008; CIAT 2009, 2010; Cardoso <i>et al.</i> 2014b; Mass <i>et al.</i> 2016
<i>U. brizantha</i> cv. La Libertad**	Sensitive	0.1 - 2.2	-	-	CIAT (2018, unpublished)
<i>U. brizantha</i> cv. Toledo	Sensitive	0.14 - 5.70	0.90 - 1.00	10 - 23	CIAT 2009; Cardoso <i>et al.</i> 2013, 2014
<i>U. hybrid</i> cv. Mulato II*	Sensitive	0.27 - 2.8	0.27 - 0.60	9 - 20	CIAT 2009; Cardoso <i>et al.</i> 2013, 2014a, 2014b; Mass <i>et al.</i> 2016
<i>U. humidicola</i> cv. Llanero	Tolerant	1.4 - 5.70	0.60 - 0.80	28 - 35	CIAT 2009; Cardoso <i>et al.</i> 2013, 2014a, 2014b; Mass <i>et al.</i> 2016
<i>U. humidicola</i> cv. Tully	Tolerant	3.10 - 6.50	0.42 - 0.90	29 - 43	Dias-filho and Carvalho 1999; CIAT 2009; Cardoso <i>et al.</i> 2013, 2014a, 2014b; Jiménez <i>et al.</i> 2015a, 2019***
<i>U. humidicola</i> CIAT16888	Tolerant	1.90 - 2.00	0.65 - 0.76	31	Cardoso <i>et al.</i> 2013, 2014
<i>U. humidicola</i> CIAT26570	Tolerant	3.60 - 5.00	0.90 - 1.20	34	Cardoso <i>et al.</i> 2013, 2014

* *U. ruziziensis* x *U. decumbens* x *U. brizantha*

** No data available on root biomass or root aerenchyma.

*** Plants were evaluated in deoxygenated stagnant agar solutions (not in waterlogged soils) during four weeks.

Table S2. Shoot and root dry mass of eight *Urochloa* genotypes after 2 weeks of growth in aerated or stagnant deoxygenated agar nutrient solution treatments

Two pooled plants per replicate ($n = 4$) were used for destructive harvest and the mean expressed on a per plant basis. Two-way ANOVA indicated differences for shoot dry mass (Gen: $P < 0.0001$, Treat: $P < 0.0001$, Gen x Treat: $P = 0.415$) and root dry mass (Gen: $P < 0.0001$, Treat: $P < 0.0001$, Gen x Treat: $P < 0.0001$). Different letters indicate statistically significant differences ($P < 0.05$, Tukey test)

Genotypes	Tolerance to waterlogging	Shoot dry mass (g per plant)				Root dry mass (g per plant)			
		Aerated		Stagnant		Aerated		Stagnant	
Marandú	Sensitive	2.06	abc	0.98	h	0.69	ab	0.29	efg
CIAT26124	Sensitive	1.65	cde	1.06	gh	0.58	bc	0.24	g
Toledo	Sensitive	1.58	cde	1.07	gh	0.58	bc	0.27	fg
Mulato II	Sensitive	1.79	cde	1.08	fgh	0.62	abc	0.37	ef
Llanero	Tolerant	2.02	abcd	1.49	efg	0.40	ef	0.34	efg
Tully	Tolerant	2.28	ab	1.54	defg	0.75	a	0.54	cd
CIAT16888	Tolerant	1.94	abcde	1.55	def	0.41	de	0.36	efg
CIAT25670	Tolerant	2.33	a	1.80	bcde	0.69	ab	0.63	abc

Table S3. Regression coefficients and *P* values of multiple regression models of six morphological, anatomical and architectural traits to predict Dry Mass of Shoots of plants grown in stagnant solutions

Plant morphological, anatomical and architectural traits data were incorporated into a multiple regression algorithm within the statistical software R. 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).

Coefficients:

	Estimate	Std. Error	t value	Pr(> t)
(Intercept)	2.935e-01	1.913e-01	1.534	0.1375
RAer	1.103e-02	6.717e-03	1.642	0.1131
RExt	2.273e-02	9.952e-02	0.228	0.8212
TRL	1.184e-04	4.546e-05	2.605	0.0152 *
RAngle	1.373e-03	3.082e-03	0.445	0.6598
NoRoots	1.024e-02	1.149e-02	0.892	0.3810
DWR	4.039e-01	4.142e-01	0.975	0.3388

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 0.1231 on 25 degrees of freedom

Multiple R-squared: 0.9013, Adjusted R-squared: 0.8776

F-statistic: 38.05 on 6 and 25 DF, p-value: 2.167e-11

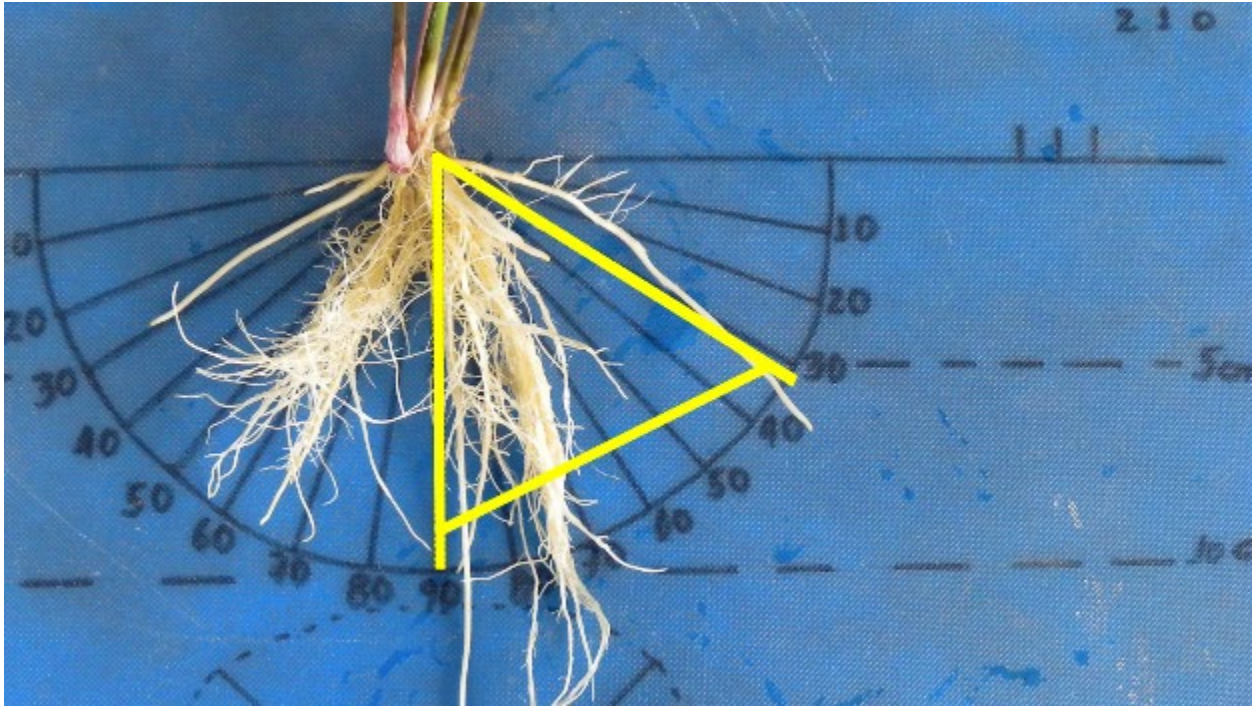


Fig. S1. Schematic representation of root angle measurements. The yellow lines drawn indicate the root angle of the basal 5 cm formed by the roots relative to the vertical axis. Roots were photographed from a nadir view at 20 cm height.

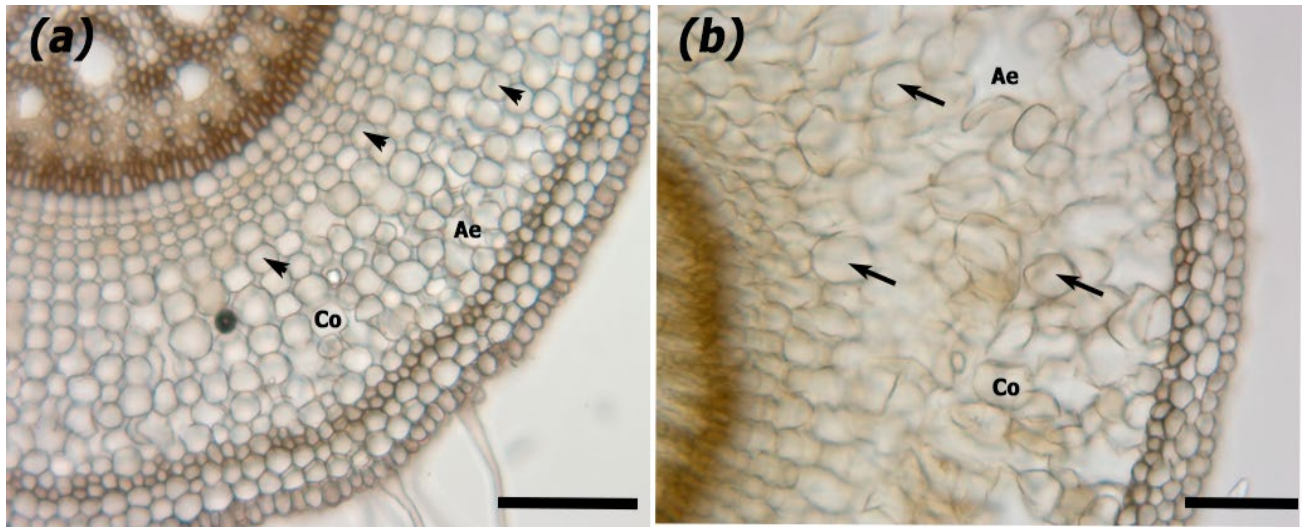


Fig. S2. Cross sections of adventitious roots at 50 mm behind the root tip of 2-week-old *U. brizantha* cv. Toledo grown in aerated (a) or stagnant conditions (b), showing increased cortical cell size in stagnant conditions. Black arrows point to enlarged cortical cells and black arrowheads to cortical cells. Average root cross-sectional area (n=4) was 2.06 mm² for controls and 4.52 mm² for stagnant treatments (see Table 4). Abbreviations: Ae= aerenchyma, Co=cortical cells. Scale Bar = 200 μ m.

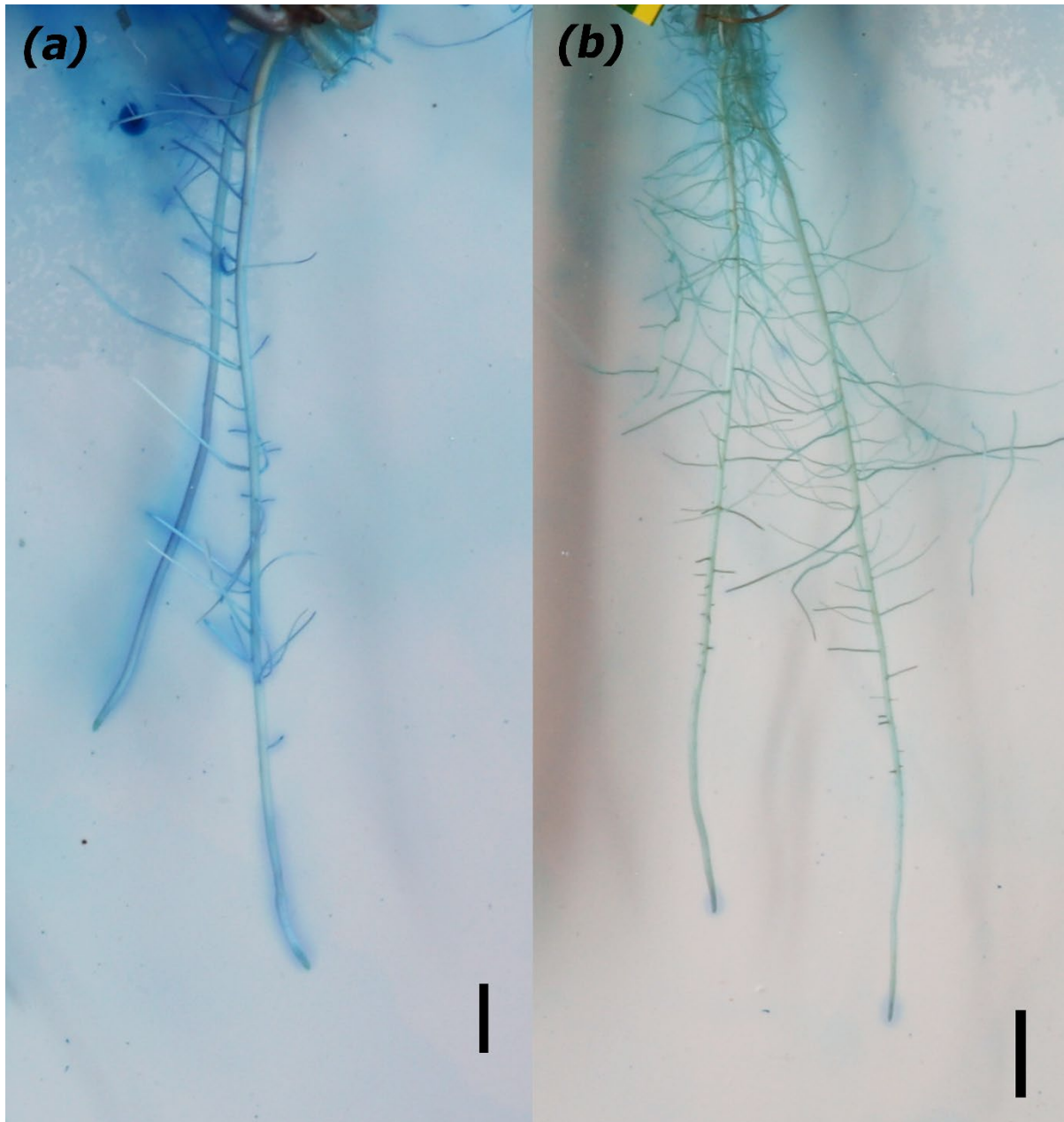


Fig. S3. Radial O₂ loss of waterlogging sensitive cv. Mulato II (a) and waterlogging-tolerant cv. Tully (b). Two adventitious roots with a length of c. 90–120 mm were selected from plants grown for two weeks in deoxygenated stagnant solutions and all other roots were trimmed off. The medium contained 0.1% (v/v) deoxygenated stagnant agar, 0.03 mM methylene blue and 0.3 mM Na₂S₂O₃. The roots were photographed after 30 min of being inserted into the solution. The assay was carried out at room temperature under white light. Blue staining indicates leakage of O₂ from roots to solution. Scale bar = 1 cm.

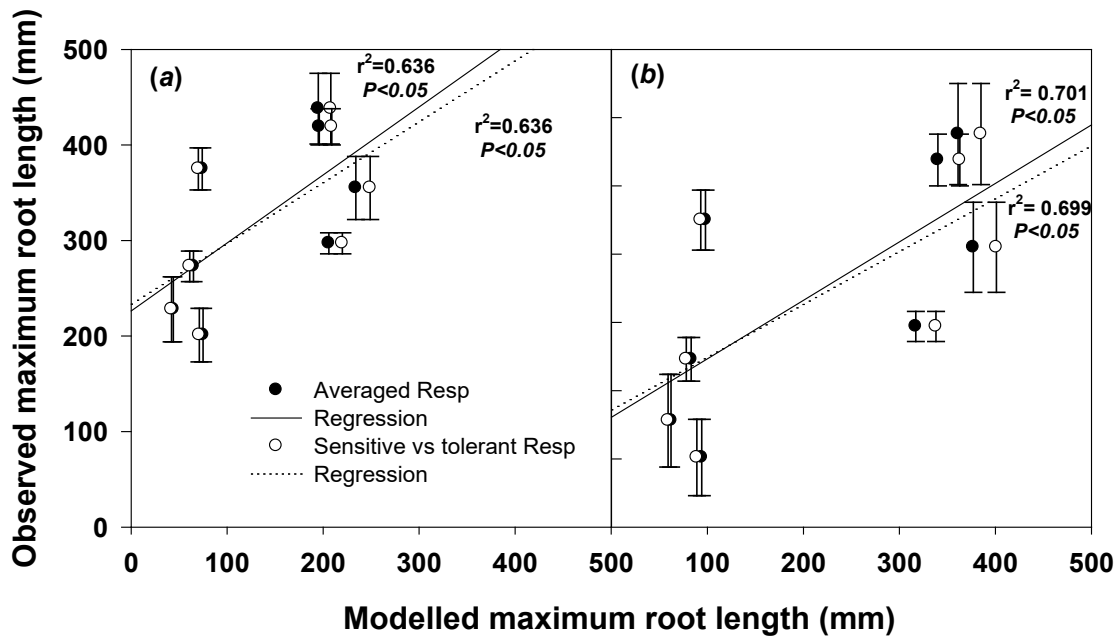


Fig. 4. Binary relationships and Pearson's correlation coefficients between observed maximum root length and modelled maximum root length of eight waterlogging contrasting *Urochloa* genotypes using the original model (Armstrong 1979; a) and a modified modelled computing differences in respiration among cortical and stellar tissues (Pedersen et al. 2020; b). r^2 = Pearson's correlation coefficients.