



Waterlogging response in *Urochloa humidicola* genotypes

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Ana María Restrepo García
Internship student



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Introduction

Forages constitute the primary feed for the majority of ruminant livestock in developing countries, where high production of meat, milk, and other livestock-derived products is generated (Fuglie et al., 2021). It is calculated that between the developing countries of Latin America, Asia, and Africa, 159 million hectares of forages are cultivated, which produce a return of around 63 billion dollars a year and that the global demand for milk and meat will be double by 2050 (Cook et al., 2020; Fuglie et al., 2021). Despite the economic and social impact of forages, and the growing demand for livestock products, there is a shortage of information about the adaptation and utilization of forages, including characteristics for their potential use under different types of stress (Fuglie et al., 2021).

Various factors can impact forage production, encompassing both biotic and abiotic influences. Abiotic stresses can cause the inhibition of plant growth or plant mortality, from an excess or deficiency in radiation, water, nutrients, and salinity (Kosová et al., 2020). These factors present and will continue to pose challenges, particularly



considering projections indicating a 2°C increase in global temperature and heightened occurrences of extreme weather events by 2050 due to global warming. This scenario raises a serious threat to sustainable food production (Peters et al., 2012).

In the context of stress caused by water, soil waterlogging is a major limitation on forage production. In this context, waterlogging will be understood when the soil reaches 100% of the hydric capacity of the soil and water remains on the surface. This condition poses a limitation on plant growth because, it generates a slow diffusion of gases, creating a barrier to the exchange of gases between the soil and the atmosphere. Consequently, oxygen levels within the root zone decrease (Cardoso & Rao, 2020; Kosová et al., 2020). In response to these challenges, plants have a series of mechanisms in response to oxygen limitations, like the production of new roots with higher proportions of aerenchyma, this intercellular air channels, connect various plant parts spatially, facilitating the swift diffusion of gases from the roots to the aerial zone of the plant. Additionally, plants develop roots with larger diameters and fewer lateral roots branching from the main root (Parent et al., 2008; Cardoso et al., 2013).

Among different forages, there have been some commercial grasses well characterized in tolerating waterlogging conditions, for example, *Urochloa humidicola*. This species has shown to maintain growth rate, leaf elongation, net photosynthesis, and leaf chlorophyll content (Dias-Filho & Carvalho, 2000) under waterlogging conditions. However, it is important for the breeding program to characterize genotypes of *U. humidicola* for these traits. For this reason, the main objective of this research is to study the response of the 65 genotypes of *U. humidicola* under three weeks of waterlogged soil conditions.

Methodology

Genotypes and growing conditions

65 genotypes of *U. humidicola* were used in this study (Complementary material 1). Seven *Urochloa* grasses well characterized under waterlogging response were used as controls (CIAT:16886, CIAT:16888, CIAT:6133, CIAT:679, Bh08:1149, Cayman and MulatoII). The soil used in this study was collected in Palmira. All genotypes were grown from vegetative propagules, from plants established on field. The propagules were allowed to grow in pots filled with 2.3 kg of soil for three weeks before the waterlogging conditions. Two pots and a plastic bag were used to simulate the flooding conditions, the pot with the soil and propagule was inside a plastic bag to avoid water drainage, and this was inside a bigger pot. The plants were flooded for three weeks.

Experimental design

A factorial combination of 65 genotypes by two conditions (waterlogging or drained) was established in a six-replicate randomized completed block. The experiment was conducted in an open area at CIAT.

Data collection

Leaf greenness (SPAD), stomatal conductance and leaf area were taken before and during waterlogging condition. SPAD and stomatal conductance values were recorded once a week for four weeks (before and during waterlogging), between 8 to 11 am, to avoid a big difference in temperature and incidence of UV rays when the data were taken. For leaf area, a picture of the plant was taken in a Photobooth before and after waterlogging and



estimated with the image software ImageJ (Schneider et al., 2012). Leaves, stems, and roots were harvested. The leaves and stems were separated, and oven dried for 72 h at 60°C to determine dry mass. Roots were washed with water and placed in falcon tubes of 50mL with alcohol at 70%. The new and old roots were separated, according to their anatomical characteristics, the new roots were white, had larger diameter and fewer lateral roots (Parent et al., 2008). Falcon tubes were stored at 4°C for later separation and analysis.

Root scanning

The roots were scanned with an EPSON Expresion 1680 dual scanner, Japan to record grey images for the determination of diameter and quantity of roots and the number of new roots. The roots were placed in an acrylic tray filled with water and arrange to avoid overlapping.

Results and discussion

Effect of waterlogging in leaf greenness (SPAD) and stomatal conductence

For both treatments, the variables measured, SPAD and stomatal conductence, show a reduction over the 3 weeks period evaluated, but neither of the weeks show a statistical difference between the treatments. This has been reported previously in plants that are tolerant to waterlogging, where SPAD values decreased in all the accessions of *U. humidicola* from the start of the experiment under drained or waterlogged soil conditions, and these values were not further reduced as the waterlogged conditions progressed (Cardoso et al., 2013).

Dry biomass and leaf area

There was a statistically significant difference in dry biomass, with stem dry weight being higher in the waterlogging treatment, while there was no difference in the leaf biomass. On the other hand, leaf area also showed significant differences, where drained soil conditions had higher leaf area, this can be seen in Figure 1. Leaf area was obtained subtracting the leaf area of the third week and of the first week.



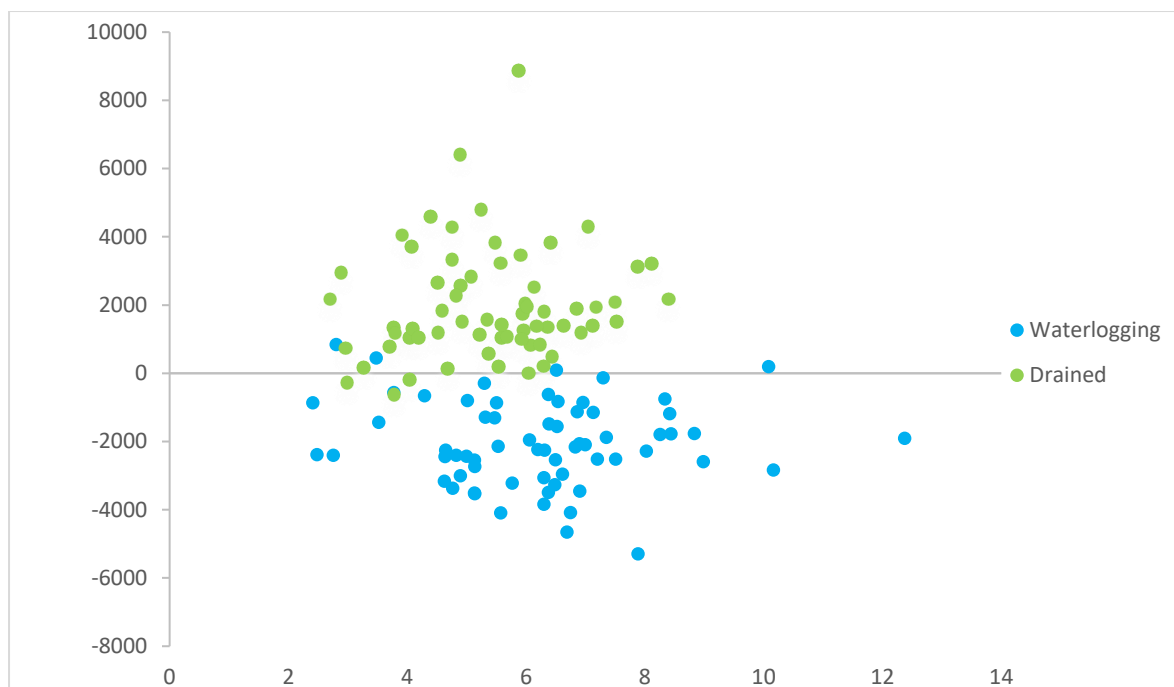


Figure 1. Scatter plot of aerial mass and foliar area, where the green dots represent the drained treatment, and the blue dots represent the waterlogging treatment.

Leaf greenness (SPAD) and foliar area

Genotypes exhibiting higher SPAD and foliar area values in the third week were chosen as prime candidates for waterlogging resistance. It is crucial for these candidates to display a rapid growth rate and maintain high levels of leaf greenness. Among the waterlogged genotypes, only three demonstrated significant growth, as indicated in Table 1. Some controls like CIAT:679 show some tolerance to waterlogging and others like Cayman, MulatoII and CIAT:16888 show great response under drained conditions.

Genotype	SPAD	Foliar area (px)	Treatment
Bh16b:1194	15.75	444.42	Waterlogging
Bh16b:0654	18.4	196.29	Waterlogging
Bh16b:0617	23.77	91.41	Waterlogging
Bh16b:1110	18.53	-131.9	Waterlogging
Bh16b:1085	23.67	-292.86	Waterlogging
Bh16b:2028	28.6	-560.35	Waterlogging
Bh16b:1051	22.93	-617.48	Waterlogging
Bh16b:0635	21.8	-660.23	Waterlogging
CIAT:679	23.6	-753.04	Waterlogging
Bh16b:0825	23.8	-798.59	Waterlogging
Bh16b:1208	19.53	-829.96	Waterlogging
Bh16b:0624	23.67	-855.28	Waterlogging
Bh16b:0855	25.2	-866.08	Waterlogging
Bh16b:1131	26.8	-868.87	Waterlogging
Bh16b:2165	18.2	8873.38	Drained
Bh16b:0825	26.1	6403.31	Drained
Bh16b:0365	26.4	4796.98	Drained



Bh16b:1987	21.1	4597.3	Drained
Bh16b:0218	20.9	4307.85	Drained
Bh16b:0021	26.8	4288.18	Drained
Cayman	18.3	4046.57	Drained
Mulatoll	22.2	3836.1	Drained
Bh16b:1208	21.9	3835.75	Drained
Bh16b:1131	30.8	3708.4	Drained
Bh16b:1411	16.5	3461.52	Drained
Bh16b:2167	19.7	3320.55	Drained
CIAT:16888	22.3	3226.75	Drained
Bh16b:1085	19.9	3203.1	Drained
Bh16b:0654	17	3131.28	Drained

Table 1. Genotypes with the higher values of SPAD and foliar area. The green labels represent genotypes that are present in both treatments and the blue labels represent some controls.

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