Development of early selection procedures of waterlogging resistant *Urochloa humidicola*

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Waterlogging (or soil flooding) occurs when soil is saturated with water. Due to slow difussivity of gases in water, the exchange of O_2 between soil and atmosphere is strongly impeded. As a result, O_2 in soil is depleted by microbial and root respiration. Unless are suited for growth under oxygen-limited conditions, their functioning is impaired due to the inhibition of aerobic respiration. Such impairment results in increased senescence of root and shoot tissue over time. A key component of tolerance to waterlogging involves the ability internally transport oxygen form aerial non-submerged tissues to surbmerged ones. Aerenchyma (tissue with air-filled cavities provides such pathway. Previous research showed that there is genotypic variation in aerenchyma formation in *U. humidicola*, and genotypes with greater aerenchyma formation under waterlogged conditions were among the more productive under such conditions (Cardoso eta l, 2013). Measurement of aerenchyma can be cumbersome and very low throughput. Measurements of air-filled porosity can be used a s a proxy for aerenchyma. Furthermore, plants grown under waterlogged conditions make difficult to collect roots for the evaluation of the extent of aerenchyma development or porosity. As such we are implementing a screening method which relies on plants grown under stagnant solutions (mimicking hypoxic to anoxic environments) and that allows with ease the collection of roots for the evaluation of the extent of porosity in them.

Evaluation of hypoxia tolerance using stagnant solutions

Vegetative propagules (containing one leaf and one node) or seedlings are grown for 7 days in 4 L pots filled with acidic low-ionic-strength nutrient solution (Wenzl et al., 2003). This standard solution has been widely used to evaluate growth and adaptation of *Urochloa* species to acid soils (Wenzl et al., 2006). After 7 days of growth in low-ionic-strength solution, plants are transferred to 4 L pots (four plants per pot)

containing either aerated (controls treatment) or stagnant 0.1% (w/v) agar nutrient solution. Standard acidic solution contained (in µM): 500 NO_3, 50 NH_4, 300 K, 300 Ca, 150 Mg, 160 Na, 5 H_2PO_4, 286 SO_4, 5 Fe-EDTA, 1 Mn, 1 Zn, 0.2 Cu, 6 H_3BO_3, 5 SiO_3, 0.001 MoO_4 and 332.4 Cl. HCl is used to adjust the pH to 4.20 (Wenzl et al., 2003). 0.001 µM. Ni is also added to the solution. In the stagnant solutions, agar at 0.1% (w/v) is added to prevent convection. Stagnant solutions are flushed with high-purity N_2 to purge out the O_2 before use, thus simulating changes in gas composition as occur in waterlogged soil conditions (Wiengweera et al., 1997). Care is taken to avoid mixing O_2 when syphoning these solutions from the preparation tanks into each individual pot. Nutrient solutions in all pots are changed weekly. Further information is described in Jiménez et al, 2019.

Plants are evaluated during two or three weeks of continuous hypoxia treatment. Aerated controls are continuously pumped with air during this time lapse. Some physiological measurements including photosynthesis, stomatal conductance, leaf greenness (SPAD) as well as non-destructively image analysis of shoot growth might be performed during the time course of the experiment.

After two or three weeks of treatments plants are harvetested. Plants from each pot are divided into green and dead leaves, stems and roots. Maximum root length, the total number of roots and stems are recorded. Excess of water from biomass material is removed, and then, fresh weigh is recorded. Samples are then oven dried at 60 ^oC for 3 days and dry weights are determined. At harvest time, root samples are collected for estimation of porosity:

Root porosity

Porosity (% gas space per unit root volume) is measured in root samples from each treatment by determining root buoyancy before and after vacuum infiltration with water (Raskin, 1983). Porosity is then calculated using the equations modified by Thomson *et al*. (1990). Four to five representative roots (100-

200 mm in length) are excised from one plant from each pot, cut into 100 mm segments. Prior to measurements of porosity are made in main roots, lateral roots are removed from them.

References

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