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Salt stress experiment in supported hydroponics examining the salt stress tolerance of durum wheat

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Genc et al., Calcium requirement of wheat in saline and non-saline conditions, Plant Soil (2010) 327:331–345 DOI 10.1007/s11104-009-0057-3 AND Genc et al., Reassessment of tissue Na+ concentration as a criterion for salinity tolerance in bread wheat. Plant, Cell and Environment (2007) 30, 1486–1498, doi: 10.1111/j.1365-3040.2007.01726.x

Protocol status: Working
We use this protocol and it's working

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Abstract

This is a standard protocol for durum wheat treatment, which was initially developed by Genc et al., 2008 and Genc et al., 2010. As durum wheat is suffering from the Ca2+ deprivation during the salt stress exposure, this needs to be compensated for, by calculating the Ca2+ activity using Visual Minteq. The wheat seedlings in this protocol are exposed to salt stress at 3rd leaf stage (xxx days after germination) with 75 mM NaCl - applied in steps of 25 mM NaCl every 12 h.

Before start

Make sure to prepare the nutrient stock solutions:
NH4NO3 - 1M
KNO3 - 1M
Ca(NO3)2 - 0.4M
MgSO4 - 0.4 M
KH2PO4 - 0.1M
NaSiO3 - 1M
NaFe(III)EDTA - 0.05M
H3BO3 - 0.05M
MnCl2 - 1M

1 Sterilize wheat seeds

Put the wheat seeds in 50% household bleech for 10 minutes.

Wash with sterile MiliQ water in laminar hood for 3-5 times

Put the seeds in 10 ml of sterile MQ and leave at 4C for over night to break the dormancy

2 Germinate the wheat seeds

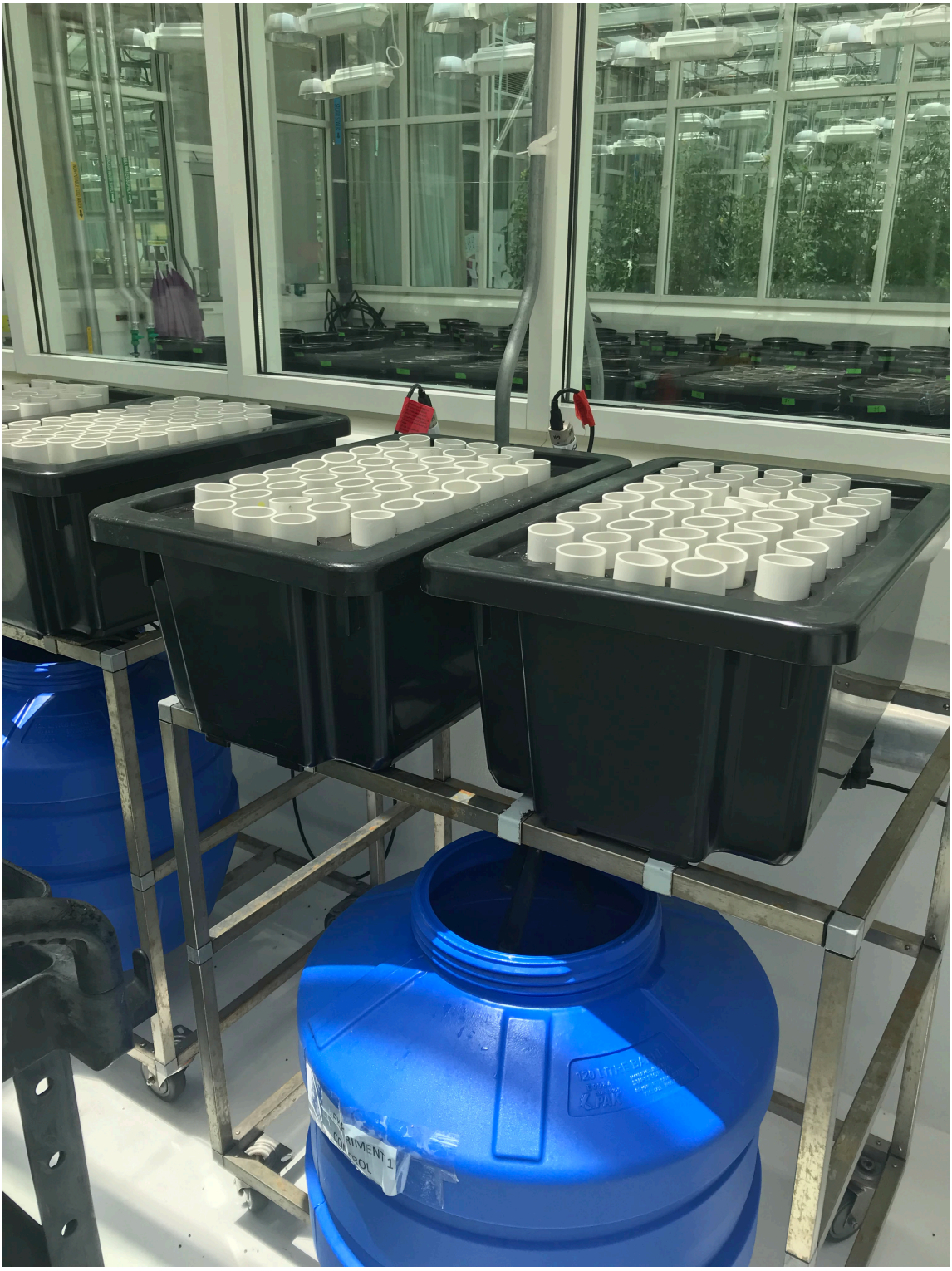
Put the sterilized seeds on autoclaved germination paper (e.g. whatmann) in sterile petri dish (7 cm diameter) and pour approximately 3-5 ml of sterile MiliQ water for germination

Seal the petridish with leukopor tape and put in the growth chamber for germination (22/20C 12/12h light/dark with 60% humidity)

3 Transfer the seedlings to larger plates (14 cm diameter) with fresh germination paper, pour 10 ml MiliQ water, seal the plates and return to the growth chamber

4 One day before transfer - Prepare the supported hydroponics setup

Fill in the PCV tubes, sealed at one end with mesh, with black plastic beads to approximately 2-3 cm from the top of the tube and place in the hydroponic racks Connect the pumps to the switch board and to the current adapter, which is connected to the time plug - make sure to install the timing on/off for 30 min. each (00:30:00 - on digital time switches)

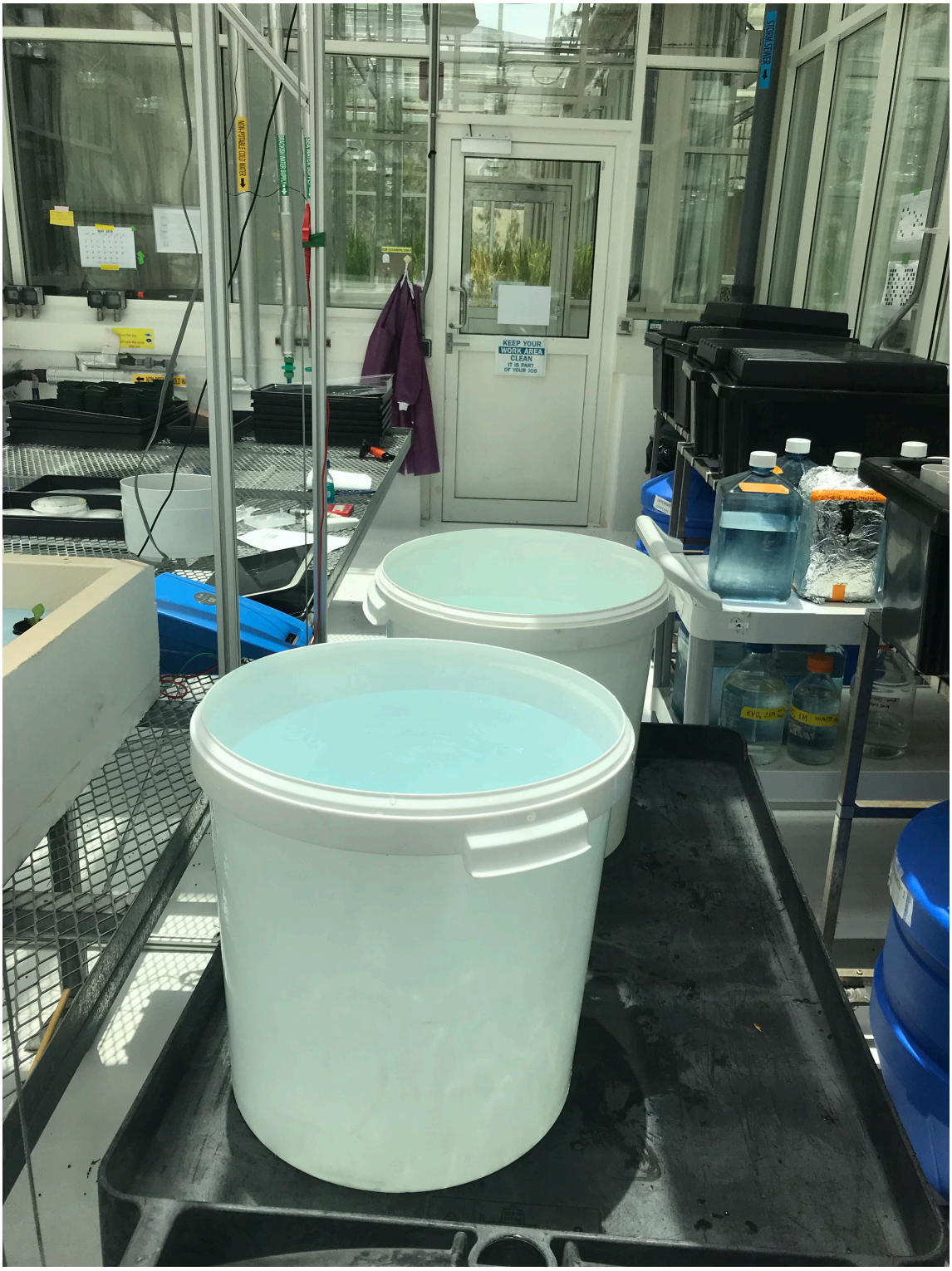


Our greenhouse setup for supported hydroponics - the blue tanks at the bottom contain 100 L of media which gets pumped up into the black containers which hold the PCV tubes filled with the black plastic beads that support the plants growing in. The Flood-and-Drain system is orchestrated by the timer switch to which the pumps are connected.

Add 100 L of tap water to the tanks below the hydroponics and let the entire system run and flush with tap water for 24h

After flushing the system - unplug the switchboard and drain the tanks from tap water

As it will take long time - at this point you also might want to collect all the MiliQ water that you will be using in the experiment - I collect 200 L for one experiment in 30 L tanks that are stored in the greenhouse compartment



MiliQ water and the solution ready to be used for adding the media into the tanks in our greenhouse setup

5 One hour before the seedlings transfer - Prepare the modified Hoogland solution media in the tank by mixing the following into 100L of MQ water

	media (mM)	stock (M)	Mole per 100 L	ml to add to tank
NH4NO3	0.2	1	0.02	20
KNO3	5	2.5	0.5	200
Ca(NO3)2	2	0.4	0.2	500
MgSO4	2	0.4	0.2	500
KH2PO4	0.1	0.1	0.01	100
NaSiO3	0.5	1	0.05	50
NaFe(III)EDTA	0.05	0.05	0.005	100
H3BO3	0.01	0.05	0.001	20
MnCl2	0.005	1	0.0005	0.5
ZnSO4	0.005	0.01	0.0005	50
CuSO4	0.005	0.25	0.0005	2
Na2MoO3	0.0001	0.01	0.00001	1
total volume of nutrient solutions	1543.5			
NaCl	75	5	7.5	1500
CaCl2	1.2	1	0.12	120

The concentrations of individual nutrients in final solution (media (mM)), the stock-solutions used (stock (M)), and amounts of ml of stock solution to add to 100 L tank to reach final concentrations

6 Seven days after the germination transfer the seedlings to supported hydroponics

During the "flush" (pumps on) interval, gently transfer the wheat seedlings to the supported hydroponics

"Brush" the plastic beads off with a spoon / spatula, put the seedling in the beads and make sure that the roots are covered with the solution

7 After emergence of 3rd leaf (approximately 1.5 weeks after transfer) replace the meda and add the first dose of salt stress at the swap

Be carefull as now you have to replace the media during half an hour of the drain cycle - so make sure to have everything ready at the end of "flood" cycle and drain the tank and add 100 L od media.

For the tank that gets the salt stress treatment, add the salt to reach final concentration of 25 mM NaCl and 0.4 mM of CaCl2. Keep adding 25 mM NaCl and 0.4 mM CaCl2 every 12h until final concentration of 75 mM NaCl and 1.2 mM CaCl2 has been reached.

8 Make sure to check on your plants every day, to control whether the tanks are not leaking and whether the cycle of flood and drain is ok

The durum wheat plants growing in the supported hydroponics setup approximately 3 weeks after germination

9 Harvest the plants 2 weeks after the application of the salt stress.

In order to prevent plants for being out of the flood-and-drain cycle for too long, the plants that are to be measured are collected into a 30 L bucket and transported to the measuring station.

transport of the plants to the measuring station

The typical traits we score during the harvesting campaign are:

- pictures using PhotoSimile from 4 angles of each tube containing one plant

The setup of making pictures from four different angle. Each tube is placed in the middle of the photo-cube on the petri-dish which has the markings at four opposite sides - each at approximately 45 degrees. There is a larger petri-dish placed under with only one marking. Photos are taken by turning the petridish every 45 degrees.

- Fresh Weight of the entire shoot

- Fresh Weight of the entire root

The roots extracted from the supported hydroponics by flushing the beads under running water

Ion accumulation (Na+ and K+) of:
- the entire root

- the last fully expanded leaf (determined by - if the leaf above is shorter - your leaf-of-interest is the last fully expanded leaf)

For ion accumulation you need to know fresh weight and dry weight of your tissue of interest. We usually do it by weighing prepared and labeled tubes individually, then weigh the tubes with fresh sample and weigh it again after at least 2 days at 65 C oven.

The ion accumulation is measured using the flame photometer by prior extraction of ions using 1% nitric acid (10 ml used for root and shoot) and incubating the tissue with nitric acid at 65C oven for at least 2 days