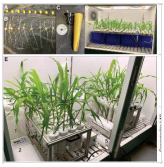

May 26, 2021

Maize hydroponics & Root multiple ion uptake kinetics [sterilization, germination, sowing, growth & uptake]

Marcus Griffiths[1], Sonali Roy[1], Haichao Guo[1], Anand Seethepalli[1], David Huhman[1], Yaxin Ge[1], Robert E Sharp[2], Felix B Fritschi[2], Larry M York[1]

[1]Noble Research Institute LLC; [2]University of Missouri - Columbia

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Marcus Griffiths
Donald Danforth Plant Science Center, Noble Research Institu...

ABSTRACT

Nutrient uptake is critical for crop growth and is determined by root foraging in soil. Growth and branching of roots lead to effective root placement to acquire nutrients, but relatively little is known about absorption of nutrients at the root surface from the soil solution. This knowledge gap could be alleviated by understanding sources of genetic variation for short-term nutrient uptake on a root length basis. A modular platform called RhizoFlux was developed for high- throughput phenotyping of multiple ion-uptake rates in maize (Zea mays L.). Using this system, uptake rates were characterized for the crop macronutrients nitrate, ammonium, potassium, phosphate, and sulfate among the Nested Association Mapping (NAM) population founder lines. The data revealed substantial genetic variation for multiple ion- uptake rates in maize. Interestingly, specific nutrient uptake rates (nutrient uptake rate per length of root) were found to be both heritable and distinct from total uptake and plant size. The specific uptake rates of each nutrient were positively correlated with one another and with specific root respiration (root respiration rate per length of root), indicating that up- take is governed by shared mechanisms. We selected maize lines with high and low specific uptake rates and performed an RNA-seq analysis, which identified key regulatory components involved in nutrient uptake. The high-throughput multiple ion-uptake kinetics pipeline will help further our understanding of nutrient uptake, parameterize holistic plant models, and identify breeding targets for crops with more efficient nutrient acquisition.

EXTERNAL LINK

https://doi.org/10.1093/plphys/kiaa080

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Griffiths M, Roy S, Guo H, Seethepalli A, Huhman D, Ge Y, Sharp RE, Fritschi FB, York LM. 2021. A multiple ion-uptake phenotyping platform reveals shared mechanisms affecting nutrient uptake by roots. Plant Physiology 185, 781–795.

DOI

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MANUSCRIPT CITATION please remember to cite the following publication along with this protocol

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KEYWORDS

plant phenotyping, nutrient uptake kinetics, hydroponics, root phenotyping, plant physiology, maize growth, maize germination

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45261

Seed sieving to consistent size

1 **(*Optional)**
For each experiment or series of experiments sieve seeds to mean seed size of parents or population used

Seed sterilization (Bleach only method)

2 12 minutes in **5 % Bleach (Sodium hypochlorite)**

00:12:00

3 Rinse x3 with autoclaved dH_2O and then leave with a dH_2O cap

Seed pre-germinatation

4 **(*Optional.) Note: stratification step was not used in the manuscript associated with this work on maize as germination was already consistent**
Stratify seeds at 4 °C dark for 5 days

4 °C dark for 5 days

120:00:00

5 Soak germination paper with 0.2 mM CaSO4. Lay seeds on soaked germination paper soaked 2” from top. Stapel genoype ID label to paper. Roll germination paper side to side & tie with cable ties. Part-submerge paper rolls in 0.2 mM CaSO4, ensuring seeds are not submerged, & cover in foil to prevent drying out.

2.5 L 0.2 mM CaSO4

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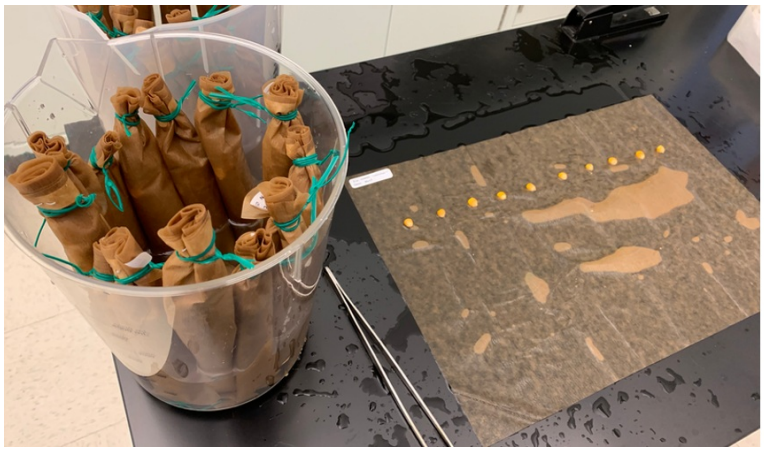


Using a 4L jug approximately 2.5 L of CaSO4 should allow a 2" gap from the line of seeds

2.5 L 0.2 mM CaSO4

6 Transfer seeds to incubator at 28 °C for 3-5 days for pre-germination

28 °C dark for 3-5 days

72:00:00

Seedling growth in hydroponic tanks

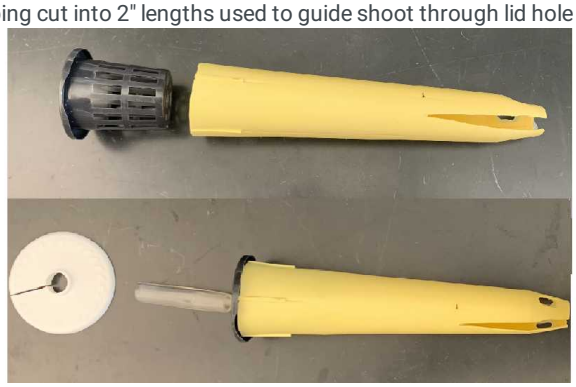
7 Set up growth chamber 28/20°C, 16/8h, 50% humidity, 210 µmol m-2 s-1 light intensity full light both fluorescent and incandescent with 1 hour dusk and dawn

28 °C day temperature 16 h

20 °C night temperature 8 h

8 Make plant hydroponics cone

1. Plastic mesh plant baskets (1.5” × 2”, Shenzhen Skywalker Electronic Limited, Shenzhen, China)
2. Plant cone-tainers (SC10 Super RL98 cell, Stuewe & Sons Inc., OR, USA). A slot about 3 mm wide was cut from the bottom of the cone to about 5 cm from the top in order to accommodate the tubing from the sampling platform described below.
3. Pot Lid 2" where shoot will emerge (Gro Pro Net)
4. PVC clear tubing cut into 2" lengths used to guide shoot through lid hole



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9 Make hydroponics tank

Tank - Quantum DG92080 Dividable Grid Container. 10.88" x 16.5" x 8"
Inlay - 250mm x 395mm acrylic inlay with rounded corners. Hole design - 24 holes 1.75" diameter (44.45mm), plus 2 x holes for aeration ***XX*** diamter.

10 Prepare 1/2 Hoagland's growth solution at pH 6 using stock solutions.

Macronutrients	**Chemical name**	**MW (g/mol)**	**Conc (µM)**	**Stock x100 (g sal / 1L)**	**mL stock to add / 1L**
KH_2PO_4	Potassium phosphate monobasic	136.086	500	6.80	10
KNO_3	Potassium nitrate	101.1032	5700	58	10
$CaCl_2$	Calcium chloride	110.984	2000	22.20	10
$MgSO_4$	Magnesium sulfate	120.3676	1000	12.04	10
$(NH_4)(NO_3)$	Ammonium nitrate	80.0434	300	2.40	10
Micronutrients	**Chemical name**	**MW (g/mol)**	**Conc (µM)**	**Stock x1000 (g sal / 1L)**	**ml stock to add / 1L**
H_3BO_3	Boric acid	61.833	46	2.84	1
$ZnSO_4 \cdot 7H_2O$	Zinc sulfate heptahydrate	287.5496	7	2.01	1
$MnCl_2 \cdot 4H_2O$	Manganese(II) chloride tetrahydrate	197.9052	9	1.78	1
$CuSO_4 \cdot 5H_2O$	Copper(II) sulfate pentahydrate	249.685	0.32	0.08	1
$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$	Ammonium molybdate	1235.9975	0.114	0.14	1
Fe(III)-EDTA ($C_{10}H_{12}N_2NaFeO_8$)	Ethylenediaminetetraacetic acid iron(III) sodium saltmol)	367.0456	150	28.26	1.94

18 L per tank

Macronutrients

[M]500 Micromolar (µM) KH2PO4 Potassium phosphate monobasic (mw 136.086)

[M]5.7 Milimolar (mM) KNO3 Potassium nitrate (mw 101.1032)

[M]2 Milimolar (mM) CaCl2 Calcium chloride (mw 110.984)

[M]1 Milimolar (mM) MgSO4 Magnesium sulfate (mw 120.3676)

[M]300 Micromolar (µM) (NH4)(NO3) Ammonium nitrate (mw 80.0434)

Micronutrients

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[M]**46 Micromolar (µM)** **H3BO3 Boric acid (mw 61.833)**

[M]**9 Micromolar (µM)** **ZnSO4·7H2O Zinc sulfate heptahydrate (mw 287.5496)**

[M]**9 Micromolar (µM)** **MnCl2 ·4H2O Manganese(II) chloride tetrahydrate (mw 197.9052)**

[M]**0.32 Micromolar (µM)** **CuSO4·5H2O Copper(II) sulfate pentahydrate (mw 249.685)**

[M]**0.114 Micromolar (µM)** **(NH4)6Mo7O24·4H2O Ammonium molybdate (mw 1235.9975)**

[M]**77 Micromolar (µM)** **Fe(III)-EDTA (C10H12N2NaFeO8) Ethylenediaminetetraacetic acid iron(III) sodium saltmol (mw 367.0456)**

pH **6**

For 18 L Hoagland's solution add 1.2 mL 1M KOH

1.2 mL **1M KOH**

11 Fill growth tank with prepared Hoaglan'ds solution pH 6. Sow plants ensuring roots are in contact with solution. Label lid with genotype information.

Provide aeration to the hydroponic tank using an airpump

- EcoPlus 1030 GPH (3900 LPH, 35W) Commercial Air Pump
- EcoPlus Air Manifold T-Style 3/8" Inlet, 8-Valve Outlet
- 3/8" tubing
- 3/16" tubing

(L) pregerminated seedlings after 3 days. (M) Seedling in cone & basket vessel, with white labeled lid. (R) Middle hydroponic tank 1 day after sowing, left tank 2 days after sowing.

12 Maintain plants in hydroponics
Check pH & add iron chelate as required (eg. every few days / weekly)
Replace Hoagland's solution with fresh as required (eg. every week / 2 weeks)

Nutrient deprivation of plants before uptake assay

13 Change nutrient solution 48hours prior to uptake assay with a largely macronutrient-free solution.

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Macronutrients	Chemical name	MW (g/mol)	Conc (µM)	Stock x100 (g sal / 1L)	mL stock to add / 1L
CaCl2	Calcium chloride	110.984	500	5.549	10
Micronutrients	**Chemical name**	**MW (g/mol)**	**Conc (µM)**	**Stock x1000 (g sal / 1L)**	**ml stock to add / 1L**
H3BO3	Boric acid	61.833	46	2.84	1
ZnSO4·7H2O	Zinc sulfate heptahydrate	287.5496	7	2.01	1
MnCl2 ·4H2O	Manganese(II) chloride tetrahydrate	197.9052	9	1.78	1
CuSO4·5H2O	Copper(II) sulfate pentahydrate	249.685	0.32	0.08	1
(NH4)6Mo7O24·4H2O	Ammonium molybdate	1235.9975	0.114	0.14	1
Fe(III)-EDTA (C10H12N2NaFeO8)	Ethylenediaminetetraacetic acid iron(III) sodium saltmol)	367.0456	150	28.26	1.94

18 L per tank

Macronutrients

[M]500 Micromolar (µM) CaCl2 Calcium chloride (mw 110.984)

Micronutrients

[M]46 Micromolar (µM) H3BO3 Boric acid (mw 61.833)

[M]9 Micromolar (µM) ZnSO4·7H2O Zinc sulfate heptahydrate (mw 287.5496)

[M]9 Micromolar (µM) MnCl2 ·4H2O Manganese(II) chloride tetrahydrate (mw 197.9052)

[M]0.32 Micromolar (µM) CuSO4·5H2O Copper(II) sulfate pentahydrate (mw 249.685)

[M]0.114 Micromolar (µM) (NH4)6Mo7O24·4H2O Ammonium molybdate (mw 1235.9975)

[M]77 Micromolar (µM) Fe(III)-EDTA (C10H12N2NaFeO8) Ethylenediaminetetraacetic acid iron(III) sodium saltmol (mw 367.0456)

pH 6 solution should already be approximately pH6

Ion uptake kinetics assay

14 Make uptake chambers with a volume capacity of 250 mL.

- 1.5” ID PVC Schedule 40 pipe Foundry Company, NC, USA)
- 1.5” hub cap fitting
- 1.5" to 2" adapter
- 3/23" hole in side for nutrient sampling pipe, tubing samples from middle of chamber, sealed with clear waterproof silicone
- 3/16" hole at bottom for nutrient filling/emptying,tubing flush with bottom of chamber for emptying, sealed with clear waterproof silicone

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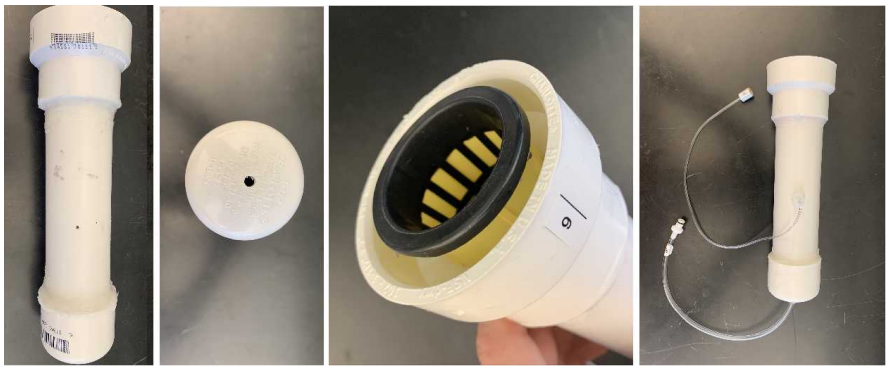
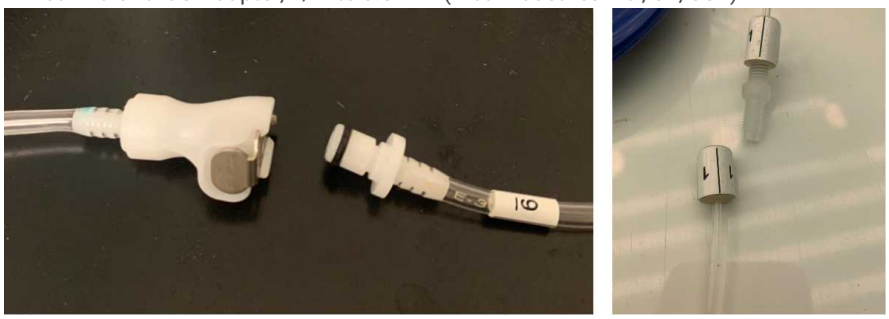


Assemble tubing per chamber
Large tubing (pump1)

- 3.17 mm ID tubing (Ismatec SC0222-LT 2-Stop 0; Masterflex SC0223-LT Tygon)
- Masterflex Hose Barb Union 1/8" (Cole-Parmer Instrument Company LLC., IL, USA)
- CPC ID 1/8" hose barb PMCD1702 & insert (PMC2202, Colder Products Company, MN, USA)

Small tubing (pump2)

- 0.51 mm ID tubing (Ismatec SC0005-LT 2-Stop 0; Masterflex SC0029-LT Tygon, Cole-Parmer Instrument Company LLC., IL, USA)
- Diba MicroBarb® Adapter, 1/4" to 0.02" ID (Diba Industries Inc., CT, USA)



(L) Tubing fittings used for filling/aeration attached to pump1. (R) Tubing fittings used for solution sampling attached to pump2.

Other tubing sizes and dimensions can be used. A larger tubing size for rapid filling and a small diameter for precise sampling was what we decided was useful.

15 Make uptake assay frame

Technical drawing of 24 hole lid **8020-lid-technicaldrawing.PDF**

Parts list for frame 80/20 extruded aluminium **8020-frame-parts-list.pdf**

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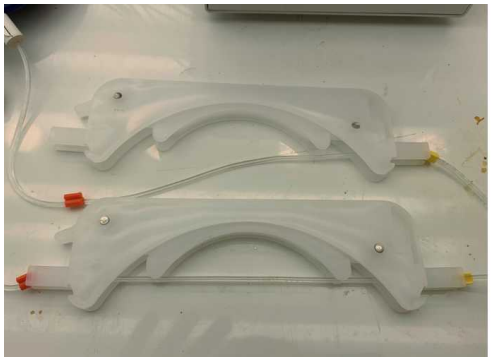
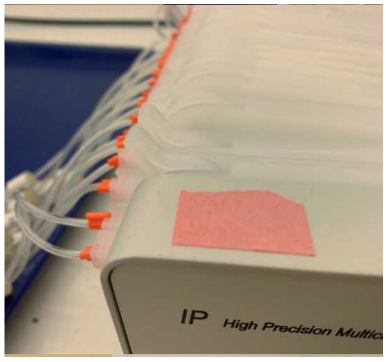
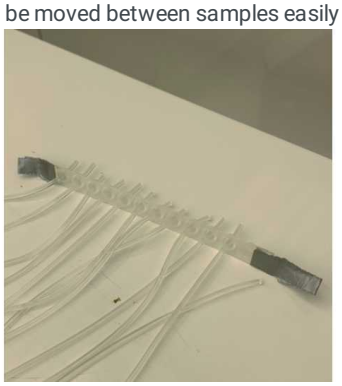
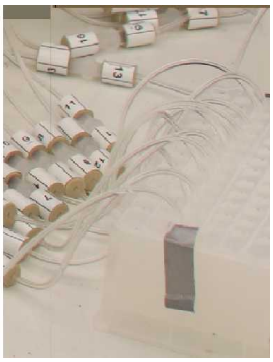
16 Set up peristaltic pumps
Attach tubing to Pump casettes
Insert casettes into Peristatlic pumps
Set tortuosity of the pump using the handles so that a steady and accurate flow rate is achieved (~12-15 notches depends on tubing size used and pump model)

(L) Tubing being attached to peristaltic pump casette. (R) Casette handle is lifted to create the tension just before use.

A 96-well microplate cover (VWR 580 International, LLC, PA, USA) was cut to size and holes drilled into the wells to make a sample tubing holder for sample collection into a deep-well collection plate. Tape was used to fasten the holder in place, so all tubings could be moved between samples easily

17 Experiment setup

Pump 1 is used to fill the chambers with nutrient solution from a header tank at max RPM via tubing connected to the bottom of each chamber. The length of time can be predetermined since the pump speed and tubing length afect this.

After chamber filling with nutrient solution. Pump 1 was used to provide continuous aeration by pumping only air into

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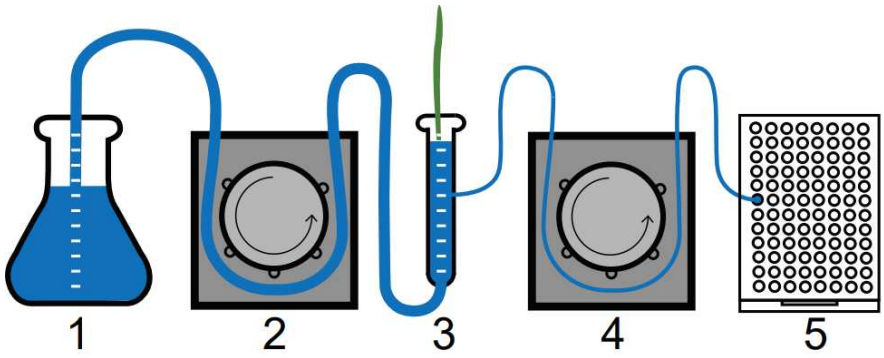
the chambers (24 rotations per minute) by lifting out the intake tubing from the header tank. (Factor for this timing during chamber filling)

Pump 2 was used for periodic sampling of the nutrient solution from the middle of the chamber into a 2 mL 96-well collection plate. 1/2 mL is required for Ion chromatography. Optionally sample for double that ammount so spare sample can be stored in the freezer. The correct sample volume was achieved by running the pump for a predetermined time



18 Transfer plants to uptake setup
Take care that the cone-tainer slot aligns with the sampling tubing inside the chamber (as described in step8)

Wait two minutes for plants to equibrilate.
Start Pump2 and timer, collect first solution sample into collection plate.
Once collected, reverse the flow of Pump 2 to ensure all solution has been expelled back into the chamber.
Pause Pump2
Move sample tubings to second row of collection plate ready for next sample.

Repeat for as many samples as needed. Typical timings will be every hour, but this depends on the sample volume, solution concentration used, and plant size.

19 After all samples have been collected clean out tubing ready for next experiment.
Pump out all remaining solution from system.

Run through tubing **0.1 % Bleach (Sodium hypochlorite)**

3x rinse with dH_2O

Run through tubing **1 % HCl**

3x rinse with dH_2O

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