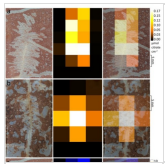

Dec 09, 2020

Citrate sampling and 2D imaging from rhizotron grown roots with ZrOH DGT gels

raphael.tiziani [1], Markus Puschenreiter[2], Erik Smolders[3], Tanja Mimmo[1], José Carlos Herrera[2], Stefano Cesco[1], Jakob Santner[2]

[1]Free University of Bolzano; [2]University of Natural Resources and Life Science Vienna; [3]Katholieke Universiteit Leuven

1 Works for me dx.doi.org/10.17504/protocols.io.bqgnmtve

raphael.tiziani

ABSTRACT

The reliable sampling of root exudates in soil grown plants is experimentally challenging. This method shows how to sample citrate from rhizotron grown plants with ZrOH DGT gels to produce high resolution citrate imaging.

The DGT technique is based on thin hydrogels with homogeneously distributed analyte-selective binding phases and is used for 2D visualization and quantification of the distribution of labile (i.e., reversibly adsorbed) analytes.

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PROTOCOL CITATION

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KEYWORDS

root exudates, citrate sampling, diffusive gradients in thin films, phosphorus deficiency, white lupin

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GUIDELINES

The production of the ZrOH gels, as well as the growth of plants in rhizotrons are well established protocols and can be found in the following publication:

Wagner, Stefan, Christoph Hoefer, Thomas Prohaska, and Jakob Santner. 2020. “Two-Dimensional Visualization and Quantification of Labile, Inorganic Plant Nutrients and Contaminants in Soil.” *JoVE*, no. 163: e61661. https://doi.org/doi:10.3791/61661.

Link:https://www.jove.com/t/61661/two-dimensional-visualization-quantification-labile-inorganic-plant

Here we discuss and show the steps which are specific for citrate sampling.

MATERIALS TEXT

ZrOH gels and plant roots grown in rhizotrons
tweezer and razor blade
glass surface with underlying millimeter paper which acts as cutting surface
ZrOH DGT gels
3 mmol L^{-1} NaN_3 (optional)
0.5 mol L^{-1} NaOH
plastic bag
freezer 4°C
Ion chromatograph or HPLC for citrate analysis

SAFETY WARNINGS

NaN_3 is acutely toxic.

ABSTRACT

The reliable sampling of root exudates in soil grown plants is experimentally challenging. This method shows how to sample citrate from rhizotron grown plants with ZrOH DGT gels to produce high resolution citrate imaging.

The DGT technique is based on thin hydrogels with homogeneously distributed analyte-selective binding phases and is used for 2D visualization and quantification of the distribution of labile (i.e., reversibly adsorbed) analytes.

BEFORE STARTING

The production of the ZrOH gels, as well as the growth of plants in rhizoboxes are well established protocolls and can be found in the following pubblication:

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Pre application

1 Once the rhizobox grown plants show roots on the side with the removable plastic plate you are ready.

For details of how to grow plants in rhizoboxes refer to Wagner et al. 2020 (https://www.jove.com/t/61661/two-dimensional-visualization-quantification-labile-inorganic-plant)

Prepare ZrOH DGT gels in the desired size. For a very detailed description of how to produce the gels please refer to S2.4 and S2.2 of Wagner et al. 2020 (https://www.jove.com/t/61661/two-dimensional-visualization-quantification-labile-inorganic-plant)

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Gel application

2 The gel size to use depends on the root which has to be sampled. Also entire sheets to sample whole root systems are possible.

If imaging is the objective we highly recommend to cut the gel in a shape which allows to remember the orientation of the gel in order to prevent confusion later. Moreover make high quality photos of the root which has to be sampled.

3 The application time of the ZrOH can be personalized and depends on the use case. However generally an application of 16-48h is okay.
for a detailed description of the gel application refer to 3.1 of Wagner et al. (2020) (https://www.jove.com/t/61661/two-dimensional-visualization-quantification-labile-inorganic-plant)

Citrate elution, calculations and image generation

4 After the application the gels can be removed and put into plastic bags together with a few drops of 3 mmol L^{-1} NaN_3, closed airtight and can be stored in the fridge at 4°C for up to approx. 41 days.

Measure the root length or surface if necessary. Alternatively you could also remove the root, remove the adherent soil and assess the fresh and dry weight.

4.1 If the objective is just a **quantification without citrate imaging the gels can now be eluted.**

Calculate the gel volume by multiplying the gel width, length and height (usually 0.4 mm when standard DGT gels such as in Wagner et al. 2020 are used).

Put the gel in the appropriate amount of 0.5 mol L^{-1} NaOH (elution solution) for 24h while shaking gently. It is of crucial importance to keep the elution solution volume to gel volume ratio constant. The ratio needs to be exactly **1 mL 0.5 mol L^{-1} NaOH for 0.196 cm^3 of ZrOH gel (or 5 mL cm^{-3} ZrOH gel).**

4.2 The elution solution can be directly analyzed for citrate by HPLC or IC or other compatible analytical techniques.

As the citrate elution has an efficiency of 89% you have to **multiply the result by 1.11 to get the real value.**

Usually the result of a HPLC is given in mg L^{-1} or mol L^{-1}. By multiplying this with the elution solution volume, a mass or amount can be obtained (mg or mmol).

This amount can then be normalized (divided) on either the gel surface, root surface, root weight or root length. Furthermore the result can be normalized by the gel application duration to get a exudation rate. The final unit could be for instance "µmol citrate cm^{-1} root length h^{-1}".

4.3 When the root geometry is simple (*i.e.* the root is not a cluster root, or does not posses a huge 3D space occupation) the result can be multiplied by a factor of 2 to give an "estimated total exudation". this can be useful when normalizing for instance with root weight.

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5 If the **objective is citrate imaging follow the next steps.**

5.1 Put the gel on a glass surface with underlying millimeter paper which acts as cutting surface together with a few drops of ultrapure sterile water (to prevent dehydration).

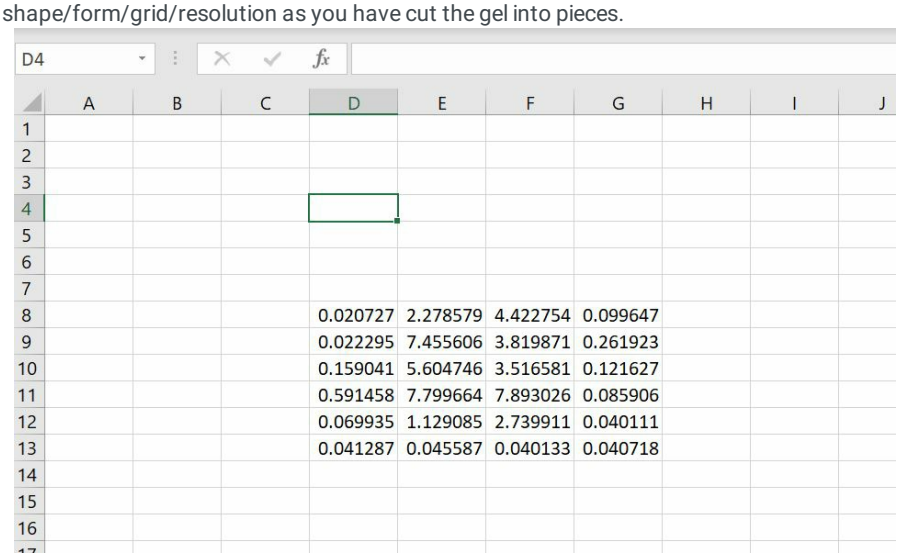
Use a razor blade to cut the gel into small pieces *i.e.* 5x5 mm pieces or whatever size you are interested in.

Put each gel piece into a small eppendorf tube together with the appropriate amount of elution solution (**see 4.1 for the elution)** for 24h. For 5x5 mm ZrOH gel pieces with 0.5 mm thickness 51µL 0.5 mol L^{-1} NaOH is needed.

5.2 After the elution the samples can be analyzed by HPLC or IC or whatever analytical technique for citrate analysis is available. Keep in mind if 51 µL volume is too small for analysis you can add water to increase the volume but you have to consider that you perform a dilution thus you have to multiply the result by the dilution factor.

5.3 The results can than be **calculated/normalized as described in 4.2.**

5.4 In order to generate a 2D citrate image you have to align the results in the same shape/form/grid/resolution as you have cut the gel into pieces.



	A	B	C	D	E	F	G	H	I	J
1										
2										
3										
4										
5										
6										
7										
8				0.020727	2.278579	4.422754	0.099647			
9				0.022295	7.455606	3.819871	0.261923			
10				0.159041	5.604746	3.516581	0.121627			
11				0.591458	7.799664	7.893026	0.085906			
12				0.069935	1.129085	2.739911	0.040111			
13				0.041287	0.045587	0.040133	0.040718			
14										
15										
16										

excel sheet example with 4x6 grid of citrate exudation rates

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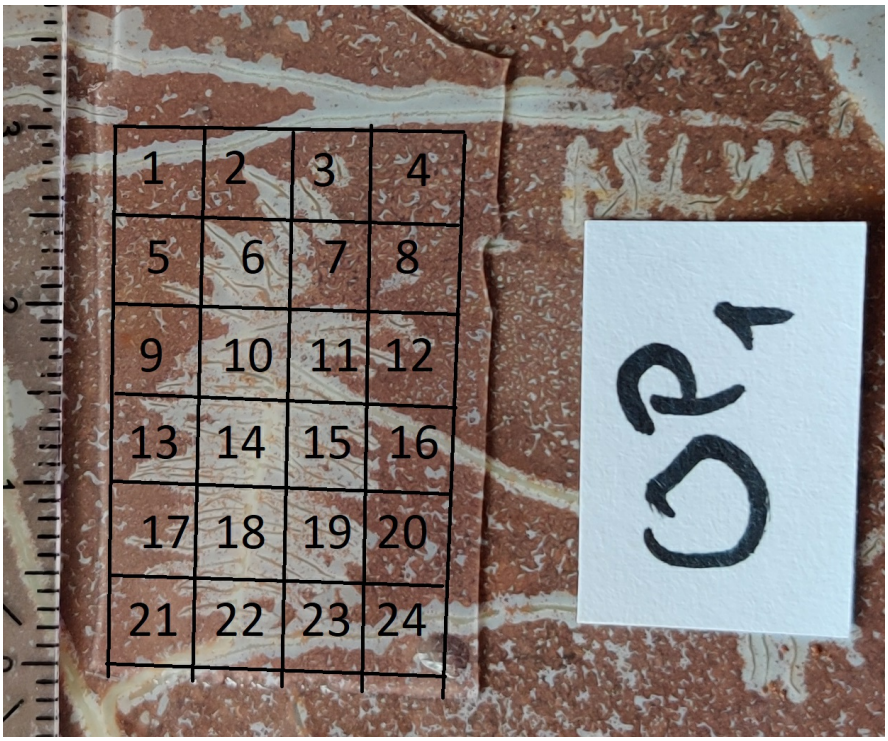

Associated root image to excel sheet shown above. the grid is 4x6 squares. Square nr. 1 corresponds to the first number in the excel sheet above and so on.

5.5 Save the excel file as textmsdos.txt

5.6 Open **ImageJ** (https://imagej.nih.gov/ij/index.html)and import the file as textimage.

Set the interpolation to "none" and image scale at 100 or 1000.

With the option luc up table you can change the color and with the option calibration bar (under tools) you can add a calibration bar.

You can play with this software a bit to generate the image you want.

Save the image in the file type you want (.jpg for instance).

5.7 Overlay the image to the root photo you took earlier with powerpoint, photoshopt, gimp or whatever software you use. Add the calibration bar and safe the file.

You should now be ready and have generated a citrate 2D image like the one below

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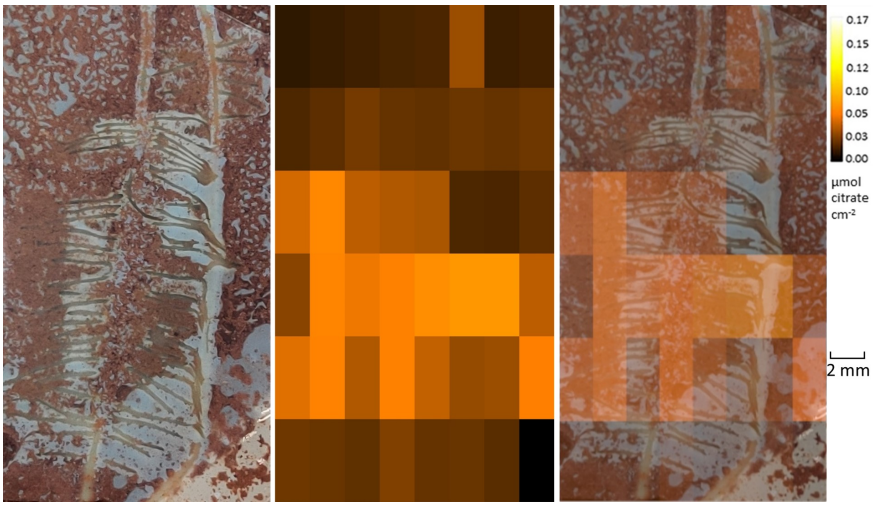

citrate 2D image

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