HYDROGEL-BASED "TRANSPARENT SOILS" FOR ROOT PHENOTYPING *IN VIVO*

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Supplementary Information

This PDF file includes:

Materials and Methods

Figs. S1 to S14

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Other Supplementary Materials for this manuscript include the following:

Movies S1 to S3

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1.0 PROCEDURE FOR MAKING TRANSPARENT SOIL

1.1 CHEMICALS

- Phytagel (Sigma-Aldrich)
- Sodium Alginate (Sigma-Aldrich)
- Magnesium chloride hexahydrate (Alfa Aesar)
- Murashige & Skoog (MS) basal salt mixture with vitamins (product code: M519 from phytotechlab.com)
- Bleach (sodium hypochlorite 5.25%/di water (1/8 volume))
- Ethanol (100%, 200 proof)
- Deionized water (DI water)

1.2 EQUIPMENT AND MATERIALS

- Class II Biosafety Cabinet
- Autoclave (Primus PSS5)
- 500 mL autoclaveable square bottle
- Support stand
- Stopcock (straight, polypropylene)
- 10 mL Syringe with BD Luer-Lok® Tip, BD Medical
- Aquarium air pump
- Stirring hotplate
- Stirring bar
- Sterilite® flip top plastic container 7 5/8"x6 ½" x4 ½" (product code: 1803)
- Sterilite® Latching box
- Fine mesh metal sieve
- Silicone tubing (1/4" ID, 3/8" OD (McMaster-Carr item #5236K87))
- 500 mL and 1L graduated cylinders
- 1 L glass bottles
- 500 mL glass bottles
- Analytical balance
- Digital camera (Nikon 5500, 100 Macro lens)
- Scissors
- Parafilm
- Aluminum foil
- Autoclave indicator tape
- Fisherbrand™ SureOne™ Thin Wall Micropoint Pipette Tips, Universal Fit (1-200 µL)
- Stainless-steel lab spoon

1.3 PROTOCOL (MOVIE S1)

It normally takes three days to make transparent soil. This first day we make all chemical solution and let them cool down; the second day we make the transparent soil and let them soak in the

nutrient solution (daily maximum TS we can make is 10L); the third day we drain out the nutrient soil and the transparent soil is ready for use. Please see the detailed steps as below:

1. Prepare the polymer solution with needed concentration for different experiments (the phytagel and sodium alginate power were mixed with a ratio of 4:1, wt).
2. Prepare $MgCl_2$ solution with needed concentration as the cross linker solution.
3. Prepare nutrient solution with required concentrations.
4. All the solutions were autoclaved with the liquids cycle (sterilization temperature: 121 ^{0}C, sterilization time: 15 min). Before autoclaving, the cap of glass bottles should be loose and covered with aluminum foil. After autoclaving, tighten the cap of bottles and let the solutions cool down to room temperature.
5. Build the automatic dropping system for transparent soil (TS) beads as shown in Movie S1. Drill a hole (3/8" OD) at the bottom of a 500 ml autoclavable square bottle. A tube (10 cm long, 3/8" OD) with a round LEGO® brick inserted in its extremity is then pulled through the drilled hole until a good seal is obtained. A stopcock is connected between this tube and another tube.
6. Autoclave all the materials used for making TS beads: the dropping system, metal sieve, spoon, flip top plastic container, paper towels.
7. Turn on the biosafety cabinet (BSC) and clean with 20% bleach and 70% ethanol before use.
8. Place all the autoclaved solutions and materials inside the BSC after surface sterilizing with 70% ethanol.
9. Surface sterilize 10 mL syringe and 2 pieces of 5 cm×15 cm Parafilm with 70% ethanol. Place them into the BSC.
10. Surface sterilize the hotplate, Aquarium air pump and support stand with 70% ethanol. Place them into BSC.
11. Connect the 10 mL syringe to the end tube of dropping system with the 2 pieces of Parafilm. If smaller size of TS beads are needed, connect a selected needle to the 10 mL syringe.
12. Turn off the stopcock.
13. Transfer the polymer solution (500 mL) into the square bottle of dropping system by passing through a fine mesh metal sieve.
14. Fix the square bottle to the support stand. For making large beads by using Luer-Lok tip without connecting needles, the bottle can be left open. For making smaller beads through needles, the bottle should be capped and connected to the air pump.
15. Place the hotplate under the dropping system. Pour 1 L $MgCl_2$ solution in a plastic container with a stirring bar and place the container on the hotplate. Stir solution at 200 rpm.
16. Turn on the stopcock and start the dropping process.
17. Adjust the stopcock to figure out a suitable dropping speed.
18. Adjust the $MgCl_2$ solution when every 200 mL gel was dropped (polymer:$MgCl_2$ ratio of 1:5, v:v).
19. When dropping process finished, leave beads in $MgCl_2$ solution for 15 min, and collect the TS beads from $MgCl_2$ solution by using metal sieve.
20. Soak the beads in 2-fold concentrated nutrient solution for at least 1h (overnight is probably best) with a ratio of beads: nutrient solution of 1:1 (v:v).
21. Filter the beads from the solution by metal sieve and dry them with the paper towels.
22. The TS beads are ready for use.

1.4 COST OF SET UP IN MAKING TRANSPARENT SOIL

It is very cheap to set up for making transparent soil. The equipment set up for making TS costs around $670, and later used price is $1.25. The cost of chemicals for making 1L transparent soil is $5.83. This price is estimated by using the chemicals (phytagel and sodium alginate) from Sigma-Aldrich Company, where we normally buy chemicals with a high price, and by considering using MS medium as the nutrient medium. The cost could reduce by selecting the products from other companies or using other nutrient medium as the choice.

Table S1. Cost of equipment set up for making transparent soil

Items	cost per each ($)	amount needed	cost ($)	reusable
Autoclaveable square bottle 500ml	15.30	1	15.30	Yes
Support stand	26.61	1	26.61	Yes
Stopcock, straight, polypropylene	31.44	1	31.44	Yes
10ml syringe	0.17	1	0.17	No
18 gauge needle	0.07	1	0.07	No
Tubing 1/4",3/8" (2 inch long)	0.16	2	0.32	Yes
Tubing 1/4",3/8" (10 inch long)	0.80	1	0.80	Yes
Parafilm	0.17	1	0.17	NO
Aquarium Air Pump,Product TitleAqua Culture: 20-60 Gallon, Double Outlet	10.87	1	10.87	Yes
Stirring hotplate	563.95	1	563.95	Yes
Stirring bar	5.41	1	5.41	Yes
Metal sieve	13.64	1	13.64	Yes
paper tower,white	4.20	0.2	0.84	No
Set up price			**669.59**	
Later used price			**1.25**	

Table S2. Cost of chemicals used for making 1L transparent soil

Items	company	size(g)	price($)	cost per gram	amount needed(gm)	cost ($)
Phytagel	Sigma-Aldrich, Inc., USA	500	172.50	0.345	9.6	3.31
Sodium Alginate	Sigma-Aldrich, Inc., USA	1000	137.00	0.14	2.4	0.33
MS medium	PhytoTechnology Laboratories, LLC™	220.5	28.97	0.13	8.86	1.16
Magnesium chloride hexahydrate	Alfa Aesar	500	51.50	0.10	10	1.03
					total	5.83

1.5 SCALE UP MAKING UP TRANSPARENT SOIL

- A system (Figure S1) was designed to produce larger amounts of TS beads. Multiple holes were drilled on the bottom of a Sterilite® small clip box and Fisherbrand™ SureOne™ Thin Wall Micropoint pipette tips (1-200 µL) were cut to fit and pushed through the holes.
- 1L of polymer solution was added into the small clip box while 5L of $MgCl_2$ solution was added into the medium clip box once. Then the small clip box was nested on the medium clip and the polymer solution can be dropped though the pipette tips.
- By using this system, 1L of TS beads can be produced in 10 mins and the cost of the set up can be reduced to $44.37. No stirring is required
- This approach is scalable to larger areas either through the use of multiple boxes or larger boxes.

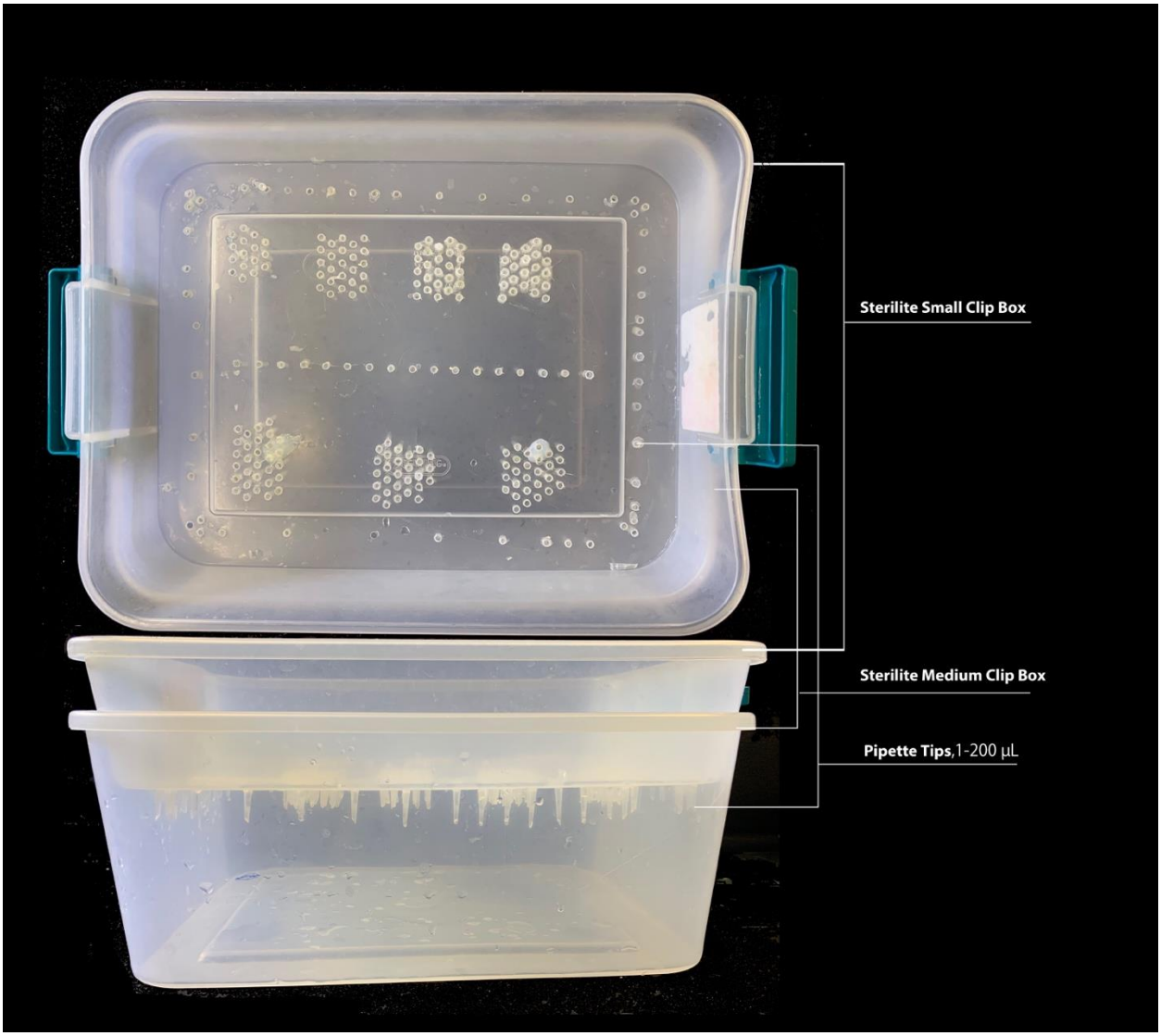

Figure S1. The system to scale up making transparent soil.

Table S3. Cost of equipment set up for scaling up making transparent soil

Items	cost per each ($)	amount needed	cost ($)	reusable
Sterilite® small clip box	8.69	1	8.69	Yes
Support stand	16.20	1	16.20	Yes
Stopcock, straight, polypropylene	0.05	100	5	Yes
Metal sieve	13.64	1	13.64	Yes
paper tower,white	4.20	0.2	0.84	No
Set up price			**44.37**	
Later used price			**0.84**	

2.0 CHARACTERIZATION OF TRANSPARENT SOIL

2.1 TRANSPARENCY ANALYSIS

The TS beads were prepared with different concentrations of polymer and crosslinker (10 gel concentrations ranging from 0.4% to 1.3%; 6 cross linker ($MgCl_2$) concentrations: 5, 7.5,10, 20, 50, 100 mM). Beads did not form well when the concentration of $MgCl_2$ is 5 mM due to the insufficient concentration of cross-linker. Beads did not form well also when $MgCl_2$ is 7.5 mM and polymer concentrations are lower than 1.0%. Therefore, 44 different types of TS beads were made by different gel and cross linker concentrations.

44 types of beads samples were placed into separate cuvettes (10 mL, plastic) with growth medium (0.5 MS and 1 MS, separately). Bubbles inside the samples were slightly removed by needles. There are 88 samples in total. All the samples were characterized by UV-Vis absorbance/transmittance (Agilent 8453 Diode Array UV-Vis spectroscopy). The transparency of TS beads is represented by the UV-Vis transmittance. According to the results from different replicates (Figure S2a and b), our TS beads showed the highest transparency in the range of wavelength close to 1080 nm. This result indicated that we should use infrared camera instead of visible camera to observe better root images *in vivo*.

The transparency of TS beads could limit the width of plant growth box for the visibility of root inside of the box. Here we showed the transparency of TS beads varying with different width of box when saturated with 0.5 MS (Figure 1c) or 1 MS solution (Figure S2c). The allowed width of growth box is calculated according to Beer-Lambert law for homogenous material.

$$T = 10^{-A} = 10^{-(\varepsilon\, l\, c)} \qquad (1)$$

Where T is the transmittance of the material; A is the absorbance of the material; ε is the molar attenuation coefficient of the material; l is the path length of the beam of light through the material; c is the amount concentration of the material.

Therefore,

$$l = \frac{log_{10}\frac{1}{T}}{\varepsilon\, c} \tag{2}$$

2.2 MECHANICAL PROPERTIES ANALYSIS

44 types of TS beads were prepared as described in 2.1. A small amount of beads were slowly added into a 20 mL measuring cylinder with 10 mL DI water until the volume of mixture reached to 12 mL. Collect the beads in the cylinder with a fine mesh metal sieve and the beads were surface dried by paper towels. This ensemble of beads was considered to have a volume of 2 mL.

The collected beads were placed into a 10 mL syringe (volume is larger than 2 ml) and the plug was put back inside the syringe. We gradually increased the load on the top of the plug and waited until the plug stopped going down and then add more weights until the bottom of plug reached to 2 ml. This experiment measured the load required to fully collapse the transparent soil. The loading (N) for each sample was recorded. Data were showed in Figure 1d, and Figure S2d.

To avoid the bottom layer of TS to be flattened by the gravity of the beads above it, we calculated the maximum loading depth of TS as follows:

$$\frac{N_1}{S_1} = \frac{N_2}{S_2} \tag{2}$$

N_1 is the gravity of loading weights; S_1 is the area of the syringe plug bottom surface; N_2 is the gravity of above beads; S_2 is the area of the interface between above layer and the bottom layer beads.

$$N_1 = mg \tag{3}$$

where m is the mass of loading weight; g is the uniform acceleration.

$$S_1 = \frac{V_1}{h_1} \tag{4}$$

where V1 is the volume of the syringe (10 mL); h1 is the height of the syringe (6.25 cm).

$$S_2 = \frac{V_2}{h} = \frac{M}{\rho h} = \frac{N_2}{g\rho h} \tag{5}$$

where V2 is the volume of above beads; h is the height of above beads; ϱ is the density of TS beads; M is the mass of above beads.

So

$$\frac{mg}{\frac{V_1}{h_1}} = \frac{N_2}{\frac{N_2}{g\rho h}} \tag{6}$$

$$h = \frac{m\, h_1}{\rho V_1} \tag{7}$$

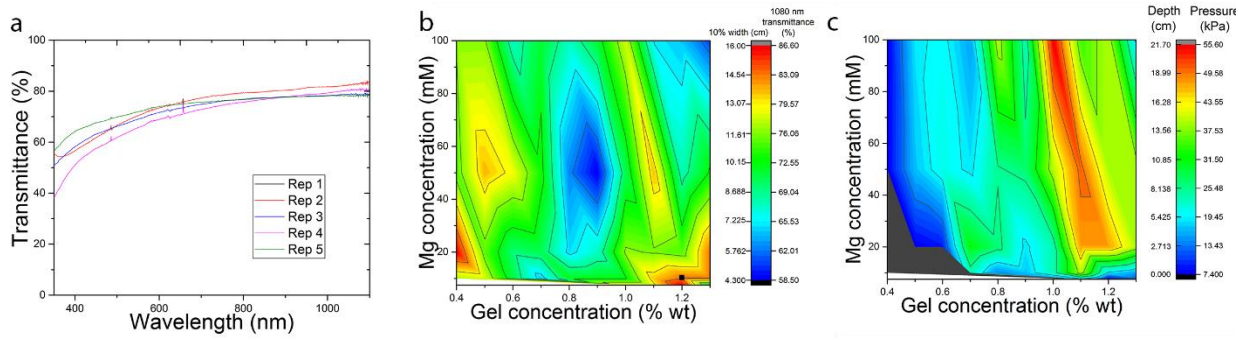

Figure S2. Transparent soil characterization. a, transmittance of beads made with 1.2% mixed gel and 10 mM $MgCl_2$ with 5 replicates which were made at a different time (Rep 1 2017 Feb 21st, Rep 2 2017 Oct 13th, Rep 3 2018 Jan 8th, Rep 4 2018 May 25th , Rep 5 2018 Jun 12th). **b,** Transmittance of 1080 nm infrared through 1 MS transparent soil made by different gel/$MgCl_2$ concentration and pot width allowed with 10% transmittance. **c,** Loading on 1 MS transparent soil system when there is no pore and pot depth allowed.

2.3 PARTICLE SIZE ANALYSIS

The first experiment was to determine the particle diameter of the TS beads (1.2% polymer concentration and 10 mM $MgCl_2$ solution) changed with the bead sizes. Five different particle sizes were made by various needles: Luer-Lok tip without needle, 16G, 18G, 22G, 25G needles. Ten TS beads for each type of beads were placed on a black paper with a 1 cm scale and an image was took by camera (Nikon 5500).

The second experiment was to measure the particle diameter of the TS beads changing with time with or without plants. The beads made through the Luer-Lok tip without needle were selected in this experiment. One set of the beads were placed in a sealed box (the same type as the one for *B. rapa* growth). Another set of the beads were sampled every day from the beads for brassica growth (more details for the plant growth will be see below). Ten TS beads from the two sets of treatments were sampled and were imaged every day for 15 days.

All the images were analyzed by the software ImageJ (https://imagej.nih.gov/ij/index.html) to determine particle diameter.

2.4 POROSITY ANALYSIS

TS beads were made in the same way as described in 2.3. 2ml of TS beads were put into a 5 mL measuring cylinder and DI water was added inside drop by drop from the top until the water level reached the top of beads. The volume of the added water were recorded as effective pore volume. Air bubbles were removed using a needle and then we added more water until the water level reached the top of beads again. The total volume of added water were recorded as total pore volume. The effective and total porosity of TS beads were calculated by effective and total pore volume divided by total volume, respectively.

2.5 NUTRIENT CONTENT OF TS BEADS

The nutrient content can be controlled by soaking the TS beads in different nutrient solutions. The TS beads were made of 1.2% gel concentration and 10 mM $MgCl_2$ solution (size controlled with Luer-Lok tip without needle). The 10 mM $MgCl_2$ solution and the $MgCl_2$ solution after cross linking were collected. The beads were soaked into MS media (1×MS for *Brassica rapa.* growth, 2×MS for *G. max* growth), or soil extract media (for *G. max* growth). The 1×MS or 2×MS solution before and after soaking were collected. After soaking, the beads were collected and dried by paper towel.

3.0 CHARACTERIZATION OF SODIUM ALGINATE

Alginate is natural unbranched polysaccharides from brown algae (1). The alginate polymer is made up of two monomeric units: b-(1→ 4) linked D − mannuronic acid (M) residues and a − (1 →4)-linked L-guluronic acid (G) residues. The basic structure of alginates consist of linear unbranched units of polymers made up of monomers arranged in blocks of M and G residues interspersed with regions containing alternating M-G sequence within the structure (2,3). Different sodium alginate products may contain different proportions and sequences of M and G residues that determine the molecular weight and physical properties of the alginate and their derived gels. Our transparent soil was formed by the mixture of phytagel and sodium alginate based on the method of ionic crosslinking with cations. It was reported that G-blocks can more tightly associate to cations compared to M-blocks (4). Therefore, we hypothesize that higher G/M ratio of alginate can provide a higher crosslink density and result in lower transparency of TS bead.

We collected five sodium alginate products from different companies or batches: one from Acros Organic with LOT number A0376873 (Ac), one from Spectrum® with LOT number ZT1147 (Sp), and three from Sigma-Aldrich® with LOT number MKBK3379V (Si1) and MKCC4541 (Si2I and Si2B).

^{13}C-NMR was conducted to determine the G/M ratio for different products with the method as reported in previous studies (5). Briefly, the samples were firstly hydrolyzed by mixing 100 mg product with 200 mL 1N H_2SO_4 in boiling water bath for 30 min, to decrease the viscosity of solution (6). The hydrolyzed polymers were then dissolved into 1ml D_2O with an adjusted pD of 7. ^{13}C NMR data were acquired at 90°C on a Bruker NMR spectrometer equipped with a triple resonance z-gradient cryoprobe operating at 600MHz. The number of scans were approximately 5000 (optimized depending on S/N), 32k points collected, and a recycle delay of 0.8s. Data were processed and analyzed using Mnova NMR software (http://mestrelab.com/software/mnova/nmr/). Assignments were based on previous work (5). The ^{13}C-NMR spectrum is shown in Figure S3a. The G/M ratio of different products is shown in Table. S4.

TS beads were made using the five different alginate products with the optimum concentration. Five types of TS beads were then submitted to UV-Vis test and mechanical property test with the methods as mentioned in 2.1 and 2.2. The absorbance of TS beads was calculated based on Beer-Lambert Law.

Results show that the G/M ratio of alginate influences the transparency of TS beads (for equal concentrations of the polymer and crosslinker solutions). The Sp product has the highest G/M ratio, but the TS beads made from Sp have the lowest transparency (Table S4, Figure S3b); meanwhile, the Si1 product has the lowest G/M ratio but the TS bead made from it have the highest transparency, across all the products. These findings support our hypothesis.

Alginate with different G/M ratio also change the mechanical properties of TS beads (Figure S3c). Generally, the loading property of TS beads seemed to increase with the increasing G/M ratio of alginate, with the exception of the Ac product.

We find a way to increase the transparency of TS beads when the G/M ratio of alginate is high, by decreasing the concentration of cross linker concentration. We conducted an experiment by using the Sp alginate to make TS beads and cross linked with four different $MgCl_2$ concentration (5, 7.5, 8, 10 mM) (Figure S3d) and the beads were submitted to UV-Vis test. We found that the transmittance of TS beads increased by 12.89% from 10 mM to 5 mM $MgCl_2$ at 1080 nm (65.45% to 78.34%), thereby allowing the match the transparency of the Si1 product. This results indicate that each laboratory might want to adapt the concentration of the $MgCl_2$ solution to the specific batch of alginate they receive.

Table S4. G/M ratio variation in different alginate products: Fg, the proportion of G-block; Fm, the proportion of M-block; G/M ratio, the ratio of G-block and M-block.

	Fg	**Fm**	**G/M ratio**
Si 1	0.413	0.587	0.7
Si 2I	0.491	0.509	1.0
Si 2B	0.500	0.500	1.0
Sp	0.622	0.378	1.6
Ac	0.484	0.516	0.9

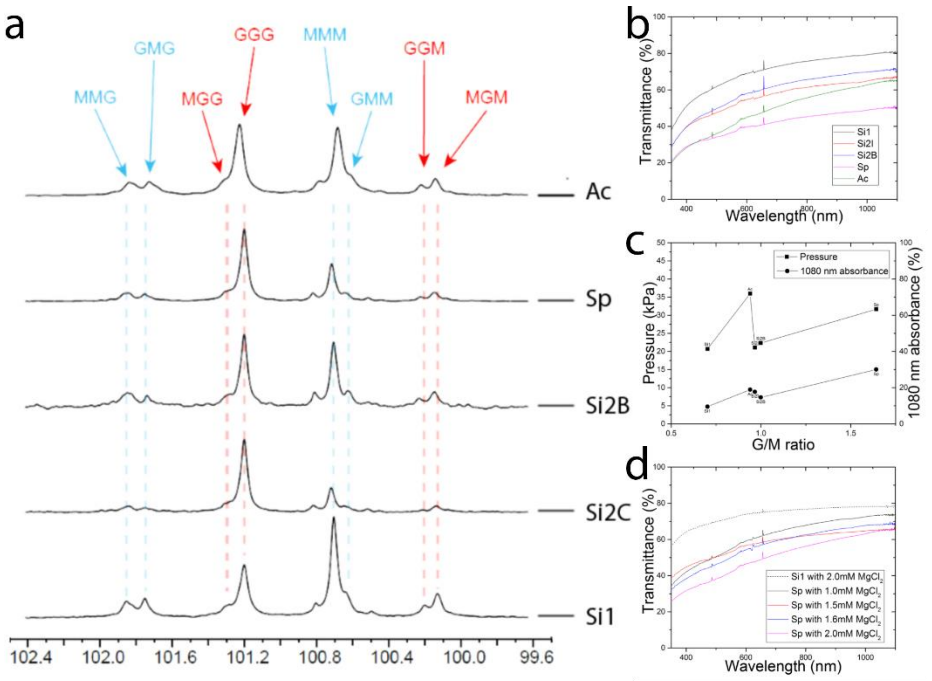

Fig S3. **a**, ^{13}C-NMR spectrum of five different sodium alginate products (Si1, Si2I, Si2B, Sp, Ac). **b,** transmittance of visible light through 1 × MS TS beads made by 1.2% gel and 10mM $MgCl_2$ and transmittance is test by UV-Vis with DI water as background. **c,** relationship between mechanical property and absorbance of TS beads, and the G/M ratio of sodium alginate. The absorbance is calculated from UV-Vis results by Beer-Lambert Law $T = 10^{-A}$. **d,** transmittance of various TS beads made by mixing gel with Sp and $MgCl_2$ of various concentration. Transmittance is test by UV-Vis with DI water as background.

4.0 APPLICATION OF TRANSPARENT SOIL IN PLANTS GROWTH

4.1 *BRASSICA RAPA* GROWTH IN TRANSPARENT SOIL

Model plant *B. rapa* (Wisconsin Fast Plants; Astro plants, dwf1) was selected to evaluate plant root development in TS media *in vivo*. Briefly, *B. rapa* seeds were placed in Petri dish and were surface-disinfected by covering with 70% ethanol for 1 minute in the biosafety cabinet and then soaking in 20% bleach for 4 minute, followed five rinses with sterile distilled water. The germination system and process of *B. rapa* was reported in our previous study (7). After 4 d of germination, *B. rapa* were transplanted in individual HOME systems and grown under 24 h light condition (light intensity of ~200 µmol m^2 s^{-1}). The HOME systems (design reported in previous study (7)) include a top node (size of 5 cm × 5 cm × 5 cm) for shoot growth, and a bottom node (size of 5 cm × 5 cm × 5 cm) for root growth.

B. rapa was grown in TS and hydroponics with 4 replicates, respectively. TS beads were made of 1.2% gel concentration and 10 mM $MgCl_2$ solution (size controlled with Luer-Lok tip), then soaked in 1 MS (1:1, v:v) for 1 hr, leading to a 0.5MS TS. Hydroponics were made of 0.5 MS solution.

Time-lapse root phenotype of *B. rapa* was evaluated during day 6 to 11 after transplanting. Images were taken at the same time each day in a dark room without any visible light. Before imaging, TS beads were saturated with 0.5 MS and the air bubbles in the pores were removed by shaking or slightly removed by inoculating loop. The root nodes for each plant were photographed by an infrared camera with flash light source which was covered with a near IR filter [preparation is given below] under the HOME system. The filter was applied to block all the visible lights. The MS solution was drained out from the node after imaging in TS treatment.

The NIR filter was custom made with Bi_2S_3 colloidal nanowires. Bi_2S_3 nanowire dispersion in toluene was used to absorb/filter out the light in the UV and Vis range because it shows very high extinction coefficient in this range (8). It was first prepared using a previously reported protocol, and then injected into a colorless glass container (4×4×0.3 cm) with two parallel flat smooth faces. According to the reported extinction coefficient, a concentration of 0.96 mM would give a transmittance lower than 5% in the UV and Vis range. The practical advantage of this filter is that it can be easily made over large areas for minimal cost.

Table S5. Cost of setting up NIR imaging.

Items	cost per each ($)	amount needed	cost ($)	reusable
Nikon DSLR D5100 Camera w/Nikon 18-55mm f/3.5-5.6 G VR Lens	524.00	1	524.00	Yes
Lab Lifting Jack Platform (8'X8')	69.55	2	139.10	Yes
NIR filter	< 1	1	< 1	Yes
Set up price			**664.10**	
Later used price			**0**	

BRASSICA RAPA GROW IN TRANSPARENT SOIL WITH DIFFERENT PORE SIZES

B. rapa was grown in various size TS beads and hydroponics with 3 replicates, respectively. TS beads were made of 1.2% gel concentration and 10 Mm $MgCl_2$. Luer-Lok tip, 18 G, 22 G , 25 G needles were used as size controlling as described in 2.3. TS beads were soaked in 1 MS (1:1, v:v) for 1 hr, leading to a 0.5MS TS. Hydroponics were made of 0.5 MS solution. Time-lapse root phenotype was evaluated during day 6 to 11 after transplanting. IR images were taken as described before.

Time-lapse images (Figure S4) showed all of TS beads with various size allow for root phenotyping *in vivo* by photography and provide different root phenotype comparing with hydroponic, while the differences of root phenotype in various size TS beads are not obvious.

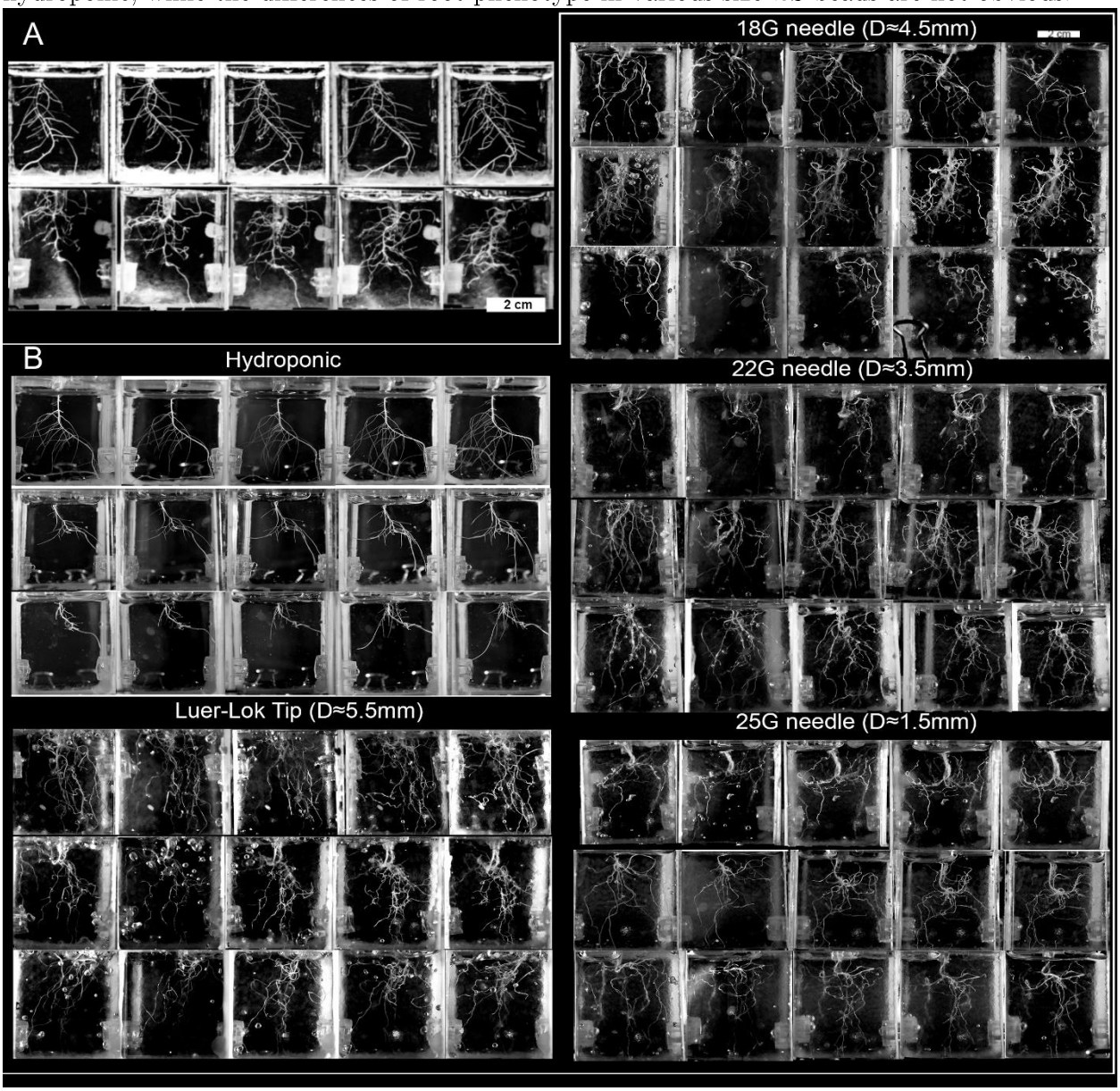

Figure S4 Time lapse image *in vivo* root phenotyping of *B. rapa*. A. Time lapse (24 hr interval)

in vivo root phenotyping of *B. rapa* growing in 0.5 MS hydroponic medium (top) and in TS (bottom) between day 7 and day 11 from transplanting. **B.** Time lapse (24 hr interval) *in vivo* root phenotyping of *B. rapa* growing in 0.5 MS hydroponic medium (line 1) and in different particle size TS (line 2-5) between day 7 and day 11 from transplanting.

4.2 GLYCINE MAX GROWTH IN TRANSPARENT SOIL

Glycine max were selected to study the root phonotype grown in TS in comparison with hydroponics and soil.

GERMINATION OF G. MAX SEEDS

Seeds (IA 2102) were obtained from the Committee for Agriculture Development, Iowa State University. *G. max* seeds were placed on a Petri dish and surface-disinfected by soaking in 100% ethanol (20 mL) with a vigorous shaking for 30 seconds, followed with one rinse with sterile distilled water, then soaking in 100% bleach with a vigorous shaking for 60 seconds, and five rinses with sterile distilled water.

Seeds were poured onto a paper towel and dried at least 20 min. Seeds were then placed on the top side of moistened germination paper with at least 1 inch space between seeds. Paper were rolled width-wise from right to left and loosely secured using a rubber band, then placed inside a Magenta® box with some distilled water to keep paper moist. Magenta box was stored in a sealed container in a dark place at a temperature of 25 ^{0}C.

After 4 days of germination, *G. max* was transplanted in three different growth media including hydroponics, TS, and soil for further growth with 9 replicates. To have equivalent nutrient levels across the three growth media, soil extract (from the same soil we used as soil treatment), was used for hydroponics, and 2-fold concentrated soil extract was used for TS preparation.

Soil was collected at 0-10 cm depth from a corn field in the Iowa State University Agronomy and Agricultural Engineering Research Farm (41°55′ N, 93°45′ W), in Boone County, IA, USA. Soils were primarily Nicollet loam (fine-loamy, mixed, superactive, mesic Aquic Hapludoll) with, on average, 24.24 g total C kg^{-1}, 1.62 g total N kg^{-1}, pH of 6.35, 22 mg mineral N kg^{-1}, and 64 mg Mehlich-3 P kg^{-1}.

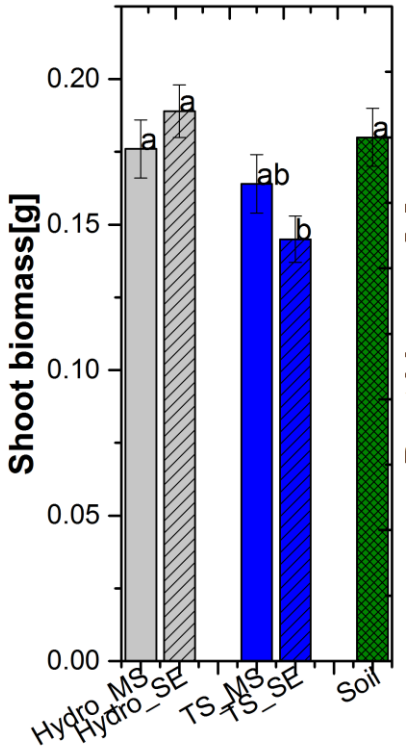

Figure S5. Biomass comparisons of *G.max* plants grown in MS hydroponics, in soil extract hydroponic, in MS transparent soil system, in soil extract transparent soil system and in soil. Error bars for biomass represent standard deviation (n=4). Different letters above the bars for each property indicate significant difference ($P < 0.05$, one way ANOVA followed by Tukey's test). Hydro, hydroponics; TS, transparent soil; SE, soil extract.

To test whether soil extract was suitable as a growth media, we compared *G. max* biomass (14 days of growth in controlled environments since transplanting) for the following treatments: hydroponics (MS media), hydroponics (soil extract), TS (MS media), TS (soil extract), and soil. We found no significant difference of shoot biomass between the different nutrient media, indicating that soil extract is not growth limiting.

G. max was planted in a designed soybean growth box (Figure S6 and Movie S2). This box is made of transparent polycarbonate sheets, which constitutes a tope node (size of 5 cm × 10 cm × 20 cm) for shoot growth, and a bottom node (size of 5 cm × 10 cm × 10 cm) for root growth. The process of setting up box is shown in Movie S2.

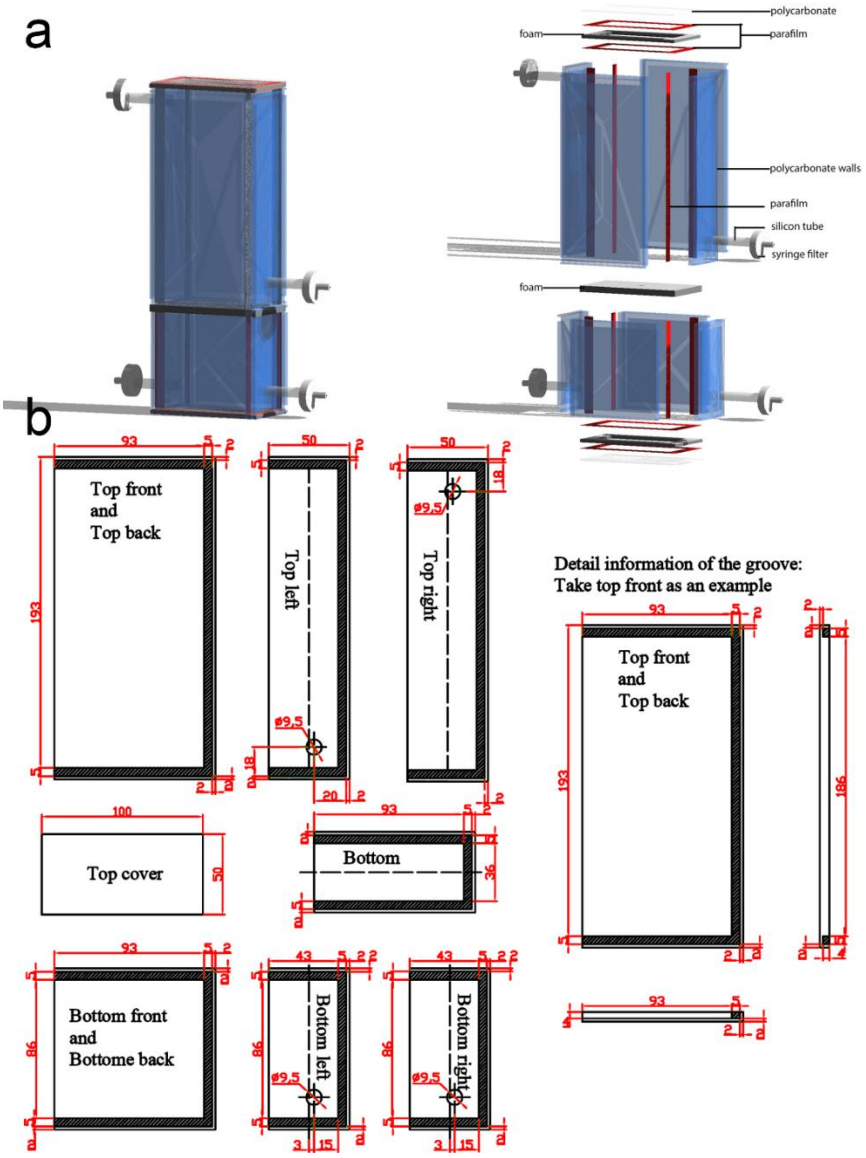

Figure S6. a, structure of soybean growth box. **b,** design of soybean growth box.

G. max was grown with a 16:8h light/dark cycle (light intensity of ~200 µmol m^2 s^{-1}) with a controlled temperature of 25 ^{0}C. The root nodes were covered by alumni foil to avoid light influence. Soil treatment was installed with a bulk density of 1.2 g m^3 and with moisture condition at 80% of field capacity.

During growing period, hydroponics and TS treatments were replenished with nutrient every 3 d. Hydroponics treatment was renewed with new soil extract solution and TS beads was saturated with soil extract solution for 1 h and drained out. Time lapse images were taken as described in 4.1 for hydroponic and TS treatment (Figure S11a). Every 2 plants in soil treatment were destructively sampled every day for root imaging (Figure S11c). Rest of plants were harvested at 12 d after transplanting .

Shoot and root samples were collected separately by cutting. For soil treatment, root samples were removed from soil by rinsing in running tap water, collected on a 2-mm mesh sieve. Roots

in hydroponics and TS were collected directly. Images of root are taken for root morphology analysis.(Figure S11b)

In another batch of *G.max* experiment, plants were germinated and grown in the same way. After harvesting, four replicates of samples for each treatment were chosen randomly for gene expression analysis (Set 1); the remaining replicates of samples were submitted for dry biomass measurement (Set 2).

All root samples were placed in a black plate with RNA free water (samples for gene expression analysis) or DI water (normal samples), dispersed using tweezers to avoid the overlap of roots, and imaged using digital camera (Nikon 5500, Japen) for root morphology analysis. After imaging and fresh biomass weighing, set 1 of root samples were frozen immediately at -80 ^{0}C for further analysis; set 2 of shoot and root samples were dried in a forced draft oven at 90 ^{0}C for 3 d to measure dry matter weights.

ROOT MORPHOLOGY ANALYSIS

The main root length, total length, average diameter, convex area and surface area of the roots were measured with the software MATLAB using the in-house software ARIA. Secondary branch numbers of root was counted by hand and secondary root density was calculated by secondary branch number divided by primary root length.

Blue line shows the path of the main root. The horizontal red line illustrates the scale of pixel to cm. This line is two cm and the number of pixels covered by this line was computed for each image individually to convert pixel to cm. The yellow circle shows the starting point of the root. This point is manually annotated. Figure S7 shows two sample images with the main root marked.

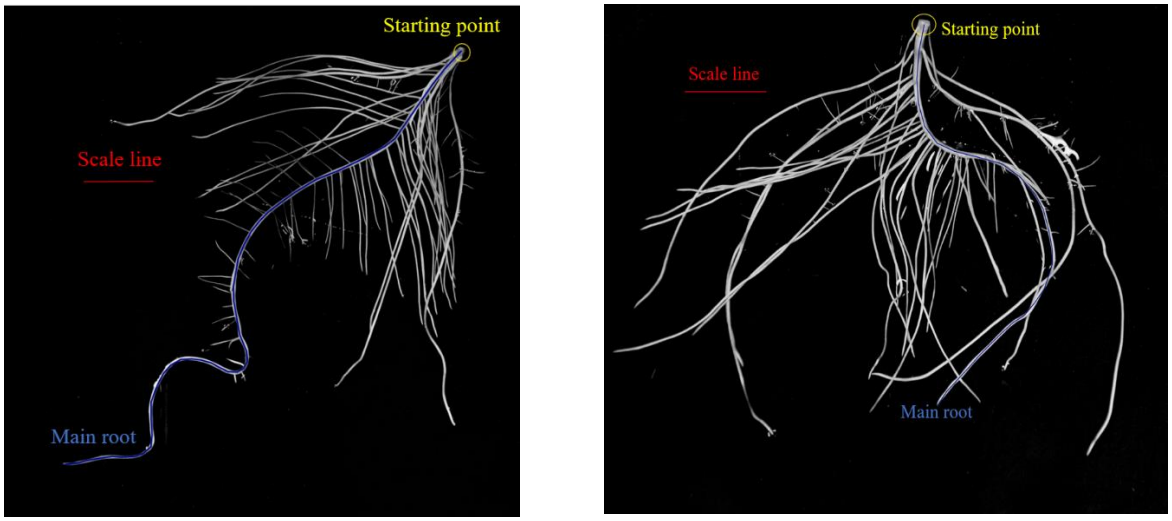

Figure S7. Two sample images of hydro hydroponic root. Yellow circle denotes the starting point of root. Horizontal red line uses for scale conversion. Blue line tracks the main root path.

These lines were detected by three threshold values for each color of red, green and blue. Yellow, red and blue colors were selected in order to automatically discern them. Each color channel was individually segmented using threshold values in the RGB color space. The blue channel result was converted into a binary image and the longest path of the binary image was calculated as the length of the main root. The longest path was computed using Djisktra algorithm (9). The extracted main root can be seen in Figure S8 .

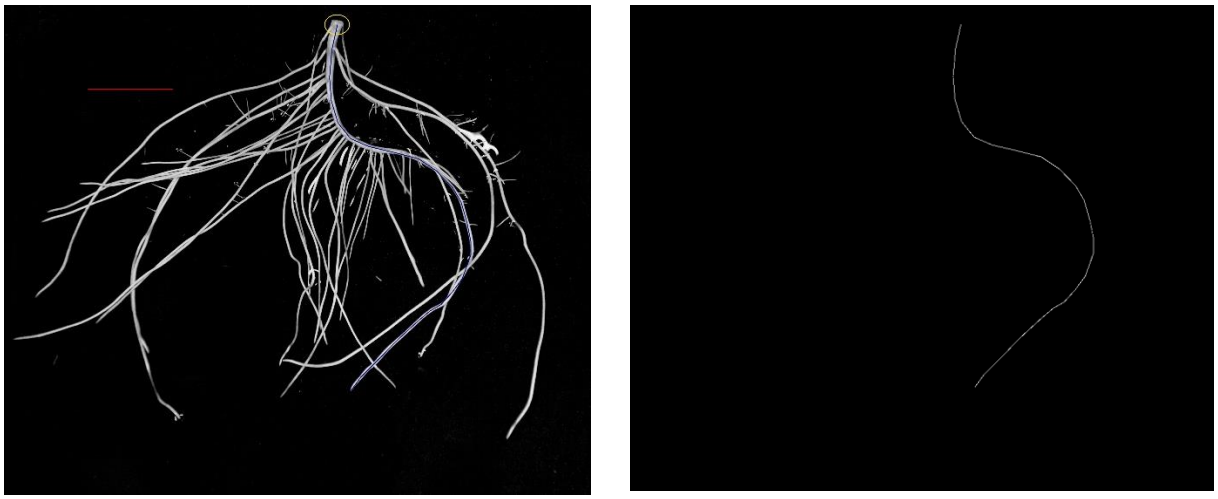

Figure S8. The main root extracted by blue line and compatible threshold values

The total number of pixels is the total area covered by the root and is called surface area. A skeletonized image was obtained from this image. Then the total number of pixels of skeleton image was computed as the total root length. The average width was calculated by division of total area by total root length (figure S9).

(a) (b)

Figure S9. Total area was obtained by counting the total number of non-zero pixels of the binary image (a) and total root length was calculated by counting the total number of non-zero pixels of the skelton image (b)

The exterior boundary location of the binary image as well the interior hole boundary was computed using Moore-Neighbor tracing algorithm (10). The nearest neighbor in the boundary location for each query point in skeleton image. The distance between two pair points is the radius

of the root for that location assuming that the root cross-section is circular. Subsequently, the surface area and volume were computed using the following equations of number 1 and 2.

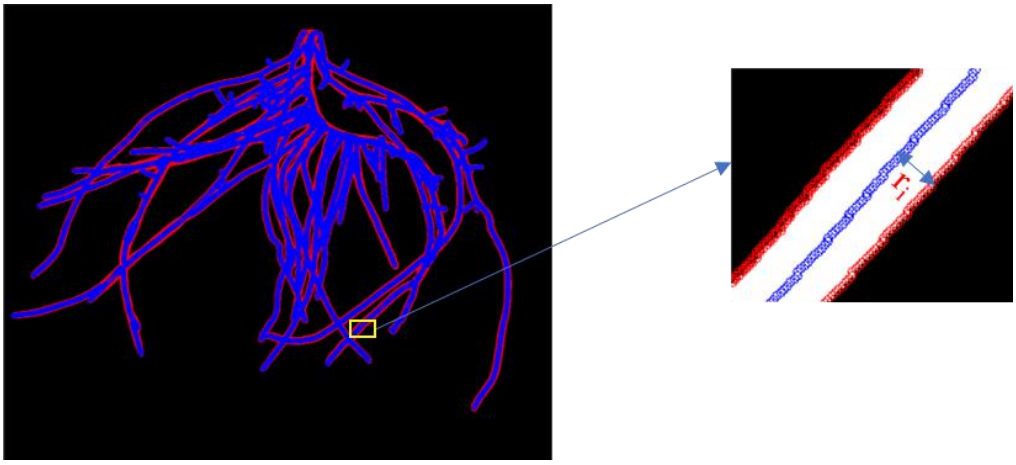

Figure S10. Boundary and skeleton points were depicted by red and blue colors, respectively. The nearest point to each blue dots were selected among red ones. The distance between these two lines was considered as the radius of that location named as r_i

$$surface\ area = \sum_{i=1}^{n} 2\pi r_i \tag{1}$$

$$volume = \sum_{i=1}^{n} \pi r_i^2 \tag{2}$$

The specific root length was obtained based on equation 3. It was classified into eight diameter classes. The percentage of length in each category of radius (e.g., <0.1mm, 0.1-0.2mm; 0.2-0.3; ... ; >0.8mm) in total root length were calculated.

$$specific\ root\ length = \frac{total\ root\ length}{volume} \tag{3}$$

All these data were checked for normality based on the Shapiro-Wilk test using R studio and transformed using the Box-Cox power transformation when necessary. The treatment effects were tested by one-way ANOVA. Differences among the least squares means for all treatment pairs were tested using the Tukey HSD test at a significant level of $P = 0.05$.

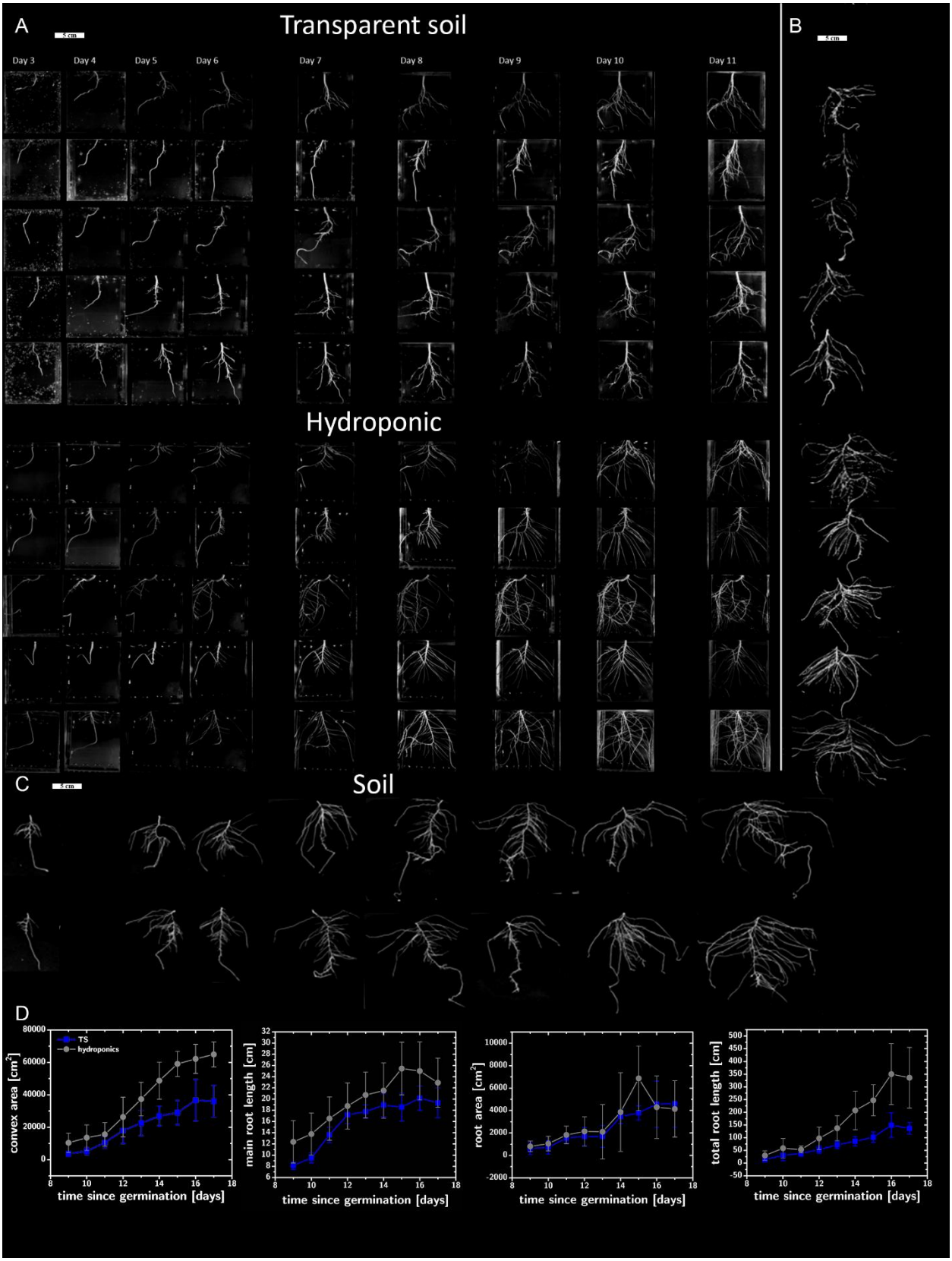
A
5 cm
Transparent soil
Day 3
Day 4
Day 5
Day 6
Day 7
Day 8
Day 9
Day 10
Day 11
B
5 cm
Hydroponic
C
5 cm
Soil
D
TS
hydroponics
convex area [cm²]
time since germination [days]
main root length [cm]
time since germination [days]
root area [cm²]
time since germination [days]
total root length [cm]
time since germination [days]

Figure S11. *G.max* Root Phenotyping in hydroponic, TS and soil. A. Time lapse (24 hr interval) *in vivo* root phenotyping of *G.max* growing in TS and in hydroponic between day 3 and day 11 from transplanting. **B.** Comparison of *G. max* roots grown in TS and hydroponic. **B.** Comparison of *G. max* roots grown in sterilized soil in different days of growth. **C.** Comparison of convex area, main root length, root area and total root length in *G.max* plants grown in hydroponics and TS. Error bars indicate standard deviation (n=5).

GENE EXPRESSION ANALYSIS OF SOYBEAN ROOTS

3 Genes related to lateral root and root tip development and 5 genes related to nutrient, water deficiency and flooding stress were selected in this study. The genes and primers were listed in Table S2. Total RNA was extracted from roots of soybean, each with four biological replicates. A total of 12 samples were isolated to extract RNA according to the manufacturer's instructions with the use of Qiagen RNeasy Plant Mini Kit. A total of 50-100 mg root were used to isolate RNA for each sample, respectively. Genomic DNA were removed from RNA samples using Invitrogen™ DNA-free™ DNA Removal kit (Catalog number: AM1906). Then about 1 µg RNA was used to configure 20 µL system to synthesize cDNA (Applied biosystem high capacity cDNA reverse transcription Kit, Catalog number: 4374966). The constitutive expression gene CYP2 (GLYMA.12G024700) was used as a reference gene and each sample was measured with three replicates. The qPCR was conducted on a QuantStudio 5 real-time PCR system (Applied Biosystems, Forster City, CA, USA) with the use of Powerup™ SYBR™ green Master Mix. Primer sets (300-800 nM final concentrations for each primer) and 5 µL cDNA SYBR Green mix were used in a final volume of 10 µL per well. The thermal profile of the qRT-PCR reactions was 95 °C for 10 min, and 40 amplification cycles of 95 °C for 15 s and 60 °C for 1 min. The QuantStudio™ Design & Analysis Software v1.4.3 software was applied to analyze the data using an Rn threshold of 0.015 to obtain the cycle threshold (Ct) values. For confirmation of primer specificity, the melting curves were verified. For each sample, three technical replicates were conducted to calculate the averaged Ct values. Relative expression was calculated by the $2^{-\Delta\Delta Ct}$ method (11) by considering soil treatment as the calibrator. One-way ANOVA was conducted to test the treatment effects on all genes relative expression level.

Gene expression data were shown in Figure S12. Results showed that relative expression of Glyma.06G070500, a gene highly expressed in lateral root and root tips, was 10-fold increase of expression in hydroponics and 4-fold increase of expression in transparent soil relative to soil treatment, verifying that roots in hydroponics were grown better than those in soil. Genes of Glyma.12G221500 (regulated by low nitrogen) and Glyma.08G053500 (regulated by phosphorus, iron, and zinc deficiency) showed no significant difference of relative expression across all three treatments, suggesting that nutrient condition could be similar in three treatments and induce no significant change in these gene expression. However, Glyma.11G121800, reported as a gene regulated by hypoxic stress, was 42-fold increases of expression in hydroponics, but was 0.14 fold change expression in transparent soil, relative to soil treatment. This indicates that transparent soil does not cause root hypoxia in plants.

Table S6. Selected genes and primer design

Gene name	Gene ID	forward primer (5' to 3')	reverse primer (5' to 3')	Description	Reference
RAN-3	Glyma.06G070500	TGGTGGTTCGAA TGGTTGGT	AGCACCCCTGTAGT ATGCAC	Involved in signal transduction and stress response. High expressed in lateral root and root tip.	Glycine max Wm82.a2.v1*; Chen et al. 2016. (12)
Rab11A	Glyma.08G133800	CAGATCTGGGAC ACTGCTGG	GATCACGCGCGAAC AAAACT	Involved in membrane trafficking and root hair tip growth. High expressed in lateral root and root tip.	Glycine max Wm82.a2.v1; Inada and Ueda 2014 (13); Blanco et al. 2009 (14)
COG8	GLYMA.02G043400	GGAACTGATCTG CAGTCGTCA	TGCTAGTTGTCTGC ACAGGG	Involved in protein trafficking. High expressed in lateral root and root tip	Glycine max Wm82.a2.v1
NAC4	Glyma.12g221500	AAAACACCGGCAC CAAGGTA	ATGGAGGAGCCATT GAAAGTT	Involved in regulating nitrogen stress. High expressed in root.	Wang et al. 2017 .(15)
GmCML39	Glyma.08g053500	TCCTATGCTTGCC GTTTCAGT	ACGGCGTGATTCCG TCAATA	Involved in regulating nutrient stress. High expressed in root and leaves.	Zeng et al. 2017. (16)
GmaxERD1-like	GLYMA.04G203300	CTCAGGTTCGTGA AGTTTTGGT	AATAAGCTGCTAGG CGTGCT	Involved in regulating drought stress.	Neves-Borges et al. 2012. (17)
nsHB	GLYMA.11G121800	ACATTGTTTATTA GCAAGGGTCAA	TGCTTCTAGAGAAT TACCCTCCA	Involved in hypoxic stress.	Nakayama et al. 2017. (18)
ADH2	GLYMA.04G240800	TGGAAACTACAAA CCCCGCA	CCAATTCCTGCACAC AAGACA	Involving in regulating flooding stress.	Valliyodan et al. 2014. (19)
CYP2	GLYMA.12G024700	CTGTGTCGGTGG CTCTGAAT	CCATAACACCGCAAT CCCCT	Internal reference	Jian et al. 2008. (20)

*https://phytozome.jgi.doe.gov/pz/portal.html#

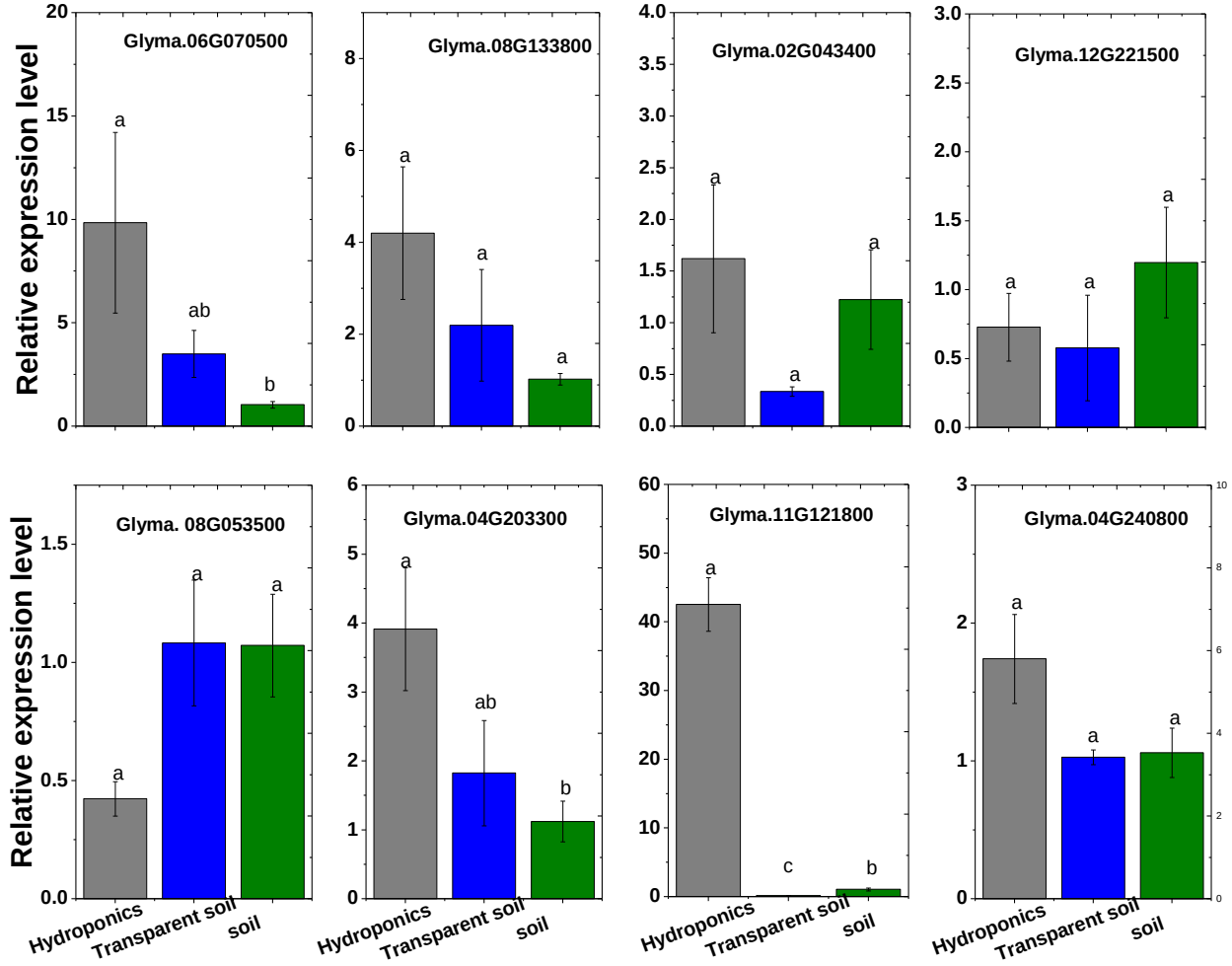

Figure S12. Expression analysis of selected genes in soybean roots under three different growth media (hydroponics, transparent soil, and soil). Real time qPCR data were normalized using soybean CYP2 gene. Data represent the means and standard errors of four independent biological samples.

4.3 *ARABIDOPSIS THALIANA* GROWTH IN TRANSPARENT SOIL

Fluorescence tagged *Arabidopsis* seed (Germplasm: CS16303, with associated constructs of Gal4-CFP, CoxIV-YFP, Cam53BD-GFP, RecA-RFP) were obtained from the Arabidopsis Information Resource Centre. Seeds were put in an autoclaved vial and were surface-disinfected by soaking in 100% ethanol for 1 min, then soaking in 20% bleach with 0.5% tween 20 for 10 min, and followed by 6-7 rinses with sterile distilled water. Seeds were sown on the gel in Petri dish. The gel was made of 0.8% agar added with 0.5 MS and 1% sucrose. The plates with seeds were incubated for 3 d at 4℃ and then were placed vertically in a growth room under conditions of 16:8 h light/dark, at 23 ^{0}C, respectively.

7 d-old plants were transferred in Petri dishes filled with TS and fitted with a glass coverslip window using silicon sealant.

Transparent soil beads were made by 1.2% gel concentration with 0.8% sucrose cross linked with 10 mM $MgCl_2$ solution (particle size controlled with Luer-Lok tip). The TS beads was then soakd in 1 MS (1:1, v/v) for 1h.

Plants were grown in TS for another 7 d. Before microscopy imaging, TS was saturated with DI water. Microscopy images of roots in TS in vivo were taken with a Leica SP5 X MP confocal microscope system (Leica Microsystems Inc., Buffalo Grove, IL, United States) and ZEISS fluorescent microscope (10x dry lens, excitation at 488 nm with 510-550 emission bandwidth). Z-stack was obtained with 0.017 mm (confocal) and 0.02 mm (fluorescent) step for the depth up to 3.26 mm. 3D model and images were analyzed using LAS X (Leica) and ZEN (ZEISS) software

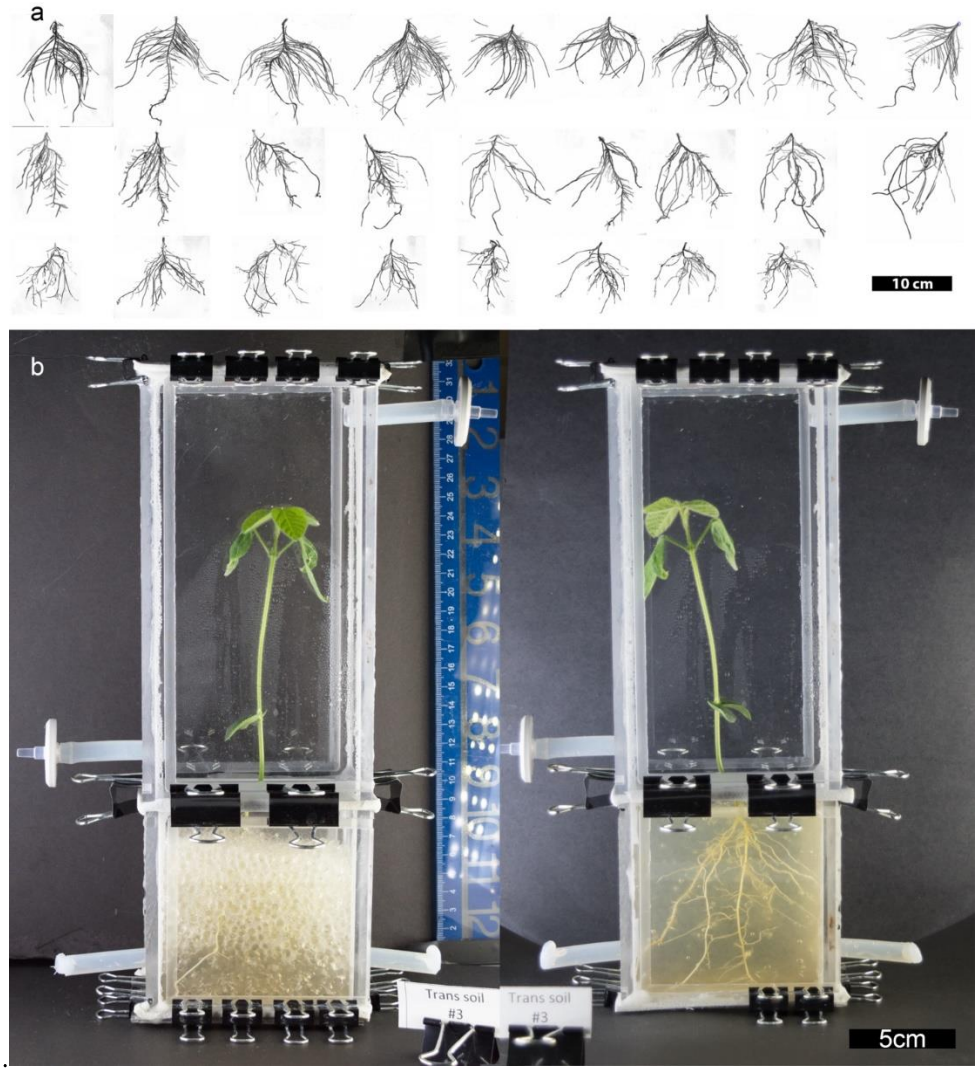

Figure S13. (a) Replicates for Figure 3b. Comparison of *G.max* roots grown in soil extract hydroponics (top row), transparent soil system (middle row) and soil (bottom row) in day 12 after transplanting **(b)** *G.max* grown in drained TS (left) and saturated TS (right).

5.0 APPLICATIONS OF TRANSPARENT SOIL

5.1 PH MONITORING AND MAPPING

TS was made by beads soaking in 2 MS solution with 0.04 g·L^{-1} Bromocresol purple (pH 8), which is purple above pH 6.8 and yellow below pH 5.2 (21). *G. max* plants were grown in hydroponics (1MS) for 7 d after 4d germination, and then transferred to the prepared TS.

Two sets of experiment were done with this treatment. In the first set of experiments, TS was saturated with autoclaved DI water and photos of roots *in vivo* were taken with both visible and infrared light. LED panels were used the light source for visible light and the flash light and NIR filter was applied for infrared light as described in 4.1. In the second set of experiments, roots grew in TS and time-lapse photos were imaged with an interval of 30 min for roots and TS beads without saturation after transplanting for 3 days.

To obtain the pH map, the visible image was color thresholded by ImageJ to isolate the areas of the image that showed a yellow color resulting from the transition of the pH indicator. The thresholded images were then converted to HSV format, and the saturation channel was extracted as a black and white image. The resulting image was overlayed on top of the NIR root photograph, and colorized in yellow. As a result, the intensity of the yellow color is proportional to the saturation level of the yellow color in the visible photograph.

Movie S3 shows the time-lapse of the color changes of TS beads.

5.2 WATER AVAILABILITY

Two type of TS beads were made for this experiment. One kind was made by soaking the TS beads in 2 MS solution with polyethylene glycol (PEG, MW= 8000, 400 g L^{-1}) for 2 days. The other kind was made by soaking TS beads in 2MS without PEG 8000. PEG 8000 was reported to decrease the water potential in previous work (22), which can decrease water availability for roots (23).

These two kinds of beads were packed in the bottom room of the HOMEs so to create two regions with different water availability (as shown in Figure S14). *G.max* was transplanted at the top of the mixed TS (n=10). After 8 days growth, roots were imaged *in vivo* with infrared camera and 4pcs high power LED infrared lamp set at the bottom of the boxes as light source. The 10 replicates are shown in Figure S14a.

We measured the fraction of roots in each half of the HOME with ImageJ software. Images were converted to 16 bit. The background was eliminated using a combination of contrast adjustment and removal of overexposed transparent soil beads and/or air bubbles. Adjusted photos are shown in Figure S14a. The projected surface area was determined for roots in each sides using thresholding for each image. The average projected root area in each side was calculated for each replicate from front and back window of the boxes and used to calculate relative value of

roots in normal water availability side. The student t-test was used to assess the significant difference in average root area between low and normal water availability sides.

5.3 HARDNESS MODIFICATION

Three different types of TS were prepared for this experiment: (1) TS1 (soft and large): 0.9% gel dropped through 10 mL Luer-Lok tip syringe without needle and cross-linked with 10 mM $MgCl_2$ solution; (2) TS2 (intermediate soft and large): 1.0% gel dropped through 18G needle and cross-linked with 10 mM $MgCl_2$ solution; (3) TS3 (hard and small): 1.0% gel dropped through 22G needle and cross-linked with 100 mM $MgCl_2$ solution.

The three types of beads were packaged in three layers in the growth box, TS1 on the top, TS2 in the middle, and TS3 at the bottom. *G. max* (n=10) was transplanted in TS at 4 d after germination, and grown for another 7 d.

The root phenotype of G. max was imaged in vivo at the end of experiment with infrared camera. 3 replicates of this experiment shown in Figure S14b.

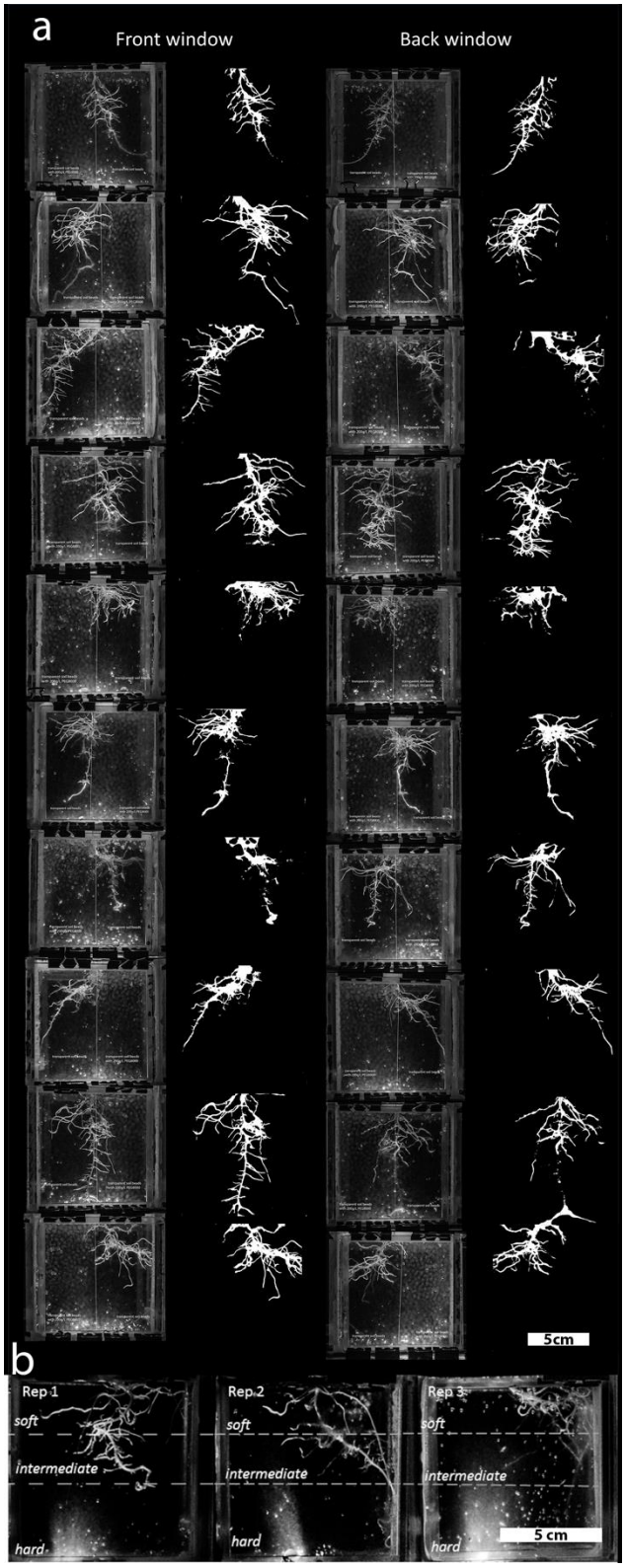

Figure S14. **(a)** Replicates of water availability experiment (left: pictures of soybean plants in TS before background removal; right: pictures of soybean plants in TS after background removal). **(b)** Replicates of hardness modification experiment.

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CAPTIONS OF MOVIES

Movie S1. The process of making transparent soil beads.

Movie S2. The process of building soybean growth boxes.

Movie S3. Time-lapse of color change caused by acidification in a pH indicator (Bromocresol purple) that was introduced into transparent soil during soybean growth in 4 d.