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Multi-chamber petaloid root-growth chip for the non-destructive study of the development and physiology of the fibrous root systems of *Oryza sativa*

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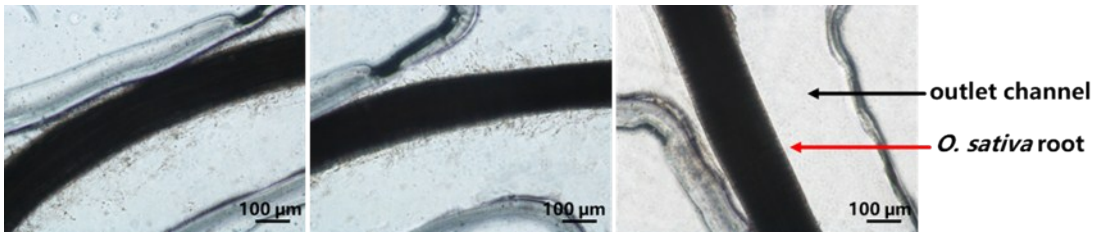
Supplementary information

1. Materials

The composition of green dye used in this study includes Tartrazine (534.36 g/mol), Erioglaucine disodium salt (792.85 g/mol), Potassium sorbate (150.22 g/mol), Gum xanthan (2×10^6~5×10^7 g/mol), α-D-Glucose (180.16 g/mol), H_2O (18 g/mol) and D-Sorbitol (182.18 g/mol); the main component of red dye is Sunset Yellow FCF (452.37 g/mol), Carmine (492.39 g/mol), Potassium sorbate (150.22 g/mol), Gum xanthan (2×10^6~5×10^7 g/mol), α-D-Glucose (180.16 g/mol), H_2O (18 g/mol) and D-Sorbitol (182.18 g/mol); the main component of a blue dye: Erioglaucine disodium salt (792.85 g/mol), Potassium sorbate (150.22 g/mol), Gum xanthan (2×10^6~5×10^7 g/mol), α-D-Glucose (180.16 g/mol), H_2O (18 g/mol) and D-Sorbitol (182.18 g/mol).

2. Supplementary data



sFigure 1. Microscopic images of *O. sativa* roots after 13 days' cultivation. Some of the roots grow into outlet channel. The diameter of *O. sativa* root is around 168±23 μm, while the width and height of outlet channel are 600 μm.

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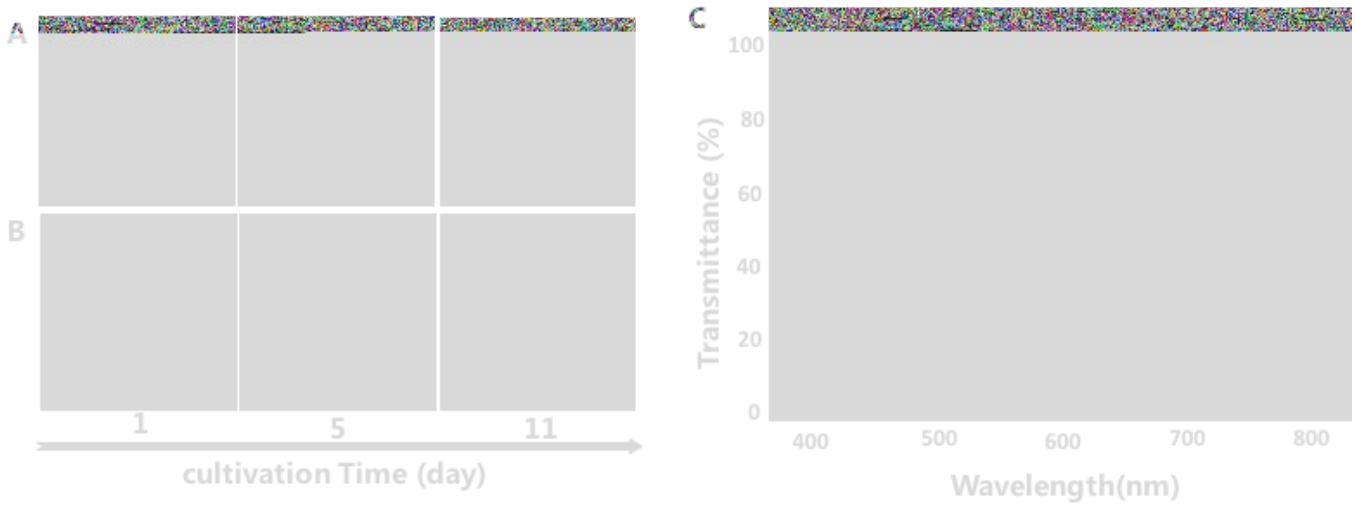
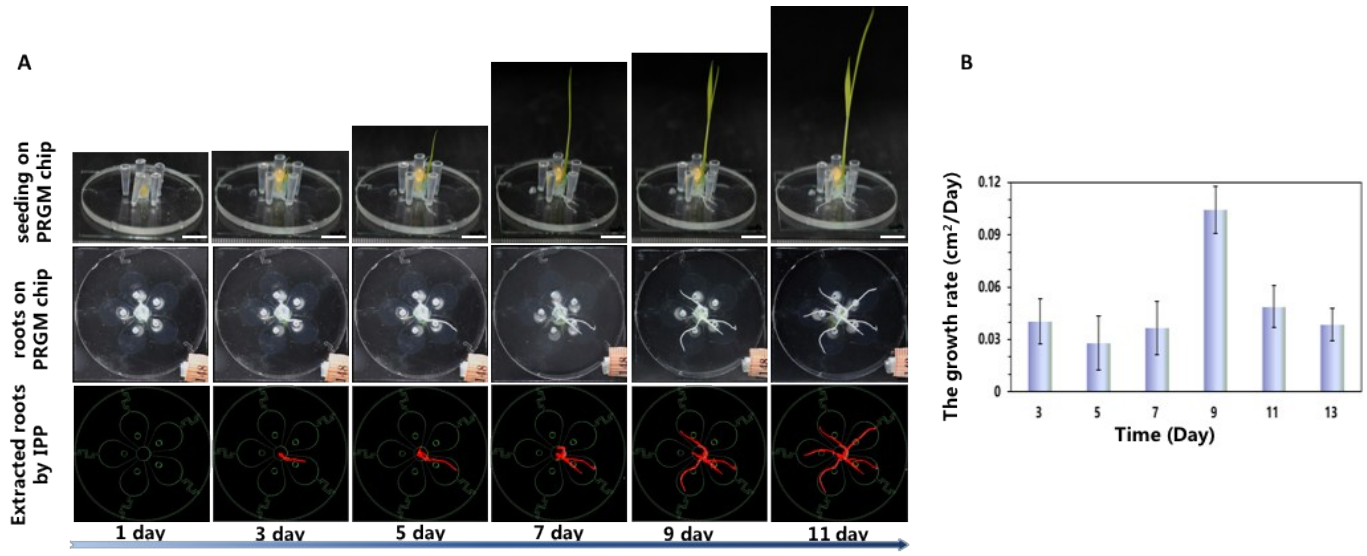
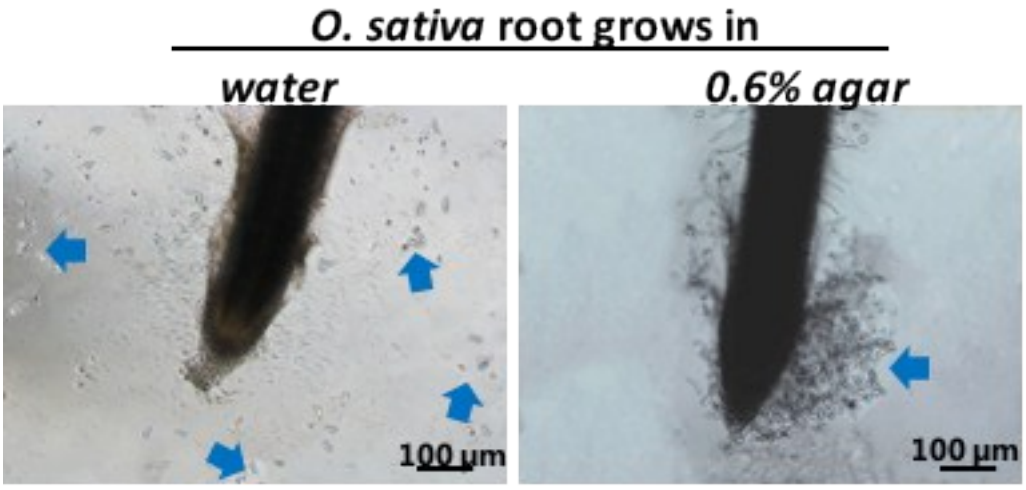


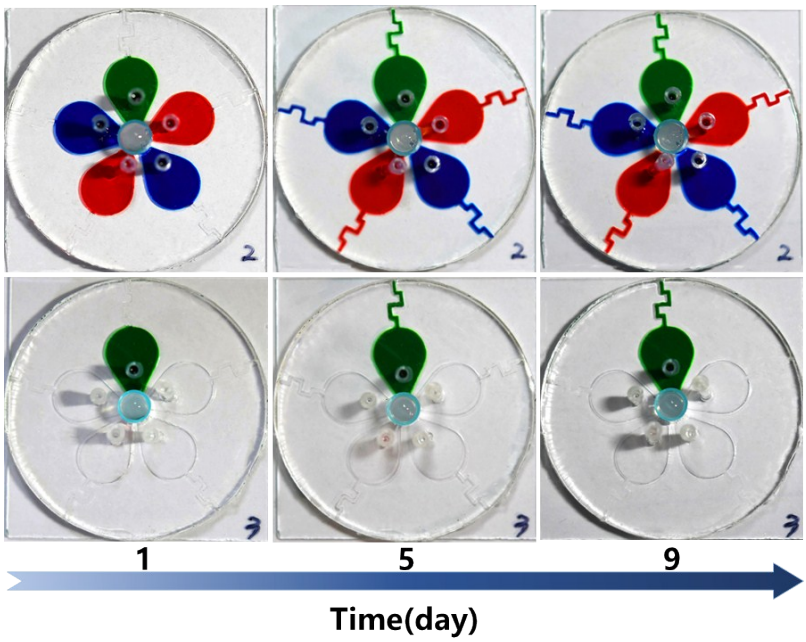
sFigure 2. The pH condition of culture medium during *O. sativa* seedling growth. A *O. sativa* seed was placed in the center germination chamber and cultured for 11 days. A pH indicator, phenol red, was used to test the pH value of culture medium (0.6% agar). A) 50 µl culture medium was taken from chip and added into a tube containing phenol red; B) Phenol red was directly added into agar medium. C) on-chip characterization the absorbance spectra of phenol red filled agar base. A *O. sativa* seed was cultured on agar base filled chip. A pH indicator phenol red was mixed in the agar base. The pH condition of culture medium during seedling growth was characterized by a spectrometer (SpectraSmart Spectrum, Taiwan).



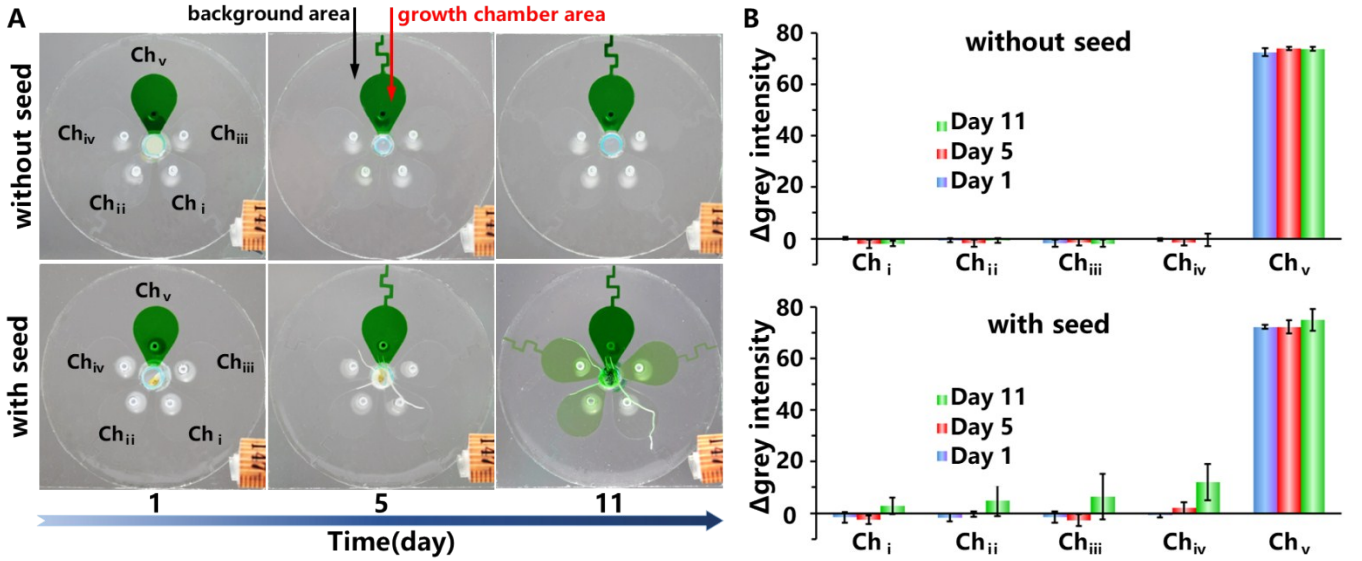
sFigure 3. Plant cultivation on petaloid root-growth microfluidic chip. A) non-destructive observation of shoot growth and fibrous root system development on-chip; B) Root development kinetic on-chip. The growth rates according to the root projection area were calculated as following: The growth rates =(RPA_n-RPA_{n-2}) / 2. The RPA_n is the root projection area of roots grown in culture medium at day n, RPA_{n-2} is the root projection area 2 days before the n^{th} day, and 2 (days) is the roots growth time.



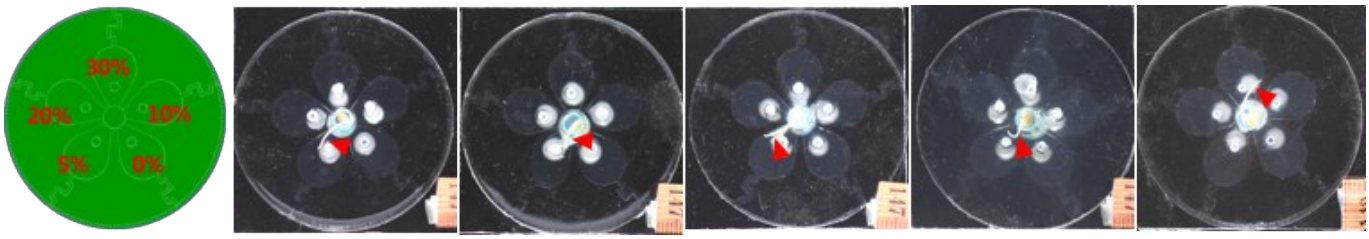
sFigure 4. Non-destructive observation of root microstructures on-chip. *O. sativa* seeds were cultivated in PRGM chip loading with water (left panel) and 0.6% agar (right panel). The roots were examined under microscopy at day 5. The blue arrow indicates the root border cells. Border cells in water medium are separated away from tip cap by water flow; while the border cells developed in agar based are closely aggregates around tip cap because of the limited flow in agar medium. Scale bar = 100 µm.



sFigure 5. Isolated root-growth chamber characterized with solution medium. The central germination chamber was perfused with 200 µL of a 0.6% agar culture solution. 60 µL of water containing different food dyes was injected into each of the five root-growth chambers; The chip was cultivated in an artificial climate incubator, and the color images were recorded by camera.



sFigure 6. Isolated root-growth chamber for establish different growth condition for single seed.
A) The central germination chamber was perfused with 200 µL of a 0.6% agar culture solution. 60 µL of 0.6% agar solution containing green food dyes was injected into one of the five root-growth chambers, while 60 µL of 0.6% agar solution was injected into each other four root-growth chambers. A prepared *O. sativa* seed was placed into the germination chamber. The chip was cultivated in an artificial climate incubator, and the color images were recorded by camera. For each of experiment conditions (without seed and with seed), six replicates were tested. After 11 days' cultivation, cross-contamination was observed from 5 of chip. In addition, it was found that cross-contamination (5 out of 6) only happened at chips with root growing into multiple growth chamber.
B) The grey value of the chip was quantified by NIH free software ImageJ. For each image, to eliminate the influence of uneven illumination, the green dye induced colormetric change can be quantified by taking a calculation defined below: Δgrey intensity = $[(G.I._{gc} - G.I._b)/G.I._b] \times 100\%$, where $G.I._{gc}$ is the grey value of the root-growth chamber, and $G.I._b$ is the grey value of neighboring background area. The results were calculated from 6 replicates.



sFigure 7. Germination and growth of a single seed under different environment conditions. 0.6% agar containing 0, 5, 10, 20 and 30% PEG6000 were injected into the root-growth chambers to impose different drought stress on a single *O. sativa* seed. The roots development was examined after 3 days' cultivation. Roots entered the growth chamber containing 5% PEG 6000.

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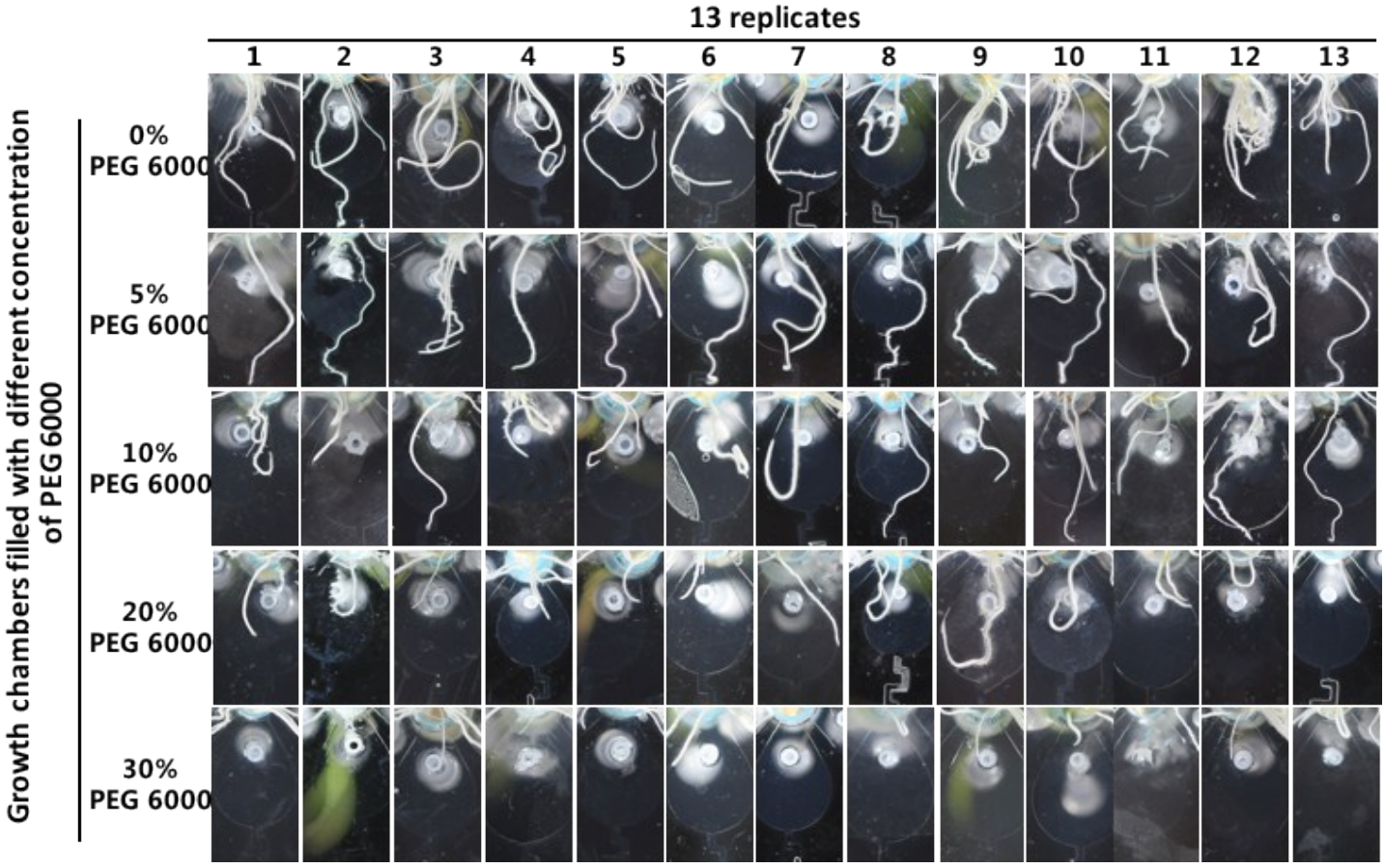


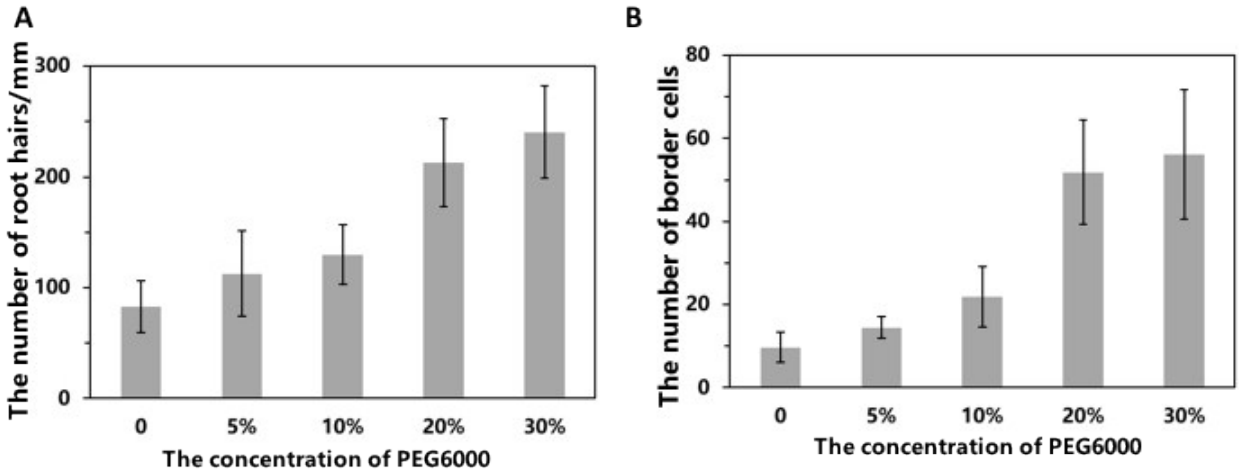
sFigure 8. Germination and growth of a single seed under different environment conditions. 0, 5, 10, 20 and 30% PEG6000 were injected into the isolated root-growth chambers to impose different drought stress on a single O. sativa seed. The experiment was repeated for 13 times.

sTable 1. The number of roots developed in growth chamber filled with different concentration of PEG6000.

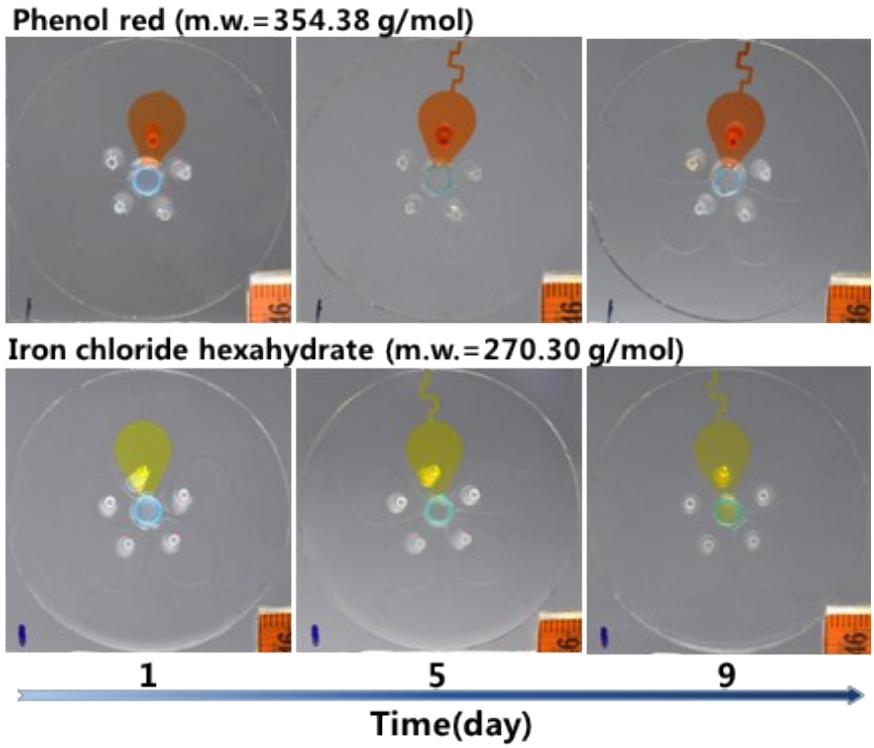
Replicates	Growth chamber filled with different concentration of PEG6000				
	0	**5%**	**10%**	**20%**	**30%**
1	4	2	2	1	0
2	3	2	2	1	1
3	3	4	2	1	1
4	4	1	2	2	1
5	3	2	2	1	0
6	3	2	2	2	1
7	3	2	1	1	0
8	3	2	2	2	1
9	4	2	2	2	1
10	3	2	2	1	1
11	3	2	1	2	0
12	4	3	1	1	1
13	4	1	1	1	1
mean	3.385	2.077	1.692	1.385	0.692
standard deviation	0.506	0.760	0.480	0.506	0.480

sTable 2. The relative root projection area developed in growth chamber filled with different concentration of PEG6000.

Replicates	Growth chamber filled with different concentration of PEG6000				
	0	**5%**	**10%**	**20%**	**30%**
1	107.826	82.174	43.478	28.696	0.000
2	110.000	70.435	29.565	16.087	6.087
3	110.100	100.873	40.425	14.783	10.870
4	101.914	92.829	31.739	29.068	7.283
5	97.391	80.000	39.130	25.217	0.000
6	98.675	68.696	40.182	28.825	22.609
7	84.384	84.228	53.478	38.261	0.000
8	82.609	74.783	53.043	43.918	8.696
9	91.304	66.522	32.174	51.739	15.652
10	90.870	79.130	48.261	36.957	11.557
11	86.087	65.652	37.826	36.522	0.000
12	130.870	71.304	47.826	14.348	4.783
13	93.409	83.648	40.447	29.428	7.643
mean	98.869	78.482	41.352	30.296	7.321
standard deviation	13.395	10.474	7.713	11.260	6.819



sFigure 9. Analysis of root microstructures from a single seedling towards different concentration PEG6000 induced drought stress. The root hairs and border cells were manually counted by a researcher who is blind to the experiment condition. A) The plot of number of root hair/mm vs. PEG 6000 concentration, n=13; B) The plot of border cells vs. PEG 6000 concentration, n=5.



sFigure 10. Isolated root-growth chamber for establish different growth condition for single seed. The central germination chamber was perfused with 200 µL of a 0.6% agar culture solution. 60 µL of 0.6% agar solution containing phenol red or iron chloride hexahydrate was injected into one of the five root-growth chambers, while 60 µL of 0.6% agar solution was injected into each other four root-growth chambers. The chip was cultivated in an artificial climate incubator, and the color images were recorded by camera.