9/10

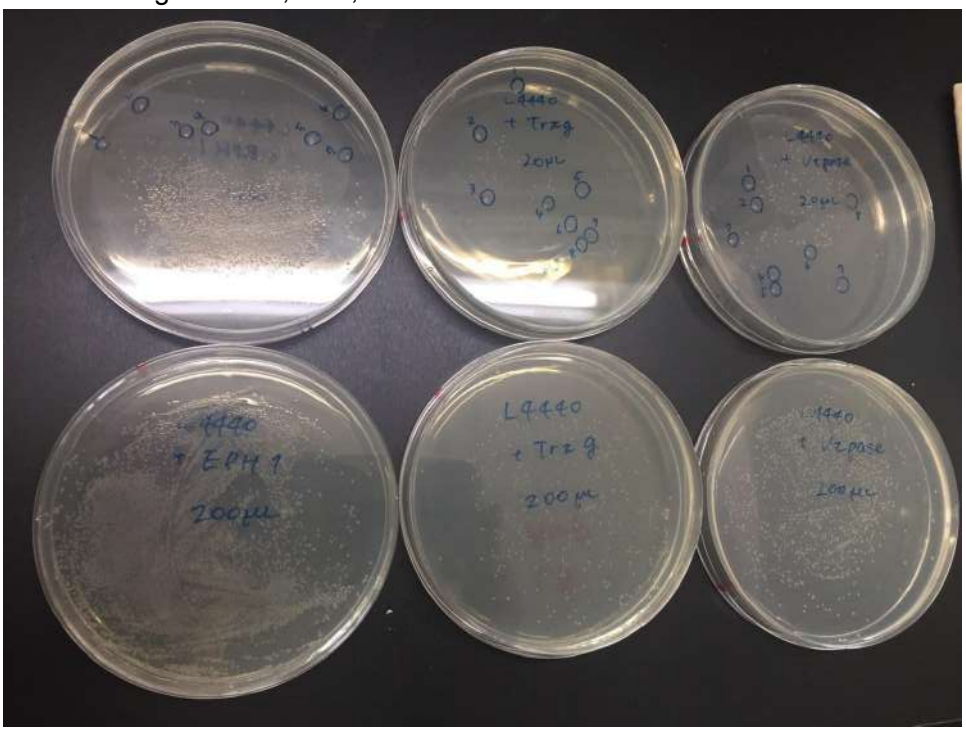
RNAi (taken over from Koga-kun.)

Transformation

1. Add 4uL of plasmid to 20uL of competent cells(DH5α)/ each sample melted on ice.
2.incubate on ice for 15 min
3.42℃ HEAT SHOCK for 45 sec
4.Add 200uL of SOC.
5.incubate at 37℃ for 40 min
6.Centrifuge 1min /8000rpm/r.t.
7.Spread onto Amp LB Xgal(20uL for each plate) Plate at clean bench. 37℃ o/n
8.Discard the supernatant and suspend until the volume reaches 50uL.
9.Spread onto Amp LB Xgal(50uL for each plate) Plate at clean bench. 37℃ o/n

9/11

We can find some white colonies on 20 uL plates.
from upper to down: 20 uL, 50 uL
from left to right: EPH1, Trxz, v-ATPase



colony PCR

1.mix each component as follows and set the samples to thermal cycler.

VTPase

x2 emerald amp	25 ul	Pipette 5 uL each for 8 colonies
DW	23 ul	
f92, m851(10uM each)	1 ul	

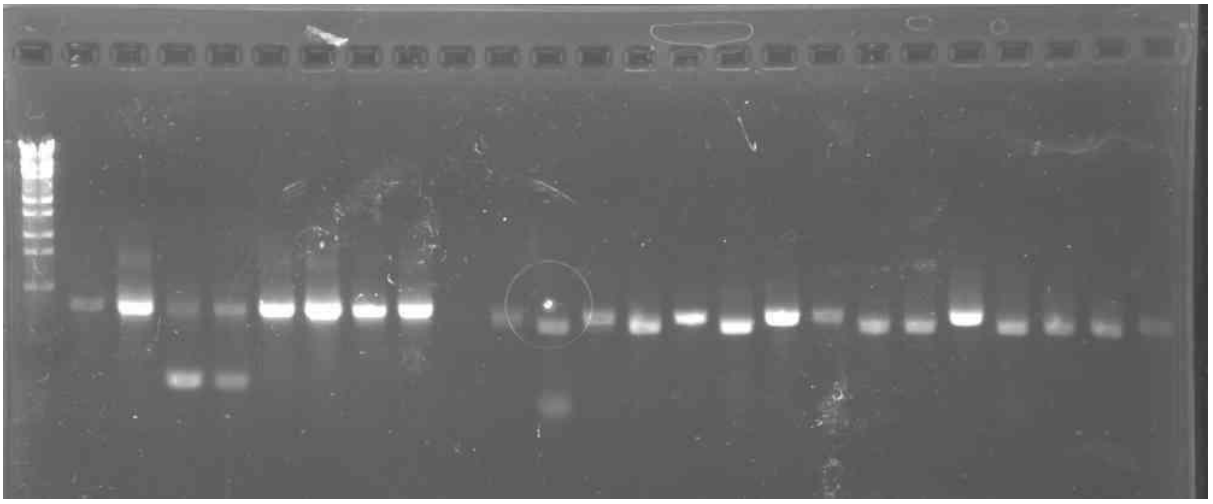
Trxz

x2 emerald amp	25 ul	Pipette 5 uL each for 8 colonies
DW	23 ul	
f96, m851(10uM each)	1 ul	

EPH1

x2 emerald amp	25 ul	Pipette 5 uL each for 8 colonies
DW	23 ul	
k006, m851(10uM each)	1 ul	

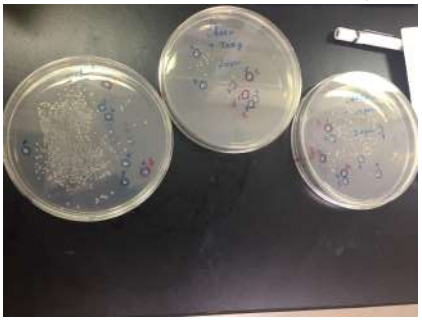
98℃ 30s→(98℃ 10s→60℃ 30s→68℃ 1min)*30cycles
2.Electrophoresis
Loaded 10uL for each sample on 1% agarose TAE gel.
100V 20min



from left to right: 1-8 EPH1, 9-16 Trxz, 17-24 v-ATPase
except size(If the insert is in the right direction.):**700 bp**(EPH1), **662 bp**(Trxz and v-ATPase**)**

candidate
EPH1 2(a),5,6(b),7,8
Trxz 2,4,6(d),8(e) (a little shorter band 5(c),7)
v-ATPase 1(f),4(h) (a little shorter band 2,3(g),5,6,7,8)

chose EPH1 2(a)&6(b) Trxz 5(c)&6(d)&8(e) vATPase 1(f)&3(g)&4(h) and put the cells into 3mL LB amp and started cultivating them at 37℃ 180rpm(shaking).



9/17

RNAi (taken over from Koga-kun.)

Culture of plasmid pR015 (for v-ATPase)

1.Add 1 mL ampicillin to each LB medium (Autoclaved, 1 L * 6 bottles).
  Replace 3 mL to the test tube(for OD reference).
2.Spread 5 mL LB medium on the plate, and suspend, add 500 uL to each 1 L LB medium(*6 bottles).
3.Mix bottle, and replace 3 mL to the test tube(for OD test).
4.Start culture at 37 ℃, 180 rpm.

OD Table

30 min	OD600 0.03
60 min	OD600 0.01
90 min	OD600 0.03
120 min	OD600 0.07
150 min	OD600 0.24
160 min	OD600 0.23
170 min	OD600 0.29
185 min	OD600 0.44
195 min	OD600 0.41
205 min	OD600 0.55

5.When OD600 becomes 0.55, add 300 uL IPTG.(13:24)
6.Incubate at 37 ℃, 180 rpm, for 4 hours.(from 13:24 to 17:24)

NGS (taken over from Shiotsu-kun.)

DNA extraction

1.Prepare 25 mL 2xLysozyme.

2xLysozyme

10 mM Tris-HCl (7.5)	500 uL 25
100 mM EDTA	10 mL 0.5
200 mM NaCl	2.5 mL 0.125
Lysozyme	50 mg 2.5
milliQ	to 25 mL to1.25ml
SDS	0.5 g（Prapare separately＊）

＊Prepare 10 % SDS.

SDS is separated from others, because SDS does not melt to the 2xLysozyme.

2. Prepare samples.
Almost all samples

2xLysozyme	500 uL
10 % SDS	100 uL
Sample	400 uL
total	1 mL

13(D,1,v), 18(C,3,s), 31(R,1,v)

2xLysozyme	250 uL
10 % SDS	50 uL
Sample	200 uL
total	500 uL

16(C,1,s)

2xLysozyme	125 uL
10 % SDS	25uL
Sample	100 uL
total	250 uL

7(D,1,s)

2xLysozyme	100 uL
10 % SDS	20 uL
Sample	80 uL
total	200 uL

25(R,1,s)

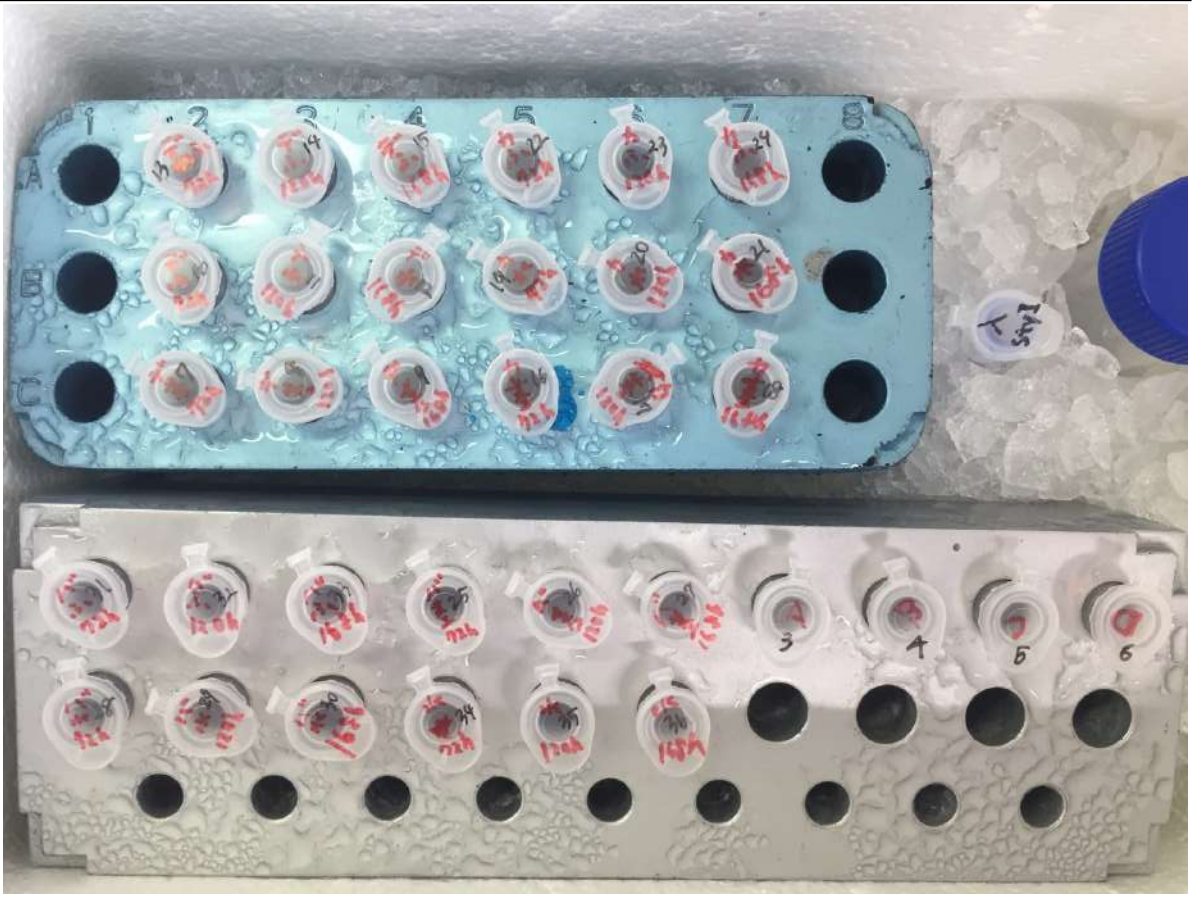
2xLysozyme	62.5 uL
10 % SDS	12.5 uL
Sample	50 uL
total	125 uL

Sample Table

1	milliQ (negative con)	—	—

2	milliQ (negative con)	—	—
3	A	Japanese anemone	3 d
4	B	Chrysanthemum	5 d
5	C	Goby	2 w 10 ℃ fridge
6	D	well water(nega con)	—
7	Delphinium	3 d	stem
8	Delphinium	5 d	stem
9	Delphinium	7 d	stem
10	Delphinium	3 d	water
11	Delphinium	5 d	water
12	Delphinium	7 d	water
13	Delphinium	3 d	vase
14	Delphinium	5 d	vase
15	Delphinium	7 d	vase
16	Carnation	3 d	stem
17	Carnation	5 d	stem
18	Carnation	7 d	stem
19	Carnation	3 d	water
20	Carnation	5 d	water
21	Carnation	7 d	water
22	Carnation	3 d	vase
23	Carnation	5 d	vase
24	Carnation	7 d	vase
25	Rose	3 d	stem
26	Rose	5 d	stem
27	Rose	7 d	stem
28	Rose	3 d	water
29	Rose	5 d	water
30	Rose	7 d	water

31	Rose	3 d	vase
32	Rose	5 d	vase
33	Rose	7 d	vase
34	water (negative con)	3 d	—
35	water (negative con)	5 d	—
36	water (negative con)	7 d	—



3. Incubate at 37 ℃, for 30 min, and store on ice.
4. Add 2 uL protein kinase to each tube.
5. Incubate at 55 ℃ overnight.

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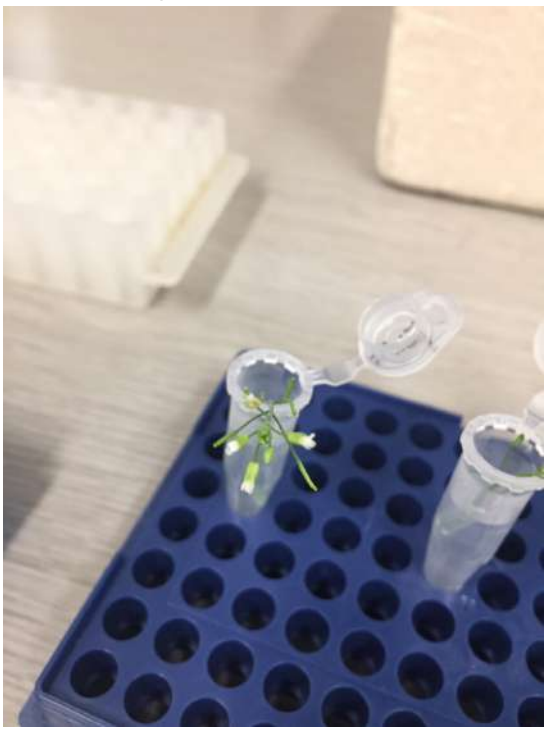
NOP-1 assay

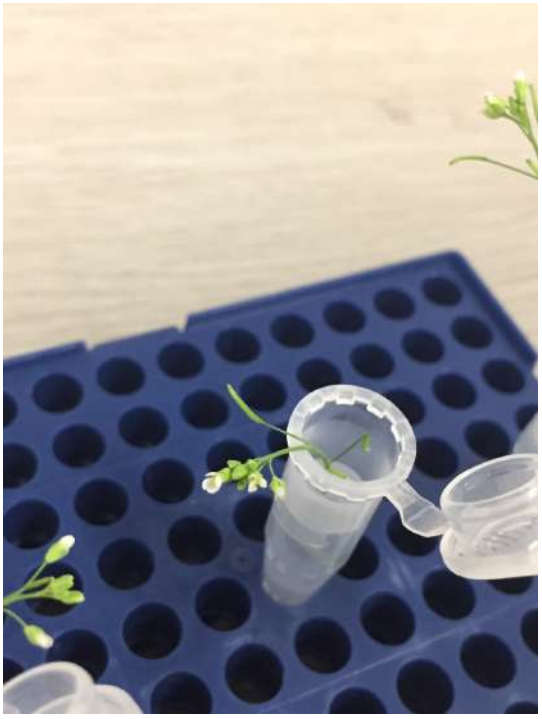
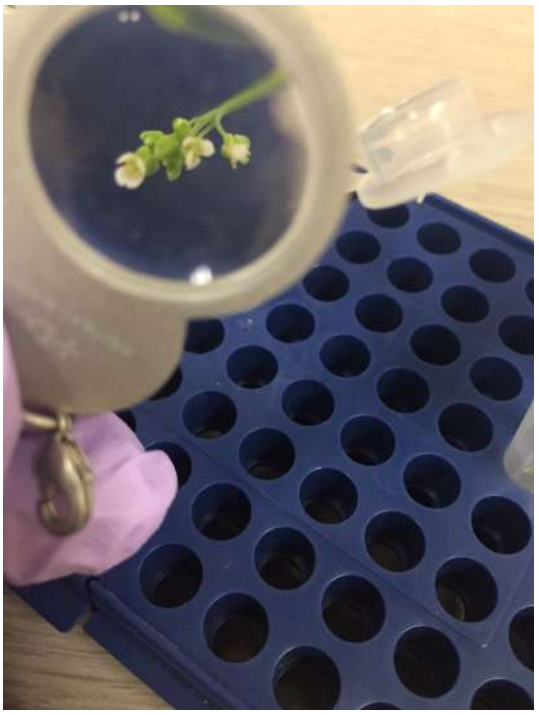
Pre-experiments

1.Add 1 mL tap water, milliQ, Krisal to each eppendorf tube.
2.Cut the tip for 2 uL at both ends.
3.Cut the arabidopsis flower about 5 cm.
4.Thread the stem through the tip.
5.Float arabidopsis with tip on the water.(9/18/11:00)
6.Store at 23 ℃, with sun-light(Turns the light off at night).

from left to right: tap water, milliQ, Krisal



tap water

milliQ


Krisal

NGS(yesterday's continuation)

Phenol-Chloroform Precipitation

1.Add 1 mL Phenol-Chloroform to each tube.
2.Vortex for 5 seconds.
3.Centrifuge at 4 ℃, 14000 rpm, 10 min.
4.Prepare new tubes.
5.Add 100 uL 4 M NaCl, and 800 uL 2-propanol to each new tube.（＊）

6.From the tube after centrifugation, take 80% of the supernatant and add the（＊）tubes.
（almost all 800 uL, 13/18/31 400 uL, 16/7 200 uL, 25 100 uL）
（for 7, it should be 160 uL, but I mistakenly took 200 uL.）
7.Perform fall mixing 40 times.
8.Store at -80 ℃ fridge in B4 room.

RNAi(yesterday's continuation)

Extraction of dsRNA

1.Add 30 mL 70 % EtOH in PBS, and mix.
(Because it doesn't melt,) incubate at 4 ℃, 10 min, and centrifuge 10 min, 10000 rpm, 4 ℃, and trash the supernatant, and vortex.
Vortex. Add 10 mL 70 % EtOH in PBS and glass beads. Remove pellets with pipet and Vortex.
2.Incubate at 4 ℃, 10 min.
3.Centrifuge 10 min, 10000 rpm, 4 ℃.
4.Trash the supernatant.
5.Add 10 mL150 nM NaCl, and mix.
6.Incubate at 4 ℃, 1 h.
7.Centrifuge for 10min 10000 rpm, 4 ℃.
8.Store the supernatant at -20 ℃ fridge.

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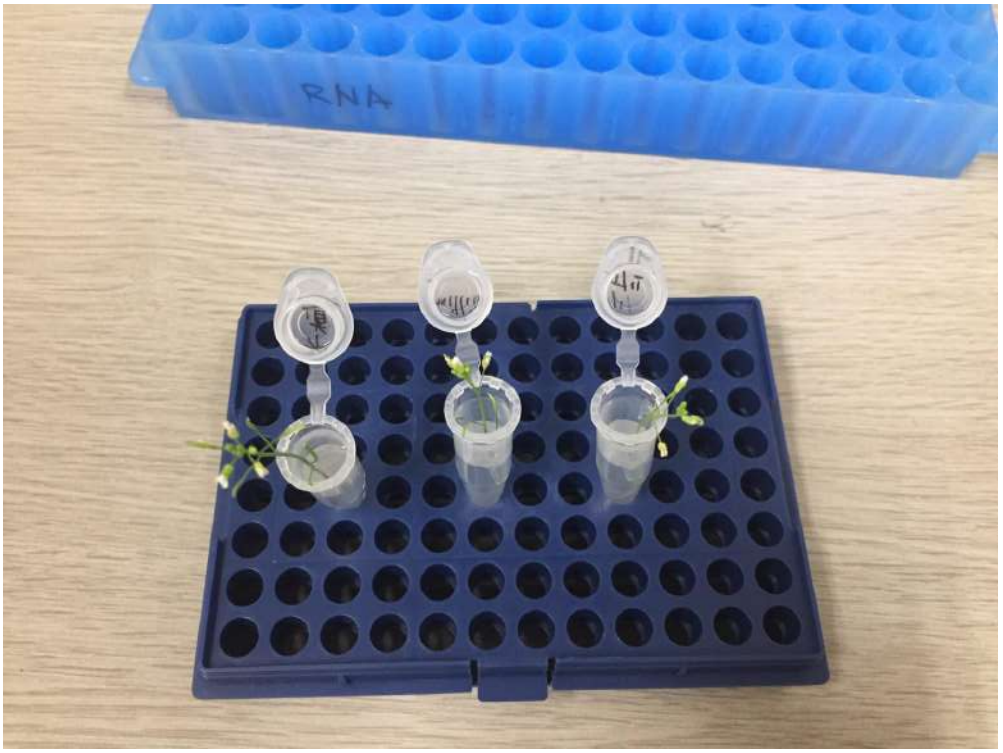
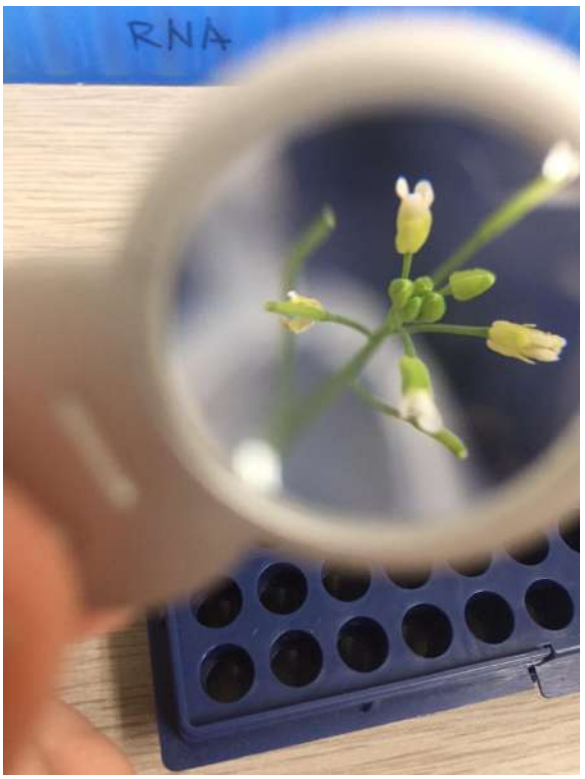
NOP-1 assay

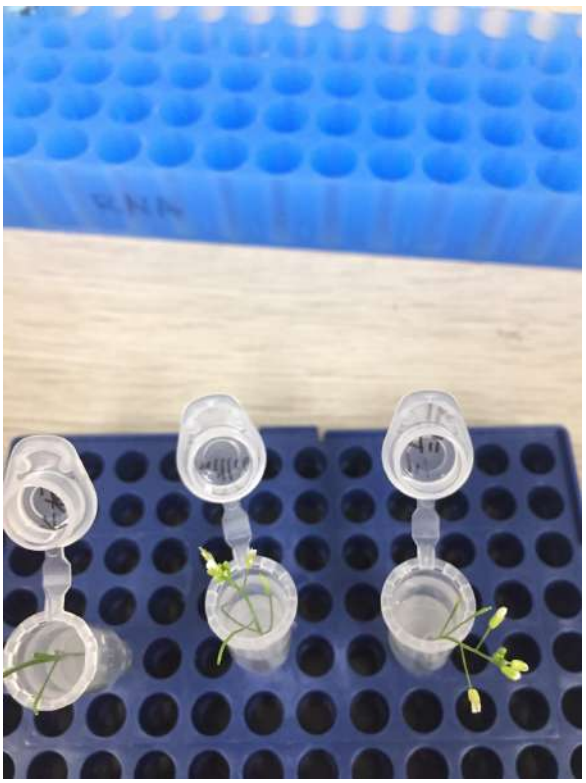
Pre-experiments(yesterday's continuation)

10:12



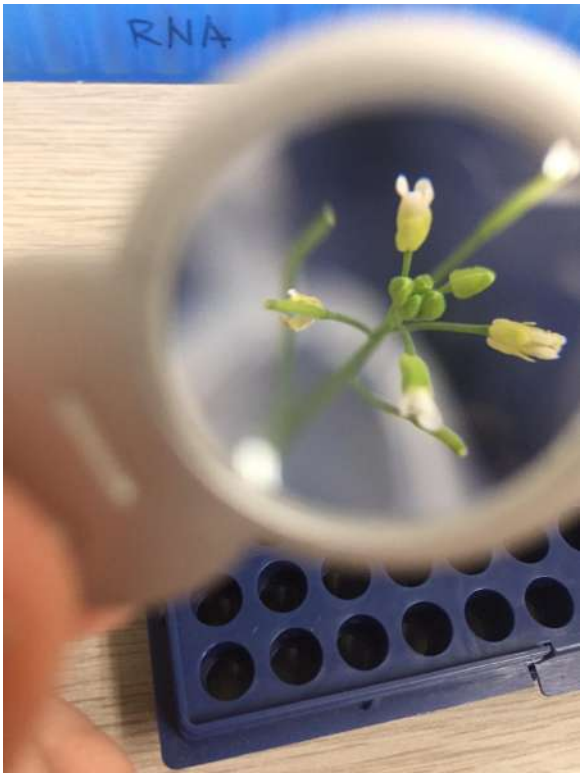
Tap water



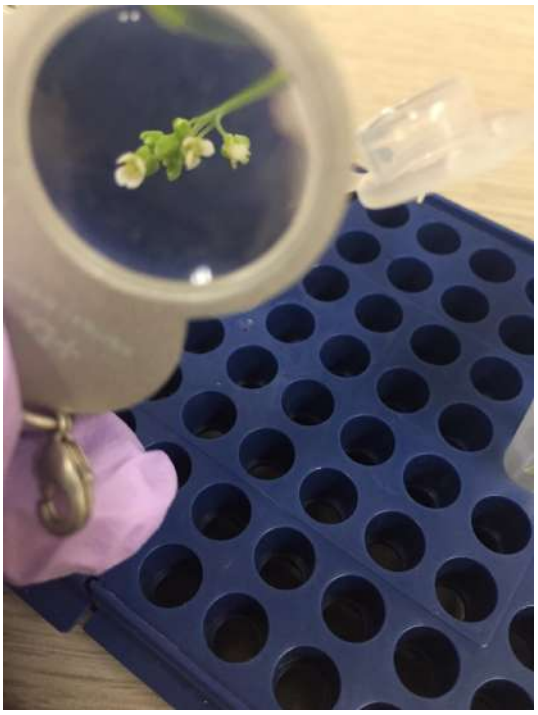

milliQ


Krisal

compare with yesterday’s pictures
from left to right:yesterday, today

Tap water




milliQ


Krisal

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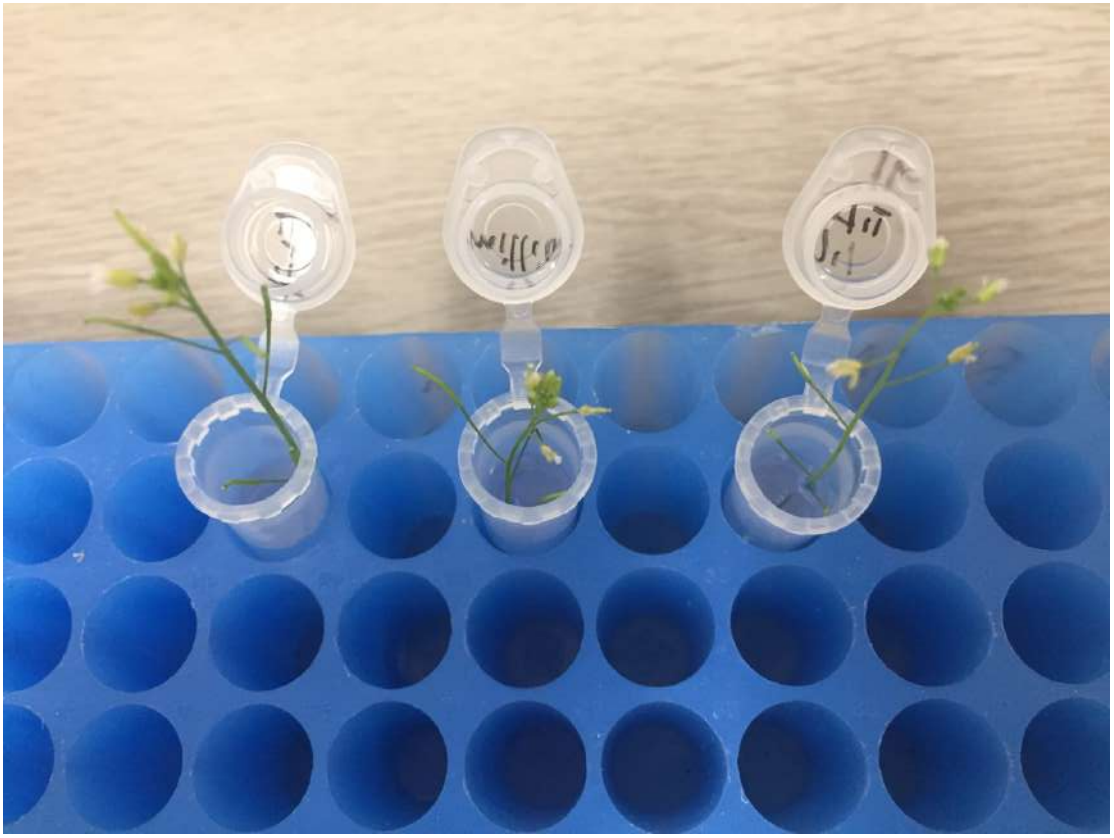
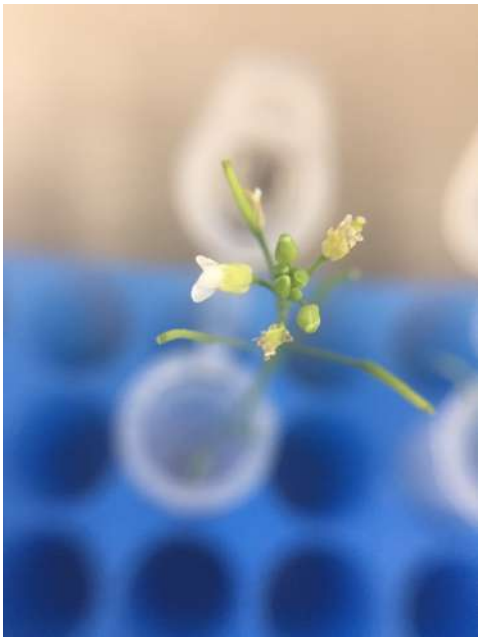
NOP-1 assay

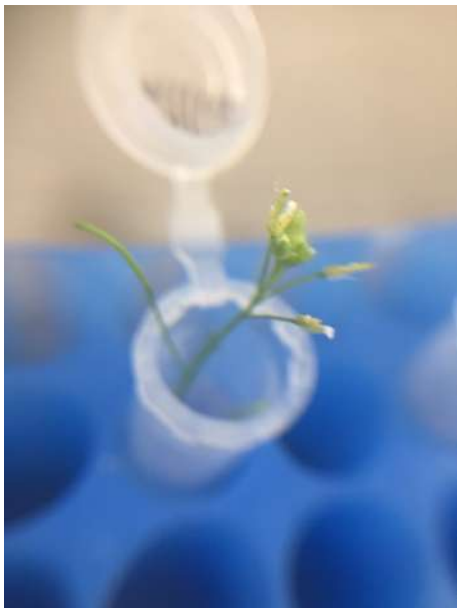
Pre-experiments(yesterday's continuation)

1

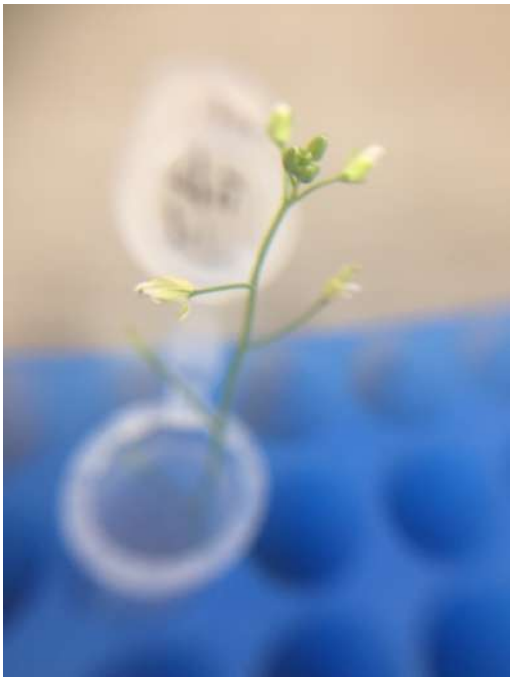




Tap water

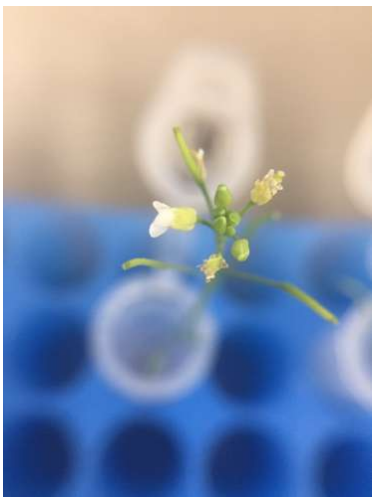



milliQ

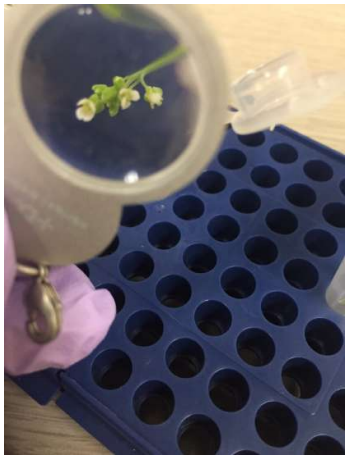



Krisal

compare to the past (from left to right: past to today)

Tap water



milliQ



Krisal

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NOP-1 assay①

Experimental Conditions

AgNO3（Positive control）	1 mM
milliQ	—
DMSO (Negative control for hormone)	(1000 times diluted, 3000 times diluted)
Dialysis Buffer (Negative control for peptide)	10 times diluted
Brz	3 μM (1000 times diluted from 3 mM) 1 μM (3000 times diluted from 3 mM)
BL	100 nM (1000 times diluted from 100 uM)
NOP-1	10 times diluted

Sample Table

1	AgNO3 (Mw=169.87)	1 mM	Positive control
2	milliQ	—	Negative control
3	DMSO (1)	1000 times diluted	Negative control
4	DMSO (2)	3000 times diluted	Negative control
5	Dialysis Buffer	10 times diluted	Negative control
6	Brz (1)	1000 times diluted	Test 1
7	Brz (2)	3000 times diluted	Test 2
8	BL	1000 times diluted	Test 3
9	NOP-1	10 times diluted	Test 4
10	NOP-1 v	10 times diluted	Test 5

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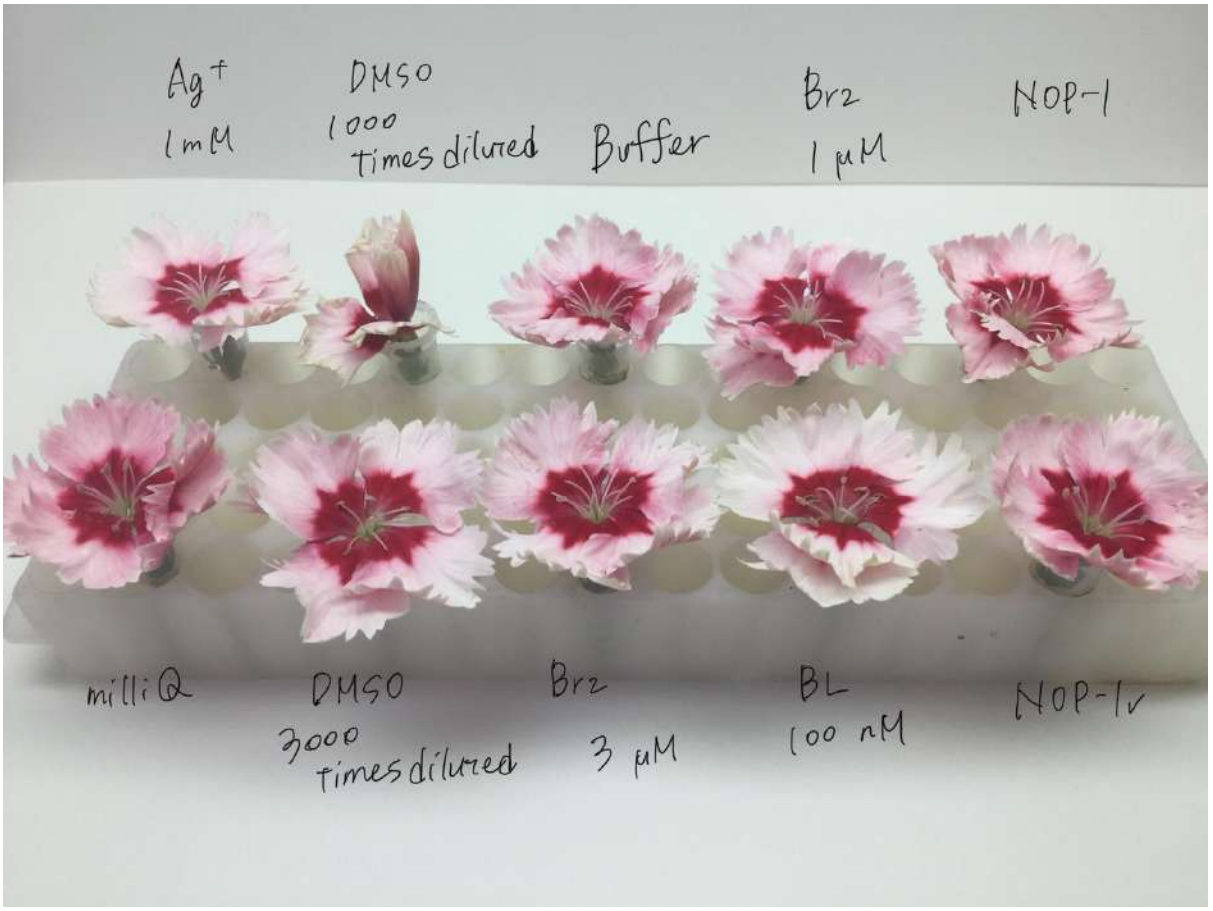
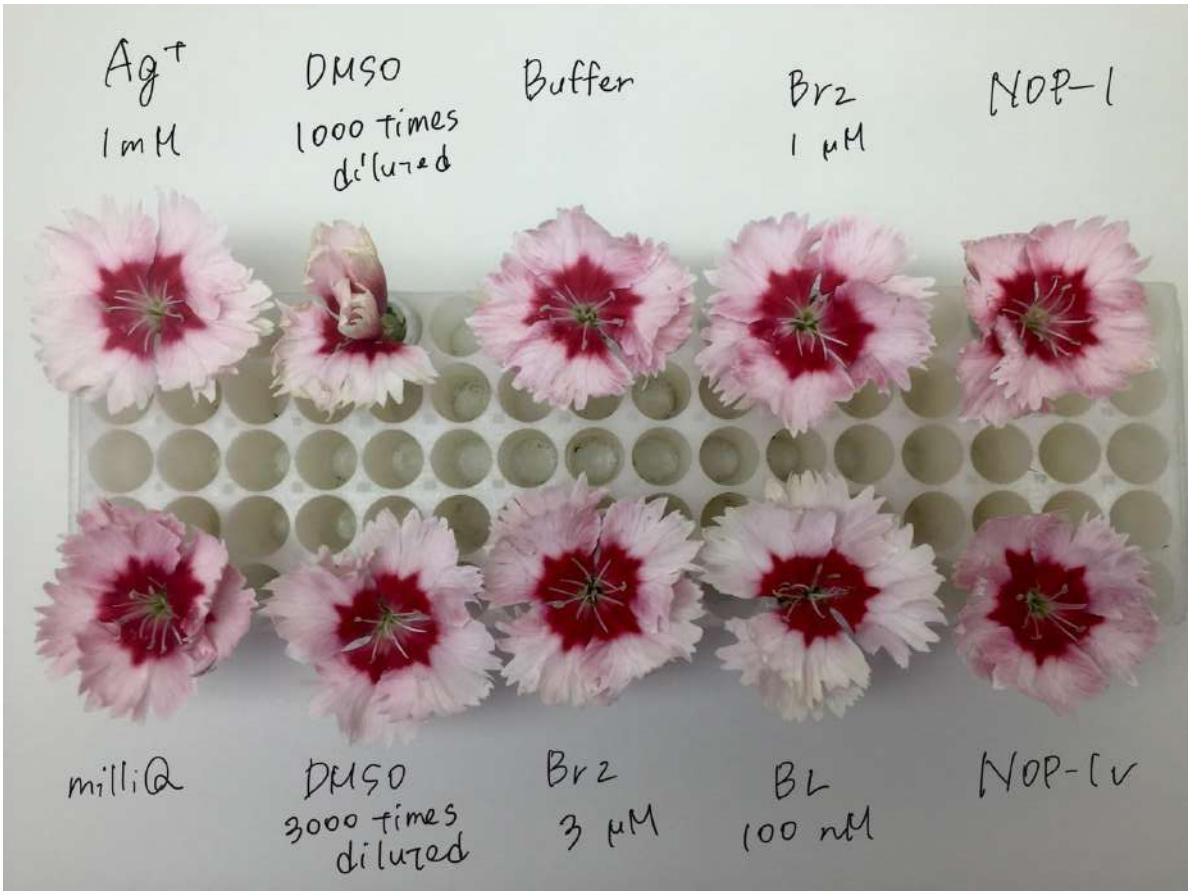

Ag+
1mM
DMSO
1000
times dilured
Buffer
Br2
1 μM
NOP-1
milliQ
DMSO
3000
times dilured
Br2
3 μM
BL
100 nM
NOP-1v


Ag+
1mM
DMSO
1000 times
dilured
Buffer
Br2
1 μM
NOP-1
milliQ
DMSO
3000 times
dilured
Br2
3 μM
BL
100 nM
NOP-1v

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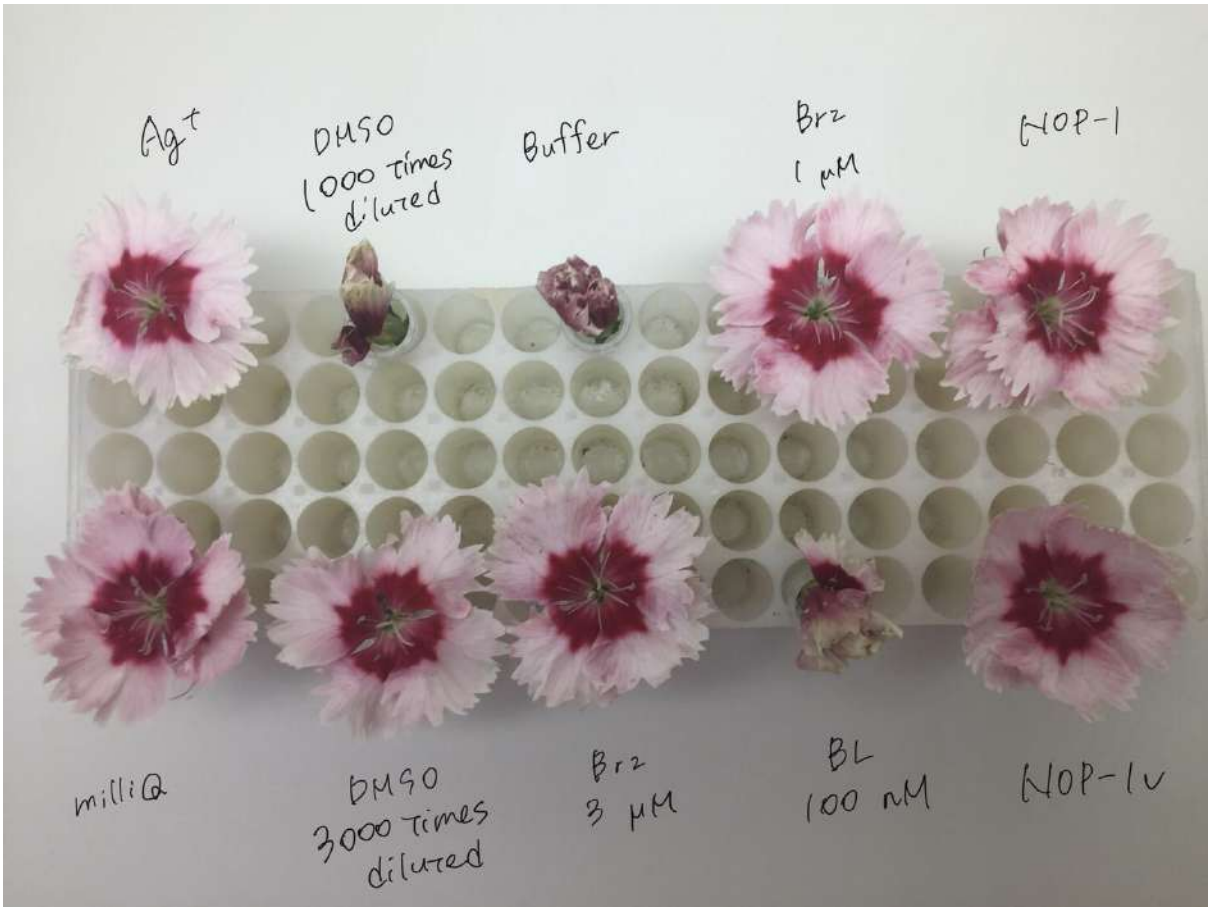
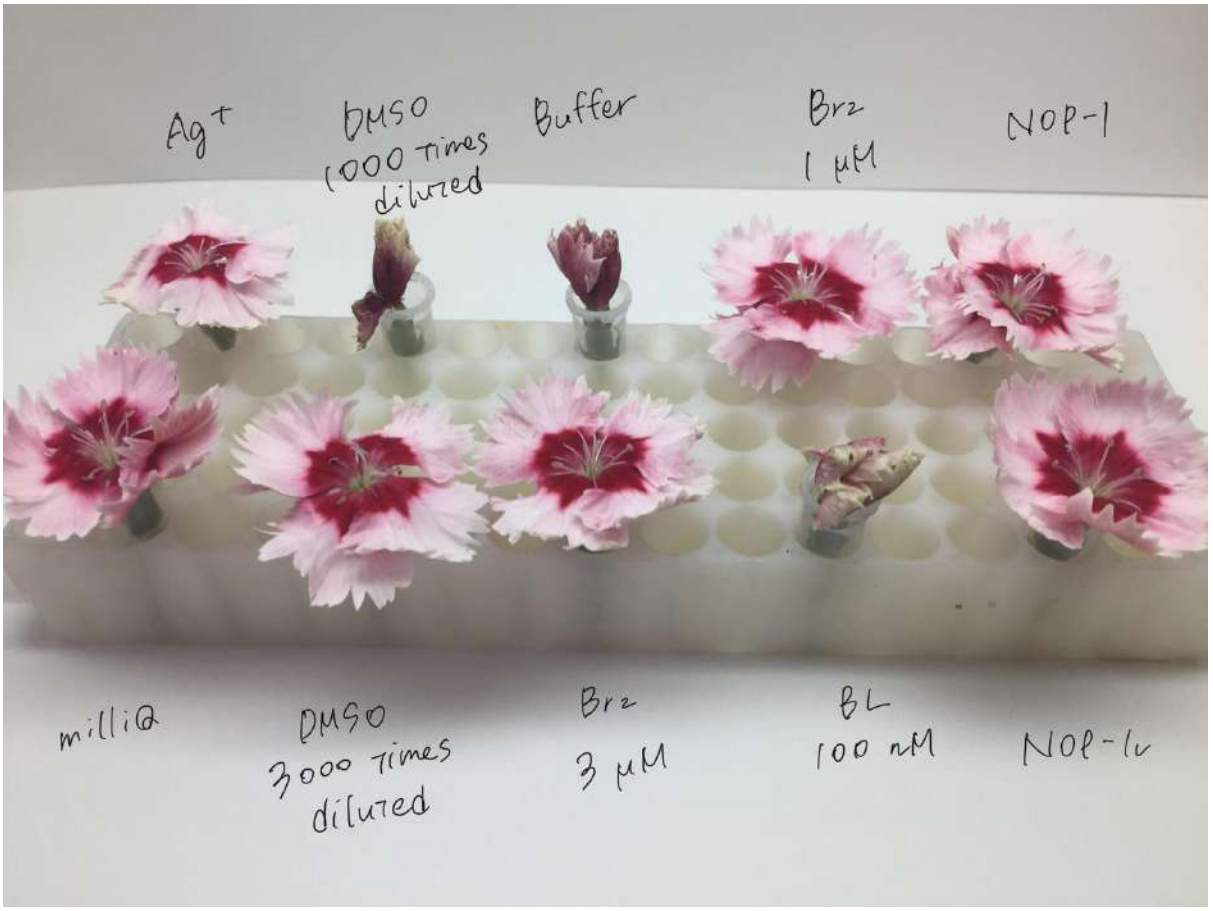
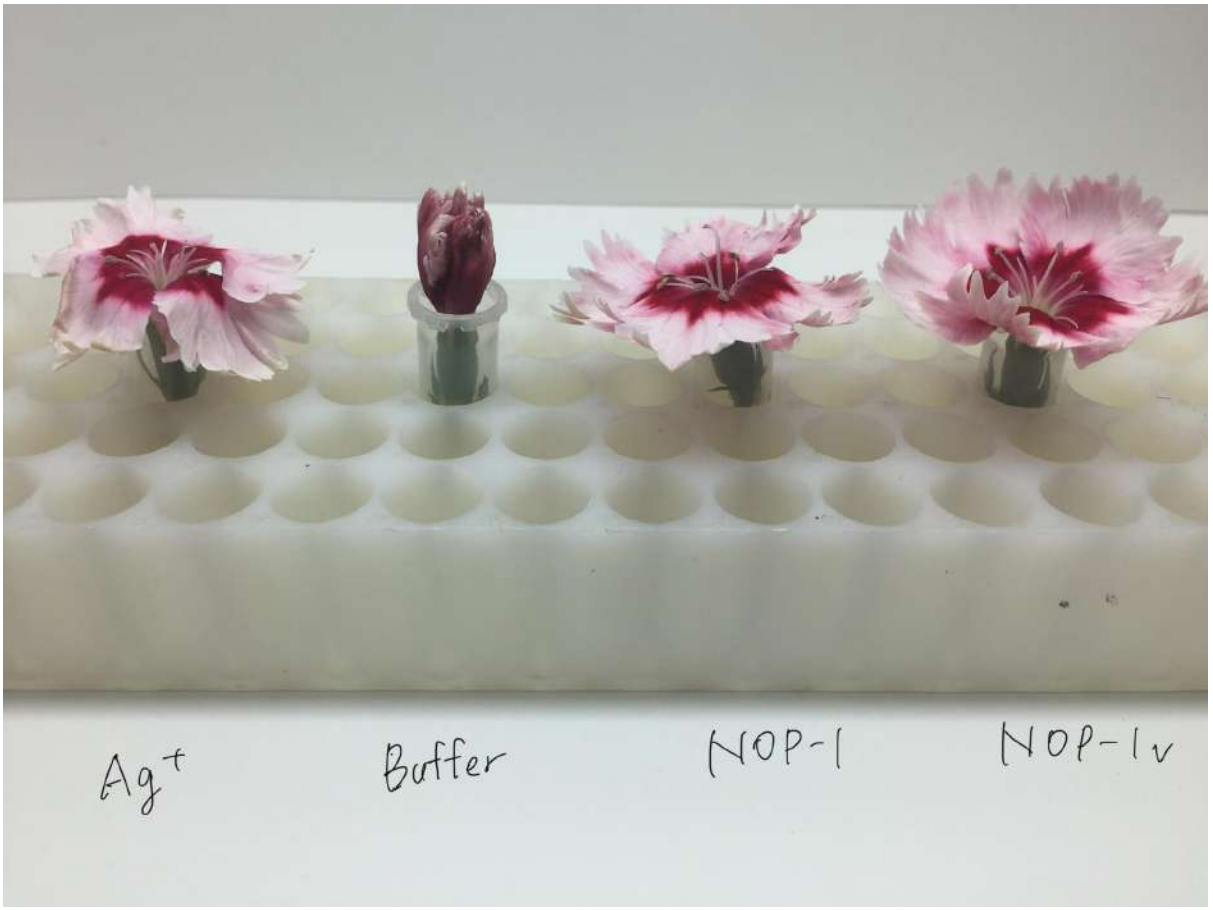

Ag^{+}
DMSO
1000 times
diluted
Buffer
Brz
1 μM
NOP-1
milliQ
DMSO
3000 times
diluted
Brz
3 μM
BL
100 nM
NOP-1v


Ag+
DMSO
1000 Times
diluted
Buffer
Brz
1 μM
NOP-1
milliQ
DMSO
3000 Times
diluted
Brz
3 μM
BL
100 nM
NOP-1v


Ag+
Buffer
NOP-1
NOP-1v

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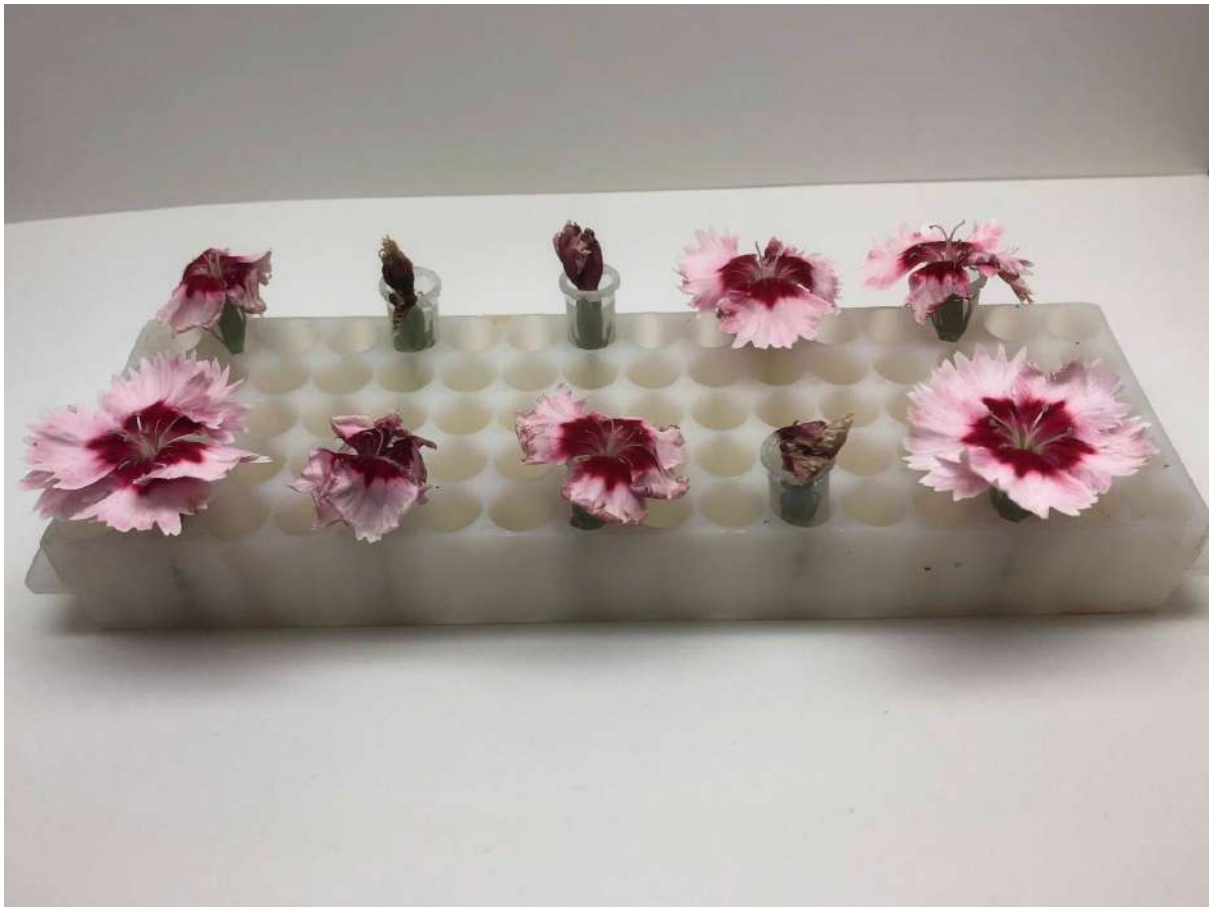

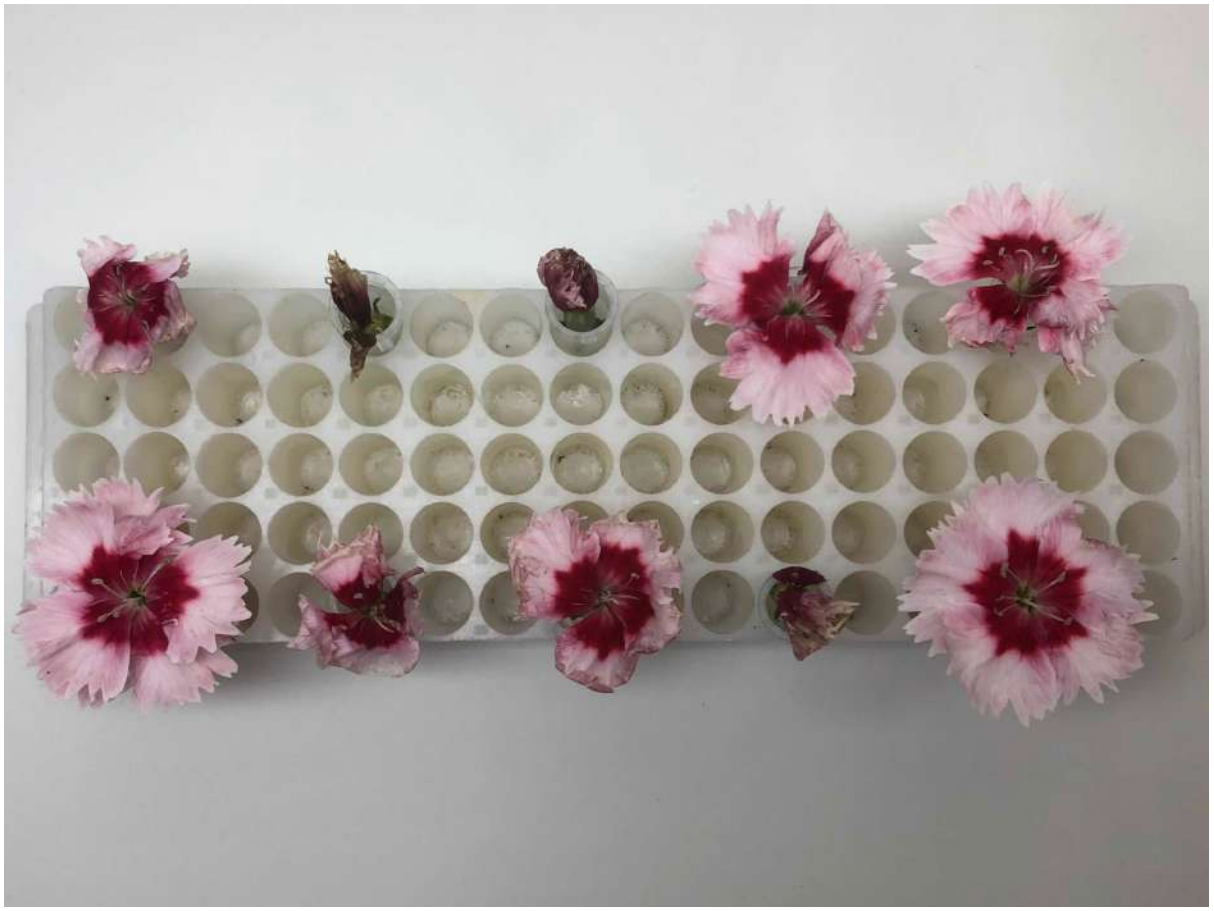

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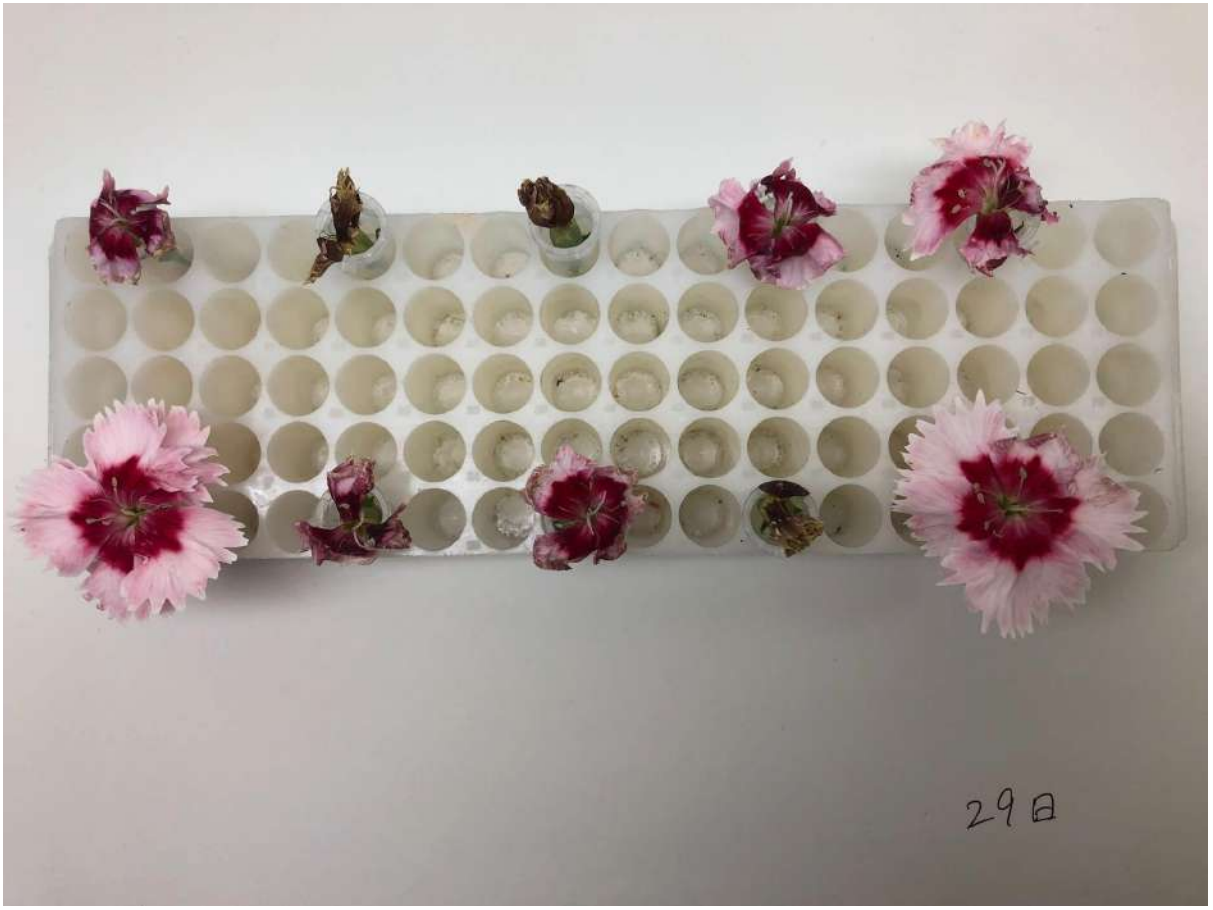
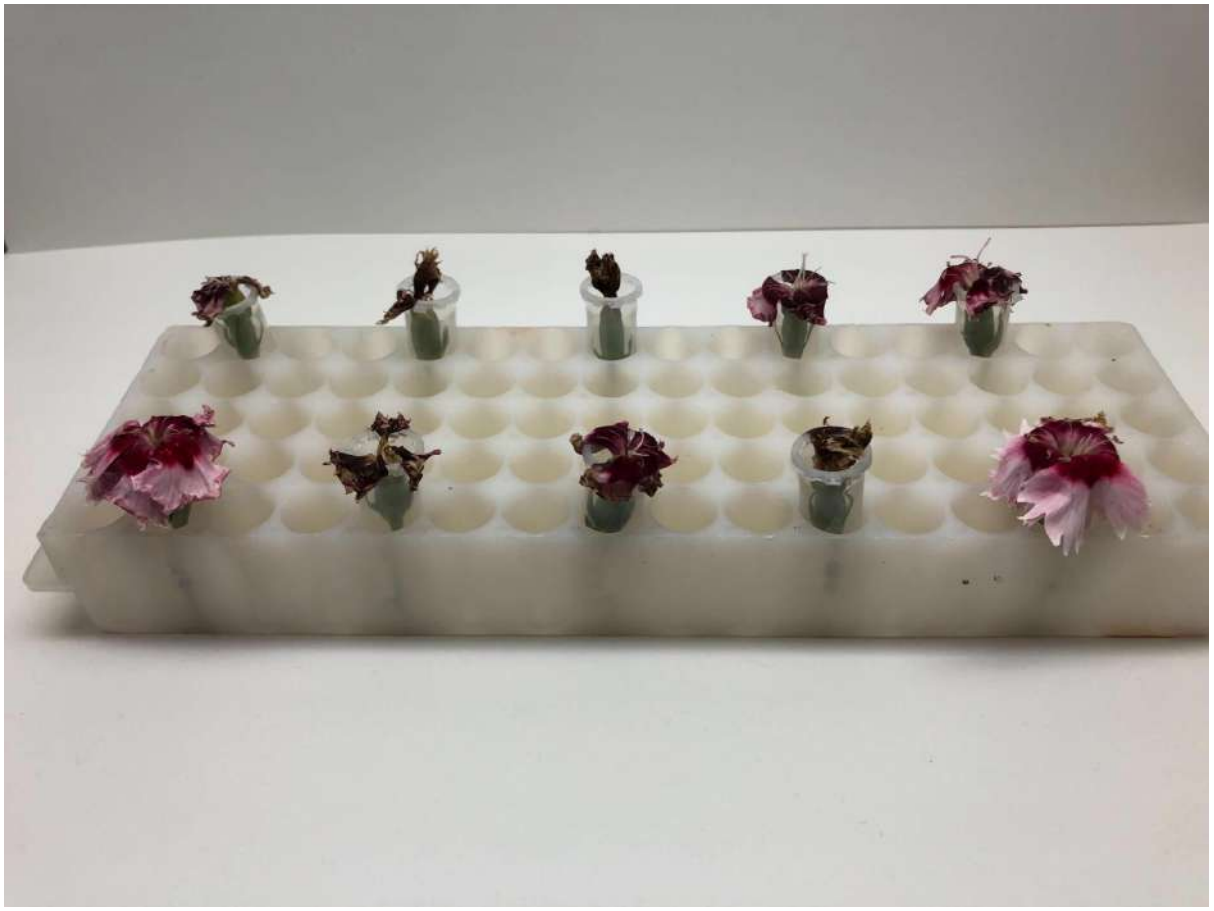
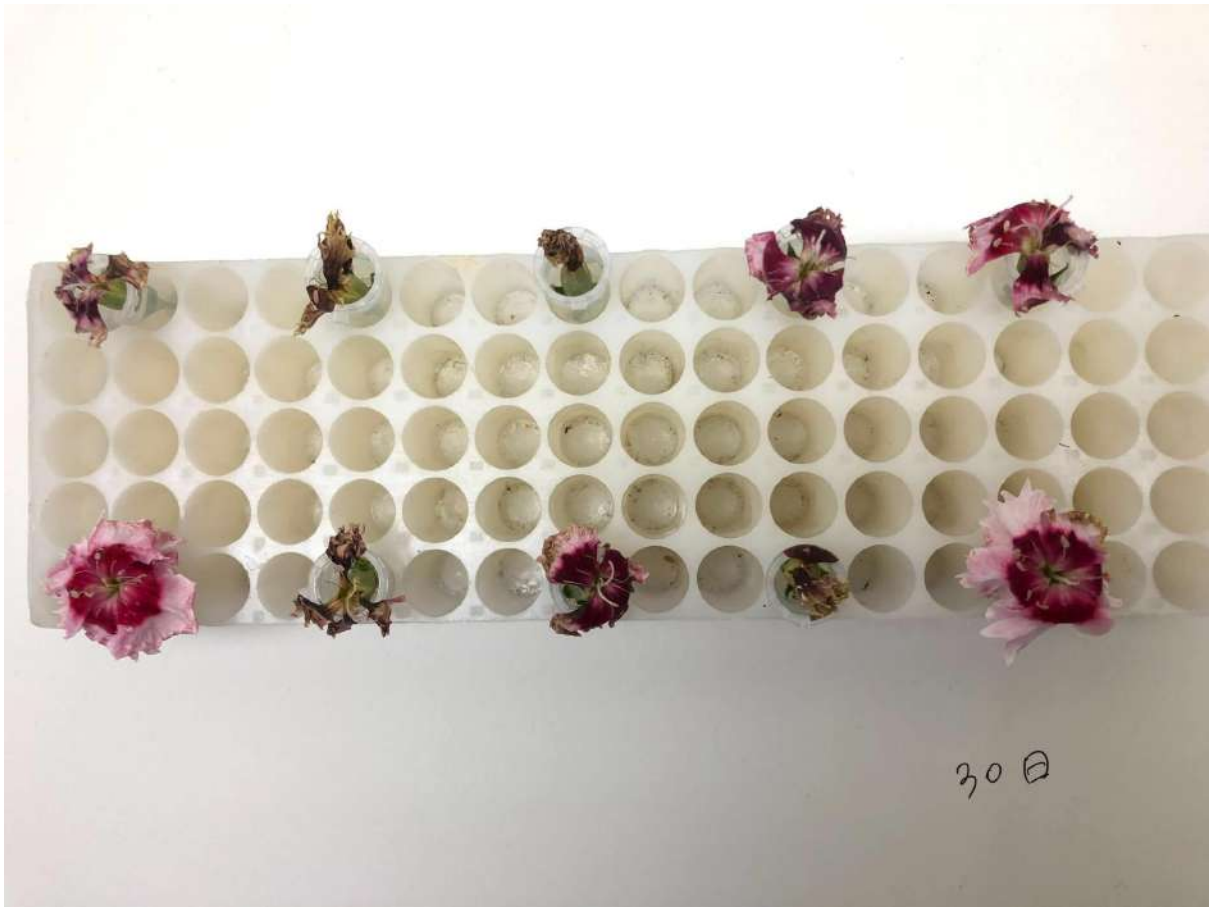
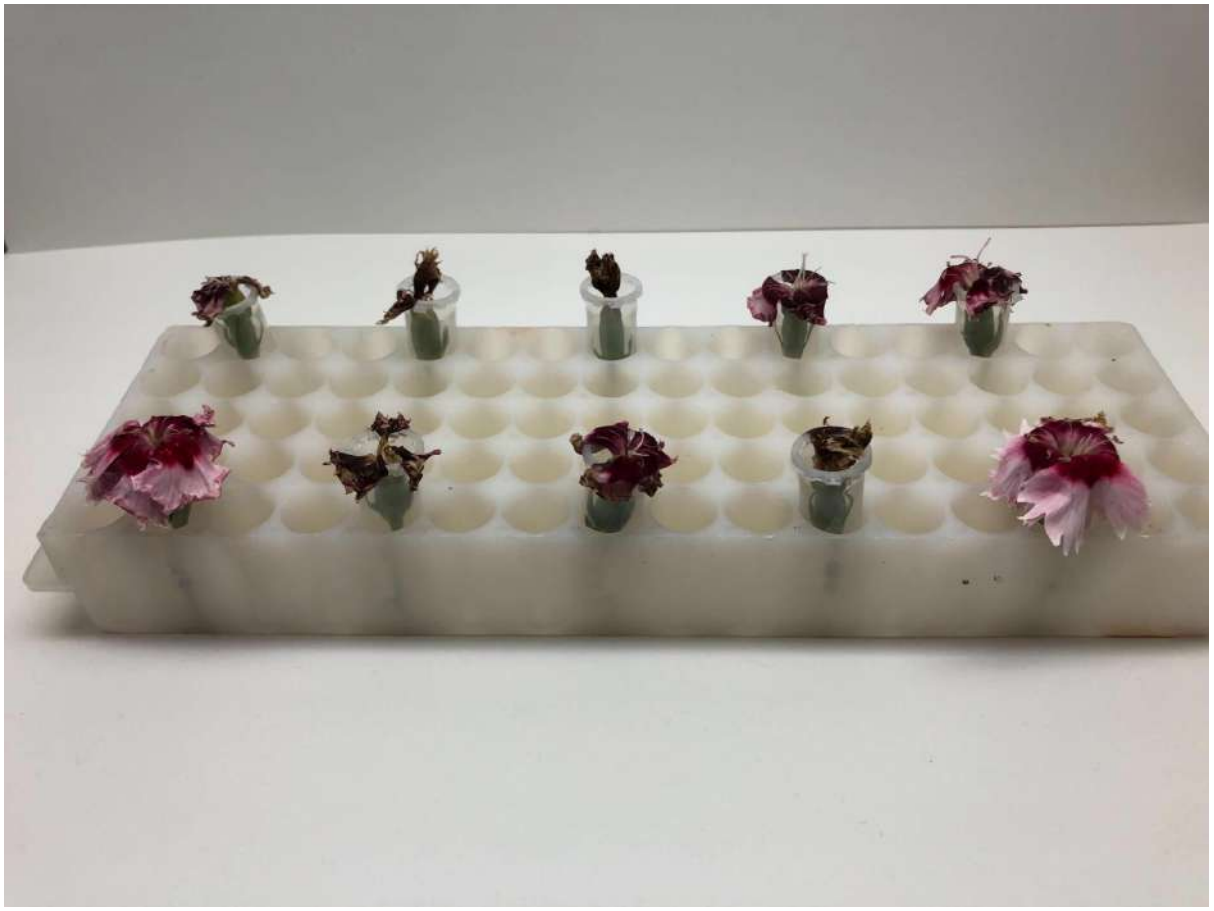


9/30




30日

10/1



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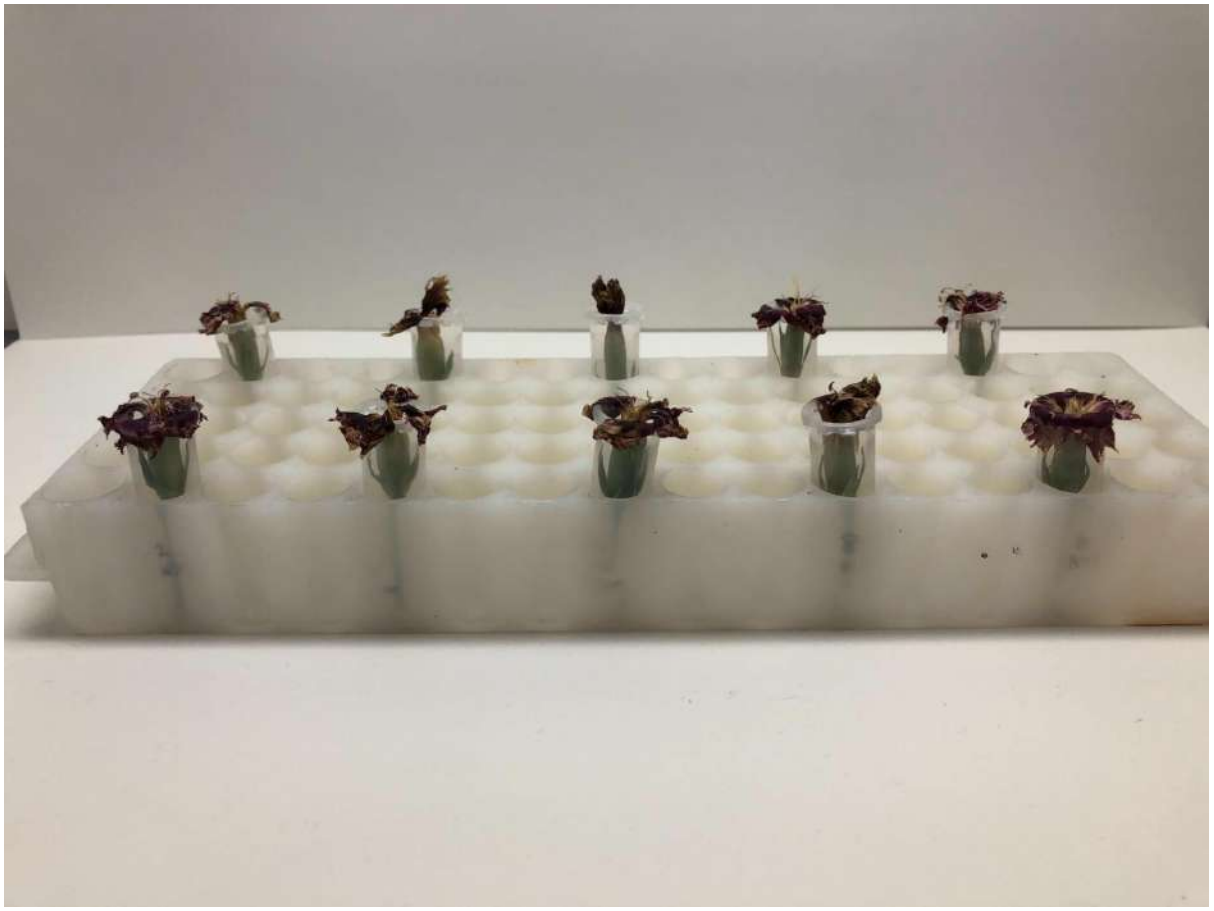
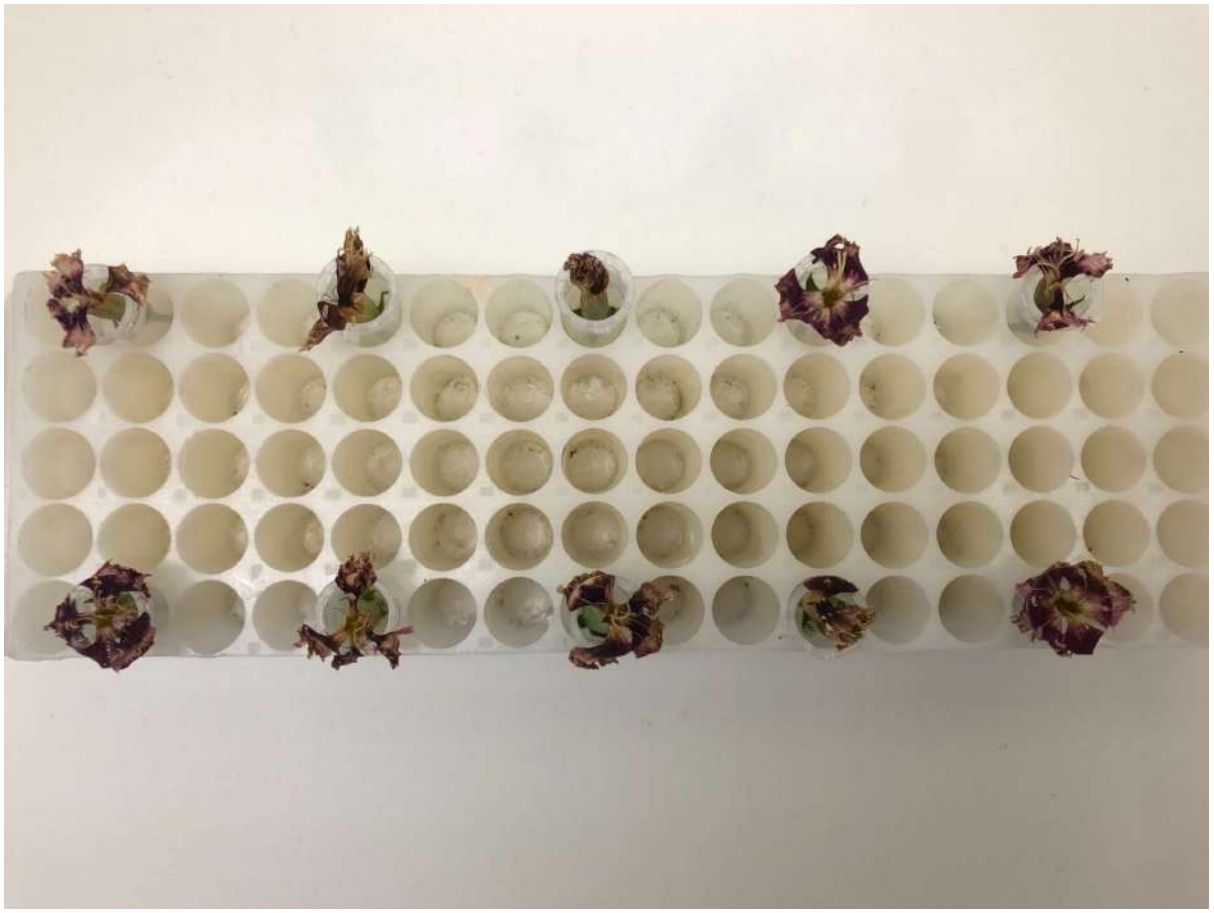




10/8

NOP-1 assay②

Experimental conditions

Chemically synthesized NOP-1 0.5 mM
Chemically synthesized NOP-1 buffer (Negative control)

E.coli synthetic NOP-1 diluted 10 times
E.coli synthetic NOP-1 buffer (Negative control)

Materials

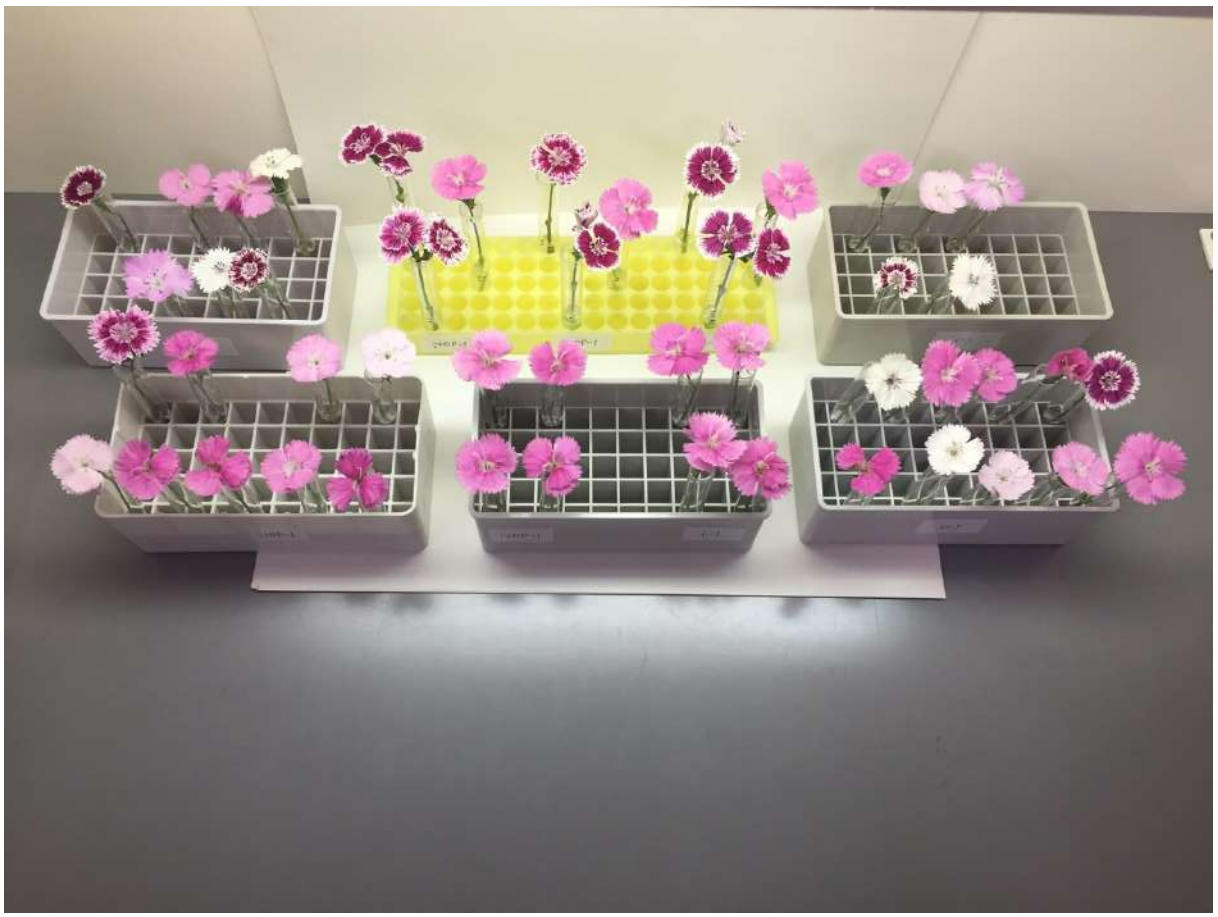
Synthetic NOP-1 peptide solution made by Mr. Tamukai.
NOP-1 peptide (Mw = 1132.41 Da) 50 mg / 500 uL 30 % acetonitrile
＊Final concentration was 0.5 mM (acetonitrile solution of Negative control was diluted in the same way).

E.coli synthetic NOP-1, NOP-1v prepared by Mr. Koga
＊Used the solution 10 times diluted.
＊The buffer for Negative control was also diluted.

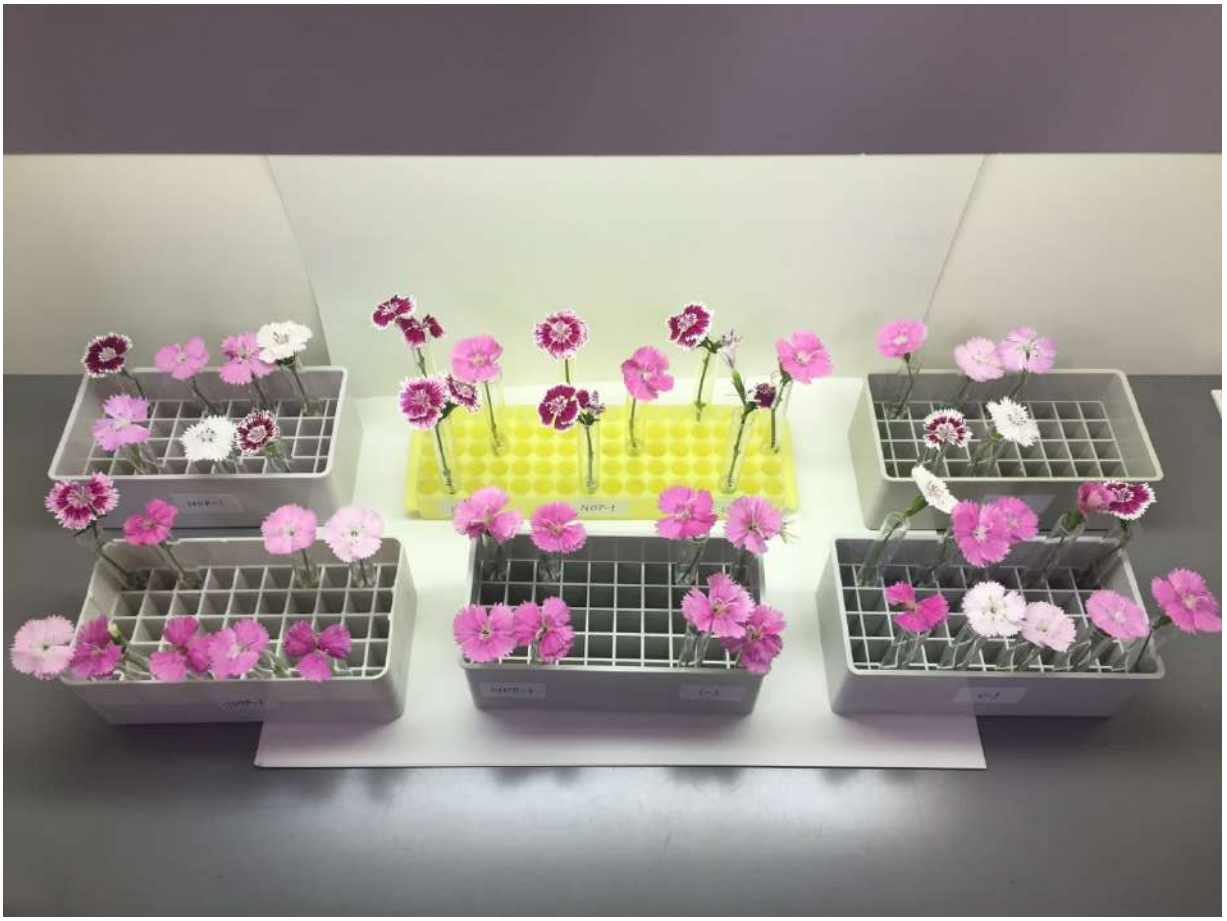
Dianthus that I bought as a potted plant

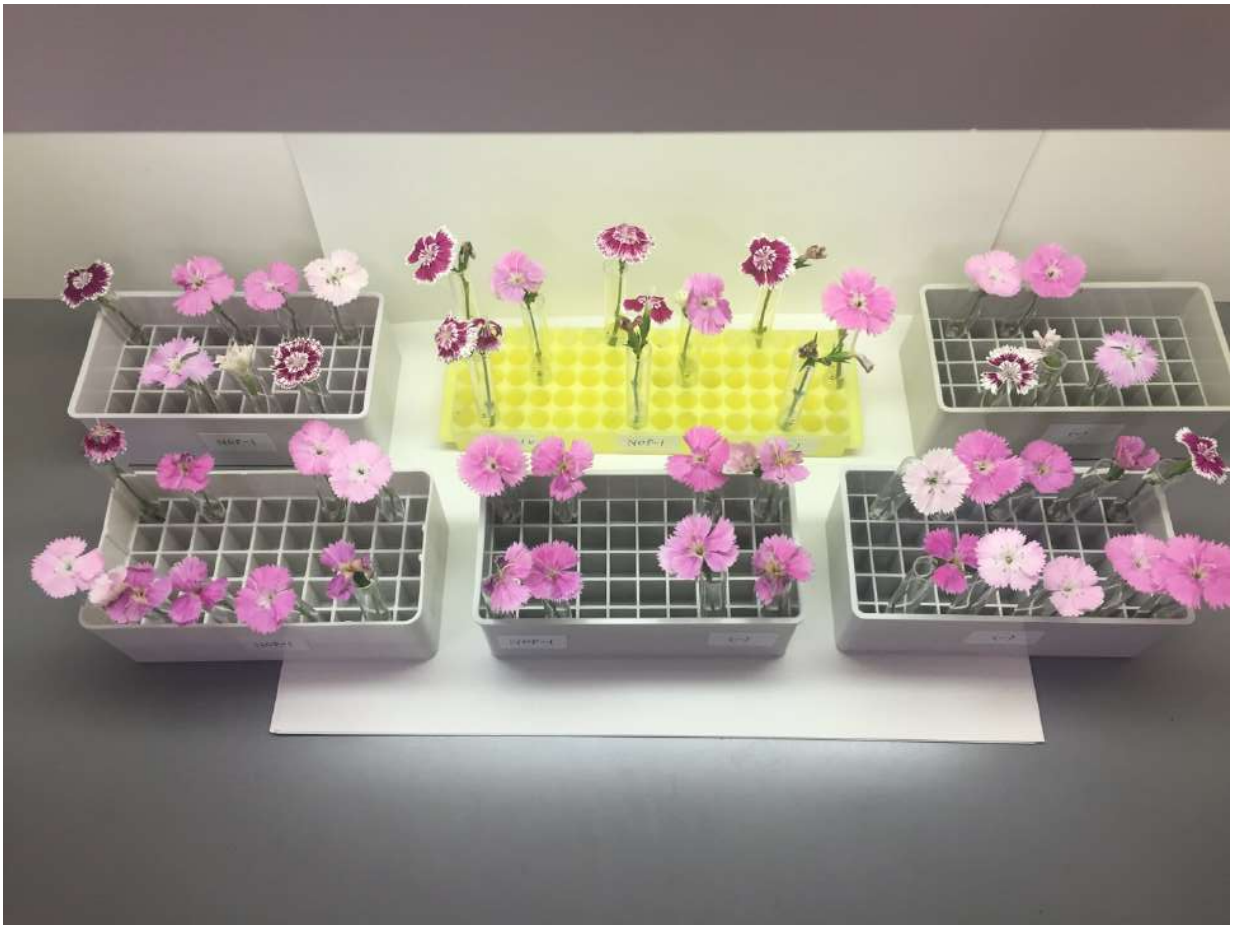

NOP-1v
NOP-1
(−)

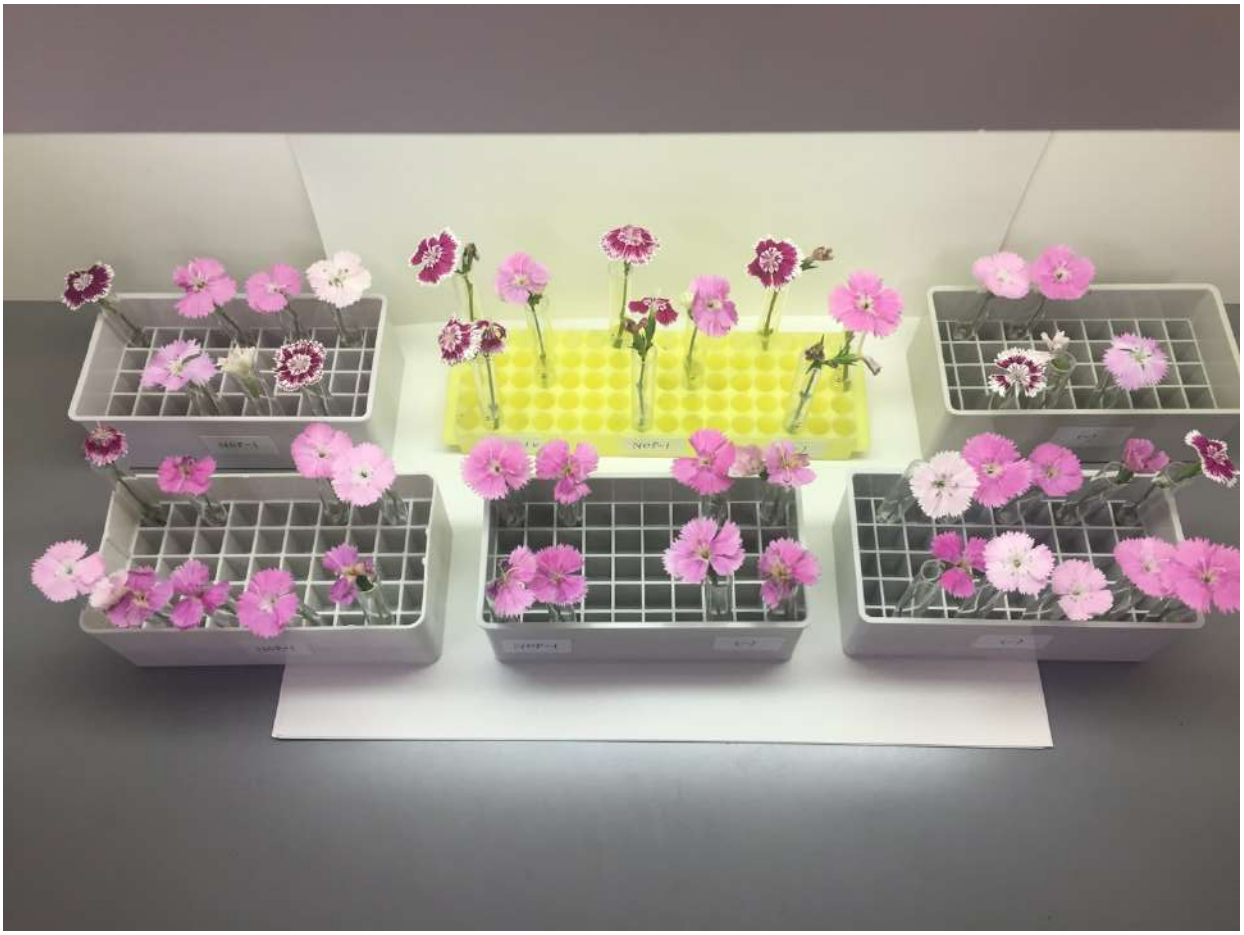
10/9



10/10







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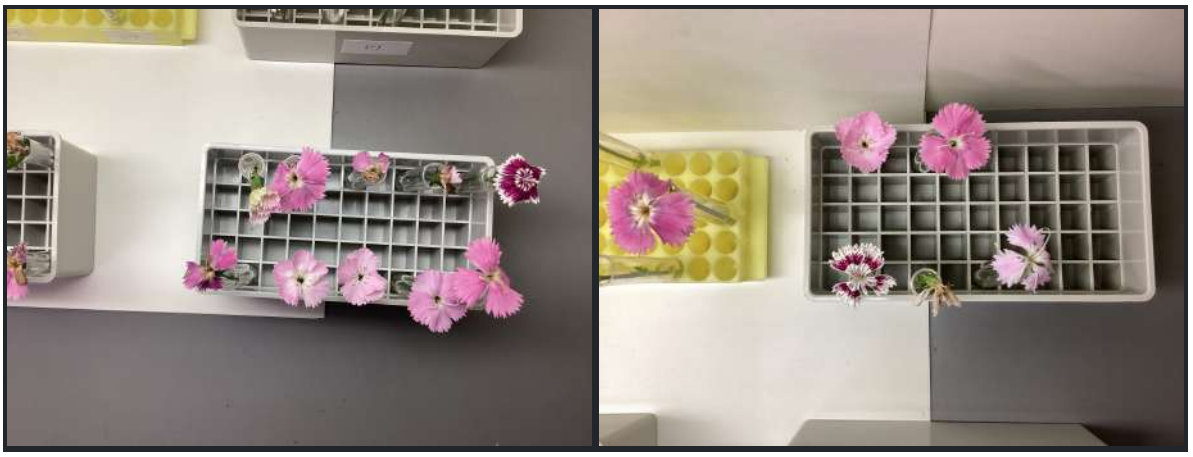

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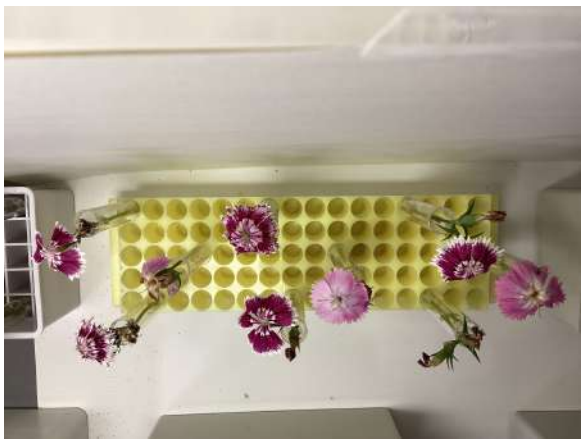


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