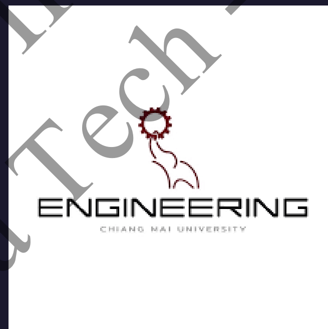
Effect of Copper Compounds Coated on *Citrullus lanatus* (watermelon) Seeds and Plasma Treatment to the Seed Quality Improvement

Thapanee Sarakonsri, Yothin Chimupala, Saranphong Yimklan, Natthawat Semakul, Chommanad Sawadeemit, Tanika Sookpasom, Lalida Shank, Dheerawan Boonyawan, Choncharoen Sawangrat, and Sa-guansak Thanapornpoonpong



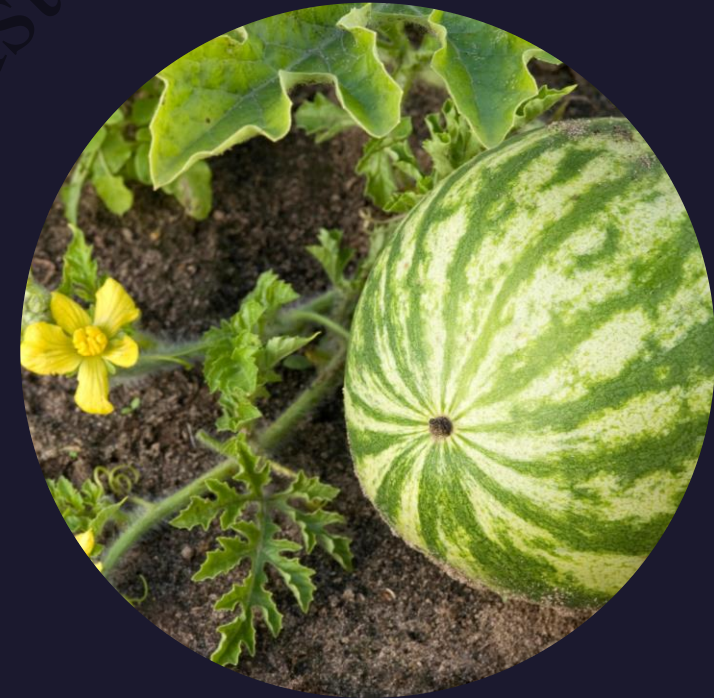
The IRN-2022 Symposium on Plasma Tech - Driving Sustainable Future

Introduction

Seeds of *Citrullus lanatus* (watermelon) have economic significance in Thailand with high production potential and export value

Low germination rate of watermelon seeds

- **Seed itself**
- **Bacteria contamination**



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Introduction

Watermelon seeds treated with a plasma via plasma dielectric barrier discharge (DBD)

(O_2 gas and Ar gas mixture)

Copper solution treatment

Increased seed quality

Copper: The Bacteria-Fighting Metal

Every day, high-touch surfaces present health risks to people in public spaces, and especially the most vulnerable in healthcare.

With naturally antimicrobial properties, copper can kill up to 99.9% of bacteria on surfaces and slow the spread of diseases.

How Antimicrobial Copper Works:

Antimicrobial copper kills bacteria in three sequential steps.

Antimicrobial copper continuously kills bacteria, never wears out, and is safe to use.

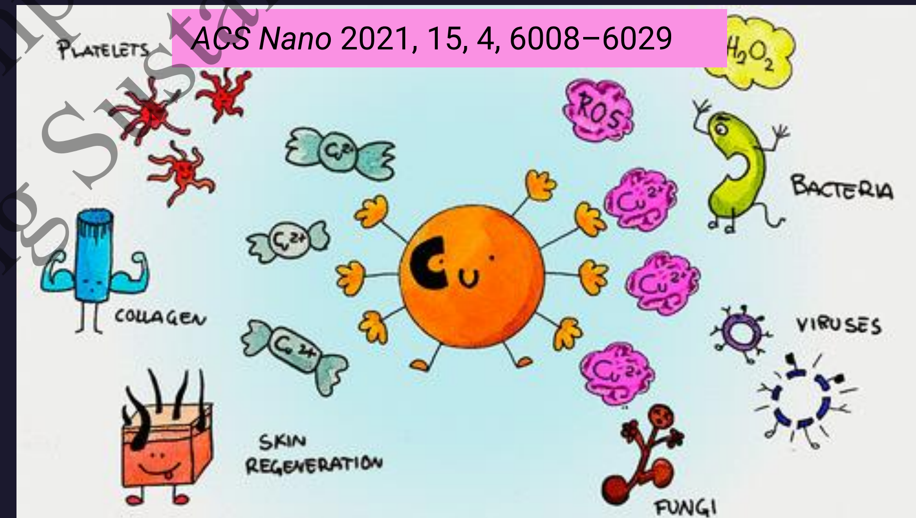
Enters — Copper ions on the surface are recognized as an essential nutrient and enter the bacteria cell.

Disrupts — Copper ions interfere with normal cell functions and membrane integrity.

Kills — When excess copper binds to the enzymes, the bacteria can no longer breathe, eat, digest or create energy.

Source: CuVerro, Copper Development Association

www.teck.com



ACS Nano 2021, 15, 4, 6008−6029

The surfaces that kill bacteria and viruses - BBC Future bbc.com

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Experiment

watermelon seeds

1

Cu solutions
0.1, 0.05 ,0.025 M
(Cu1, Cu2, Cu3)

→ Plasma treatment
1,500, 2000 V
1.0, 1.2, 1.4, 1.6 and 1.8 s/cm

→ Germination test
3, 5, and 14 days

2

Plasma treatment
1,500, 2000 V
1.0, 1.2, 1.4, 1.6 and 1.8 s/cm

→ Cu solutions
0.1, 0.05 ,0.025 M
(Cu1, Cu2, Cu3)

→ Germination test
3, 5, and 14 days

SEM TEM and FT-IR

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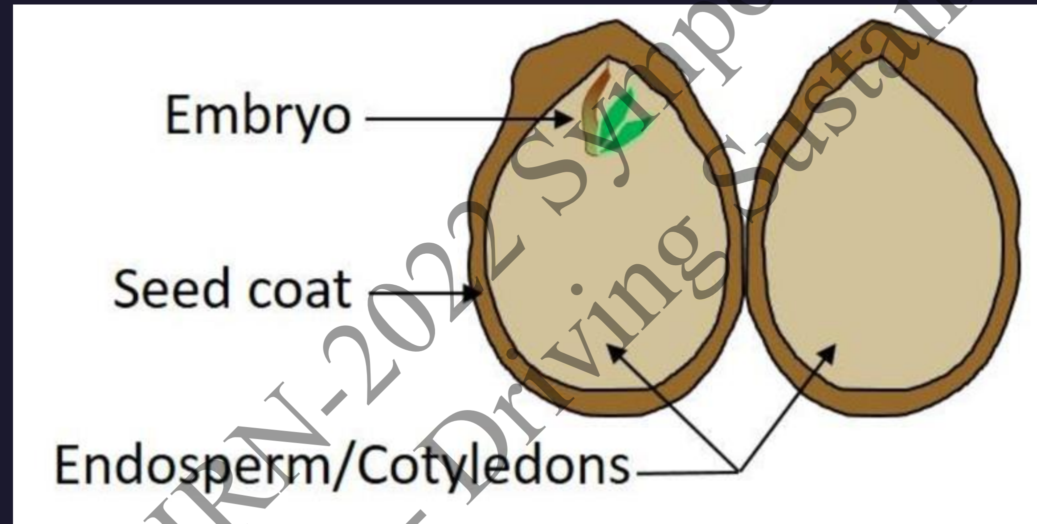
Results and Discussion

SEM observation of watermelon seed before and after plasma treatment



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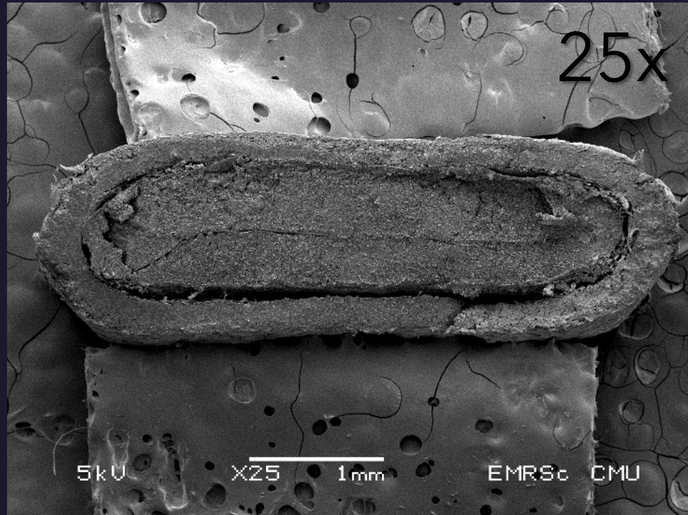
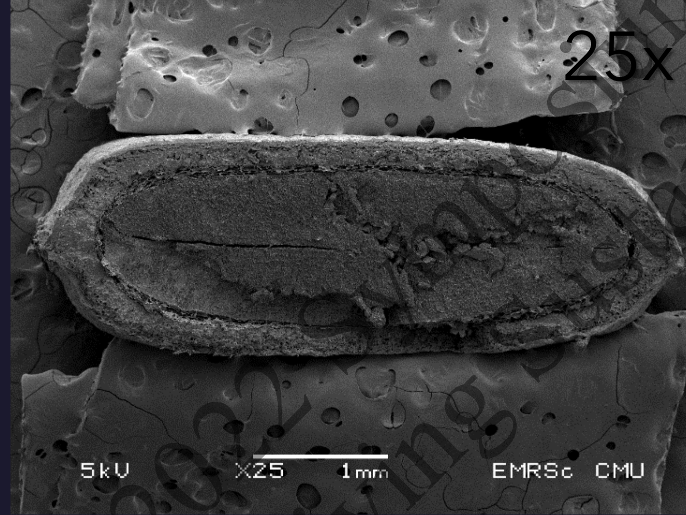
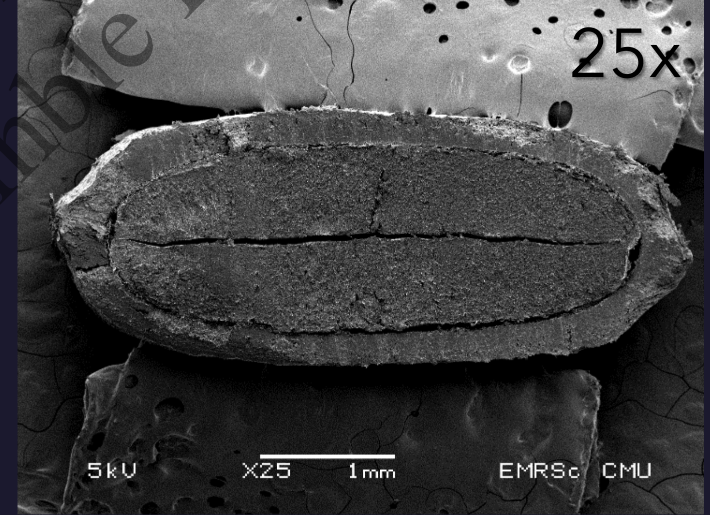
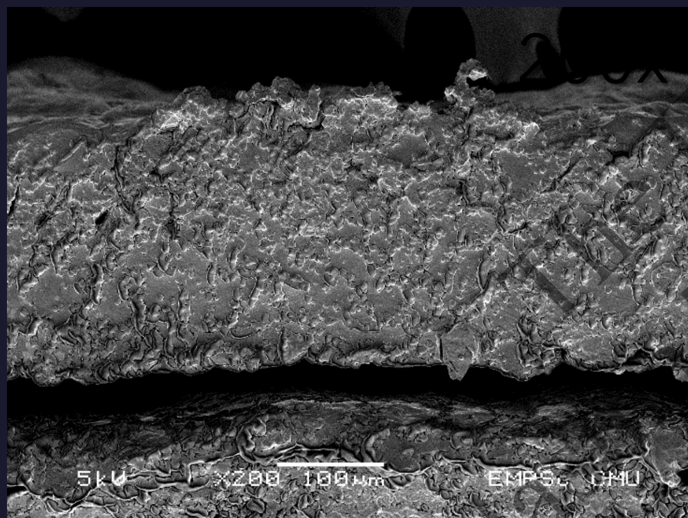
Watermelon (Seed coat) cross section

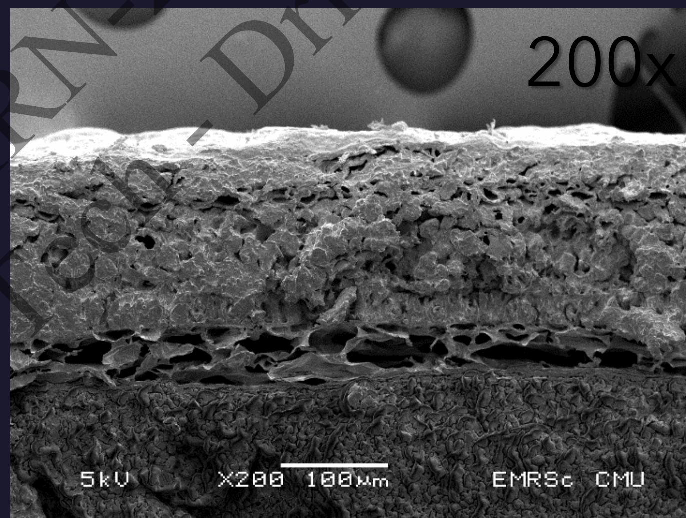
Control	Treated 1.4 s/m, 1500V	Treated 1.8 s/m, 2000V

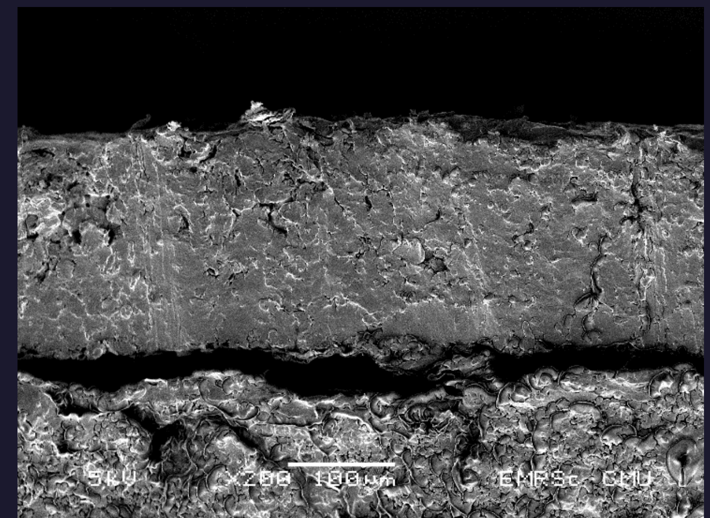






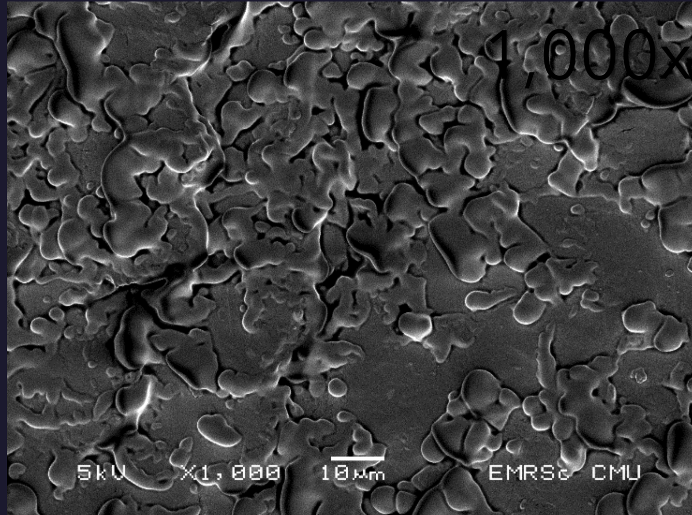


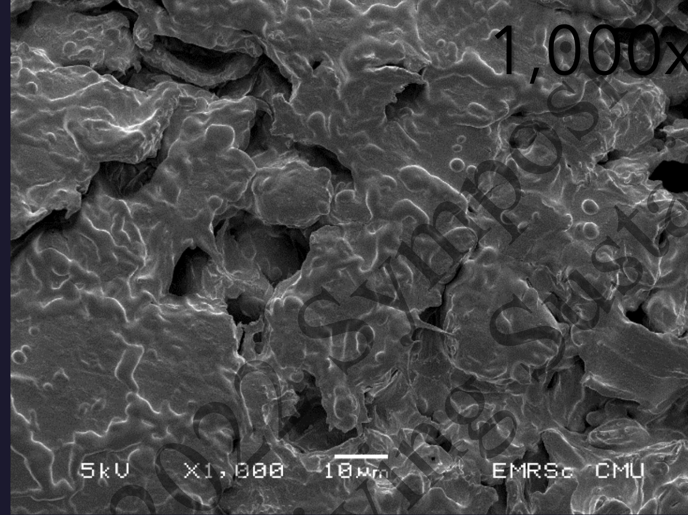


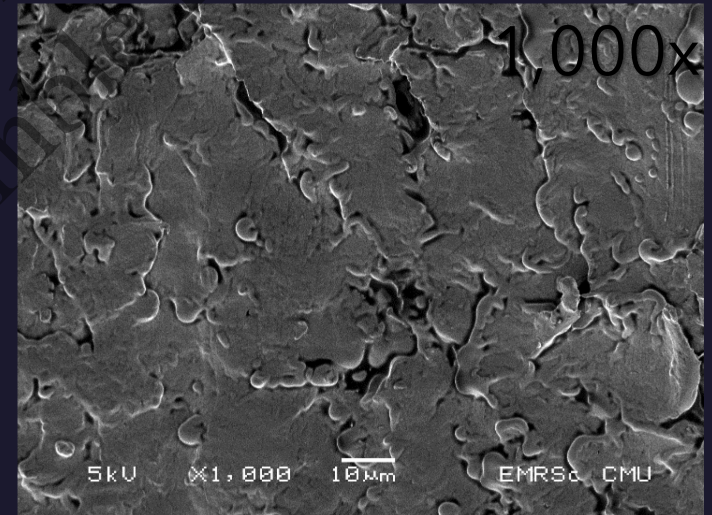


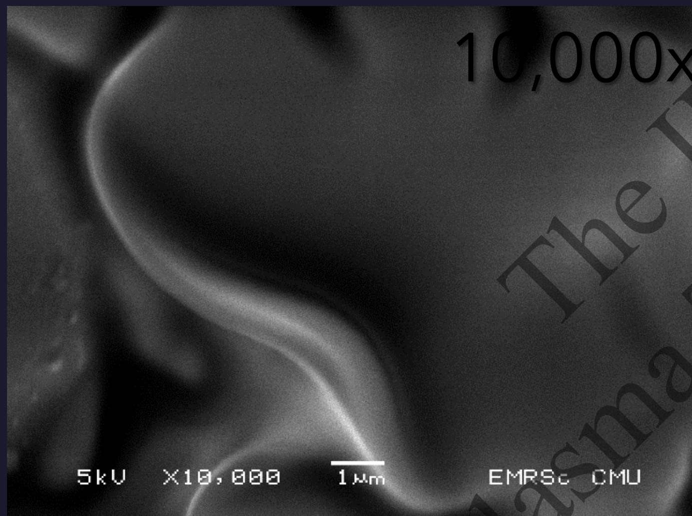
Watermelon(Seed coat)

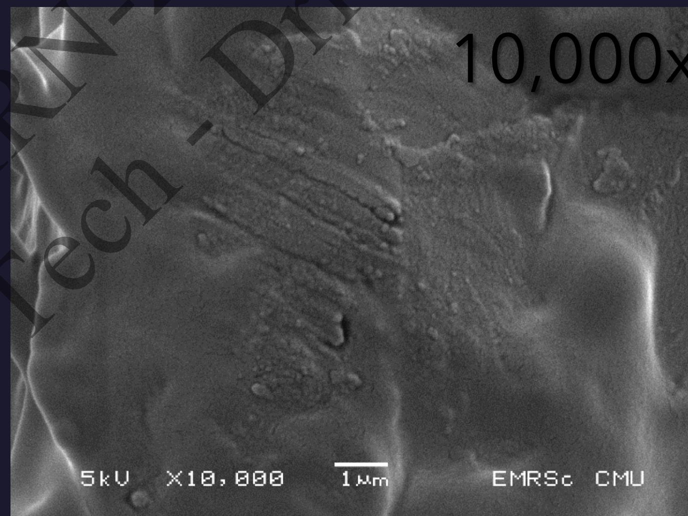
Control | Treated 1.4 s/m, 1500V | Treated 1.8 s/m, 2000V

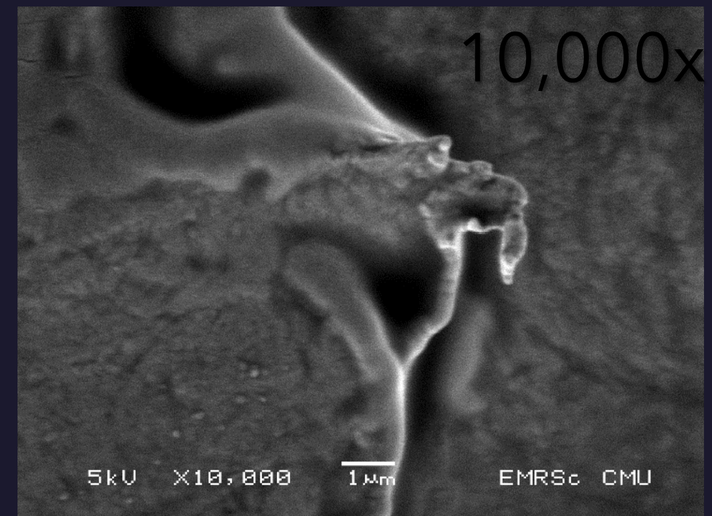












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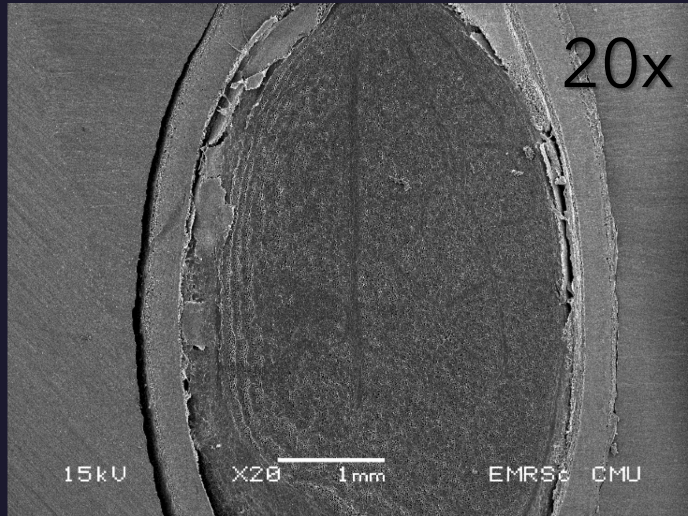
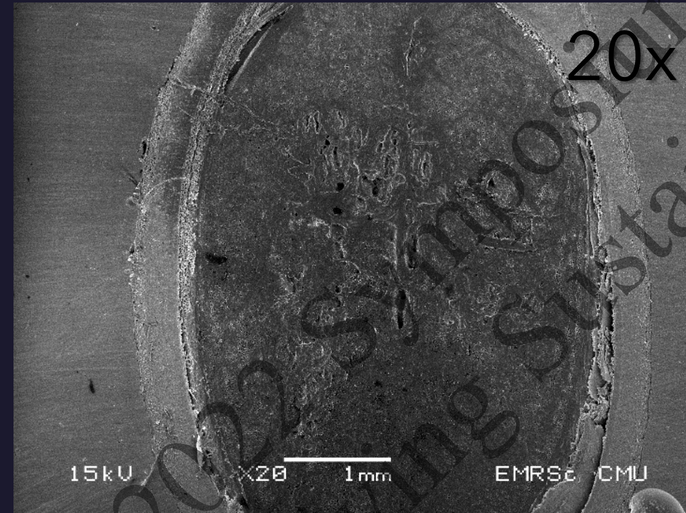
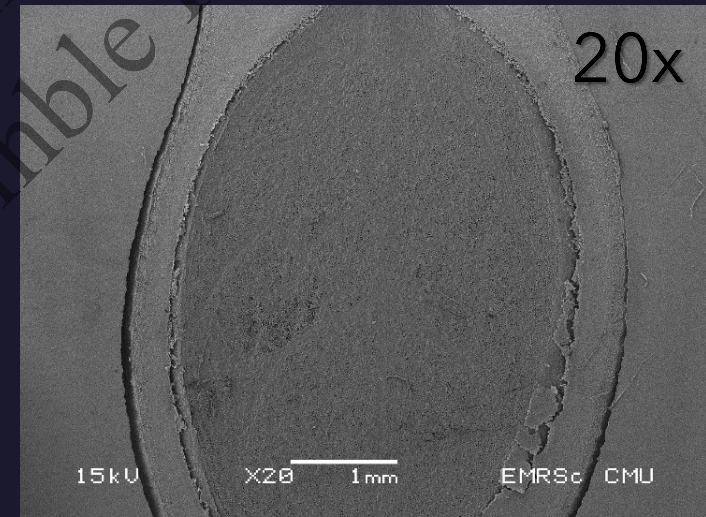
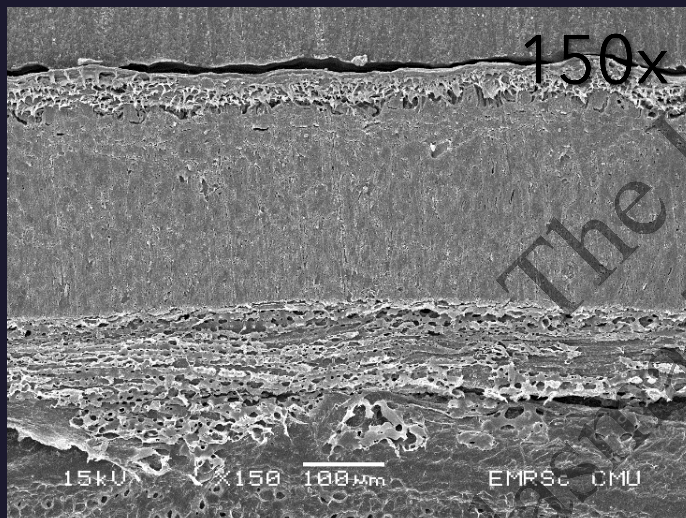
Watermelon (Seed coat) cross section

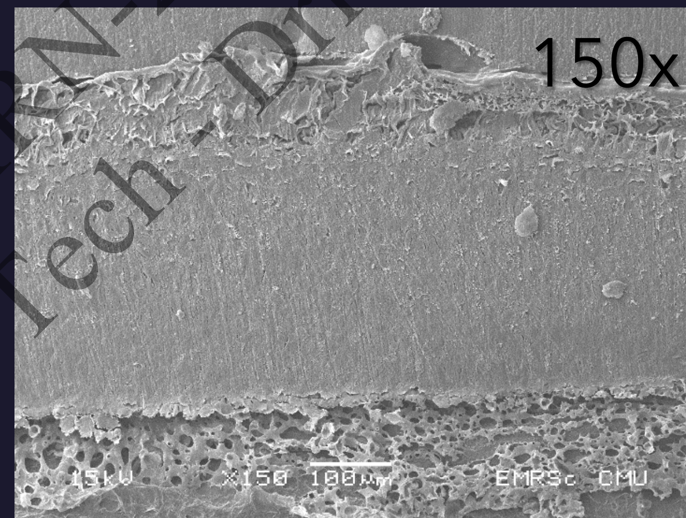
Control | Treated 1.4 s/m, 1500V | Treated 1.8 s/m, 2000V

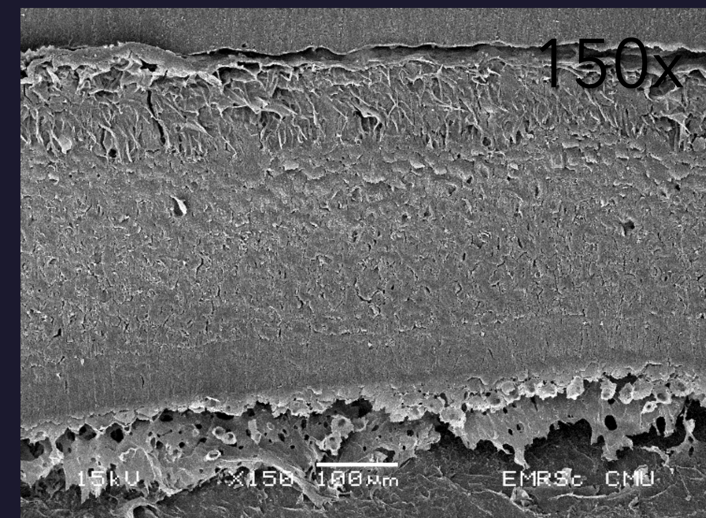












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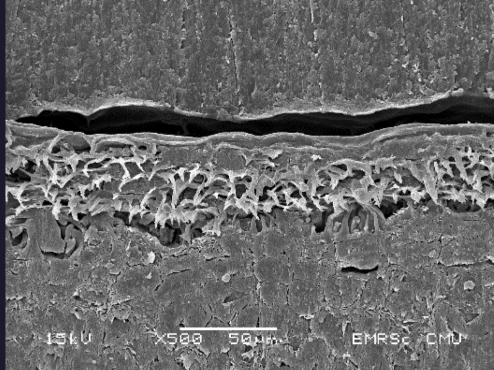
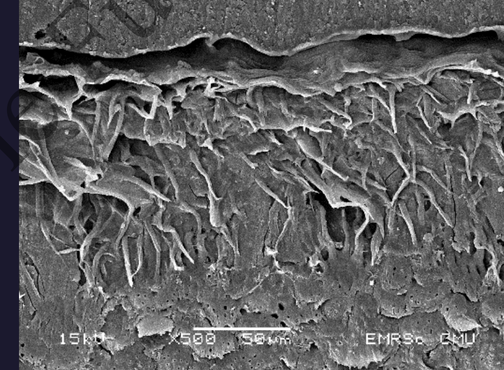
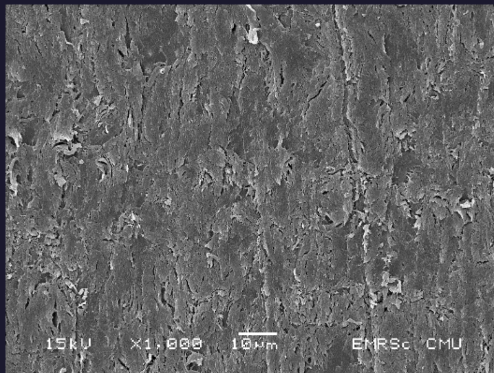
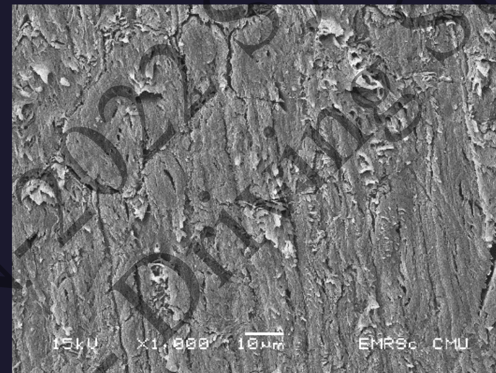
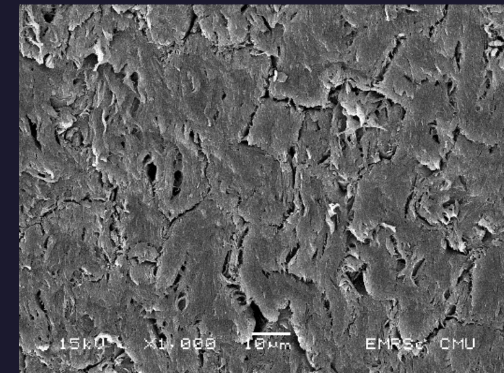
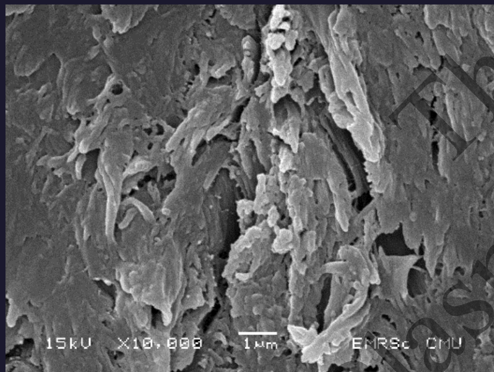
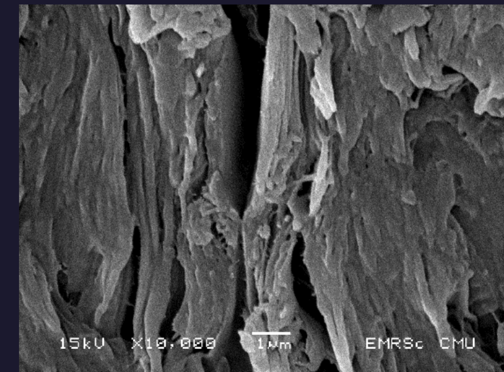
Watermelon (Seed coat)

	Control	Treated 1.4 s/m, 1500V	Treated 1.8 s/m, 2000V
500x			
1,000x			
10,000x			

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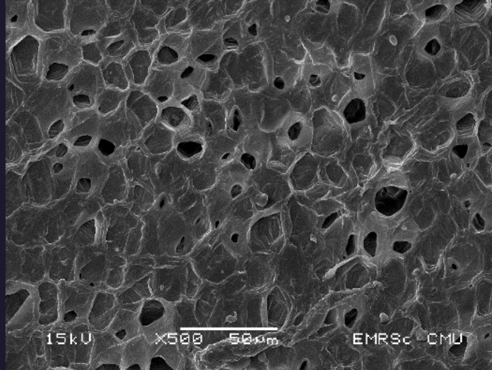
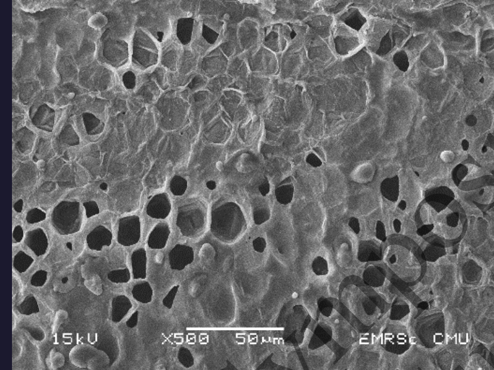
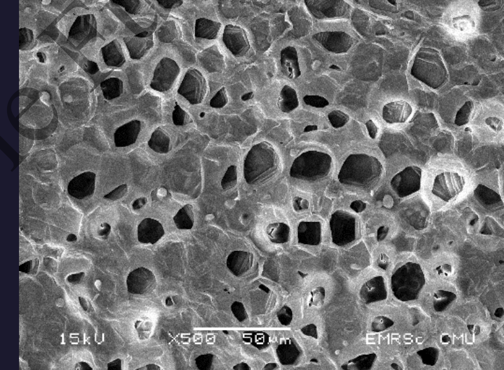
Watermelon (endosperm)

Control | Treated 1.4 s/m, 1500V | Treated 1.8 s/m, 2000V

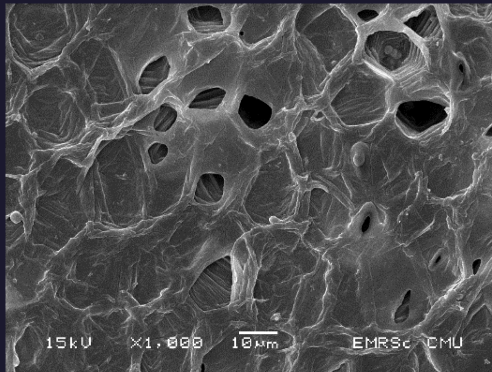
500x

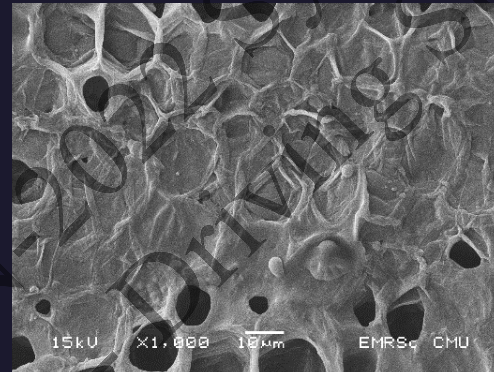


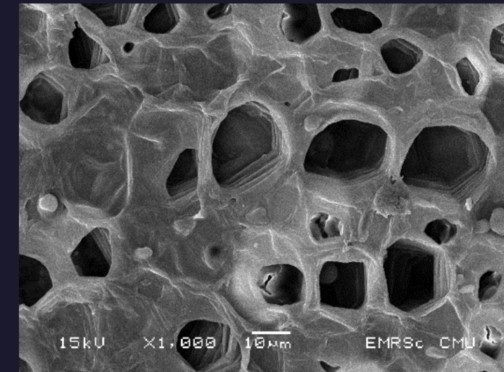




1,000x







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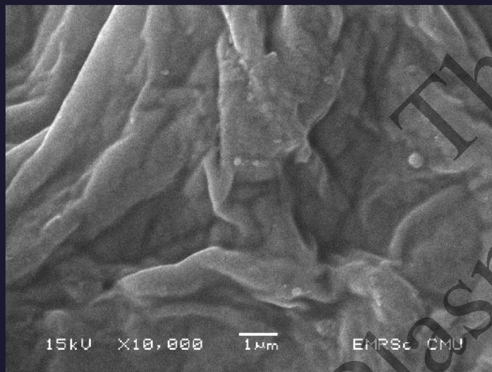

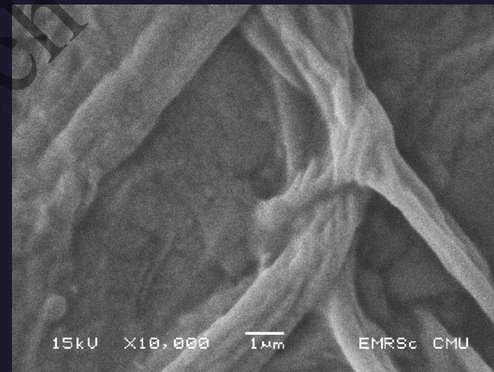

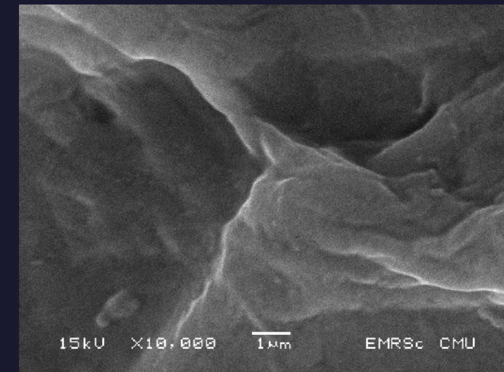

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Treatment		Radicle emergence 3 days	Seed Germination 5 days	Seed Germination 14 days	Seed Germination index
RAW		84.67 AB	70.00 AB	91.33 AB	16.16 ABC
Ar	0.4 s/cm	84.00 AB	68.67 ABC	87.33 AB	16.10 ABCD
	0.6 s/cm	82.00 ABC	73.33 A	91.33 AB	16.96 A
	0.8 s/cm	71.33 CDEF	48.00 EFGH	86.67 AB	15.31 BCDEFG
	1.0 s/cm	82.67 ABC	56.67 DEF	89.33 AB	15.71 ABCDEF
	1.2 s/cm	78.00 ABC	54.00 DEFG	92.00 AB	16.34 AB
Ar + O_2 0.2 L/min	0.4 s/cm	84.00 AB	54.67 DEFG	76.67 CD	13.58 IJ
	0.6 s/cm	89.33 A	58.00 CDE	86.67 AB	15.21 BCDEFGH
	0.8 s/cm	80.00 ABC	57.33 DE	83.33 BC	14.52 EFGHI
	1.0 s/cm	78.00 ABC	70.00 AB	88.00 AB	15.84 ABCDE
	1.2 s/cm	84.67 AB	73.33 A	89.33 AB	16.98 A
Ar + O_2 0.4 L/min	0.4 s/cm	58.67 GHIJ	35.33 IJK	70.00 DE	10.74 K
	0.6 s/cm	60.00 FGHI	25.33 K	86.67 AB	11.04 K
	0.8 s/cm	48.00 J	33.33 JK	61.33 E	9.66 K
	1.0 s/cm	52.00 HIJ	25.33 K	71.33 D	9.87 K
	1.2 s/cm	60.00 FGHI	29.33 JK	71.33 D	10.14 K
Ar + O_2 0.6 L/min	0.4 s/cm	76.67 BC	47.33 EFGH	89.33 AB	13.78 HIJ
	0.6 s/cm	56.67 GHIJ	54.67 DEFG	86.67 AB	14.24 FGHIJ
	0.8 s/cm	48.67 IJ	51.33 DEFG	90.67 AB	14.57 EFGHI
	1.0 s/cm	52.00 HIJ	50.00 DEFG	86.67 AB	13.73 HIJ
	1.2 s/cm	73.33 BCDE	45.33 GHI	90.00 AB	13.67 IJ
Ar + O_2 0.8 L/min	0.4 s/cm	54.00 GHIJ	38.67 HIJ	89.33 AB	13.99 GHIJ
	0.6 s/cm	71.33 CDEF	49.33 DEFGH	90.67 AB	14.65 DEFGHI
	0.8 s/cm	58.00 GHIJ	47.33 EFGH	91.33 AB	14.83 CDEFGHI
	1.0 s/cm	62.00 EFGH	60.00 BCD	88.67 AB	15.41 BCDEFG
	1.2 s/cm	72.00 CDE	46.00 FGHI	89.33 AB	14.87 BCDEFGHI
Ar + O_2 1.0 L/min	0.4 s/cm	74.67 BCD	48.00 EFGH	89.33 AB	14.97 BCDEFGHI
	0.6 s/cm	71.33 CDEF	52.67 DEFG	93.33 A	15.77 ABCDE
	0.8 s/cm	64.67 DEFG	52.67 DEFG	86.67 AB	15.39 BCDEFG
	1.0 s/cm	63.33 DEFGH	32.00 JK	84.67 ABC	12.83 J
	1.2 s/cm	74.00 BCD	73.33 A	85.33 ABC	16.10 ABCD
F-Test		**	**	**	**
LSD ≤ 0.05		11.38	10.95	9.36	1.49
CV%		10.06	13.16	6.70	6.42

Seed germination rate decrease when O_2 gas increase in the gases mixture

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Treatment	Radicle emergence 3 day	Seed Germination 5 day	Seed Germination 14 day	Seed Germination index
RAW	75.50 BC	52.50 AB	78.50 CDE	16.19 CD
Plasma 0.4 s/cm	63.00 D	45.50 BC	88.50 AB	17.51 ABC
Plasma 0.6 s/cm	77.50 ABC	60.50 A	88.50 AB	18.77 A
Plasma 0.8 s/cm	72.50 CD	50.50 AB	89.00 AB	17.56 ABC
Plasma 1.0 s/cm	73.00 CD	48.00 BC	86.00 ABC	17.10 BC
Plasma 1.2 s/cm	78.00 ABC	55.50 AB	92.50 A	18.63 AB
Cu	78.00 ABC	38.50 CD	92.00 A	15.20 DE
Cu1 -> 0.4 s/cm	75.00 BC	52.50 AB	91.50 A	15.13 DE
Cu1 -> 0.6 s/cm	86.50 A	62.00 A	90.00 A	15.47 DE
Cu1 -> 0.8 s/cm	79.00 ABC	50.50 AB	84.00 ABCD	14.80 DE
Cu1 -> 1.0 s/cm	79.00 ABC	52.50 AB	85.00 ABCD	14.32 EF
Cu 1-> 1.2 s/cm	82.00 ABC	28.50 D	90.50 A	14.26 EF
0.4 s/cm -> Cu1	71.50 CD	32.50 D	76.00 DE	11.86 G
0.6 s/cm -> Cu1	84.00 AB	37.50 CD	86.00 ABC	14.32 EF
0.8 s/cm -> Cu1	74.50 BC	46.00 BC	70.00 E	11.74 G
1.0 s/cm -> Cu1	79.50 ABC	38.00 CD	87.50 ABC	12.97 FG
1.2 s/cm -> Cu1	77.50 ABC	44.50 BC	80.00 BCD	12.85 FG
F-Test	*	**	**	**
LSD ≤ 0.05	10.60	11.89	9.01	1.58
CV%	9.72	17.89	7.41	7.31

1

2

Cu solution 0.1 M

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Treatment	Radicle emergence 3 day	Seed Germination 5 day	Seed Germination 14 day	Seed Germination index
RAW	78.67 ABCDEFGH	16.00 K	93.33	14.42 I
Plasma 0.4 s/cm	74.67 DEFGH	29.33 HIJ	90.67	14.65 HI
Plasma 0.8 s/cm	74.00 DEFGH	18.67 JK	92.00	14.67 GHI
Plasma 1.2 s/cm	70.67 FGHI	30.67 HI	94.67	14.93 FGHI
Cu 1	81.33 ABCDEF	62.67 BC	87.33	16.09 CDEF
Cu 2	78.67 ABCDEFGH	60.00 BCD	88.00	15.84 CDEFG
Cu 3	80.67 ABCDEF	66.67 B	91.33	16.70 ABCD
0.4 s/cm -> Cu 1	72.00 EFGHI	50.67 DE	89.33	15.91 CDEF
0.8 s/cm -> Cu 1	86.00 ABC	63.33 BC	92.00	16.86 ABC
1.2 s/cm -> Cu 1	84.67 ABCD	61.33 BCD	89.33	16.42 BCD
0.4 s/cm -> Cu 2	76.67 BCDEFGH	38.67 FGH	90.67	15.65 DEFGH
0.8 s/cm -> Cu 2	82.67 ABCDE	42.67 EFG	92.67	16.16 CDE
1.2 s/cm -> Cu 2	78.00 BCDEFGH	24.67 IJK	92.00	15.17 EFGHI
0.4 s/cm -> Cu 3	74.67 DEFGH	60.00 BCD	85.33	15.74 CDEFGH
0.8 s/cm -> Cu 3	89.33 A	84.67 A	90.67	17.75 A
1.2 s/cm -> Cu 3	86.67 AB	68.67 B	93.33	17.53 AB
Cu 1 -> 0.4 s/cm	81.33 ABCDEF	58.00 BCD	94.67	16.91 ABC
Cu 1 -> 0.8 s/cm	78.00 BCDEFGH	53.33 CDE	90.00	16.37 BCD
Cu 1 -> 1.2 s/cm	79.33 ABCDEFG	46.00 EF	92.67	15.89 CDEF
Cu 2 ->0.4 s/cm	75.33 CDEFGH	35.33 FGHI	93.33	15.78 CDEFGH
Cu 2 -> 0.8 s/cm	82.00 ABCDE	35.33 FGHI	94.00	15.82 CDEFGH
Cu 2 -> 1.2 s/cm	68.00 HI	18.67 JK	94.00	15.04 EFGHI
Cu 3 ->0.4 s/cm	72.00 EFGHI	32.67 GHI	85.33	14.23 I
Cu 3 -> 0.8 s/cm	68.67 GHI	30.00 HI	92.67	14.36 I
Cu 3 -> 1.2 s/cm	61.33 I	32.67 GHI	86.00	12.90 J
F-Test	* *	**	NS	**
LSD ≤ 0.05	10.95	10.71	6.42	1.19
CV%	8.63	14.56	4.30	4.62

2

1

Cu solutions
0.1, 0.05 ,0.025 M
(Cu1, Cu2, Cu3)

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Treatment	Radicle emergence 3 day	Seed Germination 5 day	Seed Germination 14 day	Seed Germination index 14 day
RAW	86.00 E	34.00 EFG	82.67 G	14.11 HI
Cu 1	92.67 BCD	32.00 FG	95.33 AB	15.09 DEFG
Cu 3	90.67 CDE	36.67 DEF	94.00 ABC	15.70 CD
Plasma Ar 0.4 s/cm	90.67 CDE	28.67 GH	88.00 DEF	13.57 I
Plasma Ar 0.8 s/cm	98.00 A	42.67 CD	97.33 A	16.89 AB
Plasma Ar 1.2 s/cm	95.33 ABC	34.67 EFG	87.33 EF	15.12 DEFG
Plasma Ar 0.4 s/cm -> Cu 3	94.67 ABC	55.33 AB	97.33 A	17.52 A
Plasma Ar 0.8 s/cm -> Cu 3	94.00 ABC	36.67 DEF	93.33 ABC	15.72 CD
Plasma Ar 1.2 s/cm -> Cu 3	96.00 AB	38.00 DEF	93.33 ABC	15.48 CDEF
Cu 1 -> Plasma Ar 0.4 s/cm	96.67 AB	43.33 CD	90.67 CDE	15.71 CD
Cu 1 -> Plasma Ar 0.8 s/cm	96.00 AB	36.67 DEF	85.33 FG	14.74 GH
Cu 1 -> Plasma Ar 1.2 s/cm	90.67 CDE	36.00 DEFG	84.00 FG	14.44 GH
Plasma Ar+O_2 0.4 s/cm	88.67 DE	22.00 H	92.00 BCD	14.95 EFG
Plasma Ar+O_2 0.8 s/cm	95.33 ABC	36.67 DEF	95.33 AB	15.68 CDE
Plasma Ar+O_2 1.2 s/cm	86.00 E	38.67 DEF	87.33 EF	14.91 FG
Plasma Ar+O_2 0.4 s/cm -> Cu 3	88.67 DE	59.33 A	96.67 A	17.59 A
Plasma Ar+O_2 0.8 s/cm -> Cu 3	78.00 F	49.33 BC	95.33 AB	16.78 B
Plasma Ar+O_2 1.2 s/cm -> Cu 3	74.00 F	41.33 DE	93.33 ABC	15.97 C
F-Test	**	**	**	**
LSD ≤ 0.05	5.25	7.46	4.52	0.73
CV%	3.50	11.55	2.99	2.85

0.4 s/cm Cu3

0.8 s/cm Cu1

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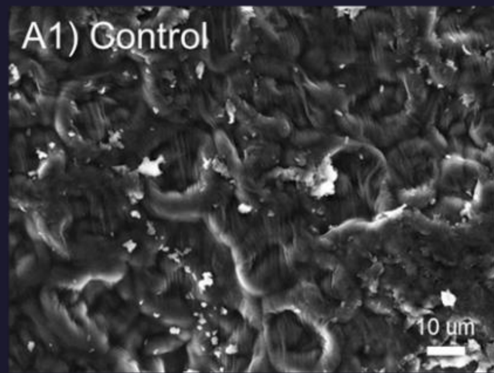
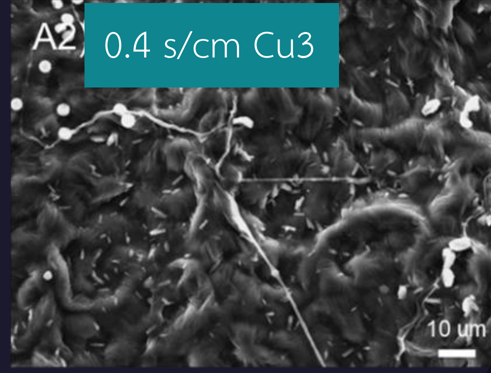
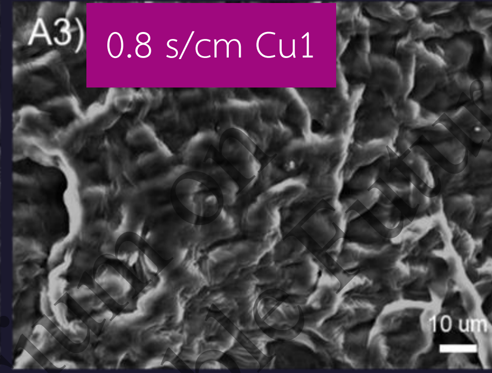
1000 X

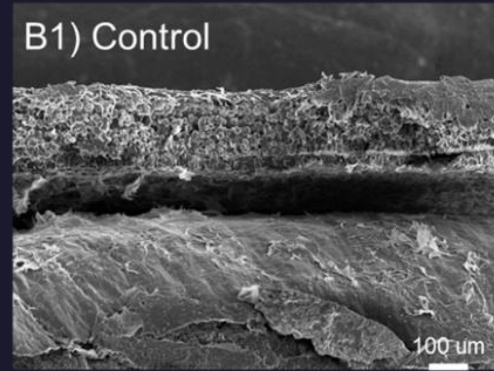


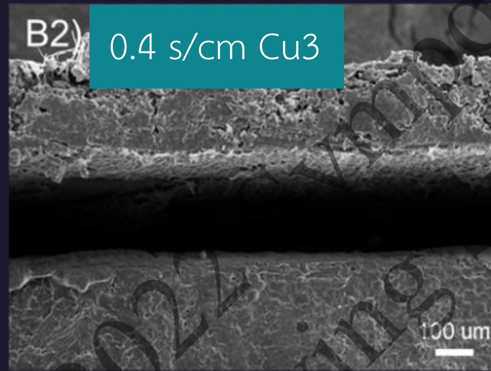


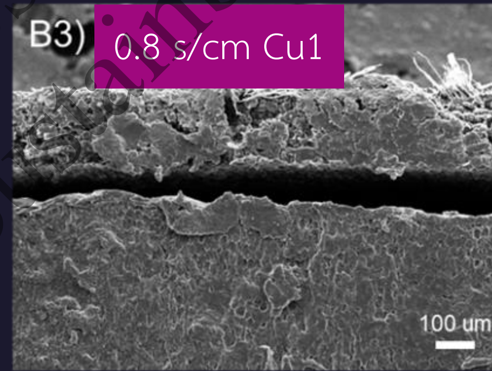


Seed coat

100 X

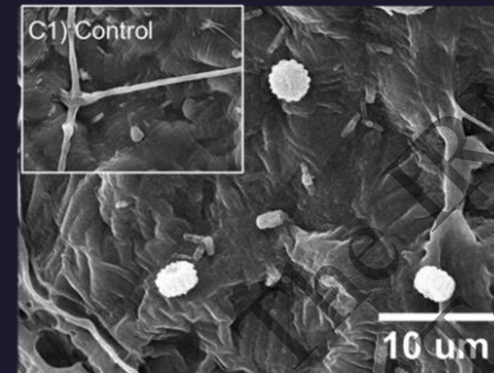




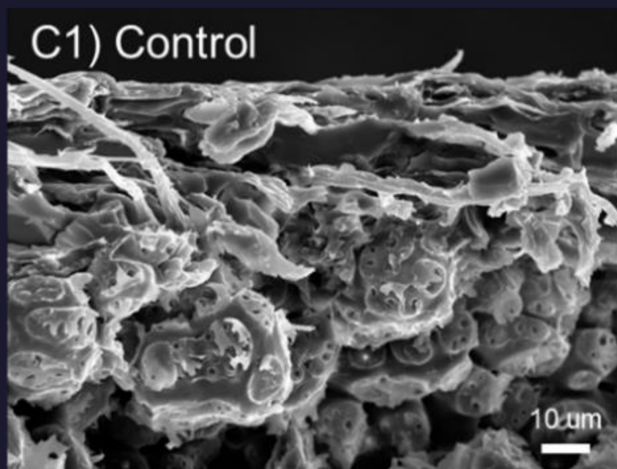


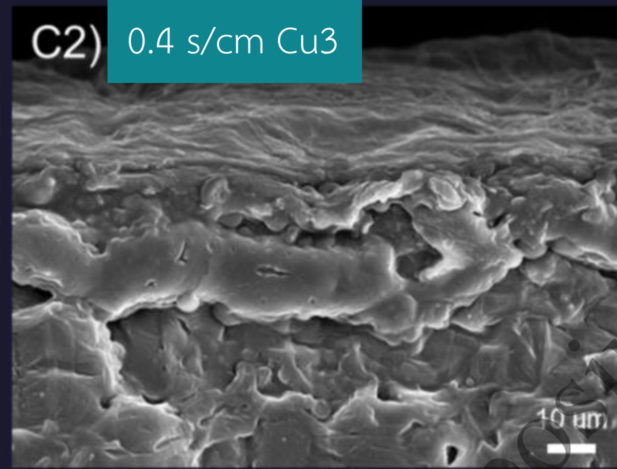
Cross section of seed coat and endosperm

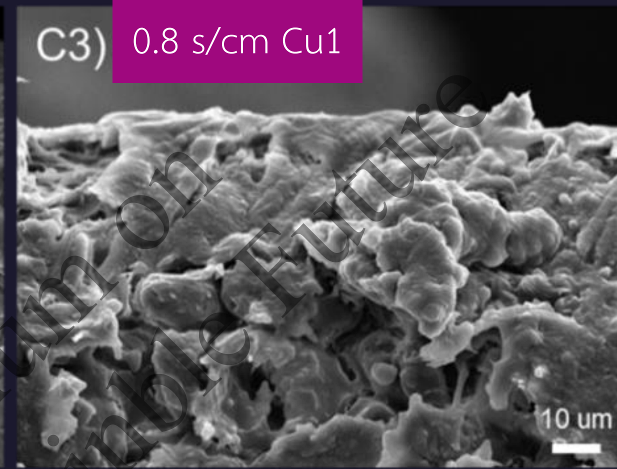
3000 X



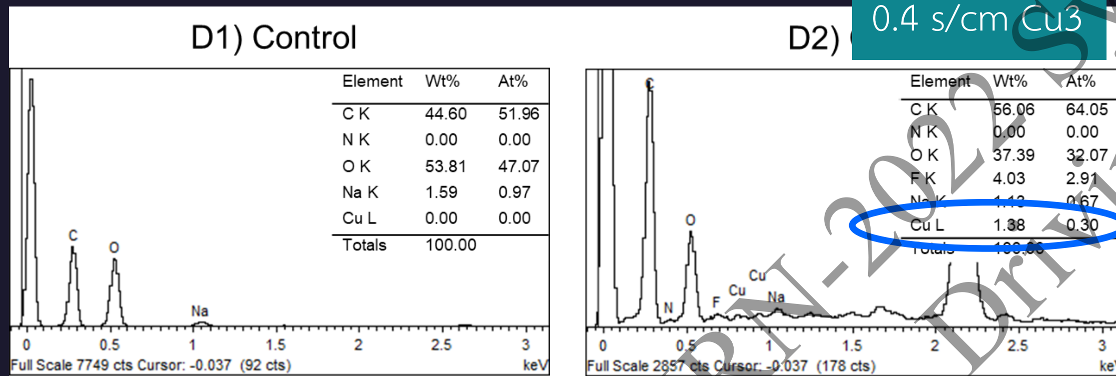
Seed coat







Cross section of seed coat endosperm at 1000 x

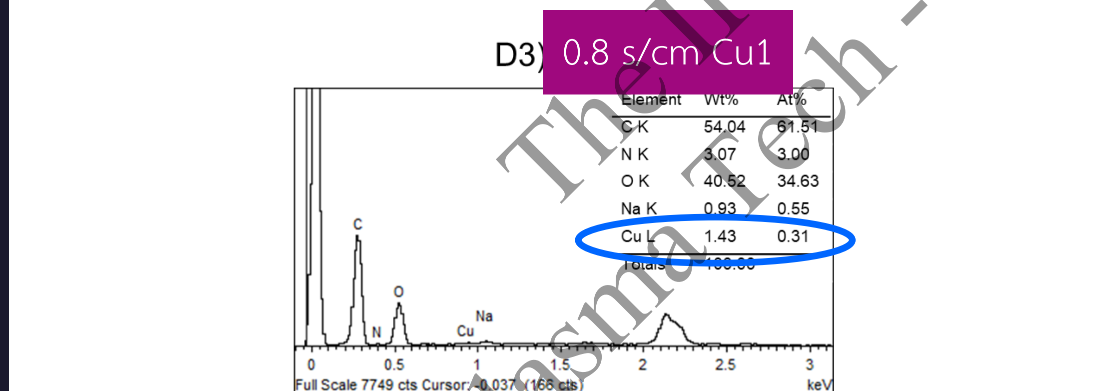


D1) Control

Element	Wt%	At%
C K	44.60	51.96
N K	0.00	0.00
O K	53.81	47.07
Na K	1.59	0.97
Cu L	0.00	0.00
Totals	100.00	

D2) 0.4 s/cm Cu3

Element	Wt%	At%
C K	56.06	64.05
N K	0.00	0.00
O K	37.39	32.07
F K	4.03	2.91
Na K	1.13	0.67
Cu L	1.38	0.30
Totals	100.00	



Element	Wt%	At%
C K	54.04	61.51
N K	3.07	3.00
O K	40.52	34.63
Na K	0.93	0.55
Cu L	1.43	0.31
Totals	100.00	

EDS spectrum indicated elemental content in seed before and after treatment

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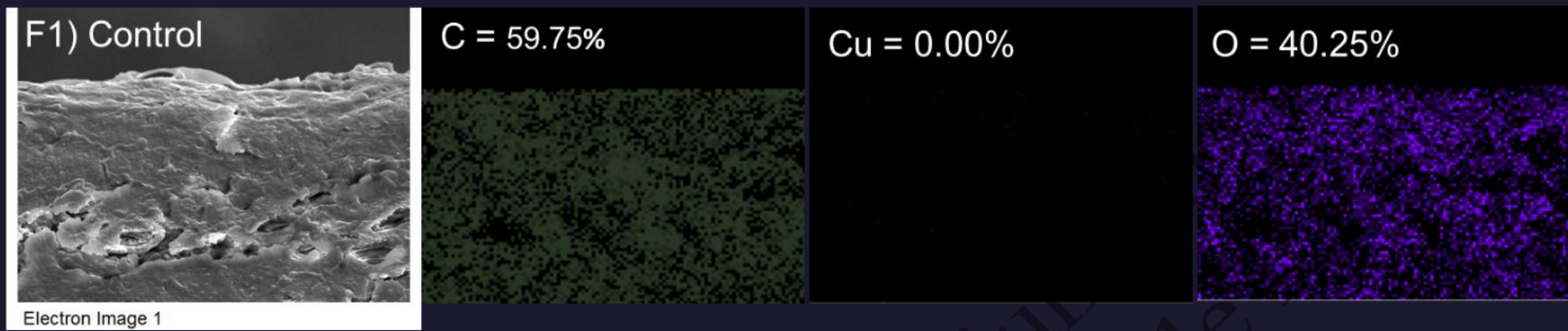


EDS-mapping control seed

0.4 s/cm Cu3

Seed Coat

C =56.06%

Cu = 1.43%

O = 37.39%

C Ka1_2

Cu Ka1

O Ka1

SED 15.0kV WD9.7mm Std.-P.C.30.0 HighVac. x100

Endosperm

C = 60.32%

Cu= 0%

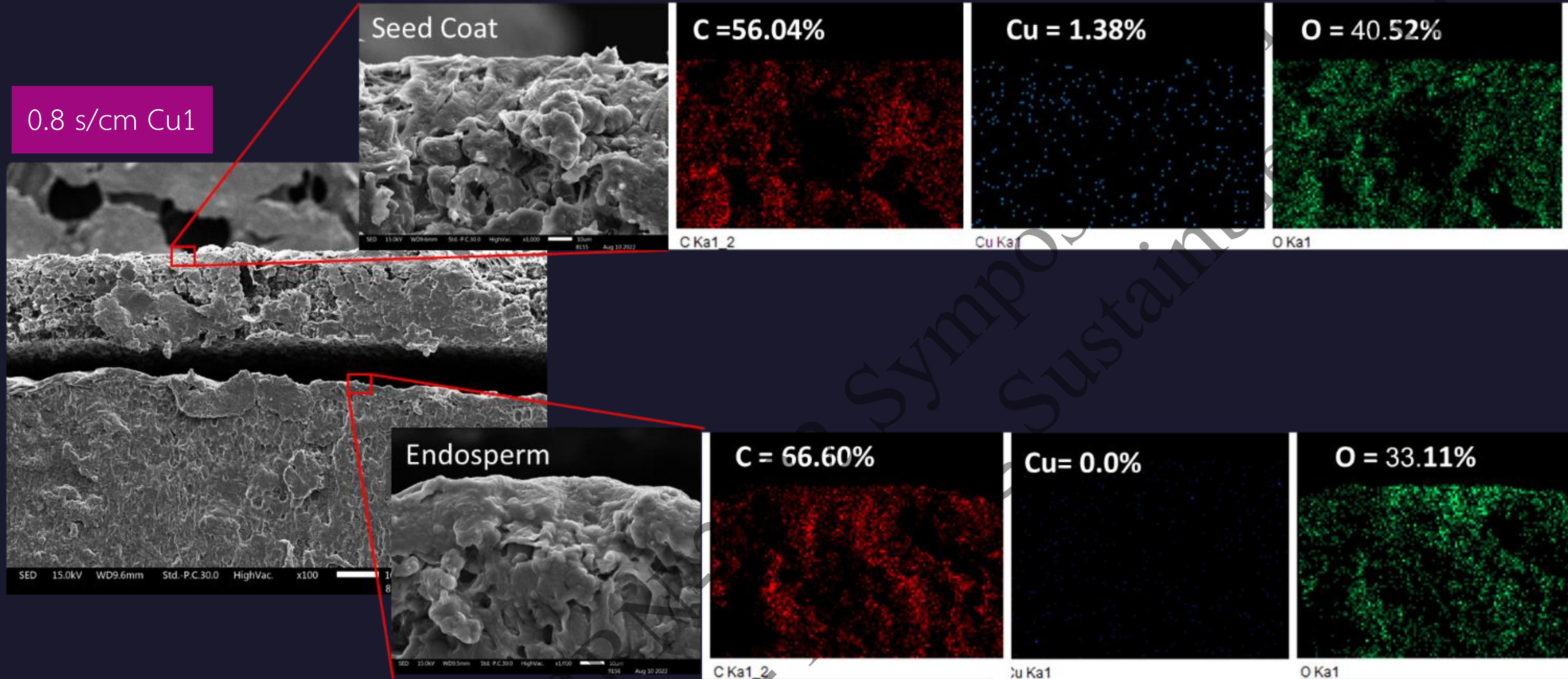
O = 35.89%

C Ka1_2

Cu Ka1

O Ka1

EDS-mapping of 0.4 s/cm Cu3



EDS-mapping of 0.8 s/cm Cu1

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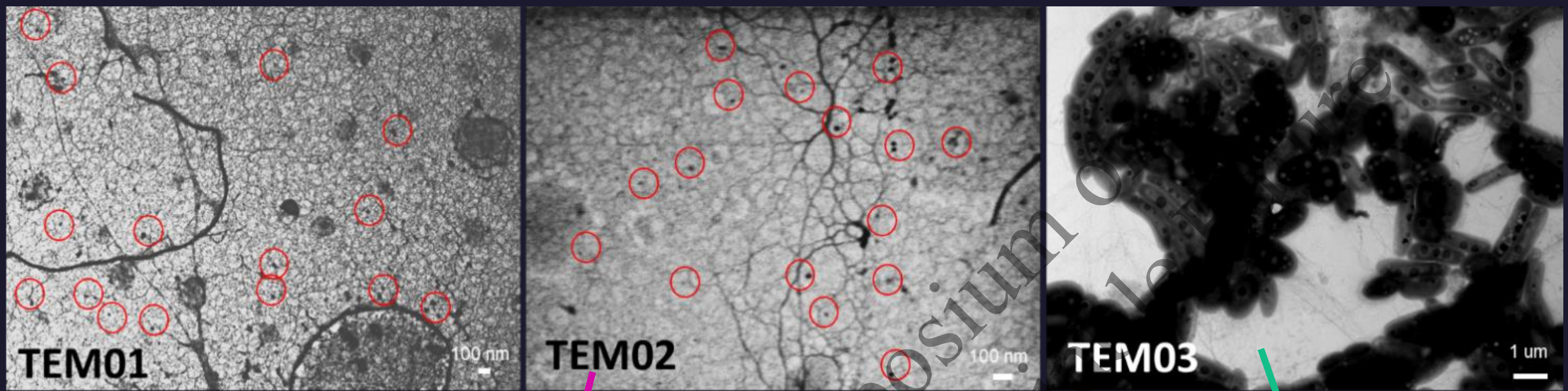
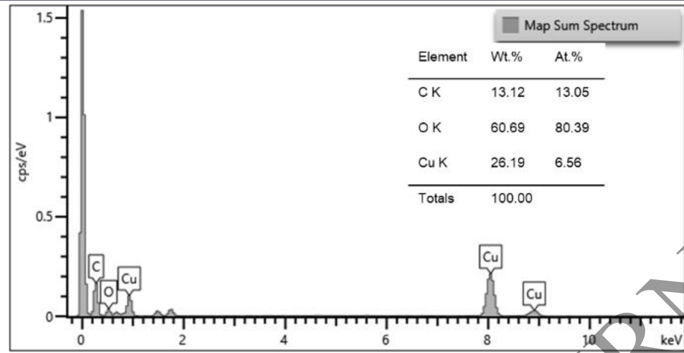
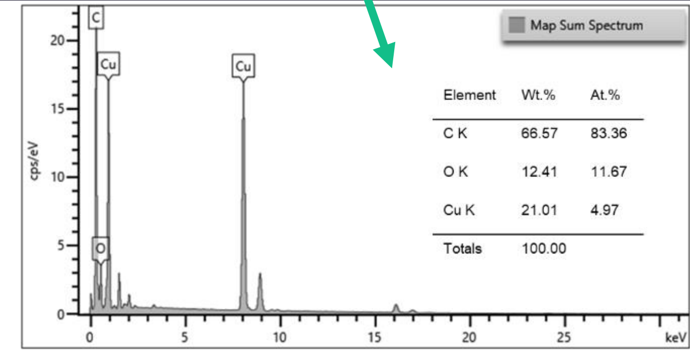
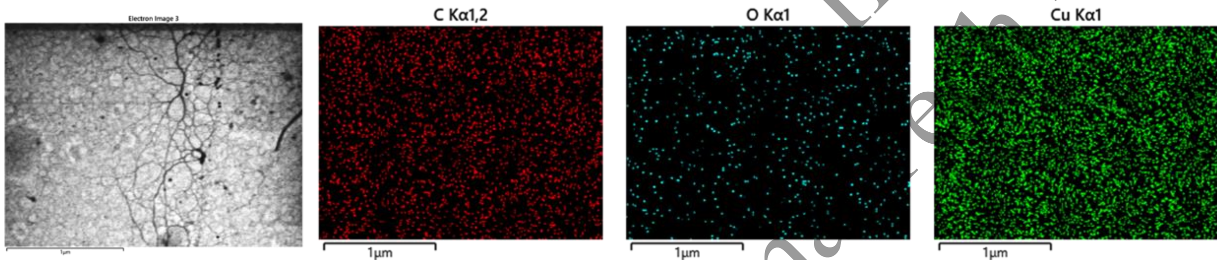
0.4 s/cm Cu3

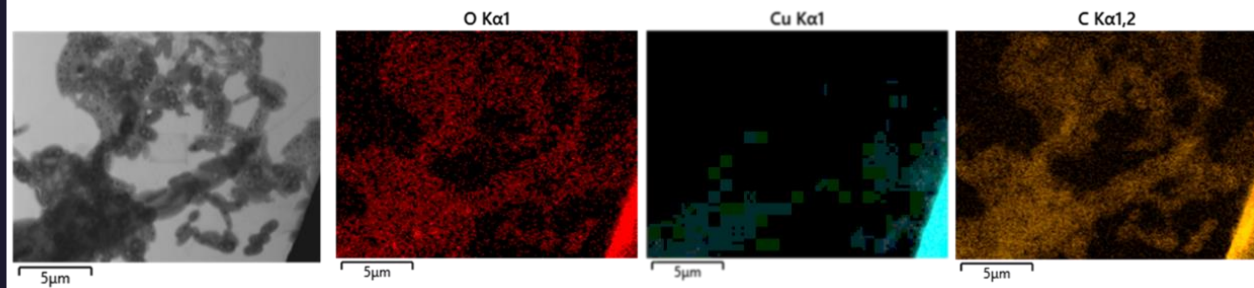


TEM images of 0.4 s/cm Cu 3 at different magnification









EDS mapping of 0.4s/cm Cu3 at TEM02 and TEM03

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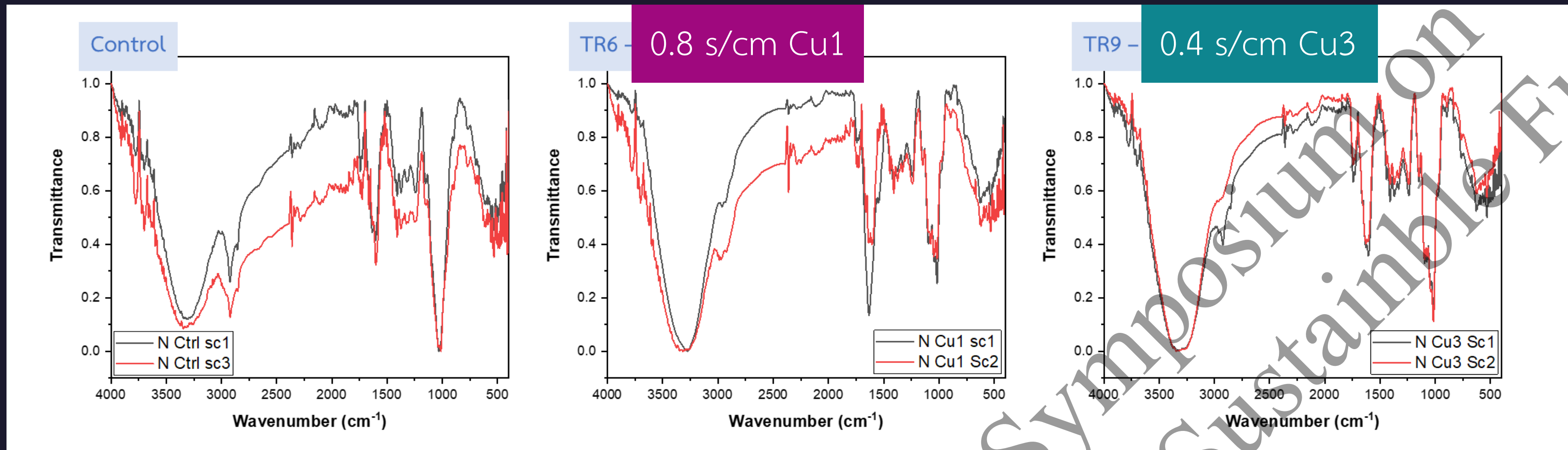
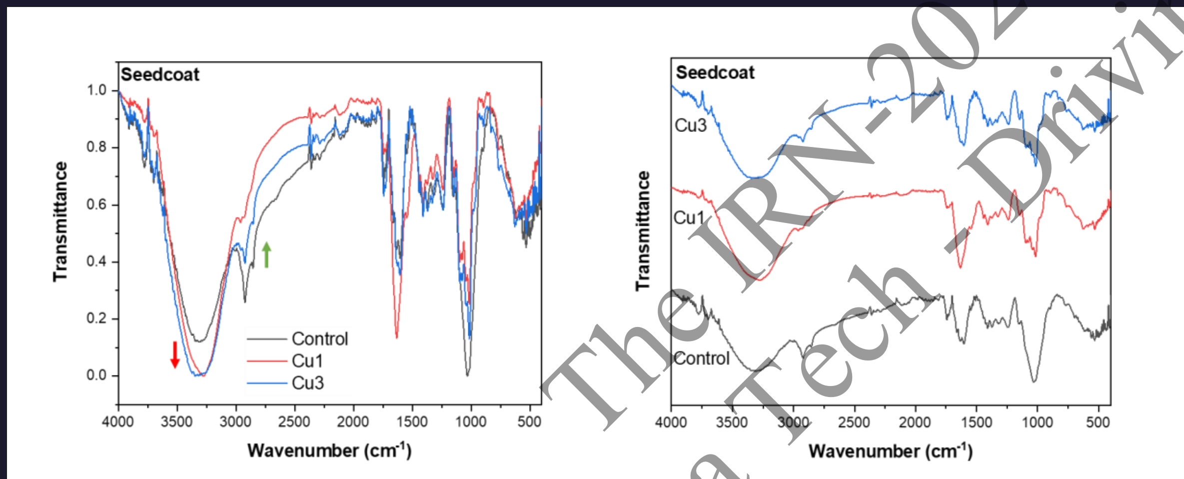
Seed coat



3250 cm^{-1} (O-H stretching), 2900 cm^{-1} (aliphatic C-H stretching), 1650 cm^{-1} (C=O stretching)



0.4 s/cm Cu3

0.8 s/cm Cu1

0.8 s/cm Cu1 and 0.4 s/cm Cu3 have higher % transmittance at 3250 cm^{-1} than control seed → higher –OH gr.

0.8 s/cm Cu1 and 0.4 s/cm Cu3 have lower % transmittance at 2900 cm^{-1} than control seed → seed coat react with plasma free radical → higher –OH gr.

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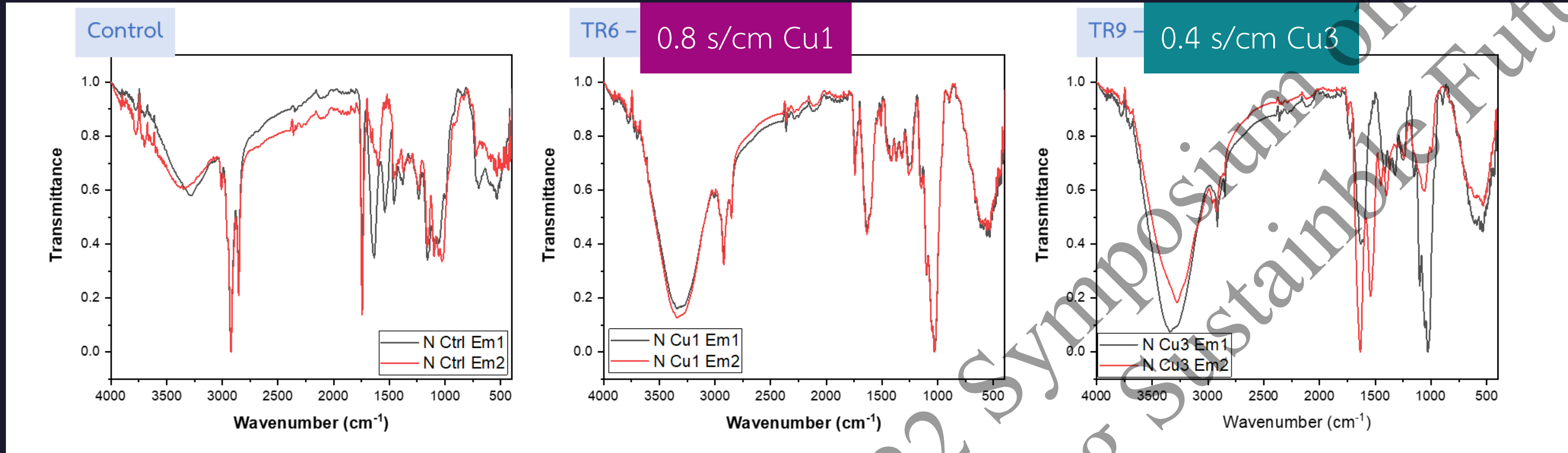
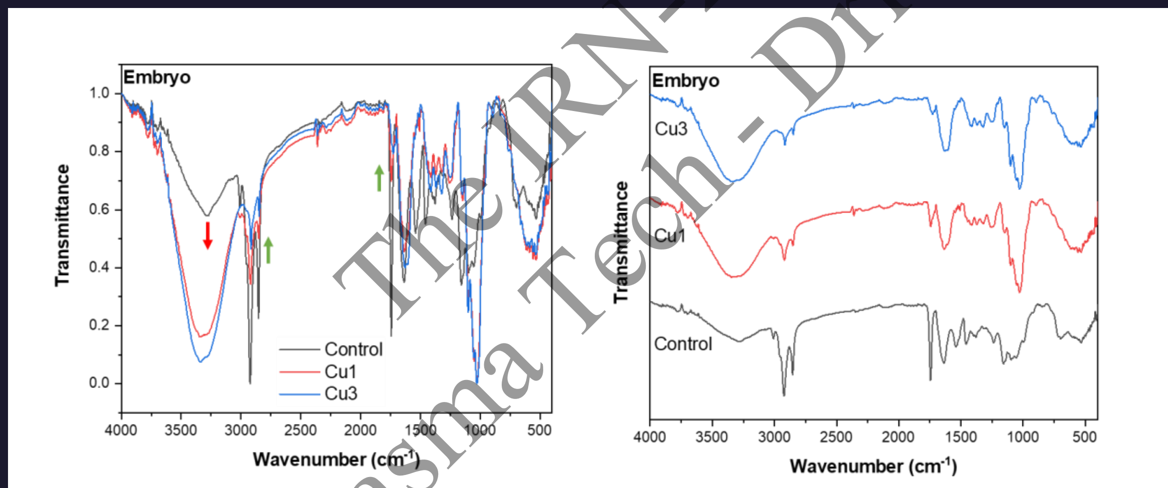
Embryo



3250 cm^{-1} (O-H stretching), 2900 cm^{-1} (aliphatic C-H stretching), 1650 cm^{-1} (C=O stretching)

High content of triglycerides was found in control seed which is the fingerprints of embryo



0.4 s/cm Cu3

0.8 s/cm Cu1

0.8 s/cm Cu1 and 0.4 s/cm Cu3 have higher % transmittance at 3250 cm^{-1} than control seed → triglyceride react with free radical

0.8 s/cm Cu1 and 0.4 s/cm Cu3 have lower % transmittance at 2900 cm^{-1} than control seed → triglyceride react with free radical

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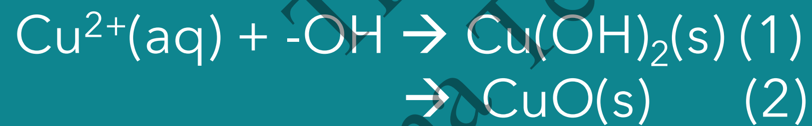
Types and intensities of free radical generated using Ar and O_2 gases mixture in different ratio

species	Wavelength (nm)	Intensity (a.u.)					
		Oxygen gas flow at 1500 v (L./min)					
		0	0.2	0.4	0.6	0.8	1.0
O	283.623	7837	6956	5545	6396	4756	5446
	380.431	8516	5948	5193	5713	4703	5067
	777.530	7949	12718	13127	16637	12685	22755
	844.646	2935	6115	6841	8795	7082	12713
O+	405.837	8109	6126	4763	5418	4242	5180
OH	309.831	64368	64368	64368	64356	64354	64351
NO	226.259	9655	4645	4620	4935	3884	3696
	236.435	17870	8325	8256	8824	7114	6622
	247.202	18604	8854	8828	9235	7629	6980
	247.202	18604	8854	8828	9235	7629	6980
	271.096	10252	5664	5230	5733	4494	4673
N2	336.577	29058	10798	9914	10145	9524	8278
	357.339	12522	7786	7238	8118	7061	6764
Ar	696.660	10367	9085	7624	7908	7331	7505
	751.424	4624	4913	4357	4717	4676	4704
	763.353	18512	18783	17147	17842	18289	17960
	772.429	10035	8971	7685	7894	7835	7490
	811.447	11530	12736	11096	12050	12397	12128
	826.096	5124	4442	3873	3931	3925	3694
	912.246	7442	7576	6818	7169	6794	7421



Confirm by XRD or Raman

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Conclusions

- It was found that increasing the O_2 ratio in the Ar mixture to ignite the plasma dielectric barrier discharge (DBD) system increased the seed nitrogen content and the O species oxidation the disinfection efficiency.
- The plasma treated seeds showed that the surface of the seed coat reacted with the plasma free radicals resulting in the formation of more hydroxyl groups (–OH) and triglyceride accumulated in the embryo reacted with the free radicals from plasma.
- The use of Ar gas mixed with O_2 influenced seed quality by causing radicle emergence, germination at 5 days and germination index of watermelon seeds tended to decrease.
- Cu compound coating had better results at 7 days germination, germination index. The distribution of Cu compound particles was found in the seed coat, not in the endosperm layer.

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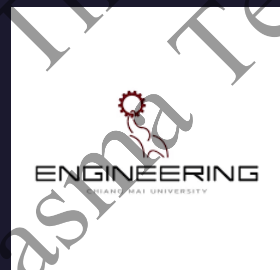
Acknowledgement

บริษัท ไดอิเล็กทริค เทคโนโลยี จำกัด



Thapanee Sarakonsri, Yothin Chimupala, Saranphong Yimklan, Natthawat Semakul, Chommanad Sawadeemit, Tanika Sookpasom, Lalida Shank, Dheerawan Boonyawan, Choncharoen Sawangrat, and Sa-guansak Thanapornpoonpong

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