

Pre-sowing treatments, seed components and water imbibition aids seed germination of *Gloriosa superba*

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Supplementary Materials

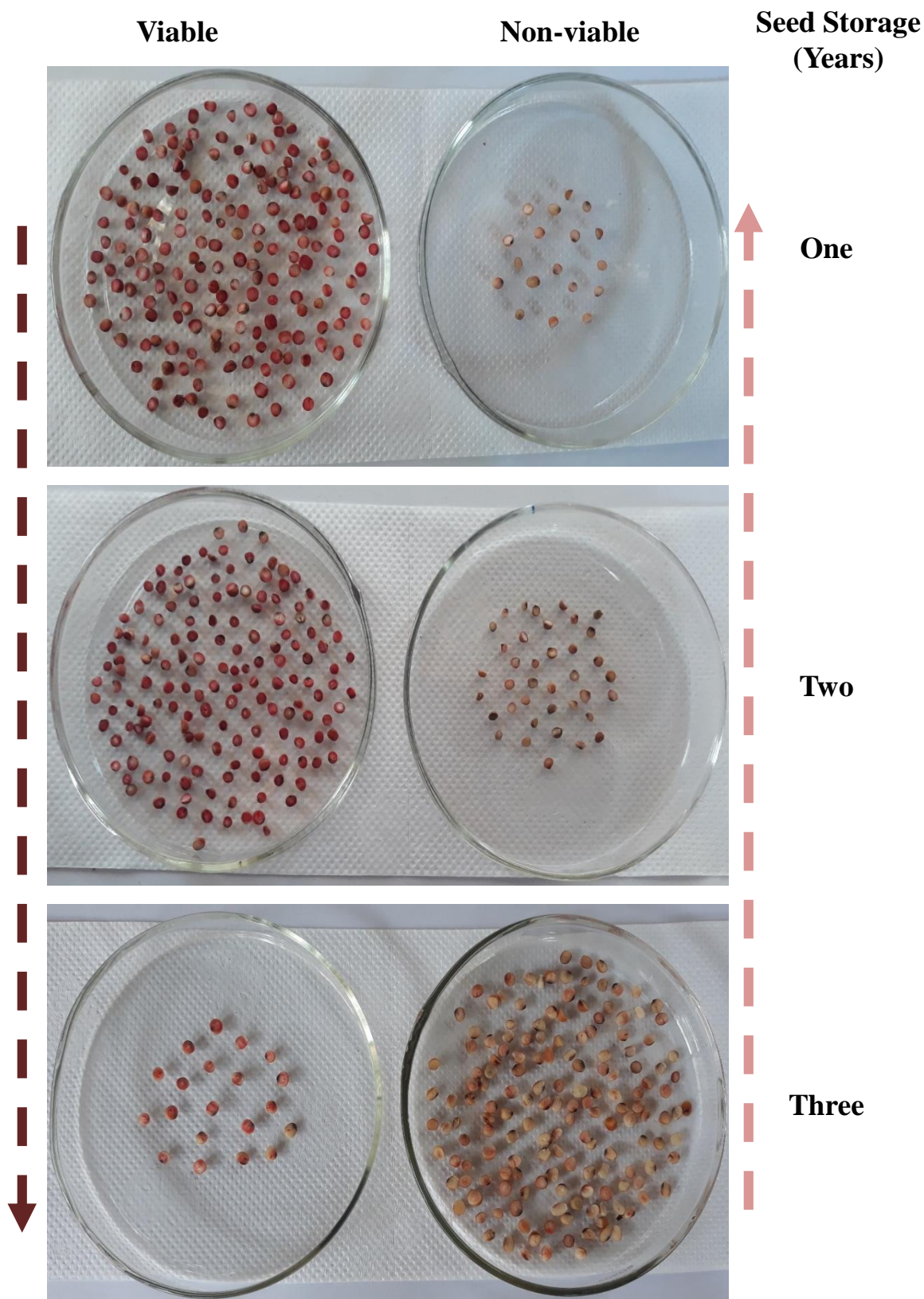


Figure S1. Effect of storage period on viability of seeds of *G. superba*: a. viable seeds embryo pink color and non-viable seeds embryo black color (after treating mechanically scarified seeds with 1% tetrazolium chloride (TZ) solution for 24 h)

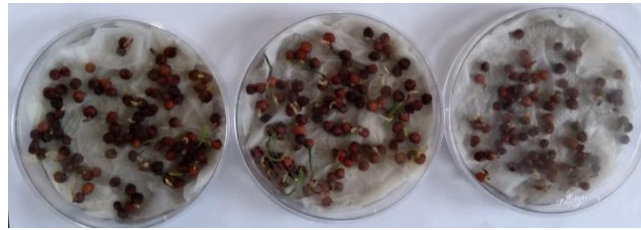
in vivo 60 DAS

in vitro 30 DAS

Control



MS



MS+
WS
24h



MS+
WS
48h



MS+
WS
72h



MS+
WS
96h



MS+
WS
120h

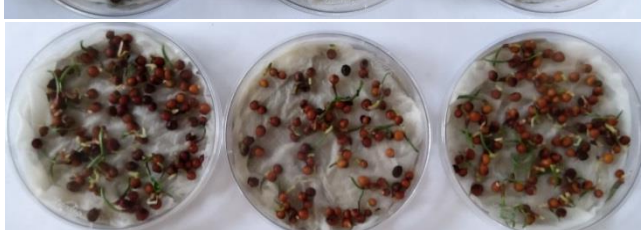
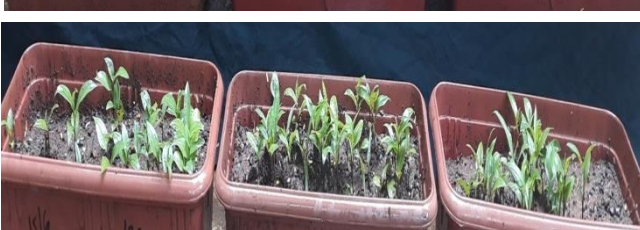


Figure S2. Germination of mechanically scarified seeds of *G. superba* in *in vivo* and *in vitro* condition after 60 days and *in vitro* condition after 30 days of sowing (n=100 in each replicate) DAS- days after sowing; Control- without mechanical scarification (MS) and water soaking (WS) at 0, 24, 48, 72, 96, 120 h

in vivo 60 DAS

in vitro 30 DAS

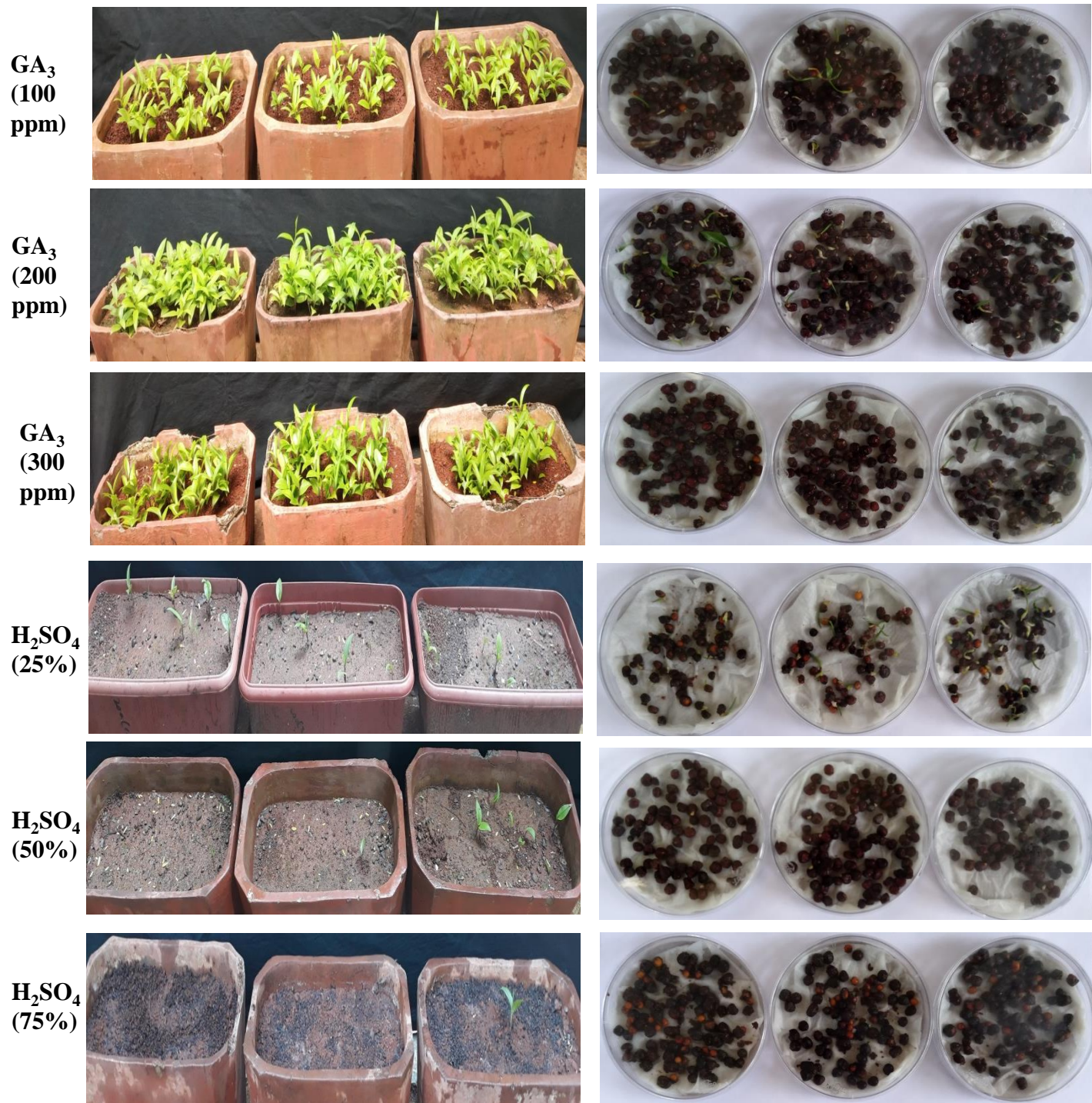


Figure S3. Influence of GA₃ and H₂SO₄ treatments on *G. superba* seed germination *in vivo* condition after 60 days and *in vitro* condition after 30 days of sowing (n=100 in each replicate) DAS- days after sowing; GA₃– treatment for 60 min and H₂SO₄ treatment for 30 min

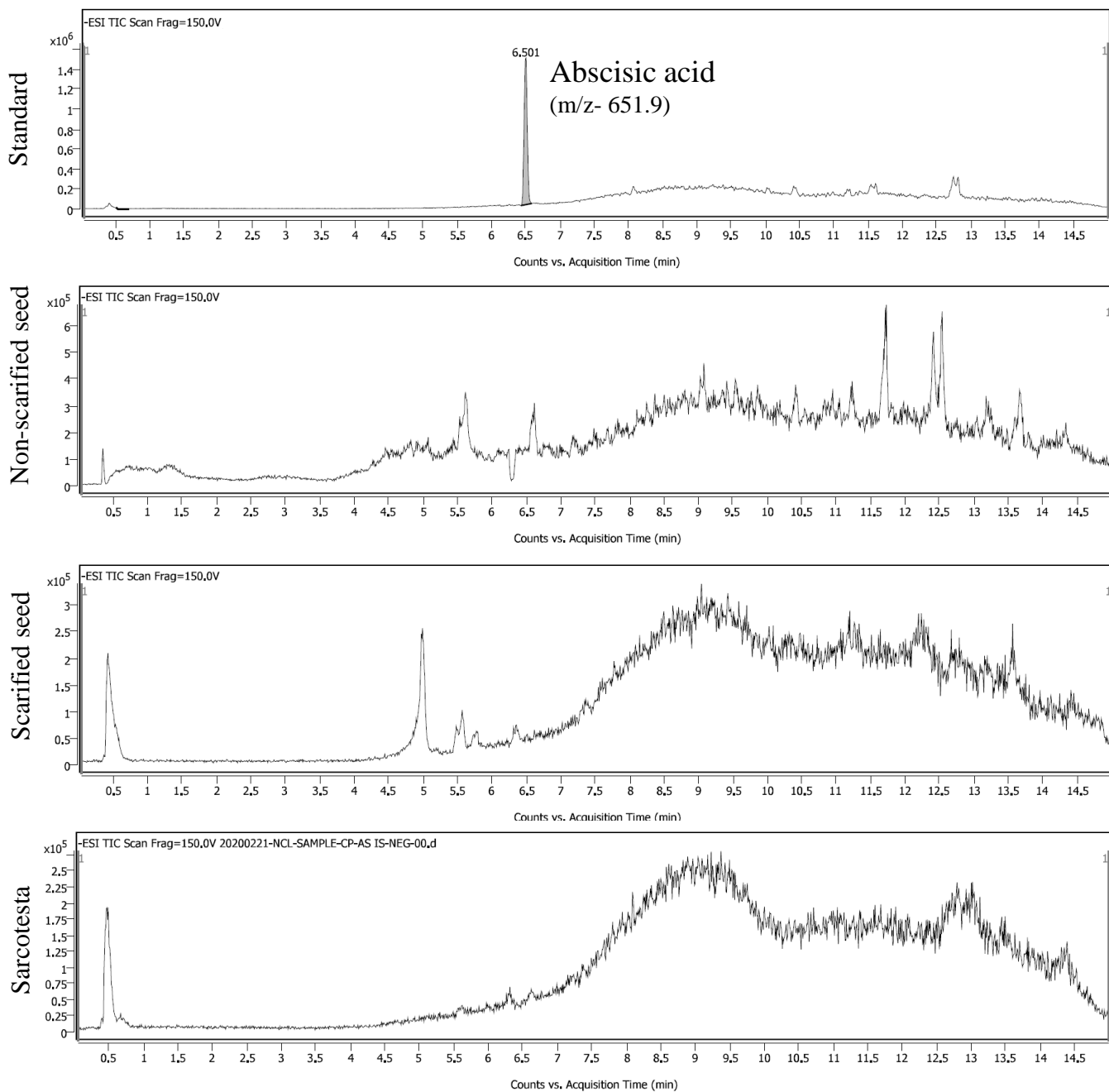


Figure S4. LC-MS chromatograph for detection of abscisic acid in non-scarified and scarified seeds and sarcotesta of seeds of *G. superba*

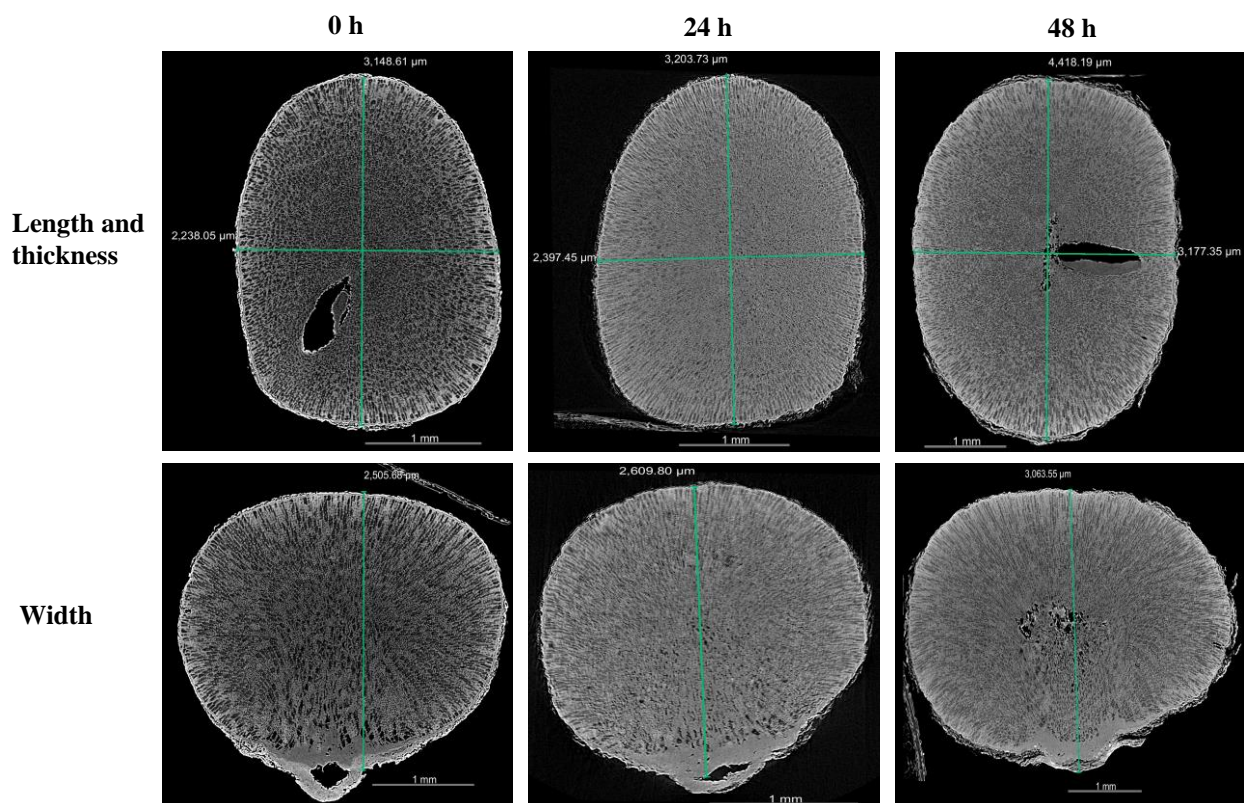


Figure S5. Illustration of Phenotypic details through micro-T of *G. superba* mechanically scarified seeds at 0, 24, and 48 h of imbibition.

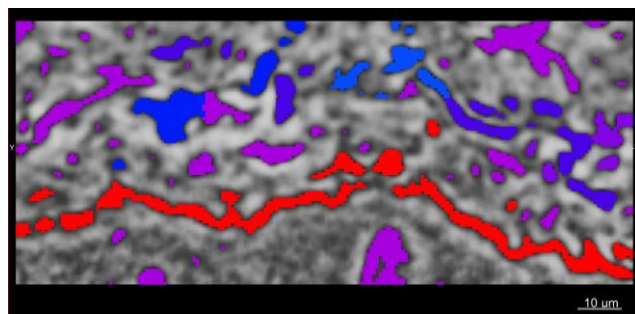
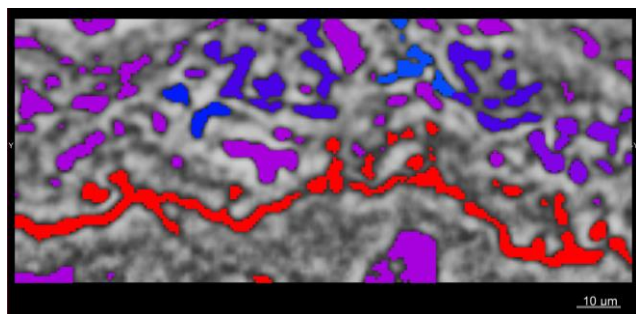
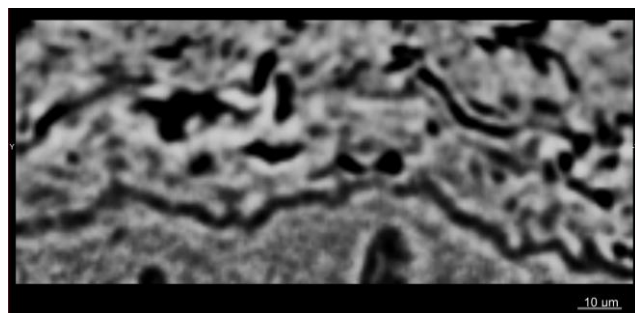
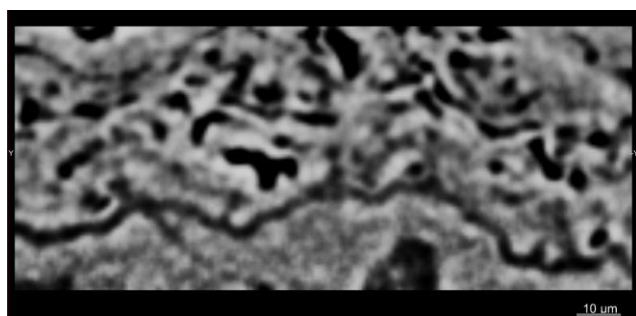
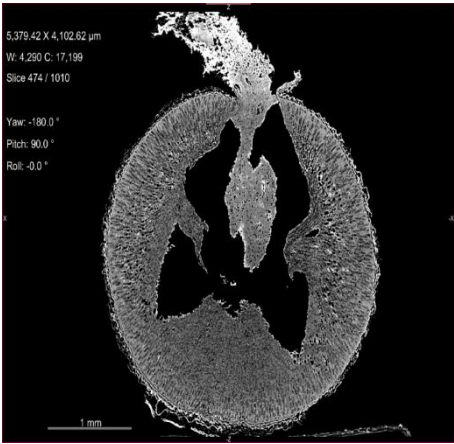


Figure S6. 2D image of pore network in tegmen of *G. superba* seed by micro-T



A



B

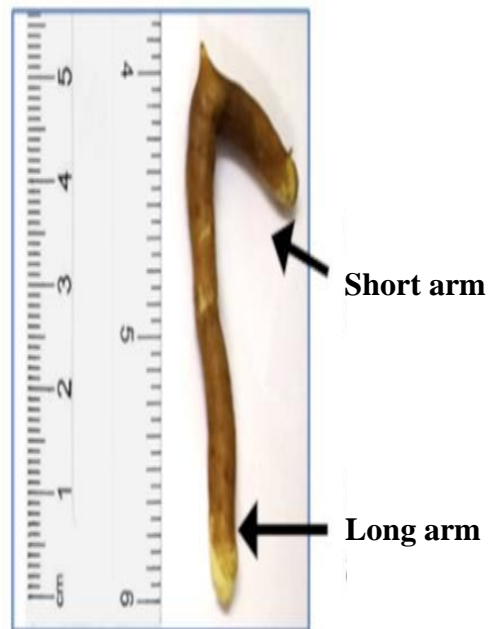


C

Figure S7. Visualization of germinated seed of *G. superba* using micro-T A) Germinated seed 2D cross-sectional image B) Germinated seed volume-rendered 3D image C) Germinated seed volume-rendered 3D image with defined grey-scale values



A



B

Figure S8. A) *G. superba* seedlings after 60 days of germination B) Tuber of *G. superba* after 120 days of germination

Supplementary Tables

Supplementary Table S1. Effect of physical and chemical treatments on seed germination of *G. superba* in *in vivo* condition after 30 days (n=100)

Treatment	Treatment Time (min/h)	Germination (%)	Minimum time required for germination (days)
		30 DAS (Mean \pm SE) ^{\$}	(days \pm SE) ^{\$}
Control (without MS and WS)*	0 h	23.00g \pm 2.98	34 ^h \pm 3
MS (without WS)	0 h	56.00 ^d \pm 1.50	17 ^d \pm 2
MS + WS	24 h	81.33^a \pm 2.31	11^a \pm 1
MS + WS	48 h	79.00 ^a \pm 1.30	13 ^b \pm 1
MS + WS	72 h	77.00 ^b \pm 1.01	11 ^a \pm 1
MS + WS	96 h	70.33 ^c \pm 1.52	11 ^a \pm 1
MS + WS	120 h	57.33 ^d \pm 1.61	11 ^a \pm 2
GA ₃ (100 ppm)	60 min	34.66 ^e \pm 1.23	14 ^c \pm 2
GA ₃ (200 ppm)	60 min	27.66 ^f \pm 1.09	18 ^d \pm 3
GA ₃ (300 ppm)	60 min	28.00 ^f \pm 1.51	24 ^f \pm 1
H ₂ SO ₄ (25%)	30 min	41.00 ^e \pm 2.12	20 ^e \pm 3
H ₂ SO ₄ (50%)	30 min	20.33 ^g \pm 0.98	37 ⁱ \pm 4
H ₂ SO ₄ (75%)	30 min	7.66 ^h \pm 1.23	29 ^g \pm 3

Notes: The values are means of 100 seeds. *MS- Mechanical scarification; WS- water soaking; DAS-days after sowing ^{\$}: The values appended with similar letters indicate non-significant differences, while those appended with different letters indicate significant differences ($p < 0.05$); SE: Standard error of the mean.

Supplementary Table S2. Effect of physical and chemical treatments on seed germination of *G. superba* in *in vitro* condition after 30 days (n=100)

Treatment	Treatment Time (min/h)	Germination (%) 30 DAS (Mean \pm SE) ^{\$}	Minimum time required for germination (days) (days \pm SE) ^{\$}
Control (without MS and WS)*	0 h	33.00 ^f \pm 1.56	37 ^j \pm 1
MS (without WS)	0 h	65.33 ^c \pm 1.23	09 ^d \pm 1
MS + WS	24 h	85.33^a \pm 3.21	06 ^b \pm 1
MS + WS	48 h	80.00 ^a \pm 2.09	08 ^c \pm 2
MS + WS	72 h	73.33 ^b \pm 1.81	05^a \pm 1
MS + WS	96 h	65.33 ^c \pm 1.72	06 ^b \pm 1
MS + WS	120 h	59.00 ^d \pm 1.31	06 ^b \pm 2
GA ₃ (100 ppm)	60 min	64.66 ^c \pm 1.23	11 ^f \pm 2
GA ₃ (200 ppm)	60 min	70.00 ^b \pm 1.29	07 ^{bc} \pm 2
GA ₃ (300 ppm)	60 min	68.66 ^b \pm 1.21	10 ^e \pm 1
H ₂ SO ₄ (25%)	30 min	46.00 ^e \pm 2.12	18 ^g \pm 1
H ₂ SO ₄ (50%)	30 min	25.00 ^g \pm 0.98	19 ^h \pm 2
H ₂ SO ₄ (75%)	30 min	10.00 ^h \pm 1.23	20 ⁱ \pm 2

Notes: The values are means of 100 seeds. *MS- Mechanical scarification; WS- water soaking; DAS-days after sowing ^{\$}: Numbers appended with similar letters indicate non-significant differences, while those appended with different letters indicate significant differences ($p < 0.05$); SE: Standard error of the mean.

Supplementary Table S3. Effect of physical treatments on water uptake capacity of *G. superba* seeds during germination (n=100)

Treatment	Treatment Time (h)	Seed diameter (mm) ^{\$}	Seed weight (mg) ^{\$}
MS (without WS)*	0 h	3.0499 ^f ± 0.0925	0.024633 ^f ± 0.001885
MS + WS	24 h	3.8607 ^e ± 0.0815	0.044006 ^e ± 0.002417
MS + WS	48 h	4.2613^a ± 0.1070	0.044309^a ± 0.002612
MS + WS	72 h	4.2319 ^b ± 0.1534	0.044262 ^b ± 0.002664
MS + WS	96 h	4.2307 ^c ± 0.1124	0.044238 ^c ± 0.002605
MS + WS	120 h	4.1445 ^d ± 0.1501	0.044153 ^d ± 0.002494

Notes: The values are means of 100 seeds. *MS- Mechanical scarification; WS- water soaking. ^{\$}: Numbers appended with similar letters indicate non-significant differences, while those appended with different letters indicate significant differences ($p < 0.05$); SE: Standard error of the mean.

Supplementary Table S4. BET surface analysis (N₂-adsorption–desorption) of *G. superba* seeds (n=6)

Sample ID	Surface area S_{BET} (m²/g)	Total Pore volume (cc/g)	Average pore size (nm)
Non scarified seed- NSS	nd	nd	nd
Scarified seed-SS	11.77	0.0098	1.67

Notes: The values are means of six seeds. * nd = not detected

Supplementary Table S5. Phenotypic details through micro-T of *G. superba* seeds (n=6)

Phenotypic details	Length (mm)	Thickness (mm)	Width (mm)	Volume (mm ³)
Scarified seed (0 h)	3.15 ^b ±0.79	2.22 ^b ±0.56	2.50 ^b ±0.63	10.70 ^c ±2.68
Seed imbibition (after 24 h)	3.20 ^b ±0.80	2.30 ^b ±0.58	2.60 ^b ±0.65	11.10 ^b ±2.78
Seed imbibition (after 48 h)	4.40^a±1.10	2.90^a±0.73	3.30^a±0.83	14.60^a±3.65

Notes: The values are means of six seeds ± SE (standard error of the mean). The values appended with similar letters indicate non-significant differences, while those appended with different letters indicate significant differences among the treatments ($p < 0.05$).

Supplementary Table S6. Porosity of *G. superba* seeds (n=6)

Porosity	Overall Porosity (%)	Connected Pores (%)	Non-connected Pores (%)
Sarcotesta	00.44 ^c ±0.10	0.00 ^c ±0.00	0.31 ^c ±0.13
Tegmen	21.01^a±5.25	17.50^a±4.38	2.10^a±0.53
Endosperm	01.30 ^b ±0.33	0.04 ^b ±0.01	1.20 ^b ±0.30

Notes: The values are means of six seeds ± SE (standard error of the mean). The values appended with similar letters indicate non-significant differences, while those appended with different letters indicate significant differences among the treatments ($p < 0.05$).

Supplementary Table S7. Effect of physical and chemical treatments on growth performance of *G. superba* in *in vivo* condition 60 days after sowing (n=100)

Treatment	Treatment Time (min/h)	Shoot length (cm)^{\$}	Tuber long arm length (cm)^{\$}	Tuber short arm length (cm)^{\$}	Vigor index (Mean \pm SE)^{\$}
Control (without MS and WS)*	0 h	6.48 ^d \pm 0.93	0.80 ^d \pm 0.03	0.56 ^c \pm 0.03	149.04 ^g \pm 11.52
MS (without WS)	0 h	6.89 ^c \pm 0.38	1.10 ^c \pm 0.16	0.59 ^d \pm 0.16	385.84 ^d \pm 18.31
MS + WS	24 h	5.50 ^f \pm 0.18	1.90^a \pm 0.69	0.94^a \pm 0.04	434.50 ^c \pm 23.05
MS + WS	48 h	8.45^a \pm 0.38	1.45 ^b \pm 0.33	0.69 ^b \pm 0.08	667.55^a \pm 34.91
MS + WS	72 h	6.85 ^c \pm 0.17	1.29 ^c \pm 0.46	0.59 ^d \pm 0.06	481.77 ^b \pm 21.72
MS + WS	96 h	6.19 ^e \pm 0.28	1.17 ^c \pm 0.87	0.53 ^f \pm 0.04	435.34 ^c \pm 21.21
MS + WS	120 h	7.61 ^b \pm 1.03	1.30 ^{bc} \pm 0.45	0.61 ^c \pm 0.11	380.50 ^d \pm 11.98
GA₃ (100 ppm)	60 min	5.61 ^f \pm 1.19	0.67 ^{ef} \pm 0.05	0.47 ^h \pm 0.02	194.44 ^f \pm 18.33
GA₃ (200 ppm)	60 min	4.64 ^g \pm 0.89	0.85 ^d \pm 0.04	0.50 ^g \pm 0.06	128.34 ^h \pm 29.27
GA₃ (300 ppm)	60 min	4.69 ^g \pm 1.18	0.70 ^e \pm 0.18	0.55 ^{ef} \pm 0.09	131.32 ^{gh} \pm 11.11
H₂SO₄ (25%)	30 min	6.21 ^e \pm 1.58	0.58 ^f \pm 0.13	0.37 ^j \pm 0.02	254.61 ^e \pm 19.32
H₂SO₄ (50%)	30 min	4.46 ^g \pm 1.69	0.60 ^f \pm 0.09	0.35 ^k \pm 0.04	90.67 ⁱ \pm 12.87
H₂SO₄ (75%)	30 min	4.57 ^g \pm 0.89	0.75 ^{de} \pm 0.05	0.45 ⁱ \pm 0.09	35.01 ^j \pm 7.43

Notes: The values are means of 100 seeds. *MS- Mechanical scarification; WS- water soaking; ^{\$}: Numbers appended with similar letters indicate non-significant differences, while those appended with different letters indicate significant differences ($p < 0.05$); SE: Standard error of the mean.

Supplementary Videos

Video S1. Non-scarified seed (NSS) structure of *G. superba* seed by micro-T Phase-3D (<https://youtu.be/-59gs3GLBVE>)

Video S2. Scarified seed (SS) structure of *G. superba* seed by micro-T Phase-3D (<https://youtu.be/85Ae12Mu8LI>)

Video S3. Detailed segmentation of pores in sarcotesta of *G. superba* seed by micro-T Phase 3D (<https://youtu.be/78xTpYN1s1o>)

Video S4. Detailed segmentation of pores in sclerotesta of *G. superba* seed by micro-T Phase 3D (<https://youtu.be/TTGOBFxS97o>)

Video S5. Detailed segmentation of pores in endotesta of *G. superba* seed by micro-T Phase 3D (<https://youtu.be/Ga0RmYzBups>)

Video S6. Pore network in sclerotesta of *G. superba* seed by micro-T Phase 2D (https://youtu.be/4AvZ-G_xZcA)

Video S7. Mapping of hydration volume percentage of phosphotungstic acid (PTA) stain inside *G. superba* non-scarified seed (NSS) by micro-T Phase 2D (<https://youtu.be/yYDayFDbbAs>)

Video S8. Mapping of hydration volume percentage of phosphotungstic acid (PTA) stain inside *G. superba* mechanically scarified seed (SS) after 24 h of water imbibition by micro-T Phase 2D (<https://youtu.be/JS0ka9FmDMY>)

Video S9. Mapping of hydration volume percentage of phosphotungstic acid (PTA) stain inside *G. superba* mechanically scarified seed (SS) after 48 h of water imbibition by micro-T Phase 2D (<https://youtu.be/KLucSZ4PB2g>)

Video S10. Germinated *G. superba* seed using micro-T in Phase 2D and 3D (<https://youtu.be/OVRcO5cFC98>)