Online Resource 1

Induction of walnut callus and physiological and molecular differences among tissues with varying degrees of browning

Plant Cell, Tissue and Organ Culture (PCTOC)

Xinlin Huang[1], Qinxiang Chang[1,2,*], Zongsuo Liang[1,*]

[1] School of Life Sciences and Medicine, Zhejiang Sci-Tech University, Hangzhou, China

[2] Department of Horticulture and Landscape Architecture, Taiyuan University, Taiyuan, Shanxi, China

* Corresponding authors: Zongsuo Liang (primary contact), Liangzs@ms.iswc.ac.cn; Qinxiang Chang, changqxkys@tyu.edu.cn.

Table S1. Individual TTC measurements.

Sample	Reaction A	Control A	Reaction mass mg	Control mass mg	ΔA	Activity
S1-1	1.024	0.090	95.4	116.0	0.934	1445.8
S1-2	1.014	0.108	94.2	111.0	0.906	1420.2
S1-3	0.954	0.095	93.1	107.0	0.859	1362.2
S2-1	0.639	0.080	87.6	101.0	0.559	940.6
S2-2	0.633	0.101	91.3	109.0	0.532	858.7
S2-3	0.607	0.073	85.3	104.0	0.534	922.6
S3-1	0.583	0.076	84.2	99.6	0.507	887.2
S3-2	0.554	0.088	89.5	95.3	0.466	766.8
S3-3	0.529	0.075	90.1	89.1	0.454	742.0
S4-1	0.445	0.075	102.0	96.7	0.370	533.4
S4-2	0.471	0.093	105.0	91.0	0.378	529.5
S4-3	0.476	0.083	108.0	95.5	0.393	535.3
S5-1	0.301	0.074	123.0	89.6	0.227	270.2
S5-2	0.306	0.072	118.0	93.1	0.234	290.4
S5-3	0.275	0.082	106.0	94.3	0.193	266.0
S6-1	0.086	0.076	121.0	117.6	0.010	NQ
S6-2	0.079	0.086	117.0	119.0	-0.007	NQ
S6-3	0.081	0.109	126.0	107.3	-0.028	NQ

NQ, not quantified. Activity units: μg TTC equivalents g^{-1} FW h^{-1}. Reaction and control samples had unequal fresh weights; no background mass correction was applied. Calculations use a 40 min incubation and a 2 mL final extract.

Table S2. Standard measurements for TTC calibration.

Nominal μg/mL	Series 1 A	Series 2 A	Mean ΔA	SD of ΔA	Delayed series 3 A
50	1.104	1.079	1.0205	0.02051	0.883
40	0.880	0.857	0.7975	0.01909	0.757
30	0.695	0.675	0.6140	0.01697	0.529
20	0.498	0.483	0.4195	0.01344	0.428
10	0.288	0.283	0.2145	0.00636	0.231
5	0.161	0.160	0.0895	0.00354	0.140

Ethyl acetate blanks: series 1, 0.069; series 2, 0.073; delayed series 3, 0.077. The fit uses the six means of individually blank-corrected series 1 and 2. Series 3 was measured after 1 h at room temperature and was not pooled.

A, absorbance at 485 nm; ΔA, series-specific blank-corrected absorbance. Nominal concentrations are expressed as TTC equivalents. The separately recorded delayed series is not part of the fitted calibration.

Table S3. Supplementary summaries of apparent TTC activity.

Grade	Mean ± SD	Pointwise 95% CI	Mean relative to S1 (%)
S1	1409.4 ± 42.8	1303.0–1515.8	100.0
S2	907.3 ± 43.0	800.4–1014.2	64.4
S3	798.6 ± 77.7	605.7–991.6	56.7
S4	532.8 ± 3.0	525.3–540.2	37.8
S5	275.6 ± 13.1	243.1–308.0	19.6

n = 3 per grade. Mean, SD, and confidence interval units are μg TTC equivalents g^{-1} FW h^{-1}. Pointwise t intervals assume independent, approximately normally distributed measurements within grades; n = 3 provides little basis for checking these assumptions. Calibration and background-related uncertainty are not included. Relative values are ratios of grade means and do not represent cell-survival percentages. S6 was not quantified.

Table S4. Recalculated two-sided pairwise Welch tests for apparent TTC activity.

Comparison	Mean difference	t	df	Raw P	Holm P
S1–S2	502.1	14.324	4.000	0.000138	0.001380
S1–S3	610.8	11.929	3.113	0.001060	0.005301
S1–S4	876.7	35.374	2.019	0.000754	0.004764
S1–S5	1133.9	43.871	2.368	0.000167	0.001506
S2–S3	108.7	2.120	3.123	0.120628	0.120628
S2–S4	374.6	15.037	2.019	0.004222	0.016889
S2–S5	631.7	24.329	2.365	0.000681	0.004764
S3–S4	265.9	5.925	2.006	0.027133	0.054266
S3–S5	523.1	11.504	2.113	0.006109	0.018326
S4–S5	257.2	33.272	2.209	0.000500	0.004000

Differences are first grade minus second grade, in μg TTC equivalents g^{-1} FW h^{-1}. Holm correction includes all ten pairwise comparisons. Welch ANOVA: $F(4, 4.11693) = 449.13707$, $P = 0.0000111872$. Reanalysis uses individual absorbances and reaction masses in Table S1 with the calibration in Table S2; no additional observations were generated.

Table S5. Primer sequences and expected amplicon lengths for RT-qPCR.

Gene and accession	Forward and reverse primers (5′–3′)	Expected product (bp)
JrSERK1 XM_018953949.2	F: TTGCCCAGGATCTCCTCCAT R: GTGGCACTATTCCCACCTGG	84
JrBBM1 XM_018958618.2	F: ATGAAGCACATGACCAGGCA R: CTTGCTTGCCATCGTCCATG	125
JrBBM2 XM_018973431.2	F: ATGAAGCACATGACCAGGCT R: TCTTGCTTGCCATCGTCCAT	126
JrLEC1 XM_018957637.2	F: TATGGAACGTAGGGGTGGGT R: GGGCTGTTGAAGGTGTTGGT	141
JrLEC2 XM_018996889.2	F: TGAGCCTGTTCCTCACAACC R: GGTTGTGGGAATGGGTGTGA	117
JrAGL15 XM_018961714.2	F: TGTGTGCTGCAACTACCGAA R: CATGTGGCTCCCTGTGTCAT	133
JrFUS3 XM_018979273.2	F: AACCAGGCTGGGTAATCACC R: CGAAATGGGTGGGGAGTGTT	101
JrWOX2 XM_018980445.2	F: CATTGTTCCCTCCTTGCCCA R: ATGATCTGCTCCCCATCCCT	129
JrEF1A XM_018973203.2	F: GCGGCCGGGTTCTATATTCA R: AGATCAAGTGGCCAGTGGTG	135
JrTIP41 XM_018953860.2	F: AGCGCTGATGAGACTAAGGG R: GCAGGATCAGATGGGTGTCC	138
JrACT XM_018950135.2	F: TCCCATTTAGCCCACCCTCT R: TCCGCCATCTTCTACCACCT	146

F, forward primer; R, reverse primer. Both sequences are written 5′–3′. JrSERK1, JrBBM1, JrBBM2, JrLEC1, JrLEC2, JrAGL15, JrFUS3, and JrWOX2 are target genes. JrTIP41 was the sole normalization reference for the reported analyses. JrEF1A and JrACT were additional reference-gene candidates and were not used in the reported normalization. Product sizes are the expected lengths supplied with the primer information; they do not demonstrate specificity or amplification efficiency.

Table S6. Reference-gene Ct measurements and descriptive statistics.

Gene	Group	Ct 1	Ct 2	Ct 3	Mean Ct	SD
JrEF1A	WT (CK)	24.11	24.24	24.18	24.177	0.065
JrEF1A	S1	24.06	24.19	24.12	24.123	0.065
JrEF1A	S2	24.17	24.31	24.24	24.240	0.070
JrEF1A	S3	24.09	24.22	24.15	24.153	0.065
JrEF1A	S4	24.20	24.34	24.27	24.270	0.070
JrEF1A	S5	24.13	24.26	24.19	24.193	0.065
JrEF1A	S6	24.05	24.18	24.11	24.113	0.065
JrTIP41	WT (CK)	25.22	25.35	25.28	25.283	0.065
JrTIP41	S1	25.16	25.30	25.23	25.230	0.070
JrTIP41	S2	25.27	25.41	25.34	25.340	0.070
JrTIP41	S3	25.19	25.32	25.25	25.253	0.065
JrTIP41	S4	25.31	25.44	25.37	25.373	0.065
JrTIP41	S5	25.24	25.37	25.30	25.303	0.065
JrTIP41	S6	25.14	25.27	25.20	25.203	0.065
JrACT	WT (CK)	23.84	23.97	23.90	23.903	0.065
JrACT	S1	23.78	23.92	23.85	23.850	0.070
JrACT	S2	23.89	24.02	23.95	23.953	0.065
JrACT	S3	23.81	23.94	23.87	23.873	0.065
JrACT	S4	23.93	24.06	23.99	23.993	0.065
JrACT	S5	23.86	23.99	23.92	23.923	0.065
JrACT	S6	23.76	23.89	23.82	23.823	0.065

Each group contains three independent biological replicates. Ct 1–3 retain the within-gene order of the measurements; these labels do not assert sample matching across genes. WT and CK denote the same uncultured control. SD is the sample standard deviation (n = 3). Only JrTIP41 was used to normalize the reported target-gene expression. No between-gene stability ranking or group-equivalence test is implied by these descriptive statistics.

Table S7. Biological-replicate ΔCt and relative expression.

Gene	Group	ΔCt 1	ΔCt 2	ΔCt 3	Fold mean	Fold SD
JrSERK1	WT	4.21	4.37	4.29	1.001	0.055
JrSERK1	S1	3.04	3.20	3.12	2.252	0.125
JrSERK1	S2	2.13	2.29	2.21	4.232	0.235
JrSERK1	S3	2.87	3.04	2.95	2.529	0.149
JrSERK1	S4	3.54	3.70	3.62	1.593	0.088
JrSERK1	S5	4.18	4.35	4.26	1.020	0.060
JrSERK1	S6	4.76	4.93	4.84	0.682	0.040
JrBBM1	WT	4.82	4.98	4.90	1.001	0.055
JrBBM1	S1	3.66	3.83	3.74	2.232	0.131
JrBBM1	S2	2.45	2.62	2.53	5.163	0.304
JrBBM1	S3	2.97	3.13	3.05	3.609	0.200
JrBBM1	S4	3.72	3.89	3.80	2.141	0.126
JrBBM1	S5	4.41	4.57	4.49	1.330	0.074
JrBBM1	S6	5.08	5.24	5.16	0.836	0.046
JrBBM2	WT	5.13	5.29	5.21	1.001	0.055
JrBBM2	S1	4.27	4.44	4.35	1.813	0.107
JrBBM2	S2	3.24	3.40	3.32	3.710	0.206
JrBBM2	S3	2.38	2.54	2.46	6.734	0.373
JrBBM2	S4	3.01	3.18	3.09	4.342	0.255
JrBBM2	S5	3.86	4.02	3.94	2.414	0.134
JrBBM2	S6	4.62	4.78	4.70	1.426	0.079
JrLEC1	WT	5.42	5.59	5.50	1.001	0.059
JrLEC1	S1	4.65	4.81	4.73	1.711	0.095
JrLEC1	S2	3.88	4.04	3.96	2.918	0.162
JrLEC1	S3	3.12	3.29	3.20	4.930	0.290
JrLEC1	S4	2.26	2.42	2.34	8.968	0.497
JrLEC1	S5	3.04	3.20	3.12	5.223	0.289
JrLEC1	S6	3.91	4.08	3.99	2.851	0.168

ΔCt = target-gene Ct minus matched *JrTIP41* Ct. Columns 1–3 represent three independent biological replicates within each group. Relative expression for each replicate = $2^{(\text{mean WT } \Delta Ct - \text{replicate } \Delta Ct)}$. Fold mean and sample SD were calculated after transformation. WT arithmetic means can slightly exceed 1 because calibration uses mean ΔCt; WT values were not forced to 1. These ΔCt values are processed measurements, not reconstructed target-gene raw Ct.

Table S7. Biological-replicate ΔCt and relative expression (continued).

Gene	Group	ΔCt 1	ΔCt 2	ΔCt 3	Fold mean	Fold SD
JrLEC2	WT	5.61	5.77	5.69	1.001	0.055
JrLEC2	S1	4.84	5.00	4.92	1.707	0.095
JrLEC2	S2	3.97	4.14	4.05	3.113	0.183
JrLEC2	S3	2.31	2.47	2.39	9.859	0.547
JrLEC2	S4	2.94	3.10	3.02	6.371	0.353
JrLEC2	S5	3.75	3.91	3.83	3.634	0.201
JrLEC2	S6	4.53	4.70	4.61	2.112	0.124
JrAGL15	WT	4.96	5.12	5.04	1.001	0.055
JrAGL15	S1	4.13	4.30	4.21	1.776	0.104
JrAGL15	S2	3.45	3.61	3.53	2.851	0.158
JrAGL15	S3	2.72	2.88	2.80	4.729	0.262
JrAGL15	S4	2.24	2.41	2.32	6.581	0.387
JrAGL15	S5	2.96	3.12	3.04	4.004	0.222
JrAGL15	S6	3.68	3.85	3.76	2.426	0.143
JrFUS3	WT	5.28	5.44	5.36	1.001	0.055
JrFUS3	S1	4.45	4.62	4.53	1.776	0.104
JrFUS3	S2	3.71	3.87	3.79	2.972	0.165
JrFUS3	S3	2.95	3.11	3.03	5.033	0.279
JrFUS3	S4	2.33	2.49	2.41	7.735	0.429
JrFUS3	S5	3.17	3.34	3.25	4.312	0.254
JrFUS3	S6	4.04	4.20	4.12	2.364	0.131
JrWOX2	WT	5.53	5.69	5.61	1.001	0.055
JrWOX2	S1	4.76	4.92	4.84	1.707	0.095
JrWOX2	S2	3.92	4.09	4.00	3.049	0.179
JrWOX2	S3	2.68	2.84	2.76	7.217	0.400
JrWOX2	S4	2.40	2.56	2.48	8.763	0.486
JrWOX2	S5	3.25	3.41	3.33	4.862	0.269
JrWOX2	S6	4.11	4.28	4.19	2.673	0.157

ΔCt = target-gene Ct minus matched *JrTIP41* Ct. Columns 1–3 represent three independent biological replicates within each group. Relative expression for each replicate = $2^{(\text{mean WT } \Delta Ct - \text{replicate } \Delta Ct)}$. Fold mean and sample SD were calculated after transformation. WT arithmetic means can slightly exceed 1 because calibration uses mean ΔCt; WT values were not forced to 1. These ΔCt values are processed measurements, not reconstructed target-gene raw Ct.

Table S8. Recalculated comparisons of ΔCt with WT.

Gene	vs WT	t	df	Raw P	Holm P
JrSERK1	S1	-17.9119	4.0000	5.70972e-05	0.000228389
JrSERK1	S2	-31.8434	4.0000	5.7973e-06	3.47838e-05
JrSERK1	S3	-19.8282	3.9851	3.92706e-05	0.000196353
JrSERK1	S4	-10.2572	4.0000	0.000509331	0.00152799
JrSERK1	S5	-0.3956	3.9851	0.712681	0.712681
JrSERK1	S6	8.2082	3.9851	0.00121986	0.00243972
JrBBM1	S1	-17.1580	3.9851	6.94974e-05	0.000277989
JrBBM1	S2	-35.1072	3.9851	4.07614e-06	2.44569e-05
JrBBM1	S3	-28.3222	4.0000	9.24785e-06	4.62393e-05
JrBBM1	S4	-16.2680	3.9851	8.57188e-05	0.000277989
JrBBM1	S5	-6.2768	4.0000	0.00328897	0.00657794
JrBBM1	S6	3.9804	4.0000	0.0163954	0.0163954
JrBBM2	S1	-12.7078	3.9851	0.000225801	0.000451603
JrBBM2	S2	-28.9346	4.0000	8.49241e-06	3.39697e-05
JrBBM2	S3	-42.1006	4.0000	1.90268e-06	1.14161e-05
JrBBM2	S4	-31.3987	3.9851	6.35163e-06	3.17581e-05
JrBBM2	S5	-19.4428	4.0000	4.12566e-05	0.00012377
JrBBM2	S6	-7.8077	4.0000	0.00145206	0.00145206
JrLEC1	S1	-11.4717	3.9851	0.000336441	0.000336441
JrLEC1	S2	-22.8939	3.9851	2.22362e-05	6.67086e-05
JrLEC1	S3	-33.1211	4.0000	4.95564e-06	1.98226e-05
JrLEC1	S4	-46.9250	3.9851	1.28564e-06	7.71381e-06
JrLEC1	S5	-35.3545	3.9851	3.96403e-06	1.98201e-05
JrLEC1	S6	-21.7447	4.0000	2.6463e-05	6.67086e-05

Two-sided independent-sample Welch t tests on ΔCt, n = 3 biological replicates per group. The t statistic is oriented as grade minus WT; negative values indicate lower ΔCt and higher relative expression. Holm adjustment covers six comparisons within each gene, not all 48 comparisons together. Degrees of freedom are Welch–Satterthwaite estimates. The small sample size limits evaluation of distributional assumptions.

Table S8. Recalculated comparisons of ΔCt with WT (continued).

Gene	vs WT	t	df	Raw P	Holm P
JrLEC2	S1	-11.7882	4.0000	0.000296355	0.000296355
JrLEC2	S2	-24.2784	3.9851	1.76214e-05	5.28643e-05
JrLEC2	S3	-50.5207	4.0000	9.18627e-07	5.51176e-06
JrLEC2	S4	-40.8759	4.0000	2.14069e-06	1.07034e-05
JrLEC2	S5	-28.4753	4.0000	9.05138e-06	3.62055e-05
JrLEC2	S6	-15.9713	3.9851	9.21573e-05	0.000184315
JrAGL15	S1	-12.2628	3.9851	0.000259501	0.000259501
JrAGL15	S2	-23.1171	4.0000	2.07502e-05	6.22506e-05
JrAGL15	S3	-34.2929	4.0000	4.31398e-06	2.15699e-05
JrAGL15	S4	-40.2992	3.9851	2.35562e-06	1.41337e-05
JrAGL15	S5	-30.6186	4.0000	6.77839e-06	2.71136e-05
JrAGL15	S6	-18.9381	3.9851	4.70822e-05	9.41645e-05
JrFUS3	S1	-12.2628	3.9851	0.000259501	0.000259501
JrFUS3	S2	-24.0356	4.0000	1.77719e-05	5.33158e-05
JrFUS3	S3	-35.6707	4.0000	3.68666e-06	1.84333e-05
JrFUS3	S4	-45.1625	4.0000	1.43755e-06	8.62529e-06
JrFUS3	S5	-31.2504	3.9851	6.47222e-06	2.58889e-05
JrFUS3	S6	-18.9835	4.0000	4.53576e-05	9.07152e-05
JrWOX2	S1	-11.7882	4.0000	0.000296355	0.000296355
JrWOX2	S2	-23.8334	3.9851	1.89617e-05	5.68851e-05
JrWOX2	S3	-43.6315	4.0000	1.64979e-06	8.24897e-06
JrWOX2	S4	-47.9181	4.0000	1.13473e-06	6.80836e-06
JrWOX2	S5	-34.9052	4.0000	4.01991e-06	1.60797e-05
JrWOX2	S6	-21.0149	3.9851	3.12075e-05	6.24151e-05

Two-sided independent-sample Welch t tests on ΔCt, n = 3 biological replicates per group. The t statistic is oriented as grade minus WT; negative values indicate lower ΔCt and higher relative expression. Holm adjustment covers six comparisons within each gene, not all 48 comparisons together. Degrees of freedom are Welch–Satterthwaite estimates. The small sample size limits evaluation of distributional assumptions.

Table S9. Integer counts for the single-factor screens (NAA).

mg L⁻¹	Rep.	E	I	S1	S2	S3	S4	S5	S6
0	1	30	18	3	3	4	4	3	1
0	2	30	18	3	4	3	4	3	1
0	3	30	17	3	3	3	4	3	1
0.5	1	30	25	9	8	4	3	1	0
0.5	2	30	26	9	9	4	3	1	0
0.5	3	30	24	9	7	4	3	1	0
1	1	30	26	10	9	4	2	1	0
1	2	30	27	10	10	4	2	1	0
1	3	30	26	10	9	4	2	1	0
2	1	30	21	5	5	4	4	2	1
2	2	30	20	4	5	4	4	2	1
2	3	30	22	5	5	4	5	2	1
4	1	30	15	2	3	3	3	2	2
4	2	30	16	2	3	3	4	2	2
4	3	30	15	2	2	3	4	2	2

E, initial explants; I, induced callus units. Each row is one recorded biological replicate. Grades S1–S6 were assigned to all induced callus units at a common assessment time; their sum equals I. Induction and conditional grade proportions in Table 3 were calculated from these counts. The original rounded percentage columns were not used as calculation inputs.

Table S9. Integer counts for the single-factor screens (6-BA).

mg L⁻¹	Rep.	E	I	S1	S2	S3	S4	S5	S6
0	1	30	16	3	3	3	4	2	1
0	2	30	17	3	3	3	4	3	1
0	3	30	16	3	3	3	4	2	1
0.25	1	30	24	8	8	4	3	1	0
0.25	2	30	25	8	9	4	3	1	0
0.25	3	30	24	8	8	4	3	1	0
0.5	1	30	25	9	9	4	2	1	0
0.5	2	30	26	9	10	4	2	1	0
0.5	3	30	26	10	9	4	2	1	0
1	1	30	21	5	5	4	4	2	1
1	2	30	20	5	4	4	4	2	1
1	3	30	21	5	5	4	4	2	1
2	1	30	14	2	2	3	3	2	2
2	2	30	15	2	2	3	3	3	2
2	3	30	14	2	2	3	3	2	2

E, initial explants; I, induced callus units. Each row is one recorded biological replicate. Grades S1–S6 were assigned to all induced callus units at a common assessment time; their sum equals I. Induction and conditional grade proportions in Table 3 were calculated from these counts. The original rounded percentage columns were not used as calculation inputs.

Table S9. Integer counts for the single-factor screens (VC).

mg L^{-1}	Rep.	E	I	S1	S2	S3	S4	S5	S6
0	1	30	22	5	4	3	5	3	2
0	2	30	23	5	4	3	5	4	2
0	3	30	22	5	4	3	5	3	2
100	1	30	26	8	9	4	3	1	1
100	2	30	25	8	8	4	3	1	1
100	3	30	26	8	9	4	3	1	1
200	1	30	27	10	10	4	2	1	0
200	2	30	26	10	9	4	2	1	0
200	3	30	27	10	10	4	2	1	0
400	1	30	26	9	9	4	3	1	0
400	2	30	27	9	10	4	3	1	0
400	3	30	26	9	9	4	3	1	0
600	1	30	21	6	6	4	3	1	1
600	2	30	22	6	6	4	4	1	1
600	3	30	21	6	6	4	3	1	1

E, initial explants; I, induced callus units. Each row is one recorded biological replicate. Grades S1–S6 were assigned to all induced callus units at a common assessment time; their sum equals I. Induction and conditional grade proportions in Table 3 were calculated from these counts. The original rounded percentage columns were not used as calculation inputs.

Table S10. Exploratory Welch ANOVA for single-factor replicate percentages.

Factor	Endpoint	F	df2	Raw P	Holm P
NAA	Induction	120.935	4.939	4.07e-05	8.14e-05
NAA	S2–S6 / I	443.498	4.803	2.3e-06	1.84e-05
NAA	S4–S6 / I	436.280	4.321	7.34e-06	3.67e-05
6-BA	Induction	155.143	5.000	1.99e-05	7.69e-05
6-BA	S2–S6 / I	268.078	4.920	6.03e-06	3.62e-05
6-BA	S4–S6 / I	600.555	4.589	1.89e-06	1.7e-05
VC	Induction	38.786	5.000	0.000589	0.000589
VC	S2–S6 / I	159.886	4.977	1.92e-05	7.69e-05
VC	S4–S6 / I	457.748	4.728	2.54e-06	1.84e-05

Numerator df = 4 for all tests; each of five concentrations has three biological replicates. Holm adjustment covers all nine omnibus tests shown. Welch ANOVA allows unequal group variances, but the small number of replicates limits assessment of distributional assumptions. No post hoc comparisons were performed. These results do not establish adjacent-level differences or an optimal combination of regulators.

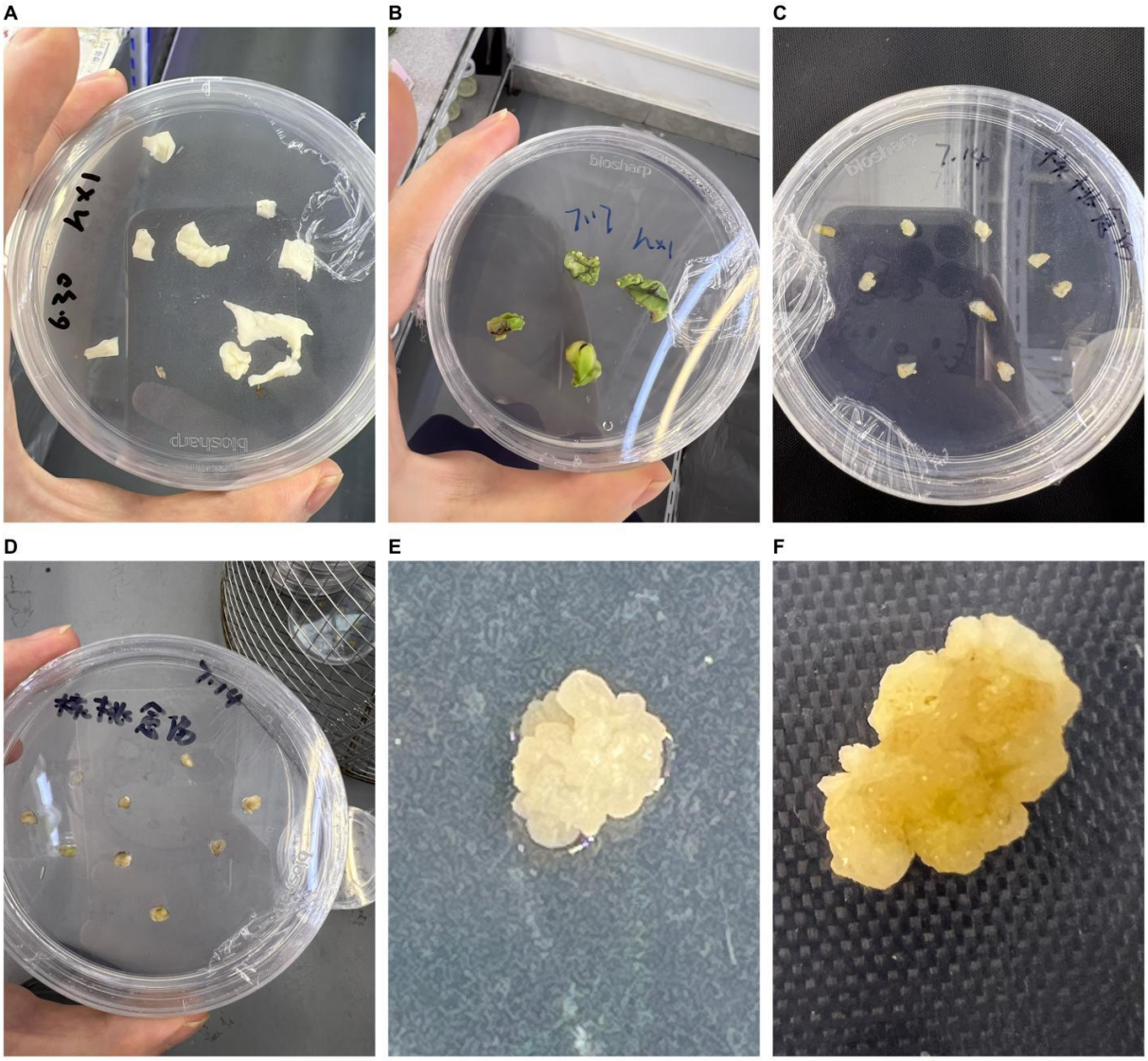


Fig. S1 Additional views of walnut explants and callus. (A) Pale explant pieces on solid medium. (B) A culture containing green folded explants. (C, D) Cultures with separated pale-to-yellow tissue pieces. (E) A pale-yellow callus mass with a lobed surface, photographed through a condensation-covered lid. (F) A yellow callus mass with an uneven surface

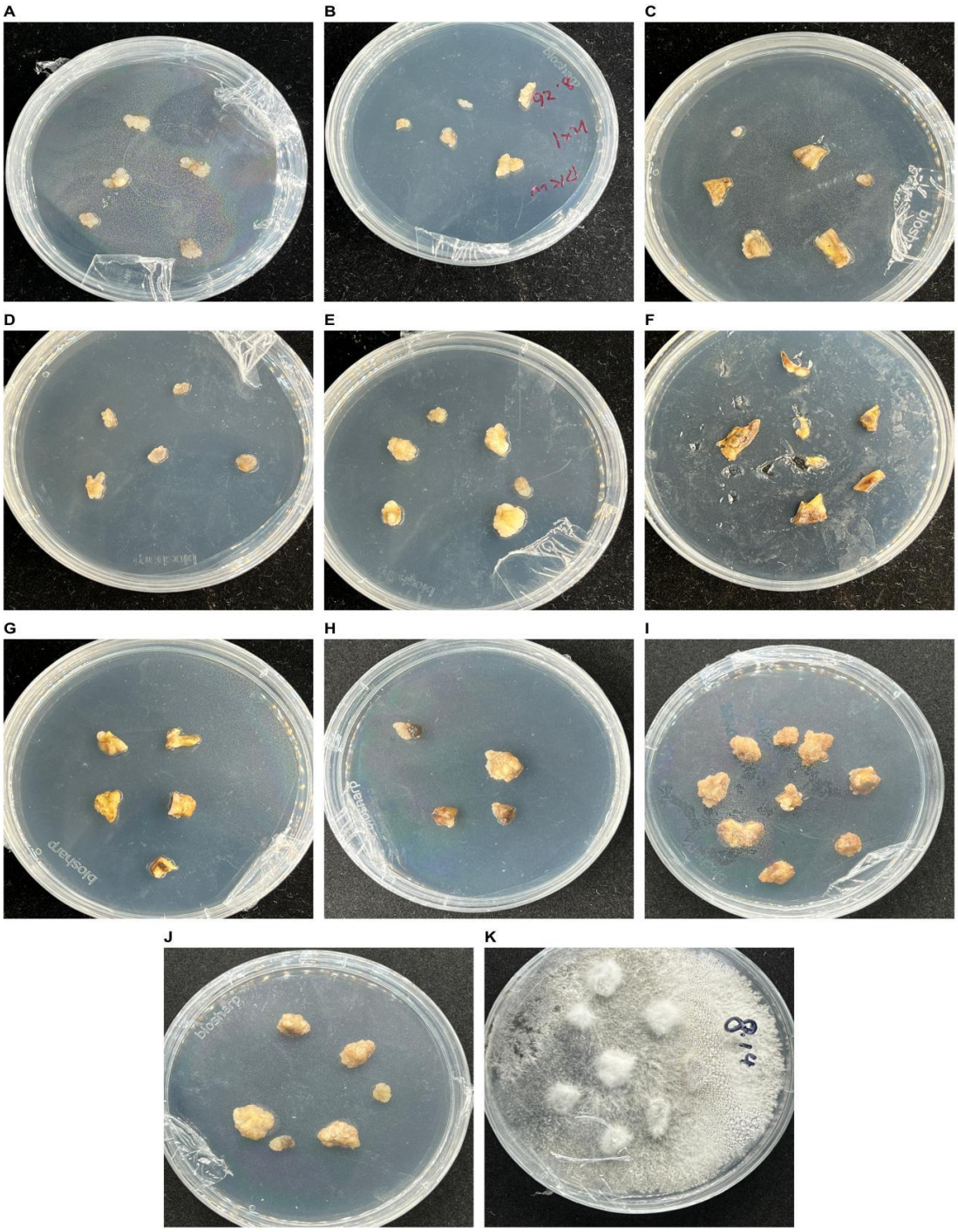


Fig. S2 Additional macroscopic appearances of walnut cultures. (A-E) Cultures containing pale-to-yellow tissue masses of variable shape. (F-H) Cultures containing tissues with localized or extensive brown discoloration. (I, J) Cultures containing yellow-to-amber nodular masses. (K) An abnormal culture with extensive white, floccose coverage, visually suggestive of microbial overgrowth; the white coverage in K is not interpreted as callus or embryogenic tissue.

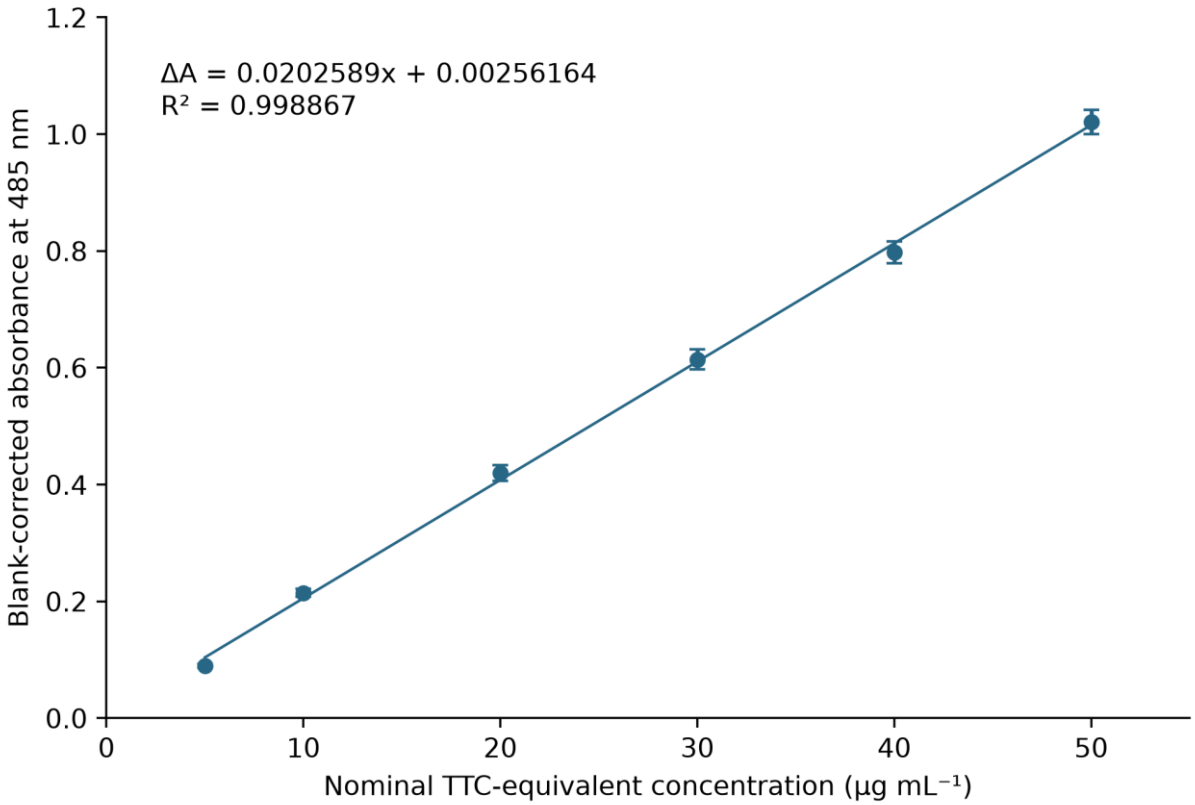


Fig. S3 TTC calibration curve. Points and error bars represent mean ± SD of two independently blank-corrected measurements at each nominal concentration. The fit uses the six concentration means: $\Delta A = 0.0202589x + 0.00256164$, $R^2 = 0.998867$. The separately recorded delayed series was not pooled. Measurements are provided in Table S2