Supplementary material

for

Quince seed mucilage as an alternative for agar in plant tissue culture

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Regression coefficients for the eight generalized linear models

Table S1: Regression coefficients, their Z values and p values of the model for root proliferation from tobacco explants. The coefficients of the hormone treatments show the effect of each treatment vs. 10 µM NAA/0.4 µM BAP; The Explant coefficient describes the effect of using stem pieces vs. buds as explants; the coefficient of the Gelling agent shows the effect of using QM vs. agar; the coefficients for Additives show the effects of using Half MS, 1/4 MS, Half sucrose or 1/4 sucrose vs. Full MS + sucrose; the last four lines show the effects of the interactions between the gelling agent and the additive combinations. See Materials and Methods for a full description of the additive combinations. The model, described in Eq. 1, assumed a negative binomial distribution for the response variable.

Effect	Coefficient	SE	Z-value	p-value
(Intercept)	2.62	0.44	6.021	0
Hormone (2 µM NAA/2 µM BAP vs. 10 µM NAA/0.4 µM BAP)	0.46	0.29	1.569	0.1167
Hormone (0.4 µM NAA/10 µM BAP vs. 10 µM NAA/0.4 µM BAP)	-0.95	0.3	-3.119	0.0018
Explant (stem piece vs. bud)	-1.86	0.25	-7.533	0
Gelling agent (QM vs. agar)	1.47	0.53	2.765	0.0057
Additive (Half MS vs. Full MS + suc)	0.92	0.54	1.716	0.0861
Additive (1/4 MS vs. Full MS + suc)	-1.56	0.59	-2.639	0.0083
Additive (Half suc vs. Full MS + suc)	-1.18	0.57	-2.063	0.0391
Additive (1/4 suc vs. Full MS + suc)	-1.19	0.57	-2.081	0.0375
QM as gelling agent : Half MS	-0.51	0.75	-0.683	0.4949
QM as gelling agent : 1/4 MS	1.69	0.79	2.148	0.0317
QM as gelling agent : Half suc	1.54	0.77	1.995	0.046

QM as gelling agent : 1/4 suc	0.2	0.78	0.26	0.7946

Table S2: Regression coefficients, their Z values and p values of the model for root proliferation from tomato explants. The coefficients of the hormone treatments show the effect of each treatment vs. 10 µM NAA/0.4 µM BAP; The Explant coefficient describes the effect of using stem pieces vs. buds as explants; the coefficient of the Gelling agent shows the effect of using QM vs. agar; the coefficients for Additives show the effects of using Half MS, 1/4 MS, Half sucrose or 1/4 sucrose vs. Full MS + sucrose; the last four lines show the effects of the interactions between the gelling agent and the additive combinations. See Materials and Methods for a full description of the additive combinations. The model, described in Eq. 1, assumed a negative binomial distribution for the response variable.

Effect	Coefficient	SE	Z-value	p-value
(Intercept)	3.96	0.4	10.023	0
Hormone (2 µM NAA/2 µM BAP vs. 10 µM NAA/0.4 µM BAP)	-0.07	0.27	-0.28	0.7797
Hormone (0.4 µM NAA/10 µM BAP vs. 10 µM NAA/0.4 µM BAP)	-1.16	0.27	-4.259	0
Explant (stem piece vs. bud)	-1.55	0.22	-7.015	0
Gelling agent (QM vs. agar)	1.77	0.49	3.623	3e-04
Additive (Half MS vs. Full MS + suc)	-0.26	0.49	-0.527	0.598
Additive (1/4 MS vs. Full MS + suc)	-1.64	0.51	-3.21	0.0013
Additive (Half suc vs. Full MS + suc)	0.64	0.49	1.302	0.193
Additive (1/4 suc vs. Full MS + suc)	-0.42	0.49	-0.852	0.3944
QM as gelling agent : Half MS	-0.41	0.69	-0.595	0.5515
QM as gelling agent : 1/4 MS	-0.2	0.71	-0.279	0.7802
QM as gelling agent : Half suc	-1.38	0.69	-2.005	0.0449
QM as gelling agent : 1/4 suc	-1.63	0.7	-2.351	0.0187

Table S3: Regression coefficients, their Z values and p values of the model for callus proliferation from tobacco explants. The coefficients of the hormone treatments show the effect of each treatment vs. 10 µM NAA/0.4 µM BAP; The Explant coefficient describes the effect of using stem pieces vs. buds as explants; the coefficient of the Gelling agent shows the effect of using QM vs. agar; the coefficients for Additives show the effects of using Half MS, 1/4 MS, Half sucrose or 1/4 sucrose vs. Full MS + sucrose; the last four lines show the effects of the interactions between the gelling agent and the additive combinations. See Materials and Methods for a full description of the additive combinations. The model, described in Eq. 1, assumed a Poisson distribution for the response variable.

Effect	Coefficient	SE	Z-value	p-value
(Intercept)	2.03	0.1	19.406	0
Hormone (2 µM NAA/2 µM BAP vs. 10 µM NAA/0.4 µM BAP)	-0.45	0.07	-6.232	0
Hormone (0.4 µM NAA/10 µM BAP vs. 10 µM NAA/0.4 µM BAP)	-0.6	0.08	-7.873	0
Explant (stem piece vs. bud)	0.28	0.06	4.442	0
Gelling agent (QM vs. agar)	0.37	0.12	3.068	0.0022
Additive (Half MS vs. Full MS + suc)	-0.28	0.14	-1.954	0.0507
Additive (1/4 MS vs. Full MS + suc)	-1.02	0.18	-5.642	0
Additive (Half suc vs. Full MS + suc)	0.07	0.13	0.516	0.6056
Additive (1/4 suc vs. Full MS + suc)	-0.52	0.15	-3.417	6e-04
QM as gelling agent : Half MS	0.15	0.18	0.827	0.4083
QM as gelling agent : 1/4 MS	0.46	0.22	2.066	0.0389
QM as gelling agent : Half suc	-0.23	0.17	-1.363	0.1729
QM as gelling agent : 1/4 suc	-0.41	0.21	-1.973	0.0485

Table S4: Regression coefficients, their Z values and p values of the model for callus proliferation from tomato explants. The coefficients of the hormone treatments show the effect of each treatment vs. 10 µM NAA/0.4 µM BAP; The Explant coefficient describes the effect of using stem pieces vs. buds as explants; the coefficient of the Gelling agent shows the effect of using QM vs. agar; the coefficients for Additives show the effects of using Half MS, 1/4 MS, Half sucrose or 1/4 sucrose vs. Full MS + sucrose; the last four lines show the effects of the interactions between the gelling agent and the additive combinations. See Materials and Methods for a full description of the additive combinations. The model, described in Eq. 1, assumed a negative binomial distribution for the response variable.

Effect	Coefficient	SE	Z-value	p-value
(Intercept)	1.44	0.16	9.062	0
Hormone (2 µM NAA/2 µM BAP vs. 10 µM NAA/0.4 µM BAP)	-0.26	0.1	-2.558	0.0105
Hormone (0.4 µM NAA/10 µM BAP vs. 10 µM NAA/0.4 µM BAP)	-0.32	0.1	-3.095	0.002
Explant (stem piece vs. bud)	0.26	0.08	3.025	0.0025
Gelling agent (QM vs. agar)	-0.13	0.21	-0.65	0.5157
Additive (Half MS vs. Full MS + suc)	-0.01	0.2	-0.033	0.9739
Additive (1/4 MS vs. Full MS + suc)	0.39	0.19	2.034	0.0419
Additive (Half suc vs. Full MS + suc)	0.46	0.19	2.421	0.0155
Additive (1/4 suc vs. Full MS + suc)	0.3	0.19	1.579	0.1143

QM as gelling agent : Half MS	0.33	0.28	1.18	0.2379
QM as gelling agent : 1/4 MS	0.34	0.27	1.257	0.2087
QM as gelling agent : Half suc	-0.13	0.27	-0.475	0.6348
QM as gelling agent : 1/4 suc	-0.23	0.28	-0.803	0.422

Table S5: Regression coefficients, their Z values and p values of the model for shoot proliferation from tobacco explants. The coefficients of the hormone treatments show the effect of each treatment vs. 10 µM NAA/0.4 µM BAP; The Explant coefficient describes the effect of using stem pieces vs. buds as explants; the coefficient of the Gelling agent shows the effect of using QM vs. agar; the coefficients for Additives show the effects of using Half MS, 1/4 MS, Half sucrose or 1/4 sucrose vs. Full MS + sucrose; the last four lines show the effects of the interactions between the gelling agent and the additive combinations. See Materials and Methods for a full description of the additive combinations. The model, described in Eq. 1, assumed a negative binomial distribution for the response variable.

Effect	Coefficient	SE	Z-value	p-value
(Intercept)	-0.87	0.31	-2.86	0.0042
Hormone (2 µM NAA/2 µM BAP vs. 10 µM NAA/0.4 µM BAP)	1.06	0.21	5.094	0
Hormone (0.4 µM NAA/10 µM BAP vs. 10 µM NAA/0.4 µM BAP)	1.66	0.2	8.342	0
Explant (stem piece vs. bud)	-0.08	0.14	-0.615	0.5383
Gelling agent (QM vs. agar)	1.16	0.3	3.798	1e-04

Additive (Half MS vs. Full MS + suc)	-0.87	0.43	-2.01	0.0444
Additive (1/4 MS vs. Full MS + suc)	-0.98	0.45	-2.182	0.0291
Additive (Half suc vs. Full MS + suc)	1.04	0.31	3.381	7e-04
Additive (1/4 suc vs. Full MS + suc)	0.66	0.32	2.078	0.0377
QM as gelling agent : Half MS	0.2	0.51	0.4	0.6894
QM as gelling agent : 1/4 MS	0.06	0.53	0.119	0.9056
QM as gelling agent : Half suc	-1.84	0.42	-4.431	0
QM as gelling agent : 1/4 suc	-0.88	0.41	-2.151	0.0315

Table S6: Regression coefficients, their Z values and p values of the model for shoot proliferation from tomato explants. The coefficients of the hormone treatments show the effect of each treatment vs. 10 µM NAA/0.4 µM BAP; The Explant coefficient describes the effect of using stem pieces vs. buds as explants; the coefficient of the Gelling agent shows the effect of using QM vs. agar; the coefficients for Additives show the effects of using Half MS, 1/4 MS, Half sucrose or 1/4 sucrose vs. Full MS + sucrose; the last four lines show the effects of the interactions between the gelling agent and the additive combinations. See Materials and Methods for a full description of the additive combinations. The model, described in Eq. 1, assumed a Poisson distribution for the response variable.

Effect	Coefficient	SE	Z-value	p-value
(Intercept)	-1.22	0.55	-2.214	0.0268
Hormone (2 µM NAA/2 µM BAP vs. 10 µM NAA/0.4 µM BAP)	0.81	0.42	1.908	0.0563
Hormone (0.4 µM NAA/10 µM BAP vs. 10 µM NAA/0.4 µM BAP)	0.75	0.43	1.758	0.0787
Explant (stem piece vs. bud)	-3.02	0.72	-4.171	0

Gelling agent (QM vs. agar)	0.34	0.59	0.575	0.5655
Additive (Half MS vs. Full MS + suc)	-1.61	1.1	-1.469	0.1418
Additive (1/4 MS vs. Full MS + suc)	-18.54	2877.8	-0.006	0.9949
Additive (Half suc vs. Full MS + suc)	0.47	0.57	0.824	0.4097
Additive (1/4 suc vs. Full MS + suc)	0.18	0.61	0.301	0.7633
QM as gelling agent : Half MS	0.76	1.29	0.589	0.5561
QM as gelling agent : 1/4 MS	-0.34	4069.82	0	0.9999
QM as gelling agent : Half suc	-0.47	0.78	-0.601	0.5476
QM as gelling agent : 1/4 suc	-0.34	0.82	-0.409	0.6824

Table S7: Regression coefficients, their Z values and p values of the model for leaf proliferation from tobacco explants. The coefficients of the hormone treatments show the effect of each treatment vs. 10 µM NAA/0.4 µM BAP; The Explant coefficient describes the effect of using stem pieces vs. buds as explants; the coefficient of the Gelling agent shows the effect of using QM vs. agar; the coefficients for Additives show the effects of using Half MS, 1/4 MS, Half sucrose or 1/4 sucrose vs. Full MS + sucrose; the last four lines show the effects of the interactions between the gelling agent and the additive combinations. See Materials and Methods for a full description of the additive combinations. The model, described in Eq. 1, assumed a negative binomial distribution for the response variable.

Effect	Coefficient	SE	Z-value	p-value
(Intercept)	0.23	0.3	0.771	0.4406
Hormone (2 µM NAA/2 µM BAP vs. 10 µM NAA/0.4 µM BAP)	1.39	0.2	6.898	0
Hormone (0.4 µM NAA/10 µM BAP vs. 10 µM NAA/0.4 µM BAP)	1.97	0.2	9.971	0

Explant (stem piece vs. bud)	-0.63	0.15	-4.097	0
Gelling agent (QM vs. agar)	1.34	0.33	4.031	1e-04
Additive (Half MS vs. Full MS + suc)	-0.8	0.38	-2.098	0.0359
Additive (1/4 MS vs. Full MS + suc)	-1.24	0.41	-3.036	0.0024
Additive (Half suc vs. Full MS + suc)	1.19	0.33	3.562	4e-04
Additive (1/4 suc vs. Full MS + suc)	0.8	0.34	2.36	0.0183
QM as gelling agent : Half MS	0.23	0.5	0.455	0.6488
QM as gelling agent : 1/4 MS	0.1	0.52	0.185	0.853
QM as gelling agent : Half suc	-1.76	0.46	-3.826	1e-04
QM as gelling agent : 1/4 suc	-1.17	0.46	-2.532	0.0113

Table S8. Regression coefficients, their Z values and p values of the model for leaf proliferation from tomato explants. The coefficients of the hormone treatments show the effect of each treatment vs. 10 µM NAA/0.4 µM BAP; The Explant coefficient describes the effect of using stem pieces vs. buds as explants; the coefficient of the Gelling agent shows the effect of using QM vs. agar; the coefficients for Additives show the effects of using Half MS, 1/4 MS, Half sucrose or 1/4 sucrose vs. Full MS + sucrose; the last four lines show the effects of the interactions between the gelling agent and the additive combinations. See Materials and Methods for a full description of the additive combinations. The model, described in Eq. 1, assumed a negative binomial distribution for the response variable.

Effect	Coefficient	SE	Z-value	p-value
(Intercept)	0.08	0.36	0.221	0.8253
Hormone (2 µM NAA/2 µM BAP vs. 10 µM NAA/0.4 µM BAP)	0.8	0.29	2.761	0.0058

Hormone (0.4 µM NAA/10 µM BAP vs. 10 µM NAA/0.4 µM BAP)	0.92	0.29	3.215	0.0013
Explant (stem piece vs. bud)	-3.09	0.41	-7.586	0
Gelling agent (QM vs. agar)	0.23	0.41	0.578	0.5632
Additive (Half MS vs. Full MS + suc)	-1.9	0.68	-2.773	0.0056
Additive (1/4 MS vs. Full MS + suc)	-36.27	13603400.34	0	1
Additive (Half suc vs. Full MS + suc)	0.65	0.39	1.679	0.0932
Additive (1/4 suc vs. Full MS + suc)	-0.01	0.42	-0.014	0.989
QM as gelling agent : Half MS	0.32	0.88	0.364	0.7157
QM as gelling agent : 1/4 MS	-0.23	19238113.26	0	1
QM as gelling agent : Half suc	-0.41	0.54	-0.749	0.4537
QM as gelling agent : 1/4 suc	-0.34	0.59	-0.586	0.5582

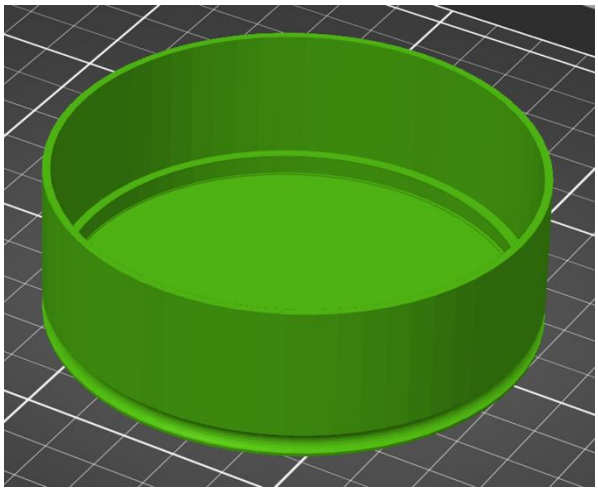

Fig. S1 3D model of the filter used for separation of the mucilage from the residues. Diameter is 91mm and the height is 30mm

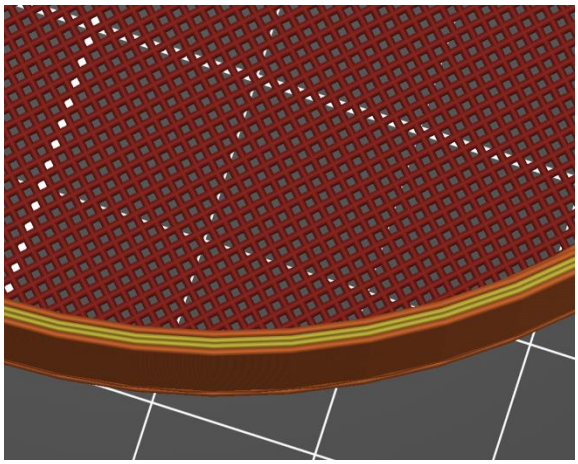

Fig. S2 A detail of the 3D model showing the mesh of the filter used for separating QM from the residues. The pore diameter is 1 mm

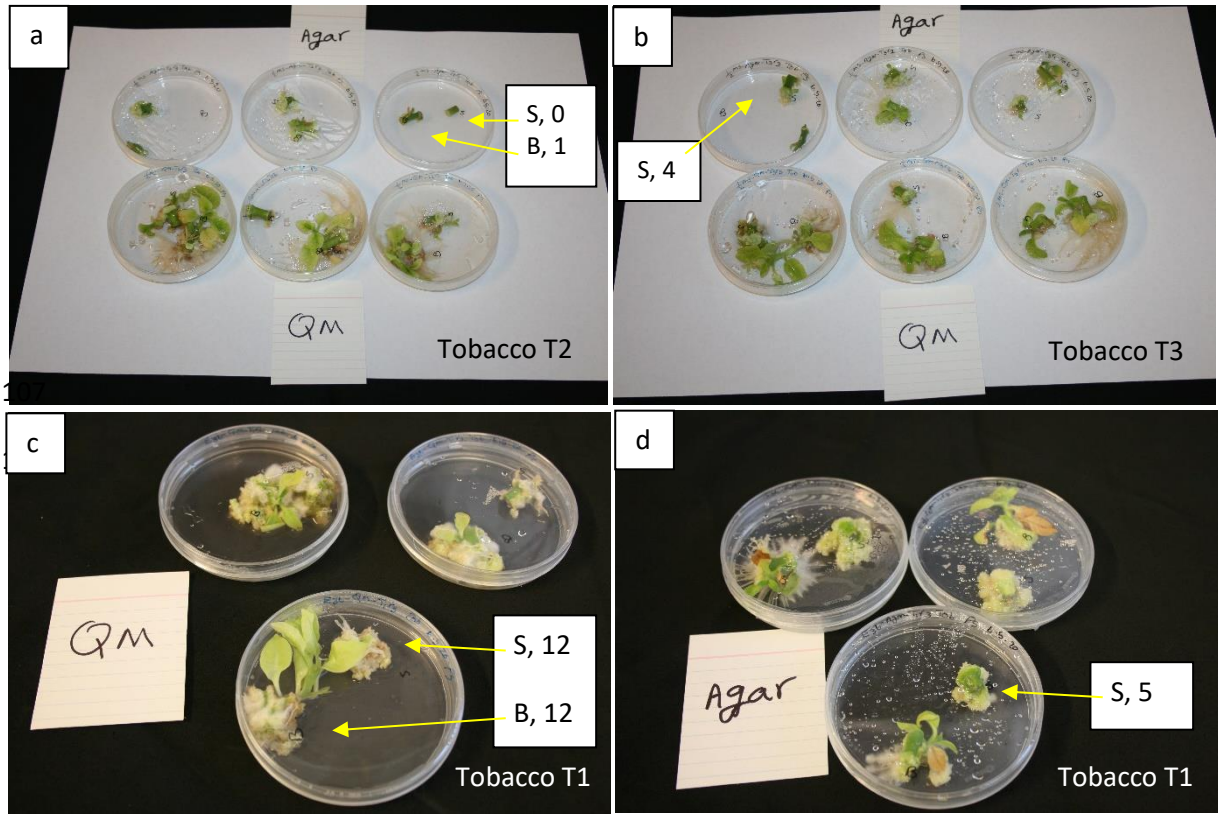

Fig. S3 Examples of the scale of callus quantification (0-12). Letters S and B represent Stem section and Bud explants, respectively and the numbers indicate the quantified callus level. The photos are taken on 30th day after culture, which is same the day that data was recorded. The designations T1, T2 and T3 show the hormone treatment (T1: NAA 10 µM + BAP 0.4 µM; T2: NAA 2 µM + BAP 2 µM; T3: NAA 0.4 µM + BAP 10 µM).

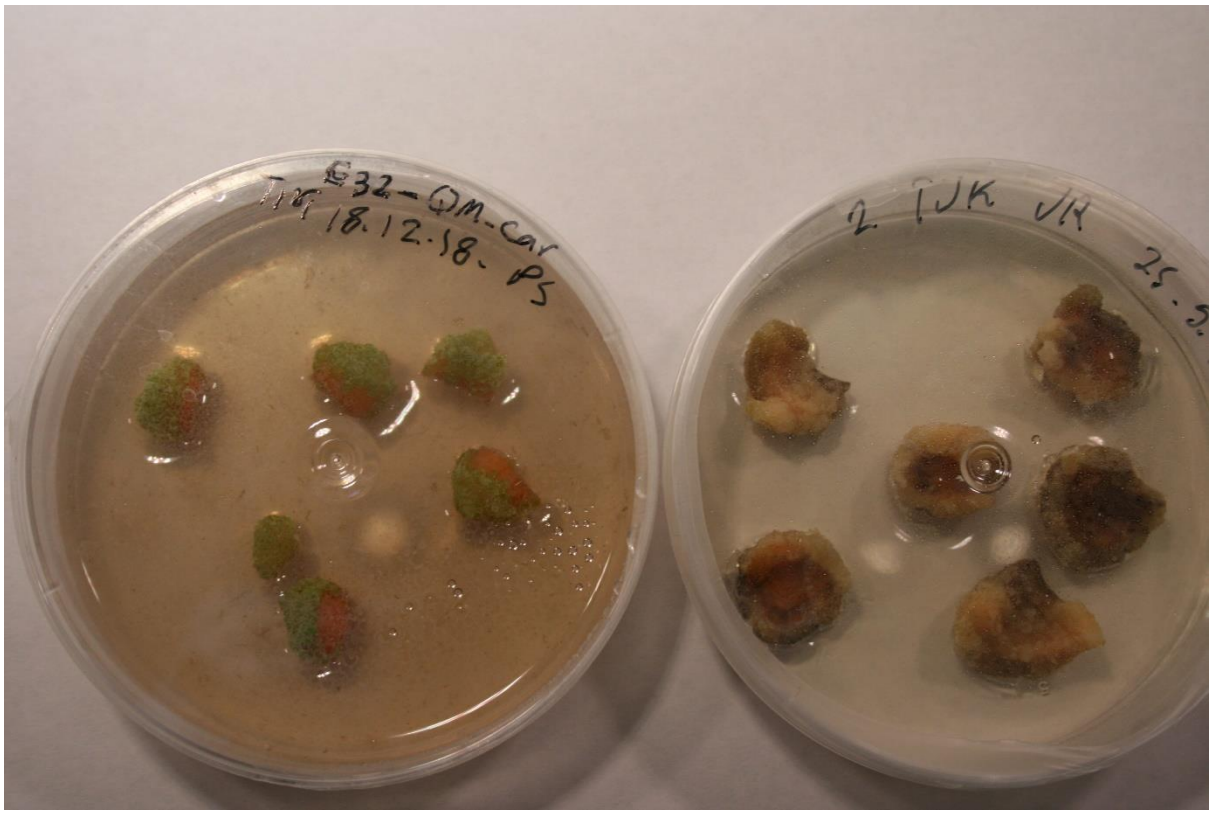

Fig. S4 Carrot explants from preliminary experiments on QM (left) and agar-based medium (right).

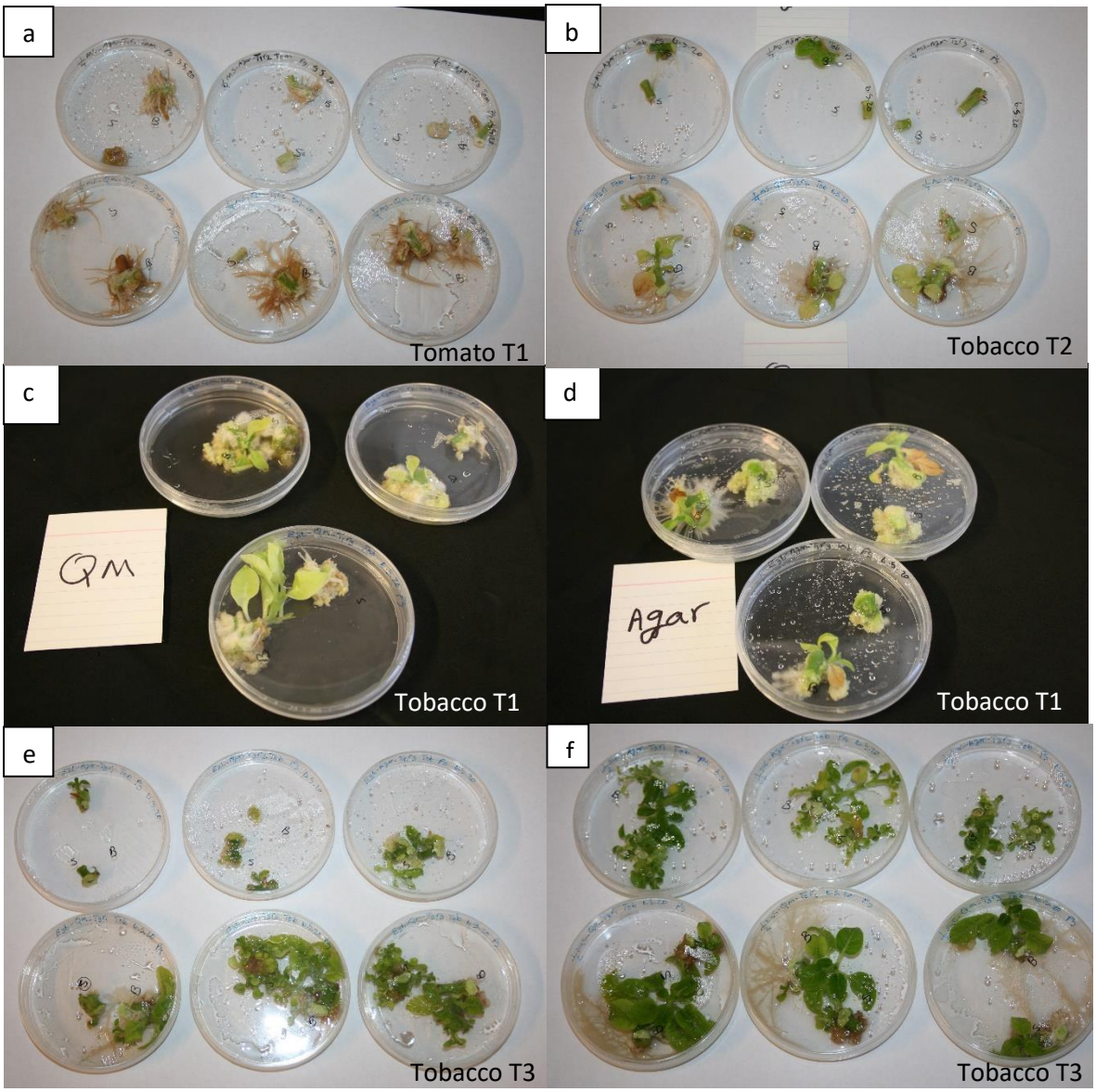

Fig. S5 Examples of the culture plates. (a) and (b), root proliferation on agar (upper row) and QM (lower row); (c) and (d), callus production on QM and agar, respectively; (e) shoot proliferation on agar (upper row) and QM (lower row); (f) proliferation of leaves on agar (upper row) and QM (lower row). The photos are taken on 30th day after culture, which is same the day that data was recorded. The designations T1, T2 and T3 show the hormone treatment (T1: NAA 10 µM + BAP 0.4 µM; T2: NAA 2 µM + BAP 2 µM; T3: NAA 0.4 µM + BAP 10 µM).

Residual Diagnostics for the statistical models

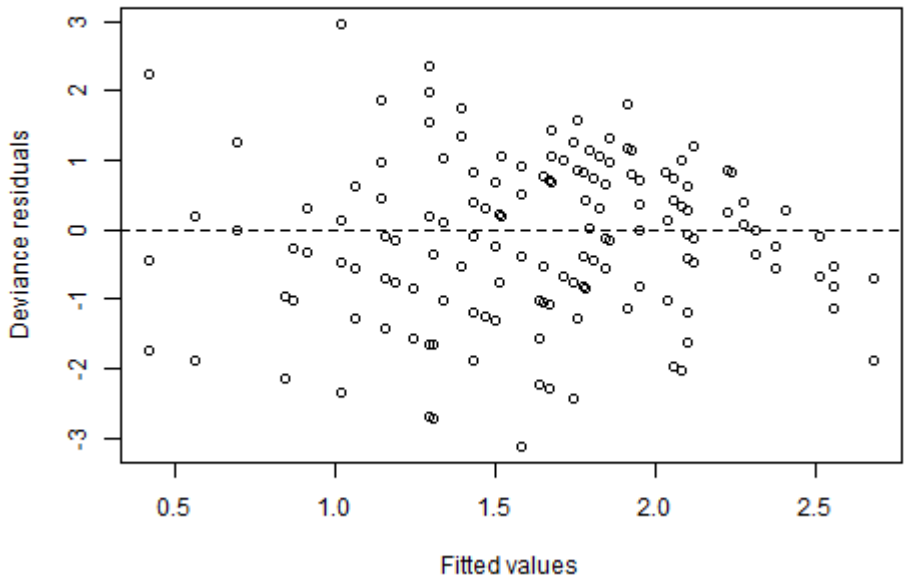

Fig. S6 Residuals of the generalized linear model for callus proliferation in tobacco as a function of the fitted values. The model, described by Eq. 1, assumed a Poisson distribution for the response variable. No clear outliers can be detected in the plot

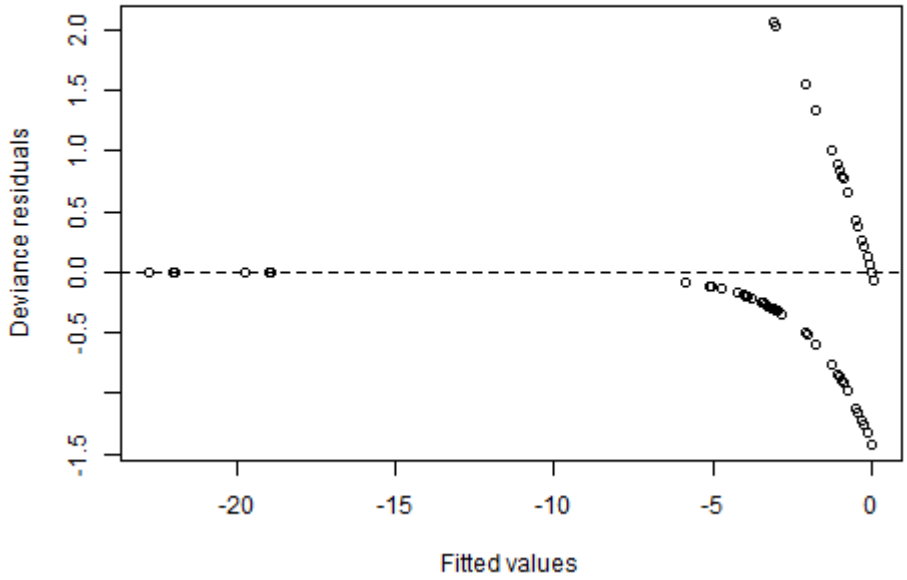

Fig. S7 Residuals of the generalized linear model for shoot proliferation in tomato as a function of the fitted values. The model, described by Eq. 1, assumed a Poisson distribution for the response variable. No clear outliers can be detected in the plot

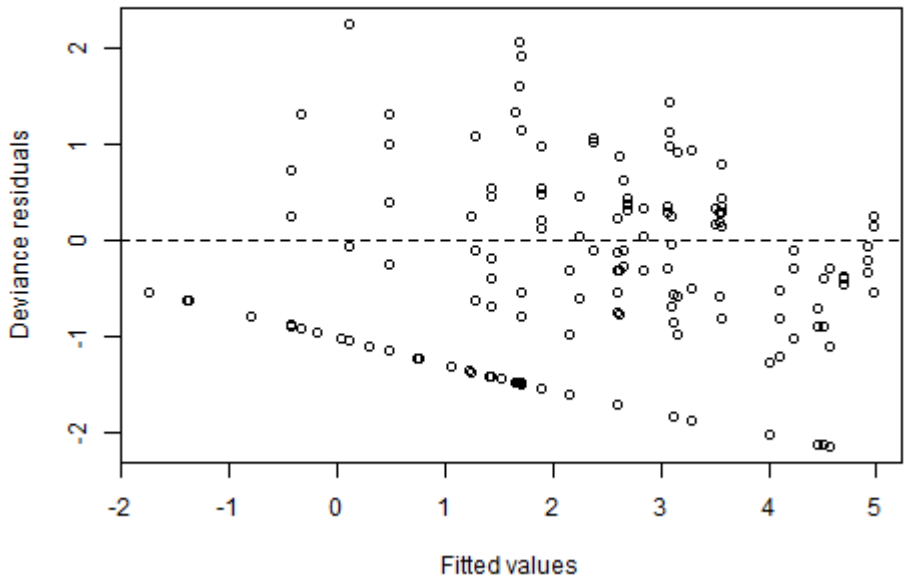

Fig. S8 Residuals of the generalized linear model for root proliferation in tobacco as a function of the fitted values. The model, described by Eq. 1, assumed a negative binomial distribution for the response variable. No clear outliers can be detected in the plot

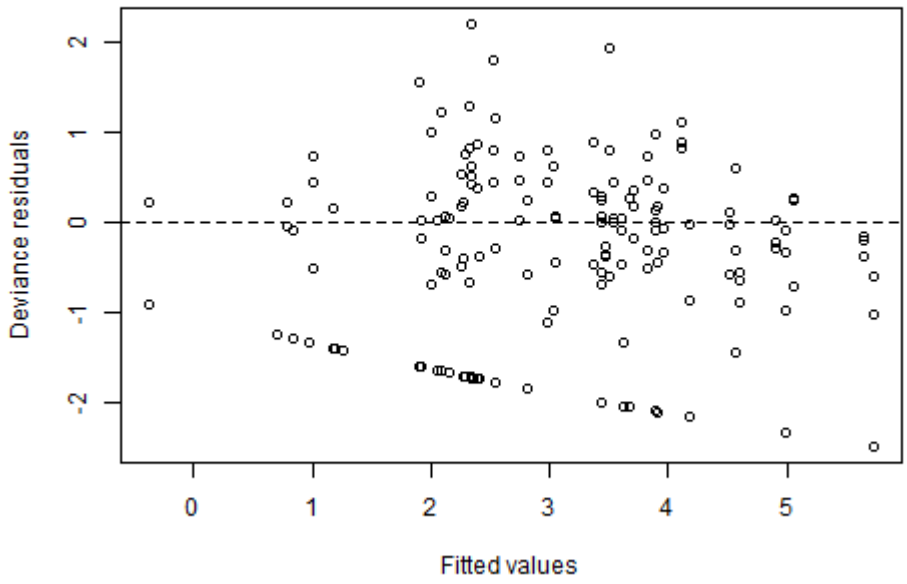

Fig. S9 Residuals of the generalized linear model for root proliferation in tomato as a function of the fitted values. The model, described by Eq. 1, assumed a negative binomial distribution for the response variable. No clear outliers can be detected in the plot

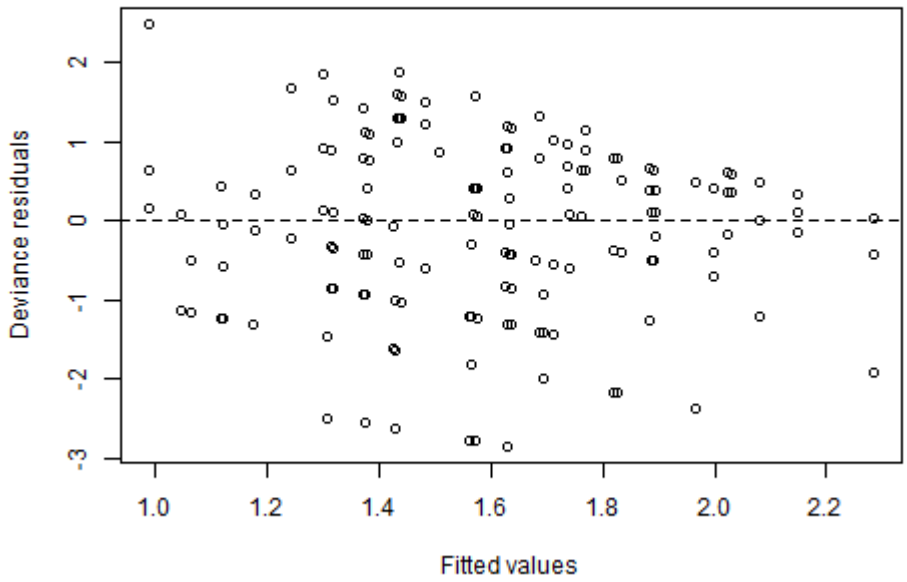

Fig. S10 Residuals of the generalized linear model for callus proliferation in tomato as a function of the fitted values. The model, described by Eq. 1, assumed a negative binomial distribution for the response variable. No clear outliers can be detected in the plot

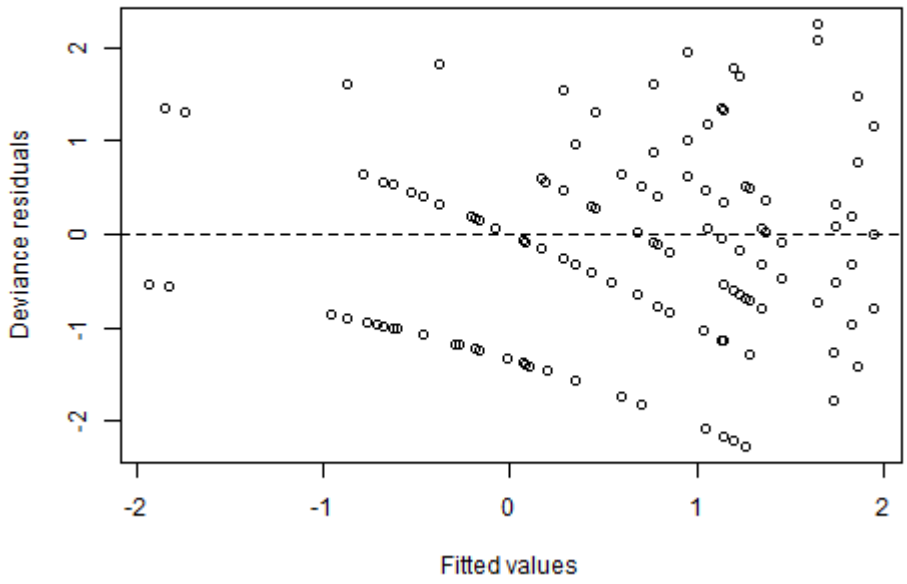

Fig. S11 Residuals of the generalized linear model for shoot proliferation in tobacco as a function of the fitted values. The model, described by Eq. 1, assumed a negative binomial distribution for the response variable. No clear outliers can be detected in the plot

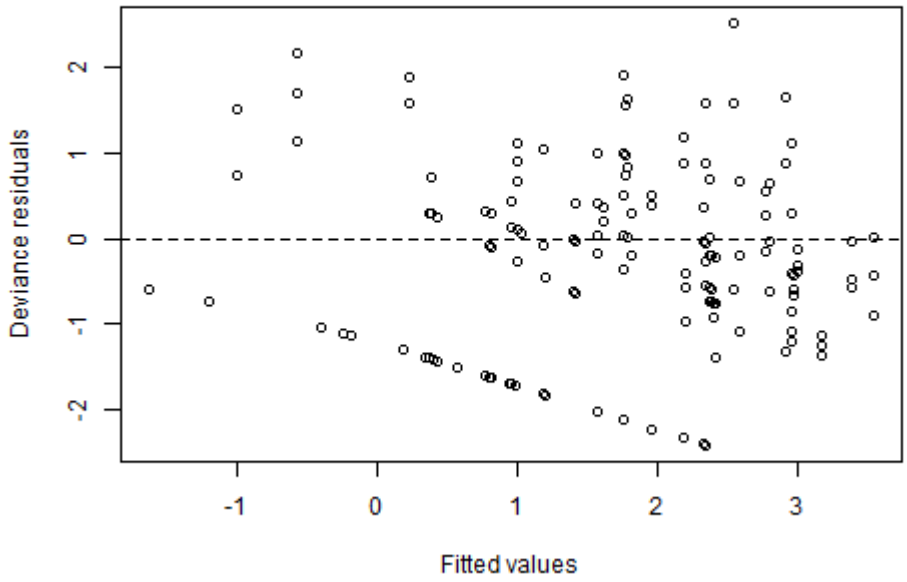

Fig. S12 Residuals of the generalized linear model for leaf proliferation in tobacco as a function of the fitted values. The model, described by Eq. 1, assumed a negative binomial distribution for the response variable. No clear outliers can be detected in the plot

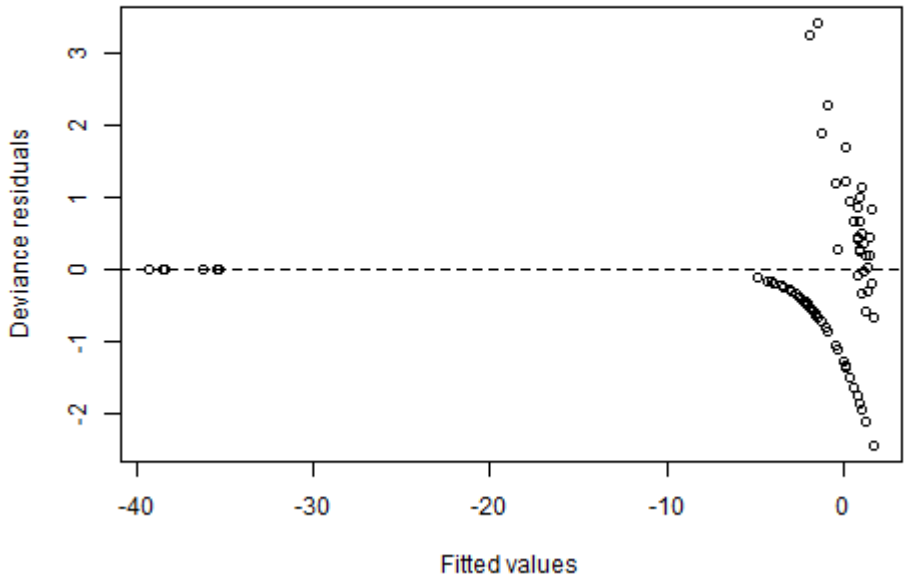

Fig. S13 Residuals of the generalized linear model for leaf proliferation in tomato as a function of the fitted values. The model, described by Eq. 1, assumed a negative binomial distribution for the response variable. No clear outliers can be detected in the plot

Overdispersion under the assumption of Poisson-distributed responses

As an alternative to the negative binomial model, we also tried fitting Poisson log-linear models to the data. For such models it is standard to conduct a test of overdispersion which we implemented with the function *dispersiontest* in the R-package AER. For tobacco, the p-values of the test (null hypothesis = there is no overdispersion in the data) were <0.0001, 0.0138, <.0.0001 and 0.2671 for Roots, Shoots, Leaves and Calluses, respectively. For tomato, the p-values were <0.0001, 1.0000, 0.02391 and 0.0001 in the same order. Thus, out of the eight models, a total of six exhibited overdispersion (at the significance level 0.05), causing us to conclude that Poisson log-linear models are not, in general, a good way to model the current data.

Interestingly, the two models for which overdispersion was not detected are the same ones for which the negative binomial models failed to converge and for which we actually resorted to the Poisson log-linear models in our analysis (see the final paragraph of Section 2.5 on statistical methodology). Thus, the previous tests for overdispersion actually go to validate our modelling choice. Additionally, we also conjecture that the convergence issues were actually *caused* by the lack of overdispersion. However, we could not verify this conjecture.