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

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Abstract

In order to reduce the expenses of plant tissue culture for plant industry, cheaper materials and/or equipment would be welcome especially in developing countries. In the present study, we tested mucilage extracted from seeds of quince (*Cydonia oblonga*) as alternative for agar in tissue culture. The seeds are currently disposed of as waste products of fruit industry. Tomato and tobacco lateral buds and stems were used as explants, and callus formation and the number of shoots, roots, and leaves were measured after thirty days of in vitro culture. The performance of quince mucilage based medium (QM) in comparison to agar was studied, varying the amounts of hormones, salts and sucrose in the medium. Statistical analyses, based on negative binomial regression, show that QM improved the production of callus, shoots, roots and leaves from tobacco explants. In tomato, results from QM and agar were similar to each other except with root production in which QM excelled. The results suggest that QM has potential for use as a gelling agent in plant tissue culture.

Keywords Agar, Jellifying agent, Mucilage, Plant tissue culture, Quince seeds

1 Introduction

The present increase of world population sets a demand for more efficient ways to produce food. Plant tissue culture can offer improvement in plant production by allowing rapid multiplication of virus-free individuals of elite varieties [1]. Plant tissue culture has a wide range of applications in improving the propagation of vegetables, eradication of diseases, and salinity resilience [2, 3], to name a few. Moreover, plant/cell tissue culture with the assets of not being dependent on climate and geographical conditions, can aid the extended production of food, as well as feed, fiber, and green energy [4]. Alongside the advantages, the high cost of chemicals and tools is a drawback of tissue culture techniques especially in large-scale production [5]. The need for cost reduction in commercial production is particularly important in developing and poor countries [6]. However, the reduction of production costs and efficient usage of resources should be achieved without lowering the quality of the production [7].

Solid or viscous medium is required in tissue culture, as explants may suffocate if placed directly in a liquid [8], and jellifying agents directly affect the costs. Agar, a



polysaccharide structured by repetition of D-galactose and 3,6-anhydro-L-galactose units, extracted from red algae like *Gelidium*, *Gracillaria* or *Pterocladia* [9], is the most common gelling agent used for this purpose [10]. The chemical costs of commercial tissue culture are dominated by the cost of agar, 77.8% [2, 11], and over-utilization of agar resources, also affecting its price, has raised the question about alternatives [8, 12, 13]. To address the high costs of gelling agents, cotton fiber [14], guar gum [12], xanthan gum [15], polyurethane foam disc, chopped coconut coir, betel nut, leaf litter [13], cassava flour, potato, and corn starch [16, 17] have been tested as alternative gelling agents/supporting materials. Moreover, several varieties of plant-based mucilage have been tested as alternatives for agar in plant tissue culture. The mucilage extracted from the seeds of Isubgol (psyllium) was tested on Java plum (*Syzygium cumini*) and Prickly-burr (*Datura innoxia*) [18]. (Isubgol) with low cost sugar source instead of sucrose were tested for turmeric tissue culture and reported to lead to 73% cost reduction [19]. Gum Katira, mucilage extracted from the bark of silk-cotton tree, was successfully used for tissue culture of Java plum and Indian siris [20]. (Isobgul) and psyllium husk were also successfully used as cheap gelling agents for in vitro culture of *Chroococcus limneticus* and *Scenedesmus quadricauda* [21]. Tragacanth gum extracted from the root sap of the tragacanth plant was successfully used as a gelling agent for in vitro culture of carnation and miniature rose [5]. Three different starches derived from potato, barley, and corn were tested as gelling agents instead of agar in tissue culture of potato [22]. Alternatives to the biologically inert agar need to be evaluated for the presence of compounds that might inhibit organogenesis or growth or adversely affect the availability of ions or growth regulators.

Numerous studies have been conducted to optimize the concentrations of Murashige and Skoog (MS; a common growth media for plant tissue culture) salts, hormones and vitamins for in vitro cultivation of different species. Full strength MS medium is usually applied for callus generation in both tobacco [23] and tomato [24, 25] whereas half-strength MS medium is better for rooting in these species [26–28]. In a recent study, the effect of MS concentration was studied together with the media pH, type of sugar (sucrose, glucose, and fructose) together with the types of explants from an endangered subspecies of *Dianthus*. The results showed that the type of explant and MS concentrations affect rooting [29]. Therefore, multiple plants species, explants and culture conditions must be tested; here, we used two plant species (tomato and tobacco) and performed all the experiments with two types of explants (buds and shoots) in a number of different compositions of the MS salts, hormones and sucrose. Quince (*Cydonia oblonga*; Rosaceae) is known as one of the earliest fruit species used by human beings [30]. Production of the quince fruit has increased within the period of 1990–2021, from 344,392 to 697562.6 tons [31]. Quince fruits are used for jam, and the mucilage extracted from the seeds has been used in folk medicine in Middle East and Asia [32]. Quince seeds release a viscous mucilage once exposed to water. Early research on quince seed mucilage showed methylated and unmethylated aldobionic acids, arabinose, and a cellulosic fraction. In a follow-up study, xylose was found from hydrolysis of aldobionic acids [33]. More recent studies reported more polysaccharides including mannose, rhamnose, glucose, galactose, and d-xylose [34]. Quince fruits have been shown to contain malic acid, sugars, tannins, vitamin C, pectin, minerals such as potassium, sodium, calcium, and phosphorus, phenolic acids and flavonoids [35–37], and ascorbic, citric, malic,

fumaric, L-shikimic, D-(-)-quinic acids are traceable in quince seeds [38]. Quince leaves have anti-diarrheic, antitussive and sedative characteristics [39]. Antiproliferative, anti-oxidant, and antimicrobial characteristics of quince fruits have been studied for wound healing [40] and proliferation of dermal fibroblasts and skin inflammatory properties [41, 42]. Quince seeds, fruit and peel, and leaves have been studied for their medicinal and pharmaceutical aspects [43]. Antimicrobial aspects of quince seeds and peel have been studied for food industry [44]. Generally, mucilage usually consists of a gelatinous, that is to say semisolid, substance. Many mature seeds produce a hydrogel also known as mucilage when they are exposed to water [45].

Quince seeds are a plentiful and presently free raw material, as the seeds are thrown away as a waste of quince jam and wine industries. The mucilage extracted from quince seeds contains flavonoids, phenolic acid and antioxidants [46]. The amount of seeds per fruit is estimated to be 5% of the fruit weight [36]. Different studies show effect of different variables on viscosity of the mucilage. For instance, if the extraction temperature, water to seed ratio and extraction time increase, the viscosity decreases although the higher the pH, the higher viscosity in quince seed mucilage [46]. The present study investigates the possibility of using the mucilage extracted from quince seeds as a cheap alternative for agar in plant tissue culture.

2 Materials and methods

2.1 Preparation of quince medium (QM)

Quince seeds were obtained from a local shop in Isfahan, Iran. 31.25 g of quince seeds were soaked in 1 L of ultrapure water at room temperature and mixed with a rotating propeller for 24 h. Mucilage was then separated from the big particles of the mixture using a combination of a Buchner funnel and 3D printed filters (Prusa i3 MK3, with 1.75 mm polylactic acid). The filters were designed with the diameter of 91 mm, the height of 30 mm, and a mesh, consisting of five net layers, with a total pore size of 1 mm, and 3D printed at the laboratories of University of Turku (Supplementary Figs. S1 and S2; the CAD file can also be found in the Supplementary Material File 1). A vacuum pump, VacuuBrand MD1 with a vacuum of 1.5 mbar, was used for this part of mucilage extraction. Remaining seed residue was removed by pumping (the same pump was used to create the vacuum) the mucilage through a VitraPOR® glass filter with porosity of one.

2.2 Explant preparation and culturing

Tomato (*Solanum lycopersicum* cv. Marmande) and tobacco (*Nicotiana tabacum* cv. Samsun) were grown in a research greenhouse, and lateral buds or 0.5–1 cm long stem segments, as indicated, were used as explants. One lateral bud and one stem segment were cultivated on each Petri dish. For surface sterilization, the explants were soaked in 1% bleach with a few drops of Triton X-100 for 25 min, rinsed three times with sterile water and kept in sterile water until transfer to the medium. No sub-culturing was done. The cultures were kept at 25 °C with photosynthetic photon flux density (PPFD) 30 $\mu\text{mol m}^{-2} \text{s}^{-1}$. The growth chamber (Weiss Gallenkamp) was set on 16 h/8 h day/night rhythm.

2.3 Preparation of culture media

All media were prepared with the Murashige and Skoog Basal Salt Mixture [47], (MS), from Sigma Aldrich, supplied with D (+)-sucrose from VWR Chemicals and 1-naphtale-neacetic (NAA) acid and 6-benzylaminopurine (BAP) from Sigma Aldrich. Either QM or agar (Merck, Germany) was used as a solidifying agent, as indicated. All the media were supplemented with the following vitamins: 0.5 mg L⁻¹ nicotinic acid, 0.5 mg L⁻¹ pyridoxine-HCl, 0.1 mg L⁻¹ thiamine-HCl, 2 mg L⁻¹ glycine, and 100 mg L⁻¹ myo-inositol. Full strength MS (4.3 g L⁻¹), half strength MS, one-fourth strength MS, full strength sucrose (30 g L⁻¹), half strength sucrose, or one-fourth strength sucrose were used, as indicated. Three sets of hormone concentrations were used:

T1: NAA 10 μM + BAP 0.4 μM;

T2: NAA 2 μM + BAP 2 μM;

T3: NAA 0.4 μM + BAP 10 μM.

After adding agar 7 g L⁻¹ (only to agar plates), media were autoclaved at 121 °C and 147.1 kPa for 20 min.

Each combination of species, solidifying agent, MS concentration, sucrose concentration and hormone concentration set was repeated on three Petri dishes.

2.4 Numerical data

After 30 days of culturing, the numbers of roots, shoots, and leaves were counted. Callus was empirically quantified with the levels 0–12, where 0 indicates no callus and 12 indicates the largest callus proliferation on one plate within the whole set of experiments (see Fig. S3 for examples of the quantification).

2.5 Statistical methods

To model the relationship between the outcome variables (the numbers of roots, shoots and leaves and the callus level) and the experimental conditions, we resorted to generalized linear models with the assumption that the responses can be modelled with the negative binomial distribution [48]. These models are functionally equivalent to the standard log-linear models typically associated with count responses, but the use of the negative binomial distribution (instead of Poisson distribution) allows for the presence of over-dispersion in the data; in biology, the negative binomial distribution is widely used in modeling RNA sequencing results (e.g., [49]). Furthermore, preliminary analyses with our data revealed that they exhibited rather strong over-dispersion (see Part 3 in Supplemental Material for more details). Even though the callus level is not a count variable but rather a discretization of a count variable, it is, nevertheless, non-negative, has ordered, roughly equi-spaced levels and attains a large enough range of values, thus remaining modellable with negative binomial regression. Now, given, e.g., the observed count of roots $Y_{i,roots}$ of the i^{th} observation, we fit the following model, separately for tobacco and tomato,

$$\log E(Y_{i,roots} | X_i) = \beta_0 + \beta_1 X_{i,gell.} + \beta_2 X_{i,supp.} + \beta_3 X_{i,horm.} + \beta_4 X_{i,explant} + \gamma_{12} X_{i,gell.} X_{i,supp.} \quad (1)$$

where *gell.* refers to the gelling agent (QM or agar), *supp.* to the supplement, i.e., combination of MS strength and sucrose concentration, and *horm.* to the hormone combination. $X_{i,explant}$ is the type of explant (lateral buds or stem segments). The model includes the main effects for each of the covariates and, additionally, the pair-wise interaction

Table 1 Comparison of QM and Agar supplemented with different levels of MS and sucrose for root proliferation in tobacco and tomato

Tobacco				Tomato			
Supplement	Effect	p-value	Significance	Supplement	Effect	p-value	Significance
Full MS and suc	4.36	0.0281	*	Full MS and suc	5.85	0.0015	**
Half MS	2.62	0.2829		Half MS	3.88	0.0281	*
1/4 MS	23.55	0	***	1/4 MS	4.8	0.0105	*
Half suc	20.39	0	***	Half suc	1.47	0.9385	
1/4 suc	5.34	0.016	*	1/4 suc	1.14	0.9996	

The "effect" column gives the average multiplicative increase in the number of roots for QM vs. Agar. The level of significance is shown as follows: dot, $p < 0.1$; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$. See supplemental Tables S1 and S2 for full compilations of the coefficients of the generalized linear models for tobacco and tomato, respectively, and supplemental figs. S5 and S6 for the plots of the error residuals as functions of fitted values for the tobacco and tomato model, respectively. The models, described by Eq. 1, assumed a negative binomial distribution for the response variable

Table 2 Comparison of QM and agar supplemented with different levels of MS and sucrose for callus proliferation in tobacco and tomato

Tobacco				Tomato			
Supplement	Effect	p-value	Significance	Supplement	Effect	p-value	Significance
Full MS and suc	1.45	0.0107	*	Full MS and suc	0.87	0.9734	
Half MS	1.68	0.0006	***	Half MS	1.22	0.8355	
1/4 MS	2.29	0	***	1/4 MS	1.23	0.7419	
Half suc	1.15	0.7929		Half suc	0.77	0.5442	
1/4 suc	0.96	0.9996		1/4 suc	0.7	0.2767	

Callus proliferation was quantified with levels 0–12. The "effect" column shows the average ratio of callus proliferation in QM versus agar. The level of significance is shown as follows: dot, $p < 0.1$; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$. See supplemental tables S3 and S4 for full compilations of the coefficients of the generalized linear models for tobacco and tomato, respectively, and supplemental figs. S3 and S7 for the plots of the error residuals as functions of fitted values for the tobacco and tomato model, respectively. The models are described by Eq. 1. The tobacco model assumed a Poisson distribution and the tomato model a negative binomial distribution for the response variable

between the gelling agent and supplement. For each categorical explanatory variable with classes, the model has $k-1$ parameters, and as we have 2 gelling agents, 5 supplement combinations, 3 hormone treatments and 2 explant types, the number of parameters (as calculated from Eq. 1) is 13, including the intercept term. Taking into account the shape parameter of the negative binomial distribution, the total number of parameters is 14. This particular model was chosen as it is at the same time parsimonious but rich enough to allow for a straightforward study of our main research question of whether QM can be used as an alternative for agar as a medium and how this depends on the used supplement. As a comparison, in some sense the next reasonable model candidate, i.e., the one with all pair-wise interactions, would include a total of 30 parameters to estimate, which would reduce statistical power and make the practical interpretation of the effects very complicated.

We fitted the model separately for all eight combinations of the outcome variable and plant species (tobacco or tomato) and the estimated regression coefficients along with the corresponding p -values are given in the Supplementary Material (Tables S1–S8). To answer the main research question, we estimated the effects of using QM vs. agar in combination with each of the supplements using contrasts, and the results are given in Tables 1, 2, 3 and 4. To interpret these, recall that the presence of the logarithmic link function in the model specification means that any additive change in the covariates leads to multiplicative effect in the expected count. Accordingly, the Effect columns in Tables 1, 2, 3 and 4 contain the exponentiated estimates of the contrasts, which thus directly correspond to the expected multiplicative increases in counts for QM vs. agar.

Table 3 Comparison of QM and agar supplemented with different levels of MS and sucrose for shoot proliferation in tobacco and tomato

Tobacco				Tomato			
Supplement	Effect	<i>p</i> -value	Significance	Supplement	Effect	<i>p</i> -value	Significance
Full MS and suc	3.17	0.0007	***	Full MS and suc	1.4	0.9845	
Half MS	3.9	0.0049	**	Half MS	3	0.8761	
1/4 MS	3.38	0.0256	*	1/4 MS	1	1	
Half suc	0.5	0.0738	.	Half suc	0.88	0.9997	
1/4 suc	1.32	0.8365		1/4 suc	1	1	

The "effect" column indicates the average ratio of the number of shoots produced on QM to the number produced on agar. The level of significance is shown as follows: dot, $p < 0.1$; *, $p < 0.05$; **, $p < 0.01$; and ***, $p < 0.001$. See Supplemental Tables S5 and S6 for full compilations of the coefficients of the generalized linear models for tobacco and tomato, respectively, and Supplemental Figs. S4 and S8 for the plots of the error residuals as functions of fitted values for the tobacco and tomato model, respectively. The models are described by Eq. 1. The tobacco model assumed a negative binomial distribution and the tomato model a Poisson distribution for the response variable

Table 4 Comparison of QM and Agar supplemented with different levels of MS and sucrose for leaf proliferation in tobacco and tomato

Tobacco				Tomato			
Supplement	Effect	<i>p</i> -value	Significance	Supplement	Effect	<i>p</i> -value	Significance
Full MS and suc	3.83	0.0003	***	Full MS and suc	1.26	0.9841	
Half MS	4.81	0.0001	***	Half MS	1.74	0.9614	
1/4 MS	4.22	0.0018	**	1/4 MS	1	1	
Half suc	0.66	0.644		Half suc	0.84	0.9933	
1/4 suc	1.19	0.9884		1/4 suc	0.9	0.9996	

The "effect" column gives the average multiplicative increase in the number of leaves for QM vs. Agar. The level of significance is shown as follows: dot, $p < 0.1$; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$. See Supplemental Tables S7 and S8 for full compilations of the coefficients of the generalized linear models for tobacco and tomato, respectively, and Supplemental Figs. S9 and S10 for the plots of the error residuals as functions of fitted values for the tobacco and tomato model, respectively. Both models, described by Eq. 1, assumed a negative binomial distribution for the response variable

For example, based on the third row of Table 3, when using ¼ MS as an additive, QM produces on average 3.38 times as many shoots as agar in tobacco, and this effect is statistically significant at significance level of 0.05 (the *p*-value is approximately 0.03).

The negative binomial models for callus levels in tobacco and for shoots in tomato failed to converge and were replaced with the Poisson log-linear model. Based on the residual plots (see Figs. S6 and S7 in the Supplementary Material), this simpler and more restrictive model fits the data well in both cases. See also the discussion of over-dispersion in Part 3 of the Supplementary Material.

3 Results

The suitability of quince mucilage as a solidifying agent in plant tissue culture was studied with tomato and tobacco bud and shoot explants by comparing root, callus, shoot and leaf proliferation during 30 days of culturing (without sub-cultures) on either QM or agar-based media. Three hormone combination sets, three concentrations of sucrose and three concentrations of MS salt mixture were tested with both types of solidifying agents. The description of the results will focus on the comparison between QM and agar with different concentrations of MS salts and sucrose.

The QM, prepared simply by using the same seed mass per volume ratio every time, was viscous rather than solid in comparison to agar, but explants stayed on the surface of the medium (Fig. S4).

3.1 Root proliferation

The average root proliferation was higher in QM than on agar in both tobacco and tomato in most experimental treatments (Fig. 1; see Fig. S5 for photographs), and the differences were statistically significant ($p < 0.01$) for tobacco in $\frac{1}{4}$ MS and $\frac{1}{2}$ sucrose and for tomato in full MS (Table 1). In both tomato and tobacco explants, hormone concentration sets T1 (auxin > cytokinin) and T2 (balanced concentrations of auxin and cytokinin) were equally efficient whereas T3 (auxin < cytokinin) yielded significantly fewer roots than T1 (Tables S1 and S2). Similarly, less roots were produced from stem than from bud explants of both species (Tables S1 and S2). Sucrose concentration did not have consistent effects on root production (Tables S1 and S2) but, compared to agar, reducing sucrose concentration in QM had a negative impact on root production in tomato (Fig. 1B; Table S2). The concentrations of mineral nutrients did not have consistent interaction with the gelling agent in root production (Tables S1 and S2).

3.2 Callus proliferation

A balanced auxin/cytokinin ratio (T2; NAA 2 μ M + BAP 2 μ M) was chosen for the evaluation of callus proliferation, and the results show, in general, similar callus proliferation on both QM and agar for both species (Fig. 2; see Fig. S4 for photographs). In tobacco, callus proliferation showed a decreasing trend with reduction of concentrations of both mineral nutrients (MS) and sucrose (Fig. 2A; Table S3). In the case of decreasing mineral nutrients, the decline in callus proliferation was milder on QM than on agar (Fig. 2; Table 2; Tables S3 and S4), but a negative interaction effect of the combination of QM and the lowest sugar concentration, reflecting the stronger decrease in callus formation in $\frac{1}{4}$ sucrose, compared to the media with full MS and sucrose, in QM than in agar (Fig. 2A), was found for tobacco (Table S3). Stem explants produced somewhat more callus than bud explants in both species (Tables S3 and S4).

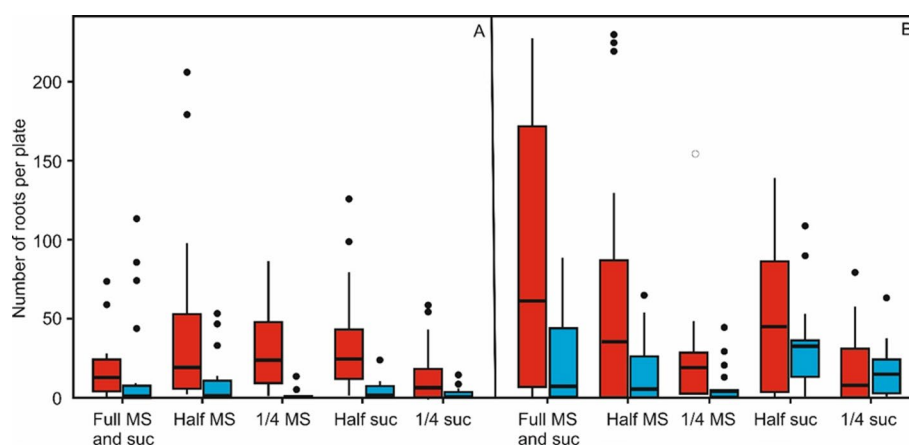


Fig. 1 Root proliferation on media with quince extract (QM; red boxes) or agar (blue boxes) as the gelling agent, in tobacco (A) and tomato (B) with different strengths of MS and sucrose. See Materials and Methods for the definitions of, “full”, “half” and $\frac{1}{4}$ in different media. The half and $\frac{1}{4}$ MS were supplemented with full strength sucrose, and half and $\frac{1}{4}$ sucrose contained full strength MS. The thick line in each box shows the median, the box is drawn between the upper and lower quartiles, the whiskers show the minima and maxima and the separate points show outliers. Results from all hormone concentration sets and both explant types have been pooled by combining the respective datasets. Each boxplot item represents results from nine plates (three plates per each hormone treatment with one stem and one bud explant on each plate)

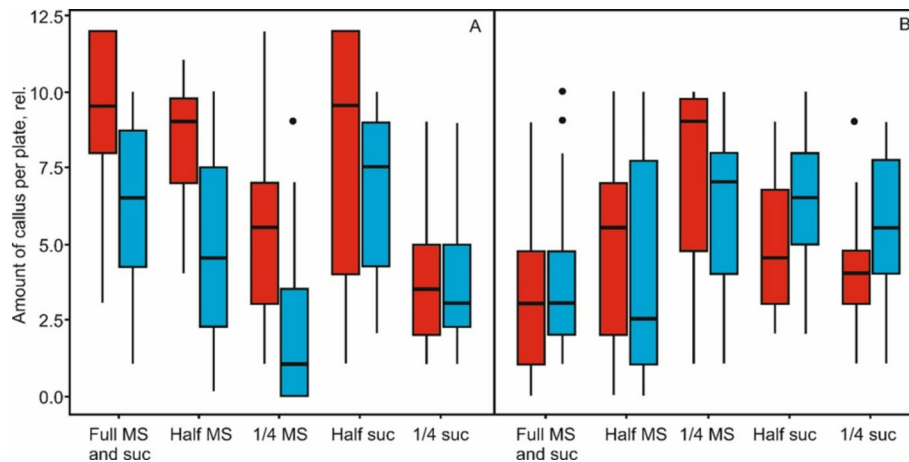


Fig. 2 Callus proliferation on QM (red boxes) and agar (blue boxes) from tobacco (A) and tomato (B) explants with different strengths of MS and sucrose. See Materials and Methods for the definitions of “full”, “half” and $\frac{1}{4}$ in different media. The half and $\frac{1}{4}$ MS were supplemented with full strength sucrose, and half and $\frac{1}{4}$ sucrose contained full strength MS. Callus was quantified with levels of 0–12, where zero means no callus and twelve shows the highest amount of callus produced in one plate in the experiment. The thick line in each box shows the median, the box is drawn between the upper and lower quartiles, the whiskers show the minima and maxima and the separate points show outliers. Results from all hormone concentration sets and both explant types have been pooled by combining the respective datasets. Each boxplot item represents results from nine plates (three plates per each hormone treatment with one stem and one bud explant on each plate)

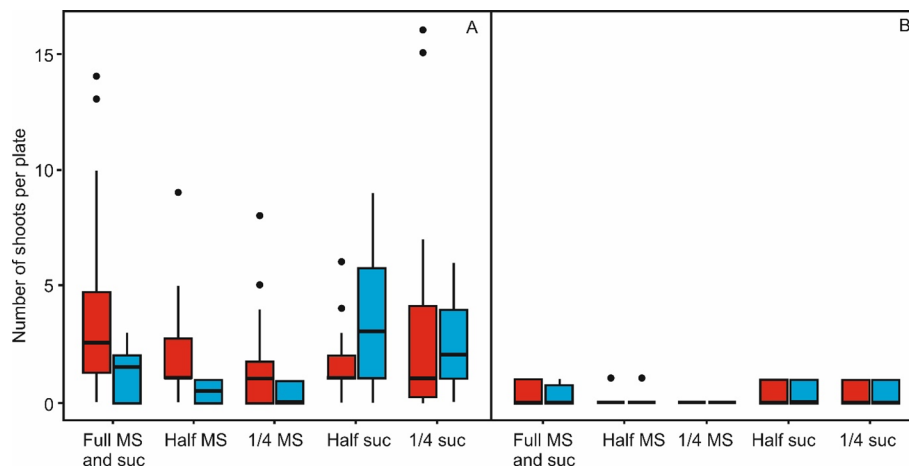


Fig. 3 Shoot proliferation on QM (red boxes) and agar (blue boxes) from tobacco (A) and tomato (B) explants with different strengths of MS and sucrose. See Materials and Methods for the definitions of “full”, “half” and $\frac{1}{4}$ in different media. The half and $\frac{1}{4}$ MS were supplemented with full strength sucrose, and half and $\frac{1}{4}$ sucrose contained full strength MS. The thick line in each box shows the median, the box is drawn between the upper and lower quartiles, the whiskers show the minima and maxima and the separate points show outliers. Results from all hormone concentration sets and both explant types have been pooled by combining the respective datasets. Each boxplot item represents results from nine plates (three plates per each hormone treatment with one stem and one bud explant on each plate)

3.3 Shoot proliferation

Shoot production from tomato explants was similar in QM and agar but with tobacco explants, a significantly larger number of shoots were produced on QM in full MS medium (Fig. 3; Table 3; see Fig. S4 for photographs). However, significant negative effects of the combination of QM and the two lowest sucrose concentrations were noted for tobacco explants (Table S5). Shoot production from tomato explants was weak in half

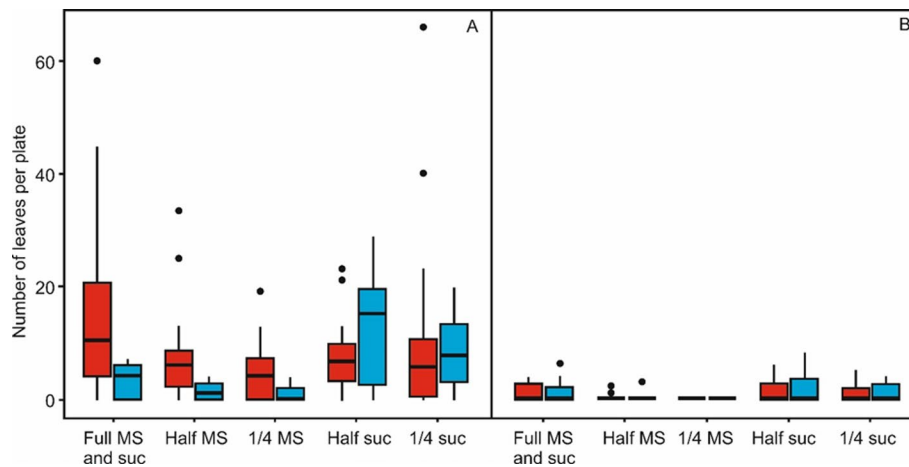


Fig. 4 Leaf proliferation QM (red boxes) and agar (blue boxes) in tobacco (**A**) and tomato (**B**) with different strengths of MS and sucrose. See Materials and Methods for the definitions of “full”, “half” and $\frac{1}{4}$ in different media. The half and $\frac{1}{4}$ MS are supplemented with full strength sucrose, and half and $\frac{1}{4}$ sucrose contain full strength MS. The thick line in each box shows the median, the box is drawn between the upper and lower quartiles, the whiskers show the minima and maxima and the separate points show outliers. Results from all hormone concentration sets and both explant types have been pooled by combining the respective datasets. Each boxplot item represents results from nine plates (three plates per each hormone treatment with one stem and one bud explant on each plate)

or $\frac{1}{4}$ MS, irrespective of the gelling agent (Fig. 3). Hormone concentration sets T2 and T3 (with a higher cytokinin concentration than set T1) led to production of more shoots in tobacco (Table S5), whereas the results from tomato did not show differences between the hormone concentration sets (Table S6). For tobacco, explant type did not matter but for tomato, bud explants produced significantly more shoots (Tables S5 and S6).

3.4 Leaf proliferation

QM had a positive effect, in comparison to agar, for leaf proliferation from tobacco explants in full, half and $\frac{1}{4}$ MS, if the medium was supplemented with full sugar content (Fig. 4; Table 4; see Fig. S4 for photographs). However, when compared to agar, the combination of QM and low sucrose concentration had a negative effect on leaf proliferation in tobacco (Table S7). Less leaves were produced from tomato explants than from tobacco (Fig. 4), and there was no significant difference between QM and agar (Table 4) in tomato. In both species, hormone concentration sets T2 and T3 were significantly more favorable for leaf proliferation than set T1, and bud explants produced more leaves than stem explants (Tables S7 and S8).

Results from stem and bud explants have been combined in Figs. 1, 2, 3 and 4 to get an overview of the behavior of two physiologically different materials, but differences between the explant types were found (Tables S1–S8). Bud explants produced significantly more roots (Tables S1 and S2) and leaves (Tables S7 and S8) whereas stem explants produced more callus (Tables S3 and S4); in shoot proliferation, bud explant was more efficient in tomato only (Tables S5 and S6).

4 Discussion

The present set of experiments was carried out to test the behavior of quince seed mucilage as a gelling agent for plant tissue culture. Agar was used as the control. Cultivation of bud and stem explants of tobacco and tomato was tested. As the growth medium

might affect the availability of mineral nutrients, sucrose or hormones, different compositions of the growth medium were tested, always comparing QM and agar as the gelling agent. In general, production of roots, shoots, leaves and callus was fairly similar with QM and agar (Figs. 1, 2, 3 and 4), but also differences in responses to different growth media were observed.

QM was found to outperform agar in root production especially with tobacco explants (Fig. 1; Table 1). As expected, based on earlier data [50–52], more roots were produced from both tobacco and tomato explants when media with higher auxin/cytokinin ratio were used (Tables S1 and S2). Thus, the better root production on QM than on agar may suggest that NAA is more available from QM than from agar. Furthermore, shikimic acid, a precursor of auxin, is present in quince seeds [38, 43]. If this compound leaks to QM, it might promote root growth by boosting auxin biosynthesis. However, shikimic acid was not measured in the present study. Differences between species and auxin varieties are well known, as indole acetic acid at 0.05 μM is optimal for *Berberis asiatica* [53] whereas 2 mg L⁻¹ of NAA (11 mM) is optimal for blueberry [54]. The finding that sucrose concentration affected root production only in tobacco (Fig. 1) is consistent with the literature showing that sucrose concentration has a species-dependent optimum for root production [55–57]. The better performance of tobacco on QM with low sucrose (in terms of root proliferation) but a negative interaction between low sucrose concentrations and QM in tomato further highlights the species-dependent sugar optimums and suggests that reaching a species-dependent optimum requires more sucrose on QM than on agar.

Significant interactions between gelling agent and MS concentration (Tables S1 and S2) indicate that mineral nutrients are equally available for root production from QM and from agar medium. Earlier results for *Syzygium alternifolium* show that production of healthy plants with functional roots may not succeed with a low concentration of MS salts [55].

The response of callus production to the auxin/cytokinin ratio varies between plant species [58]; here a balanced hormone ratio performed best (Tables S3 and S4), in line with earlier studies with e.g., Turkish crocus [59]. Callus proliferation was fairly equal on QM and agar (Fig. 2) except that the negative effect of lowering mineral nutrient concentration was counteracted by QM whereas the negative effect of lowering sucrose concentration was enhanced by QM (Table S3). *Albizia procera* explants produced the largest amounts of callus when the medium was supplemented with half MS and 3 $\mu\text{g L}^{-1}$ of BA [51]. In taro (*Colocasia esculentum*) the highest amount of callus was produced on 3% sucrose with micro-agar supplemented with half strength MS medium with TDZ, 2,4-D and L-glutamine [60].

Results from shoot and leaf proliferation were, for obvious reasons, similar with each other (Figs. 3 and 4) and showed the expected trend with stronger shoot/leaf production in lower auxin/cytokinin ratio in both species (Tables S5–S8). QM showed a better shoot/leaf production than agar in tobacco, but the weak shoot/leaf production from tomato explants was independent of the gelling agent (Figs. 3 and 4; Tables 3 and 4). The combination of QM and low sugar concentration had a negative interaction effect on shoot/leaf production in tobacco (Tables S5 and S7). A big difference between tobacco and tomato is not surprising, as different levels of shoot production have been observed even between two species of tobacco [61, 62]. A study on blue honeysuckle reported that

axillary shoot proliferation was improved with $\frac{3}{4}$ strength MS whilst half strength MS led to an increase in apical shoot necrosis [63]. In *Dianthus giganteiformis*, the number of shoots regenerated from terminal bud explants decreased when sucrose was reduced to one third. However, the length of regenerated shoots from shoot cuttings increased at one third of sucrose strength [29]. The same study reported a higher rooting percentage from explants on full strength MS media. Nonetheless, no difference was found in the number of axillary buds or in the number and length of shoots when MS strength was halved [29]. Tests with combinations of different levels of auxin and various strengths of MS medium showed that half strength MS together with IBA produced the highest rooting in Motihari variety of tobacco [27].

QM compares well with results from other alternatives of agar. Cotton fibers outperformed agar in maintenance of *Agrostis* and *Taxus* callus and were equal to agar in shoot formation in *Artemisia* and *Agrostis* [14]. Xanthan gum performed as well as agar in caulogenesis and rhizogenesis of *Albizia lebbeck*, and androgenesis of *Datura innoxia* and somatic embryogenesis of *Calliandra tweedii* occurred equally well on agar and guar gum media [15]. Immature seed germination and plant regeneration of *Cymbidium aloifolium* happened quicker on agar than on media with polyurethane foam, chopped coconut coir, betel nut or leaf litter as supporting material, but plants raised on polyurethane foam discs or coconut coir were healthier than those raised on agar [13]. Corn or rice flour-based media displayed higher shoot regeneration than agar-based media, and cassava flour and potato starch showed better outcomes when a small amount of agar was added to the medium [16]. Corn starch performed equally to agar in shoot production by plum cultures [17], and (Isubgol) performed as well as agar for caulogenesis, rhizogenesis and seed germination of *Syzygium cuminii* and for androgenesis of *Datura innoxia* [18]. In *Curcum longa*, (Isubgol) based media produced less but longer shoots and roots than agar [19]. Gum katira and agar were equally good for shoot and root formation of *Syzygium cuminii* and somatic embryogenesis of *Albizia lebbeck* [20]. A combination of tragacanth gum and agar produced more and longer shoots and leaves from rose explants than agar alone [5]. Potato tissue culture produced more leaves on agar but reached a higher fresh weight on potato starch and a higher dry weight on corn starch than on agar [22].

In our experiments, the effects of hormones and additives to the growth media are shown in Tables S1–S8 in Supplementary Material. Figures S6–S3 show plots of the error residuals versus the fitted values for each of the eight models. Such plots for count data regression models are notoriously difficult to interpret and, for example, the “curves” of points visible in several of the plots are simply artefacts of the discrete nature of the data and not indicative of any lack of fit. Indeed, the current residual plots appear rather as what one would expect from typical examples of well-fitting count data. Looking at the residual magnitudes, a large majority of them is contained within the interval from -3 to 3 , with only few instances outside of it. Thus, based on these diagnostics, we conclude that there is no reason to suspect that the used models would not fit the data.

In conclusion, the data show that QM is a potential low-cost alternative for agar in plant tissue culture. QM has a particularly good potential for root production compared to agar and may therefore be of specific advantage for low-cost production of secondary metabolites where only roots are needed. The negative interactions found between low sucrose concentrations and QM suggest that the sucrose concentrations cannot

be directly transferred to QM from agar-based recipes. The present results only yield a proof of a concept, as only one source of the quince seeds was used, and standardization of the raw material will be needed for establishing QM for plant tissue culture. The present data show that QM is a promising cheap alternative for Agar but more work is needed before using QM can be established as an industrial method.

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1007/s44372-025-00311-3>.

Supplementary Material 1.

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Author contributions

PS and ET conceptualized the work; JV did formal analysis; PS and HM did all experimental work; ET provided resources; ET and KL supervised the work; PS wrote the original draft; all authors reviewed and edited the manuscript.

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Availability of data and materials

The datasets generated during and/or analyses during the current study are available in the Mendeley Data repository, <https://doi.org/10.17632/jzkcvc37g.1>.

Declarations

Ethics approval and consent to participate

The authors declare that they do not have any conflict of interest (financial or non-financial). No human participants and/or animals were involved in this research. Thus, there are no informed consents included. The quince seeds used in the study were bought from a local shop in Isfahan, Iran, and the tomato and tobacco plants were grown from commercial seeds in a research greenhouse owned and managed by the University of Turku. The cultivation of the plants was done according to the standards of the Plant Molecular Biology unit of the Department of Life Technologies of the University of Turku under the supervision of the corresponding author, following the guidelines of the Plant Molecular Biology unit.

Competing interests

The authors declare no competing interests

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