The study on mechanical properties of Phytagel medium

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Abstract. Background: The mechanical properties of plant culture medium such as Phytagel affect plant growth and development. Given that the mechanical properties of Phytagel medium are vital for biomechanical experiments designing, a systematic study on mechanical properties of Phytagel medium with different concentrations were carried out here to better understand the response of plant to mechanical stimulation. Results: Uniaxial compression test was conducted for the mechanical strength and Young’s modulus. The variation of concentrations of media results in different mechanical strength. The linear-regression analysis of the breaking load shows that there is a lack of fit of the linear regression model to the observed data points for all these Phytagel concentrations ($R^2 = 0.9708$). The spline regression model, however, fits well to the Young’s modulus for Phytagel medium data. The rheological measurements from the oscillation tests (frequency sweep from 0.1 to 20 Hz at 1% strain) indicate that increasing the Phytagel concentrations results in a stiffer structure at 0.5%-1.2%. Conclusions: Although the biological effects of Phytagel on plant along with mechanical power are expected to check, this contribution provides a useful reference in biomechanical experiments to choose the best Phytagel concentration for the culture of plants and tissues.

1. Introduction
Physical laws and processes have profoundly impacted plant morphogenesis [1], mechanical stimulus can influence plant architecture by triggering organ initiation and causing alterations in the stem or root growth direction. Plants have evolved various responsive behaviors to adapt to these stimuli [2-4]. In plant biomechanical experiments, the mechanical properties of plant growth medium such as the stiffness, elasticity and surface roughness, influence the root system architecture [5, 6]. Varying amounts of Phytagel have been used to regulate the mechanical strength of plant growth medium [7]. Murashige and Skoog medium (MS) is a plant growth medium used in the laboratories for cultivation of plant cells, tissues and organs. It was originally developed for the in vitro culture of tobacco tissues. Since MS is a mixture of inorganic salts, it is mixed into the Agar or Phytagel as the culture medium to
provide macro elements, trace elements and vitamins for the plant growth [8]. Phytigel concentrations of 0.5%-2.0% in half strength MS medium were commonly used in the research of mechanical response of root behavior [9]. Composition of plant culture medium can change its rheological properties and then the growth behavior of plant living in it. In many cases the rheological character is the determinant of growth medium performance. However, the lack of mechanical properties of Phytigel medium is one of the main barriers in applying quantitative study of the effect of stiffness on root development.

The application of high stability, high transparency Phytigel as a curing agent in plant culture medium has received an upsurge of interest. Research in plant development has advanced greatly by application of gelling agent in plant growth medium [10]. In recent decades, Phytigel has been mainly used in root cultivation because soil particles bound to roots are difficult to remove without injury to the roots [9]. Previously, Phytigel has been used to improve the food quality [11], and it is fabricated from a bacterial substrate and as an agar substitute composed of glucuronic acid, rhamnose and glucose [11]. Phytigel has been considered as green composites and an economical alternative to agar as a gelling agent. Half strength MS medium, sucrose and Phytigel were blended to form an interpenetrating network-like cross-linked complex.

Successful plant tissue culture depends on properties of growth medium, especially the mechanical property. Phytigel belongs to a complex fluid. The accurate measurement on the rheological characteristics of Phytigel growth medium can make the application in plant or tissue culture to be perfect. Therefore, it is essential to study mechanical properties of Phytigel in depth. In this paper, the mechanical properties of different composites of culture medium containing a range of Phytigel concentrations were investigated through tests and comparison. A systematic study of Phytigel were performed using uniaxial compression testing, together with rotational rheometer to provide a better understanding of the effect of Phytigel concentration on the mechanical properties of culture medium. Uniaxial compression test was performed to determine the mechanical strength of Phytigel media. Dynamic mechanical properties of low Phytigel concentration (0.5%-1.2%) were detected by frequency sweep tests. Quantitative measures were preliminarily reported for different concentrations of Phytigel. The results indicate that Phytigel should be regarded as the best regulator for the mechanical property of plant growth medium.

2. Materials and methods

2.1. Phytigel medium preparation

The standard plant growth medium consists of 1/2 MS basal salt (phytotechnology laboratories M519) and 15 g/l sucrose. Phytigel was added to 1/2 MS medium in various ratios from 0.5% to 2.0% w/v of fluid medium, and then they were blended at room temperature on high speed to eliminate any lumps before microwave heating. The pH of all media was adjusted to 5.8 with KOH 1 mM. The melted Phytigel media were sterilized by high pressure steam (1.05 kg/cm, 121.3 °C for 20 minutes). Homogeneous Phytigel media with a series of concentration from 0.5% to 2.0% were poured into the petri dishes (diameter = 9 cm, thickness = 12 mm). The Phytigel specimens were cut to dimensions (length = 20 mm, width = 40 mm and thickness = 12 mm). These rectangle specimens were under the condition of 23 °C and saturation moisture content.
2.2. Uniaxial compression test
Uniaxial compression experiment was conducted using Electron Puls E1000 machine (capacity, 1000 newtons) with Console Bulehill 3 software, and began when the load force touches the sample top area and reached 0.001 N. Loading rate was 1 mm per min and load force was increasing at the beginning, reaching the maximum, then declining. When the load force begins to decline, the test was finished. In this study, various amounts of Phytagel have been used to regulate the stiffness of plant growth medium. Phytagel media were tested from 0.5% to 2.0%. The minimum of three specimens with each Phytagel concentration was tested to obtain average values for Young’s modulus and breaking load. The tensile properties of Phytagel mediums could not be measured due to their low stretch ability.

2.3. Dynamic oscillatory tests
The Phytagel specimens were prepared in the same way as described above. Homogeneous Phytagel media with concentration from 0.5% to 1.2% were poured into the petri dishes (diameter = 9 cm, thickness = 2 mm). A hole puncher with 2 cm diameter was pushed vertically into the Phytagel layer to make round Phytagel. The rheological properties were measured using a rotational rheometer (Haake MARS, Schwerte, Germany) and 20 mm parallel plates. Because Phytagel media with high concentrations exhibited high hardness from uniaxial compression test and rotational rheometer can’t test high-hardness materials, rheological behavior of 0.5%-1.2% Phytagel media were carefully documented to determine complex modulus, elastic modulus and viscous modulus from dynamic oscillatory tests. All rheology measurements were performed at 25 °C. The round sample was placed between the plates and aligned with plates, and the gap was adjusted to 2 mm. A standard loading sequence was used to ensure both samples were subjected to a consistent and controllable loading protocol. The specimens were scanned from low frequency (0.1 Hz) to high frequency (20 Hz). The number of measuring points is 21. The data of frequency sweeps for each sample at different concentration are plotted as storage and loss moduli, viscous modulus and complex viscosity.

2.4. Statistical analysis
Multiple samples with various ratios of Phytagel were used in each type of experiment. Each experiment was repeated independently for three times. The results of all experiments were subjected to statistical analysis. Means and standard deviations were determined and confidence intervals were evaluated. Statistical differences were analyzed by one-way analysis of variance (ANOVA) followed by the Student’s t-test, and P-value ≤ 0.05 was significant.

3. Results and discussion

3.1. Breaking strength
Gelling agent concentrations were varied to study the effect on the breaking strength properties. Their breaking load was analyzed by load-displacement curves (Figure 1). There was no evidence of any kind of physical phase separation based on the visual observation.
Figure 1. Breaking load of Phytagel media with concentration from 0.5%-2.0%

The R² and the slope of the linear regression (black solid line) was calculated from data points in the breaking load.

Load-displacement data indicated that Phytagel can increase the mechanical strength of culture medium. As the Phytagel concentration (x) increased, value of breaking load (y) increased linearly: \( y = 13.726x - 3.494 \) (R-squared value of 97.08%). In terms of breaking load, the highest value was measured for 1.7% concentration, indicating a more brittle structure for concentrations higher than 1.7%. The lowest Phytagel concentration has the lowest value, suggesting a comparatively more ductile structure. These results seem to indicate that the incorporation of Phytagel in 1/2 MS liquid medium was regarded as good mechanical structure, and the best level for designing plant biomechanical experiments is from 0.5% to 1.7%. All tested Phytagel media demonstrated that high concentration of Phytagel enhanced the resistance to fracture and damage tolerance and dosing with water did not affect the breaking force for all samples.

3.2. Young’s modulus

Because the breaking load reflects the maximum force loading on surface of samples when the samples are broken, it was expected that the Young’s modulus would provide more information for the mechanical strength of sample.

Young’s modulus \( E \) describes compression elasticity or the tendency of an object to deform along an axis when compressional forces are applied along that axis. It is defined as the ratio of positive stress and the positive strain of elastic material under stress.

\[
E = \frac{\text{STRESS}}{\text{STRAIN}} = \frac{\sigma}{\varepsilon} = \frac{F/A_0}{\Delta L/L_0} = \frac{FL_0}{A_0\Delta L},
\]

where

\( F/A_0 \): the force per unit area, \( A_0 \): cross-sectional area;
\( E \): Young's modulus; 
\( F \): the force loaded on a sample under compression; 
\( A_0 \): original cross-sectional area through which the force is applied; 
\( \Delta L \): the amount by which the length of the object changes; 
\( L_0 \): original length of the object.

As Table 1 shows, when the percentage of Phytagel increased from 0.5% to 2.0%, Young’s modulus increased from 0.01530 to 0.12251 MPa. With the same medium composition, Phytagel at 1.7% generally tended to result in a higher mechanical strength than others.

| The concentration of Phytagel media | Young’s modulus (MPa) |
|------------------------------------|-----------------------|
| 0.5%                               | 0.01530 ±0.00057      |
| 0.6%                               | 0.02063 ±0.00111      |
| 0.7%                               | 0.02653 ±0.00179      |
| 0.8%                               | 0.04017 ±0.00151      |
| 0.9%                               | 0.05000 ±0.00681      |
| 1.0%                               | 0.04093 ±0.00114      |
| 1.1%                               | 0.04150 ±0.00125      |
| 1.2%                               | 0.04838 ±0.00144      |
| 1.3%                               | 0.05755 ±0.00321      |
| 1.4%                               | 0.06244 ±0.00079      |
| 1.5%                               | 0.07601 ±0.00844      |
| 1.6%                               | 0.08685 ±0.00268      |
| 1.7%                               | 0.12251 ±0.01596      |
| 1.8%                               | 0.10214 ±0.01265      |
| 1.9%                               | 0.09657 ±0.00978      |
| 2.0%                               | 0.08869 ±0.01597      |

3.3. Further modelling
An analysis of repeated measures design was done using the data provided. As a result, Phytagel with different concentration have different influence on the stiffness of the plant culture media with a P-value of 0.0007 (< 0.05). To establish the relationship between Phytagel concentration level and stiffness of the culture media, further modeling using the spline regression is subsequently applied to the data for Phytagel (Figure 2). With Phytagel at 5%-1.7 % Young’s modulus also showed a steady increase and that was consistent with the result of breaking load. It should be noted that the slope of modeling curve from 0.5%-1.2% was small, and the slope from 1.2%-1.7% was steep. In other words, stiffness increased slowly when Phytagel concentration increased to 1.2%, and stiffness increased rapidly when Phytagel concentration increased from 1.3% to 1.7%. It could be concluded that mechanical strength of Phytagel medium have a transition point at 1.2%. Generally speaking, high concentration of Phytagel would enhance extrusion force for plant tissues when they were living in it. Phytagel of 1.0%-1.2% have been widely used in plant tissue culture, and the reason for that may be those Phytagel level provide a suitable mechanical stress for plant development. Plant morphogenesis depends on the integration of both mechanical and biochemical signals. For the published literatures of plant morphogenesis, the cause of formation of phenotype could be estimated from these data.

Figure 2. Summary of the model fitting to the Young’s modulus for Phytagel medium.

\[ y = 0.01341804 + 0.03376900X + 0.05079903X^2 + 0.04575657X^3 + 0.13323091 (X - 0.011)^3 + 0.09362697(X - 0.014)^3 + 0.08838223(X - 0.017)^3 \]

where \( y \) denotes the predicted stiffness of Phytagel medium and \( X \) represents the concentration level of Phytagel medium. The above model is statistically significant with \( P \)-value < 0.0001 and adjusted R-squared value of 89.6%.

3.4. Rheological properties
Phytagel properties were evaluated from dynamic oscillatory tests for a frequency sweep from 0.1 to 20 Hz [12]. The data of frequency sweeps for each media with different concentration are plotted as \( G^* \) (Complex modulus), \( G_s \) (Elastic modulus, Storage modulus, Pa), \( G_l \) (Viscous modulus, Loss modulus,
Pa). $G_s$ measures the stored energy, representing the elastic portion, and $G_l$ measures the energy dissipated as heat, representing the viscous portion. Mechanical strength was expressed as complex modulus. The mathematical forms of these equations are given as below:

Storage Modulus: \[ G_s = \frac{\sigma_0}{\varepsilon_0} \cos \delta \]

Loss Modulus: \[ G_l = \frac{\sigma_0}{\varepsilon_0} \sin \delta \]

stress and strain are represented by $\sigma_0$ and $\varepsilon_0$, respectively, and phase lag between stress and strain is represented by $\delta$.

Complex Modulus: \[ G^* = G_s + iG_l \]

Figure 3. The variation of moduli (Storage and loss moduli, viscous modulus, and complex viscosity) for all the Phytagel medium with different concentration among various frequency (0.1-20 Hz). Master curves are constructed by using time – concentration superposition, referenced to the labeled concentrations, respectively. The reference concentrations are 0.5% for A, 0.6% for B, 0.7% for C and 0.8% for D. 0.5% -1.2% from left to right, up to down.
Phytagel medium combine the characteristics of elastic solids and Newtonian fluids based on the results of dynamic mechanical properties of all the Phytagel concentration. Complex modulus with higher value indicated a stiffer structure. Of the samples A-H representing media with 0.5%-1.2% Phytagel concentration with almost increasing complex modulus from 3101.22 ± 1308.73 Pa to 8607.205 ± 1357.059 Pa, sample A of 0.5% exhibited the lowest gel strength and sample F of 1.0% exhibited the highest gel strength (Figure 3). Rheological results showed that the mechanical response of Phytagel media was viscoelastic and well structured. The storage modulus is higher than the loss modulus. Whether structural integrated Phytagel media presented elasticity or viscidity depended on which modulus was predominant under the specific frequency. Almost each modulus exhibited frequency dependence during the dynamic oscillatory tests, indicating of structural relaxation.

With the increase of Phytagel concentration, the relative increase of complex modulus was observed. It was also observed for all Phytagel concentrations that elastic modulus varies very little with frequency, suggesting little structural relaxation is occurring with time. 0.8%-1.2% exhibited increase of viscous modulus at high frequency (10-20 Hz), especially 0.9%-1.1%. It is generally accepted that the chain relaxation governs the response at low frequencies while segmental dynamics dominate the response at high frequencies.

![Figure 4](image_url)

**Figure 4.** A schematic representation of relationship of phase angle and complex modulus. When oscillatory frequency was 0.1, viscoelastic properties of Phytagel media from 0.5% to 1.2% were reflected by phase angle.

Another characteristic of viscoelastic properties is reflected by phase angle. As the amount of Phytagel increased from 0.5% to 1.2%, the structure of media changed irregularly. In frequency of 0.1 Phase angle for all the samples were < 10°, indicating all samples could be considered as ideal gel. Phase angle of zero presented a perfect gel while the value between 0 and 45° suggested some degree of viscous damping, which could facilitate structural relaxation. Of the samples labeled 0.5%-1.2% in
Figure 4, Phytagel concentration increased from 0.5% to 0.8%, and the media exhibit increasing elasticity and stiffness. It was interesting that Phytagel medium of 0.9% was less stiff but more elastic than that of 0.8%. As Phytagel concentration increased from 1.0% to 1.2%, elasticity increased very little and stiffness decreased.

4. Discussion

Uniaxial tension and compression experiment was a common test for mechanical properties of materials [13]. Only compression test could be carried out for Phytagel because of its poor tensile properties. Previous studies raised concerns about the mechanical strength of Phytagel, for instance, when Phytagel was mixed in Soy Protein, its tensile and moisture properties were significantly improved [11, 14]. Measurement of rheological properties can provide microscopic structure for better understanding of the application features. There are various methods for determining rheological characteristics of gel materials. However, for the gel material one of the most sensitive methods is the dynamic oscillatory test. Oscillation testing included variable amplitude and variable frequency tests [15]. Amplitude sweep measurement was taken before frequency sweep measurement to make sure the response was under critical strain and within the linear viscoelastic region (LVER). Subsequent frequency sweep tests were performed between 0.1 and 20 Hz using a constant strain within the LVER. Oscillatory rheological experiments can systematically investigate nonlinear viscoelastic material responses. This study employed a combination of two types of mechanical experiments to characterize the properties of Phytagel medium. There are two factors determining the application of high concentration of Phytagel: medium moisture content, stiffness or hardness. Phytagel media were cross-linked complex solid which could congeal the liquid medium.

If the measurement is only made through compression test, elastic relaxation time scales will be lost. In addition, yield stress and strain were evaluated from these two kinds of tests. The results demonstrated how such an approach can be used to quantify and compare the properties of different gel systems.

Linear viscoelasticity theory is only valid when the total deformation is quite small [16]. Single shear rate can only provide little information and cannot reach the relaxation time scales. In contrast, frequency sweep test was useful for a broad class of complex fluids and soft matter because strain amplitude and frequency can be varied independently allowing a broad spectrum of conditions to be attained.

Previous studies considered Basal medium, plant growth regulators, and Phytagel concentration as factors affecting initiation frequency [17]. When the new biomechanical experiments are designed, it is essential to investigate the properties of the materials and chose the best Phytagel concentration to optimize the experiments.

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References

[1] Niklas K J 2013 Biophysical and size-dependent perspectives on plant evolution J Exp Bot. 64
[2] Valentine T A, Hallett P D, Binnie K, Young M W, Squire G R, Hawes C and Bengough A G 2012 Soil strength and macropore volume limit root elongation rates in many UK agricultural soils Ann Bot. 110 259-70

[3] Acuña T L B and Wade L J 2013 Use of genotype × environment interactions to understand rooting depth and the ability of wheat to penetrate hard soils Ann Bot. 112 359-68

[4] Jin K, Shen J, Ashton R W, White R P, Dodd I C, Phillips A L, Parry M A and Whalley W R 2015 The effect of impedance to root growth on plant architecture in wheat Plant Soil 392 323-32

[5] Zhu L, Wang B, Wang Y, Liu J, Yang X and Fu X 2014 A micromechanics model for turgor pressure of arabidopsis thaliana protoplast J Plant Growth Regul. 33 751-6

[6] Massa G D and Gilroy S 2003 Touch modulates gravity sensing to regulate the growth of primary roots of Arabidopsis thaliana Plant J: Cell Molecul. Biol. 33 435-45

[7] Buer C S, Masle J and Wasteneys G O 2000 Growth conditions modulate root-wave phenotypes in Arabidopsis Plant Cell Physiol. 41 1164-70

[8] Murashige T and Skoog F 1962 A revised medium for rapid growth and bioassay with tobacco tissue culture Physiol. Plant 15 473-97

[9] Yamamoto C, Sakata Y, Taji T, Baba T and Tanaka S 2008 Unique ethylene-regulated touch responses of Arabidopsis thaliana roots to physical hardness J. Plant Res. 121 509-19

[10] Stolz A, Bender P L, Faller J E, Silverberg E C, Mulholland J D, Shelus P J, Williams J G, Carter W E, Currie D G and Kaula W M 1976 Earth rotation measured by lunar laser ranging Science 193 997-9

[11] Lodha P and Netravali A N 2005 Characterization of Phytagel® modified soy protein isolate resin and unidirectional flax yarn reinforced “green” composites. Polym. Compos. 26 647-59

[12] Sivaramakrishnan H P, Senge B and Chattopadhyay P 2004 Rheological properties of rice dough for making rice bread J. Food Eng. 62 37-45

[13] Oliver W C and Pharr G M 1992 An improved technique for determining hardness and elastic modulus using load and displacement sensing indentation experiments. J. Mater Res. 7 1564-83

[14] Huang X and Netravali A N 2006 Characterization of nano-clay reinforced phytagel-modified soy protein concentrate resin Biomacromolecules 7 2783-9

[15] Hyun K, Wilhelm M, Klein C O, Cho K S, Nam J G, Ahn K H, Lee S J, Ewoldt R H and McKinley G H 2011 A review of nonlinear oscillatory shear tests: Analysis and application of large amplitude oscillatory shear (LAOS). Progr. Polym. Sci. 36 1697-753

[16] Dealy J M and Wissbrun K F 2012 Melt rheology and its role in plastics processing: theory and applications Springer Science & Business Media

[17] Li X, Huang F and Gbur Jr E 1998 Effect of basal medium, growth regulators and Phytagel concentration on initiation of embryogenic cultures from immature zygotic embryos of loblolly pine (Pinus taeda L.) Plant Cell Rep. 17 298-301