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ORIGINAL RESEARCH

Developed applicability of a bacterial cellulose matrix as a gelling substitute for plant tissue culture media

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Abstract Bacterial cellulose (BC) is a natural biodegradable, eco-friendly fiber, lying within the nanoscale range. It is reputable for its various physical and chemical qualities, like high hydrophilicity, immense crystallinity, ease of sterility, being toxin-free, and extremely pure. Adding to its wide applicability in different fields, this study evaluated the applicability of a developed gelling substitute for plant tissue culture media. The BC matrix was characterized under the acronym PLATIBACGEL (PLAnt TIssue Culture BActerial Cellulose GEL), formed by *Komagataeibacter hansenii* AS.5, preisolated from rotten apple waste. Scanning electron microscope, Fourier-transform infrared, X-ray diffractometer, and tensile strength analyses confirmed the formation of purified, porous, and heterogeneous densely packed multiple network polymers possessing cellulose properties. The water holding capacity (WHC) values of wet and dried BC membranes were 9179% and 226.9%, respectively, and the water absorption rate (WAR) of dry BC membranes was higher than that of wet membranes. Using BC as a tissue culture gelling agent, six genotypes from tomato and wheat seeds were cultured in vitro, for guaranteeing explant genetic diversity, over seven treatments. Treatment 5, included PLATIBACGEL as the main constituent, improved and sustained all in vitro seed germination, root penetration, and plant support. Likewise, repeated tomato micropropagation subcultures were successful. Results demonstrated applying PLATIBACGEL as a promising, reusable, cheap, and reliable alternative plant micropropagation media gelling agent. Wherefore, plant cellular developers and tissue-culturists can utilize bio-polymers like BC for better understanding plant cell response to different in vitro culturing conditions, with expected beneficial returns on gelling agents industry and markets as well.

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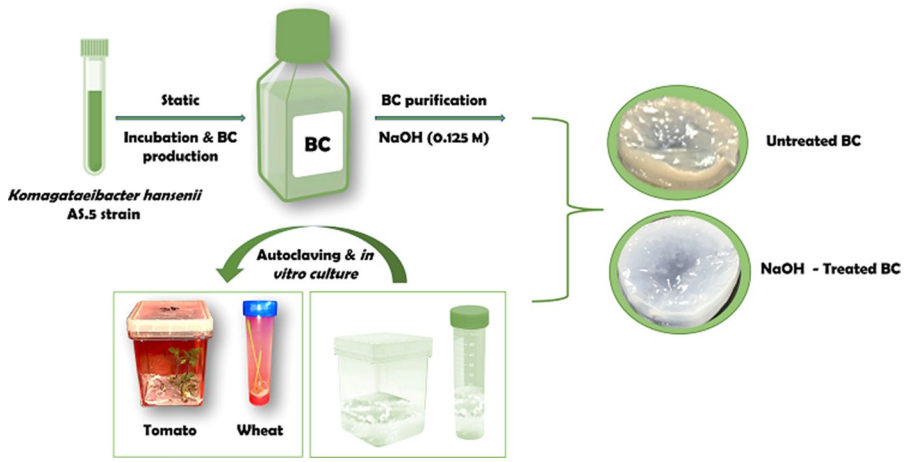
Graphical abstract



Keywords Bacterial cellulose · Gelling agents · Plant tissue culture · *Komagataeibacter hansenii* · PLATIBACGEL

Introduction

Gelling agents are polymeric structures composed of some colloidal polysaccharides or certain proteins that originate from microbes, algae, or plants. They can solidify or stabilize the medium by forming continuous three-dimensional molecular networks. They are commonly added to liquid media to convert them into solid or semisolid structures (Das et al. 2015). Adding gelling agents to liquid media can give them firmness and influence their diffusion characteristics. The medium's viscosity is the primary factor determining its diffusion rate, which is almost dependent on the concentration and physicochemical characteristics of the applied gelling agent (Palaniraj and Jayaraman 2011). An ideal gelling agent must be colorless, odorless, and preserve moisture effectively.

Most gelling agents have limited pH and temperature ranges, implying that outside their optimal ranges, they become ineffective in achieving the required purposes. Although some applied gelling agents have a wide working temperature and pressure range, most of them can be easily degraded by various microorganisms; thus, indicating the need for other alternative gelling agents with more powerful properties (Das et al. 2015; Jain et al. 2005).

Both gelatin and agar were discovered in the 1950s, and xanthan was discovered from the bacterium *Xanthomonas campestris* after several decades (Bellini et al. 2015). Furthermore, carrageenan and gellan gum were discovered in two successive years, viz., 1977 and 1978, respectively (Jamshidian et al. 2014; Razavi et al. 2014). Another alternative gelling agent known as isubgol was reported in 1997, followed by guar gum in 2005 (Babbar and Jain 1998; Fialho et al. 2008; Kirchmajer et al. 2014; Naik et al. 2020).

Among all these gelling agents, agar is the most frequently used in plant tissue culture due to its desirable characteristics of being clear, stable, and resistant to degradability by plant enzymes. However, there are reports of adverse effects of agar, such as inhibition of growth, impairment of vitrification, and batch-to-batch variability. In contrast, agar can be the most expensive gelling agent based on the price of basic tissue culture components (Dhawale et al. 2021; Puchooa et al. 1999).

In an effort to reduce the cost of commercial micropropagation, other alternatives, such as methylcellulose, potato and corn starches, alginate, gellan gum, gelatin, pectin, and microcrystal cellulose have been used instead of agar (Bornman and Vogelmann 1984; Calleberg et al. 1989; Gorinova et al. 1993; Hassan and Moubarak 2020; Mbanaso and Roscoe 1982).

According to our current research, a substitute gelling agent that is proposed for use in the field of plant tissue culture, and would be a promising alternative is bacterial cellulose (BC).

In general, cellulose is a linear organic polysaccharide discovered in 1838 by Payen. It is known for its insolubility in water and hydrophilic behavior. It is also nondegradable in physiological environments due to the lack of appropriate enzymes that can break down its beta acetal linkage (Wang et al. 2019). It is commonly found in the cell walls of plants, especially in the stem, branches, and woody parts, and other microorganisms such as bacteria, fungi, and algae (Mohammad et al. 2014; Poddar and Dikshit 2021). In 1886, Brown reported the first production of cellulose from the bacterium *Acetobacter xylinum* (Brown 1886; Rusdi et al. 2022). BC is a polysaccharide containing hundreds to thousands of chain β (1–4) D-glucose units. It has a high Young's modulus value (Hsieh et al. 2008), high water uptake capacity, and high aspect ratio (Trovatti et al. 2010). It is produced from different Gram-negative and Gram-positive bacterial species and has different structures, morphology, and properties (Jonas and Farah 1998). However, its purification and macromolecular characteristics can differ (Wang et al. 2019). Both BC and plant cellulose have the same chemical structure, but the former has better fibers that can reach > 50 nm in length (Phillips and Williams 2000).

The microbial production of BC depends on using one of three incubation methods: static, shaking, and bioreactor cultures, which result in different microscopic morphology, properties, and microstructures. Incubation under static conditions results in the accumulation of a gelatinous cellulosic membrane that floats on the nutrition solution, whereas incubation under shaking conditions results in the formation of sphere-like, pellet-like, asterisk-like, or irregular masses (Pandit and Kumar 2021; Rani and Appaiah 2011; Watanabe et al. 1998). The final required application and the required physical and mechanical characteristics are almost determined and help to select the convenient incubation method (Wang et al. 2019).

Gottlieb Haberlandt proposed the first in vitro plant tissue culture method in 1902 (Haberlandt 1902; Laimer 2003). The proposal was a theoretical explanation for the in vitro application of tissue cultures based on the totipotency of plant cells. He was trying to understand the functions and relationships among cells of multicellular organisms. His studies depended on the cultivation of isolated plant cells in a nutrient solution (Loyola and Vázquez 2006; Smith 2012). However, the first truly successful plant cell culture method was developed in 1922 when Robbins cultivated the tips of roots and stems, overcoming the problem of medium sterilization (Aldowigh 2022; Robbins 1922). The discovery of in vitro plant cell culture allows the cultivation of wild plants, such as those containing bioactive compounds that are intensively used in pharmaceutical fields. Such plants are considered endangered medicinal plants as they face destructive harvesting practices and overharvesting for the production of medicines (Mulabagal and Tsay 2004).

Applying in vitro-propagated plants helps obtain a uniform, sterile, and easily cultivated plants possessing active constituents that can be accurately identified and characterized (Khan et al. 2021; Miura et al. 1987). Moreover, the compounds obtained from tissue culture can be easily purified as they can be easily extracted, reducing production costs and processing costs (Mulabagal and Tsay 2004). In addition, the physiological processes of in vitro-cultured plants could be controlled to a greater extent because of the discovery of plant growth regulators, including plant hormones, which was the major factor that revolutionized the development of this technology (Dias et al. 2016; Roberts 2012).

The present study aimed to use BC as an innovative gelling agent, namely PLATIBACGEL (PLAnt TIssue Culture BActerial Cellulose GEL), for plant cell culture applications. The aim also extended to evaluate its potentiality for the in vitro cultivation of different genotypes, such as tomato and wheat, as they represent two economic plant species.

The research objectives also extended to provide the field of plant tissue culture with an inexpensive and promising gelling agent, such as PLATIBACGEL. It is a solid material that can resist changes in pH and temperature ranges, cannot be degraded by plant enzymes, and can be insusceptible to microbial contamination.

Although BC production from microbes has recently received much attention and application in different fields, our research hypothesis was that producing PLATIBACGEL from BC is a promising alternative for ordinary media gelling agents. It could be used in in vitro seed germination and plant tissue culture. Primarily, polymer technology could be integrated into broader disciplines. Products such

as PLATIBACGEL can be applied in plant cell biology and development to better understand plant cell responses under different in vitro culture conditions. The use of PLATIBACGEL represents an inexpensive and simple handling procedure for plant tissue culturists, and the field is still open for further investigations and applications.

Materials and methods

Strain and preinoculum

The BC membrane used in this study was synthesized using *Komagataeibacter hansenii* AS.5 (*K. hansenii* AS.5) (ac: MH109871) strain that was isolated from rotten apple samples, as reported in our previous study (Saleh et al. 2019). The standard Hestrin–Schramm (HS) medium was used for preinoculum preparation, which consisted of (in g/L) 20 D-glucose, 5 peptone, 5 yeast extract, 2.7 Na_2HPO_4, and 1.15 citric acid, with pH being adjusted to 6.0 ± 0.02. After sterilization, the medium was inoculated with a single colony of *K. hansenii* AS.5 and incubated at 30 °C and 200 rpm for 2 days.

Biosynthesis and purification of BC

As reported in our previous study (Saleh et al. 2020), the optimized medium was used for BC production using the following composition (in g/L): 25 glucose, 13 yeast extract, 0.15 $MgSO_4$, and 2 KH_2PO_4 and 7.18 ml/L ethanol with subsequent sterilization at 15 psi, 121 °C for 20 min. The optimized parameters were as follows: pH 5.5 ± 0.02, inoculum size of 7%, production temperature of 20 °C, and incubation period of 9 days. After the completion of fermentation, the observed BC pellicle on the air–liquid interface was harvested and washed 3–4 times with distilled water to eliminate the residues of medium components. The BC pellicle was soaked in 0.125 M NaOH for 30 min at 90 °C to remove microbial contaminants and other impurities and then rinsed several times with distilled water and once with a washing liquid of neutral pH, after which bright-white BC was obtained (Hsieh et al. 2016).

Determination of dry and wet weights of BC membranes

After the purification of BC, its wet weight was immediately recorded, followed by drying it at 70 °C until a constant weight was obtained. The BC membranes' wet and dry weights were recorded in g/L (Khattak et al. 2015).

Thickness of dry and wet BC membranes

The thickness of the wet and dry BC membranes was measured using an electronic digital micrometer. Ten different points of each membrane were selected for the measurements, and the average of the obtained readings was calculated (Semjonovs et al. 2017).

Water holding capacity (WHC) of dry and wet BC membranes

To determine WHC; wet BC samples were webbed by a soft tissue and their weight was recorded (W_h = hydrate weight). The BC samples were dried for 24 h at 70 °C until a constant weight was achieved (W_d = dry weight) to remove the water completely. Finally, the WHC was calculated using the following formula: $WHC\ (\%) = (W_h - W_d)/W_d$. The dried BC samples were first immersed in a beaker containing distilled water for 12 h at room temperature under 100 rpm, after which they were weighed (W_h = hydrate weight) and considered as wet BC samples before their subsequent drying under the above mentioned drying conditions. Results were calculated as the average of three samples (Barshan et al. 2019).

Water absorption rate (WAR) of dry and wet BC membranes

The WAR of dry or wet BC samples was determined according to the shake method (Schrecker and Gostomski 2005) with a few modifications. Each BC sample was cut into three pieces (5 mm^2) and immersed in distilled water at room temperature for 24 h at 100 rpm. The WAR of each BC membrane was analyzed by continuously measuring its weight, calculated as $WAR\ (\%) = (W^1 - W)/W \times 100$, where W^1 (g)

was the weight of BC after absorbing water, and W (g) was the weight of the BC sample (dry or wet).

Characterization of BC dry membrane

The surface morphology of the BC membrane was examined under a scanning electron microscope (SEM) (JEOL JSM 6360 LA®, Japan) at a magnification of 5000×, and an applied voltage of 10 kV. The functional groups and chemical bonds of BC samples were analyzed by Fourier-transform infrared spectroscopy (FT-IR) (JASCO FTIR-4100 E®, Japan), operated in the absorption mode at a wavenumber range of 4000–500 cm^{-1} and a resolution of 1 cm^{-1}. The dried samples' crystallinity was determined using X-ray diffractometer (XRD) patterns collected on an XRD (X PERT PRO-PAN® Analytical-Netherlands) with a back monochromatic and a Cu anticathode. Samples were scanned with 2θ ranging from 5° to 80°, at a scan rate of 5° per min. The mechanical properties, including tensile strength, elongation at break, and Young's modulus, of dry BC membranes, were investigated using a universal tensile testing machine (Universal Testing Machine, model: AG-I/50 N-10 kn®, Japan). The BC membrane was cut into rectangular strips (10 cm long and 1 cm wide) for measurement, with a gage length of 20 mm. Each test was performed using three specimens, and the average of the results was recorded.

In vitro culture and propagation

Two major genetically different crops representing two different plant families have been used for testing PLATIBACGEL as a successful plant tissue culture media gelling agent. Explants from tomato (*Solanum lycopersicum*), family *Solanaceae*, and wheat (*Triticum aestivum* L.), family *Poaceae*, were used to ensure that PLATIBACGEL would work for different plants of various genetic origins.

Explant material

Tomato (S. lycopersicum) tissue culture

Five different F1 tomato seed genotypes (TG1; Determinate: Cheyenne E448, TG2; Determinate: GS 12, TG3; Determinate: Nairouz (TH 99806), TG4; Determinate: Tomaland (TH 01308), and TG5; Indeterminate: Tyrmes), obtained from Syngenta®, Egypt, were used as an explant sources in this experiment. This hypothesis was proposed depending on the genetic variation between the two plant species, derived from irrelevant families, and was put under investigation. Although there is no complete gurantee that PLATIBACGEL would work for "all" plant species, however, choosing two plants owning different genetic composition, could represent a proper practice for ensuring wide application. At least in this case, applying PLATIBACGEL on tomato and wheat would give better resolution than applying it on a model plant, while there would be no warranty for PLATIBACGEL applicability if model plants were used too. Of course, we look forward to applying it on more plant types as well.

Wheat (T. aestivum L.) tissue culture

Wheat "Giza 168" local genotype (WG) seeds, obtained from the Egyptian Agricultural Research Center (Giza, Egypt), were used as an explant source in this experiment.

In vitro seed germination

For plant tissue culture and in vitro seed germination process, seeds were washed thoroughly with distilled water, soaked in tween® 20 solutions, and then washed again before sterilization using a 5% commercial bleach solution (Clorox®; active ingredient sodium hypochlorite) for 5 min. Next, the seeds were soaked in 70% alcohol for 3 min and washed and rinsed 4–6 times with sterilized deionized distilled water before being cultured in plant tissue culture containers (Magenta®, USA, tissue culture glass jars, and/or transparent Falcon Flat Base®, USA). For tomato plantlets, subcultures of shoot segments were performed every 4 weeks as required.

Tissue culture media preparation

As this study aimed to evaluate PLATIBACGEL gelling agent as a potential alternative to classical gelling materials, a set of treatments has been practiced, as shown in Table 1. Modified Murashige and Skoog

Table 1 Treatments and conditions for tomato and wheat plant in vitro culture and propagation

Treatment	PLATIBACGEL membrane		Medium composition				
	Treated*	Untreated	MS medium (without vitamins)	Agar (8 gl^{-1})	Sugar (Sucrose, 30 gl^{-1})	Rooting hormone (RH; 1 mg l^{-1} IAA)	Shooting hormone (SH; 2 mg l^{-1} GA3)
T1	+	−	+	−	−	−	−
T2	+	−	+	−	+	−	−
T3	−	+	+	−	−	−	−
T4	+	−	−	−	+	−	−
T5	+	−	−	−	−	−	−
T6	+	−	+	−	+	+	+
T7	−	−	+	+	+	−	−

*Treated BC membrane is a washed membrane treated with NaOH and rinsed to remove BC-producing bacteria

T1: Treated PLATIBACGEL membrane + MS medium—sugar

T2: Treated PLATIBACGEL membrane + MS medium + sugar

T3: Untreated PLATIBACGEL membrane + MS medium—sugar

T4: Treated PLATIBACGEL membrane + sugar

T5: Treated PLATIBACGEL membrane

T6: Treated PLATIBACGEL membrane + MS medium + sugar + Rooting Hormone (RH) + Shooting hormone (SH)

T7: Normal Agar + MS medium + sugar (control)

(MS) (Murashige and Skoog 1962) basal medium without vitamins (Duchefa®, Netherlands) but containing sucrose (30 gl^{-1}) and agar (8 gl^{-1}) (Sigma®, Germany) was used. In contrast, medium containing 1 mg l^{-1} indole-3-acetic acid (IAA) and 2 mg l^{-1} gibberellic acid (GA3) (Sigma®, Germany) was used as required for specific treatments (MS and/or any additives were supplied as a solution, left to be absorbed by dry PLATIBACGEL), as shown in Table 1. Plant tissue culture tools were sterilized in a dry oven, and media pH was adjusted to 5.8 before sterilization by autoclaving (121 °C and 15 psi for 20 min). The presence of any contamination was regularly monitored and reported. All plants were cultured under aseptic conditions in a laminar flow hood (Thermo Fisher Scientific®, UK) and subsequently incubated at 18–23 °C in a 16:8 h light:dark photoperiod, according to plant species growth requirements.

Statistical analysis

For both tomato and wheat in vitro growth, phytotechnical parameters, such as plant germination percentage (PG %), plant root length (RL, cm/plant), plant shoot length (SL, cm/plant), shooting percentage (St, %), and rooting percentage (Rt %), were recorded. The number of shoots per explant (NSE) was regularly monitored and recorded every 2 weeks for wheat cultures only. One hundred explants were used as replicates for every treatment to facilitate calculation processes. Differences among mean values were considered significant at P-value < 0.05 in multiple ranges. The least significant difference compared and resolved differences among the mean values of replication using the IBM SPSS® software, 1997, according to Duncan's multiple range test (Duncan 1955).

Results and discussion

Weights and thickness of dry and wet BC membranes

As reported previously, *K. hansenii* AS.5 was isolated from rotten apple samples and used as a BC producer. After 1 week of incubation in HS media, the initial production weights were 2.48 and 139.4 g/L for dry and wet BC membranes, respectively. The one-variable-at-a-time (OVAT) method was applied to enhance BC production to reach 3.75 and 338.4 g/L for dry and wet BC membranes, respectively, using modified

Yamanaka media. Finally, statistical optimization achieved 6.30 and 582.7 g/L for dry and wet BC membranes, respectively (Khera et al. 2019; Saleh et al. 2020). The different optimization strategies positively affected the weight of the produced BC membranes, which is consistent with previous studies (Barshan et al. 2019; Lin et al. 2014). The BC thickness was influenced by the culture and environmental conditions, as shown by the obtained results. The BC thickness in the initial production reached 0.18 and 1.6 mm for dry and wet BC membranes, respectively. With OVAT optimization, the thickness reached 0.26 and 3.7 mm for dry and wet BC membranes, respectively. After statistical optimization, the respective BC thickness was 0.35 and 6 mm. Thus, the weight and thickness of the synthesized BC membranes are proportionally matched.

WHC and WAR% of dry and wet BC membranes

WHC and WAR are important parameters that play a vital role in different BC applications, such as biomedical, cell culture, and active ingredient carriers (Gorgieva 2020; Lin et al. 2013). For wet BC, the average wet weight of the BC membrane was 12.323 g, and its dry weight was 0.1328 g. Based on this result, the WHC was 9179%. The average wet weight of BC membrane for dry BC was 0.0425 g, and its dry weight was 0.0130 g. According to this result, the WHC was 226.9%. A comparison between dry and wet BC membranes based on WHC showed that the WHC reached 173 and 690 times of dry weight for dry and wet BC membranes, respectively. Table 2 shows the WAR values measured at different time points for dry and wet BC membranes produced by *K. hansenii* AS.5. The WAR values for dry and wet BC membranes gradually increased with time until reaching constant weights after 60 min. The WAR of dry BC membranes was higher than that of wet BC membranes, but it was similar to that reported in previous studies (Dikshit and Kim 2020; Feng et al. 2015). The WHC and WAR depend on BC network structure, such as the morphology, pore size, high surface area per unit mass and fiber homogeneity. The BC membranes mechanism of water holding depends on the numerous hydroxyl (OH) groups of fibrils. These groups interact to form intramolecular and intermolecular hydrogen bonds between β-D-glucopyranose monomers to form amorphous and crystalline domains. The absorbed water molecules render the BC membrane hydrophilic (Rebelo et al. 2018; Ul-Islam et al. 2012).

Table 2 WAR% of dry and wet BC membranes at different time points

Time (min)	WAR (%)	
	Dry BC	Wet BC
0	0	0
5	54.7	16.6
10	115.6	26
20	143.6	37.6
30	155.9	39.8
40	170.3	40.8
50	171	41
60	172	41

Characterization of dry BC membrane

SEM analysis

The detailed morphological structure of dry BC membrane was investigated using SEM. As shown in Fig. 1A (1, and 2), the pellicle BC membrane obtained from *K. hansenii* AS.5 is purified, porous, and heterogeneous. It is densely packed in a tridimensional interconnected network structure originating from randomly oriented nanocellulose and microcellulosic fibers (Bagewadi et al. 2020; Saleh et al. 2020). The BC membranes surface purification was confirmed by applying NaOH solution for the treatment process to remove all impurities and microbial contaminants (Bilgi et al. 2016; Lee et al. 2015).

FT-IR analysis

The chemical structure (functional groups and molecular bonding) of the BC membrane obtained from *K. hansenii* AS.5 was evaluated by FT-IR spectroscopy at the wave number of 4000–500 cm^{-1}, as shown in Fig. 1B. A band of intense absorption in the BC spectrum at 3354.3 cm^{-1} was attributable to the presence of the OH group of cellulose type I (Fan et al. 2016; Saleh et al. 2020), and a strong absorption band at 2897.1 cm^{-1} was also attributable to the presence of CH_2 stretching vibrations (Rozenberga et al. 2016). The cellulose absorption spectrum is the

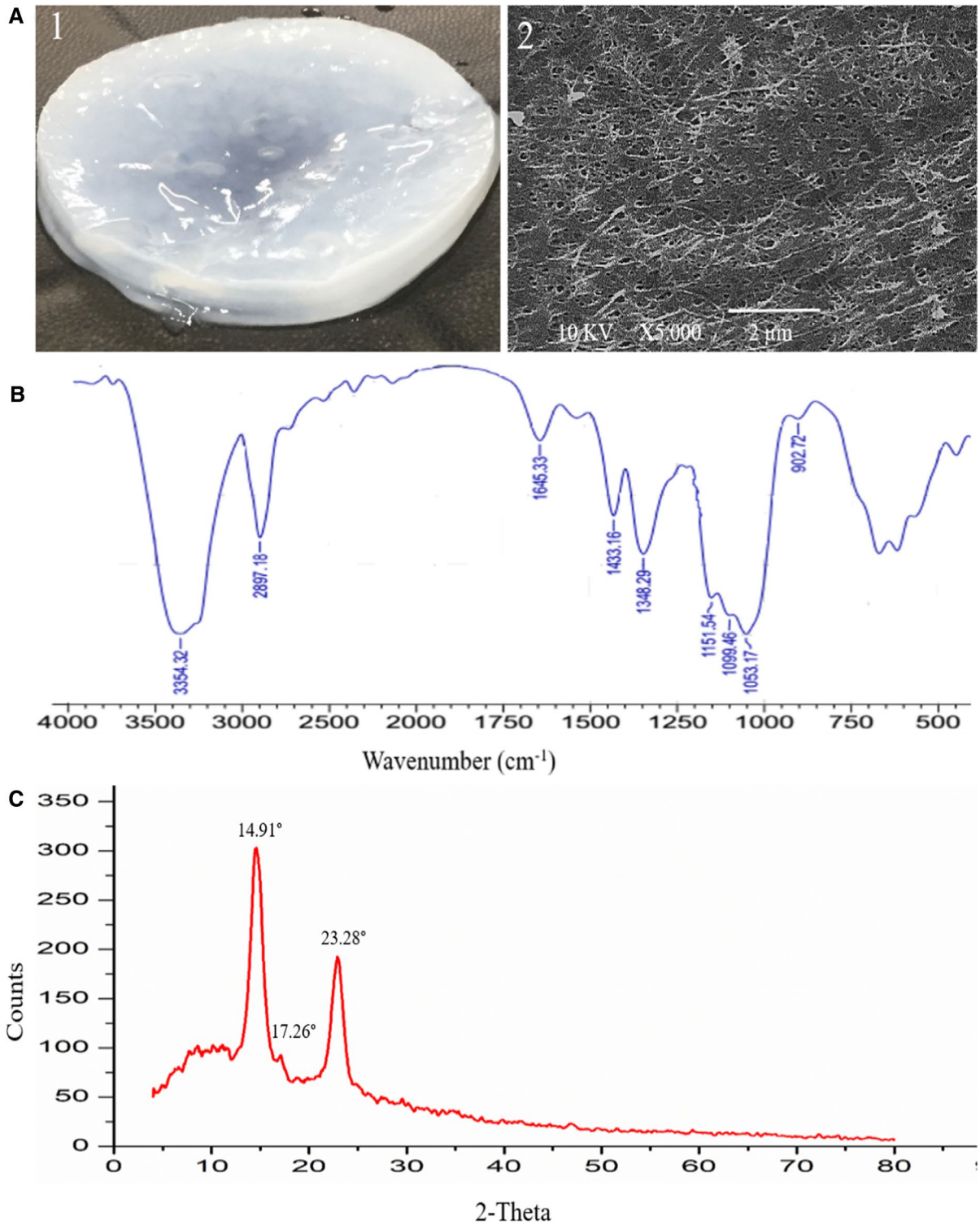


Fig. 1 **A** Morphological structure (1) Macroscopic photograph, and (2) SEM, **B** FT-IR, and **C** XRD analysis of BC membrane obtained from *K. hansenii* AS.5

band at 1645.3 cm^{-1} assigned to the carboxyl functional group (Lin et al. 2016). Bands also appeared at 1433.1 cm^{-1} (asymmetric angular deformation of C–H bonds), 1348.2 cm^{-1} (symmetric angular deformation of C H bonds), 1151.5 cm^{-1} (asymmetric stretching of C–O–C glycoside bonds), 1099.4 and 1053.1 cm^{-1} (stretching of C–OH and C–C–OH bonds in secondary and primary alcohols), respectively, and 902.7 cm^{-1} (angular deformation of C–H bonds) (Saleh et al. 2020). Furthermore, the FT-IR data analysis revealed the presence of a crystalline region and the purity of the synthesized BC membrane (Castro et al. 2012).

XRD analysis

As depicted in Fig. 1C, the XRD pattern of the dry BC membrane showed different reflections at 14.91°, 17.26°, and 23.28°, respectively, displaying a typical pattern of cellulose Iα; these data are similar to those reported previously (Fang and Catchmark 2014; Rozenberga et al. 2016; Saska et al. 2011; Tyagi and Suresh 2016). The crystallinity index (CI) was calculated as the intensity of the main peak ratio and the count number of the adjacent minimum (Segal et al. 1959), yielding a CI of 86.7%. The CI of the BC membrane depends on the strain type used for BC production, such as CI = 83% for *K. rhaeticus* (Machado et al. 2016), CI = 79.3% for *K. intermedius* (Lin et al. 2016), and CI = 87% for *Gluconacetobacter xylinum* (Zhijiang and Guang 2011).

Mechanical strength

The mechanical strength of the dry BC membrane produced by *K. hansenii* AS.5 was evaluated. Results showed that, the average tensile strength, Young's modulus, and elongation at break of the dry membrane were 59.7 ± 1.7 MPa, 2534 ± 3.42 MPa, and 1.1% ± 0.51%, respectively. Different studies have reported different mechanical properties of BC according to the type of microbial strain used (Dikshit and Kim 2020), incubation time (Zhang et al. 2020), additive concentrations (Sun et al. 2020), and cultivation conditions (Wang et al. 2019). Table 3 shows a brief comparison between some various BC properties, obtained from different bacterial strains.

In vitro seed germination and propagation of tomato and wheat

A biotechnological approach was followed in this study to produce an inexpensive and reliable plant tissue culture matrix that can act as a solidifying material for plant tissue culture media. Designing a matrix for supporting or hardening plant tissue culture medium using a BC-derived gelling agent under the acronym PLATIBACGEL has been challenging for the research team. It was expensive and overexploited when agar was predominantly used as the gelling agent for microbial and plant cell culture media (Das et al. 2019). Others have voiced concerns regarding agar applicability, especially for its limited resources overuse, and considerations include exorbitant pricing (Hassan and Moubarak 2020; Hegele et al. 2021). Previous studies have investigated the use of

Table 3 Comparison of the mechanical properties of BC membranes obtained in this study and other studies

Strain	Tensile strength (MPa)	Young's modulus (MPa)	Elongation at break (%)	References
K. hansenii AS.5	59.7 ± 1.7	2534 ± 3.42	1.1 ± 0.51	Present study
K. intermedius FST213-1	44 ± 8.3	4100 ± 1.6	1.24 ± 0.58	Lin et al. (2016)
Lactiplantibacillus plantarum AS.6	73.3	7838	1.6	Saleh et al. (2022)
Gluconacetobacter xylinus 23769	39.1 ± 9.8	2650 ± 0.50	1.54 ± 0.52	Lin et al. (2016)
K. xylinus ATCC 53582	36.9 ± 5.3	1220 ± 0.21	8.06 ± 2.1	Nascimento et al. (2021)
Gluconacetobacter intermedius SNT-1	74 ± 4.7	2000 ± 2.8	0.8 ± 0.04	Tyagi and Suresh (2016)
Acetobacter xylinum AGR 60	107.03 ± 7.38	11,201 ± 521.3	1.48 ± 0.15	Sintharm et al. (2022)
Gluconacetobacter xylinum BRC-5	96	6500	5	Cai and Kim (2010)

alternative gelling agents such as gelrite (Gehad M Mohamed et al. 2021), gum katira (Jain and Babbar 2002), isubgol and sago starch (Bhattacharya et al. 1994), guar gum (Babbar et al. 2005), locust bean gum (LBG) (Gonçalves and Romano 2005), and xanthan gum (Jain and Babbar 2006). Consequently, this has widened the applicability of plant tissue culture techniques as an effective tool in biotechnology.

For instance, in our study, simply to calculate how much one liter of a medium would cost, in comparison to one liter prepared by agar (based on the agar used in our investigation, originated from Sigma; https://www.sigmaaldrich.com/US/en/product/sigma/a8678), one g of agar costs $1.78. Assuming that one agar-gelled media liter would need about 6–8 g of good quality tissue culture grade agar to solidify, then, one liter would approximately cost about $12. In comparison, it would cost about $0.2 to produce a 1 L solidified medium, using PLATIBACGEL, in a standard tissue culture Magenta container, filled with 40 ml of medium. In addition, PLATIBACGEL is reusable, and re-autoclavable, while agar, and most of other gelling agaents are «one-use-only» media solidifiers, and would lose thier gelling properties after being applied in a tissue culture process.

Micropropagation has been the most popular, widely used, and reliable plant tissue culture technique for establishing aseptic cultures and propagating many plants in vitro (Gupta et al. 2020). When establishing a plant tissue culture protocol using new materials, there are often some problems. As long as the totipotency of plants to propagate and regenerate in vitro is sufficient, as long as this is reflecting how sufficient is the used medium, and optimal are the conditions. This has been inspected through seven different treatments using five tomato F1 genotypes and one wheat genotype as unrelative members of two dissimilar plant families to guarantee the genetic diversity among used explant sources. Therefore, we attempted to determine the appropriate procedure to achieve our study goal. Selecting the appropriate medium and certainly the composition of inorganic salts, plants, types and concentrations of growth regulators, and sugar sources are considered extremely important factors in the experimental output. However, the selection of the medium-solidifying agent is a difficult issue. Depending on the type of the solidifying agent used, there are severe effects on processes such as cell division, proliferation, and division frequency of adventitious bud/adventitious embryo

Table 4 Tomato and wheat genotypes' root length (RL, cm/plant), shoot length (SL, cm/plant), and number of shoots per explant (NSE "only for wheat") as influenced by different PLATIBACGEL and medium treatments*

Plant species/Genotype	T1			T2			T3			T4		
Phytotechnical parameter	RL	SL	NSE	RL	SL	NSE	RL	SL	NSE	RL	SL	NSE
TG1	3.21b	12.56a	–	3.62a	13.11a	–	1.02c	2.00c	–	4.21a	12.33a	–
TG2	3.00b	10.32	–	3.55a	12.24a	–	0.72c	1.42c	–	3.82a	10.60b	–
TG3	2.89b	12.00a	–	2.24b	13.21a	–	0.71c	1.56c	–	3.22a	12.11a	–
TG4	3.10a	10.21	–	2.11b	10.56b	–	0.21c	0.38c	–	2.98b	11.82a	–
TG5	2.91a	12.10a	–	2.45b	11.20aa	–	0.30c	0.89c	–	2.66b	10.13b	–
WG	4.33b	16.20b	3.00b	4.87b	15.11b	2.00b	1.88c	4.74d	1.00c	4.22b	15.68b	2.00b
Plant species/Genotype	T5			T6			T7					
Phytotechnical parameter	RL	SL	NSE	RL	SL	NSE	RL	SL	NSE			
TG1	4.98a	14.38a	–	3.12a	10.35b	–	3.21a	14.11a	–			
TG2	3.55a	13.32a	–	2.18b	12.81a	–	2.95a	12.39a	–			
TG3	3.10a	11.49a	–	3.35a	13.22a	–	2.11b	11.89a	–			
TG4	4.11a	14.00a	–	2.96a	12.20a	–	2.88b	12.11a	–			
TG5	4.24a	13.21a	–	2.49b	12.33a	–	2.93a	11.49a	–			
WG	5.98a	19.00a	4.00a	3.99b	13.30c	1.00c	4.38b	14.33b	2.00b			

*Values in the same column having different letters are significantly different at $P \leq 0.05$ based on Duncan's multiple range test (Duncan 1955)

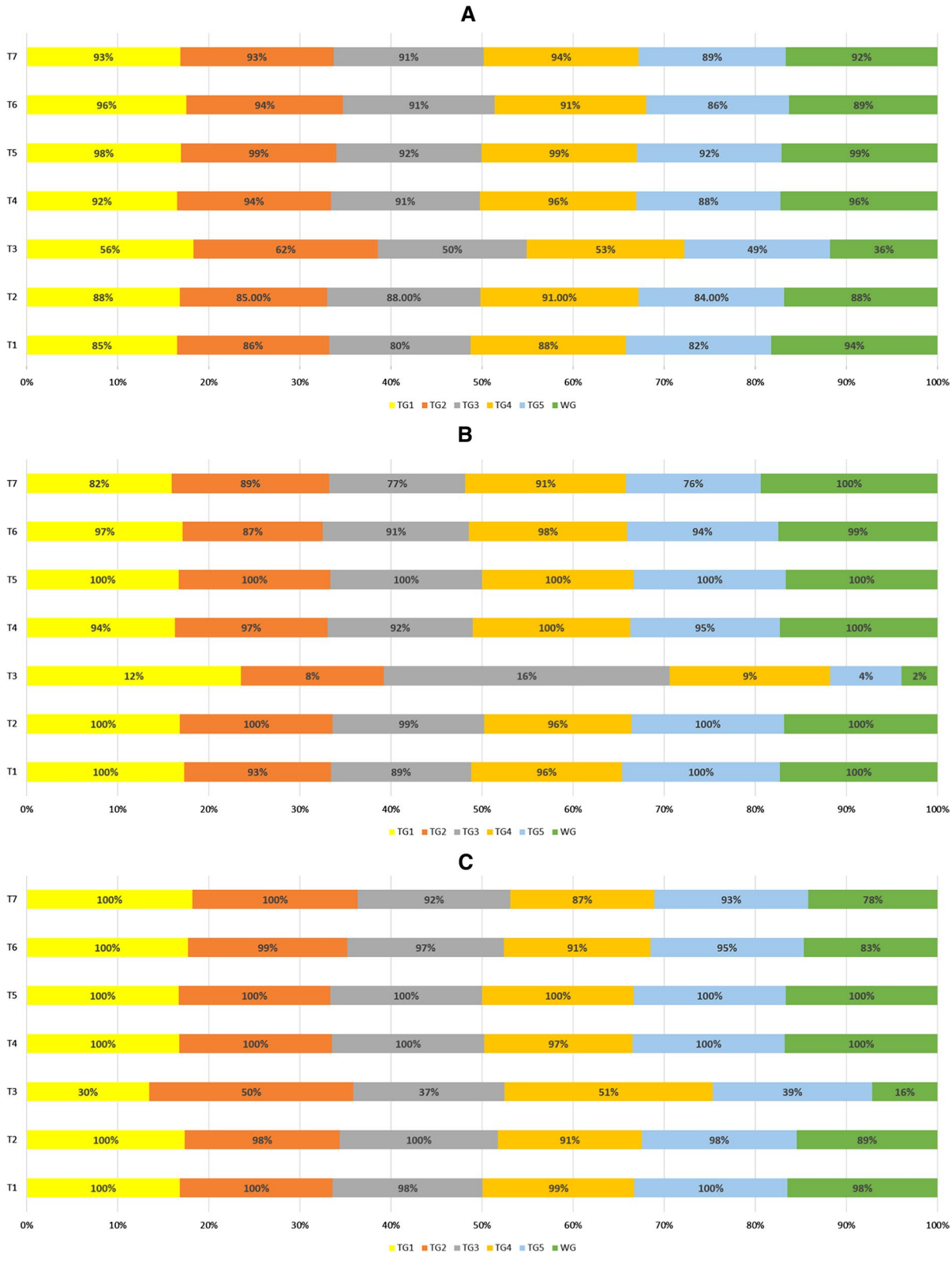
differentiation and cell differentiation and secondary metabolite production. Although in vitro propagation has been extremely well elucidated in the literature, it is worth developing a customized system that meets the requirements of the culture (Shimomura and Kamada 1986).

Table 4 reveals the effects of using PLATIBACGEL as a newly tested plant tissue culture media gelling agent on RL (cm/plant) and plant SL (cm/plant) of in vitro-cultured tomato and wheat seeds, in addition to the NSE of only wheat seeds. Plant RL (cm/plant) and SL (cm/plant) varied significantly among the five tomato genotypes and the "Giza 168" wheat genotype, which were affected by the seven PLATIBACGEL treatments. RLs ranged from 2.8 cm/plant for TG3 to 3.21 cm/plant for TG1 as affected by treatment 1, whereas WG was 4.33 cm/plant for the same treatment. In treatment 2, TG4 showed the lowest value of 2.11 cm/plant, whereas TG1 showed the highest value of 3.62 cm/plant, and WG was 4.87 cm/plant. Furthermore, RL was the shortest among tomato genotypes, as influenced by treatment 3, where it ranged from 0.21 cm/plant for TG4 to 1.02 cm/plant for TG1; the same trend could be observed for WG, which was 1.88 cm/plant. In contrast, treatment 4 showed significant differences among tomato genotypes from TG5 (2.66 cm/plant) to TG1 (4.22 cm/plant), and simultaneously the WG reached 4.22 cm/plant. Considerably increased values were recorded among genotypes under treatment 5, where the tomato genotypes under TG1 (4.98 cm/plant), TG2 (3.55 cm/plant), TG3 (3.10 cm/plant), TG4 (4.11 cm/plant), and TG5 (4.24 cm/plant) showed the highest RL values among all treatments, including for the wheat genotype (WG = 5.98 cm) also. The RL values of the genotypes were significantly influenced by treatment 6, ranging from 2.18 cm/plant (TG2) to 3.35 cm/plant (TG3) in tomatoes and 3.99 cm/plant in wheat. Similarly, treatment 7 influenced the genotypes' RL, ranging from 2.11 cm/plant for TG3 to 3.21 cm/plant for TG1 in tomatoes and 4.38 cm/plant in wheat. Moreover, the SL values showed the same variation patterns as those of RL values, as affected by genotypes and treatments, significantly ranging from 0.38 cm/plant (TG4) for treatment 3 to 14.38 cm/plant (TG1) for treatment 5 in tomatoes. Similarly, the WG showed the lowest SL (4.74 cm/plant) with treatment 3 and the highest SL (19.00 cm/plant) with treatment 5 (Table 4).

Table 4 and Fig. 4C show the NSE measured only for wheat, with values recorded as 3.00, 2.00, 1.00, 2.00, 4.00, 1.00, and 2.00 for treatments T1, T2, T3, T4, T5, T6, and T7, respectively.

Researchers have always been exploring other gelling aid replacements to address regular gelling agent problems. The functional properties of solidifying materials in plant tissue culture media have been reported for several years (Cassells and Collins 1999; Raina 2017; Shimomura and Kamada 1986). The efficiency of sago (from *Metroxylon sagu* Rottb.) and isubgol (from *Plantago ovata* Forsk.) as support matrices in gelling agents, filter paper, nylon cloth, polystyrene foam, and glass wool cloth has been evaluated for propagating chrysanthemum (*Dendranthema grandiflora Tzvelev*) plantlets, with optimistic results (Bhattacharya et al. 1994). A study conducted in 2002 demonstrated the use of gum katira, an insoluble gum derived from the bark of *Cochlospermum religiosum*, as an inexpensive gelling agent (Jain and Babbar 2002). Furthermore, the natural hydrocolloid extracted from the seeds of the carob tree (*Ceratonia siliqua* L.), namely "LBG," was analyzed for its potential as plant tissue culture media (Gonçalves and Romano 2005). Recently, guar gum has also been explored in this context (Das et al. 2019). Studies were also extended to investigate a wide range of plant species and crops in an attempt to screen the best ingredients to be used in explants and medium components. Our study has also produced convincing results consistent with those obtained with bananas (Kacar et al. 2010), wheat (Malik et al. 2017), kiwi (Mardiana et al. 2018), oil and date palms (Al-Mayahi and Ali 2021; Palanyandy et al. 2020), and rice (Gehad M. Mohamed et al. 2021; Repalli et al. 2019).

Figure 2A shows the changes in the germination percentage (PG%) of tomato and WGs due to different PLATIBACGEL and medium treatments. The PG% for all tomato genotypes was relatively high; however, the lowest value was recorded with treatment 3 and TG3 in tomato (50.00%), whereas the same treatment also resulted in a relatively low percentage, with WG for wheat at 36.00%. The highest PG% was recorded with treatment 5 in either tomato or WG, reaching 99.00%.


A
T7 93% 93% 91% 94% 89% 92%
T6 96% 94% 91% 91% 86% 89%
T5 98% 99% 92% 99% 92% 99%
T4 92% 94% 91% 96% 88% 96%
T3 56% 62% 50% 53% 49% 36%
T2 88% 85.00% 88.00% 91.00% 84.00% 88%
T1 85% 86% 80% 88% 82% 94%
0% 10% 20% 30% 40% 50% 60% 70% 80% 90% 100%
TG1 TG2 TG3 TG4 TG5 WG
B
T7 82% 89% 77% 91% 76% 100%
T6 97% 87% 91% 98% 94% 99%
T5 100% 100% 100% 100% 100% 100%
T4 94% 97% 92% 100% 95% 100%
T3 12% 8% 16% 9% 4% 2%
T2 100% 100% 99% 96% 100% 100%
T1 100% 93% 89% 96% 100% 100%
0% 10% 20% 30% 40% 50% 60% 70% 80% 90% 100%
TG1 TG2 TG3 TG4 TG5 WG
C
T7 100% 100% 92% 87% 93% 78%
T6 100% 99% 97% 91% 95% 83%
T5 100% 100% 100% 100% 100% 100%
T4 100% 100% 100% 97% 100% 100%
T3 30% 50% 37% 51% 39% 16%
T2 100% 98% 100% 91% 98% 89%
T1 100% 100% 98% 99% 100% 98%
0% 10% 20% 30% 40% 50% 60% 70% 80% 90% 100%
TG1 TG2 TG3 TG4 TG5 WG

◂**Fig. 2** Tomato and wheat genotypes (TG1, TG2, TG3, TG4, TG5, and WG); **A** Plant germination percentage (PG %), **B** Shooting percentage (St), **C** Rooting percentage (Rt %), as influenced by different PLATIBACGEL and medium treatments (T1, T2, T3, T4, T5, T6, and T7)

Figure 2B shows the shooting percentage (St%) of tomato and wheat genotypes affected by different PLATIBACGEL and medium treatments. The lowest St% of 2.00% was recorded with WG, as affected by treatment 3. Moreover, the same treatment also resulted in relatively low St% among all tomato genotypes, reaching 12.00%, 8.00%, 16.00%, 9.00%, and 4.00% for TG1, TG2, TG3, TG4, and TG5, respectively, successfully.

Figure 2C illustrates the effect of different PLATIBACGEL and medium treatments on tomato and wheat genotypes' rooting percentage (Rt %). In vitro-cultured plantlets that successfully germinated and efficiently produced shoots could also root easily. All germinated explants showed significant differences in rooting percentage among treatments and genotypes. Primarily, 100.00% Rt % was recorded for most genotypes with different treatments, except for treatment 3, which showed the minimum Rt %, reaching 30.00% with TG1 in tomato and 16.00% for WG in wheat.

Comparing PLATIBACGEL with other gelling agents is not oriented to its concentration in the medium, rather than being focused on its quality and performance. Based on our study results, treatment 5 (T5) maintained all the used explants for in vitro seed germination and even for tomato subcultures (Fig. 3C). T5 consisted of only PLATIBACGEL without any additives. Interestingly, tomato explants cultured on PLATIBACGEL medium could survive for almost 1 year without any subculturing processes (Fig. 3D). Seeds cultured on the agar gelling medium could hardly survive for a few weeks. However, the uniqueness of our results was that PLATIBACGEL was a plant supporting network and, "by a way or another," a nutrition-supplying medium. Nevertheless, these results could not be interpreted as of now and represent another serious question to be answered later through further in-depth investigations. The inexpensiveness of BC-derived gels, in general, is due to their low maintenance requirements. A primary advantage of using PLATIBACGEL is its capability to sustain clear and clean, without any contamination, or even very limited contamination percentage (< 1%) among cultures, and also maintaining its aqueous stability for holding water or moisture inside the medium for long periods, as well as its durability and low degradation. Furthermore, PLATIBACGEL has additional benefits, such as ease of handling, re-autoclaving (rewashing and sterilization), and high absorbance of solutions (in our case MS and/or any additives were supplied as a solution, left to be absorbed by dry PLATIBACGEL). Although these unique characteristics may be relatively weird, the factors that receive more attention are its lower cost production than that of other gelling agents because it can be produced from different environmental waste raw materials, with impressive competitiveness.

Since our findings revealed that T5 treatment, which consisted of BC only, could sustain the plant in vitro growth, meaning that it could do this without adding any further supplements to the medium; this condition was used for two reasons: the first one is to investigate if the bacterial strain can support the growth of the plant and interact positively as a plant growth enhancer. The second one depended on the success of the first assumption, and hence, would reduce the cost required for washing, and cleaning the BC matrix from the bacterial cells, incorporated inside its holes.

Explants were stimulated toward shoot and root formation, resulting in good average lengths in tomato and wheat, which could grow well on media and proliferate actively. Among T1, T2, T4, T5, T6, and T7, the performance of the in vitro-germinated seeds and grown explants was found to be very similar to their development pattern. Concerning PLATIBACGEL medium alone (T5), with no supplementation of plant tissue culture media, or any other nutritive additives to the culture conditions, it was observed that it could provide the best results for all the measured phytotechnical parameters. Tomato seedlings sprouted and shot up densely, and wheat plantlets showed an average of 3–5 branched offshoots per explant (Fig. 4). This indicates the reliability of PLATIBACGEL when combined or augmented with other plant growth regulators and supplements. In contrast, among the seven tested treatments, T3, representing the untreated PLATIBACGEL medium, showed significant differences from all other treatments and was characterized by its lowest potentiality to maintain cultured explants in vitro. This

Fig. 3 In vitro propagation of tomato seeds; **A** In vitro propagation of control tomato seeds cultured on agar gelling medium (T7) 6 weeks after culture incubation, **B** In vitro propagation of tomato seeds cultured on PLATIBACGEL gelling medium (T5) 3 weeks after culture incubation, **C** In vitro propagation of tomato shoots subcultured on PLATIBACGEL gelling medium 2 weeks after culture incubation, **D** PLATIBACGEL gelling medium showing no contamination, purposely left for almost 1 year after tomato seed culture incubation

Fig. 4 In vitro propagation of wheat seeds; **A** In vitro propagation of wheat seeds cultured on PLATIBACGEL medium 1 week after culture incubation, **B** Lengths of micropropagated wheat plantlets 4 weeks after in vitro culture incubation, **C** Branched wheat offshoots from micropropagated plantlets on PLATIBACGEL medium (T5) 4 weeks after culture incubation

considerable difference could be attributable to the microbial content of the untreated cellulose membrane. It probably inhibited growth and suppressed the continuity of plant cell division progression under in vitro culture conditions due to competitive feeding purposes or other enzymatic reactions responsible for securing microbes' nutritious requirements over any other organism.

Although not relatively representing a new material in the industry, the benefit of using BC-derived gel in plant tissue culture media could be attributable to the reuse and application of the material itself.

Our findings revealed that PLATIBACGEL could work successfully as a substitute gelling agent for plant tissue culture media to replace some conventional gelling agents like agar. Although several previous studies have investigated other alternative gelling agents, with respect to other resources and materials, BC membranes have not been investigated precisely in such discipline. Therefore, our research is a pioneering step on the revolutionary pathway for

future generations in the industry of media gelling agents and their production technology.

Besides its functionality and controllability of contamination in plant tissue cultures, it requires further extensive experiments. Future perspectives indicate some obstacles, such as achieving regular satisfactory thickness, the appropriateness of PLATIBACGEL as a BC membrane to be shaped easily and conveniently in plant tissue culture vessels, and its ability to be mixed with other different medium components. The manufacturing technology still faces these challenges on a large scale to apply PLATIBACGEL in the gelling agent market. Moreover, strategies concerning evaluating its reliability for producing whole in vitro plantlets, starting from callus regeneration till reaching the open field, require additional research. There are also concerns about its limitations and applicability in semisolid media. Future research must provide solutions to these obstacles, leading to further productive studies.

Conclusion

BC has been used in different environmental, medical, and industrial applications. The present study was concerned with using BC as a biopolymer matrix, under the acronym PLATIBACGEL, as a promising alternative for traditional media gelling agents for use in vitro seed germination and plant culture techniques. The physical and mechanical properties evaluated in this study, besides in vitro seed germination and tissue culture processes, revealed that the BC membrane could successfully aid the in vitro culture of two different plant species using seven different combinations and/or shifts between basic medium, agar/BC membrane, nutrients, and hormones. To examine the applicability of PLATIBACGEL on multiple plants, five tomato F1 genotypes, and one wheat genotype were used. Treatment 5 (T5) maintained all the used explants for in vitro seed germination and even for micropropagation subcultures. T5 treatment consisted of only PLATIBACGEL without any supplements. Importantly, tomato explants cultured on PLATIBACGEL medium could survive for almost 1 year without any subculturing processes. Seeds cultured on the agar-gelled medium could hardly survive for a few weeks. Furthermore, BC is a promising, inexpensive, and reliable alternative gelling agent for use in plant cell culture, with optimistic applications in the plant cell culture field. In addition, products such as PLATIBACGEL can be used in plant cellular biology and development to clarify plant cell responses to different in vitro culture conditions. However, the prospects of PLATIBACGEL require supplementary investigations to answer deeper significant questions about challenging perspectives such as its in vitro applicability on wider plant types, technical production procedures, and marketing of this promising material.

Patent

PLATIBACGEL is registered as a patent through the Egyptian Patent Office, Ministry of Scientific Research, Academy of Scientific Research and Technological Applications (ASRAT), Egypt, under the Access Number “1314/2021”.

Author contributions GAGA: methodology, investigation, formal analysis, experimental details, and writing the original draft. AKS: methodology, investigation, formal analysis, and writing the original draft. THT: conceptualization, investigation, writing—review and editing. WKE: conceptualization, final review, and editing. YRA-F: final review and editing.

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Declarations

Conflict of interest The authors declare that they have no known conflict, competing interests, or personal relationships that could have appeared to influence the work reported in this paper.

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