Original article

Effects of different gelling agents on the different stages of rice regeneration in two rice cultivars

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ABSTRACT

Plant tissue culture technology offers a solution for meeting the increasing commercial demand on economically important plants such as rice, a widespread dietary staple. However, significant genotype-specific morphogenetic responses constitute a considerable on rice regeneration in plant biotechnology contexts. Aside from genotype dependency, the components of the nutrient media including gelling agents have an important impact on regeneration efficiency. The current study explores the effect of different gelling agents on various stages of rice regeneration in two Egyptian rice cultivars-Sakha104 and Giza178. Media solidified with varying concentrations of a variety of gelling agents (agar, bacto agar, gelrite and phytagel) were tested for their impact on the frequency of callus induction, shoot regeneration and rooting. The results indicated gellan gum (gelrite and phytagel) was superior to agar products (agar and bacto agar) for callus induction. By contrast, no significant differences were found between different gelling agents for shoot regeneration. Gellan gum and media solidified with bacto agar were found to lead to significantly higher root regeneration than agar. The Sakha104 cultivar showed better responses than Giza 178 for callus induction and similar performance to the Giza 178 cultivar for root regeneration irrespective of the gelling agent. This work provides insights into the impact of different gelling agents on the morphogenetic response of two rice cultivars and can be used to help maximize the frequency of rice regeneration.

1. Introduction

From Spanish paella to katsudon in Japan, rice is consumed in almost every culture around the globe. It is a staple food for more than half the world's population and supplies 20% of daily calories worldwide (Fahad et al., 2019). From an economic standpoint, rice provides a livelihood for a fifth of the global population. On the basis that rice was the most significant nourishment crop for billions of individuals, the United Nations assigned 2004 as the year of rice. However, the current rate of grain production is not sufficient to fulfill the anticipated requirements for a rising population. Rice yield is growing at nearly 1% per year, which is far beneath the 2.4% per year increase that has been projected to be required to satisfy demands by 2050 (Ray et al., 2013). This challenge is complicated by a lack of availability of appropriate lands, limited water sources and more importantly, climate change. As a result of these constraints, one of the most feasible solutions is to improve rice productivity by developing superior genotypes that may be tolerant to biotic and abiotic stresses and possess an increased yielding capacity (Khan, 2019).

Transgenic technology enables breeders to design new cultivars by introducing desirable genes into current commercial lines providing an opportunity to maximize yields (Sanagala et al., 2017). Previous investigations on rice transformation have demonstrated low rates of regeneration and recovery of transgenics despite optimization attempts, and confirm the recalcitrant nature of rice (Ge et al., 2006; Lin and Zhang, 2005). Over the years, persistent efforts to produce high throughput regeneration systems by various research groups have resulted in considerable success. However, significant genotype-specific morphogenetic responses remain a

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significant constraint in rice tissue culture. Despite the development of efficient protocols for rice regeneration, in vitro recovery of some rice cultivars is troublesome due to genotype-reliance (Yaqoob et al., 2021). Variations in tissue culture responses indicate that genotypic differences exist between different rice cultivars (Repalli et al., 2019; Feng et al., 2018). Consequently, optimizing effective regeneration systems for individual cultivars is a crucial step before applying transformation techniques. Besides the genotype dependency, the components of the nutrient media (basal mineral salts, organic supplements, growth regulators and gelling agents) have an important effect on regeneration efficiency (Sadhu et al., 2020; Davoudi et al., 2019; Samiei et al., 2019; Venkataiah et al., 2016).

One of the most important factors that affect the chemical and physical characteristics of the culture medium in vitro, is the type and concentration of gelling agent. Gelling agents make the medium firm and influence the diffusion characteristics of the medium. Consequently, solidifying agents can significantly impact the morphogenetic response, growth, and development of tissue cultured plant material (Das et al., 2015). Moreover, they can contribute to the occurrence of hyperhydricity (also known as vitrification) which is a common physiological disorder that causes shoots and leaves to become brittle, with a glassy appearance (Amer and Omar, 2019). Two types of gelling agents are typically used in plant tissue culture: agar and gellan gum. Agar is a polysaccharide extracted from seaweeds, whereas gellan gum (such as gelrite and phytagel) is a bacterial polysaccharide. To our best knowledge, in spite of the important applications in plant tissue culture, there are no comprehensive studies comparing the effect of different types and concentrations of gelling agents on the stages of rice regeneration from callus induction through to shoot regeneration and rooting.

Egypt is the largest producer of rice in both Africa and the Near East region and ranks first in the productivity of rice farms worldwide with an average yield of 9.5 ton/ha (El-Shahway et al., 2016). The high productivity was obtained through the development of new improved varieties that have been released for cultivation in the past few decades. Previously, we investigated many factors affecting rice regeneration and developed an efficient protocol for two selected Egyptian rice cultivars, namely, Sakha104 and Giza178 (Amer, et al., 2017). These genotypes are widely cultivated in Egypt because of desirable features such as their high yield and good taste. While the Giza178 cultivar (Indica/Japonica type) is resistant to blast and tolerant to drought, Sakha104 (Japonica type) is sensitive to both of them (Gaballah et al., 2021). There is a need to explore other factors that might maximize the frequency of regeneration. The primary aim of this study was to investigate the influence of conventional agar (agar and bacto agar) as well as modern gelling agents like gelrite and phytagel on different regeneration stages of these two important Egyptian rice cultivars.

2. Material and methods

2.1. Plant material and surface sterilization of seeds

The seeds were collected from the Rice Research Program, Field Crop Research Institute, Agricultural Research Center, Ministry of Agriculture and Land Reclamation, Egypt from rice cultivars Sakha104 and Giza 178. Selected standardised mature seeds were submerged in 70% ethanol for 2 min and then sterilized in 50% industrial chlorox (5% NaOCl) with 0.1% Tween 20 for 30 min. The seeds were then washed in sterilized distilled water three times and then blotted dried on a sterilized filter paper (90 mm).

2.2. Callus induction

Aseptic mature seeds were cultured on MS medium (Murashige and Skoog 1962) solidified with different concentrations of either agar (6, 7, 8, and 9 g/L), bacto agar (6, 7, 8, and 9 g/L), gelrite (1.5, 2, 2.5, and 3 g/L), or phytagel (1.5, 2, 2.5, 3 g/L). All media were adjusted to pH 5.8 and supplemented with 3% sucrose, 300 mg/L casein hydrolysate and 2 mg/L 2,4-dichlorophenoxy acetic acid (2,4-D). The media were sterilized by autoclaving at 121 °C for 20 min. The media (20 mL) were distributed in petri dishes (9 cm in diameter) that were sealed with parafilm. Each petri plate contained five seeds. The cultures were incubated at 27 °C under dark conditions for 3 weeks. The responded explants (seeds) that successfully induced calli were scored.

2.3. Shoot regeneration

The produced calli were cultured on MS medium solidified with different concentrations of either either agar (6, 7, 8, and 9 g/L), bacto agar (6, 7, 8, and 9 g/L), gelrite (1.5, 2, 2.5, and 3 g/L), or phytagel (1.5, 2, 2.5, 3 g/L). All media were adjusted to pH 5.8 and supplemented with 3% sucrose, 100 mg/L casein hydrolysate, 2 mg/L kinetin, and 0.2 mg/L naphthalene acetic acid (NAA). The media were sterilized by autoclaving at 121 °C for 20 min. The media (50 mL) were distributed in 350 mL glass jars (6 cm in diameter) that were covered with plastic lids. Each jar contained two explants. The cultures were incubated at 27 °C under 16/8 h (light/-dark) photoperiods for 5 weeks. The responded explants (calli) that managed to form shoots were scored.

2.4. Root regeneration

The developed shoots were cultured on MS medium solidified with different concentrations of either agar (6, 7, 8, and 9 g/L), bacto agar (6, 7, 8, and 9 g/L), gelrite (1.5, 2, 2.5, and 3 g/L), or phytagel (1.5, 2, 2.5, 3 g/L). All media were adjusted to pH 5.8 and supplemented with 3% sucrose. The culture vessels and the growth conditions were the same as those described above for shoot regeneration, except that the results of root induction were scored after 3 weeks.

2.5. Statistical analysis

The IBM SPSS Statistic Subscription was used as a statistical research (IBM, Armonk, New York, USA). The significance level was assessed using the variance analysis (ANOVA). Three replicates of 25 explants per replicate were included in each treatment. The analysis for each procedure has given the mean and standard errors (SE). Data are shown as means ± SE and contrasted by Tukey's test at $p \leq 0.05$.

3. Results

The present study represents a comprehensive evaluation of the effect of various gelling agents, i.e., agar, bacto agar, gelrite and phytagel on different stages of rice regeneration. In the callus induction step, statistical analysis revealed that there was a significant effect from the treatments (both from the type and concentration of gelling agent) on the callus induction frequency in both tested cultivars (Table 1). The results showed differences in the percentage of induced calluses between the two tested Egyptian rice cultivars across the same type and concentration of gelling agents. In addition, the Egyptian rice cultivars showed different callus induction frequencies when using different types and concentrations of gelling agents. On the media solidified with agar,

Table 1
Effect of different types and concentrations of gelling agents on callus induction.

Gelling agent	Concentration (g/L)	Callus induction (%)	
		Sakha104	Giza178
Agar	6.0	70.3 ± 1.2 F-a	64.0 ± 1.0 F-b
	7.0	78.3 ± 0.9 DE-a	71.3 ± 0.9 CD-b
	8.0	80.3 ± 1.5 D-a	73.0 ± 1.2 BC-b
	9.0	71.7 ± 1.3 F-a	65.3 ± 0.7 EF-b
Bacto agar	6.0	71.7 ± 0.9 F-a	63.0 ± 1.2 F-b
	7.0	78.7 ± 0.7 DE-a	70.7 ± 0.9 CDE-b
	8.0	81.0 ± 1.2 CD-a	73.0 ± 1.7 BC-b
	9.0	79.3 ± 0.9 DE-a	72.3 ± 1.2 C-b
Gelrite	1.5	81.7 ± 0.9 CD-a	79.3 ± 0.7 A-a
	2.0	89.3 ± 1.3 A-a	81.3 ± 0.9 A-b
	2.5	88.3 ± 0.9 A-a	80.3 ± 0.7 A-b
	3.0	82.3 ± 1.5 BCD-a	72.0 ± 1.2 C-b
Phytagel	1.5	74.7 ± 0.9 EF-a	66.0 ± 1.0 DEF-b
	2.0	81.0 ± 1.0 CD-a	72.3 ± 0.7 C-b
	2.5	87.3 ± 0.9 AB-a	80.3 ± 1.5 A-b
	3.0	86.0 ± 0.6 ABC-a	78.3 ± 0.9 AB-b

Values represent mean ± Standard error of three independent experiments with 25 replicates each. Values marked with either the same uppercase letters within a column or the same lowercase letters within a row were not found to differ significantly using Tukey's test ($p \leq 0.05$).

Table 2
Effect of different types and concentrations of gelling agents on shoot regeneration.

Gelling agent	Concentration (g/L)	Shoot regeneration (%)	
		Sakha104	Giza178
Agar	6.0	81.0 ± 1.2 C-a	80.3 ± 0.9 BC-a
	7.0	89.3 ± 0.9 AB-a	88.3 ± 1.5 A-a
	8.0	91.0 ± 1.5 A-a	88.7 ± 1.2 A-a
	9.0	88.3 ± 0.9 AB-a	86.3 ± 0.9 A-a
Bacto agar	6.0	84.3 ± 0.7 BC-a	80.0 ± 1.0 BC-b
	7.0	90.0 ± 1.2 AB-a	87.7 ± 0.9 A-a
	8.0	90.3 ± 0.9 AB-a	89.0 ± 1.2 A-a
	9.0	91.0 ± 1.5 A-a	89.0 ± 1.0 A-a
Gelrite	1.5	92.0 ± 1.2 A-a	86.3 ± 0.9 A-b
	2.0	93.0 ± 1.0 A-a	89.0 ± 1.2 A-a
	2.5	92.0 ± 1.0 A-a	90.3 ± 1.5 A-a
	3.0	90.3 ± 1.5 AB-a	88.3 ± 0.9 A-a
Phytagel	1.5	84.3 ± 0.9 BC-a	79.0 ± 1.2 C-b
	2.0	90.7 ± 1.2 A-a	88.7 ± 1.5 A-a
	2.5	92.0 ± 1.2 A-a	87.3 ± 0.9 A-b
	3.0	91.3 ± 1.5 A-a	85.7 ± 1.2 AB-b

Values represent mean ± Standard error of three independent experiments with 25 replicates each. Values marked with either the same uppercase letters within a column or the same lowercase letters within a row were not found to differ significantly using Tukey's test ($p \leq 0.05$).

the callus induction frequency ranged from 70.3 ± 1.2% to 80.3 ± 1.5% for the Sakha104 cultivar and from 64.0 ± 1.0% to 73.0 ± 1.2% for the Giza178 cultivar. MS medium solidified with either 7 g/L or 8 g/L agar yielded the highest frequencies of callus induction, with no significant differences between the different cultivars at these concentrations. Out of the different concentrations of bacto agar evaluated, the media containing 7 g/L, 8 g/L or 9 g/L were the most favorable for callus induction with no significant differences between these groups, whereas the medium supplemented with 6 g/L proved to be the least favorable in both cultivars (Table 1). For gelrite, the callus induction frequency varied from 81.7 ± 0.9% to 89.3 ± 1.3% for the Sakha104 cultivar, with the maximum frequency being achieved at 2 or 2.5 g/L with no significant differences between these concentrations. In the Giza178 cultivar, the maximum efficiencies (81.3 ± 0.9%, 80.3 ± 0.7% and 79.3 ± 0.7%) were observed on MS media solidified with 1.5, 2.0 or 2.5 g/L gelrite with no significant differences between these optimal concentrations. For phytagel, the best callus induction frequency was obtained on media containing either 2.5 or 3.0 g/L in both cultivars with no significant differences observed between them. Taken together, the best response in terms of callus induction was obtained when either gelrite was used at a concentration of 2.0 or 2.5 g/L or phytagel was used at a concentration of 2.5 or 3.0 g/L. In addition, it was observed that the callus induction ability of Sakha104 was higher than that of Giza178, irrespective of the type or concentration of the solidifying agent.

The shoot regeneration frequency ranged between 81.0 ± 1.2– 93.0 ± 1.0% for the Sakha104 cultivar and ranged between 79.0 ± 1.2–90.3 ± 1.5% for the Giza178 cultivar (Table 2). Except for the lowest concentration of agar, bactoagar and phytagel, there were only slight differences between treatments in both cultivars. Analysis of variance indicated no significant differences between the four tested gelling agents at their optimum concentrations. No trends were observed to show a connection between the gelling agent and shoot regeneration percentages.

The root regeneration frequency was greatly affected, however, by different concentrations of each gelling agent. The results in Table 3 revealed that the rooting response ranged from 79.3 ± 1.2% to 85.3 ± 1.2% for the Sakha104 cultivar and from 78.0 ± 1.0% to 84.3 ± 0.9% for the Giza178 cultivar when agar was used. However, a significantly higher root regeneration fre-

Table 3
Effect of different types and concentrations of gelling agents on root regeneration.

Gelling agent	Concentration (g/L)	Root regeneration (%)	
		Sakha104	Giza178
Agar	6.0	79.3 ± 1.2 C-a	78.0 ± 1.0 D-a
	7.0	85.3 ± 1.2 B-a	84.3 ± 0.9 BC-a
	8.0	84.7 ± 0.9 BC-a	84.3 ± 1.3 BC-a
	9.0	79.7 ± 1.2 BC-a	78.7 ± 1.2 CD-a
Bacto agar	6.0	94.3 ± 0.9 A-a	93.3 ± 1.5 A-a
	7.0	95.0 ± 1.0 A-a	94.0 ± 1.2 A-a
	8.0	93.7 ± 1.2 A-a	94.7 ± 1.2 A-a
	9.0	85.0 ± 1.2 BC-a	86.0 ± 1.0 B-a
Gelrite	1.5	93.7 ± 1.5 A-a	93.3 ± 1.2 A-a
	2.0	94.3 ± 0.9 A-a	95.0 ± 1.2 A-a
	2.5	93.7 ± 1.2 A-a	95.3 ± 0.9 A-a
	3.0	85.3 ± 1.2 B-a	83.3 ± 1.5 BCD-a
Phytagel	1.5	94.0 ± 1.2 A-a	93.0 ± 1.2 A-a
	2.0	94.3 ± 0.9 A-a	94.3 ± 1.5 A-a
	2.5	92.3 ± 1.2 A-a	93.3 ± 0.9 A-a
	3.0	84.3 ± 1.3 BC-a	83.7 ± 1.2 BCD-a

Values represent mean ± Standard error of three independent experiments with 25 replicates each. Values marked with either the same uppercase letters within a column or the same lowercase letters within a row were not found to differ significantly using Tukey's test ($p \leq 0.05$).

quency was obtained when bacto agar, gelrite, or phytagel were used. 6, 7 and 8 g/L of bacto agar and 1.5, 2.0 and 2.5 g/L of both gelrite and phytagel were shown to be the best conditions to maximize the frequency of root regeneration in both cultivars. At the same time, the results showed that there were no significant differences between the root regeneration frequency of the Sakha104 cultivar compared to the Giza178 cultivar. Fig. 1 represents a regeneration scheme for rice stages from mature seeds up to fully rooted plantlets.

In this study, clear differences were observed between different gelling agents concerning the physical characters of the media across all three stages of rice regeneration. Media solidified with gellan gum (gelrite and phytagel) were more transparent compared to media solidified with either type of agar (agar and bacto agar) (Figs. 2 and 3). In addition, they were more rigid and brittle.
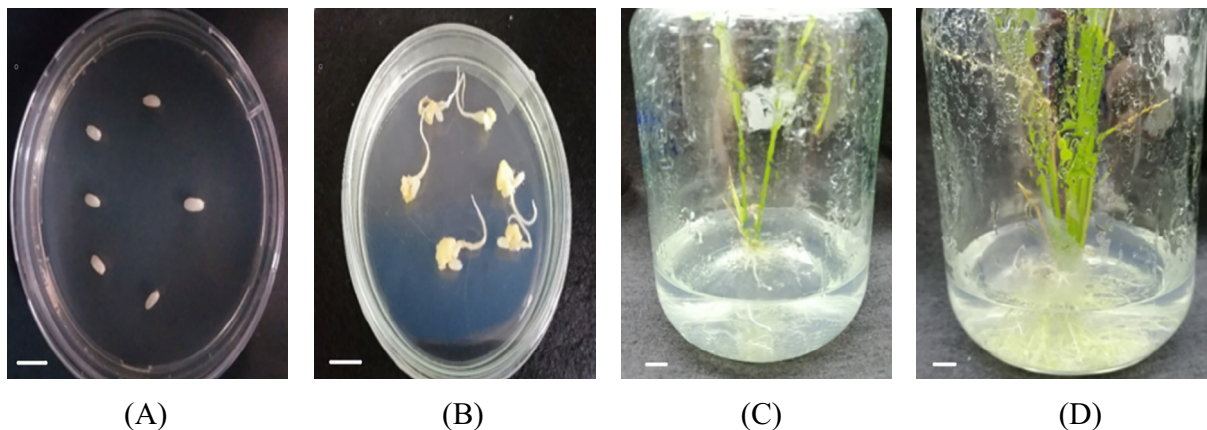
Fig. 1. Regeneration cycle of rice. A: mature seeds, B: callus induction, C: shoot regeneration, D: full plantlet formation with extensive root system (scale bar = 10 mm).
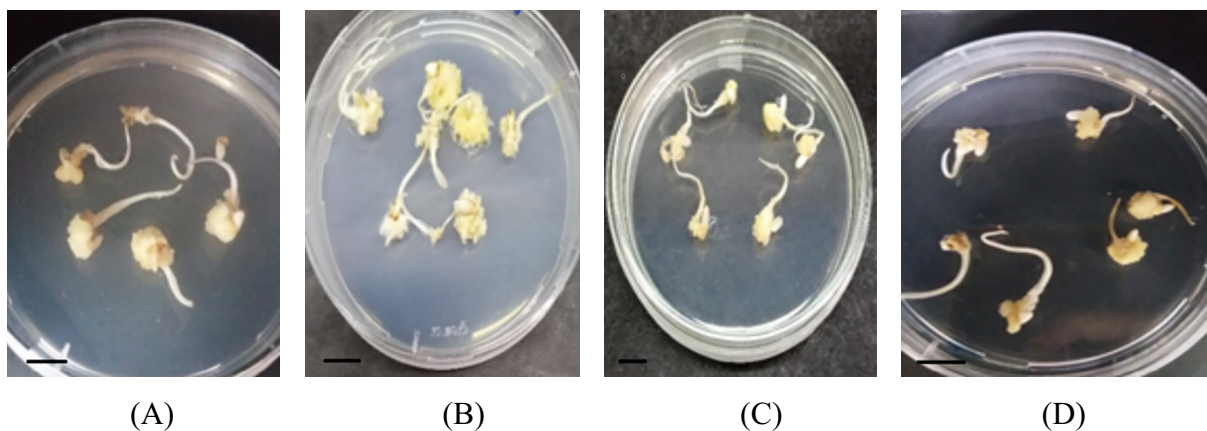

Fig. 2. Callus induction of rice in media solidified with A: 8 g/L agar, B: 8 g/L bacto agar, C: 2 g/L gelrite and D: 2.5 g/L phytagel (scale bar = 10 mm).
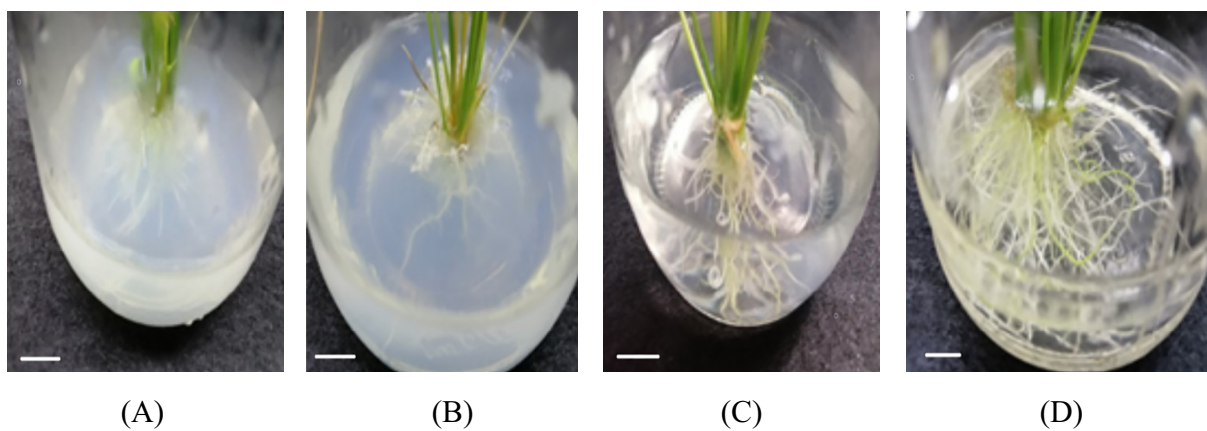

Fig. 3. Rooting of rice in media solidified with A: 7 g/L agar, B: 7 g/L bacto agar, 2 g/L gelrite and D: 2 g/L phytagel (scale bar = 10 mm).

4. Discussion

The present investigation studied the influence of different concentrations of agar, bacto agar, gelrite and phytagel on callus induction, shoot regeneration and subsequent root regeneration of two Egyptian rice cultivars: Sakha104 and Giza178. Irrespective of the cultivar, we observed that all of the four tested gelling agents supported the morphogenetic response of rice to varying degrees (Tables 1, 2 and 3). The differing effects of gelling agents are likely caused by physiochemical characteristics, such as diffusion rate of nutrients, elemental and organic impurities and gel strength (Jain et al., 2009). Gelling agents offer plants direct physical contact with nutrients and promote growth (Nery et al., 2021). The composition of gelling agents will directly determine plant growth by preferring the binding of certain nutrients over others. Our findings correspond to several studies which show that the gelling agent itself causes fluctuations in the response to plants with otherwise identical nutrient media (Sah et al., 2014; Jain et al., 2009). Moreover,

differences in morphogenetic responses were reported even between different brands of the same gelling agent (Sulusoglu, 2014). Solidifying agents from different manufacturers may differ in terms of the presence of impurities, level of solidification and overall composition. This may explain the differential response between bacto agar and agar in our rooting experiment (Table 3). In addition, it was reported previously that the same gelling agent at various concentrations has a profound influence on water retention and the regulation of the moisture regime of the medium (Repalli et al., 2019). For this reason, supplementation of gelling agents to the correct concentration is needed to meet different requirements at different stages of plant tissue culture i.e., callus induction, shoot regeneration and rooting.

Although the results clearly showed that all of the four gelling agents supported callus induction, media that was solidified with gelrite or phytagel at their optimum concentrations demonstrated significantly higher performance than media that was solidified with agar or bactoagar (Table 1). The superiority of gellan gum (gelrite and phytagel) over agar products may be attributed to the impurities found in agar. Agar consists of two polysaccharides: linear agarose, and a heterogeneous mixture of smaller molecules called agropectin, with agarose accounting for about 70% of the mixture (Das et al., 2015). An explant growth and the proliferation of calves can be inhibited by the Agar that contains agropectins and some other organic impurities. In contrast, gellan gum, which is a water-soluble anionic polysaccharide, is a highly purified natural gelling agent with a consistent quality. It therefore contains none of the contaminating impurities found in agar. Phytagel and gelrite have both been reported to be free from such impurities, with one grade satisfying a variety of plant tissue culture needs. Moreover, substantially smaller quantities are able to produce gels of comparable hardness to agar (Sah et al., 2014). Our results are in agreement with those reported by Sah et al. (2014) who demonstrated the advantage of phytagel over agar for callus induction frequency in rice (using the Kitaake cultivar). Juturu et al. (2016) also found that medium solidified with phytagel gave 30% higher callus fresh weight using the Swarna rice cultivar. In addition to phytagel, gelrite was found to show higher efficiency for callus induction as compared to agar using Swarna and Mahsuri rice cultivars (Jadhav et al., 2011).

In contrast to the callus induction step, where gelrite and phytagel performed better than agar, solidifying media with gellan gum was not found to enhance the frequency of shoot regeneration in either rice cultivar (Table 2). Our results showed that no statistical differences were found between the four tested gelling agents when used at their optimum concentrations. Conflicting data have been observed in previous studies regarding this point. While some researchers have reported higher regeneration frequencies on media solidified with gellan gum (Manokari et al., 2020; Rodrigues et al., 2017; Sun et al., 2008), other investigators have demonstrated the opposite (Repalli et al., 2019; Mitić et al., 2012). Combining both kinds of gelling agents (gellan gum and agar) proved to be the best (Sah et al., 2014). Lastly, similar to our results, Tsao and Reed (2002) reported that the three different gelling treatments (agar, gelrite, and a combination) did not produce significant differences in the regeneration frequency for different blackberry genotypes tested. The variability in these results suggests that the effect of a given gelling agent may be dependent on the specific plant species or genotype (cultivar). Different genotypes may show different sensitivity to different gelling agents. This is also a possible explanation for our observation that there are clear differences in the callus induction frequency between the two rice cultivars (Table 1). The Sakha104 cultivar showed significantly higher callus induction frequency than Giza178 cultivar cultured on the same medium. The two chosen cultivars represented different germplasms of Egyptian rice and consequently derive from a different genetic background. Our results suggest that the predisposition toward gelling agents may be primarily genotype-dependent. Variations might occur among species and between different varieties or cultivars within the same plant species, thus necessitating studies that test other plant species, varieties, and cultivars. The present findings are consistent with previous observations that different cultivars of rice exhibit a variable tendency for callus induction (Zaidi et al., 2006; Ge et al., 2006; Nishimura et al., 2005). Genotypes, as widely accepted, are considered to be a major limitation which restricts the successful regeneration of rice cultivars (Khan et al., 2019). A lot of literature has been published on optimized tissue culture protocols for rice, but they are mainly genotype-dependent.

In this study, even though Sakha104 cultivar showed a better response than Giza 178 for callus induction, it displayed equal performance with the Giza 178 cultivar in root regeneration (Tables 1 and 3). Regeneration is usually described as a broad spectrum of phenomena. Regeneration can range from restoring a wound in single tissues to developing an entirely new structure, which might be composed of multiple tissues, organs, or even individuals (Ikeuchi et al., 2016; Sena, 2014). Totipotency is the term used to refer to regeneration collectively. Even though all plant species can be totipotent, it can get difficult to identify the culture conditions and stimuli required to express totipotency (Nishimura et al., 2005). *De novo* organogenesis is a way to grow an entire plant where the roots and shoots subsequently develop after forming ectopic apical meristems in plant cuttings or explants. Alternatively, plants can also be developed through somatic embryogenesis, whereby isolated protoplasts or cells first develop cellular structures similar to zygotic embryos (Ikeuchi et al., 2016). Both these mechanisms of regeneration occur directly from parental tissues or indirectly via the development of a callus. The exogenous complement of plant hormones, in which a high proportion of auxin to cytokinin usually contributes to root recovery, may improve in vitro regeneration capability, but a low proportion supports shoot regeneration (Amer et al., 2019). Most recently, modern imaging and genomic research is used to study the molecular foundations of *de novo* organogenesis in plants. In short, they discovered that several genes that were up-regulated during early phases of shoot development, were the same genes that respond to cytokinin induction (Hwang et al., 2002; Rashotte et al., 2003; To et al., 2004; Che et al., 2006) and these genes differed from those involved in the development of the new root meristems caused by the accumulation of auxin (Che et al., 2006; Goh et al., 2012; Liu et al., 2014). Together, it could explain our findings as mentioned earlier: the presence of two separate groups of genes involved in the two developmental pathways. It is likely that a complicated genetic mechanism, which distinct groups of genes may control, is involved in response to in vitro tissue culture. Usually, Researchers typically use "regeneration capacity" to describe the efficiency of regeneration of the whole plant (regenerable or recalcitrant). This would be better if the regenerative ability of each particular developmental pathway is determined independently i.e., formation of shoots, roots and callus.

5. Conclusion

The present study represents a systematic comparison of the morphogenetic response by two Egyptian rice cultivars to various types and concentrations of gelling agents. While callus induction and root regeneration were significantly affected by choice of gelling agents, there were no significant differences between gelling agents on the shoot regeneration stage. In this respect, media solidified with gellan gum (gelrite and phytagel) were superior for callus induction over media solidified with agar products (agar and bacto agar). In addition to gellan gum, media gelled with bacto agar

showed a better response to root regeneration compared with agar. Regardless of the type of gelling agent, Sakha104 cultivar was found to have a better response than Giza 178 in terms of callus induction but an equal performance in terms of root regeneration. The results of this research provide scientific insights into the impact of gelling agents on the morphogenetic response of different rice cultivars and can be used to maximize the frequency of rice regeneration in plant tissue culture.

Declaration of Competing Interest

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

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