Food Research 7 (5) : 275 - 282 (October 2023)
Journal homepage: https://www.myfoodresearch.com

FOOD RESEARCH

FULL PAPER

The effect of plant growth regulators, carbon sources and gelling agent on the callus regeneration of Malaysian rice MR219 (*Oryza sativa* L.)

[1,2]Md Saad, N., [3]Teo, C.H., [4]Ab Rahman, Z. and [1,*]Zainal, Z.

[1]*Department of Biosciences and Biotechnology, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, UKM, 43600 Bangi, Selangor, Malaysia*
[2]*Plant Biotechnology Centre, Agro-Biotechnology Institute, National Institutes of Biotechnology Malaysia, Jalan Bioteknologi, 43400 Serdang, Selangor, Malaysia*
[3]*Centre for Research in Biotechnology for Agriculture, University of Malaya, Lembah Pantai, 50603 Kuala Lumpur, Malaysia*
[4]*Biotechnology and Nanotechnology Research Centre, MARDI Headquarters, MARDI HQ, Persiaran MARDI-UPM, 43400 Serdang, Selangor, Malaysia*

Article history:
Received: 1 March 2023
Received in revised form: 19 April 2023
Accepted: 7 August 2023
Available Online: 24 October 2023

Keywords:
Carbon source,
Indica rice MR219,
Embryogenic callus,
Shoot regeneration

DOI:
https://doi.org/10.26656/fr.2017.7(5).096

Abstract

The development of a highly efficient tissue culture system for indica rice is crucial to speed up the implementation of gene editing in breeding programmes as well as to explore the roles of indica genes. However, because indica rice types are recalcitrant to *in vitro* response, there are only a few reports on the successful transformation process on indica rice due to recalcitrant response to *in vitro* response. The present study attempted to identify the effects of plant growth regulator (PGR), carbon sources and different concentrations of gelling agent on embryogenic callus induction and rice regeneration of *Oryza sativa* var MR219. The PGR used were 1 or 2 mg/L 6-benzylaminopurine (BAP) and 1-Naphthaleneacetic acid (NAA) and the carbon sources were 30 g/L sucrose and maltose. Three different gelling agent concentrations (3 mg/L, 6 mg/L or 9 mg/L) were evaluated. The highest frequency of friable callus induction (87%) was observed in callus induction media (CI) containing Murashige and Skoong media (MS) supplemented with 3 mg/L 2,4-dichlorophenoxy acetic acid (2,4-D) and 0.3 mg/L kinetin. Histological studies of the calli showed the presence of embryogenic calli with small intercellular spaces compared to non-embryogenic areas which developed on the hormone MS media. The highest regeneration frequency (50%) was achieved when friable calli were cultured on MS medium containing 3% sucrose, 1 mg/L BAP, and 1 mg/L NAA with 9% phytagel. The callus grown on sucrose media showed the highest number (66.13%) of albino plantlets, while the number of albino plantlets on maltose media was smaller (20%). In conclusion, this optimised mature embryo-derived callus of MR219 rice is responsive to regeneration and could be amenable to genetic transformation studies.

1. Introduction

Rice is a vital commodity crop that provides daily calories for about 30 million people in Malaysia, with rising demand annually. Thus, food safety assurance must be considered, especially in developing countries such as Malaysia, to ensure adequate food supply to meet the population's needs. Abiotic stress and biotic stress affect plant development and growth (Liu *et al.,* 2018), so various studies have been conducted to improve rice yield via conventional breeding and genetic manipulation or CRISPR-based genome editing. Conventional breeding such as hybrid methods can improve yields and rice genetics but require at least two years to produce new varieties with the desired characteristics. In addition, efforts have been made to increase rice productivity and quality by combining tissue culture techniques with genetic transformation by methods of direct or indirect DNA transfer (Adlak *et al*., 2019). *Agrobacterium*-mediated is commonly used in rice to effectively produce transgenic rice (Jain *et al*., 2022; Yaqoob *et al*., 2017). Several varieties of rice have been successfully genetically transformed so far; these include transgenic rice plants expressing the *Cry1Ac:: asal* fusion proteins have been developed to provide

*Corresponding author.
Email: *zz@ukm.edu.my*

eISSN: 2550-2166 / © 2023 The Authors.
Published by Rynnye Lyan Resources

FULL PAPER

resistance to lepidopteran and hemipteran insects (Boddupally *et al*., 2018). These transgenic rice plants displayed dramatically increased insect resistance, especially against the chewing (lepidopteran) and sucking (hemipteran) types. The constitutive overexpression of the rice chitinase in transgenic rice enhances resistance to a wide range of fungal pathogens (Karmakar *et al*., 2016). Embryogenic callus and shoot regeneration are the most critical steps in the genetic manipulation of rice plants (Jain-Raina and Babbar, 2011), however, genetic transformation is still challenging for both Indica and Japonica rice due to their low regeneration rate after transformation (Liang *et al*., 2021). Indica rice is more recalcitrant than Japonica rice in genetic transformation as it showed low callus induction, proliferation, and regeneration (Zuraida *et al*., 2010; Sundararajan *et al*., 2020). Nevertheless, several protocols have been reported to induce callus and regeneration but only achieve low-yield regeneration, thus nature remains recalcitrant to both routine *in vitro* regeneration and genetic transformation. The optimal formulation of micro and macro compositions may effectively reduce this recalcitrant problem. Moreover, the type of explant sources such as hypocotyls, young leaves, immature embryos (Leelavathi *et al*., 2004), mature embryos and anthers (Ali *et al*., 2021; Maharani *et al*., 2020) may influence the success rate of obtaining embryogenic callus and regeneration.

Explant growth and development *in vitro* are impacted by various parameters that affect the efficacy of callus induction and regeneration of rice, including the type of explant employed, culture medium components, gel-forming agents, and Agrobacterium strains used (Singh *et al*., 2020). Each explant typically requires a different culture media consisting of carbon sources, elemental compounds, inorganic and organic salts, and plant hormones (auxins and cytokines). The addition of phytohormones such as auxin stimulates cell division, as well as proliferation from the explants (Mengs *et al*., 2018). Auxin stimulates the expression of the CDC2 gene which encodes a regulatory protein (protein kinase) for the cell cycle. The auxin treatment of explants is a vital plant growth regulator of somatic embryogenesis in plants (Wójcik *et al*., 2020). The auxin synthetic 2,4-D can generate DNA hypermethylation to maintain cells in a state of highly active mitosis (Joshi and Kumar, 2013). Furthermore, the carbon source provides the energy for development including shoot proliferation and root induction (Martinez *et al*., 2021). Media supplemented with sucrose is optimum for growth and biomass accumulation in shoot cultures, followed by maltose, glucose and fructose (Praveena and Veeresham, 2014).

This research aimed to develop media formulation and optimization of PGR and carbon sources and phytagel concentrations for maximum embryogenic callus and proliferation from MR219 seed explants. Thus, leading to a more effective *in vitro* method for indica rice that may be employed in gene transformation for crop improvement.

2. Materials and methods

2.1 Rice materials and explant preparation

The explant source was 4-month-old seeds of the rice variety Malaysia MR219 supplied by MARDI Seberang Perai, Penang. First, the husks were carefully removed to avoid damage to the embryo and the seed was surface sterilised with the fungicide solution, Benomyl (Benofun 50 WP, Zagro Chemicals) for 30 mins with agitation. After being washed in distilled water to remove any debris and chemical residue, the explants were sterile-treated in 70% ethanol for 30 seconds while being gently shaken. Finally, the explants were sterilised with 50% bleach supplemented with 0.015% Tween-20 (Bio-Rad, USA) with agitation for 1 hr. The sterile explants were rinsed 5 times with sterile water and then leave them dried for 15 mins in laminar airflow.

2.2 Embryogenic callus induction

MS media with vitamins (Duchefa Biochemie, Netherlands) was the primary medium used in the callus induction media. Sterile seeds were cultured on callus induction media code (C1-C12) supplemented with 2,4-D (1.0, 2.0, 3.0 mg/L), kinetin (0.3, 1.0 and 1.5 mg/L), 3% sucrose (Duchefa Biochemie, Netherlands) and 3 g/L phytagel (Duchefa Biochemie, Netherlands) at 25°C in a dark room for 6-8 weeks. All media were acidified to a pH of 5.8 before being autoclaved at 121°C for 15 mins. Calli were produced after 3–4 weeks and recorded as the percentage of callus induction, then subcultured every 2 weeks onto fresh media.

2.3 Histological analysis

The rice calli cultured for six weeks on C10 media were selected and fixed in fixation solution solution for 24 hrs according to the protocol by Vega *et al*. (2009). Fixed calli were then horizontally embedded in paraffin wax using the tissue embedding system EG1150 (Leica Biosystem, Germany) after being dehydrated using a different dilution of ethanol from 70% to 100%). The paraffin-embedded calli were cut into 6-8 µm sections using a rotary microtome (Leica Biosystem, Germany) for Hematoxylin and Eosin staining (Surgipath, Leica). The sections were viewed under a light microscope, virtual slide system (Olympus, Japan) and Olympus VS-ASW software.

https://doi.org/10.26656/fr.2017.7(5).096 © 2023 The Authors. Published by Rynnye Lyan Resources

2.4 Rice regeneration and acclimatization in different types of carbon sources, concentrations of phytagel and plant growth regulators

Six-week-old calli were transferred straight to regeneration media containing PGR, BAP (1.0 and 2.0 mg/L), and NAA (1.0 and 2.0 mg/L) together with 3% sucrose or 3% maltose and phytagel (3%, 6%, or 9%). Calli were incubated at 25±2°C under 16 hrs photoperiod and 8 hrs dark conditions for 3-6 weeks. After 6 weeks in regeneration media, the regeneration frequency was calculated as a percentage. Plantlets with healthy roots were transferred into pots with nutrient soil for acclimatization *ex vitro* (25±2°C). Briefly, the rice plantlets were removed from the regeneration medium and the roots were washed in tap water to remove excess phytagel, then transferred to plastic bags with peat soil and maintained in a greenhouse. The grains were harvested after 120 days.

2.5 Collecting and analysing data

After 4 weeks, the percentages of callus formation were found. After 6 weeks in the regeneration medium, the number and percentage of new shoots were counted. The percentages of callus and regeneration were calculated as follows:

$$\text{Callus formation rate (\%)} = \frac{\text{Number of explants producing calli}}{\text{Total number of explants plated}} \times 100$$

$$\text{Healthy plantlets regeneration (\%)} = \frac{\text{Total number of healthy plantlet}}{\text{Total number of calli regenerated}} \times 100$$

$$\text{Albino plantlets regeneration (\%)} = \frac{\text{Total number of albino plantlet}}{\text{Total number of calli regenerated}} \times 100$$

Data from the media code C1-C12 and R1-R18 were analysed with MINITAB 20 software and presented as means of standard errors. A one-way analysis of variance (ANOVA) was employed to identify statistically significant deviations.

3. Results and discussion

3.1 Callus induction

Embryogenic callus formation was observed after 14 days of incubation in all callus induction media, as shown in Figure 1A. The combination of 3 mg/L 2,4-D and 0.3 mg/L kinetin in MS media C10 and an incubation period of 4 weeks resulted in an enhanced callus induction rate of 87% (Table 1) compared to the previously reported 85% (Zuraida *et al*., 2011) and 84% (Sivakumar *et al*., 2010). Two-week-old embryogenic calli were subcultured on a fresh CI media C10 for callus proliferation (Figures 1A and 1B) and were densely arranged globular, nodular, and bright yellow tissues (Figures 1C and 1D). Ali *et al*. (2007) and Ebrahimi and Payan (2013) reported that the best somatic embryogenesis can be obtained from a combination of 2,4-D and cytokinins. However, another study showed differences in the callus production capacity of Indica rice, even when cultured on the same media (Ge *et al*., 2006). Ozawa and Kawahigashi (2006) and Wang *et al*. (2018) reported that different rice cultivars differed in their capacity for callus formation and regeneration, with cultivars with a high capacity for callus formation exhibiting high levels of *Os22A* gene expression.

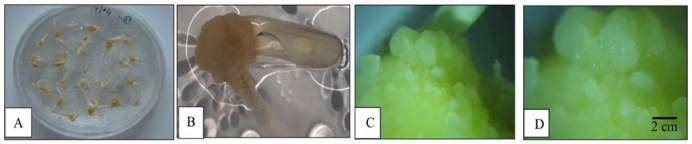

Figure 1. Somatic embryogenesis in rice MR219. A) Rice embryos were obtained after 2 weeks in callus induction media containing hormone 3 mg/L 2,4-D and 0.3 mg/L kinetin, B) Microphoto of callus (100× magnification), C) Somatic embryogenic callus obtained after 8 weeks in callus induction media and ready for use in Agrobacterium-mediated transformation (40× magnification), D) Somatic embryogenic callus under a magnification of 100×.

3.2 Histological observation of callus formation in rice

The cross-section of rice friable yellowish calli at week six is shown in Figure 2A-D, revealing the presence of proembryogenic (pc) cells in the peripheral zone (Figure 2A) and two types of cells (Figure 2B). Figure 2C shows non-embryogenic calli have loosely arranged cells and large spaces, indicating that the non-embryogenic calli have greater water retention capacity compared to embryogenic calli (Akaneme and Eneobong, 2008). Figure 2D shows compact embryogenic calli with small intercellular spaces. This observation is akin to changes in calli development observed in vanilla plants (Palama *et al*., 2010), where embryogenic calli are composed of huge clusters of

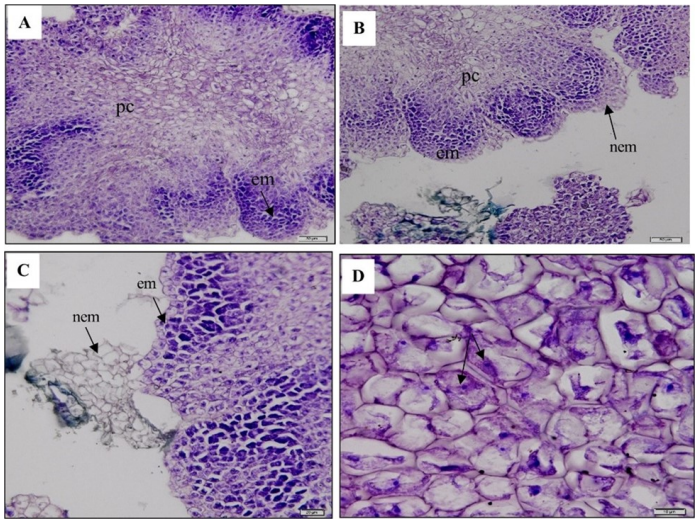

Figure 2. Light micrographs of calli produced by mature seed explants of MR219 on C10 media (A-D). (A) Friable callus derived from rice with proembryogenic cells (pc), (B) Embryogenic (em) and non-embryogenic (nem) masses within the callus tissue, C) Cells in non-embryogenic calli are more spread out than in embryogenic calli, D) The arrow indicates proembryo cells.

https://doi.org/10.26656/fr.2017.7(5).096

© 2023 The Authors. Published by Rynnye Lyan Resources

Table 1. Callus formation performance of MR219 in response to varying PGR concentrations and combinations. Analysis using the Tukey method and 95% confidence.

Media code	Concentration of 2,4-D (mg/L)	Concentration of Kinetin (mg/L)	Average number of explants inducing callus	Callus formation rate (%) Mean±SEM
C1	1	0	12.00	40±1.15^{c}
C2	1	0.3	1.33	4±0.33^{d}
C3	1	1	1.33	4±0.33^{d}
C4	1	1.5	1.67	5±0.33^{d}
C5	2	0	19.33	64±1.33^{b}
C6	2	0.3	21.67	72±1.20^{ab}
C7	2	1	5.67	19±1.20^{d}
C8	2	1.5	2.33	8±0.33^{d}
C9	3	0	17.00	57± 0.57^{b}
C10	3	0.3	25.67	87±1.20^{a}
C11	3	1	3.67	12±1.20^{d}
C12	3	1.5	3.33	11±0.88^{d}

Values with different superscripts are statistically significantly different (p<0.05). In future, this media formulation (C10) can be used for genetic transformation process.

cohesive cells. The results of tissue sections of embryogenic callus in *Oryza sativa* showed characteristics large nucleus, cells are isodiametric and small with a dense cytoplasm. In addition, close arrangement occurred in the interior and also the embryogenic cell clusters composed of small cells with vigorous division. The embryogenic cells adhere to meristematic cells in that they divide vigorously, have a dense cytoplasm with several starch grains, a large nucleus with a prominent nucleolus, few vacuoles, thin cell walls, and a high metabolic rate (Quiroz-Figueroa *et al*., 2006; Mesbah *et al*., 2021). Additionally, proembryogenic cells comprised of vertically dividing tiny cells were found inside the actively dividing callus. These cells have the potential to function as totipotent stem cells to induce somatic embryogenesis or organ development. These proembryogenic cells readily respond to the auxin or cytokinin ratio, resulting in organ differentiation or somatic embryogenesis. This distinction is typically dictated by the direction and location of division of pre-procambial cells (Zhu *et al*., 2020). In *Oryza sativa*, the embryogenic calli inner epidermal cells were loosely dispersed, irregularly organised, and had a lot of intercellular space. They were elongated or large, highly vacuolated large parenchymal cell clusters with less nucleus, which was similar to the result reported by Vega *et al*. (2009) and Ming *et al*. (2019). Hence, the histology analysis would allow us to identify the embryogenic callus for efficient plantlet regeneration and development.

3.3 Shoot regeneration and acclimatization of rice plantlets

After 3-4 weeks of culturing somatic embryos on a regeneration medium, the number of healthy plantlets (Figure 3) and albino plantlets produced were recorded (Table 2), showing that the media combination of R1, R2, and R3 did not show any regeneration of rice plants. Most embryogenic calli cultured on regeneration media supplemented with 3 g/L phytagel produced dormant calli, embryogenic calli that only turned green but did not produce plant regeneration. The same observations were reported by Ali *et al*. (2007), where the authors stated that part of the callus of Indica and Japonica rice turned into green dormant callus without plantlet regeneration when cultured on a low phytagel concentration medium (0.3%). However, when calli were transplanted to a higher phytagel concentration medium, most green calli produced shoots, indicating that higher phytagel concentrations allow callus differentiation to occur compared to lower phytagel concentrations. Poraha *et al*. (2016) found that the regeneration frequency at a phytagel concentration of 5.2 g/L was higher than 2.6 g/L, similar to the present study which showed that the regeneration frequency of Indica rice var MR219 in 9 g/L phytagel (50%) was significantly higher than in 3 g/L phytagel (Table 2). The 9 g/L phytagel medium showed more regeneration compared to the medium with 3 g/L and 6 g/L phytagel. The only medium with 6 g/L phytagel that produced shoots was

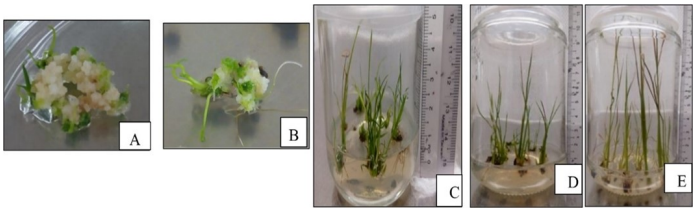

Figure 3. A) Green shoot on the surface of callus after transfer to regeneration media for 2 weeks. (B) Green shoot production after 6-8 weeks of culture, (C-E) Multiple shoots and roots developed on MS media enhanced with 30 g/L maltose, 1 mg/L BAP, 2 mg/L NAA and 3 g/L phytagel.

https://doi.org/10.26656/fr.2017.7(5).096 © 2023 The Authors. Published by Rynnye Lyan Resources

Table 2. Percentage of regeneration, number of healthy plantlets and number of albino plantlets produced for MR219 variety rice.

Type of sugar	Concentration of phytagel (g/L)	PGR (mg/L)		Regeneration percentage (%)±SE	Observation		Media code
		BAP	NAA		Healthy shoots percentage (%)	Albino shoots percentage (%)	
Sucrose (30 mg/L)	3	1	1	-	-	-	R1
		2	1	-	-	-	R2
		1	2	-	-	-	R3
	6	1	1	-	-	-	R4
		2	1	-	-	-	R5
		1	2	11±0.07[b]	93	6.9	R6
	9	1	1	50±0.23[a]	48.4	51.6	R7
		2	1	33±0.17[a]	33.7	66.13	R8
		1	2	22±0.11[a]	48.9	51	R9
Maltose (30 mg/L)	3	1	1	6±0.06[b]	33.3	66.7	R10
		2	1	3±0.03[b]	100	0	R11
		1	2	11±0.07[b]	100	0	R12
	6	1	1	0±0.00[b]	0	0	R13
		2	1	3±0.03[b]	71.4	28.5	R14
		1	2	11±0.07[b]	0	100	R15
	9	1	1	3±0.03[b]	0	100	R16
		2	1	11±0.09[b]	80	20	R17
		1	2	11± 0.05[b]	80	20	R18

Values with different superscripts are statistically significantly different (p<0.05).

R6 where a combination of 1 mg/L BAP and 2 mg/L NAA was used as PGR. Different phytagel concentrations influence the water and moisture content in the media (Repalli *et al*., 2019).

The MR219 variety showed the highest regeneration (50%) in the R7 medium compared to R8 and R9 media which showed only 33% and 22% regeneration, respectively. The number of green plantlets produced from the R9 medium (48.9% green plantlets) was also higher compared to R7 and R8 media. However, most albino plantlets (66.13% albino plantlets) were observed in the R8 medium. The R7 and R8 treatment mediums produced 48.4% and 33.7% green plantlets respectively and albino plantlets of 51.6% and 51% plantlets each. This study showed that shoot regeneration increased when cultured on a medium with a high concentration of phytagel (6 or 9 g/L phytagel). Callus cultured on a medium with higher gel concentration enhances shoot regeneration (Jain and Babbar, 2002). There was no difference in MR219 regeneration on R7, R8 and R9 media. The shoot regeneration in R7 media is shown in Figure. 2. Overall, fewer albino plantlets than green plantlets were observed in the medium containing maltose as the carbon source, with 100% plantlets observed in R11 and R12 medium, no albino plantlets in R12 and 100% albino plantlets on R15 and R16. The R11 and R12 media were more suitable for plantlet regeneration as evidenced by the higher regeneration percentage of green plantlets and no albino plant regeneration. Even though albino plantlets lost the ability to produce roots (Figure 4), they grew well and formed numerous new shoots, which shows that they can obtain sugar and nutrients from the regeneration media. The albino state occurs due to cytogenetic variations caused by hormonal processing in the media. Albino plants are difficult to root and cannot survive when propagated *ex vitro* (Dewir *et al*., 2018). They experience complete chlorophyll loss, possibly due to differences in genotype and environmental stresses, resulting in genome modifications that reduce chlorophyll biosynthesis and ultimately damage the photosynthetic system (Dewir *et al*., 2018; Kumari *et al*., 2009). A gene that encodes rice's lone Octotricopeptide Repeat Protein (RAP) regulates the albino leaf phenotype. RAP is crucial for the initial stages of chloroplast formation in addition to rRNA maturation (Kleinknecht *et al*., 2014). Carbon sources are important in tissue culture media for growth, cell development and biosynthetic processes (Chutipaijit and Sutjaritvorakul, 2018). Maltose-containing media showed no, or fewer albino plants produced compared to sucrose-containing media. Although both are water-soluble disaccharides, sucrose consists of a combination of glucose and fructose, while maltose consists of two glucose molecules. Park *et al*. (2013) reported that maltose improved the osmotic pressure stability in culture media compared to sucrose during callus culture in regeneration media. The morphogenetic response of

https://doi.org/10.26656/fr.2017.7(5).096
© 2023 The Authors. Published by Rynnye Lyan Resources

differentiation by plants to different carbohydrates may be due to their differential role in vascular differentiation, and different enzymes in metabolism (Yaseen *et al*., 2013).

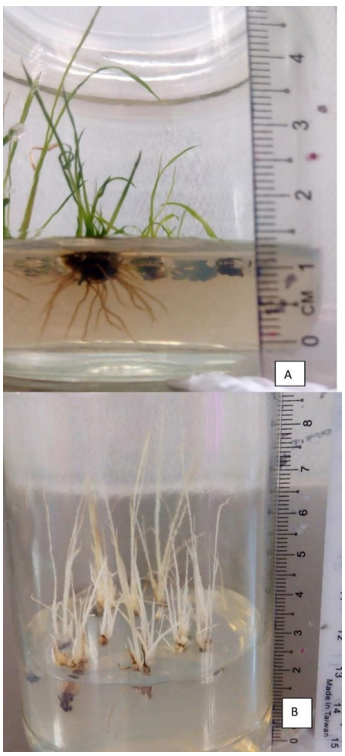

Figure 4. Rice regeneration as evidenced by the somatic embryogenic callus at 12 weeks in R7 media. (A) Green shoot and rooting plantlets. (B) Albino plantlets with no root.

PGR factors as well as the carbon source affect the production of albino plants. According to Park *et al*. 2013, a high maltose concentration will increase the production of albino plants, while a low maltose concentration leads to more green plant production in *Oryza sativa*. For MR219, the development of albino plantlets was enhanced when sucrose was used as the carbon source in the regeneration medium rather than maltose at the same concentration. Rooted shoots established wells during the acclimatization after transfer to large pots for growth in the greenhouse (Figure 5). Acclimatized plants showed a survival rate of plantlets of 90% after 4 weeks in the greenhouse, with no variation with respect to morphological growth characteristics. The *in vitro* plants produced grains comparable to the control plants and the seeds could be used for the genetic transformation of rice.

Figure 5. Acclimatization of MR219 seeds from R12 tissue culture regeneration media. A) Regenerated plantlet acclimatized in the greenhouse. B) and C) Harvested seeds from tissue culture rice. The *in vitro* grown plant was not significantly different at $p>0.05$.

4. Conclusion

MS medium containing 3.0 mg/L 2,4-dichlorophenoxy acetic acid (2,4-D) and 0.3 mg/L kinetin induced more calli production, but the maximum regeneration frequency (50%) was observed when friable calli were cultured on MS medium containing 3% sucrose, 9% phytagel, 1 mg/L BAP and 1 mg/L NAA. However, regeneration albino plantlets were also produced, thus sucrose promoted the growth of more albino plantlets compared to maltose. These findings expand our understanding of embryogenic callus morphology and development in the rice cultivar MR219, which will aid in identifying the embryogenic callus for effective plantlet regeneration and development with a p-value <0.05.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

This work was sponsored by the Ministry of Science Technology and Innovation through the Science fund (02-05-19-SF0008) and partly funded by GUP-2021-044. We are grateful to the MARDI, Seberang Perai for providing the rice seeds used in this work.

References

Adlak, T., Tiwari, S., Tripathi, M. K., Gupta, N., Sahu, V.K., Bhawar, P. and Kandalkar, V.S. (2019). Biotechnology: An advanced tool for crop improvement. *Current Journal of Applied Science and Technology*, 33(1), 1-11. https://doi.org/10.9734/cjast/2019/v33i130081

Akaneme, F.I. and Ene-obong, E.E. (2008). Tissue culture in *Pinus caribaea* Mor. var. Hondurensis barr. and golf. II: Effects of two auxins and two cytokinins on callus growth habits and subsequent organogenesis. *Agro-Science*, 4(1), 14-23. https://doi.org/10.4314/as.v4i1.1516

Ali, A., Naz, S., Iqbal, J., Ali, A. and Al, E.T. (2007). Effect of different explants and media compositions for efficient somatic embryogenesis in sugarcane (*Saccharum officinarum*). *Pakistan Journal of Botany*, 39(6), 1961–1977.

Ali, J., Nicolas, K.L.C., Akther, S., Torabi, A., Ebadi, A.A., Marfori-Nazarea, C.M. and Mahender, A. (2021). Improved anther culture media for enhanced callus formation and plant regeneration in rice

 © 2023 The Authors. Published by Rynnye Lyan Resources

(*Oryza sativa* L.). *Plants,* 10(5), 839. https://doi.org/10.3390/plants10050839

Boddupally, D., Tamirisa, S., Gundra, S.R., Vudem, D.R. and Khareedu, V.R. (2018). Expression of hybrid fusion protein (*Cry1Ac:: ASAL*) in transgenic rice plants imparts resistance against multiple insect pests. *Scientific Reports*, 8(1), 8458. https://doi.org/10.1038/s41598-018-26881-9

Chutipaijit, S. and Sutjaritvorakul, T. (2018). Improvement of Plant Regeneration Frequency from Carbon Sources in Aromatic Rice (*Oryza sativa* L.). *Iranian Journal of Science and Technology, Transactions A: Science*, 42, 1131-1137. https://doi.org/10.1007/S40995-017-0169-1

Dewir, Y.H., Naidoo, Y. and Teixeira da Silva, J.A. (2018). Thidiazuron-induced abnormalities in plant tissue cultures. *Plant Cell Reports*, 37, 1451-1470. https://doi.org/10.1007/s00299-018-2326-1

Ebrahimi, M.A. and Payan, A. (2013). Induction of callus and somatic embryogenesis from cotyledon explants of *Fagonia indica* Burm. *Journal of Medicinal plants and By-product*, 2(2), 209-214. https://doi.org/10.22092/JMPB.2013.108596

Ge, X., Chu, Z., Lin, Y. and Wang, S. (2006). A tissue culture system for different germplasms of indica rice. *Plant Cell Reports*, 25, 392-402. https://doi.org/10.1007/s00299-005-0100-7

Jain, N., Khurana, P. and Khurana, J.P. (2022). A rapid and efficient protocol for genotype-independent, Agrobacterium-mediated transformation of indica and japonica rice using mature seed-derived embryogenic calli. *Plant Cell, Tissue and Organ Culture,* 151(1), 59-73. https://doi.org/10.1007/s11240-022-02331-3

Jain-Raina, R. and Babbar, S.B. (2011). Evaluation of blends of alternative gelling agents with agar and development of xanthagar, a gelling mix, suitable for plant tissue culture media. *Asian Journal of Biotechnology*, 3(2), 153–164. https://doi.org/10.3923/ajbkr.2011.152.164

Joshi, Rohit and Kumar, Paramod. (2013). Regulation of somatic embryogenesis in crops: A review. *Agricultural Reviews*, 34(1), 1–20.

Karmakar, S., Molla, K.A., Chanda, P.K., Sarkar, S.N., Datta, S.K. and Datta, K. (2016). Green tissue-specific co-expression of chitinase and oxalate oxidase 4 genes in rice for enhanced resistance against sheath blight. *Planta*, 243(1), 115–130. https://doi.org/10.1007/s00425-015-2398-x

Kleinknecht, L., Wang, F., Stübe, R., Philippar, K., Nickelsen, J. and Bohne, A.V. (2014). RAP, the sole octotricopeptide repeat protein in Arabidopsis, is required for chloroplast 16S rRNA maturation. *Plant Cell*, 26(2), 777–787. https://doi.org/10.1105/tpc.114.122853

Kumari, M., Clarke, H.J., Small, I. and Siddique, K.H. (2009). Albinism in plants: a major bottleneck in wide hybridization, androgenesis and doubled haploid culture. *Critical Reviews in Plant Science,* 28(6), 393-409. https://doi.org/10.1080/07352680903133252

Leelavathi, S., Sunnichan, V.G., Kumria, R., Vijaykanth, G.P., Bhatnagar, R.K. and Reddy, V.S. (2004). A simple and rapid Agrobacterium-mediated transformation protocol for cotton (*Gossypium hirsutum* L.): Embryogenic calli as a source to generate large numbers of transgenic plants. *Plant Cell Reports*, *22*(7), 465–470. https://doi.org/10.1007/s00299-003-0710-x

Liang, Y., Biswas, S., Kim, B., Bailey-Serres, J. and Septiningsih, E.M. (2021). Improved transformation and regeneration of indica rice: disruption of SUB1A as a test case via CRISPR-Cas9. *International Journal of Molecular Sciences,* 22(13), 6989. https://doi.org/10.3390/ijms22136989

Liu, Y., Li, L., Zhang, L., Lv, Q., Zhao, Y. and Li, X. (2018). Isolation and identification of wheat gene *TaDIS1* encoding a RING finger domain protein, which negatively regulates drought stress tolerance in transgenic Arabidopsis. *Plant Science,* 275, 49-59. https://doi.org/10.1016/j.plantsci.2018.07.017

Maharani, A., Fanata, W.I.D., Laeli, F.N., Kim, K.M. and Handoyo, T. (2020). Callus Induction and Regeneration from Anther Cultures of Indonesian Indica Black Rice Cultivar. *Journal of Crop Science and Biotechnology*, 23(1), 21–28. https://doi.org/10.1007/s12892-019-0322-0

Martinez, M. E., Jorquera, L., Poirrier, P., Díaz, K. and Chamy, R. (2021). Effect of the carbon source and plant growth regulators (PGRs) in the induction and maintenance of an *in vitro* callus culture of *Taraxacum officinale* (L) weber Ex FH Wigg. *Agronomy,* 11(6), 1181. https://doi.org/10.3390/agronomy11061181

Mengs, B., Chimdessa, M. and Abraha, E. (2018). *In vitro* propagation of sweet potato *(Ipomoea batatas* (L.) Lam.) through lateral bud culture. *International Journal of Innovative Pharmaceutical Sciences and Research,* 6(7), 1-12.

Mesbah, H.A., Nassar, A.E.S.M. and El-Sheikh, M.A. (2021). Histological studies on somatic embryogenesis in rice (*Oryza sativ*a L.). *African Journal of Biological Sciences,* 17(1), 73-81. https://doi.org/10.21608/AJBS.2021.153144

Ming, N.J., Mostafiz, S.B., Johon, N.S., Zulkifli, N.S.A. and Wagiran, A. (2019). Combination of plant

 © 2023 The Authors. Published by Rynnye Lyan Resources

growth regulators, maltose, and partial desiccation treatment enhance somatic embryogenesis in selected malaysian rice cultivar. *Plants*, 8(6), 144. https://doi.org/10.3390/plants8060144

Ozawa, K. and Kawahigashi, H. (2006). Positional cloning of the nitrite reductase gene associated with good growth and regeneration ability of calli and establishment of a new selection system for Agrobacterium-mediated transformation in rice *(Oryza sativa* L.). *Plant Science*, 170(2), 384–393. https://doi.org/10.1016/j.plantsci.2005.09.015

Palama, T.L., Menard, P., Fock, I., Choi, Y.H., Bourdon, E., Govinden-Soulange, J. and Kodja, H. (2010). Shoot differentiation from protocorm callus cultures of *Vanilla planifolia* (Orchidaceae): proteomic and metabolic responses at early stage. *BMC Plant Biology*, 10, 82. https://doi.org/10.1186/1471-2229-10-82

Park, S.G., Ubaidillah, M. and Kim, K.M. (2013). Effect of maltose concentration on plant regeneration of anther culture with different genotypes in rice (*Oryza sativa* L.). *American Journal of Plant Sciences,* 4 (11), 2265. https://doi.org/10.4236/ajps.2013.411279

Poraha, R., Poeaim, A. and Pongjaroenkit, S. (2016). Callus induction and plant regeneration on optimization of the culture conditions in Jow Haw rice (Oryza sativa L.). *Journal of Agricultural Technology*, 12(2), 241–248.

Praveena, C. and Veeresham, C. (2014). Multiple shoot regeneration and effect of sugars on growth and nitidine accumulation in shoot cultures of *Toddalia asiatica*. *Pharmacognosy Magazine*, 10(39), S480–S486. https://doi.org/10.4103/0973-1296.139777

Quiroz-Figueroa, F.R., Rojas-Herrera, R., Galaz-Avalos, R.M. and Loyola-Vargas, V.M. (2006). Embryo production through somatic embryogenesis can be used to study cell differentiation in plants. *Plant Cell, Tissue and Organ Culture,* 86, 285-301. https://doi.org/10.1007/s11240-006-9139-6

Repalli, S.K., Geda, C.K., Pradhan, N.S. and Rao, G.N. (2019). Influence of additional nutrients and gelling agents on *in vitro* response of selected Indica rice varieties. *International Journal of Biology,* 11(4), 26–35. https://doi.org/10.5539/ijb.v11n4p26

Singh, A., Chaudhuri, B. and Roychoudhury, A. (2020). Influence of night temperature on rice yield and quality. In Roychoudhury, A. (Eds.) Rice Research for Quality Improvement: Genomics and Genetic Engineering, p. 579-590. Singapore: Springer. https://doi.org/10.1007/978-981-15-4120-9_24

Sivakumar, P., Law, Y.S., Ho, C.L. and Harikrishna, J. (2010). High frequency plant regeneration from mature seed of elite, recalcitrant Malaysian indica rice (*Oryza sativa* L.) CV. MR 219. *Acta Biologica Hungarica*, 61(3), 313–321. https://doi.org/10.1556/ABiol.61.2010.3.7

Sundararajan, S., Rajendran, V., Nayeem, S. and Ramalingam, S. (2020). Physicochemical factors modulate regeneration and Agrobacterium-mediated genetic transformation of recalcitrant indica rice cultivars - ASD16 and IR64. *Biocatalysis and Agricultural Biotechnology*, 24, 101519. https://doi.org/10.1016/j.bcab.2020.101519

Vega, R., Vásquez, N., Espinoza, A.M., Gatica, A.M. and Valdez-Melara, M. (2009). Histology of somatic embryogenesis in rice (*Oryza sativa* cv. 5272). *Revista de Biologia Tropical,* 57(Suppl. 1), 141-150.

Wang, M., Mao, Y., Lu, Y., Wang, Z., Tao, X. and Zhu, J.K. (2018). Multiplex gene editing in rice with simplified CRISPR-*Cpf1* and CRISPR-*Cas9* systems. *Journal of Integrative Plant Biology*, 60(8), 626–631. https://doi.org/10.1111/jipb.12667

Wójcik, A.M., Wójcikowska, B. and Gaj, M.D. (2020). Current perspectives on the auxin-mediated genetic network that controls the induction of somatic embryogenesis in plants. *International Journal of Molecular Sciences*, 21(4), 1333. https://doi.org/10.3390/ijms21041333

Yaqoob, U., Kaul, T. and Nawchoo, I.A. (2017). Development of an efficient protocol for Agrobacterium mediated transformation of some recalcitrant indica rice varieties. *Indian Journal of Plant Physiology*, 22(3), 346–353. https://doi.org/10.1007/s40502-017-0304-1

Yaseen, M., Ahmad, T., Sablok, G., Standardi, A. and Hafiz, I.A. (2013). Review: Role of carbon sources for *in vitro* plant growth and development. *Molecular Biology Reports*, 40(4), 2837–2849. https://doi.org/10.1007/s11033-012-2299-z

Zhu, J., Zhao, X., Lin, G., Yan, L., Tong, D. and Lian, Y. (2020). Morphological and Histological Analysis of Somatic Embryogenesis in *Haworthia cooperivar. Plant Gene and Trait,* 11(6), 1-9. https://doi.org/10.5376/pgt.2020.11.0006

Zuraida, A. R., Naziah, B., Zamri, Z. and Sreeramanan, S. (2011). Efficient plant regeneration of Malaysian indica rice MR 219 and 232 via somatic embryogenesis system. *Acta Physiologiae Plantarum*, 33(5), 1913–1921. https://doi.org/10.1007/s11738-011-0739-3

Zuraida, A., Roowi, S. and Subramaniam, S. (2010). Regeneration of Malaysian Indica rice (*Oryza sativa*) variety MR232 via optimised somatic embryogenesis system. *Journal of Phytology*, 2(3), 30–38.

 © 2023 The Authors. Published by Rynnye Lyan Resources