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RESEARCH ARTICLE

DEVELOPMENT OF FRIABLE EMBRYOGENIC CALLI FROM GINGER SHOOTS OF VARYING MATURITY

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Abstract: Ginger is a vegetative propagated monocot with medicinal properties and is cultivated mainly in the tropics as a spice crop. The lack of seed setting limits genetic improvement in this crop to clonal selection and mutation breeding. Therefore, assistance from modern technologies, like genetic transformation and genome editing, can be used to broaden the genetic base and improve traits like yield, quality, and climate resilience in ginger. Callus induction is a crucial step in Agrobacterium-mediated genetic transformation, genome editing, production of somaclonal variants, *in vitro* production of phytochemicals, etc. Production of friable embryogenic calli requires fine-tuning the hormonal composition in the callus induction media in a genotype- and explant-specific manner. In this study, we have optimised the callus induction protocol for the variety IISR Varada using five different explant tissues, like leaf lamina, immature shoot tip, immature shoot base, mature shoot tip, and mature shoot base. We found that the shoot bases from the mature and immature shoots of IISR Varada were capable of responding to the exogenous application of 2,4-D and developed calli with an induction rate of 75%. The mature shoot tip also responded to a combination of 2,4-D and BAP.

Keywords: Genetic engineering, Genome editing, Tissue culture, Ginger, Zingiber officinale

INTRODUCTION

Ginger (Zingiber officinale Rosc.) is a highly valued aromatic perennial herb, generally cultivated as a spice crop for its underground stem, rhizome. It is used in culinary and many traditional medical preparations across the globe. It is highly susceptible to bacterial and fungal diseases and the cultivated ginger does not show natural variants resistant to these deadly pathogens (Prasath *et al*., 2014). Modern plant breeding technologies such as genetic engineering and genome editing are promising tools for introgressing disease resistance-like desirable traits into ginger lines. In direct embryogenesis, a low number of shoots per explant, a limited number of tissues that respond, etc., hinders the generation of a sufficient number of embryos required for genetic transformation (Mehaboob *et al*., 2019). Thus calli-mediated indirect organogenesis became the backbone of transgenic and genome editing technologies in many crop plants (Khatun *et al*., 2003; Sahoo *et al*., 2011; Turhan, H., & Baser, 2004; Liu *et al*., 2015). The primary prerequisite for genetic engineering and gene editing is the development of friable embryogenic calli, which allows easy transformation and large-scale regeneration.

Callus is defined as the mass of fast-dividing cells, derived from a somatic tissue or an embryo as an explant under *in vitro* conditions. Embryogenic calli are friable, curd-like masses of cells that can be easily infected by Agrobacterium or used for transformation through PEG, particle bombardment-like methods (Du 2019, Song *et al*., 2020, Folling and Olesen, 2002, Suwanaketchanatit, 2007). The optimised protocols for efficient and cost-effective callus production specific to crop and variety of choice are important for making genetic modifications, mass multiplication and long-term storage of genetic resources and in vitro production of phytochemicals (Oliveira *et al*., 2018). Studies in various crop plants have resulted in many protocols for callus development and it is generally accepted that callus induction can be accomplished by manipulating the external supply of auxin:cytokinin ratio in the medium. For instance, combinations of 2,4-D and BAP or 2,4-D alone have been used in the optimisation of callus induction in different crops (Praveen and Ashalatha, 2014; Yasuyuki *et al*., 1967; Roy *et al*., 2011; Sahoo *et al*., 2011). Callus induction in the family Graminae was mainly for Agrobacterium-mediated transformation, while in families having valuable secondary metabolites such as Zingiberaceae, it was intended mainly for phytochemical production (Anasori and Asghari 2009).

Though the Zingiberaceae family plants respond quickly to in vitro regeneration protocols, several

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attempts were made to develop efficient callus induction protocols in different ginger varieties. Red ginger (Zingiber officinale var. Rubrum) responded well to a 1:1 ratio of 2,4-D and kinetin in 2 weeks with a 70% callus induction rate at a 16/8 hrs light-dark cycle (Widyastuti *et al*., 2024). The number of days to callus induction, induction rate, callus size and quality of calli vary depending on the genotype and hormonal concentrations. For example, leaf explants of the cultivar Santarampur Local were found to produce friable creamy white calli in 21 days with 1 mg/L 2,4-D, but the number of days rose by one week at higher and lower concentrations of 2,4-D in MS medium (Solanky *et al*, 2013). In a different attempt, shoot tips placed in an inverted pose induced well sized calli in semi-solid MS medium supplemented with different combinations of 2, 4-D and BAP (Ibrahim *et al*., 2015). In yet another study, MS media with 0.5% 2,4-D produced friable calli in the ginger variety Maran (Babu *et al*., 1992), but 0.5 mg/l dicamba was more effective in the variety Suruchi (Sultana *et al*., 2009). The extend of callus induction is intune with the variety and explants and hormonal composition (Gurav *et al*, 2020).

It was shown that picloram in red ginger and many other crops yielded a high rate of callus induction (Gnasekaran *et al*., 2023; El-Mageid *et al*., 2019; Habibah *et al*., 2019; Salma *et al*., 2019). Mango ginger, belonging to a related genus, Curcuma produced calli in 2.0 mg/L 2,4-D and 0.5 mg/L BAP (Raju *et al*., 2013). Different concentrations and combinations of PGRs have led to the formation of different types of calli; for instance, with a 2:1 ratio of 2,4-D to BAP has resulted in friable calli with an 88% callus induction rate. (Abd El-Hameid *et al*., 2020). Apart from normal somatic explants, reproductive structures like ovaries and anthers were also attempted as explants for callus induction (Babu *et al*., 1996; Samsudeen *et al*., 2000). This diversity in hormonal combinations for callus induction among different crops and explants reiterates the need for optimisation of callus induction in a variety- and explant- specific manner.

In this study, we have evaluated different explants like leaf lamina, immature shoot base, immature shoot tip, mature shoot base, and mature shoot tip and various combinations of 2,4-D and BAP in MS basal media for the variety IISR Varada.

MATERIALS AND METHODS

Plant Material and explant selection

Healthy, disease-free rhizomes of ginger variety IISR-Varada were used for germination in coir pith in plastic trays with mild irrigation to retain the moisture. Five different types of explants were used in this study – immature shoot base, immature shoot tip, mature shoot base, mature shoot tip and leaf lamina. Shoots obtained 3 weeks after sprouting are denoted as immature and 3 months after sprouting, they are denoted as mature. Immature shoot base and immature shoot tip explants were made from 10-15cm long shoots of 3 weeks old plants grown in coir pith under shade. To get mature shoot explants, the sprouted rhizomes were planted in the grow-bags with potting mixture treated with 1% Bavistin and were grown under direct sunlight for 60 days. Mature shoot base and mature shoot tip explants were obtained from these ginger plants after removing the outer leaf sheaths up to the 4th leaf from the tip. Leaf lamina explants were obtained from 60-days-old plants.

Surface Sterilisation of Explants

The cut shoots were wiped with 70% ethanol and then washed with Tween 20 (2-3 drops per 100ml distilled water) for 5 minutes. Washed thrice in 100ml distilled water and submerged the explants in 70% ethanol in a sterile conical flask inside a laminar air flow chamber for 30 sec. After washing off the ethanol completely by rinsing with distilled water, the explants were treated with 0.1% mercuric chloride ($HgCl_2$) solution for 3 minutes and the explants were cleaned with sterile distilled water by repeated washing.

Preparation of Culture Medium

The callus induction media consisted of Murashige and Skoog (MS) medium with different combinations of the plant growth regulators (PGRs) BAP and 2,4-D (Murashige and Skoog, 1962). Sterilised explants were aseptically inoculated onto the prepared MS medium supplemented with the PGR combinations. To ensure adequate contact, the explants were placed on the surface of the solidified medium. The inoculated explants were incubated under dark condition at a temperature of 25 ± 2°C.

The cultures were regularly monitored for callus formation, and subculturing was performed every 3 weeks onto fresh medium to promote callus growth. The explants were monitored for callus initiation at regular intervals (weekly) for up to 6-8 weeks. The duration of callus development and callus type (texture and colour of the callus) were noted. Callus induction frequency (percentage of explants forming callus) was calculated by counting the number of explants showing callus formation.

RESULTS AND DISCUSSION

Ginger is an asexually propagated crop amenable to mass production under controlled conditions and hydroponics. Such cultivation possibilities make it suitable for the production of secondary metabolites. Cell culture and protoplast regeneration are future perspectives of secondary metabolites from ginger. Towards this and for the development of GM and GEd ginger production, the optimisation of high-quality calli production methods specific to leading varieties of ginger is essential. In this study, we focused mostly on the responsiveness of various

tissues of ginger variety IISR Varada to hormonal compositions to yield friable calli. We have chosen 5 different tissue types: 1. Immatrue shoot base 2. Immatrue shoot tip 3. Mature shoot base 4. Mature shoot tip 5. Leaf lamina. (Figure 1)

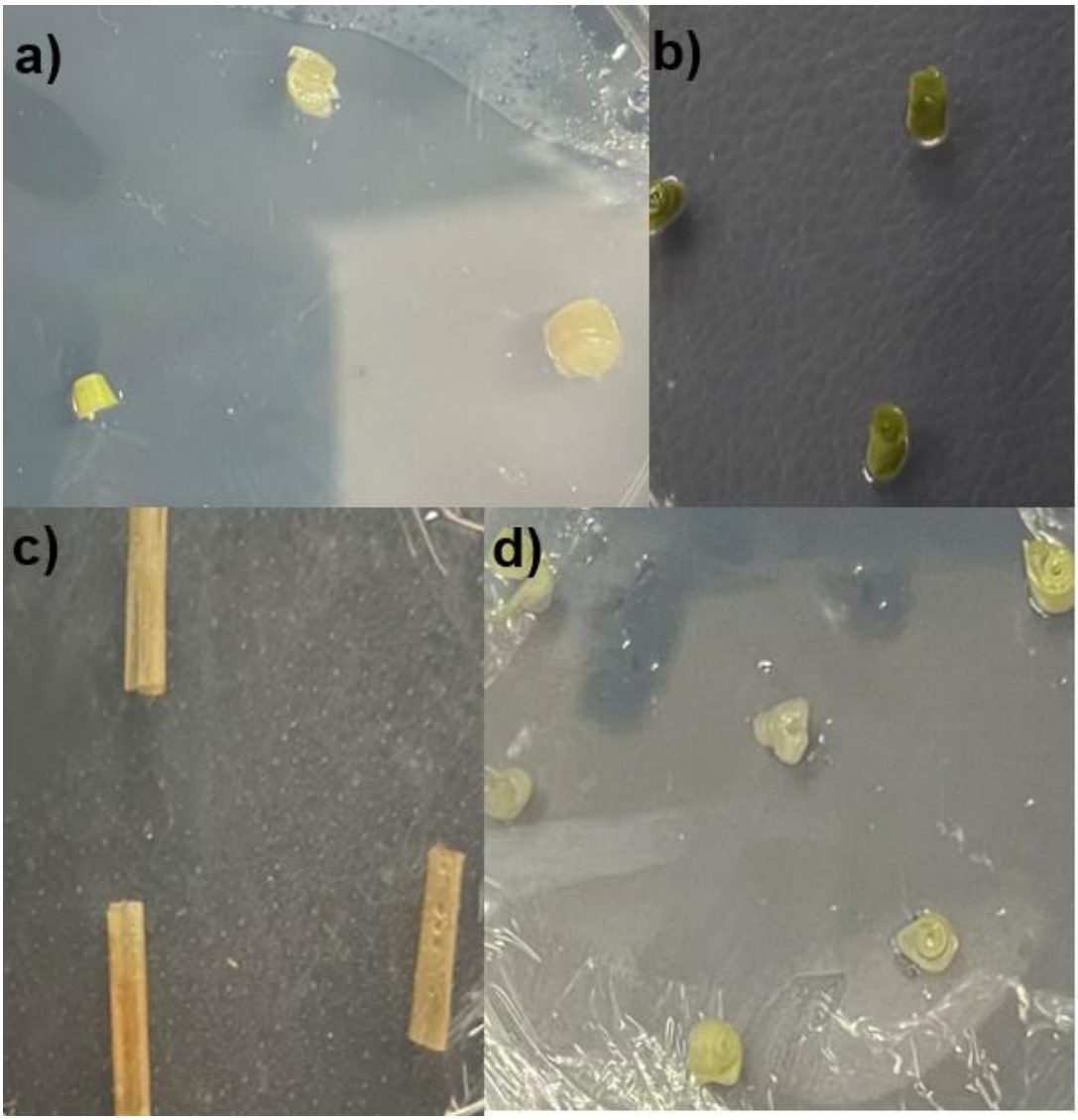

Figure 1: Different explants used for callus induction in IISR Varada a) 0.5mg/L 2,4-D (Immature shoot base) b) 0.5mg/L 2,4-D (Immature shoot tip) c) 3mg/L 2,4-D (Mature Leaf sheath) d) 1mg/L 2,4-D and 1mg/L BAP (Mature shoot base)

In both immature and mature shoots, the green portion differentiating to become leaf lamina were considered as shoot tip, while the light coloured basal portion was considered as shoot base. In 0.5mg/L 2,4-D containing MS media plates, shoot base explants were developed into friable calli with 75% callus induction in immature shoot bases and 25% in mature shoot bases (table 1). Subculturing of these calli gave rise to embryogenic calli (Figure 2a, Figure 2b). At a higher concentrations of 2, 4-D (3mg/L) a callus induction like reponse was observed in immature shoot base explants (Figure 2c). Other tissues including mature shoot tip, immature shoot tip and leaf lamina did not responds to any of the hormonal compositions tested in this study even after subculturing 2-3 times. An intermediate ratio between Auxin and cytokinin promotes callus induction (Skoog and Miller, 1957). The ginger shoot base used in this study contains the apical meristem and developing leaf, which has a higher level of cytokinin compared to mature shoots. So, a slight exogenous dose of 2,4-D alone was sufficient to bring auxin:cytokinin ratio to a balance and induce calli. Meanwhile, the mature shoot base responded to a combination of 2,4-D and BAP, though the calli were not friable but slimy/hairy callus induction was observed (Figure 2d, Table1). At the same concentrations, the green shoot tips did not respond in both young and mature shoots.

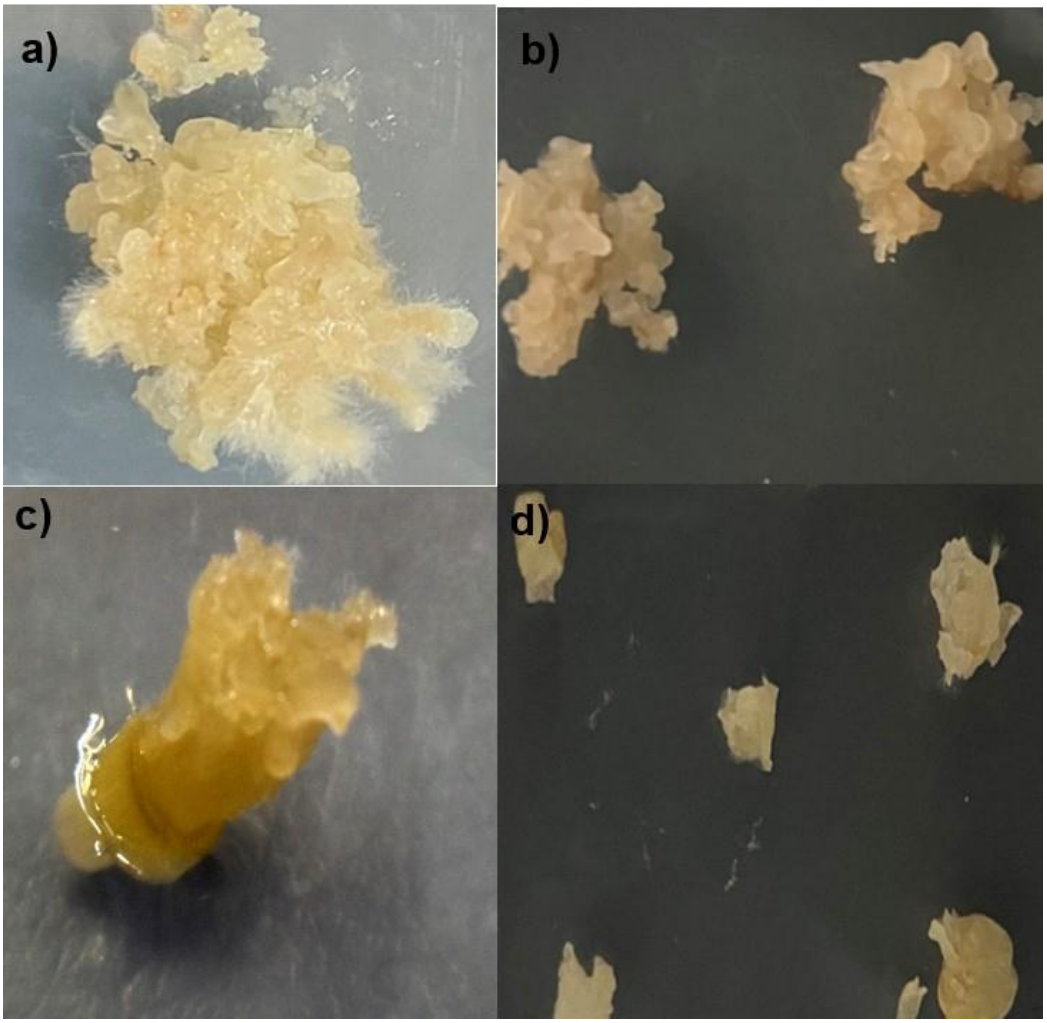

Figure 2: Friable embryogenic calli produced from shoot base explants of IISR Varada a) 0.5mg/L 2,4-D (Immature shoot base - I Subculture) b) 0.5mg/L 2,4-D (Immature shoot base - II Subculture) c) 3mg/L 2,4-D (Immature shoot base) d) 1mg/L 2,4-D and 1mg/L BAP (Mature shoot base)

Table 1. MS medium supplemented with different concentrations and combinations of plant growth regulators (PGR).

Treatment	Basal Media	PGR concentration	Explant Response	
			Young Shoot base (lower)	Mature Shoot base (lower)
T1	MS	2,4-D (0.5mg/L)	75% CI	25% CI
T2	MS	2,4-D (1mg/L)	Nil	Nil
T3	MS	2,4-D (3mg/L)	25% tissue callus like	Nil
T4	MS	2,4-D (1mg/L) + BAP(0.5mg/L)	Nil	Nil
T5	MS	2,4-D (1mg/L)+BAP (1mg/L)	Nil	100% response (slimy calli)
T6	MS	2,4-D (1mg/L)+BAP(1.5mg/L)	Nil	Nil

CONCLUSION

The explant that responded to combinations of 2, 4-D and BAP in this study was the basal shoot where in young meristematic portion is covered with differentiating leaf sheath. So, it can be concluded that the shoot apical meristem or the undifferentiated leaf sheath tissues at the base of the shoot are giving raise to the observed calli rather than the shoot tip that is differentiating to form leaf lamina. Thus, this study pin points to the exact tissue that respond to callus induction in ginger and the hormonal requirement at different maturities of explants.

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Declaration of interest statement

Authors hereby state that they have no conflict of interest

Authors Contribution

Conceptualization of research work and designing experiments (DPS) Excecution of lab experiments and data collection (NSK, SA and MB) Analysis of data and interpretation (DPS, NSK) Preparation of manuscript (DPS, TES).

REFERENCES

Abd El-Hameid, A. R., Abo El-kheir, Z. A., Abdel-Hady, M. S. and Helmy, W. A. (2020). Identification of DNA variation in callus derived from Zingiber officinale and anticoagulation activities of ginger rhizome and callus. *Bulletin of the National Research Centre*, **44**: 1-8.

Google Scholar

Anasori, P. and Asghari, G (2009). Effects of light and differentiation on gingerol and zingiberene production in callus culture of Zingiber officinale Rosc. *Research in Pharmaceutical Sciences*, **3**(1): 59-63.

Google Scholar

Andersen, A. N., Fisher, A., Hoffmann, B. D., Read J. L. and Richards, R. (2004). Use of terrestrial invertebrates for biodiversity monitoring in Australian rangelands, with particular reference to ants. *Austral Ecology*, **29** (1): 87-92.

Google Scholar

Babu, K. N., Samsudeen, K. and Ratnambal, M. J. (1992). In vitro plant regeneration from leaf-derived callus in ginger (Zingiber officinale Rosc.). *Plant cell, tissue and organ culture*, **29**: 71-74.

Google Scholar

Babu, K. N., Samsudeen, K., Ratnambal, M. J. and Ravindran, P. N. (1996). Embryogenesis and plant regeneration from ovary derived callus cultures of ginger (Zingiber officinale Rosc.). *Journal of Spices and Aromatic Crops*, **5**(2): 134-138.

Google Scholar

Du, D., Jin, R., Guo, J. and Zhang, F. (2019). Infection of embryonic callus with Agrobacterium enables high-speed transformation of maize. *International Journal of Molecular Sciences*, **20**(2): 279.

Google Scholar

El-Mageid, I. S. (2019). Evaluation of genetic stability by using protein and ISSR markers during callus development stage of some date palm (Phoenix dactylifera L.) cultivars under effect of 2, 4-D and Picloram. *Middle East J. Appl. Sci*., **9**: 483-493.

Google Scholar

Folling, L. and Olesen, A. (2002). Transformation of wheat (Triticum aestivum L.) microspore-derived callus and microspores by particle bombardment. *Plant Cell Reports*, **20**: 1098-1105.

Google Scholar

Gnasekaran, P., Rahman, Z. A., Chew, B. L., Uddain, J., Solayappan, M., Chear, N. J. Y. and Subramaniam, S. (2023). Picloram enhanced the callus induction, growth kinetics, antioxidant potentials, and secondary metabolites production of Zingiber officinale var. rubrum callus cultures. *Plant Cell, Tissue and Organ Culture (PCTOC)*,**155**(3): 843-859.

Google Scholar

Gurav, S. S., Gurav, N. S., Patil, A. T. and Duragkar, N. J. (2020). Effect of explant source, culture media, and growth regulators on callogenesis and expression of secondary metabolites of Curcuma longa. *Journal of Herbs, Spices & Medicinal Plants*, **26**(2): 172-190.

Google Scholar

Habibah, N. A., Widiatningrum, T., Anggraito, Y. U., Rahayu, E. S., Mukhtar, K., Wijayanti, N. and Mustafa, F. (2019). Growth of Elaeocarpus grandiflorus callus cultures in MS medium with various concentrations of growth regulators. In *Journal of Physics: Conference Series* (Vol. **1321**, No. 3, p. 032037). IOP Publishing.

Google Scholar

Ibrahim, D. A., Danial, G H., Mosa, V. M. and Khalil, B. M. (2015). Plant regeneration from shoot tips-derived callus of ginger (Zingiber officinale Rosc.). American Journal of Experimental Agriculture, **7**(1): 55-61.

Google Scholar

Khatun, M. M., Ali, M. H. and Desamero, N. V. (2003). Effect of genotype and culture media on callus formation and plant regeneration from mature seed scutella culture in rice. *Plant Tissue Cult*., **13**(2): 99-107.

Google Scholar

Liu, G, Gilding, E. K. and Godwin, I. D. (2015). A robust tissue culture system for sorghum [Sorghum bicolor (L.) Moench]. *South African Journal of Botany*, **98**: 157-160.

Google Scholar

Mehaboob, V. M., Faizal, K., Shamsudheen, K. M., Raja, P., Thiagu, G and Shajahan, A. (2019). Direct organogenesis and microrhizome production in ginger (Zingiber officinale Rosc.). *J. Pharmacogn. Phytochem*.,**8**: 2880-2883.

Google Scholar

Roy, M., Hossain, M., Biswas, A., Biswas, M. K. and Islam, R. (2011). Plant Regeneration through Somatic Embryogenesis from Leaf Sheath Derived Callus of Sugarcane (Saccharum officinarum L.) var.

Isd-16. *Plant Tissue Culture and Biotechnology*, **21**(2): 143-149.

Google Scholar

Murashige, T. and Skoog, F. (1962). A revised medium for rapid growth and bio assays with tobacco tissue cultures. *Physiologia plantarum*, **15**(3).

Google Scholar

Oliveira, L. D., Oliveira, A. D., Machado, C. D. A., Cardoso, M. N., Santana, F. V., Miranda, I. C. D. and Ledo, A. D. S. (2018). Induction, growth kinetics and morpho-histological characterization of Neem callus. *Journal of Agricultural Science (Toronto)*, **10**(6): 283-290.

Google Scholar

Prasath, D., Karthika, R., Habeeba, N. T., Suraby, E. J., Rosana, O. B., Shaji, A. and Anandaraj, M. (2014). Comparison of the transcriptomes of ginger (Zingiber officinale Rosc.) and mango ginger (Curcuma amada Roxb.) in response to the bacterial wilt infection. *PLoS One*, **9**(6): e99731.

Google Scholar

Praveen, R. P. and Nair, A. S. (2014). Callus induction and multiplication of internodal explants of Myxopyrum smilacifolium Blume. *Int.J.Curr.Microbiol.App.Sci*., **3**(10): 612-617.

Google Scholar

Sahoo, K. K., Tripathi, A. K., Pareek, A., Sopory, S. K. and Singla-Pareek, S. L. (2011). An improved protocol for efficient transformation and regeneration of diverse indica rice cultivars. *Plant Methods*, **7**: 1-11.

Google Scholar

Salma, U., Kundu, S., Ali, M. N. and Mandal, N. (2019). Somatic embryogenesis-mediated plant regeneration of Eclipta alba (L.) Hassk. and its conservation through synthetic seed technology. *Acta Physiologiae Plantarum*, **41**: 1-10.

Google Scholar

Samsudeen, K., Babu, K. N., Divakaran, M. and Ravindran, P. N. (2000). Plant regeneration from anther derived callus cultures of ginger (Zingiber officinale Rosc.). *The Journal of Horticultural Science and Biotechnology*, **75**(4): 447-450.

Google Scholar

Skoog, F. and Miller, C.O. (1957). Chemical regulation of growth and organ formation in plant tissues cultured in vitro. In *Symp. Soc. Exp. Biol*., **11**: 118–130.

Google Scholar

Solanky, R.U., Patel, S.R. and Patel, J.R. (2013). In vitro regeneration of ginger (zingiber officinale rosc.) through callus culture. *AGRES-an International e-Journal*, **2**(2):196-202.

Google Scholar

Song, Y., Bai, X., Dong, S., Yang, Y., Dong, H., Wang, N. and Li, S. (2020). Stable and efficient agrobacterium-mediated genetic transformation of larch using embryogenic callus. *Frontiers in Plant Science*, **11**: 584492.

Google Scholar

Soundar Raju, C., Kathiravan, K., Aslam, A. and Shajahan, A. (2013). An efficient regeneration system via somatic embryogenesis in mango ginger (Curcuma amada Roxb.). *Plant Cell, Tissue and Organ Culture (PCTOC)*, **112**: 387-393.

Google Scholar

Sultana, A. Z. R. A., Hassan, L., Ahmad, S. D., Shah, A. H., Batool, F., Islam, M. A. and Moonmoon, S. (2009). In vitro regeneration of ginger using leaf, shoot tip and root explants. *Pak. J. Bot*., **41**(4): 1667-1676.

Google Scholar

Suwanaketchanatit, C., Piluek, J., Peyachoknagul, S. and Huehne, P.S. (2007). High efficiency of stable genetic transformation in *Dendrobium* via microprojectile bombardment. *Biol. Plant*, **51**:720–727.

Google Scholar

Turhan, H. and Baser, I. (2004). Callus induction from mature embryo of winter wheat (Triticum aestivum L.). *Asian Journal of Plant Sciences*, **3**: 17-19.

Google Scholar

Widyastuti, D. A., Santosa, D., Nuringtyas, T. R. and Rohman, A. (2024). Callus induction of red ginger (Zingiber officinale var. rubrum) with 2, 4-D and Kinetin combination to enhance total phenolic and flavonoid content. In *IOP Conference Series: Earth and Environmental Science* (Vol. **1364**, No. 1, p. 012051).

Google Scholar