Journal Homepage: - www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)

Article DOI: 10.21474/IJAR01/18770
DOI URL: http://dx.doi.org/10.21474/IJAR01/18770

RESEARCH ARTICLE

IN VITRO CALLUS INDUCTION AND REGENERATION OF ORYZA SATIVA L. VAR. MTU1075 (PUSHYAMI)

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Manuscript Info

Manuscript History
Received: 20 March 2024
Final Accepted: 27 April 2024
Published: May 2024

Key words:-
MTU1075, 2,4-D, Callus Induction, Regeneration

Abstract

In vitro inducement of callus and plant regeneration capacity were investigated using mature embryos of the Indian rice (Oryza sativa L.) variety MTU1075. The investigation was conducted using callus induction media (Murashige and Skoog, 1962) with varying concentrations of 2, 4-D, namely 1.0, 1.5, 2.0, 2.5, 3.0, and 3.5mg/l. It shows the maximum degree of callus induction at 2 mg/l 2,4-D. The maximum callus induction rate was 86%. Plant regeneration efficacy of in vitro generated plantlets was studied then successfully transferred into soil conditions.

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Introduction:-

Plant regeneration is the process by which an adult tissue or organ, a mass of disorganized calli, or even a single cell can regenerate into a whole plant. The term "plant regeneration" describes the physiological replacement, repair, or rejuvenation of plant tissue. (Ikeuchi et al., 2016). A number of factors influence the capacity for plant regeneration, such as the kind of explant used (Minutolo et al., 2020), the composition of the basic medium (Chimdessa,2020), and the application of a plant growth regulator (Gerdakaneh et al., 2020). After wheat and maize, rice, is the most significant cereal crop in the world and the main dietary meal of people in India and is a perennial grass of the genus Poaceae. (Ray JK 1985). It supplies 50–80% of the overall daily caloric intake of almost three billion people and accounts for fifty percent of all dietary carbohydrates, particularly in Asian countries. (Khush GS 2005). Conventional rice breeding has yielded notable improvements in quality, quantity, and resilience to disease, among other vital agricultural traits. In the future, rice breeding is expected to continue to play a significant role in achieving these goals. (Sun ZR et al 1990). Rice is the Asian Subcontinent's principal food, accounting for around 70% of calories and half of the protein consumed among populations. To safeguard hunger and malnutrition, it is vital to increase rice output and productivity.(Bishwajit et al. 2013).

In cultivating rice, procedures involving tissue culture are applied for somaclonal variations and gene transfer to develop distinct varieties. (Ram, H.H. et al, 1998). The first phase in gene transformation is to induce callus through indirect organogenesis. Callus produced from juvenile and mature embryos or scutellum-derived in rice can be regenerated, however various issues have been noted with shoot regeneration. (Chu, Q.R.et al,1990). Dehusked rice is frequently employed and readily available explant. (Raina, S.K., 1989). Several hormones may trigger a rapidly dividing, undifferentiated lump known as a callus. (Naqvi, S.M.S.,2002).

In vitro conditions, varying genotypes have been shown to respond variably for induction of callus at various levels of 2, 4-D hormone. (Elwafa, A.A.A. et al, 1999). Improvising media composition, mainly by altering plant growth

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regulators, may raise the efficacy rate of callus induction. (Zhu, Y., et al,1997). Because various genotypes behave variably during callus induction, an attempt was conducted to produce adequate callus by altering 2, 4-D levels, in vitro with MTU1075 cultivar. The study aimed to identify an appropriate medium and culture conditions for inducing callus, which can also be applied further for abiotic stress studies.

MTU1075 is a semi dwarf variety with long slender grain and shows resistant towards leaf blast, BLB, WBPH.

Materials and Methods:-

Plant Material:

Mature seeds of MTU1075 (Pushyami) were obtained from IIRR- INDIAN INSTITUTE OF RICE RESEARCH, Rajendranagar and cultivated and maintained in a greenhouse at Osmania University, CPMB. They were used as explants in current study to optimize embryogenic callus production and as target material to optimize regeneration protocols.

Surface Sterilization:

Manual dehusking of Rice seeds was done, Further process was carried out inside flow cabinet, cleaned using surfactant Extran (2-3% V/V) for few minutes, subsequentlywashed with sterile distilled water. These seeds were then soaked in sterile water overnight. Next day, they were sterilized using mercuric chloride (0.1% w/v) for 7-8 minutes with continuous gentle manual shaking followed by rinses with distilled water and blot-dried on autoclaved filter paper (Whatman paper) until dried completely. These seeds were transferred onto MS media (Murashige, T.; Skoog, F. 1964) (with various doses of 2,4-D hormone as related cultivars shown highest induction%. (Anjana Priyadarshani.K et al, 2024).

Optimization of Efficient Callus Induction and Regeneration:

To examine the influence of 2,4-D on rate of callus induction for MTU1075, surface-sterilized explants were administered onto MS- callus induction medium (CIM) (Table 1) enriched with 1.0, 1.5, 2.0, 2.5, 3.0 and 3.5 mg/L of 2,4-D individually. (Table 2). The pH of media was set prior to autoclaving for 5.8. The laminar flow unit has been sterilized with UV radiation for 5-15 minutes before the procedure begins. To prevent contamination, hands were sanitized with absolute alcohol, and the surface of the laminar unit was wiped with spirit. UV light was applied for 5-10 minutes to autoclaved and sterile Petri plates, forceps, and media.Followed by autoclaving, growth regulators and other supplements were introduced to the media at a somewhat higher average temperature inside the cabinet. Upon solidification of media the 10-14 seeds were inoculated per petri plate and subjected to dark at 22-25°C. After 10-12 days, newly formed calli were subcultured on fresh medium plates (Fig 1B) and incubated again for 6-8 days. Two rounds of sub culturing for about 6-8 days each were performed to obtain significant amount of calli (Fig 1C). Results of callus induction were noted as frequencies after 3-4 weeks of incubation. The ideal concentration of 2,4-D in medium was employed to examine the regeneration capability in MTU1075. MS-Shooting medium (SM) (Table 3) was prepared with two different combinations of hormones, one with (2mg/L kinetin and 1mg/L NAA)(Aadarsh et al,2010) and other with (2.5 mg/L BAP, 1 mg/L kinetin, 0.5 mg/L NAA) (Yadav et al,2023). The healthy calli were shifted onto shooting medium in bottles for further. These bottles were incubated at 22-24C with 16 hours under light of 2000 lux units followed by 7-8 hours of dark. Once the greening of callus starts, they were shifted onto fresh MS-SM media bottles. The regenerated shoots are inoculated on rooting medium. Rooting media components were tabulated (Table 4) and 2.5% Gelrite (Aadarsh et al,2010) and 2.0% Gelrite (Anjana Priyadarshani.K et al, 2024) were used. Incubation for 16/8 hours of light/dark at 22-25C about 10 days were done until visible root mass was observed. Well-rooted plantlets were pulled out carefully avoiding excess media and placed in smaller pots stuffed with sterilized 1:1 vermicompost and soil rite, enclosed using polythene bag for 2 days and eventually holes were introduced to bag for aeration. Slowly the bag is removed and plantlets were subjected to natural environment inside net house. Adequate amount of sunlight and temperature was ensured.

Below formula was used to calculate the callus induction % and regeneration frequency. (Zaidi et al., 2006)

$$\text{Callus induction frequency}(\%) = \frac{\text{No. of seeds producing calli}}{\text{No. of seeds cultured}} \times 100$$

$$\text{Plant regeneration}\,(\%) = \frac{\text{No. of calli producing plants}}{\text{No. of plants planted}} \times 100$$

Results and Discussion:-

The primary goal of the current research was to establish a viable callus – based indirect regeneration approach for MTU1075 Indica rice variety.The primary goal of this study is to figure out the precise route for explant sterilization, callogenesis and indirect organogenesis in this cultivar.

The callus was developed and grown on MS media that contained various concentrations of 2, 4-D. After 7-10 days of inoculating on CIM media, callus formation was observed.The highest rate of induction occurred with 2.0 mg/l of 2, 4-D (Fig 1A), which is consistent with earlier results of Libin, A., et al. 2012. When developed embryos were incubated on MS medium with 2,4-D (2.0mg/l), (2.5mg/l), and (3.0mg/l) approximately 86% of the embryonic calli were generated. The observed features of embryogenic callus were fragile, creamy to slight yellowish in colour. The highest induction of calli (Table 5) was observed as 86% at 2.0mg/l, 2.5mg/l and 3.0mg/l. Likewise, to the present investigation, the impact of 2, 4-D was tested in various rice cultivars, and 2mg/L concentration was suggested for prominent induction of callus formation. (Pandey, S.K. et al, 1994). In 1994, Pandey et al. studied callus induction of matured dehusked rice seeds and found it was most effective at 2 mg/L of 2, 4-D. (2.0 mg/L) of 2,4-D was discovered to be optimal dosage for inducing callus formation and proliferation.(Sarker et al 2016). Further increase in concentration of auxin shown any relatable increase in callus induction.

Previous studies by Aadarsh et al,2010 with (2mg/L kinetin and 1mg/L NAA) and Yadav et al,2023 with (2.5 mg/L BAP, 1 mg/L kinetin, 0.5 mg/L NAA) found enhanced regeneration frequency at mentioned hormonal concentrations in closely related cultivars to MTU1075. Moreover, both were considered in present work. The highest regeneration of 54% (Table 6) was observed with (2mg/L kinetin and 1mg/L NAA). Typically, seven to eight days were required to stimulate callus formation and regeneration. After 8-10 days of exposure to SM, small greenish shoot formation was noticed (Fig 1D). When subcultured onto same media more proliferation and development of shoots were observed (Fig 2A). They were subcultured repeatedly for every 7-8 days to obtain healthy plantlets. Prominent root development was observed in every calli with shoots even without any hormones (Fig 2B). The root development was faster within 4-5 days in media with 2% Gelrite (Mohamed, Gehad M., et al. 2021). comparatively to 2.5% Gelrite in media (7 days). The frequency of root formation did notchange with concentration of solidifying agent.Healthy plants were obtained after proper acclimatization of plantlets (Fig2C) and shifted to pots (Fig2D).

TABLES

Table 1:- Basic composition of CIM.

MS - Callus induction medium (CIM) per Litre - pH 5.8	
MS salts with vitamins	4.4g
Maltose	30g
Inositol	100mg
Casein hydrolysate	200mg
Tryptophan	50mg
Gelrite	3g

Table 2:- Hormonal concentrations.

Media	Hormonal Concentrations
MS-CIM	2,4-D-1.0mg/l
MS-CIM	2,4-D-1.5mg/l
MS-CIM	2,4-D-2.0mg/l
MS-CIM	2,4-D-2.5mg/l
MS-CIM	2,4-D-3.0mg/l
MS-CIM	2,4-D-3.5mg/l
MS-SM	2mg/L kinetin and 1mg/L NAA
MS-SM	2.5 mg/L BAP, 1 mg/L kinetin and 0.5 mg/L NAA.

Table 3:- Shooting medium composition.

MS- Shooting medium(SM) per Litre pH-5.8	
Ms salts + Vitamins	4.4g
Maltose	30g
Inositol	100mg
Gelrite	3g

Table 4:- Rooting medium composition.

MS- Rooting medium per Litre pH-5.6-5.8	
Ms salts	2.0g
Maltose	36g
Inositol	50 mg
Gelrite	2 g/2.5g

Table 5:- Callus induction % with different levels of hormone.

Hormone concentration	Number of explants	Number of calluses formed	Callus induction %
2,4-D-1.0mg/l	100	32	32%
2,4-D-1.5mg/l	100	56	56%
2,4-D-2.0mg/l	100	86	86%
2,4-D-2.5mg/l	100	85	85%
2, 4-D-3.0mg/l	100	86	86%
2,4-D-3.5mg/l	100	63	63%

Table 6:- Regeneration%.

Hormones (SM)	Calli inoculated	Shoots produced	Shoot Induction %
2.5 mg/L BAP, 1 mg/L kinetin, 0.5 mg/L NAA	100	43	43%
2mg/L kinetin and 1mg/L NAA	100	54	54%

Figures

Figure 1:-

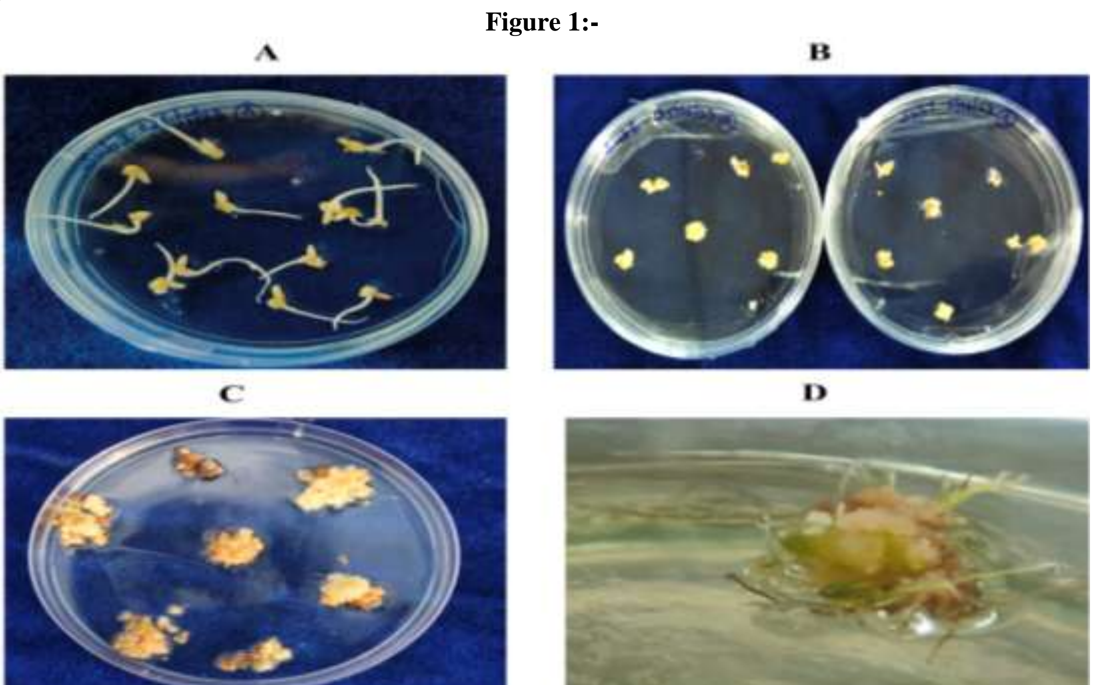

Fig 1:- A) Initiation of callus from explant of MTU1075. B) First subculture of calli onto CIM-2.0mg/l medium. C) Second subculture of callus. D) Initiation of shooting from callus.

Figure 2:-

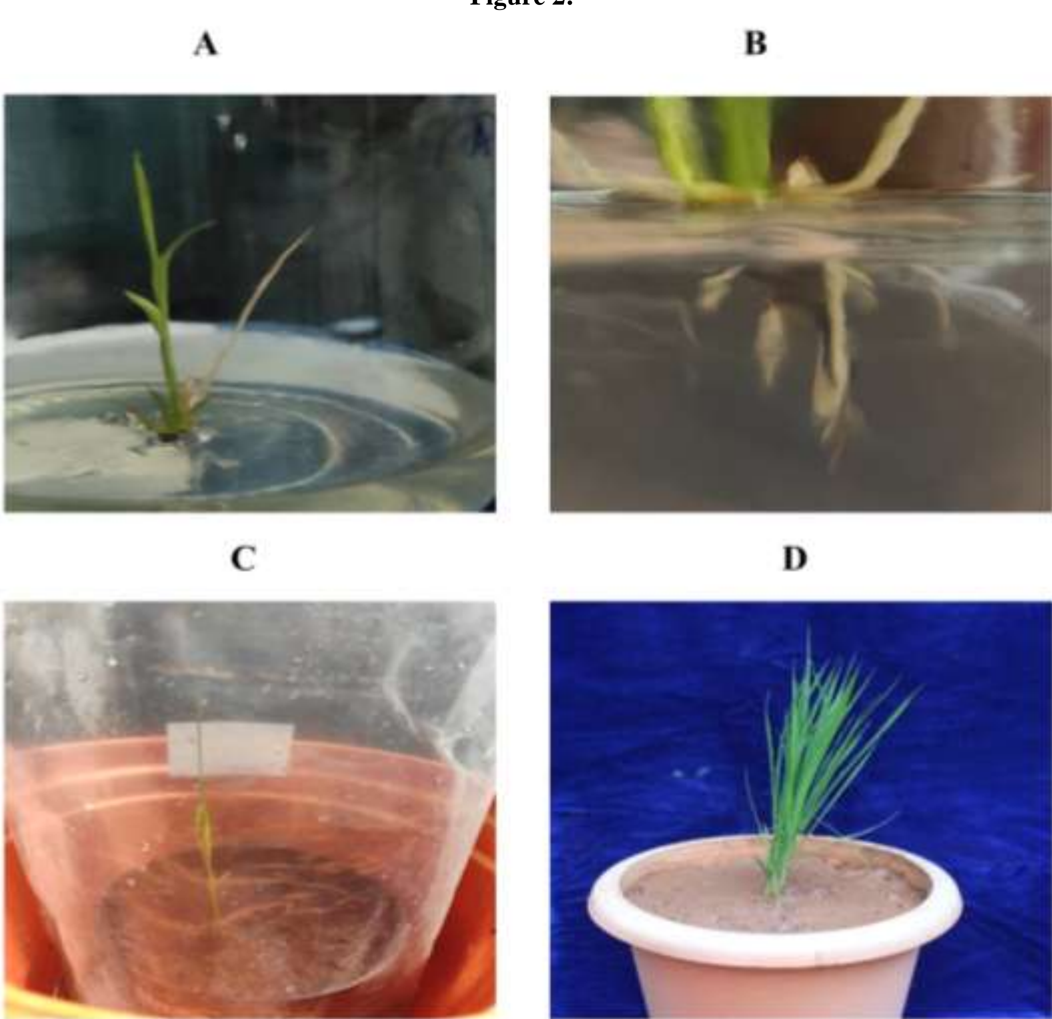

Fig 2:- A) Shoot proliferation. B) Root development from shoots. C) Acclimatization of plantlets with polythene bags in net house. D) Fully grown plantlets of Rice cultivar.

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