Official Publication of Pakistan Society of Biomass and Bioenergy
Journal of Biomaterials and Bio Products Technology
ISSN: 2790-2595 (Print), 2790-2609 (Online)
http://www.jbbt.org

CALLUS PRODUCTION EMBRYOGENESIS AND REGENERATION OF PLANTS IN SOME LOCAL VARIETIES OF RICE

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ABSTRACT

The plant matter was separated from the three varieties of Basmati rice (Basmati 2000, Basmati 385, and Super Basmati) to achieve one of the key goals. The seeds were surface sterilized, then inoculated with 2, 2, 5, and 3.5 mg/L of 2, 4-D on N6 medium to observe what would happen. The greatest incidence of callus acceptance recurrence and the fastest development, after just three weeks, were seen in the medium containing 2 mg/L of 2,4-D. Basmati 2000 performed best among the three varieties in terms of callus acceptance and plant recovery. It was observed that the callus aesthetic permeated all three collections. It was possible to tell apart the immobile, non-embryoid calli from the densely packed, massive embryoid calli. Over the course of three weeks, we gradually replaced the calli with maintenance medium (N6). Both 6-benzyl aminopurine (6-BAP) and naphthalene acidic corrosive (NAA) forms of basic salts and vitamins were usable in the setting of MS. When it came to revitalizing plants, the Basmati 2000 mixture performed the best, followed by the Basmati 385 and Super Basmati mixes. The calli were vaccinated and then put on recovery media, and after three weeks, roots began to develop. The growth-promoting effects of both BAP and NAA-containing media were seen, although none was enough for full recovery on its own. Callus acceptance was highest in all three varieties when grown in N6 medium with 2 mg/L of 2, 4 D. When grown on MS medium supplemented with 1 mg/L of NAA and 2 mg/L of BAP, however, Basmati 2000 and Super Basmati produced much better yields. Treatment with 1 mg/L of NAA and 5 mg/L of BAP resulted in the greatest recovery in the Basmati 385 variety.

Keywords: Basmati 2000, Basmati 385

INTRODUCTION

The pursuit of genetic variety in rice, a staple crop of significant importance in developing nations, has the potential to provide widespread access to the advantages offered by scientific and technological advancements. This might positively impact the lives of hundreds of millions of people in need. The advancement of cellular research and atomic breeding technology has been associated with the genetic enhancement of rice. The dissemination of non-native rice species has been associated with the use of tissue culture methodologies such as anther culture, advancements in life support

systems, and the utilization of somaclonal variants.

Plants have the ability to undergo rejuvenation via mechanisms known as prolonged embryogenesis or organogenesis. In order to modify a plant, the use of in vitro tissue culture techniques may be necessary. In order to cultivate a whole plant inside a laboratory setting, it may be necessary to establish distinct evidence of living cells or tissues.

This research examined the effects of excessive moisture on three commonly cultivated plant species in the Punjab region. Shimada et al. (1969) and Vasil (1982) conducted studies on tissue culture challenges pertaining to rice cultivation in Pakistan. However, it is worth noting that no study has been conducted so far on the specific varieties developed in the region of Punjab. Regenerative rice cultures have been established with several forms of explants, including developed seeds, scutellum, and root-activated callus. The study demonstrated that there are significant variations in the formation and healing process of calluses across individuals.

For many decades, scholars in the disciplines of genetics, botany, and plant pathology have used callus and cell samples, alongside other plant components such as stems, flowers, roots, and eggs, in their research endeavors. This phenomenon might be attributed to the pluripotent nature of plant cells, which enables them to differentiate into many specialized cell types within the plant organism. The remarkable flexibility and capacity for growth from many cell types are retained by plants when cultivated in a laboratory setting. This phenomenon may arise due to physiological, physical, or restorative causes. Schwann (1939) concurred with the notion that every cell within a multicellular organism had the capacity for autonomous growth, provided it is provided with suitable conditions. The term "totipotent cell" was used by Morgan in 1901 (Krikorian &Berquam, 1969) to designate a solitary cell with the capacity to develop into a whole organism.

The present discourse aims to provide a concise overview of the historical development of tissue culture techniques specifically applied to the cultivation of cereal crops. 2.1

Larue (1949) is credited with pioneering the successful cultivation of corn endosperm. Carew and Schwarting (1958) as well as Roberts and Road (1955) both successfully

generated a viable callus from mature rye plants. Norstog's research on community development behavior (Norstog, 1967, 1970) expanded upon his earlier study conducted in 1961. The research conducted by Yamaguchi et al. (1970) used wheat embryos as a model system to investigate the acceptance of callus and subsequent separation and development of cells in suspension.

2.2 Tissue Culture Utilizing Rice

The first attempts to cultivate rice using juvenile hybridized eggs and isolated root organ cultures were conducted by Fujiwara and Ojima in 1955, followed by Amemiya et al. in 1956. Since the 1960s, Japanese researchers have been endeavoring to develop methods for cultivating rice without the use of traditional seeds. The first effective callus formation was achieved by Furuhashi and Yatazawa (1964) by the use of rice stem hubs. Subsequently, the authors emphasized the significance of yeast extract in their study (Yatazawa et al., 1967). The approach used by Yamada et al. (1967b) was utilized for the cultivation of rice calli inside a laboratory setting. The study conducted by Yamada et al. (1967a) revealed that the application of indole-3-acetic acid (IAA) and 2,4-dichlorophenoxyacetic acid (2,4-D) positively influenced the formation of callus in base and shoot node 3. Plants were successfully regenerated by the use of several callus procedures, including seed callus (Tamura, 1968; Nishi et al., 1968), root callus (Kawata and Ishihara, 1968), and dust callus (Niizeki and Oono, 1968).

In this study, coleoptile fragments derived from Infica rice (namely, the Kasturi variety) were used in an effective methodology aimed at achieving a high frequency of acceptance of embryogenic callus and subsequent plant regeneration. The coleoptile pieces were utilized at various developmental stages, specifically 3, 4, 5, and 7 days post-germination.

The coleoptile sections were cultivated in MS basal medium supplemented with 2,4-D (4.50-18.0 M), kinetin (2.32 mu M), and 3% sugar. As a result, the cut ends of the sections underwent a transformation, forming a rigid callus. Embryogenic calli were cultured in a Murashige and Skoog (MS) medium supplemented with a concentration of 2.25 millimolar (mM) 2, resulting in the development of viable embryos. The use of a medium containing a 4-dimensional structure, a concentration of 2.32 millimolar kinetin, 490 micromolar L-tryptophan, and 3% (w/v) sugar resulted in a notable increase in callus acceptance and substantial fetal

development. According to Sahrawat and Chand (2001), The embryogenic callus clumps were transferred to a Murashige and Skoog (MS) medium supplemented with 2.85 micrograms per liter (mu M) of indole-3-acetic acid (IAA), 17.77 mu M of 6-benzylaminopurine (6 BAP), and 3% (w/v) of sugar. Consequently, this resulted in the regeneration of plant life.

In their study, Gul et al. (2000) examined the response of four rice varieties, namely Basmati-385, JP-5, Pakhal, and Swat-Il, to various cultural circumstances in the context of in vitro callus organization and plant regeneration. The efficacy of culture conditions supplemented with 2 mg/L 2,4 D and 0.2 mg/L kinetin, as well as those devoid of these hormones, was assessed to determine their impact on callus growth. Pakhal (70%) and Basmati-385 (58%) exhibited significant callus elongation during cultivation on a growth hormone supplemented medium. For

The Kn levels used for recovery in the MS medium are MSK2 (2.0 mg/L) and MSK2 (0.2 mg/L). The experiment included the use of Kn and IAA at concentrations of 0.5 mg/L and MSKS at a concentration of 5 mg/L. The MSKS medium, namely MSKS medium (not MSK2), demonstrated superior performance across all genes in terms of wound healing. The therapeutic methods that exhibited the highest levels of cruelty were the JP-S type (64%), followed by Swat-II (51%), Pakhal (44%), and Basmati-385 (30%).

The topic of interest is rice cultivation in the Chorotega region of Costa Rica. In their study, Shankhdhar et al. (2001b) enhanced the development and proliferation of Pusa Basmati I embryos by a specific protocol. This included subjecting the embryos to a dark environment at a temperature of 26-41°C for a duration of four weeks. Additionally, the researchers supplemented the MS medium with certain components, including 2 mg/L of 2,4-D, 3% sucrose, and 8 g/L agar. The concentration of 2,4 D in the medium decreased as the fetal callus developed from the developing embryo. The manipulation of the MS-IAA-6BAP and kinetin genes in the eggs resulted in the development of robust and vigorous plants. When exposed to a concentration of 0.5 mg/L of BAP, the plantlets exhibited optimal self-arrangement.

The study conducted by Biswan and Mandal (1999) investigated the behavior of several varieties of in vitro-cultivated indica rice

with regard to callus acceptance and plantlet healing. The obtained findings were compared to those of Taipei 309, a strain of japonica rice that exhibits robust growth in a laboratory environment. Annada extracted the highest number of plantlets from each seed call, and subsequently obtained the greatest number of plantlets.

The plant species known as Oryza sativa L. has the ability to undergo callus formation. cv. The present study investigated the impact of 2, 4D alone (at pH 0 and 1) and in conjunction with BAP (at a concentration of 0.5 g/L) on Super Basmati seeds. Following fertilization, the compact calliwere transferred to a growth medium for a duration of four weeks, after which they were then transferred to a healing media. The concentrations of NAA and BAP used for the purpose of recovery ranged from 0 to 0.1 mg/L and 10 mg/L, respectively. The frequency of callus formation in Super Basmati varied from 54.6% when cultured on N6 medium to 87.7% when cultured on MS media. A marginal enhancement was seen in the proliferation of the callus.

N6 has a moderate level of effectiveness when compared to MS. When examined in isolation from the conventional media, it was shown that the application of 2,4-D at a dosage of 2 mg/1 yielded the highest rate of callus development and acceptance. According to Rashid et al. (2001), the inclusion of BAP and 2,4-D in the callus acceptance medium resulted in a significant reduction in the rate and frequency of callus acceptance.

The objective of this research endeavor was to enhance an existing technique that has previously shown the ability to induce callogenesis in rice. The experiment included three distinct varieties of rice, namely Rachna Basmati, Basmati 2000, and 370. The highest callogenesis was seen in Basmati 370 when cultivated on N6 medium supplemented with 2, 4-Dic 1.2, and 3mg/L of the hormones. In the process of callogenesis, no discernible difference was seen between Rachna Basmati and Basmati 2000. The callus acceptance rate of Basmati 370 was shown to be decreased in comparison to Rachna Basmati, Basmati 2000, and Basmati 370. The study conducted by Rashid et al. (2003) examined the development of callus in three different varieties, with Rachna Basmati demonstrating the most favorable results.

To further investigate soma-clonal variation in two varieties of rice (Oryza sativa L), a single round of sexual reproduction was conducted on R-0 rescued plants, sandwiched

between two cycles of cultivating callus cultures obtained from embryos. Following a single round of tissue culture, the regenerated plants exhibited diminished regularity and aesthetic quality compared to the initial stock. By using R-1 embryos as explants in a subsequent cycle, it seemed that the heritable trait of cellular salt resistance shown in the first cycle might be transmitted to the offspring. The extent and kind of somaclonal variation is contingent upon the characteristics of the R-0 parental plant and the specific circumstances of the culture environment (Lutts et al., 2001). Research findings have shown that the level of somaclonal diversity seen in certain lineages cultivated from calli subjected to salt treatment is significantly reduced.

This study used immature seed callus derived from four distinct varieties of Australian rice to investigate the regenerative potential of plants within a laboratory setting. The manner in which the media portrays a state of unyielding acceptance.

The strains exhibited the ability to cultivate calluses when exposed to varying concentrations of 2,4-D in MS medium, ranging from 0.5 mg/L to 2.0 mg/L. The recently regenerated shoots were placed in a growing mixture without the typical plant development nutrients. The discovered plant specimens exhibited a wide range of common and diverse morphologies.

According to the study conducted by Azria and Bhalla in 2000, According to our study findings, callus derived from the cultivation of several rice embryo types has sufficient flexibility to be used in genetic modification studies.

In their work, Nouri and Arzani (2001) used MS, LS, and N6 medium to investigate the callus acceptance and plant regeneration potential of 18 distinct rice genotypes derived from immature eggs. The measurement of plant healing rate was conducted by transferring the calli from the acceptance medium to the recovery medium. Due of its rapid regrowth, Japonica rice emerged as the predominant variety for cultivation. According to the findings of this research, MS and N6 media were identified as the most suitable growth mediums for cultivating rice seedlings in a laboratory setting.

The seeds of Basmati 370, Basmati 385, and KS 282 rice varieties were cultivated on a growth medium consisting of MS medium supplemented with 2.0 mg/L 2,4-D. Among

the tested plant varieties, KS 282 had the greatest success rate (33.0%) in callus formation, followed by Basmati 385 (17.6%) and Basmati 370 (6.5%). Various combinations of auxin and cytokinin were used to facilitate the transfer of calli from Murashige and Skoog (MS) medium. The plant regeneration was seen to be maximum after a period of 90 days of maintenance when the MS medium was supplemented with 0.5 mg/L NAA and 1.0 mg/L BAP for Basmati 370, resulting in a regrowth rate of 57.14% for Basmati 385. In a study conducted by Rashid et al. (2003), it was shown that KS 282 exhibited the highest recovery rate of 75% when subjected to lesser dosages (0.4 mg/L NAA and 0.8 mg/L BAP) on the same medium.

A research was conducted using three indica rice (Oryza sativa L.) varieties to investigate the impact of genetics on callus acceptance and the recovery of green plantlets in response to different medium combinations.

The Kamal District includes the surrounding areas including IR-72 and IR-54. A total of 15 distinct media combinations were used in order to assess the scutellar callus inference in developing embryos that had undergone incision. The combinations included MS, N6, R2, SKI, and other derivative forms. The acceptance of callus, as well as its subsequent recovery, is influenced by several factors including the acceptance medium, the recovery medium, and the combinations of genotype, media, callus acceptance, and recovery. Additionally, the interactions between genotype, recovery, callus acceptance, and medium may also impact the acceptance and recovery processes. The Kamal region had the best percentage of recovery (47.5% in IR-72) when the callus-inducing agent SKIm was synergistically paired with the recovery-enhancing medication MS. A significant proportion of individuals who had MMS(N)-MMS(N) therapy prior to the administration of IR-54 (25%) exhibited successful regeneration. Although genetics and the composition of the basal medium used for callus acceptance were significant factors contributing to the recovery response, a comprehensive examination of variance indicated a crucial interaction between the media used for callus and plantlet recovery (Khanna and Raina, 1998).

Scholars have conducted investigations on the genetic mechanisms behind the regenerative capacity of the seed callus in the

resilient rice cultivar known as Joshu. An experiment was conducted to assess the regenerative capacity of the progeny resulting from a cross between Joshu and two cultivars known for their ability to heal from injury, namely Moritawane and Norin 1. The findings indicate that the tall recovery ability seen in Joshu is governed by two distinct factors: a dominant character and a latent character. High recovery capacity is shown when either one or both of these features are exhibited. Furthermore, this research could not identify any discernible disparities in aptitude between different generations. (2021). The Impact of Social Media on Mental Health: A Review of the Literature)

Scientists have used groome change, chromosomal transformation, and plasmon transformation techniques to assess the somatic diversity of offspring plants belonging to the R-0, R-1, and R-2 generations.

The progeny of R-2 plants exhibited the largest proportion (52.4%) of quality modifications, with albinos and other chlorophyll-deficient mutants accounting for a range of 20 to 33. The study demonstrated that callus cells exhibit a significant variation in chromosomal count, which is influenced by the aging process. According to Chatterjee (1998), the findings indicated that the use of tissue culture techniques may result in a substantial occurrence of somaclonal variation.

The effects of isopentenyl adenine (2ip) on wheat tissue and plant healing were investigated by researchers by cultivation of the chemical under several environmental conditions. The embryogenesis and plant recovery rates of rice calli were significantly enhanced upon transfer to a conventional tissue culture recovery medium, subsequent to preculturing at certain 2ip ratios. The inclusion of 2ip in the preculture medium significantly influenced the subsequent stage of cell separation in the context of 2,4-D assistance. By altering the concentration of 2,4-D and 2ip in the growing media, it is possible to differentiate the processes of plant development and plant healing. Zhu et al. (1996) provide several methodologies for the use of 2ip, 2, 4-D, and KT in the context of grain recovery.

on this study, grain and root explants were used on a Murashige and Skoog (MS) medium to assess the regenerative capacity and healing ability of nine indica rice varieties and one indica-cross line. The outcomes seemed to be contingent upon an individual's genetic makeup. The recognized

percentage of callus healing in corn cultivation increased significantly from 24% to 93.3%. According to the study conducted by Rahim et al. (1991), the plant regeneration rates observed in plants using grain calli varied between 6.2% and 18.6%.

The study included the cultivation of callus societies derived from 7-week-old embryos of several indica rice varieties. The morphogenic calli rates observed ranged from 10% to 47%. It was observed that these plants exhibited the ability to undergo healing processes.

We conducted experiments involving callogenesis and plant resurrection using three distinct growth media. The use of Murashige and Skoog basal media, namely MSC for callogenesis and MSR for recovery, proved to be the most effective medium combination among the seven types evaluated for plant revival. The rates of callogenesis were not commensurate with the regenerative capacity of plants. The CR-1113 and CR-5272 lines had the highest levels of greenness among the plants. According to Valdez et al. (1997), the findings of their research indicate a potential correlation between genetic variations in several plant species and their capacity for regeneration.

The investigation included an examination of the morphological characteristics of rice (Oryza sativa L.) callus cultivated in a laboratory setting, as well as an assessment of the regenerative capacity of the plant from this callus tissue. A model was developed to characterize the many morphological characteristics shown by rice calluses. In a study conducted by Kucherenko (1993), a total of 33 rice calluses were collected and visually shown via drawings. Subsequently, these calluses were categorized into four distinct categories according to their respective healing capacities.

MATERIALS AND METHODS

The study's main objective was to develop and analyze greenhouse-grown rice callus samples. The probe took place at ABP. I'll be visiting the National Agribusiness Research Center in Islamabad between December 2022 and January 2023.

ORIGINAL SOURCE

Islamabad's NARC's Plant Breeding Program is where we got our seeds for the Basmati 2000, Basmati 385, and Super Basmati varieties of rice.

PROCEDURE FOR STERILIZATION

The sterilization procedure described by Rashid et al. in 1996 involves cleaning dishware and sanitizing the explant's exterior.

Explant Sterilization

A laminar flow cover was used throughout the explant sterilization process to ensure that no contamination might occur. We autoclaved water in carafes and dropped precisely chosen sound seeds within. Seeds were soaked for one minute in 70% ethanol before being rinsed in purified water that had been heated in an autoclave. After soaking the seeds for 20 kilometers in a 50% clorox solution, they were vigorously shook for the whole duration. Clorox is a commercial bleach that has 5.25 percent sodium hypochlorite in it. The Clorox treatment was followed by three thorough rinses in filtered water from an autoclave. The seeds were allowed to germinate, and then moved to a petri dish using sterile filter paper.

Forming Content for Media

A combination of salts and vitamins from Murashige and Skoog (1962) and Chu (1978), as well as sucrose and plant growth regulators, served as the basis for the callus refining protocol. Table 1 displays the current inventory status of MS and N6 media components.

Table No.1: The Individual Parts That Make Up MS and N6 MediaMS(mg/L) N6(mg/L)

No.	Components	MS(mg/L)	N6(mg/L)
1.	**Macronutrients(A stock solution)**		
	Ammonium nitrate	1650	-
	Potassium nitrate	1900	2830
	Calcium chloride	370	185
	Magnesium sulphate	170	400
	Monopotassium dihydrogen phosphate	-	283
2.	**Micronutrients (B stock solution)**		
	Boric acid	6.2	1.6
	Cobalt chloride.$6H_2O$	0.025	-

	Copper sulphate	0.025	-
	Mangnese sulphate. H_2O	15.6	3.3
	Iron sodium chelate	43	43
	Potassium iodide	0.83	-
	Sodium molybdate	0.25	0.25
	Zinc sulphate. $7H_2O$	8.6	1.5
3.	**Vitamins (C stock solution)**		
	Thiamine- HCL	0.5	10
	Pyridoxin- HCL	0.5	0.5
	Nicotinic acid	0.5	0.5
	Myo- inositol	100	-
4.	**Carbon source(sucrose)**	**30g/L**	**30g/L**

1. Macronutrients (Readily Available). The ratio of ammonium nitrate to potassium nitrate is 1650:1900:2830.370 mM Calcium Chloride 185 mMSulfate of magnesium, 170-400Dihydrogen phosphate, monopotassium – 283

Ingredients for B Stock Solution, MicronutrientsSodium borate6.2 1.6 Chloride of cobalt.Sulfate of copper (CuSO4) and manganese (MnSO4), each at 0.025 percent in 6H2O. H2O 15.6 3.3 Chelated iron (sodium) 43The ratio of potassium iodide to sodium molybdate is 0.83 to 0.25. Sulfate of zinc. 7H2O 8.6 1.5

Vitamin C (as a stock)HCL Thiamine.5 HCL Pyridoxin.5 HCLHalf a half of nicotinic acid Inositol 100 Myo-Sugar, the fourth carbon source30g/L 30g/L.

Disc sterilization

Wrapped test tubes containing ober surgical rebellious and distilled water are autoclaved at 15 pressure for 20 minutes at 121 °C.

INDUCTING CALLUSES

Seed Inoculation One seed was placed in each test tube. Societies were able to interact and advance in a natural climate-controlled area. They were strung vertically, 10 inches apart, from regular electric fluorescent lamps that produced a steady, strong light of around 2000 lux. The environment was kept at 25 degrees Celsius (3 degrees Fahrenheit) for the duration of the development period. After

vaccination, callus acceptance was restored in all genotypes within

four to five days. All varieties were tested for callus development rate and quality two to three weeks after vaccination. Method of Trade and Cultural Infiltration

To assure the cleanliness of the swabs, we moved them inside a laminar-flow cabinet. The calli were collected in Petri plates, and the embryogenic callus was then cultured in a maintenance medium similar to the callus acceptance media. For three weeks, the institutions depended on media created for upkeep.

REGROWTH OF PLANTS

Murashige Skoog's (1962) salts, vitamins, and 3.0 percent (w/v) sucrose were used to assess the plant recovery potential of four genotypes. Six-benzyl amino purine (BAP) and indole acidic corrosive (IAA) were used in two different combinations as growth regulators. Carefully separating embryogenic (E) calli from non-embryogenic (NE) callus, the latter were then divided into smaller pieces and inoculated on the recovery medium shown inside the glass vials, resulting in a complete plant recovery. The recovery medium, at a volume of 50 ml each combination, was individually poured into glass containers measuring 55 mm in diameter and 120 mm in depth. In each shock, around four or five calli received a cap immunization. For a period of two to three weeks, the test tubes and jars were stored in an environment similar to that required for callus acceptance.

BAP and NAA make up the two combinations tested for their efficacy as a recovery cocktail (Table No. 2).

No.	**Conc. of NAA (mg/L)**	**Conc. of BAP (mg/L)**
1.	1	2
2.	1	5

NAA and BAP Concentrations in Two Different MS Media

No. NAA Concentration (mg/L)BAP Concentration (mg/L)

1. 1 2 2. 1 5

These were used in conjunction with MS salts and served in 2% sugar arrangements.

3.4 BECOMING A GLASS HOUSE

First, all of the plants that had been rescued were moved into the greenhouse, where the humidity and temperature could be carefully maintained. As time went on, the temperature

adapted progressively to its new surroundings. After these plants hardened, they were transplanted into the wild, where they thrived despite the fact that no one knew whether or not they were disease-free (Figs. 7, 8, and 9).

RESULT AND DISCUSSION

The objective of this study was to ascertain the most effective combination of the growth regulators benzyl amino purine (RAP) and naphthalene acidic corrosive (NAA) in order to enhance the regeneration process of three distinct varieties of rice (Oryza aiva 1): Basmati 385, Basmati 2000, and Super Basmati.

Prior to commencing tissue culture tests, it may be necessary to sterilize the explants. In their study, Rashid et al. (1996) provided a description of the sterilization procedure used in this inquiry. The researchers utilized a combination of 70% ethanol and 50% clorox to sterilize the rice seeds. The most pronounced rate of callus development was seen when a 50% concentration of Clorox was used. Li, et al. (1992) conducted a study. According to the available research, seedlings subjected to a 30-minute treatment with Clorox 45% (v/v) were seen to exhibit germination.

The study conducted by Shankhdar et al. (2001a) investigates the role of 2,4-D in the induction of a callus. The available evidence from previous studies strongly indicates that the addition of 2,4-dichlorophenoxyacetic acid (2,4-D) is a necessary need for the process of dedifferentiation in rice callus prior to its successful acceptance. Consequently, a solution of 2,4-D was administered to N6 medium including ure seeds of several kinds at concentrations of 2, 2.5, 3.0, and 3.5 mg/L. In a previous study conducted by Naheed Gul al (2000), it was shown that the inclusion of growth hormone in the medium resulted in enhanced callus formation. Consequently, in the present study, plantlets were cultured on N6 medium without 2,4-D, as an alternative to callus cultivation.

This observation suggests that 2, 4-D exhibits effects on the genome, providing a possible explanation for the significant role of 2, 4-D in callus formation, as reported by Fan et al. (2000).

The greatest quality callus was seen when a concentration of 2 mg/L of 2,4-D was used. Consequently, the presence of different concentrations of 2, 4-D led to alterations in the quality of the callus. The callus acceptance rates for Basmati 2000, Super

Basmati, and Basmati 385 were found to be 85%, 80%, and 60%, respectively. According to Rashid et al. (2001) and Shankandar et al. (2001b), the application of 2,4-D at a dose of 2 mg/L on MS medium yielded the most favorable rates of callus acceptance in rice. Nevertheless, as shown by the data presented in Table 3, higher dosages of the substance were found to impede the process of cell division. The frequency of the three accepted calluses exhibited significant variation due to the distinct genetic ability of each type to absorb calluses (Schaeffer et al., 1979). Among the several varieties tested, the Basmati 2000 shown considerable promise, but the Basmati 385 variety did not demonstrate similar potential. The consistency of the callus's appearance is evident across many breeds, as seen in Figures 1, 2, and 3. The promotion of cell division and proliferation is attributed to the auxin properties of 2,4-D. The process of mRNA and protein conjugation is subject to external influences. Hence, it is plausible that a concentration below the ideal range would not sufficiently permeate the combination of mRNA and proteins, but a quantity beyond the optimal range might potentially intensify the alteration (Malik and Srivastava, 1985).

Table No. 3. Effects of different concentrations of 2, 4- D on callus induction.

2,4- D Concentration (2 mg/L)	Callus Induction Frequency (%)		
	Basmati 2000	Basmati 385	Super Basmati
2.0	85.0	60.0	80.0
2.5	81.0	54.5	75.0
3.0	74.2	51.7	69.0
3.5	70.0	50.0	67.5

Table No.4. Callus induction frequency of Super Basmati on N6 medium with 2 mg/L 2,4- D

No. of Replicates	Total seeds culture	Callus Indeed	Percentage Frequency (%)
1.	120	90	75
2.	96	70	72.9
3.	144	103	71.5

4.	144	105	73

Mean = 73.1%

Table No.5. Callus induction frequency of Basmati 385 on N6 medium with 2 mg/L 2,4- D

No. of Replicates	Total seeds culture	Callus Indeed	Percentage Frequency (%)
1.	72	37	52
2.	144	73	57
3.	96	42	55
4.	144	72	51

Mean = 53.75%

Table No.6. Callus induction frequency of Basmati 2000 on N6 medium with 2 mg/L 2,4- D

No. of Replicates	Total seeds culture	Callus Indeed	Percentage Frequency (%)
1.	168	129	76.78
2.	144	126	87.5
3.	120	105	87.5
4.	144	102	70.8

Mean = 80.6%

Table No.7. Comparison of Average Callus Induction (%) of Basmati 2000, Super Basmati and Basmati 385.

Variety	Average Callus Induction (%)
Basmati 2000	80.6
Super Basmati	73.1
Basmati 385	53.75

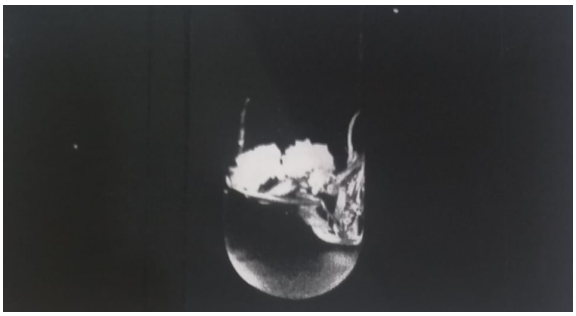

Fig. 1. Embryogenic Callus in Basmati 2000 on N6 at 2 mg/L of 2,4- D

Fig.2. Embryogenic Callus in Super Basmati on N6 at 2 mg/L of 2,4- D.

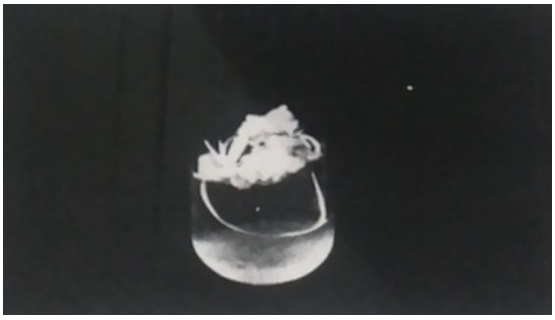

Fig.3. Embryogenic Callus in Basmati 385 on N6 at 2 mg/L of 2,4- D.

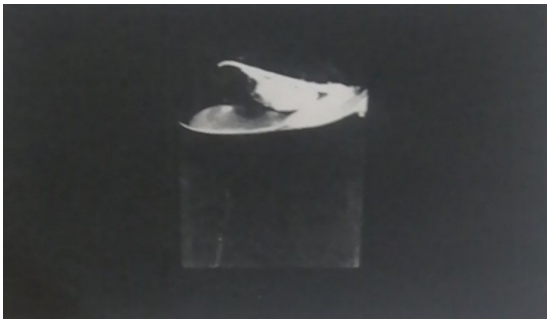

Fig.4. Non- Embryogenic Callus

Embryogenic and atypical calli are the two groups to compare.Calli were divided into embryogenic (EC) and non-embryogenic (NEC) groups according to their morphological characteristics. In Figs. 1, 2, and 3, you can observe the embryonic rice grains that gave rise to the EC calli, which have a compact spherical shape and a white to velvety look. When cultivated in the same N6 media, embryonic cells (ECs) may form structures that resemble, or are identical to, embryoid bodies. Friable, unrestrained calli were indicative of NEC (Fig. 4).

The process of callus reproduction is of interest

Important for a successful regeneration is the elimination of non-embryogenic or brown/dark sections of embryogenic callus. A 21-day period ended with the appearance of an embryoid. The best results for callus production and growth in the cultured medium were achieved when 2,4-D was used at a dosage of 2 mg/L. The varying mixtures all produced desirable results. Tables 8, 9, and 10 indicate the growth of the assortments used in the display displays. Katiyar et al. (1999) observed that the rate of callus proliferation differed from one genotype to the next. Genotypic uniqueness in the persistence of callus acceptance in Basmati cultivars was thoroughly described by Rashid et al. (2000). According to studies by Chen and Lin (1976) and Tsai and Lin (1977), the frequency with which a callus is accepted depends on the particular varietal type.

Table No.8. Maintenance of embryogenic calli of Basmati 2000 on N6 2 mg/L 2,4- D.

No. of Calli	Contamination	Browning	Growth and Proliferation	Growth(%)
25	4	3	18	72
40	6	5	30	75
40	4	7	29	72.5

Mean = 73%

Table No.9. Maintenance of embryogenic calli of Super Basmati on N6 2 mg/L 2,4- D.

No. of Calli	Contamination	Browning	Growth and Proliferation	Growth(%)
30	4	5	21	70

35	5	6	24	68.5
40	7	4	29	72.5

Mean = 70%

Table No.10. Maintenance of embryogenic calli of Basmati 385 on N6 2 mg/L 2,4- D.

No. of Calli	Contamination	Browning	Growth and Proliferation	Growth(%)
25	5	3	12	60
30	4	6	20	66
40	5	10	25	62.5

Mean = 62.8%

PLANT REGENERATION

On a modified Murashige and Skoog (MS) medium supplemented with 2 g/L of casine hydrolysate, 3% sugar, and 3% sorbitol, various varieties of calli were cultivated in anticipation of the harvest. Table 2 exhibits the two distinct NAA+BAP formulations utilized in the study. Both auxins and cytokinins played equally important roles in the regeneration of plantlets.The data presented in Table 7 pertain to the total number of green-spotted calli and the subsequent number of regenerated plants derived from these calli under varying BAP and NAA concentrations. Rashid et al. (2000) discovered that the close proximity of a high concentration of BAP had a positive effect on plant development. However, the experiment produced contradictory results, which may be attributable to the unique characteristics of the plant varieties utilized. All genotypes displayed significant variation in their recovery capacities. According to Kyungsoo et al. (2003), the lack of agar in the recovery medium had a negative impact on the growth of the seedlings. The initial allocation period of four days for verdant space has been extended to ten days. Basmati 2000 and Super Basmati exhibited recovery rates of 80.6% and 73.1%, respectively, when subjected to a standard treatment consisting of the application of 1 mg/L of NAA and 2 mg/L of BAP, according to the results of the experiment. These results are illustrated in Figures 5 and 4. In contrast to the results reported by Rashid et al. (2000), the application of 1 mg/L of NAA and 5 mg/L of

BAP to Basmati 385 resulted in the greatest recovery to the initial level (Table 8, Figure 6). No one made the effort to observe the revival.

Initially, the seeds produced little root development; however, after four weeks, they had effectively established a solid foundation. Four weeks were required for the plants to reach the appropriate level of readiness for transplanting into the greenhouse environment. In addition, it is plausible that the presence of a sediment remediation expert would aid in the recovery process. The duration of recovery for calli immunized on an agar-based recovery medium is significantly longer than for calli immunized on a gel-based recovery medium.

Table No.11. Regeneration of calli in all the three varieties at 1 mg/L NAA and 2 mg/L BAP.

Variety	Total Calli Cultured	Calli Left after contamination	Blackening	Green Spots	Plants Formed	Percentage(%)
Basmati 2000	55	49	7	9	39	71
Basmati 385	60	50	18	8	20	33.33
Super Basmati	50	48	6	7	34	68

Table No.12. Regeneration of calli in all the three varieties at 1 mg/L NAA and 5 mg/L BAP.

Variety	**Total Calli Cultured**	**Calli Left after contamination**	**Blackening**	**Green Spots**	**Plants Formed**	**Percentage (%)**
Basmati 2000	40	36	4	6	24	60
Basmati 385	60	50	11	16	23	38
Super Basmati	38	32	10	6	22	57.8

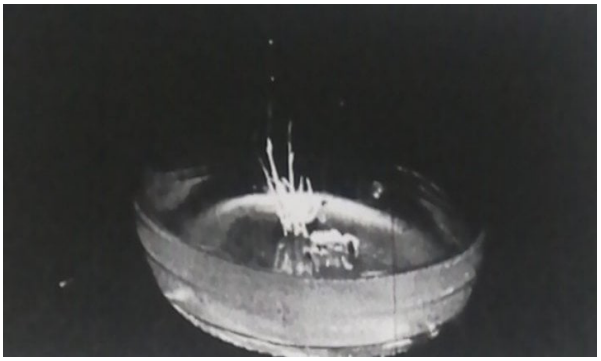

Fig.5. Regeneration of callus in Super Basmati

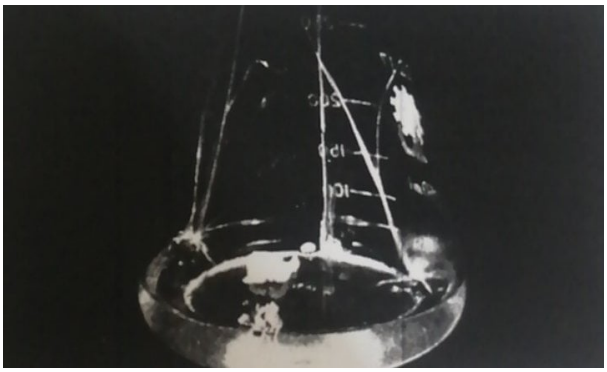

Fig.6. Regeneration of callus in Basmati 2000

Fig.7. Regeneration of callus in Basmati 385

GLASSHOUSE ASSESSEMENT OF THE PLANT REGENERATED FROM CALLI

In order to conduct a comparative analysis of the growth patterns shown by different plant species, a total of eight plants were transplanted inside the confines of the conservatory. The control plants of all cultivars propagated from seeds exhibited a significant level of development, but the tissue-refined plants had comparatively

lower levels of development. There exist two potential rationales for this phenomenon: either the plants' heightened susceptibility renders them exceptionally amenable to the regulated environment of a greenhouse, or the plants first manifest reduced growth rates which subsequently escalate after they establish a stable foundation. The results of the study showed that the calli-grown plants of Basmati 2000 shown the highest level of growth compared to the other kinds examined. Conversely, the calli-grown plants of Basmati 385 demonstrated the lowest level of development. Certain specimens that have been retrieved exhibit a lack of growth, maybe due to an underdeveloped root structure that hinders their ability to collect essential water and nutrients from the surrounding soil. According to Su et al. (1992), the somaclones exhibited a somewhat limited growth in terms of height.

Table No.13. Glasshouse assessement of the somaclones of Basmati 2000

No. of Plants	Basmati 2000			
	Control		Somaclone	
	Height(cm)	No. of Tillers	Height(cm)	No. of Tillers
1	125	5	92.5	5
2	122.5	7	90	3
3	130	7	105	3
4	132.5	6	80	2
5	95	3	105	2
6	131	7	87.5	2
7	140	8	100	3
8	130	6	98.75	2

Table No.14. Glasshouse assessement of the somaclones of Basmati 385

No. of Plants	Basmati 2000	
	Control	Somaclone

	Height(cm)	No. of Tillers	Height(cm)	No. of Tillers
1	75	4	27.5	1
2	80	5	32.5	2
3	62.5	3	13.75	1
4	72.5	3	17.5	1
5	52.5	0	27.5	1
6	0	4	12.5	2
7	20.5	2	0	0
8	72.5	3	8.75	1

Table No.15. Glasshouse assessement of the somaclones of Super Basmati

No. of Plants	Basmati 2000			
	Control		Somaclone	
	Height(cm)	No. of Tillers	Height(cm)	No. of Tillers
1	30	4	30	1
2	47.5	5	27.5	1
3	20	2	7.5	1
4	25	3	0	0
5	30	3	10	2
6	29	4	0	0
7	31	5	0	0
8	30	5	0	0

Fig.8. Growth of somaclone of Basmati 2000

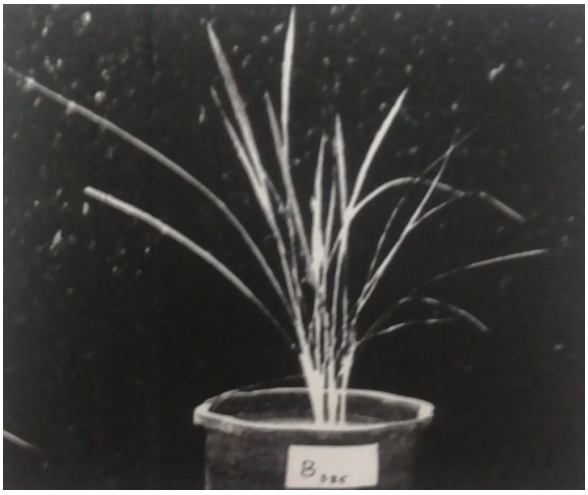

Fig.9. Growth of somaclone of Basmati 385

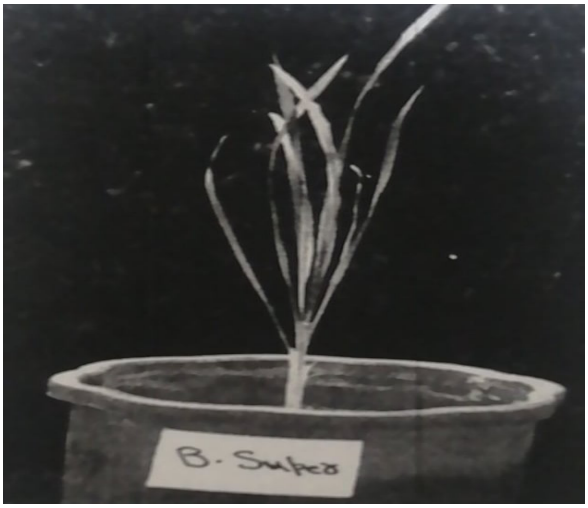

Fig.10. Growth of somaclone of Super Basmati

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