The Bioscan 18(1): 45-50, 2023
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES
www.thebioscan.com

EFFECT OF MUTAGEN ON SOMATIC EMBRYOGENESIS AND PLANT REGENERATION OF LOCAL AROMATIC RICE CV. "DUBRAJ"

SUDHANIRJHARINI SWAIN, KAILASH C. SAMAL AND SWAPAN K. TRIPATHY*
Department of Agricultural Biotechnology, College of Agriculture, OUAT, Bhubaneswar - 751 003,INDIA
e-mail: swapankumartripathy@gmail.com
ORCID ID:htpp://orcid.org/0000-0002-3443-4961

KEYWORDS
In vitro mutagenesis
Ethyl methanesulfonate (EMS)
Callusing

Received on :
11.01.2023

Accepted on :
26.03.2023

***Corresponding author**

ABSTRACT

Establishment of an efficient *in vitro* protocol *via* somatic embryogenesis is an essential prerequisite in harnessing the advantage of cell and tissue culture for genetic improvement in aromatic indica rice. Besides, the present investigation was undertaken to develop an efficient *in vitro* mutagenesis system with early callus induction and rapid plant regeneration in a popular aromatic rice variety Dubraj. The maximum embryogenic calli (90%) with excellent callus growth was acchieved in MS medium supplemented with 2.5mg/l of 2, 4-D + 0.5mg/L Kn. Such calli on transfer to MS + 2.5 mg/l BAP and 1.0 mg/l NAA resulted maximum morphogenetic response (86%) with production of well developed green plantlets after two weeks of culture. However, addition of EMS (0.005%) to the above medium still maintained optimum callus growth and regeneration of green plantlets although with reduced callusing (47%) and regeneration response (62.0%). Such a regeneration system can be routinely used for isolation of desirable in vitro mutants for aromatic rice breeding.

INTRODUCTION

Rice belongs to genus *Oryza* that has 24 different species among which two are cultivated *i.e*, *Oryza sativa* and *Oryza glaberrima*. *Oryza sativa* is cultivated everywhere throughout the world while the Oryza glaberrima has been cultivated in West Africa. Asian cultivated rice (*Oryza sativa* L.) is a diploid species with a small genome (Moin *et al*., 2017) and it has two major sub-species, indica and japonica. The sub-species, indica is most widely cultivated in South and South East Asia while the sub-species japonica is mostly grown in the temperate region of Asia (Glaszmann *et al*., 1987). The aromatic rice is a small but distinct subpopulation of indica subspecies (Garris *et al*., 2005). This includes the indigenous short and medium grain fragrant rices which fetch high price in international trade, possess the excellent aroma, taste and many rare alleles for stress tolerance but have low yield potential (Parimala *et al*., 2020), which has to be genetically improved (Samal *et al*., 2014, Das and Khanda, 2020). The major limitations in the genetic improvement of these cultivars are their cross incompatibility with other rice groups and the undesirable linkage in the resultant recombinants (Das *et al*., 2003). To overcome these difficulties, induction of mutation through *in vitro* culture can be a way forward for genetic improvement of these genotypes while maintaining their original genetic make-up (Gosal and Kang, 2012).

However, aromatic varieties being under indica sub-species, are mostly recalcitrant to *in vitro* culture compared to japonica lines. An appropriate media composition with suitable hormonal combination and concentration can achieve a highly efficient plant regeneration system amenable for recovery of induced mutants through *in vitro* culture. Mutagenesis is an important strategy for induction of mutation (Maluszynski *et al*., 2000; Da Luz *et al*., 2016) enabling studies of functional genomics and the development of new genotypes. In rice, recovery of induced mutants have been reported from immature embryos (Ookawa *et al*., 2014), mature caryopsis derived calli (Serrat *et al*., 2014) and cell suspension cultures (Chen *et al*., 2013). Ethyl methane sulfonate (EMS) causes mutations (Singh *et al*., 2015) through the alkylation of guanine that mismatches with thymine resulting in transitions of G/C to A/T (Talebi *et al*., 2012). The present investigation was undertaken to develop an efficient somatic embryogenic regeneration system amenable for *in vitro* mutagenesis for genetic improvement of aromatic rice.

MATERIALS AND METHODS

In vitro culture

The mature seeds of local aromatic variety "Dubraj" were dehusked manually and the kernels were surface sterilized with 70% alcohol for 2 minutes followed by treating with 4% Sodium hypochlorite solution containing 2 drops of Tween-20 for 15 minutes. Finally, the seeds were sterilized with 0.05% Mercuric chloride solution for 5 minutes followed by washing thrice with double distilled sterile water. The kernels were then blot dried on sterilized filter paper for aseptic inoculation on cultured medium. Murashige and Skoog (MS) medium (1962) supplemented with various plant growth regulators along with 100 mg/L myo-inositol, 3% sucrose (w/v) and 0.8%

agar-agar (Himedia, India) was used for in vitro culture. The pH of the medium was adjusted to 5.8 before adding agar to the medium. About 20 ml of molten media was transferred to 25x150 mm glass culture tubes and were plugged with non-absorbent cotton. The cultures were sterilized at 121ºC and 15 psi for 20 minutes. Following inoculation of explants, the cultures were incubated in culture room at 25+ 1ºC under dark for four weeks. Data on callus induction was taken after four weeks of inoculation. Different concentration of 2,4-D(1.0-3.5mg/L) alone as well as its combination with Kn (0.5mg/L and 1.0mg/L) in MS medium were tested for callusing response and growth of callus. Percentage of callus induction was calculated as follows:

$$\text{Callus induction frequency}(\%) = \frac{\text{Number of kernels produced cwlli}}{\text{Number of kernels inocultured}} \text{X100}$$

Fresh well developed calli were taken in a sterile petri dish and cut into small pieces(3mm dia) and then aseptically inoculated on shoot regeneration medium containing NAA at 1.0mg/L alone and different concentration of BAP. (0-3.0mg/L) alone as well as combination with NAA (0.5mg/L and 1.0mg/L). Green shoot primordia were developed on the surface of calli within 2-3 weeks and the shoots elongated subsequently. After four weeks of culture the percentage of shoot regeneration efficiency was calculated as follows:

$$\text{Shoot regeneration efficiency}(\%) = \frac{\text{Number of calli produced shoots}}{\text{Number of calli cultured}} \text{X100}$$

In Vitro Mutagenesis

The *in vitro* induced Scutellum derived embryogenic calli were used for *in vitro* mutagenesis. The composition of in vitro culture medium was formulated for effective callus induction and plantlet regeneration referred to as MS media with EMS solution. Phytohormones used included 2,4-dichlorophenoxy acetic acid (2,4-D),α-napthalene acetic acid (NAA) under auxin and 6-benzyl amino purin (BAP) and Kinetin (Kn: β-furfury1 amino purine) under cytokinins. Casein hydrolysate (as organic additive), proline and adenosine sulphate (ADS) were added to optimize the callus induction medium (CIM) and regeneration (R) medium for effective callus induction and plant regeneration respectively.

Before EMS treatment,the sterilized kernels were plated on filter paper soaked with liquid callus induction medium for 36h until emergence of coleptile.Thereafter,such kernels were treated with filter sterlized EMS-added liquid medium in flasks and shaken on an orbital shaker(150rpm) for overnight followed by transfer(without washing) to semi solid callus induction medium with a view to induce mutation in the primary callus cells. Before the calli were transferred to regeneration medium, the calli were partially desiccated by placing it on two layers of Whatman-1 filter paper in a Petri dish at 25±1°C in dark for 48 hours to allow loss of 40 to 65% water content. Following desiccation, somatic embryogenic calli were transferred to the regeneration medium supplemented with 2.5mg/L BAP + 1.0mg/L NAA. Effect of mutagen for callus induction and its carry-over effect on regeneration of plantlets were assessed. MS media supplemented with NAA (2.0mg/L), Kn (0.5 mg/L) and sucrose (50 g/L) and agar-agar (7g/L) for root initiation. The resulting plantlets with well developed roots were removed from the culture vessels, washed thoroughly under tap water and planted on autoclaved sandy loam soil. The plantlets were grown in green house for seven days before transfer to net house for plant establishment and follow-up growth till maturity.

Statistical analysis

Three sterilized kernels were placed on each culture tube per treatment (hormone combination) in each replication and the experiment was laid out in CRD with twenty four replicates. Similarly, CRD with twenty four replicates was followed for plantlet regeneration. Observations were recorded for callus induction frequency(CIF %), relative growth of callus, extent of necrosis, embryogenic callusing response, morphogenetic potential and plant establishment. Data sets were analyzed statistically by the Duncan's multiple range test (Duncan, 1955). Means followed by the same letter within columns were considered not significantly different at $P \leq 0.05$.

RESULTS AND DISCUSSION

In vitro culture of seeds is limited by plant genotype and hormonal composition of medium (Jain, 1997) to sustain growth of calli, subsequent plant regeneration and survival as

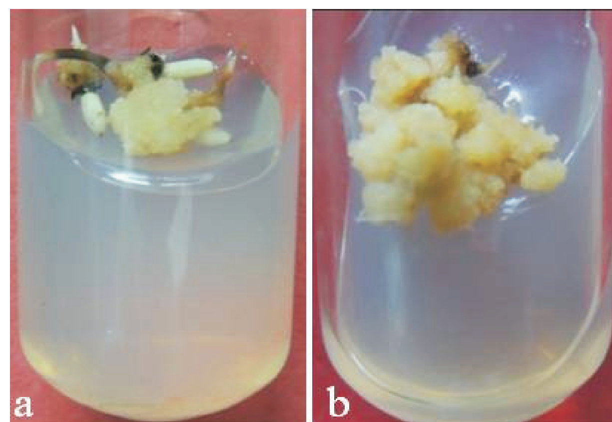


Fig ure1: Callus induction showng 28 d old callus in primary culture (a) and proliferation of callus after 10d in first sub-culture(b) of cv. Dubraj in MS + 2.5mg/L 2,4-D + 0.5mg/L Kn

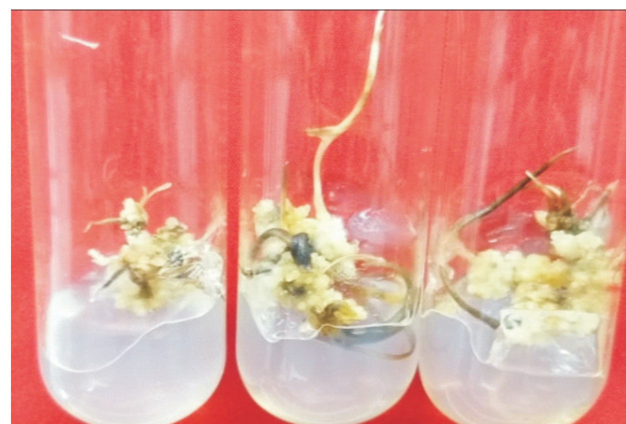


Figure 2: Emergence of occasional albino shoot followed by necrosis in cv. "Dubraj" using MS + 1.0mg/L NAA after two weeks of culture

Table 1: Response of different phytohormones for callus induction in cv. Dubraj after 28 days of culture

Media type*	2, 4 D (mg/l)	Kinetin (mg/l)	Callus induction (%)	Callus growth**	Callus morphology
M1	1	0	12.50 h	+	Pale yellow, moderate granular, somewhat compact and dry
M2	2	0	38.50 f	+	Pale yellow, moderate granular, dry and compact
M3	2.5	0	55.50 e	+ +	Creamy, somewhat smooth, soft and mucilaginous
M4	3.5	0	65.25 d	+ +	Soft, white, occasionally necrotic
M5	1	0.5	82.00 b	+ + +	Creamy , Large granular, dry and compact
M6	2	0.5	88.00 ab	+ + +	Creamy, moderate granular, dry and compact
M7	2.5	0.5	90.00a	+ + + +	Creamy, granular, friable, well developed (rapid proliferation)
M8	3.5	0.5	85.00 b	+ + +	Creamy , small granular, compact and dry
M9	1	1	63.22 d	+ +	Creamy white, large granular, somewhat compact and mucilaginous
M10	2	1	69.23 c	+ +	Creamy white, large granular, somewhat compact, dry
M11	2.5	1	54.63e	+ +	Pale yellow, moderate granular, somewhat compact and dry
M12	3.5	1	27.51 g	+	Pale yellow, moderate granular, dry and compact

*Basal medium used- MS medium; **Callus growth: + =Small size; + + = Moderate size; + + + = Good size, + + + + = excellent growth. Means followed by the same letter within columns were considered not significantly different at P 0.05.

Table 2: Effect of duration of primary culture on callus growth and nature of callus in cv "Dubraj" using MS medium supplemented with 2.5mg/L 2,4-D and 0.5mg/L kinetin

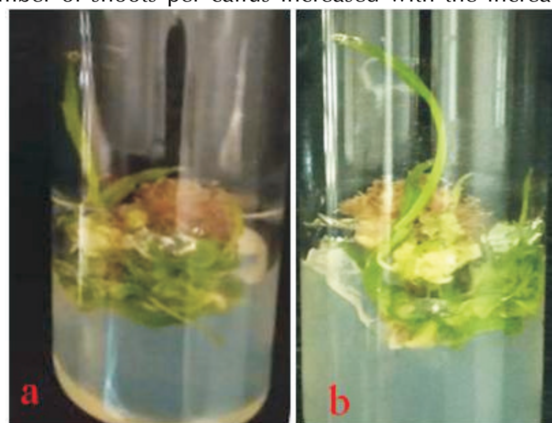
SL. No.	Culture period	Size of callus in M7 media (mm)	Nature of callus
1	After 14days of culture	1.5+0.001 c*	White, bit soft
2	After 21 days of culture	2.5+0.010 b	Pale white, moderate granular
3	After 28 Days of culture	3.7+0.021 a	Creamy white, nodular, friable without any necrotic spots
4	After 42 days of culture	4.0+0.030 a	Dull white, nodular, friable, but often necrotic

Means followed by the same letter within columns were considered not significantly different at P d" 0.05. *Values are mean + S.E.

fertile plants. It is often difficult to induce embryogenic calli and to regenerate plants from these callus cultures specially those belonging to Indica subspecies (Jain, 1997). The recalcitrant nature of this subspecies has, in fact, been a major limiting factor in transfer of valuable genes (Toenniessen, 1991). The MS (Murashige and Skoog, 1962) medium is widely used as the basal medium for callus induction in rice culture. Besides, available report revealed that 2,4-D was potent enough for callus induction than NAA and it was highest in case of 3mg/l 2,4-D alone in Malaysian wetland rice variety MR220-CL2(Mostafiz and Wagiran, 2018). In the present investigation, 12 treatments were formulated and tested for callus induction. Different combinations and concentration of plant growth regulators such as 2-4-D and Kinetin were supplemented to the MS basal medium. Calli protruded from the scutellum were clearly visible 7-8 days onwards. Frequency of callus induction was shown to increased to the tune of 65.25% with the concentration of 2,4-D alone. Calli induced in different treatments, varied in textures, size and colour (Table 1). However, combination of 0.5mg/L Kinetin with increased concentration of 2,4-D (2.5mg/L) was recorded (after 28 days of incubation) optimum in terms of callusing response (CIF: 90%) as well as callus growth. (Table 1, Fig 1). This confirms the findings of Tripathy *et al.* (2018) in upland rice. The calli developed were mostly creamy, nodular and friable as well as rapidly proliferating and embryogenic in nature. Such calli are preferred for high throughput plantlet regeneration and often targeted for genetic transformation. While longer period of primary culture (42 days) resulted necrotic calli not suitable for plantlet regeneration(Table 2). In the present investigation, supplementation of 2,4-D with higher concentration of Kinetin (1.0mg/L) did not revealed any dividend. In contrast, some researchers have reported highest callus induction (59.44%) from MS+2mg/l 2,4-D followed by MS+2 mg/l 2,4-D+0.5 mg/l kinetin (Joya et al. 2019). In an another report, 2.0mgL-1 2, 4-D was found best concentrations for callus induction (85%), callus size (4.533mm) and callus weight (81.847mg) in 10.66days (Mukherjee *et al.*, 2015).

Effect of plant growth regulators on shoot induction and plant regeneration

Different concentrations and combinations of auxins (NAA) and cytokinins (BAP) were used for shoot regeneration from 28d old callus induced in primary culture using MS+ 2.5mg/L 2,4-D + 0.5mg/L Kn. NAA alone at 1.0mg/L produced occasional albino shoot formation which became necrotic after few days(Fig 2), while BAP alone did not induce any plantlet regeneration. However, addition of NAA was shown to be essential along with BAP to produce green plant regeneration. Frequency of plantlet regeneration as well as number of shoots per callus increased with the increase in



Figurte 3: Formation of profuse shoot initials (a) followed by emergence of green shoots in cv. "Dubraj" using MS + 2.5mg/L BAP + 1.0mg/L NAA after two weeks of culture.

Table 3: Effect of growth regulators on shoot regeneration from 28-day old calli derived from cv. Dubraj using MS medium with varying concentration of BAP and NAA

Media type*	BAP	NAA	Morphological sign for onset of regeneration (at 8days of culture)	Time taken for shoot for mation (days)	Shoot regeneration frequency (%)
T1	0	1	Occasional albino shoots, later become necrotic		
T2	1	0	No sign of green spots	—	—
T3	2	0	No sign of green spots	—	—
T4	2.5	0	No sign of green spots	—	—
T5	3	0	No sign of green spots	—	—
T6	1	0.5	Greenish spots and shoots	12	42
T7	2	0.5	Greenish spots and shoots	9	30
T8	2.5	0.5	Healthy Green shoots	10	50
T9	3	0.5	Greenish shoots and spots	7	60
T10	1	1	Greenish shoots	15	64
T11	2	1	Greenish shoots	16	67
T12	2.5	1	Green well developed shoots	14	86
T13	3	1	Greenish shoots	15	80

*Basal medium used- MS medium

Table 4: Effect of EMS on callus induction and plant regeneration in cv. Dubraj cultured on MS-medium

Treat-ment	EMS (%)	Callus response† (%)	Nature of callus	Plant regene-ration response‡ (%)	No. of shoots /callus
T-1	0	89.9+2.02a*	Profuse callus proliferation	86.0+1.32 a *	8
T-2	0.0025	54.2+1.05b	Healthy callus	70.2+1.24 b	6.5
T-3	0.005	47.0+1.03b	Well developed callus	64.0+1.03 b	7.2
T-4	0.0075	12.0+0.05c	Fragile callus	50.0+1.33 c	4.3
T-5	0.01	2.5+0.001d	Necrotic callus	13.2+0.08 d	2.1

Means followed by the same letter within columns were considered not significantly different at P 0.05. *Values are mean + S.E. †- MS + 2.5mg/l 2,4-D + 0.5mg/l Kn for callus induction, ‡- MS + 2.5mg/l BAP + 1.0mg/l NAA for plantlet regeneration

Figure 4: Regenerated plants acclimatized under green house condition following in vitro mutagenesis with 0.005% EMS in MS medium

concentration of BAP with a constant level of 0.5mg/L NAA(Table 3). However, on verification, combination of BAP with still higher concentration (1.0mg/L) was shown to be effective enough for regeneration response and highest being achieved at 2.5mg/L + 1.0mg/L NAA (86.0%) after two weeks of culture (Table 3, Fig 3). Besides, continuous production of shoots was achieved by serial subculturing of shoot clumps in the same medium. The plant hormone auxin (NAA) is implicated in many central developmental processes of plants, including cell division, cell elongation, vascular differentiation, root initiation, and apical dominance (Benkova *et al*., 2003; Leyser, 2001; 2006; Reinhardt *et al*., 2000). However, our study revealed that BAP with a low concentration of NAA is essential to maintain undifferentiated cells in shoot apical meristem and to promote cell differentiation. Hence, MS medium supplemented 2.5 mg/l BAP and 1.0 mg/l NAA was selected as an appropriate concentration for multiple shoot regeneration systems. Similar results were reported by Sikder et *al*. (2006) in rice and they achieved the highest regeneration frequency, as well as shoot length in MS media supplemented with 0.05mg/l NAA+5mg/l BAP. However, Tripathy et al. (2018) got maximum frequency of plantlet regeneration at MS + 2.0mg/L BAP + 0.5mg/L NAA. In contrast, 45 days-old calli cultured with increasing order of BAP alone showed better regeneration as against the 25-day old callus (Tyagi et *al*. (2007) and in fact 1.5mg/l BAP gave best result.

Differential response for regeneration was also reported earlier using different combination and concentration of phytohormones. Combination of 2 mg/L BAP(6-benzylaminopurine), 2 mg/L Kin (Kinetin) and 0.5 mg/L NAA supplemented MS medium was shown to have potential to promote regeneration to the tune of 82% in Malaysian wetland rice variety MR220-CL2(Mostafiz and Wagiran, 2018). In an another report, MS + 8 mgL- ' Kn + 0.5 mgL- ' NAA) was found to be best in shoot length (6.00cm) with highest shoots initiation in BRRI Dhan 29(Mukherjee *et al*., 2015). Similarly, Joya *et al*. (2019) had shown maximum plant regeneration response(47%) in MS+4 mg/ml BAP+1.2 mg/ml kinetin+0.5 mg/ml NAA, followed by MS +1 mg/ml BAP+1 mg/ml Kinetin+1 mg/ml NAA

In Vitro Mutagenesis

Creation of genetic variability is the key to varietal development of cereal crops (Singh *et al*., 2015). In this context, mutations are the significant source of genetic variability (Oladosu *et al*.,

2016) which can open up the study of functional genomics and the development of new genotypes (Luz *et al.*, 2016). Matured dehusked seed culture is often used in rice for creation of novel genetic variants (somaclonal variation) and in fact, long term callus cultures result proportionately higher frequency genetic variants than short term cultures. In this context, addition of chemical mutagen (at very low concentration) to the medium still expands the spectrum of genetic variation in culture. An efficient callus induction and reproducible rapid regeneration system can achieve the success of *in vitro* mutagenesis. The present investigation was undertaken to develop an efficient *in vitro* mutagenesis system with early callus induction and rapid plant regeneration in a popular aromatic rice variety Dubraj.In our study,we preferred lower concentrations of EMS for pretty longer time (overnight) to target mutagenesis at callus inititation stage in contrast to earlier reported 0.2%.EMS treatment of scutellum derived calli for 2.0h prior to plantlet regeneration (Serrat *et al.*,2014). The highest callus induction (89.9%) was recorded in mutagen (EMS) free MS medium supplemented with 2.5mg/l 2, 4-D and 0.5 mg/l kinetin) (Table 4). Addition of EMS at low concentration (0.0025 to 0.010%) to the above MS medium supplemented with 2.5mg/l 2, 4-D and 0.5 mg/l kinetin decreased the callus induction frequency (CIF%). Callus induction frequency and callus growth were achieved maximum (54.0%) at 0.0025% EMS and thereafter, it decreased with increasing concentration of EMS (Tripathy *et al.*, 2022). This might be due to subsequent increased mutagenic damage at cellular level at higher concentration of EMS. The callus so induced were mostly fragile / brunt in nature (necrotic) after few days in culture. Similar was the case for plantlet regeneration which was highest (70%) at the above low concentration of EMS(0.0025%) as compared to mutagem-free medium(86.0%). The plant regeneration frequency decreased with increase in concentration of EMS (Tripathy *et al.*, 2022), and it was minimum at 0.01% EMS. This might be due to carry-over effect of mutagen to plant level that caused drastic morphogenetic selectivity for plantlet regeneration (Tripathy *et al.*, 2022), and a single plant may carry a number of mutations in different genes (Serrat-Gurrera 2016).

Considering the importance of developing rice mutants to create genetic variability (Singh *et al.*, 2015), it is imperative to assess LD50 concentration of the mutagen (Talebi *et al.*, 2012). In the present investigation, addition of EMS at 0.005% to the callus induction medium reduced the callusing response (47%) nearly to 50% compared to that in EMS-free medium (89.9%). Hence, 0.005% may be considered optimum for in vitro mutagenesis.

Acclimatization and plant establishment

The ultimate success of propagated plantlets lies in its growth in the external environmental conditions in pots. A set of 550 regenerated plants with well-developed roots were initially transferred to paper cups (covered with perforated polythene bags) containing sterilized distilled water for three days in culture room for primary hardening. Thereafter the plants were raised in pots (filled with autoclaved sandy loam soil) under green house condition for seven days before transfer to net house for plant establishment and follow-up growth till maturity. During the process 80% were survived and those were checked for induction of any mutant trait(s) for further study (Tripathy *et al.*, 2022).

ACKNOWLEDGEMENT

The authors are thankful to Department of Biotechnology (DBT), Ministry of Science and Technology, Govt. of India for providing financial assistance for *in vitro* culture facility at OUAT, Bhubaneswar.

REFERENCES

Benková E., Michniewicz M., Sauer M., Teichmann T., Seifertova D., Jürgens G., Friml J. 2003. Local, efflux-dependent auxin gradients as a common module for plant organ formation. *Cell.* **115:** 591-602.

Chen Y-L., Liang H-L, Ma X-L., Lou S-L, Xie Y-Y, Liu Z-L, Chen L-T and Liu Y-G. 2013. An efficient rice mutagenesis system based on suspension-cultured cells. *J. Integrative Plant Biology*. 2013, **55(2):** 122–130

Da Luz, Pinheiro A.M., Silva M.L., Monteiro M.C., Prediger R.D., Maia C.S.F. and Fontes-Jr E.A. 2016. Ethnobotany, phytochemistry and neuro pharmacological effects of *Petiveria alliacea* L. (Phytolaccaceae): a review. *J. Ethnopharmacol.* **185:**182–201.

Das S. and Khanda C.M. 2020. Origin and evolution of aus type fragrant rice (*Oryza sativa* L.). *Oryza*.**57(3):** 169-180.

Das S.R., Roy J. K., Kar M. and Das S. 2003. Aromatic rices of Orissa. In: Singh, R.K. and Singh, U.S. (Eds.), A treatise on the scented rices of India. Kalyani publishers, New Delhi, India, pp. 355-375

Duncan D.B. 1955. Multiple range and multiple F tests. *Biometrics* **11:** 1-42.

Garris, A.J., TaiT, H., Coburn, J., Kresovich, S and Mc Couch S.R.2005. Genetic structure and diversity in *Oryza sativa* L. *Genetica* **169:** 1631-1638.

Glaszmann J.C. 1987. Isozymes and classification of asian rice varieties. *Theor. Appl. Genet.* **74:** 21-30.

Gosal SS and Kang MS. 2012. Plant tissue culture and genetic transformation for crop improvement. In: Improving crop resistance to abiotic stress; Tutejan., Gill.S.S. Tiburcio A F., Tuteja .R. Eds; **Wiley- VCH Verlag Gmbh & Co. KGaA : Germany; pp. 357-397.**

Jain R.K. 1997. Effect of some factors on plant regeneration from indica rice callus and protoplasts- a review. *Ind. J. Exp. Biol.* **35:** 323-331.

Joya SD, Sultana S, Ferdous J, Qayum M.A. and Hoque M.E. 2019. Response to Callus induction and regeneration of newly released BRRI rice varieties. *Bangladesh Rice J.* **23 (2) :** 17-25, 2019.

Leyser O. 2006. Dynamic integration of auxin transport and signaling. *Curr Biol.* **16:** R424–R433.

Luz D., Pinheiro A.M., Silva M.L., Monteiro M.C., Prediger R.D., Maia C.S.F. and Fontes-Jr E.A. 2016. Ethnobotany, phytochemistry and neuropharmacological effects of *Petiveria alliacea* L. (Phytolaccaceae): a review. *J Ethnopharmacol.* **185(2016):** 182-201.

Maluszynski M., Nichterlein K., Van Zanten L., Ahloowalia S. 2000. Officially released mutant varieties - The FAO/IAEA Database (INIS-XA–291). *International Atomic Energy Agency* (IAEA), No. 2, Dec. 2000.

Mostafiz SB and Wagiran A. 2018. Efficient callus induction and regeneration in selected indica rice. *Agronomy.* **8(77):**1-18.

Moin M., Bakshi A., Madhav M.S. and Kirti P.B. 2017. Expression profiling of ribosomal protein gene family in dehydration stress responses and characterization of transgenic rice plants overexpressing RPL23A for water-use efficiency and tolerance to drought and salt stresses. *Front. Chem.* **5**:97.

Mukherjee A, Islam M.R., Nasiruddin K.M., Banerjee P. 2015. Study on callus initiation and plantlet regeneration ability of Some rice genotypes. *Int. J. Sci. and Tech. Res.* **4(10):** 354-360.

MurashigeT. and Skoog F. 1962. A revised medium for rapid growth

and bio-assays with tobacco tissue cultures *Physiologia Plantarum* **15(3):** 473-497.

Oladosu, Y., Rafii, M.Y., Abdullah, N., Hussin, G., Ramli, A., Rahim, H.A., Miah, G., Usman, M. 2016. Principle and application of plant mutagenesis in crop improvement: a review. *Biotech and Biotechnol*. Equip. **30(1):** 1-16.

Ookawa T., Inoue K., Matsuoka M., Ebitani T., Takarada T., Yamamoto T., Ueda T., Yokoyama T., Sugiyama C., Nakaba S., Funada R. , Kato H., Kanekatsu M., Toyota K., Motobayashi T., Vazirzanjani M., Tojo S. and Hirasawa T. 2014. Increased lodging resistance in long-culm, low-lignin gh2 rice for improved feed and bioenergy production. *Sci Rep.* **4:** 6567

Parimala, G., Raju,C.D., Subba, R.V and Uma, M.K. 2020. Canonical root analysis and clustering for characterization and evaluation of aromatic rice germplasm based on morphological characters. *The Bioscan.* **15(3):** 371-374.

Reinhardt, D. , Mandel, T and Kuhlemeier, C. 2000. Auxin regulates the initiation and radial position of plant lateral organs *Plant Cell* **.12:** 507-518

Samal, K.C., Rout, G.R., Das, S.R. 2014. Study of genetic divergence of indigenous aromatic rice (*Oryza sativa* L.) :Potentials and consequences on farm management in traditional farming. *J Agric Sci.* **4 (4) :** 176-89.

Serrat, X., Esteban, R., Guibourt, N., Moysset, L., Nogués, S. and Lalanne, E. 2014. EMS mutagenesis in mature seed-derived rice calli as a new method for rapidly obtaining TILLING mutant populations. *Plant Methods.* **10:** 5.

Serrat-Gurrera X.. 2016. Applied biotechnology to improve Mediterranean rice varieties. A doctoral thesis submitted to University of Barcelona. *Bercelona* (2016).

Sikder M.B.H., Sen P.K., Al-Mamun M.A., Ali R. and Rahman S.M. 2006. *In vitro* regeneration of aromatic rice (*Oryza sativa* L.). *Int. J. Agric. Biol*. **6:** 759-752.

Singh, S., Sharma, R.K., Singh, P. and S. K. Chakravarti,S.K. 2015. Gamma ray and EMS induced genetic variability in M2 population of aromatic rice. *The Bioscan*. **9(1&2):** 250-256.

Talebi, A.B., Talebi, A.B. and Shahrokhifar, B. 2012. Ethyl methane sulphonate (EMS) induced mutagenesis in malaysian rice (cv. MR219) for lethal dose determination. *American J. Plant Sciences.* **3:** 1661-1665.

Toenniessen, G.H. 1991. Potentially useful genes for rice genetic engineering, In: Rice Biotechnology, Khush GS and Toenniessen GH (Eds.), *CAB International and IRRI, Wallingford/Manila*. pp. 253-280.

Tripathy Swapan, K., Maharana, M., Panda, S., Sahoo, B., Sahoo D.B., Behera, S.K. and Chakma, B. 2018. Exploring efficient callusing and rapid regeneration system in upland rice (*Oryza sativa* L.). *Rice Genomics and Genetics.* **9(2):** 7-14.

Tripathy, S.K., Panda, S. and Kajal Kiran, 2022. *In vitro* chemical mutagenesis of manipuri fragrant black rice cv.'Chak Hao-Amubi. *Agric Res J.* **59 (3):** 537-544.

Tyagi, H., Rajasubramanium, S. and Dasgupta, I. 2007. Regeneration and Agrobacterium-mediated transformation of popular indica rice variety ADT39. *Current Science.* **93:** 678–683.