In vitro Callus induction of aromatic rice depends on the concentration of 2, 4-D

Md. Niuz Morshed Khan*

Department of Biotechnology, Bangladesh Agricultural University, Mymensingh, Bangladesh.

*Corresponding Author Email: rumman899@gmail.com

Doi: 10.2478/mjhr-2019-0007

Abstract:

Due to growing population, there is an increasing demand of rice production but its productivity is lessened day by day. Aromatic rice has a great demand during festivals in many countries. Kalijira is one of them not only Bangladesh but also all over the world due to its attractive flavor, fine grain and good taste which is generally used to prepare dishes in different special occasions. But there are some limitations to cultivate aromatic rice Such as lack of high yielding variety, fine grain quality, disease or pest resistant, stress and salt tolerance variety and proper cultural management. To overcome this problem tissue culture can be used. However, the lack of a simple and efficient protocol for callus induction in this cereal crop. In this study we tried to find out the potentiality of aromatic rice variety named kalijira for callus induction from mature embryo and to find out the suitable concentration of 2, 4-D for callus induction and proliferation. The highest callus induction were observed when the media was supplemented with 2 mg/L of 2, 4-D and the frequency of callus induction was lowest in 0.5 mg/L concentration of 2, 4-D. This study will be useful for selecting suitable concentration of growth regulator (2, 4-D) for callus induction in future that will be useful for not only national but also international plant breeders.

Keywords: Callus induction, 2, 4-D, Embryogenic callus, Aromatic rice.

1.0. Introduction:

Rice (*Oryza sativa* L.) is a monocot plant that comprised of two subspecies, Indica and Japonica is being cultivated for more than 10,000 years [1]. Aromatic rice is special group that covers 2% of the national rice of Bangladesh and 12.5% of the total transplant Aman rice cultivation [2]. It has a great demand during festivals in many countries. Kalijira is the most important aromatic rice variety of Bangladesh and the rest of the world due to its attractive flavor, fine grain and good taste. This rice is generally used to prepare dishes such as polau and biryani like food in special occasions. The yield of aromatic rice was lower (1.5-2.0 t ha⁻¹) but it is profitable for its low cost of cultivation generated compared to other varieties grown in the area [3]. But there are some limitations to cultivate aromatic rice Such as lack of high yielding variety [3], fine grain quality, disease or pest resistant, stress and salt tolerance variety and proper cultural management. The conventional breeding techniques are time consuming and self in-compatibility act as barrier for distant hybridization and fertilization. The aromatic variety can be improved through tissue culture techniques. It is very essential to efficient regeneration through in vitro micropropagation for utilizing biotechnology in crop improvement [4]. Now-a-days rice plant has found to be regenerated from root [5], leaf [6], inflorescences, mature and immature embryos [7], anther [8] and protoplasts [9] through formation of callus. But for callus induction of rice, seed is better explant than its other organ parts like nodes and tips [10]. In recent years successful callus induction and plant regeneration from rice seed has been reported by several researchers [1, 11]. The plant growth regulators are usually used to manipulate the tissue culture, which are almost similar in action with natural plant hormones [12]. Callus production and plant regeneration from dehusked rice is limited by factors like genotype of plant [13], media composition [14], growth regulators [15, 5] and culture condition which influence the culture efficiency. Considering the circumstances above, the present study was undertaken to find out the potentiality of aromatic rice (*Oryza sativa* L.) variety kalijira for callus induction from mature embryo and to find out the suitable concentration of 2, 4-D for callus induction and proliferation.

2.0. Materials and methods

This experiment was conducted at Biotechnology Laboratory, Bangladesh Agricultural University, Mymensingh, Bangladesh. For this experiment, MS medium supplemented with different concentration of auxin (2,4-D) was used for callus induction of "kalijira" variety.

2.1. Description of the experimental site

In this experiment field grown mature seeds of aromatic rice (*Oryza sativa* L.) variety 'kalijira' were used for callus induction. The explants were collected from Barisal district, Bangladesh.

2.2. Methods

2.2.1. Preparation of Stock solution

At first stock solution preparation was done for preparing of culture media. Freshly prepared stock solutions were needed which contained different constituents. So, different types of stock solutions (I, II, III, IV, V and VI), organic compounds and growth regulators were prepared separately and stored at refrigerator (Table 1).

Table 1: Stock Solution required for media preparation

SL. NO.	Ingredient	Amount (g/200ml)
I	NH ₄ NO ₃	16.5 gm
	KNO ₃	19 gm
II	MgSO ₄ .7H ₂ O	7.4 gm
	MnSO ₄ .4H ₂ O	0.446 gm
	ZnSO ₄ .7H ₂ O	0.172gm
	CuSO ₄ .5H ₂ O	0.0005 gm
III	CaCl ₂ .2H ₂ O	8.8 gm
	CoCl ₂ .6H ₂ O	0.0005 gm
	KI	0.0166 gm
IV	KH ₂ PO ₄	3.4 gm

	H ₃ BO ₃	0.124gm
	Na ₂ MoO ₄ .2H ₂ O	0.005gm
V	Na ₂ EDTA.2H ₂ O	0.746 gm
	FeSO ₄ .7H ₂ O	0.556 gm
VI	pyridoxine HCL (VitB ₆)	0.001 gm
	nicotinic acid (VitB ₃)	0.001gm
	thiamin HCL (VitB ₁)	0.002 gm
	Inositol	0.02 gm
VII	pyridoxine HCL (VitB ₆)	0.001 gm
	nicotinic acid (VitB ₃)	0.001gm
	thiamin HCL (VitB ₁)	0.002 gm
	Inositol	0.02 gm
	Glycin	0.04 gm

2.2.2. Preparation of MS Culture Media

To prepare 200 ml of MS medium 100 ml distilled water was taken in a 500 ml beaker, 4 ml of stock solution I was added in the beaker then 2 ml of stock solution II, III, IV, V & VI was added. 3% sucrose was added. The final volume was adjusted to 200 ml. 0.7 % TC Agar was added after adjusting the PH at 5.7-5.8. Finally media was autoclaved at 121°C for 20 minutes at 15 Psi.

2.2.3. Sterilization of Plant Materials

The dehusked seeds were washed with 10% savlon for 3 min and then washed three times with sterilized distilled water. After washing with sterilized water the explants were dipped at 70% ethanol for one minute and washed three times with sterilized distilled water. After washing with sterilized water the explants were dipped at 50% sodium hypochlorite for 30 minutes and washed three times with sterilized distilled water. All of these works were done in laminar air flow cabinet.

2.2.4. Inoculation of seeds

After surface sterilization the explants were kept onto sterile filter paper placed inside the Petridis. After removal of water from seeds surface, these were inoculated into the culture Petri dish with sterilized forceps and niddle. The explants were inoculated into the MS medium supplemented with 0.5, 1.0, 1.5, 2.0, 2.5, 3.0 mg/L of 2, 4-D in each petridish for callus induction. The plates were then incubated in the culture shelves at dark, the temperature and RH were maintained at 25±2°C and 78% respectively.

2.3. Data collection

2.3.1. Callus Induction

Data were collected by counting the calli induced and inoculated seed. Data of callus induction were collected after three weeks of inoculation. All the calli originated from a single seed was considered as one. The frequency of callus induction was calculated as below:

$$\text{Callus induction frequency} = \frac{\text{No of callus produced}}{\text{No. of seeds inoculated}} * 100\%$$

2.3.2. Color of callus

After four weeks of incubation the color of callus was observed visually.

2.3.3. Intensity of callus

After four weeks of incubation the intensity of callus was observed visually and graded “+++” for maximum size of calluses, “++” for medium size of calluses and “+” for small size calluses.

3.0. Results and Discussion

3.1. Results

In vitro regeneration of plant via calli offers unique facilities of reproducible protocol as well as recovery of somaclonal variants, which can be utilized for the future crop improvement programs. Thus, induction of calli from different explants and subsequent regeneration of complete plants could play an important role.

3.1.1. Treatments used for callus induction

During conducting this research 2, 4-D was used as growth hormone. Different concentration of 2, 4- D were applied for the initiation of callus. In control no hormone was used. MS media was supplemented with different concentration of 2,4- D ranging from 0.5 mg/ L to 3.0 mg/ L for callus induction of rice.

3.1.2. Callus induction frequency

It was noticed that MS medium supplemented with only 2.0 mg/L of 2, 4-D produced highest percentage of callus that is 90% and the MS medium supplemented with only 0.5 mg/L of 2, 4-D produced lowest percentage of callus that is 60%. In those media which were supplemented with 1.0 mg/L, 1.5 mg/L, 2.5 mg/L, 3.0 mg/L showed callus induction frequency of 70%, 70%, 80%, 80% respectively. The callus induction frequency with MS media supplemented with different concentration are shown in Figure 1.

Figure 1: Callus induction percentage of different concentration of 2,4-D.

3.1.3. Intensity of callus

In 0.5 mg/L and 1.0 mg/L hormone concentration callus size was same with the seeds that were inoculated. In 1.5 mg/L, 2.0 mg/L and 3.0 mg/L hormone concentration callus size was double than the seeds while In 2.0 mg/L and 2.5 mg/L hormone concentration callus size was three times of the seeds.

3.1.4. Days of callus initiation

2.0 mg/L of 2, 4-D took less time (9 days) than any other concentration of 2, 4-D. Other concentration 0.5 mg/L, 1.0 mg/L, 1.5 mg/L, 2.5 mg/L and 3.0 mg/L took 13 days, 14 days, 12 days, 10 days and 11 days respectively.

Table 2: Intensity and type of callus of different concentration of 2,4-D.

Treatment 2,4-D (mg/ L)	% of callus induction	Intensity of callus	Days to callus induction	Type of callus	Color
0	0%	-	-	Non embryo genic	Creamy Yellowish
0.5	60%	+	13		
1.0	70%	+	14		
1.5	70%	++	12		
2.0	90%	+++	9		
2.5	80%	+++	10		
3.0	80%	++	11		

(- = No Callus, + = Poor callus, ++ = Good callus, +++ = Very good callus)

3.1.5 Color of callus

Creamy yellowish color callus was observed during this research. (Table 2)

3.1.6 Types of callus

The types of callus of different treatment are same and non-embryogenic. The high amount of polyploidization, rough endoplasmic reticulum, polysome, poly-nucleolus, and incomplete cell wall together with abnormal partitioning in non- embryogenic cells, as compared to embryogenic cells. In contrast, vacuolation of cytoplasm, perfect cell wall and partitioning structure, and the high proportion of nucleus/cytoplasm area were recognized in embryogenic cells [16].

0.5 mg/L 2,4-D

1.0 mg/L 2,4-D

1.5 mg/L 2,4-D

2.0 mg/L 2,4-D

2.5 mg/L 2,4-D

3.0 mg/L 2,4-D

Figure 2: Callus induction in different concentration of 2,4-D

3.2. Discussion

In any in vitro regeneration technique, callus induction is the primary step. Under present study the callus induction frequency of kalijira, a special aromatic rice variety with different concentration of 2, 4-D was conducted. The callus induction frequency shows significant difference under different concentration of 2, 4-D. The frequency of callus induction was highest when seeds were inoculated in media fortified with 2 mg/L concentration of 2,4-D that support the previous study [1, 17] and the frequency of callus induction was lowest in 0.5 mg/L concentration of 2,4-D. All the calli were non-embryogenic, friable and creamy yellowish. 2mg/L of 2, 4-D concentration took less time for callus initiation and 0.5mg/L of 2,4-D concentration took more time.

4.0. Conclusion

The present study was conducted to assess the callus induction efficiency of the aromatic rice variety Kalijira on MS medium. The different concentration (mg/L) of 2, 4-D for callus induction were used as follows: 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0. The control replica shows no callus, but other treatments showed callus induction of varying frequency. Among them 2.0 mg/L 2, 4-D has found to induce highest frequency of callus (90%). Other treatments (2.5 and 3.0 mg/L 2, 4-D) showed better result (both 80%). The color of all calli found from different 2, 4-D treatments were creamy yellow and texture of all calli were friable. So, it can be concluded that 2.0 mg/L 2, 4-D was suitable for callus induction and proliferation from mature embryo of Kalijira variety.

5.0. Acknowledgement

Thanks to Bangladesh Agricultural University (BAU), Bangladesh for all experimental support.

References

- [1] M.N.M. Khan, M.M. Islam, and M.S. Islam, "Studies on in vitro response to callus induction and gene transfer technique of five high yielding indica rice varieties", *Journal of Scientific Agriculture*, Vol. 3, 41-45, 2019. DOI: 10.25081/jsa. 2019.v3.5465
- [2] A. Roy, M.A.R. Sarkar, and S.K. Paul, "Effect of age of seedlings at staggered transplanting and nutrient management on yield performance of aromatic

fine rice (cv. BRRI dhan38)", SAARC Journal of Agriculture, Vol. 16, 49-59, 2018. DOI: [org/10.3329/sja.v16i1.37422](https://doi.org/10.3329/sja.v16i1.37422).

[3] S.K. Paul, L.S. Nahar, N.C. Paul, and M. Begum, "Influence of weeding regime on the performance of aromatic Boro rice (*Oryza sativa* L.)", Archives of Agriculture and Environmental Science, Vol. 4, 133-140, 2019. DOI: [org/10.26832/24566632.2019.040202](https://doi.org/10.26832/24566632.2019.040202).

[4] S. Paul, and A. Roychoudhury, "Comparative Analyses of Regeneration Potentiality of Eight Indigenous Aromatic Indica rice (*Oryza sativa* L.) Varieties", Int. J. Sci. Res. in Biological Sciences, Vol. 6, 2019. DOI: [org/10.26438/ijrsbs/v6i1.5564](https://doi.org/10.26438/ijrsbs/v6i1.5564)

[5] R. Abiri, M. Maziah, N.A. Shaharuddin, Z.N.B. Yusof, N. Atabaki, M.M. Hanafi, M. Sahebi, P. Azizi, N. Kalhori, and A. Valdiani, "Enhancing somatic embryogenesis of Malaysian rice cultivar MR219 using adjuvant materials in a high-efficiency protocol", International Journal of Environmental Science and Technology, Vol. 14, 1091-1108, 2017. DOI: [10.1007/s13762-016-1221-y](https://doi.org/10.1007/s13762-016-1221-y).

[6] W. Wernicke, R. Brettell, T. Wakizuka, and I. Potrykus, "Adventitious embryoid and root formation from rice leaves", Zeitschrift für Pflanzenphysiologie, Vol. 103, 361-365, 1981. DOI: [org/10.1016/S0044-328X\(81\)80135-3](https://doi.org/10.1016/S0044-328X(81)80135-3).

[7] J.W. Heyser, T.A. Dykes, K.J. DeMott, and M.W. Nabors, "High frequency, long term regeneration of rice from callus culture", Plant Science Letters, Vol. 29, 175-182, 1983. DOI: [org/10.1016/0304-4211\(83\)90141-4](https://doi.org/10.1016/0304-4211(83)90141-4).

[8] S.K. Tripathy, D. Swain, P.M. Mohapatra, A.M. Prusti, B. Sahoo, S. Panda, M. Dash, B. Chakma, and S.K. Behera, "Exploring factors affecting anther culture in rice (*Oryza sativa* L.)", Journal of Applied Biology & Biotechnology, Vol. 7, 87-92, 2019. DOI: [10.7324/JABB.2019.70216](https://doi.org/10.7324/JABB.2019.70216).

[9] Y. Yamada, Z.Q. Yang, and D.T. Tang, "Plant regeneration from protoplast-derived callus of rice (*Oryza sativa* L.)", Plant cell reports, Vol. 5, 85-88, 1986. DOI: [article/10.1007/BF00269240](https://doi.org/10.1007/BF00269240).

[10] Z.A. Rahman, A. Ramli, H. Hosni, R. Kamaruzaman, Z.A. Seman, A.N. Othman, Z. Zainal, J. Uddain, and S. Subramaniam, "Efficient plant regeneration of Malaysian aromatic rice (*Oryza sativa* L.) through somatic embryogenesis", Emirates Journal of Food and Agriculture, Vol. 27, 857-863, 2015. DOI: [10.9755/ejfa.2015-07-535](https://doi.org/10.9755/ejfa.2015-07-535).

[11] A. Libin, P.J.H. King, K.H. Ong, J.K. Chubo, and P. Sipen, "Callus induction and plant regeneration of Sarawak rice (*Oryza sativa* L.) variety Biris", African Journal of Agricultural Research, Vol. 7, 4260-4265, 2012. DOI: [10.5897/AJAR12.587](https://doi.org/10.5897/AJAR12.587).

[12] V.M. Jiménez, "Regulation of in vitro somatic embryogenesis with emphasis on to the role of endogenous hormones", Revista Brasileira de Fisiologia Vegetal, Vol. 13, 196-223, 2001. DOI: [org/10.1590/S0103-31312001000200008](https://doi.org/10.1590/S0103-31312001000200008).

[13] B. Pawar, K.A.L.E. Prashant, J. Bahurupe, A. Jadhav, K.A.L.E. Anil, and S. Pawar, "Proline and glutamine improve in vitro callus induction and subsequent shooting in rice", Rice Science, Vol. 22, 283-289, 2015. DOI: [org/10.1016/j.rsci.2015.11.001](https://doi.org/10.1016/j.rsci.2015.11.001).

[14] M. Chakraborty, P.S. Reddy, M.L. Narasu, G. Krishna, and D. Rana, "Agrobacterium-mediated genetic transformation of commercially elite rice restorer line using nptII gene as a plant selection marker", Physiology and molecular biology of plants, Vol. 22, 51-60, 2016. DOI: [10.1007/s12298-015-0334-y](https://doi.org/10.1007/s12298-015-0334-y).

[15] S. Binte Mostafiz, and A. Wagiran, "Efficient callus induction and regeneration in selected Indica rice", Agronomy, Vol. 8, 77, 2018. DOI: [org/10.3390/agronomy8050077](https://doi.org/10.3390/agronomy8050077).

[16] B.E. Moghaddam, and R.M. Taha, "Cellular behavior in embryogenic and non-embryogenic sugar beet calluses", In Vitro Cellular & Developmental Biology-Plant, Vol. 41, 465-469, 2005. DOI: [10.1079/IVP2005641](https://doi.org/10.1079/IVP2005641).

[17] Z.Y. Roly, M.M. Islam, M.P.E. Shaekh, M.S.I. Arman, S.M. Shahik, D. Das, M.M.E. Haamem, and M. Khalekuzzaman, "In vitro callus induction and regeneration potentiality of aromatic rice (*Oryza sativa* L.) cultivars in differential growth regulators", International Journal of Applied Sciences and Biotechnology, Vol. 2, 160-167, 2014. DOI: [org/10.3126/ijasbt.v2i2.10313](https://doi.org/10.3126/ijasbt.v2i2.10313).