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

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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 transplanted 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 organs like nodes and tips [10]. In recent years successful callus induction and plant regeneration from seed rice 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 when 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       | NH₄NO₃    | 16.5 gm         |
|         | KNO₃      | 19 gm           |
| II      | MgSO₄.7H₂O | 7.4 gm          |
|         | MnSO₄.4H₂O | 0.446 gm        |
|         | ZnSO₄.7H₂O | 0.172 gm        |
|         | CuSO₄.5H₂O | 0.0005 gm       |
| III     | CaCl₂.2H₂O | 8.8 gm          |
|         | CoCl₂.6H₂O | 0.0005 gm       |
|         | KI        | 0.0166 gm       |
| IV      | KH₂PO₄    | 3.4 gm          |
H$_2$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  
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:

\[ \text{Callus induction frequency} = \frac{\text{No of callus produced}}{\text{No. of seeds inoculated}} \times 100\% \]

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.
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 embryogenic | Creamy         |
| 0.5                   | 60%                   | +                   | 13                       | Non embryogenic | Creamy Yellowish |
| 1.0                   | 70%                   | ++                  | 14                       | Non embryogenic | Creamy         |
| 1.5                   | 70%                   | ++                  | 12                       | Non embryogenic | Creamy         |
| 2.0                   | 90%                   | +++                 | 9                        | Embryogenic    |                |
| 2.5                   | 80%                   | +++                 | 10                       | Embryogenic    |                |
| 3.0                   | 80%                   | ++                  | 11                       | Embryogenic    |                |

(- = 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-nucleous, 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].

Figure 1: Callus induction percentage of 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.

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