

In vitro regeneration and conservation of aromatic rice of South Assam

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Abstract : *In vitro* technique for induction and regeneration by application of different concentration of nutrient gradient supplied to MS medium has been established in an elite aromatic rice landrace Biroin of Karimganj district of South Assam. Callus induction and regeneration have much importance in crop improvement programme for developing high yielding varieties choosing appropriate parental superior donor and traditional landraces are the best option to solve the problem. Keeping this in view, present paper deals with the observation of induction effect of different concentration of 2,4-D which showed remarkable callus induction at the concentration of 1.0, 2.0, 2.5, 3.0 and 3.5 mgL⁻¹ with 75-90% and 78% callus growth. A combination of BA, Kn and NAA with concentration of 3.0, 0.5 and 0.5 mgL⁻¹ revealed 78% outcome of regeneration frequency. So, this method could have great prospect in future plant breeding to conserve the beneficial genes present in the rice landraces creating pollution free environment.

Key Words: Biroin rice, aromatic feature, regeneration, 2,4-D, growth regulators

I. INTRODUCTION

Tissue culture has more positive impact through somaclonal variation to develop a new and promising germplasm (Ram and Singh, 1998). Different techniques of *in vitro* study like root culture (John and Prathapasenan, 1999), leaf culture (Boissot *et al.*, 1990), anther culture (Faruque *et al.*, 1998) and grain culture (Chakravorty, 2016) have been adopted since last few decade for sustainable agriculture and *in vitro* conservation. *In vitro* regeneration and callus production *via* somaclonal variation is very much significant in rice landraces because all the essential beneficial genes are getting abolished from farmers' field due to urbanization, industrialization and cultivation of poor quality high yielding varieties (HYV) (Chakravorty 2014). Also, with the change of climatic conditions, *in vitro* approach of conservation of rice germplasm is the best option for today's agricultural research to avoid pollution and the rice improvement of the existing germplasm is only possible when proper genetic manipulation through agro-morphological and biotechnological approaches is implemented (Yang *et al.* 1999). Also, to accomplish the need of *in vitro* conservation, there is a primary and urgent need to establish a challenging *in vitro* regeneration method because this work is still limited to a number of genotypes (Tagichi-Shiobara *et al.*, 1997). With this point of view, the present study aims at observation of hormonal effects on some aromatic rice landraces of South Assam in inducing callus, their *in vitro* regeneration using different nutrient media have been remarked which is very much useful and significant in any crop improvement programme choosing the appropriate donor and beneficial genes for future plant breeding programme.

II. MATERIALS AND METHODS

Mature dehusked grain were collected from the farmers field of Bagbari village of Kaliganj area of Karimganj district, South Assam in 2015-16 and their pre and post agro-morphological evaluation were done through *in vitro* culture of these grains. Laboratory works were done at the plant tissue culture laboratory of Sripat Singh College in assistance of University of Kalyani, Nadia. The collected grains were washed in running tap water vigorously using detergent made with 1%(v/v) tween 20 and rinsed many times with distilled water. This was followed by wasing with bavistin (20min) and rinsing repeatedly with distilled water. After that the seeds were treated with 4% (v/v) sodium hypochlorite solution for 3mins.. Completing the surface sterilization, the seeds were then dried and inoculated to culture media of MS (Murashige and Skoog, 1962) as a basal media with different concentration of 2,4-D (Table 1) for induction of callus.

The influence of different growth regulators like NAA, Kn and BA in different combination (Table 2) were studied on the explants to note their regeneration capability at the pH 5.8. Standardized medium was used for callus induction following inoculation by combined treatment of dark and light

phase for four weeks duration and three weeks respectively. Study of regeneration was done in regeneration culture room at 25°C with a photoperiod of 12 hr duration at 2000lux light at sterile condition. Repeated thorough and minute observations on frequency of development of culture proliferation and plantlet regeneration were done and the data was recorded after repeated treatment and at the end of five weeks a visually recorded data was put to statistical analysis.

III. RESULTS AND DISCUSSION

4.1 Results of Descriptive Statics of Study Variables

Treatment of seed with different concentration of 2,4-D (1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5 and 5.0 mgL⁻¹) showed a good remarkation of callus development within 10-14days. Remarkable induction of callus was found for 2,4-D treatment at 1.0, 2.0, 2.5, 3.0 and 4.5 mgL⁻¹ which gave a good reflection of 75-90% callus induction. Treatment with 2.0 mgL⁻¹ gave 90% callus induction. For rice invitro study, MS has been found promising (Niroula et al., 2005) along with N6 medium (Rashid, 2004).

After that, the calli were transferred to the regeneration media and after 9-10 days, visualization of green spot confirmed its regeneration capability and full grown regeneration root and shoot developed after 5-7 weeks. A combination of, BA, Kn and NAA gave the good picture of root generation followed by combination of treatment of 3.0, 0.5 and 0.5 mgL⁻¹ respectively in MS medium reflected the remarkable regeneration efficiency with 78% outcome with average 3 shoots per callus were recorded. In this way somaclonal variation can be explained following this, although this technique is very limited in such a case, yet this angle of study do have impact in reducing time, energy, economy of the crop and overall avoiding the pollution hazards.

Table 1: Results of induction and development of Callus by 2,4-D in aromatic Biroin rice

Sl. No.	2,4-D concentration gradient(mgL-1)	Callus induction in percentage	Degree of Callus	On first culture (callus growth)
1	1.0	78	Vg	78±0.015
2	1.5	78	Vg	78±0.019
3	2.0	90	Vg	80±0.020
4	2.5	85	G	80±0.021
5	3.0	78	G	50±0.031
6	3.5	75	G	52±0.028
7	4.0	78	G	55±0.025
8	4.5	78	Fg	55±0.030
9	5.0	78	Fg	56±0.015

Vg=Very good; G=Good : Fg=Fairly Good

Table 2: Findings the effects on regeneration frequency and average no. of explants /plant of aromatic Biroin rice by different combination of different growth regulators

Growth regulators	Concentration in mgL-1	Regeneration frequency%	Average no. of shoot per explant
BA+NAA	0.5+0.5	10	1±0
	10.+0.5	10	1±0
	1.5+0.5	10	1±0
	2.0+0.5	15	2±0
	3.0+0.5	20	2±0
Kn+NAA	1.0+0.5	10	1±0
	1.0+1.0	10	2±0
	1.0+1.5	10	3±0
	1.0+2.0	0	0±0
	1.0+2.5	0	0±0
	1.0+3.0	0	0±0
BA+Kn+NAA	0.5+0.5+0.5	10	1±0
	1.0+0.5+0.5	25	1.5±.23
	2.0+0.5+0.5	45	1.8±.25
	3.0+0.5+0.5	78	3.0±.15

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