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Research Article

Experimental Morphogenic studies on some Landraces of Rice (*Oryza sativa* L.) growing in Jharkhand State, India

Fauziya Basri*, Sony Kumari, Sonam kumari, Sangeetha Samad, Siyone Bage, Pinki Kumari, H.P.Sharma.

Laboratory of Plant Physiology and Biotechnology

Department of Botany, Ranchi University, Ranchi, Jharkhand, India - 834001.

ABSTRACT

Cultivation of landraces of rice is declining globally due to introduction of high yielding varieties. In this study some of the local landraces of rice of Jharkhand has been subjected to callus induction and regeneration. MS medium supplemented with different concentration/combination(s) of phytohormones have been used. MS medium supplemented with 2, 4- D and NAA showed pronounced callusing while supplementation with IAA resulted only in germination. Regenerated plantlets were obtained in MS + IAA (2mg/L) + Kinetin (4 mg/L) and MS + NAA (1mg/L) + BAP (2mg/L).

The protocol can be helpful for conservation and preservation of these local cultivars which are on the verge of extinction. These local varieties carry desirable traits that can be utilized in advanced breeding program.

Key words: Landraces, Callus induction, Regeneration, Rice, Growth regulators.

INTRODUCTION

Rice (*Oryza sativa* L.) is a very important crop which feeds over half of the global population[1]. Rice is erect annual grass, 1 to 1.2 m tall, belongs to family Poaceae. In Asia it covers half of the arable land used for agriculture in many countries[2]. Over two billion people in this continent alone derive 80% of their energy needs from rice, which contains 80% carbohydrate, 7-8% proteins, 3% fat and 3% fibre[3,4]. It holds second position in terms of consumption after wheat. In global scenario India and China have major share in rice production.

In the state of Jharkhand, India, is dominated by tribal population, large numbers of land races of rice are cultivated that are associated with their customs and cultures. In recent past it has been observed that due to introduction and cultivation of high yielding varieties, cultivation of local varieties of rice are under the process of decline. Other factors adding tothe problem are developmental activities leading to encroachment of agriculture lands thereby encouraging the practice of maximum yield from the available land area. If this practice remains continuous, local varieties will soon be lost forever. Importantly, land races having many good features, such as grain size, aroma, colour, rich in nutrients, disease resistance, drought tolerant, grow easily on inhospitable conditions and are source of valuable genes. Some of the local varieties show medicinal properties in addition to nutritional values; therefore it comes under the category of nutraceutical.

Therefore, it is imperative to find ways and means to protect, conserve these valuable land races and also to take up some measures for qualitative and quantitative improvement. Generally, plant breeding is one of the oldest methods employed for the improvement. This traditional method has its own limitations; therefore, some alternative strategy needs to be worked out.

In this perspective tissue culture technique is very effective to bring desired changes[5]. Identification and

selection of local varieties of rice are very important step before to start any improvement programme. In the present investigation four local varieties, namely Dahiya, Dani goda, Karhani and Neta have been undertaken for experimental morphogenic studies i.e. callus induction and regeneration.

MATERIAL AND METHODS

Plant material

Seeds of under mentioned four varieties of rice were obtained from Gene Campaign, Ranchi, Jharkhand, India and Birsa Agriculture University, Kanke, Ranchi, Jharkhand, India.

(a) Dahiya: An upland variety sown by broadcasting. Crop maturation is 90-100 days, the variety is non-lodging type, and grain colour is white. The variety is reported for its high calorific value derives its name from "Daah" a local bird known for its energy. The variety is drought tolerant and resistant to bacterial leaf blight.

(b) Dani goda: It is an upland variety sown by broadcasting. A non-lodging cultivar, showing maturity period of 69-73 days. Grain colour is red and the variety is resistant to bacterial leaf blight.

(c) Karhani: An upland variety, with red kernel and sown by broadcasting. It is also a non-lodging cultivar with crop duration of 123 days. It is widely used by local people to prepare a fermented drink, locally known as 'Hadia'. In addition to its use as alcoholic drink, it is also used to control jaundice by the ethnic communities. The variety is also known to have some medicinal properties.

(d) Neta: A midland variety sown by broadcasting. Variety is of lodging type, showing maturity of 110 days. It is highly nutritious and regarded as tonic for good health.

Methodology

In the present investigation seeds were selected as explants. Seeds were dehusked manually and washed with distilled water 2-3 times. Subsequently, seeds were surface sterilized using 70% alcohol for 1min followed by treatment with 0.01%(w/v) $HgCl_2$ aqueous solution for 15-20 min. Finally, treated seeds were washed with autoclaved double distilled water under laminar airflow hood to remove traces of sterilizing chemical before inoculation.

MS medium was prepared, which was supplemented with different concentrations of phytohormones; Indole -3- acetic acid (IAA), Naphthalene-1- acetic acid (NAA), 2,4- Diclorophenoxy acetic acid (2,4-D) and Kinetin (KN) either singly or in combinations for callus induction and regeneration[6]. The pH of the medium was adjusted to 5.8 using N/10 NaOH or N/10 HCl and about 10-15 ml was dispensed into each culture tube.

The culture tubes were sterilized at 15 lbs per sq. inch for 20 min. The inoculated culture tubes were maintained at controlled conditions; light intensity 2000-3000 lux, light duration 16h light and 8h dark, temperature 25±3°C and relative humidity was 60%. Results were photographed and recorded routinely.

The frequency of callus induction was calculated as:

$$\text{Callus induction frequency (\%)} = \frac{\text{No. of explants producing callus}}{\text{No. of explants plated}} \times 100$$

Plant regeneration from plated calli was calculated with the following formula:

$$\text{Plant regeneration (\%)} = \frac{\text{No. of calli produced plants}}{\text{No. calli plated for regeneration}} \times 100$$

RESULTS AND DISCUSSIONS

In order to induce callus, sterile seeds were inoculated in MS medium, which was supplemented with different concentrations of auxins; IAA (2, 4, 6 ,8, 10, 12 mg/L), NAA (2, 4, 6, 8, 10, 12 mg/L) and 2,4-D (1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5. 5.5, 6 mg/L). However, for regeneration seed-derived callus was transferred into regeneration medium; MS supplemented with IAA (2mg/L) + KN (4 mg/L) and MS + NAA (1 mg/L) + BAP (2 mg/L).

At all chosen concentration of IAA only germination resulted no callus induction was found. The scutellar end of seeds showed hypertrophy within 3-4 days followed by emergence of coleoptiles and callusing. Profuse growth of callus was seen within 4-6 weeks. The nature of the callus was compact, nodular and embryogenic.

The cultivars Dani goda, Karhani and Neta formed prolific, compact, nodular and big in size whereas callus from Dahiya were smaller in size. The colour of the callus was either white or creamy (Fig.4 and 5). The percentage of callus was highest at 3.5mg/L 2-4, D in all the varieties. Genotypic differences were seen regarding percentage of callusing in local varieties- Karhani (96%), Dani goda (95%), Dahiya (50%) and Neta (38%) as shown in Table 1. Maximum callus induction was obtained with NAA combination. 98% callus induction was observed in Neta (8 and 10 mg/l NAA) and in Karhani (10 mg/l NAA) as shown in Table 2. Earlier workers have also reported the influence of genotype on callus induction[7,8].

In terms of fresh and dry weight variety Neta gave the best result (0.05gm) followed by Dani goda ,

Karhani and Dahiya (Fig 1). Moreover the weight of calli induced by NAA was found to be higher than that induced by 2,4-D irrespective of genotype.

The seed derived callus when transferred to regenerating media; MS + IAA (2mg/L)+ KN (4mg/L), the regeneration percentage was highest in variety Dani goda (63%) followed by Neta (61%), Karhani (60%) and Dahiya (45%) as shown in Fig 2. In other combination MS + NAA (1mg/L) + BAP (2mg/L), variety Karhani gave the best result (67%) followed by Dani goda (62%), whereas both Neta and Dahiya showed 58% regeneration frequency (Fig 3). Generally, within 4-6 days shoots were seen to arise from the embryogenic callus followed by root regeneration. In addition to complete plant other responses were also seen, such as roots only, shoots only, necrotic or no response (Fig 5 and 6). Such variation may be attributed to genotypic variation as reported by previous works of Revathi *et. al.*2011, Islam *et. al.*2004, Saklan Naqvi *et. al.*(2005); Quiroz-Figueroa *et al.*, 2006.[9,10,11,12]

CONCLUSION

The present investigation was carried out to find out callogenic and regeneration potentialities as well as suitable media composition for callus induction and regeneration of some local cultivars of rice. Both genotype and media composition and their interaction effect callus induction and regeneration. Factors like media composition, growth regulators, genotype, age of explants etc. effect regeneration efficacy.

Table 1
Effect of different concentration of 2, 4- D for induction of callus from four varieties of rice.

Variety	No. of Explant	Callus induction frequency										
		Concentration of 2,4-D(mg/L)										
		1	1.5	2	2.5	3	3.5	4	4.5	5	5.5	6
Dahiya	24	-	-	25± 4.16[a]	50 ± 4.16[a]	50 ± 8.33[a]	50± 12.5[a]	25 ± 10.48[a]	41.66± 4.16[a]	25 ± 12.5[a]	25 ± 1.66[a]	25 ± 2.83[a]
Dani goda	24	70.83 ± 4.17[b]	66.66 ± 6.36[b]	90.9± 4.76[b]	91.66 ± 4.16 [b]	87.5 ± 4.16[b]	95 ± 4.76[b]	58.33 ± 8.33[b]	54.16 ± 4.16[b]	63.63± 9.43[b]	62.5 ± 4.16[b]	83.33 ± 4.17[b]
Karhani	24	87.5 ± 4.16[c]	66.66 ± 4.16[b]	77.27± 4.54[c]	68.8 ± 4.54[c]	81.8 ± 4.54[b]	95.65 ± 1.5[b]	58.33 ± 4.17[b]	37.5 ± 6.26[c]	45.45 ± 4.5[c]	83.3 ± 4.17[c]	66.66 ± 4.16[c]
Neta	24	33.33 ± 4.17[d]	27.27 ± 4.54[c]	27.27± 6.94[a]	33.3 ± 8.3[d]	33.3 ± 12.5[c]	37.9 ± 3.13[c]	25 ± 8.33[a]	16.66 ± 4.16[d]	29.16 ± 4.16[a]	29.16 ± 4.1[a]	33.37 ± 4.34[d]

*Each value represents mean ± standard deviation from 3 replicates having twenty four explants per replicate. Values denoted by different letters differ significantly. # Observations were taken after four weeks of inoculation in respective media.

Table 2
Effect of different concentration of NAA for induction of callus from four varieties of rice.

Variety	No. of Explant	Callus induction frequency					
		Concentration of NAA(mg/L)					
		1	2	4	6	8	10
Dahiya	24	-	29.17 ± 4.16[a]	25 ± 4.16[a]	9 ± 4.54[a]	8.3 ± 4.17[a]	25 ± 8.3[a]
Dani goda	24	12.5 ± 4.18[b]	5.67 ± 2.29[b]	97.22 ± 2.41[b]	75 ± 4.16 [b]	50 ± 4.16[b]	41.66 ± 4.16[b]
Karhani	24	4.17 ± 6.07[c]	41.67 ± 4.16[c]	83.33 ± 8.33[c]	83.33 ± 4.17[c]	83.33 ± 8.33[c]	98.16 ± 2.41[b]
Neta	24	-	5.56 ± 2.40[b]	91.67 ± 8.33[d]	91.66 ± 4.16[d]	98.16 ± 2.40[c]	98.48 ± 2.63[c]

*Each value represents mean ± standard deviation from 3 replicates having twenty four explants per replicate. Values denoted by different letters differ significantly. # Observations were taken after four weeks of inoculation in respective media.

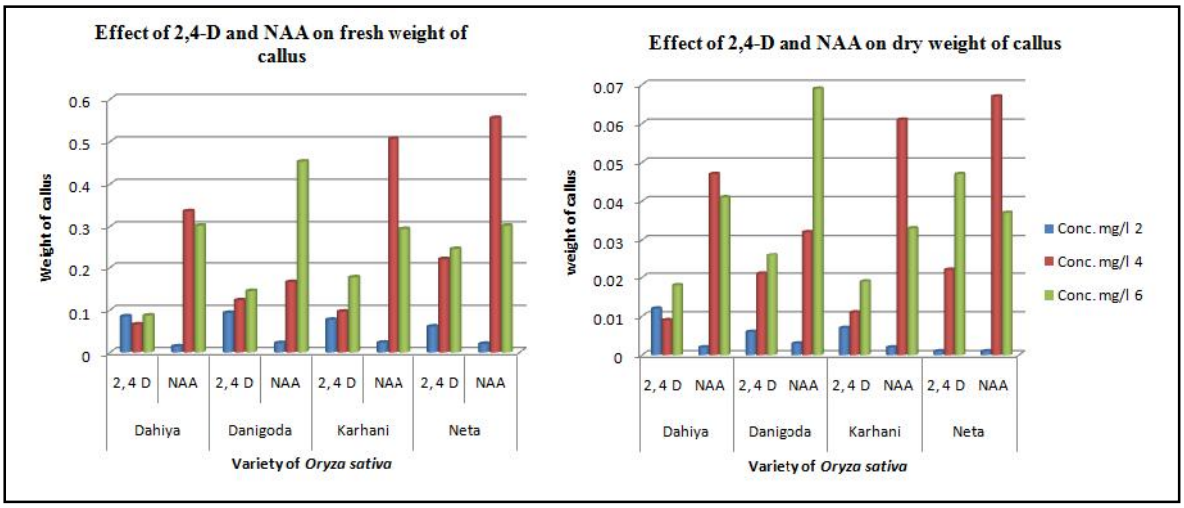

Fig 1
Comparison between calli weight (in gm) induced on MS + 2, 4-D and MS + NAA.

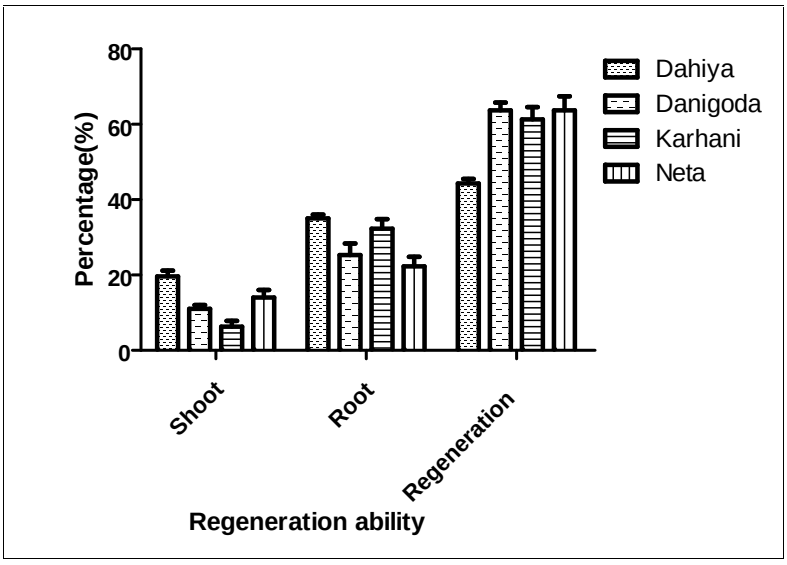

Fig 2
Regeneration ability of different varieties of rice on MS + IAA (2mg/L) + Kinetin (4 mg/L).

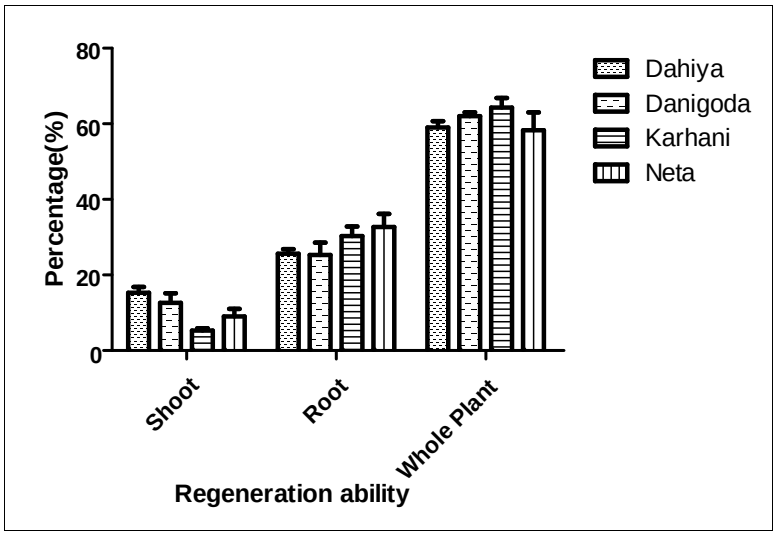

Fig 3
Regeneration ability of different varieties of rice on MS + NAA (1mg/L) + BAP (2mg/L).

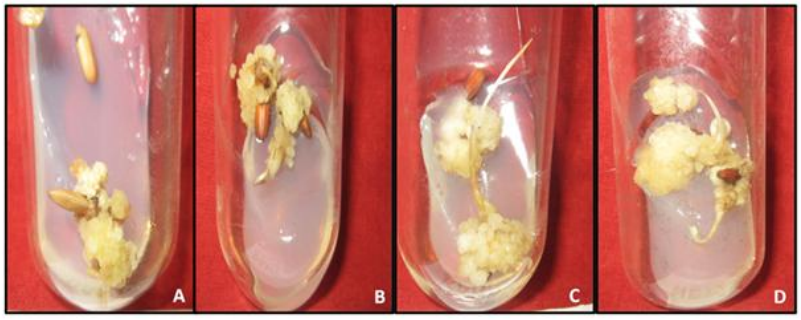

Fig 4
Callus induction in local varieties of rice in MS + 2,4-D (6mg/L).
(A) Dahiya; (B) Dani goda ; (C) Karhani (+ coleoptile); (D) Neta (+ coleoptiles).

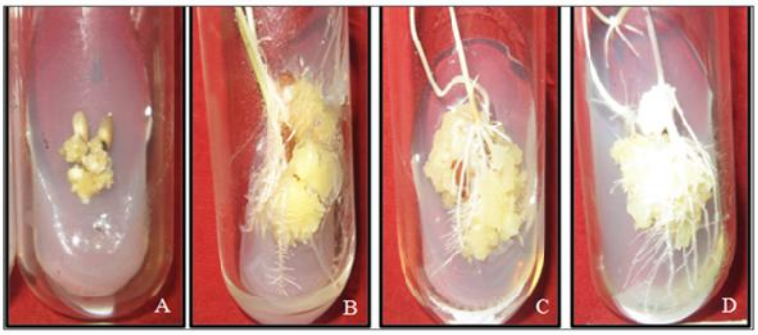

Fig 5
Callus induction in local varieties of rice in MS + NAA (6mg/L).
(A)Dahiya; (B) Dani goda; (C) Karhani (+ coleoptile); (D) Neta (+ coleoptiles).

Fig 6

Regeneration from seed derived callus of different local varieties of rice in MS + IAA (2mg/L) + KN (4mg/L). (A) Karhani: friable callus only; (B) Dani goda: roots only with necrotic tissues; (C) Dani goda: multiple shoots only with necrotic tissues; (D) Neta : multiple shoots with a few roots;(E) Dani goda: multiple green and albino shoots;(F) Neta: multiple albino shoots with a few roots.

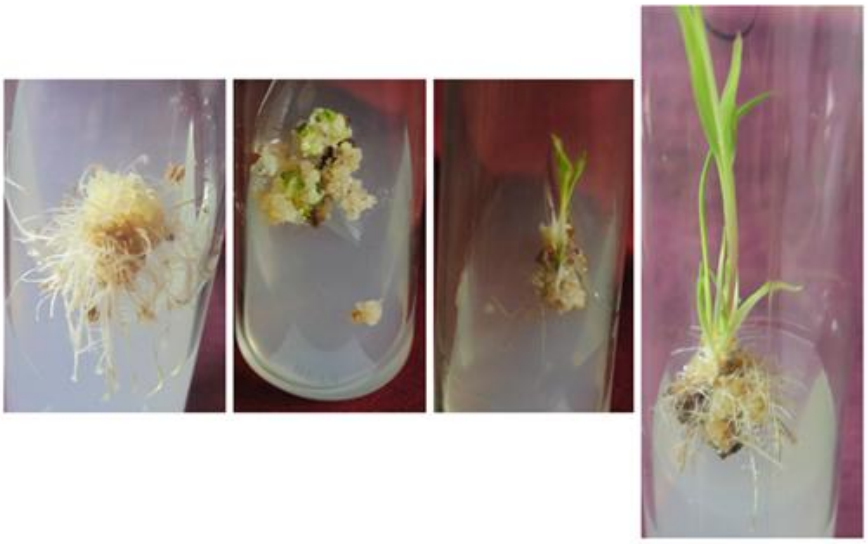

Fig 7

Regeneration from seed derived callus of different local varieties of rice in MS + NAA (1mg/L) + BAP (2mg/L). (A) Dani goda: roots only; (B) Neta: green spots developed; (C) Dani goda: shoots and roots with necrotic tissues; (D) Dani goda : multiple shoots with roots.

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