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RESPONSE OF MATURE ENDOSPERM OF SOME VARIETIES OF RICE (Oryza *sativa* L.) FOR THEIR CALLUSING AND DIFFERENTIATION

R. P. SINGH[1*], H. P. SHARMA[2], P. C. THAKUR[3] and S.N. ORAON[4]

[1*]Department of Botany, Chas College, Chas, Bokaro – 827013, Jharkhand (INDIA)
[2] University Department of Botany, Ranchi University, Ranchi- 834008, Jharkhand (INDIA)
[3]Department of Botany. Chas College, Chas, Bokaro-827013, Jharkhand (INDIA)
[4]Department of Botany, B.S. College, Lohardaga, Jharkhand (INDIA

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***Corresponding Author**

ABSTRACT

Rice is one of the most important cereals and it is a staple food crop of the most of the Asian countries. It fulfils 40% of the nutritional needs of a vast population of the world. In comparison to the population explosion of the world the production of rice, has not been reached up to the mark. In this regard, it is a high time, that the production of rice should be increased scientifically. But before going in detailed studies such as genetic engineering, protoplast culture, anther culture etc, it is essential to screen the rice varieties from its mature endosperm level first. In this perspective four varieties of rice had been undertaken for the response of their mature endosperm on different concentrations of 2, 4-D for their callusing and upon joint concentration of IAA and KN for their differentiation. Out of the four varieties of rice, the mature endosperm of Kanak and Pusa-Basmati showed their best callusing and differentiation in comparison to Tulsi and Birsa-101. For the best callusing, the concentration 4mg/l of 2, 4-D was confirmed. On the other hand the combined concentration of IAA (2mg/l) + KN (4mg/l) was proved as the best differentiating concentration of the growth regulators.

INTRODUCTION

Although in terms of area, the cultivation of rice comes next to wheat, but rice ranks first regarding the nutritional values among the different food crops. Rice provides more calories in respect to other cereals (De Datta 1981).

Rice is an annual herb, which belongs to the family *Poaceae*. Its inflorescence is of Spikelet subtype of the type Racemose. Its flower has got six stamens and one pistil. It has got two perianths, which become modified into two lodicules.

India has the largest rice growing area with 42 million hectares, which is followed by People's Republic of China. About 90 million tons per hectare is the annual production of rice in India (Ahmed Ilyas *et al*. 2006).

On worldwide perspective rice production has to be increased to cope with the global population explosion (Athawal 1972). In India, to maintain the present level of self sufficiency annual rice production has to be increased to 115 million tons by 2020 (Ahmed llyas et al. 2006). Hence, the most modern technology should be adopted to explore artificially induced genetic variability in rice improvement programme. In past time many investigators have worked upon rice tissue culture (Bajaj and Bidani 1980, Zafar *et al*. 1992, Ghosh *et al*. 1993, Datta *et al*. 1996, Usha Rani and Reddy 1996, Khanum *et al*. 1997, Cooking *et al*. 2003, Jelodar et *al*. 2003). Some scientists

had worked upon rice mature endosperm (Sharma 1986, Nakano et al. 1975, Nag 1970).

MATERIALS AND METHODS

In the present investigation two lowland (Kanak and Pusa Basmati) and two upland (Tulsi and Birsa-101) varieties of rice of Jharkhand had been undertaken. The seeds of the above varieties of rice had been collected from Birsa Agriculture University, Kanke, Ranchi.

MS (Murasige and Skoog 1962 medium) was used as basal medium for the present investigation. Analytical grade chemicals and double glass distilled water were used for the preparation of medium. To solidify the medium 1.0% Difco Bacto Agar was used. The p^H of the medium was maintained at 5.8 before autoclaving using 0.1 N NaOH or 0.1 N HCl. About 20 ml. of the hot medium was dispensed into each culture tube (15.0 x 2.5 cm). Later on all the tubes were plugged with nonabsorbent cotton wrapped in cheese cloth. After some time, the culture tubes were autoclaved at 1.06 kg/sq. cm for 15 minutes.

Since the explant (mature endosperm) was not grown in *in-vitro* condition, there was a need for their surface sterilization. The surface sterilized mature endosperm was placed on sterile petridishes under aseptic condition. Mature endosperm of these varieties were cut into many pieces and two pieces of endosperm were implanted upon the nutrient medium in each culture tube under aseptic condition and maintained at the temperature 25+ 5^0C & the R.H. 80%. The calli, which were developed from the mature endosperm were sub-cultured for their differentiation in the differentiating medium.

RESULTS AND DISCUSSION

The inoculated explants (mature endosperm) induced callusing on MS medium supplemented with different concentrations of 2, 4-D. The induced callus was mostly granular, friable and pale yellow (Fig. 1).

It was observed that, when the concentration of 2, 4-D was 2mg/1, Kanak showed 40% response, which was followed by Pusa Basmati, Tulsi and Birsa-101. However, when the concentration of 2.4-D was 4mg/1 the best callusing 81% was induced by Kanak, which was followed by Pusa Basmti 75%, Tulsi 66% and Birsa – 101 62%. But at higher concentration of 2, 4-D tendency of callusing was gradually declined (Table – 1).

On the contrary, Sharma (1986) reported that at higher concentration of 2, 4-D excellent callusing was induced in some varieties of rice. However similar to our findings Zagorska *et al*. 1997 reported that the lower concentration of 2, 4-D is the most potent for the induction of callus.

From the Table 1, it was also inferred that the low-land varieties of rice responded better percentage of callusing than the upland varieties of rice.

Table – 2 clearly shows the varietal differences in the differentiation. At the strength 2 mg/l of IAA and 4 mg/l of KN, the mature endosperm derived calli of Kanak and Pusa Basmati started their differentiation fast i.e., from the first week of inoculation. However, at the same concentration of above growth regulators Tulsi and Birsa – 101 started their differentiation from the second week and their rate of differentiation was comparatively slow. It was also seen that numbers of differentiated roots were more than the number of shoots. In case of Pusa Basmati, it was observed that number of roots was many, but number of shoots was only two but both the shoots were coiled (Fig.– 02). More or less similar condition was observed in Kanak, Tulsi and Birsa – 101.

Hence, it became clear that the combined strength of IAA (2 mg / l) + KN (4mg/l) was the best strength as upon this strength, the mature endosperm derived calli of all the varieties of rice undertaken had differentiated very well. Similar to our findings, Sharma (1986) reported that MS +

IAA (2 mg/l) + KN (4 mg/l) was the best differentiating medium for the differentiation of mature endosperm derived callus of some varieties of rice.

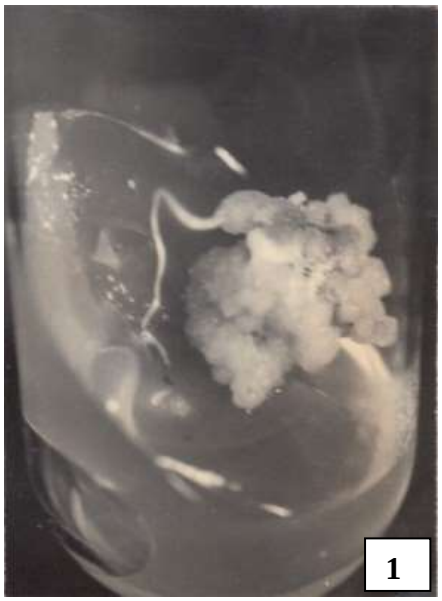

Fig. – 1. Mature endosperm derived callus of Pusa Basmati

Fig – 2 Differentiation in Mature Endosperm derived Callus in Pusa Basmati

TABLE- 1 Percentage of culture showing callus on MS + different concentrations of 2.4-D + YE (3000 mg/l) from the mature endosperm of the different varieties of rice

Varieties	Concentrations of 2, 4-D				
	2 mg /l	4 mg / l	6 mg /l	8 mg / l	10 mg /l
Kanak	40%	81%	71%	69%	62%
Pusa-Basmati	30%	75%	67%	73%	56%
Tulsi	15%	66%	65%	64%	50%
Birsa – 101	10%	62%	60%	61%	48%

Data recorded after 4 weeks of culture, YE - Yeast Extract

CONCLUSION

From the above discussion, it was concluded that best strength of 2, 4-D was 4 mg/l for the callusing as upon this concentration, the mature endosperm of all the varieties of rice undertaken induced their best callusing (Kanak 40 %, Pusa Basmati 30%, Tulsi 15% and Birsa-101 -10%). Regarding, differentiation, it was concluded that mature endosperm derived callus of Kanak and Pusa Basmati induced their best differentiation 42% and 36% respectively, at the combined strength of IAA 2 mg / l + KN 4 mg / l. It was followed by Tulsi 30% and Birsa -101, 25 %. Hence, finally it is concluded that lowland varieties of rice responded better for callusing as well as differentiation in comparison to upland varieties of rice.

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TABLE-2 Differentiation of Mature Endosperm derived callus in different varieties of rice, subcultured on MS + IAA (2 mg/l) + KN (4 mg / l)

SL No.	Varieties	% of differen-tiation of roots and shoots	Mean no. of roots per culture	Mean length of roots (cm.)	Mean no. of shoots per culture	Mean length of shoots (cm.)	Rate of Differen-tiation
1.	Kanak	42	25.1± 0.2	2.2±0.1	3.5±0 .1	1.7± 0.2	Fast
2.	Pusa-Basmati	36	20.1±0.1	1.6 ±0.2	2.1 ± 0. 1	1.8± 0.1	Fast
3.	Tulsi	30	10.2 ±0.4	1.5±0.1	1.6± 0.3	1.0± 0.3	Average
4.	Birsa-101	25	8. 3 ±0.3	1.3 ±0.2	1.4 ± 0 .2	0.8 ± 0.2	Average

Data recorded after 4 weeks of the culture, mean ±

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