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Research Article

Effect of growth regulators on callus induction in Rice embryo culture

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Abstract

An experiment was conducted to study the effect of growth regulators on callus induction in rice embryo culture. In this experiment, caullogenesis was initiated from the matured seeds of seven indica rice varieties *viz*., TRY 1, TRY 2, Pokkali, CSR 10, W.Ponni, BPT 5204 and IR 29. The medium used for this callus induction was Murashige and Skoog (1962) commonly referred as MS medium with six different combinations of growth regulators *viz*., MS+2,4-D 25mMl^{-1}, MS+2,4-D 20mMl^{-1}, MS+2,4-D 15mMl^{-1}, MS+2,4-D 10mMl$^{-1,}$ MS+2,4- D 15mMl^{-1}+ KIN 2.5mMl^{-1}, MS+2,4-D 10mMl^{-1}+ KIN 2.5 mMl^{-1} Among the above combination of the growth regulators, MS + 2 mMl^{-1} 2,4,D + 0.5 Kinetin performed well irrespective of all the genotypes. Analysis of variation showed that the genotype and medium are significantly different from each other and the interaction between Genotype X Medium also highly significant. Among the seven genotypes, Pokkali was significantly at (1%) superior for callus induction (63.57%) followed by W.Ponni (53.96%). The genotypes TRY 2 (51.70 %), TRY 1 (50.87) and CSR10 (49.22) are significantly at (5%) on par with each other. The well developed callus was transferred to regeneration medium for regeneration. The medium combination of MS +Kin 5mMl^{-1} + BAP 5mMl^{-1} + NAA 0.5mMl^{-1} recorded maximum average regeneration frequency of 57.25 percentage and higher response than the others. Among the varieties, Pokkali showed maximum regeneration frequency of 39.41 per cent and CSR 10 recorded minimum regeneration frequency of 20.23 per cent.

Key words: Rice genotypes, callus, medium, growth regulator and embryo.

Introduction

Rice is the staple food for more than 40 percent of the world population. The rice production has been increasing worldwide by large-scale adoption of modern high-yielding rice varieties and improved cultural practices. Even though the rice production meet out the current demand, there is a urgent need to double the production in mid 21st century because the current global population of 6.4 billions is expected to reach 7.5 billions by 2020, 9.0 billions by 2050 AD. So variation is essential to develop the new varieties with adoption to the new environment. *In vitro* technique is one of the efficient tool in rice breeding especially for the creation of variation through genetic manipulation. According to Ocono (1978, 1982) a very high frequency of modifications are induced *in vitro* cultures and some of them are very useful.

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The different growth hormones are used for the different crops. Slight variation in the doses is necessary for the different genotypes of the same crop also. The standardization media for the callus induction and regeneration are very important in tissue culture technique. Hence the study was carried out to standardize the effect of growth regulators in rice embryo culture both for callus induction as well as regeneration.

Materials and Methods

Seven rice varieties *viz.,* Pokkali, CSR10, TRY1, TRY(R)2, BPT 5204, White Ponni and IR29 were used to study the effect of growth regulators for callus induction as well as regeneration. The nutrient medium chosen for the study was Murashige and Skoog (MS) medium (1962) supplemented with different concentration of growth regulators in 0.8% agar. The pH of the medium is 5.5-5.8 The medium was transferred into the test tube (10 ml each) and plugged with nonabsorbent cotton. The cotton

plugged test tube was autoclaved at 1.01kg/cm pressure at 121°C for 20 minutes. The medium was allowed to cool at room temperature and stored at 10°C. The explant used for this callus induction study was mature embryo. Mature embryos from dehusked seeds were surface sterilized with 70 % alcohol for 30 sec followed by 15 % of common bleach for 20 minutes then finally rinsed several times with the sterile distilled water before inoculation into the callus induction media.

The media combination used for the callus induction were MS+2,4-D 25mMl^{-1}, MS+2,4-D 20mMl^{-1}, MS+2,4-D 15mMl^{-1}, MS+2,4-D 10mMl^{-1} , MS+2,4-D 15mMl^{-1}+ KIN 2.5mMl^{-1}, MS+2,4-D 10mMl^{-1}+ KIN 2.5mMl^{-1} (Agrawal *et al*.. 2006).

The observation recorded were number of days to callus induction, callus formation ability in different cultivars,, the effect of growth regulators on callus formation ability, callus induction frequency (CIF, %) (number of calli / number of inoculated explants×100%) (Aditya and Baker 2006). The well developed callus was transferred to the regeneration medium. The different concentrations of growth regulators used for regeneration were MS+15mMl^{-1}KIN, MS+0.5mMl^{-1}NAA +10mMl^{-1}BAP, MS+10mMl^{-1}KIN + 0.5mMl^{-1}NAA and MS+5mMl^{-1}KIN + 5mMl^{-1}BAP+ 0.5mMl^{-1}NAA. The experiment was laid out in a Factorial CRD design (Gomez and Gomez , 1984) with three replications.

The data obtained with percent values were subjected to arc sin transformation and analysed using the AGRES (Agricultural statistics) computer package. Level of significance (P value) was determined using the standard Analysis of Variance (AVOVA) (Panse and Sukhatme, 1964). Differences among mean values were assessed by LSD (Least Significant Difference) test.

Result and Discussion

In vitro technique is the one of the important biotechnological tool to create the variation through the means of somaclonal variation. In this technique there is a strong need to understand the factors affecting the callus induction in many aspects for the repeatable success in rice tissue culture. The callus was induced by using the mature embryo as explant and the medium used was MS with different hormonal combinations. This is the only explant available through out the year and the good embryogenic callus production was also high. Similarly Niroula *et al*. (2005) and Agrawal *et al*. (2006) reported the regeneration via somatic embryogenesis for callus obtained from matured embryos and recorded development of high level of good embryogeneic calli. The embryos facing upside down were produced good callus and the regeneration ability was also higher than the embryos placed horizontally. Hodges (1989) and Agrawal *et al*. (2006) showed similar findings.

The days to callus initiation showed significant difference between the medium and the genotypes (Table 1). The significant difference between the medium and genotypes indicate the medium and the genotypes used in this experiment are effective. Similar findings were also reported by Pushpam and Sree Rangasamy (2000).

Increasing the 2, 4,-D concentration beyond 15mMl^{-1} the callus induction frequency also reduced and beyond 25mM^{-1} there was no callus induction. Using the higher concentration of 2,4-D *ie.,* more than 10mMl^{-1} was not desirable, because it produced only the necrotic callus depending upon the increased 2,4-D concentration hence the possibility of subsequent plant regeneration got reduced (Table 2). The lower level of 2,4-D concentration *ie.,* less than or equal to 5mMl^{-1} increase the shoot and root growth from the embryo and no callus induction was found. The results indicate 2,4D level of 10mMl^{-1} is optimum for the callus induction through rice embryo irrespective of all the genotypes. The optimum level of 2,4 D (15mMl^{-1}) would help to development of good callus (Table2).

Among the six combination of medium used, the maximum of callus induction frequency was recorded in the medium of MS + 2,4 D (10mMl^{-1}) + KIN (2.5mMl^{-1}) (87.16 per cent) irrespective of all the genotypes followed by MS + 2,4 D (15mMl^{-1}) + Kin (2.5mMl^{-1}) (69.36 per cent) and MS+ 2,4 D (10mMl^{-1}) (54.13 per cent) (Table 3) irrespective of the genotypes. Similar finding was observed by the Agrawal and *et a*l. (2006).

Among the seven genotypes studied, Pokkali (63.57 per cent) responded well followed by White Ponni (53.96 per cent). The genotypes TRY 2 (51.70 %), TRY 1 (50.87) and CSR10 (49.22) are significantly at (5%) on par with each other (Table 3 & Fig.1) irrespective of the medium. Pokkali is well responded genotype than the others. Pokkali is a saline tolerant variety and the somaclones obtained through this

technique could be further used for the development of saline tolerant varieties.

The effect of genotype, medium and genotype x medium interaction were found to be significant. In a diallel analysis of callus induction, Quimio and Zapata (1990) found that effect of genotypes and genotype x medium interaction was significant. Optimum level of hormone combination was found to be MS + 2,4 D (10.0 mMl^{-1}) + KIN (2.5mMl^{-1}). Similar optimum level of 2,4-D and KIN had also been reported by Oard and Rutger (1989) and Agrawal *et al*. (2006).

The green islets were found when the 30 days old callus was transferred to the regeneration medium. Significant effect was found between the medium, variety and interaction of medium x variety. Similar significant effect of variety and variety x media interaction was also found by Agrawal and coworkers (2006) and Bregitzer and Poulson (1995).

Among the seven combinations of growth regulators tried only four media composition *viz.,* MS +Kin 5mMl^{-1} + BAP 5mM^{-1}+NAA0.5mMl^{-1} ; MS + BAP 10mMl^{-1} + NAA0.5mMl^{-1}; MS + 2.5mMl^{-1} NAA + 5mMl^{-1} BAP and MS +5mMl^{-1} IAA + 20mMl^{-1} BAP showed for regeneration of green islets. Among the above four combinations, MS +Kin 5mMl^{-1} + BAP 5mMl^{-1}+NAA0.5mMl^{-1} performed better with average regeneration ability of 57.25 per cent followed by MS + BAP 10mMl^{-1}+ NAA0.5mMl^{-1} (35.93 per cent) irrespective of the genotypes. While comparing the varieties Pokkali performed with maximum regeneration frequency of 39.41 per cent and CSR 10 recorded minimum regeneration frequency of 20.23 per cent irrespective of the medium (Table 4 & Fig. 2).

In the good performing regeneration media MS +Kin 5mM^{-1} + BAP 5mMl^{-1}+NAA0.5mMl^{-1} recorded the maximum regeneration frequency of 75.02 per cent in the variety Pokkali followed by 67.95 per cent in W. ponni. Among the seven varieties TRY1 recorded minimum regeneration frequency of 41.81 per cent in MS +Kin 5mMl^{-1} + BAP 5mMl^{-1} + NAA 0.5mMl^{-1} where as it recorded higher regeneration frequency of 49.29 per cent in the medium MS + BAP 10mMl^{-1} + NAA0.5mMl^{-1} This result was conforming the earlier results of Agrawal *et al*. (2006) and Aditya and Baker (2006).

The standardization of growth hormones for callus induction as well as regeneration is more important to create large amount of variation with in the short time .

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Table 1. Days to Callus Initiation

Genotype	MS + 2,4 D ((25m Ml^{-1})	MS + 2,4 D (20m Ml^{-1})	MS+ 2,4 D (15mMl^{-1})	MS+ 2,4 D (10mMl^{-1})	MS + 2,4 D (15mMl^{-1}) + KIN (2.5mMl^{-1})	MS + 2,4 D (10mMl^{-1}) + KIN (2.5mMl^{-1})	Mean
TRY1	20.33t	16.00r	12.33klmn	9.67fg	9.67f	8.67ef	12.78 d
TRY(R)2	21.00t	14.67q	11.33ij	8.33de	7.67cde	6.33b	11.56 bc
POKKALI	14.67q	11.67ijkl	7.67de	6.33 bc	5.67 ab	4.67 a	8.44 a
BPT5204	17.67s	13.33nop	11.33ijk	9.67fg	8.67ce	7.33d	11.33b
W.PONNI	14.67q	14.33pq	11.33ijk	10.67gh	10.00gh	9.67fg	11.78 c
CSR 10	22.33u	17.67s	13.67opq	12.67lmno	11.00hij	11.67ijkl	14.83 e
IR29	24.67v	17.67s	14.67q	13.00mno	12.00jklm	12.33klmn	15.72 f
Mean	19.33f	15.05e	11.76d	10.05c	9.24b	8.67a	**12.35**

	SED	CD(0.05)	CD(0.01)
Genotypes(G)	0.19	0.38	0.50
Treatment (T)	0.20	0.41	0.54
GxT	0.50	1.00	1.33

* The mean having the same letter following is not significantly different at 0.01 probability level by Least Significant Difference Test (LSD)

Table.2. The visual observations on callus induction in different media after 20-25 days

Media	Shoot formation	Callus formation
MS+ 2,4 D (25mMl^{-1})	Less germination albino	Poor
MS + 2,4 D (20.0mMl^{-1})	Albino	Poor to better
MS + 2,4 D (15.0mMl^{-1})	Green	Better
MS + 2,4 D (10.0mMl^{-1})	Green	Fair
MS + 2,4 D (15.0mMl^{-1}) + KIN (2.5mMl^{-1})	Green and albino	Fair
MS + 2,4 D (10.0mMl^{-1}) + KIN (2.5mMl^{-1})	Albino	Good

Table .3.Callus Induction Frequency (CIF) in seven *indica* rice varieties after 20-25 days

Genotype	MS + 2,4 D (25m MI^{-1})	MS + 2,4 D (20mMI^{-1})	MS+ 2,4 D (15mMI^{-1})	MS+ 2,4 D (10mMI^{-1})	MS + 2,4 D (15mMI^{-1}) + KIN (2.5mMI^{-1})	MS + 2,4 D (10mMI^{-1}) + KIN (2.5mMI^{-1})	Mean
TRY1	15.05 (22.80)rs	38.43 (38.31)no	47.87 (43.78)klm	54.67 (47.68)jk	64.73 (53.57)i	81.51 (64.54)def	50.87 (45.49) cd
TRY(R)2	14.56 (22.35)rs	40.73 (39.59)mn	41.93 (40.33m)n	46.95 (43.25)klm	73.78 (59.25)gh	92.24 (74.07)ab	51.70 (45.98) bc
POKKALI	32.79 (34.93)op	50.68 (45.39)kl	64.12 (53.24)i	63.61 (52.92)i	77.15 (61.47)efg	93.49 (75.33)a	**63.57** (52.87) a
BPT5204	5.44 (13.48)t	17.73 (24.70)r	29.65 (32.96)p	42.72 (40.82)lmn	61.56 (51.69)ij	84.43 (66.92)cd	40.25 (39.38) f
W.PONNI	13.43 (21.48)rs	28.42 (32.22)p	51.06 (45.61)k	68.76 (56.03)hi	74.92 (59.98)fgh	87.10 (69.02)cd	53.96 (47.27) b
CSR 10	12.17 (20.40)rs	25.91 (30.56)p	47.13 (43.35)klm	54.22 (47.43)jk	68.04 (55.58)hi	87.89 (69.67)bc	49.22 (44.55) d
IR29	11.53 (19.37)s	24.67 (29.74)	47.01 (43.28k)lm	48.00 (43.86)klm	65.38 (53.97)i	83.48 (66.03)cde	46.67 (43.09) e
Mean	**14.99** (22.77) g	**32.37** (34.67) f	**46.97** (43.26) e	**54.13** (47.37) c	**69.36** (56.39) b	**87.16** **(69.00)a**	**50.89** (45.51)

	SED	CD(0.05)	CD(0.01)
Genotypes(G)	0.95	1.89	2.50
Treatment (T)	0.88	1.75	2.32
GxT	2.33	4.63	6.13

* Values in parentheses indicate the transformed arc sin values
* The mean having the same letter following is not significantly different at 0.01 probability level by Least Significant Difference Test (LSD)

Table .4. Regeneration Frequency from the rice calli

Genotypes	MS +KIN (5mMl^{-1}) +BAP (5mMl^{-1})+ NAA (0.5mMl^{-1})	MS + BAP (10mMl^{-1}) + (NAA0.5m Ml^{-1})	MS + 2.5mMl^{-1} NAA + 5mMl^{-1} BAP	MS +5mMl^{-1} IAA + 20mMl^{-1} BAP	Mean
TRY1	41.81 (40.28) cde	49.29 (44.59) c	28.17 (32.05) gh	11.00 (19.36) lm	30.92 (33.15)
TRY(R)2	63.21 (52.67) b	30.00 (33.08) hi	31.27 (34.00) hi	12.67(20.85) kl	33.51 (34.67)
POKKALI	75.02 (60.05) a	49.05 (44.45) c	25.89 (30.58) ij	7.67(16.07) no	39.41 (37.79)
BPT5204	63.06 (52.58) b	27.78 (31.76) hi	14.67 (22.52) k	5.89(14.02) op	27.85 (30.22)
W.PONNI	67.95 (55.52) b	35.73 (36.70) fg	12.21(20.44) klm	4.33(12.00) pq	33.45 (33.14)
CSR10	44.00 (41.55) cd	22.49 (28.30) j	11.67(19.97) klm	2.78 (9.54) q	20.23 (24. 84)
IR29	45.68 (42.52) cd	37.21 (37.59) ef	9.00 (17.44) m	2.44 (8.93) q	22.62 (26.06)
Mean	**57.25 (49.31)**	35.93 (36.64)	18.98 (25.29)	6.6 (14.39)	29.71 (31.40)

	SED	CD(0.05)	CD(0.01)
Genotypes(G)	0.59	1.18	1.60
Treatment (T)	0.78	1.56	2.08
GxT	1.56	3.12	4.15

* Values in parentheses indicate the transformed arc sin values
* The mean having the same letter following is not significantly different at 0.01 probability level by Least Significant Difference Test (LSD)

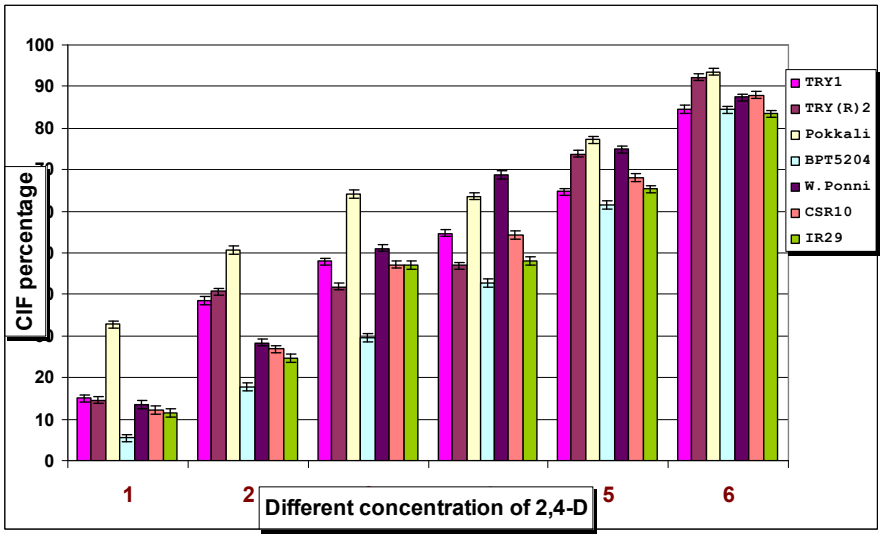

Fig.1.Callus Induction Frequency (CIF) in seven *indica* rice varieties after 20-25 days

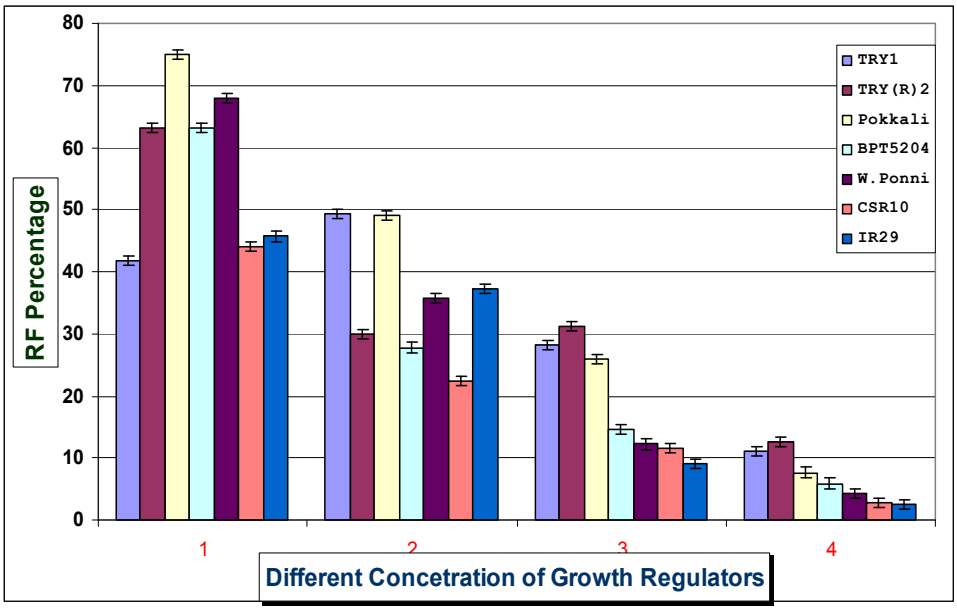

Fig. 2. Regeneration Frequency from the rice calli