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Influence of Media Type and Carbon Source on Callus Induction and Regeneration Response of Different *Indica* Rice Genotypes

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Abstract

Suitable carbon source and an ideal media type are essential pre-requisites for rice tissue culture and transformation experiments. Maltose is found to be the best carbon source and MS media is found to be the ideal media type for the results obtained from callus induction and regeneration capabilities of seven *indica* rice genotypes taken under study. Varied tissue culture response of selected genotypes is found conspicuous at different sugar concentrations.

Keywords: Carbon source; Media type; Rice tissue culture; *Indica* rice genotypes

Introduction

Indica rice varieties are known to be recalcitrant to culture and several attempts are made throughout the years to improve tissue culture protocols for effective callus induction and regeneration- *in vitro* [1-3]. Several factors of the culture media play a critical role in tissue culture response of the various rice genotypes. Types of the media used, and carbon sources are two vital factors among them. Moreover, genotypic differences do exist in response to the culture media [4-7]. In this study attempts were made to find the suitable media for rice tissue culture between the two widely used media *viz.*, MS and N6. Response of the cultivars towards varied carbon sources and concentrations is studied. Browning of callus in long standing rice tissue cultures is one of the major concerns which hamper the regeneration capacities of the rice cultivars [8,9]. This is observed in rice cultures grown on sucrose, compared to maltose. This study envisaged to find out the ideal concentrations of sucrose and maltose to be used for ideal green plant regeneration. This study holds importance because the choice of best responding variety to suitable sugar concentration in an amenable media is an essential pre-requisite for gene transformation experiments and efficient regeneration of the successful transformants. Results obtained were tabulated and the consequences are discussed.

Materials and Methods

Genotypes

The rice genotypes selected for the study were elite *indica* rice cultivars Swarna, Gayatri, Samba Mahsuri, Pooja, Tapaswini, Sahabhagidhan and Anjali. Swarna, a widely grown variety in eleven states of India, is highly popular with a yield potential of 8.0 t/ha [10]. It is also being widely grown in Bangladesh and Myanmar suggesting its wide adaptability [11]. Gayatri, a high yielding cultivar released from NRRI, is widely grown in shallow and medium low land ecology in Eastern India. Samba Mahsuri (BPT 5204) is one of the India's most popular and highly prized rice varieties because of its high yield and excellent cooking quality [12].

Pooja, a high yielding cultivar released from NRRI, is widely grown in shallow and medium low land ecology in Eastern India [13]. Shabhagidhan is a popular variety suitable for upland, rainfed direct seeded as well as transplanted conditions. It is released for cultivation in states of Jharkhand and Odisha. It bears golden husked long bold grains and has an average productivity of 3.8-4.5 t/ha [14]. Tapaswini is an elite *indica* rice variety with a yield potential of 5.0 t/ha [15,16]. Anjali is an upland early maturing rice variety released from NRRI [17]. Characteristic features of seven different genotypes taken in study are given in Table 1 [18].

Mature dehusked grains of the selected genotypes were washed with sterile distilled water and were surface sterilized successively with, 70% ethanol for two min, sodium hypochlorite (contains 4% (v/v) active chlorine) for 15 min and with 0.1% (w/v) aqueous mercuric chloride solution for 5 min with intermittent repeated washings with sterile distilled water [19].

The kernels were inoculated in culture tubes in two separate experiments containing semisolid callus induction (CI) medium in two separate media namely MS [20] and N6 [21], each supplemented with 2,4-D (2.0 mg l^{-1}) and Kn (0.5 mg l^{-1}) were evaluated for their potential to support callus induction and subsequent green plant regeneration, of the callus induced on these two media from different genotypes. Calli developed on these media were transferred onto MS regeneration medium supplemented with phytohormones [NAA (0.5 mg l^{-1})+Kn (0.5 mg l^{-1})+BAP (1.5 mg l^{-1})]. Callus induction (CI) and regeneration frequencies (RF) were recorded and statistical analyses were performed using SAS software [6].

S. No	Name of Variety	Duration (in days)	Grain type	Yield Q/ha.	Year of notification	Parentage
1	Swarna	150	Short bold	72	1983	Vasista × Mahsuri
2	Gayatri	160	short bold	46	1988	Pankaj/Jagannath
3	Samba Mahsuri	145	medium slender	55	1979	GEB-24 × T(N)1 × Mahsuri
4	Pooja	150	medium slender	46	1990	Vijaya × T.141
5	Tapaswini	135	medium slender	55	1996	Jagannath × Mahsuri
6	Shabhagidhan	100	long bold	41	2009	IR 55419-04*2. Way Rarem (IR 55419-04 (IR 12979-24-1 (Brown)/UPRLRi5
7	Anjali	90	short bold	32	2003	RR-19-2 × RR-149-1129

Table 1: Characteristic features of seven different genotypes taken in study.

Results and Discussion

Influence of media types on callus induction and regeneration response of different rice genotypes

This experiment was conducted with an objective of identifying suitable medium for raising the somatic cell cultures in *indica* rice so that the identified medium can be effectively used for rice tissue culture and transformation purposes. The genotypes showed varied response on both the media with respect to callus induction (Table 2).

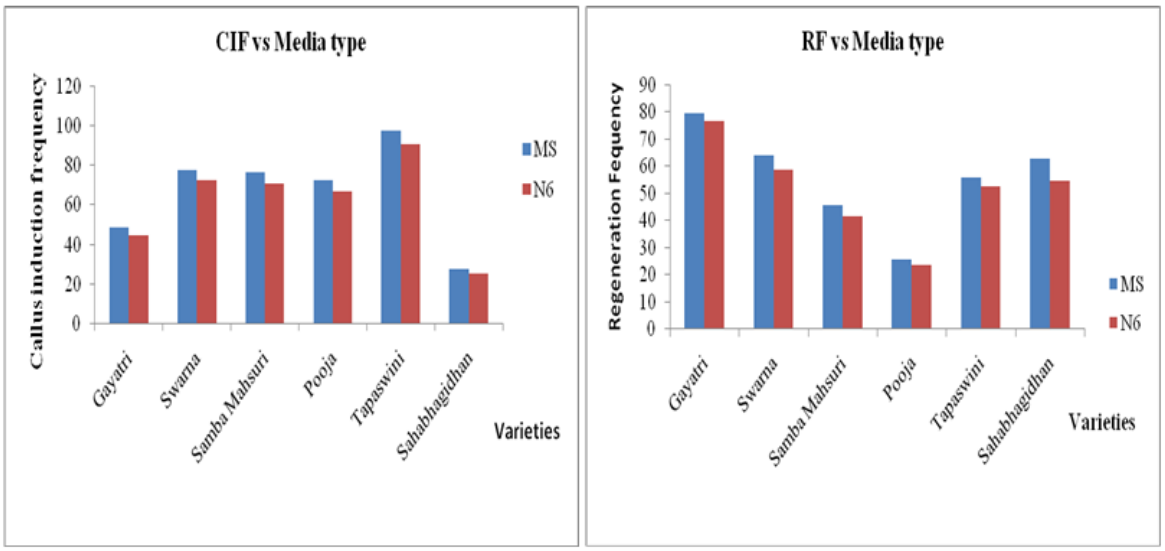
MS medium, in general, proved to be better for early callus induction and calli induced on this medium showed good levels of green plant regeneration in all the genotypes tested compared to N6 medium (Figure 1).

Even though, compositions of the both the media are distinctive, ratio of nitrogen between nitrate form and ammonium form during plant utilization in the media was found to play a critical role in somatic embryogenesis of monocots [22,23] and the results showed that rice genotypes responded better on MS medium compared to N6 medium.

S. No	Genotype		CI (%)		RE (%)	
			MS	N6	MS	N6
1	Gayatri	F	48.6	44.5	79.6	76.4
		SD	1.2	0.9	0.9	1.2
		SE	0.6	0.5	0.5	0.6
2	Swarna	F	77.6	72.53	63.76	58.56
		SD	0.8	1.23	1	0.85
		SE	0.4	0.7	0.58	0.49
3	Samba Mahsuri	F	76.3	70.76	45.5	41.73
		SD	0.9	1.05	0.79	1.15
		SE	0.5	0.61	0.45	0.66
4	Pooja	F	72.5	66.67	25.76	23.43
		SD	0.9	1.16	0.96	1.26
		SE	0.5	0.67	0.55	0.73
5	Tapaswini	F	97.4	90.73	55.6	52.6
		SD	1.1	0.9	1.11	0.91
		SE	0.6	0.52	0.64	0.52
6	Sahabhagidhan	F	27.5	25.46	62.63	54.6

		SD	1	0.7	0.97	1.15
		SE	0.6	0.4	0.56	0.66
CI: Callus Induction; RE: Regeneration; F: Frequency; SD: Standard Deviation; SE: Standard Error						

Table 2: Influence of media type on callus induction and regeneration response of *indica* rice varieties.



CIF: Callus Induction Frequency; RF: Regeneration Frequencies

Figure 1: Callus Induction and regeneration frequencies of different genotypes VS Media type.

Most desirable strategy to obtain better regeneration is to use the most appropriate medium [24]. MS medium is the best suitable medium as per the results obtained. The analysis of variance conducted with the six varieties suggests that the differences between media, genotypes and interaction were significant for both callus induction and green plant regeneration. (Table 3).

A. Callus induction					
Source of Variation	**% of total variation**	**p-value**	**p-value summary**	**Significant?**	**p-value**
Interaction	0.1153	0.011	*	Yes	--
Media	1.24	<0.0001	****	Yes	--
varieties	98.5	<0.0001	****	Yes	--
ANOVA table	**SS**	**DF**	**MS**	**F (DFn, DFd)**	**p-value**
Interaction	20.09	5	4.019	F (5,24)=3.813	P=0.0110
Media	216.1	1	216.1	F (1,24)=205.0	P<0.0001
varieties	17164	5	3433	F (5,24)=3257	P<0.0001
Residual	25.29	24	1.054		
B. Regeneration					
Source of Variation	**% of total variation**	**p-value**	**p-value summary**	**Significant?**	**p-value**
Interaction	0.3267	0.0009	***	Yes	--

Media	1.648	<0.0001	****	Yes	--
Varieties	97.77	<0.0001	****	Yes	--
ANOVA table	**SS**	**DF**	**MS**	**F (DFn, DFd)**	**p-value**
Interaction	32.48	5	6.495	F (5,24)=6.078	P=0.0009
Media	163.8	1	163.8	F (1,24)=153.3	P<0.0001
Varieties	9720	5	1944	F (5, 24)=1819	P<0.0001
Residual	25.65	24	1.069		
df: Degrees of freedom; ns: Non-significant; MS: Mean square; SS: Sum-of-squares					

Table 3: Analysis of variance on the media type for *in vitro* response.

Effect of carbon sources on callus induction and plant regeneration

This experiment was conducted with the objective of identifying the ideal carbon source for rice tissue culture which can be employed later for transformation purposes. Two sugars i.e., sucrose and maltose were tested at four different levels as the carbon sources in the MS callus induction medium and the responses of six genotypes, Gayatri, Swarna, Samba Mahsuri, Pooja, Anjali, and Sahabhagidhan were studied.

a) Callus induction										
S. No	**Varieties**	**%**	**Carbon source**							
			Maltose	**Sucrose**						
			3%	**4.50%**	**6%**	**9%**	**3%**	**4.50%**	**6%**	**9%**
1	Gayatri	CIF	50.7	56.4	50.6	48.56	46.63	49.8	47.36	45.36
		SD	2.05	2.2	2.2	1.72	3.85	3.9	4.05	4.1
		SE	1.18	1.27	1.27	0.99	2.22	2.25	2.34	2.36
2	Swarna	CIF	79.8	69.4	65.73	64.53	70.5	66.6	62.7	61.63
		SD	2.05	2.1	1.33	1.92	3.75	3.85	4.05	3.65
		SE	1.18	1.2	0.76	1.1	2.16	2.22	2.33	2.1
3	Samba Mahsuri	CIF	78.5	75.56	74.66	68.53	68.53	67.43	64.4	62.46
		SD	2.26	1.82	1.97	1.85	4.35	3.85	4.3	4.26
		SE	1.3	1.05	1.14	1.06	2.51	2.22	2.48	2.46
4	Pooja	CIF	74.36	78.53	69.6	67.36	66.67	70.53	65.46	61.6
		SD	2.05	1.7	2.35	2.35	4.152	3.8	4.3	3.91
		SE	1.18	0.98	1.35	1.35	2.39	2.19	2.48	2.26
5	Anjali	CIF	49.4	50.66	58.53	48.7	44.34	48.56	52.6	40.6
		SD	2.1	1.97	1.7	2.2	3.75	4.1	3.9	4.05
		SE	1.21	1.14	0.98	1.27	2.16	2.37	2.25	2.33
6	Sahabhagidhan	CIF	38.56	42.8	51.6	38.43	38.4	41.53	44.56	36.46
		SD	1.8	1.95	2.15	1.88	4.2	4.11	4.25	3.91
		SE	1.03	1.12	1.24	1.08	2.42	2.37	2.45	2.25

CIF: Callus induction frequency; SD: Standard Deviation; SE: Standard Error										
b) Regeneration										
S. No	**Varieties**		**Carbon source**							
			Maltose	**Sucrose**						
			3	**4.5**	**6**	**9**	**3**	**4.5**	**6**	**9**
1	Gayatri	REF	15.57	27.26	34.63	33.56	13.8	25.53	23.4	12.6
		SD	2.15	2.57	2.31	1.94	2.56	2.6	3.02	2.2
		SE	1.24	1.48	1.34	1.12	1.47	1.52	1.74	1.27
2	Swarna	REF	34.63	28.43	26.53	24.8	26.57	28.37	24.57	23.57
		SD	2.15	1.75	1.76	2.05	1.91	1.95	2.11	1.87
		SE	1.24	1.01	1.02	1.18	1.1	1.12	1.21	1.08
3	Samba Mahsuri	REF	34.46	32.46	30.7	24.13	28.6	26.13	24.76	22.76
		SD	1.88	1.7	1.9	3.19	2.61	2.11	1.95	2.1
		SE	1.08	0.98	1.1	1.84	1.51	1.22	1.12	1.21
4	Pooja	REF	26.53	27.26	25.13	24.86	25.5	26.73	27.1	22.76
		SD	2.15	2.04	2.05	2.45	2.16	1.85	1.75	2.05
		SE	1.24	1.17	1.18	1.41	1.25	1.06	1.01	1.18
5	Anjali	REF	14.1	15.56	17.16	17.6	16.63	18.56	20.66	22.73
		SD	2.21	2.21	2	3.75	2	1.8	2	2.15
		SE	1.27	1.27	1.15	2.17	1.18	1.06	1.18	1.24
6	Sahabhagidhan	REF	37	36.6	39.83	35.3	36.7	37.46	38.7	32.53
		SD	1.96	4.35	1.79	2.38	2.05	2.05	1.96	2.3
		SE	1.13	2.51	1.03	1.37	1.18	1.18	1.13	1.32
REF: Regeneration frequency; SD: Standard Deviation; SE: Standard Error										

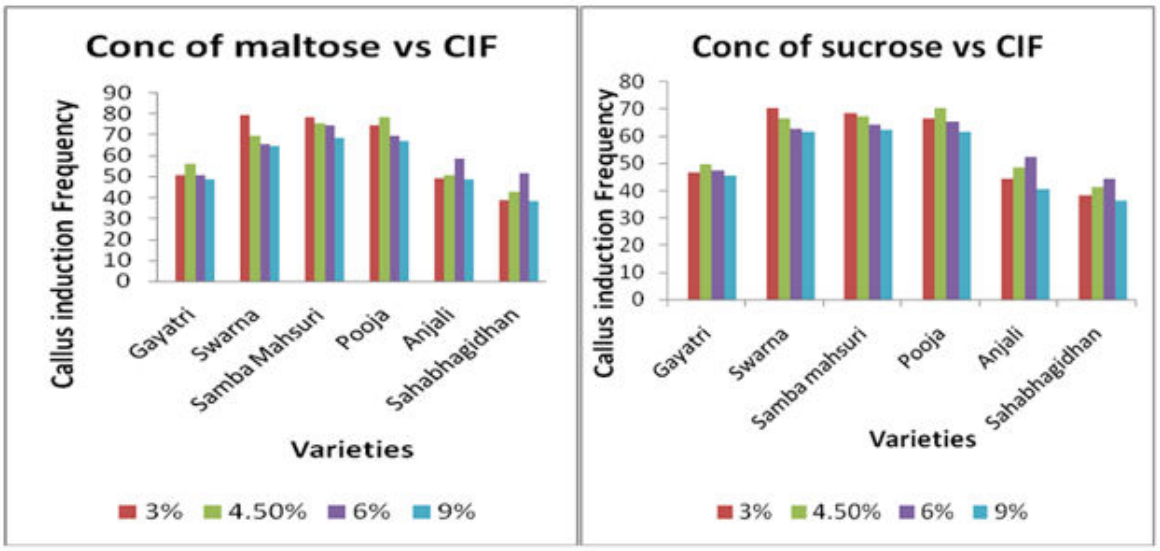
Table 4: Effect of sugars on *in vitro* response in different *indica* rice varieties.

Genotypic differences were observed for both callus induction (Figures 2 and 3) and regeneration (Table 4) in response to different concentrations of sucrose and maltose in the callus induction medium.

Addition of higher levels (>6%) of sucrose to the callus induction media did not show any significant enhancement of callus induction when the pooled data of all the six genotypes was taken into consideration.

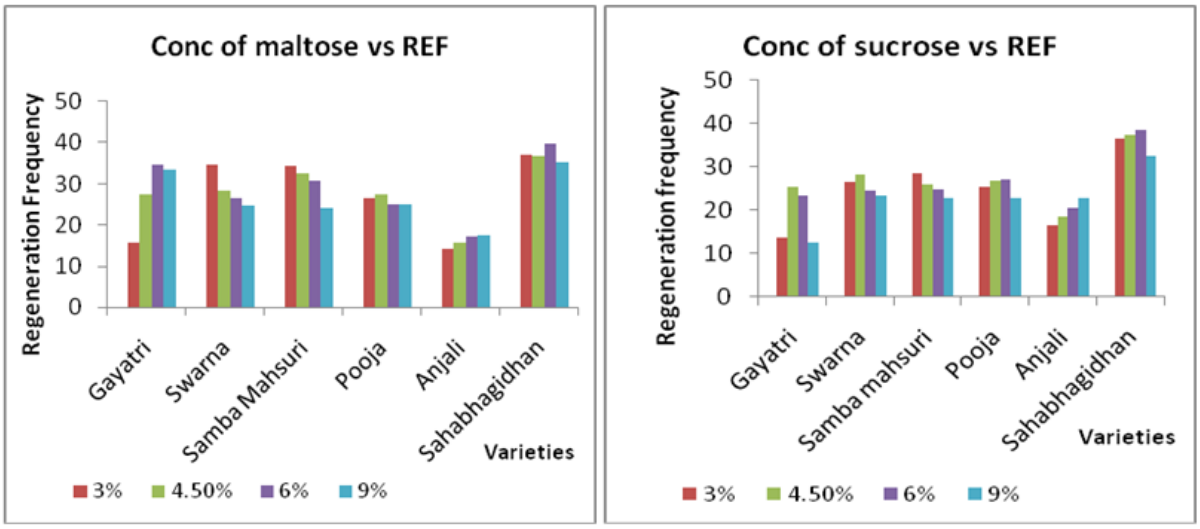
The analysis of variance of the data suggests that the differences between various concentrations of sucrose and maltose and genotypes were significant for both callus induction, and green plant regeneration (Table 5).

A level of maltose at 3% was found to be ideal for genotypes like Swarna and Samba Mahsuri for obtaining high rates of callus induction and regeneration, where as 4.5% of maltose was ideal in case of Gayatri and Pooja for inducing a high rate of callus induction, but the result varied with respect to regeneration i.e., Gayatri showed good regeneration at 9.0% sucrose while Pooja showed good regeneration at 6% maltose. For genotypes like Anjali and Sahabagidhan, 6% maltose was better for callus induction while 9% of sucrose yielded higher levels of regeneration in case of Anjali and 6% maltose in case of Sahabhagidhan. As compared to sucrose, maltose proved to be better, for the callus induction, although genotypic differences were conspicuous.



CIF:Callus Induction Frequency

Figure 2: Callus induction frequencies of 6 different varieties at 4 different maltose and sucrose concentrations.



REF:Regeneration Frequency

Figure 3: Regeneration frequencies of 6 different varieties at 4 different maltose and sucrose concentrations.

This can be attributed to the reduced rate of hydrolysis of the maltose to glucose and glucose-1-phosphate [25-27] thereby providing a readily metabolized carbon source over a long period in culture and improvement of osmotic stability of the culture medium in comparison to sucrose [28]. This is in congruence with earlier research works of Kumria et al. [29], Zaidi et al. [5] and Bagheri et al. [30].

Callus induction with maltose					
Source of Variation	**% of total variation**	**p-value**	**p-value summary**	**Significant?**	
Interaction	7.863	<0.0001	****	Yes	

% maltose	3.896	<0.0001	****	Yes	
Varieties	86.68	<0.0001	****	Yes	
ANOVA table	SS	DF	MS	F (DFn,DFd)	p-value
Interaction	967.2	15	64.48	F (15,48)=16.08	P<0.0001
% maltose	479.2	3	159.7	F (3,48)=39.83	P<0.0001
Varieties	10661	5	2132	F (5,48)=531.6	P<0.0001
Residual	192.5	48	4.011		
Callus induction with sucrose					
Source of Variation	**% of total variation**	**p-value**	**p-value summary**	**Significant?**	
Interaction	3.538	0.1717	ns	No	
Sucrose %	3.858	0.0002	***	Yes	
Varieties	84.69	<0.0001	****	Yes	
ANOVA table	SS	DF	MS	F (DFn, DFd)	p-value
Interaction	347.5	15	23.17	F (15, 48)=1.431	P=0.1717
Sucrose %	378.9	3	126.3	F (3,48)=7.800	P=0.0002
Varieties	8319	5	1664	F (5,48)=102.7	P<0.0001
Residual	777.3	48	16.19		
Regeneration with maltose					
Source of Variation	**% of total variation**	**p-value**	**p-value summary**	**Significant?**	
Interaction	24.91	<0.0001	****	Yes	
Maltose %	1.346	0.0262	*	Yes	
Varieties	67.33	<0.0001	****	Yes	
ANOVA table	SS	DF	MS	F (DFn,DFd)	p-value
Interaction	1047	15	69.77	F (15,48)=12.44	P<0.0001
Maltose %	56.56	3	18.85	F (3,48)=3.361	P=0.0262
Varieties	2828	5	565.7	F (5,48)=100.8	P<0.0001
Residual	269.2	48	5.609		
Regeneration with sucrose					
Source of Variation	**% of total variation**	**p-value**	**p-value summary**	**Significant?**	
Interaction	13.7	<0.0001	****	Yes	
Sucrose %	6.402	<0.0001	****	Yes	
Varieties	72.95	<0.0001	****	Yes	
ANOVA table	SS	DF	MS	F (DFn,DFd)	p-value
Interaction	441.8	15	29.45	F (15,48)=6.312	P<0.0001
Sucrose %	206.4	3	68.79	F (3,48)=14.74	P<0.0001
Varieties	2352	5	470.3	F (5,48)=100.8	P<0.0001

Residual	224	48	4.666		
MS: Mean square; SS: Sum-of-squares; df: degrees of freedom					

Table 5: Anova on response of genotypes to different levels of sugars.

There was significant influence regarding the level of sucrose or maltose in the callusing media [31] and also on the regeneration potential of the calli depending on the genotypes (Table 4). However, browning of the callus is observed due to the ethylene produced under the influence of sucrose on regeneration of calli. This is in congruence with the research studies of Hidekazu Kobayashi and Hitoshi Saka [32]. This is one of the reasons why maltose is preferred over sucrose [33].

Conclusion

Ideal carbon source and an amenable media are essential pre-requisites for best tissue culture response of selected *indica* rice genotypes. The results of this study showed that MS media and maltose are best sources for tissue culture response. However, variation in concentrations of the sugar source is to be judged in response to type of genotype chosen. This study holds importance in improvement of protocols for rice tissue culture and future transformation experiments for crop improvement.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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