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Identification of Callus Induction Potential of Nine Different Genotypes of Indica Rice

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Abstract

Callus induction potential of nine economically important indica rice genotypes of Tamil Nadu and neighboring areas was examined on different callus induction medium. Nine genotypes include Improved White Ponni(I W Ponni), BPT-5204, TKM-9, CR1009, ADT-39, ADT-43, KKL(R) -1, CO(R)-48 and Savitri/ Ponmani. These rice genotypes were having distinct useful agronomical trait like resistance to various pest, tolerance to salinity and drought conditions. Most of the indica rice genotypes having good grain quality and nutritional properties are susceptible to pest and diseases causing loss in annual yield. The yield could be enhanced by genetic improvement by rice transformation to enable them to withstand biotic and abiotic stress. First step towards this approach is to produce a pleuripotent cell mass, callus induced from scutellum of mature rice seeds. MS basal medium with various 2, 4-Dichlorophenoxy acetic acid (2, 4-D) concentrations (2mg/L, 2.5mg/L, 3mg/L, 4mg/L and 5 mg/L) was used for these studies. Four indica rice genotypes I. W. Ponni, ADT- 43, BPT-5204 and TKM-9 found to be potent for callus induction from scutellum of mature seed at the concentration of 2mg/l to 5 mg /l 2,4-D. The varieties ADT-39, KKL (1) R, CO(R) 48, CR1009 and Savitri/Ponmani were recalcitrant and no embryogenic calli was obtained on either of the 2, 4-D concentration. 34 % of healthy calli obtained from I W Ponni 20 % of healthy callus induction obtained from BPT-5204 and 10 % of healthy calli from each of the ADT-43 and TKM-9. These indica rice genotypes could be used for various plant science researches.

Keywords *- Callus Induction; scutellum; Indica* rice genothype.

1. INTRODUCTION

Indica rice varieties show large genetic diversity and large numbers of varieties are being used commercially (FAO 2012). The varieties in Tamil Nadu and Pondicherry in commercial use includes Improved White Ponni(I W Ponni), BPT-5204, TKM-9, CR1009, ADT-39, ADT-43, KKL(R) -1, CO(R)-48 and Savitri/Ponmani. These varieties with agronomically beneficial traits could be employed for advance research in plant sciences to control and minimize the agricultural production loss caused by various biotic and abiotic factors. The transformation in *indica* rice cultivars is difficult due to the recalcitrant regeneration system in *indica* rice (Lin and Zhang 2005). Therefore, optimization of callus induction and regeneration system in *indica* rice cultivars is the first and foremost step towards crop improvement. Callus induction is the key step towards selecting the genotype for the tissue culture based research and crop improvement particularly in genetic transformation. Embryogenic callus, rather than direct tissues such as shoot spices, immature inflorescences, roots and leaves are used for genetic transformation and regeneration of rice plants because the callus culture, compared with organogenesis, is more suitable for gene delivery

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are used for genetic transformation and regeneration of rice plants because the callus culture, compared with organogenesis, is more suitable for gene delivery and regeneration of transgenic rice plants. Successful application of available genetic transformation methods in rice is possible when efficient and reproducible plant regeneration protocols are available for the particular cultivar (Jain, 1997). Therefore, the identification and screening of useful cultivars for callus growth and plant regeneration *in vitro* are prerequisites in genetic improvement programs (Abe and Futsuhara, 1986). Scutellum from the mature seed of rice is found to the best explants for callus induction and plant regeneration as its potential to proliferate actively and high frequency of plant regeneration (Khaleda et al. 2006, Abe and Futsuhara, 1986). However, callus induction as well as regeneration potential is affected not only by genotype and the type of explants but also by the composition of the culture medium including plant growth regulators and by the culture conditions. 60 -100 per cent of the cultured seeds formed callus at all the concentrations of 2, 4 -Dichorophenoxy acetic acid (2,4-D) used and among the different auxin analogues used to induce somatic embryogenesis 2, 4-D is the most efficient and therefore used in majority of embryogenic and tissue culture systems. (Pandey *et al*. 1994; shankhdhar *et al*. 2002; Tam and Lang, 2003; Naqvi *et al*. 2005. Jaseela *et al*. 2009). Tissue culture protocol for callus induction had been established for various indica rice cv shown in Table 5. Some of these indica rice varieties were found to be recalcitrant were briefly studied to investigate the mechanism underlying the recalcitrance of the rice varieties. It was reported that Polyamines (PAs), spermidine (Spd) and spermine(Spm) and their precursor putrescine (Put) are ubiquitous organic polycations and are involved in various plant growth and developmental processes including somatic embryogenesis. It reveals that recalcitrant nature of varieties were associated with high Putrescine: spermidine ratio (Rajam et al. 2001). Favorable modiûcation of cellular PA titers and their Put:Spd ratio by the addition of exogenous PAs (Put, Spd) or their biosynthesis inhibitor, diûuoromethylarginine (DFMA) led to the induction/promotion of plant regeneration in poorly responding genotypes. Recaltritrant indica rice genotype, Khalsa-7 (KH-7), when applied exogeneous Spermidine and diûuoromethylarginine caused induction of plant regeneration (Rajam et al. 2001). The indica rice genotypes used in present study were selected on the basis of their distinct argonomically important traits such as parentage, biotic and abiotic resistance characteristic features showed in Table 1.

The objective of the present study is to determine the callus induction potential of the above mentioned nine different genotypes of Indica rice on media containing different concentration of 2, 4-Dichlorophenoxy acetic acid. Genotypes having potential to induce good type of callus would be evaluated and could be used in future for various tissue culture based studies and crop improvement program using valuable genes of agricultural interest.

2. EXPERIMENTAL METHODS

Plant material

Mature seeds of nine different genotypes I W Ponni, BPT5204, TKM-9, CR1009, ADT-39, ADT-43, KKL(R) -1, CO(R)-48 and Savitri/Ponmani of *indica* rice were procured from Pondicherry Agro Service and Industries Corporation Limited (PASIC), Puducherry . All seed varieties used in this investigation showed >90% germination.

Callus induction

Mature healthy seeds were dehusked, rinsed with water and these were then surface sterilized with 1% of sodium hypochlorite solution containing few drop of Tween20. These were then gently inverted periodically for 15 minutes ensuring the contact of the solution with all seeds. 100-150 seeds were placed in 50 ml conical tubes and these were filled with 35ml of the solution. The solution was drained and in aseptic conditions these surface sterilized seeds were rinsed with sterile water three times. Seeds were blotted on sterile tissue paper in sterile conditions. These were then air dried for 20-25

jminutes in sterile conditions. The seeds were inoculated with their endosperm end anchored in the medium. The callus induction media was prepared by using MS basal medium (Table 2) with different concentration of 2, 4-Dichlorophenoxy acetic acid (2, 4-D) 2mg/l, 2.5mg/l, 3mg/l, 4mg/l and 5mg/l. The media was sterilized in autoclave at 15 lb pressure for 20 minutes. The sterilized media was distributed in 90 mm petriplates and 25-30 seeds were inoculated per plate under aseptic conditions. The seeds were inoculated on callus induction media and cultured in the dark at 27±1 %C for 4 weeks, with subculture being done every 15 days of interval and a regular evaluation of the plates was done for the contaminations. After a 4 weeks culture, explants were examined for callus induction ability (SCI: total number of explants with scutellum derived calli per total number of explants cultured ×100%), % of white or cream or yellow calluses that belong to callus type (WYC: number of white or cream or yellow calluses with compact or friable structure per number of explants cultured ×100%), and % of callus browning (CB: number of callus browning per number of explants cultured ×100%).The seeds were inoculated on callus induction media and cultured in the dark at 27±1 %C for 4 weeks, with subculture being done every 15 days of interval and a regular evaluation of the plates was done for the contaminations. After a 4 weeks culture, explants were examined for callus induction ability (SCI: total number of explants with scutellum derived calli per total number of explants cultured ×100%), % of white or cream or yellow calluses that belong to callus type (WYC: number of white or cream or yellow calluses with compact or friable structure per number of explants cultured ×100%), and % of callus browning (CB: number of callus browning per number of explants cultured ×100%).

Table 1. Characteristics of the genotypes used in this study (Shaikh et al., 2012).

Variety	Parentage	Characteristics
I W PONNI	Taichung 65/2 x Mayung Epos 80	Resistance to brown spot &blast.
C R 1009	Pankaj x Jaganath	Resistant to blast, RTV
ADT39	IR 8 x IR 20	Resistant to blast, bacterial blight, sheath rot.
SAVITRI/ PONMANI	Pankaj x Jaganath	Tolerant to all pest and diseases
ADT 43	IR 50 X I W Ponni	Resistance to stem borer and gall midge
TKM-9	TMK 7 x IR8	Resistant to BLB, drought tolerant
BPT5 204	Pure line	Moderately resistance to BLAST, RTV
CO(R) 48	CO43 X ASD19	Tolerant To Stem borer. Moderately resistant to blast.
KKL(R) 1	CR1009 x ADT 39	Moderately resistant to sheath rot

Table 2. Composition of Basal MS medium (Murashing and Skoog et al., 1962) used for this experiment

A. Macro Stock (10X) Soln.:

Components	Amount Per litre in MS (g/L)	Amount of per litre of stock (g/L)
NH_4NO_3	1.65	16.50
KNO_3	1.9	19.00
$CaCl_2 \cdot 2H_2O$	0.44	4.40
$MgSO_4 \cdot 7H_2O$	0.37	3.70
KH_2PO_4	0.17	1.70

B. Micro Stock (100X) Soln.:

Components	Amount Per litre in MS (g/L)	Amount of per litre of stock (g/L)
H_3BO_3	0.0062	0.62
$MnSO_4 \cdot 4H_2O$	0.0223	2.230
$ZnSO_4 \cdot H_2O$	0.0086	0.860
KI	0.000083	0.0083
$NaMO_4 \cdot 2\ H2O$	0.00025	0.025
$CuSO_4 \cdot 5H_2O$	0.000025	0.0025
$CoCl_2 \cdot 6H2O$	0.000025	0.0025

C. Iron Stock (10X) Soln.:

Components	Amount Per litre in MS (g/L)	Amount of per litre of stock (g/L)
$FeSO_4 \cdot 7H_2O$	0.0278	0.278
$Na_2EDTA \cdot 2H_2O$	0.0373	0.373

D. Vitamin Stock (100X) Soln:

Components	Amount Per litre in MS (g/L)	Amount of per litre of stock (g/L)
Nicotinic acid	0.0005	0.05
Pyridoxine•H Cl	0.0005	0.05
Thiamine•HCl	0.0001	0.01
Myo-inositol	0.01	1
Sucrose	-	30

3. RESULTS

Callus induction was observed for indica rice cv. I W Ponni, ADT-43, BPT-5204 and TKM-9 at concentration of 2mg/l, 2.5mg/l, 3mg/l, 4mg/l, 5 mg/l of 2, 4 -D supplemented with MS basal media (Table 3 and 4). Increase in the concentration of 2, 4-D to 5 mg/l showed no callus induction in indica rice cv. ADT 39, CO (R)48, CR1009, SAVITRI, KKL(R)1 (Table 3 and 4). 34 % of healthy calli obtained from I W Ponni at 2mg/l concentration of 2,4-D, 30 % calli at 2.5 mg/l of 2,4-D , 24 % calli at 3 mg/l , 8 % calli at 4 mg/l and 2 % calli at 5 mg/l of 2,4-D(Fig. 1). ADT-43 was observed to induce 10 % of healthy calli at 2mg/l of 2, 4-D, 8 % at 2.5 mg/l, 5 % at 3 mg/l but no callus induction at 4mg/l and 5 mg/l of 2, 4-D. 20 % of healthy callus induction obtained from BPT-5204 at 2mg/l 2, 4-D, 18 % at 2.5 mg/l, 10 % at 3mg/l , 6 % at 4 mg/l of 2,4-D but no calli were obtained at 5 mg/l 2 , 4-D. TKM-9 induces 10 % healthy calli at 2mg/l of 2,4-D, 8 % at 2.5 mg/l, 5 % at 3 mg/l, 3 % calli at 4mg/l but no calli were obtained at 5mg/l of 2,4-D (Table 4). Necrosis or Callus browning was observed at more higher concentration of 2, 4-D in all four indica cv. I W Ponni, ADT-43, BPT5204 and TKM-9. In I W Ponni callus browning increases from 10 % at 2mg/l of 2,4-D to 28 % at 5mg/l (Table 3 and 4). In ADT-43, callus browning at 2mg/l was observed about 15 % increase to 25 % at 5 mg/l of 2,4-D. BPT-5204 callus browning at 2mg/l of 2,4-D was observed about 30 % increases to 47 % at 5mg/l. In TKM-9 callus browning increases from 19 % to 35 % at 2mg/l to 5mg/l of 2, -D respectively (Table 3 and 4).

4. DISCUSSION

In the present studies indica rice varieties Improved white ponni, ADT 43, BPT5204, TKM-9, KKL (R)1, TKM-9, Savitri, CO(R) 48, ADT-39 and CR1009 were evaluated on MS medium supplemented with 2 mg/l, 2.5mg/l, 3 mg/l, 4 mg/l and 5 mg/l of 2,4-D(Table 4). The indica rice varieties have problems associated with regeneration due to recalcitrant nature of most of the varieties and affects its tissue culture (Lin and

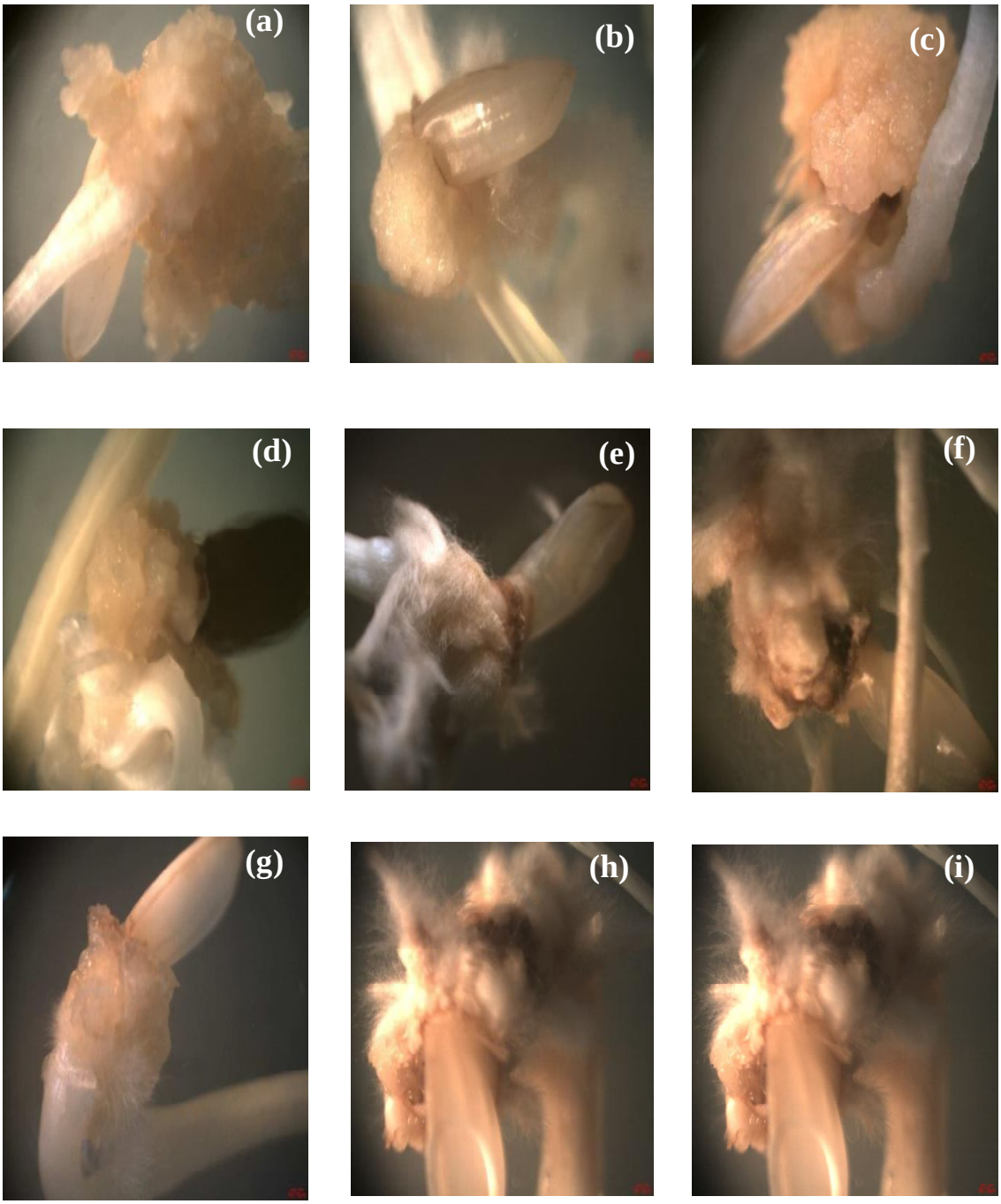

Fig. 1: Callus induction from the scutellum of different varieties of rice on the MS supplemented with 2mg/L of 2, 4-D. A) I. W. Ponni B) ADT-43 C) BPT-5204 D) TKM-9 E) CR1009 F) CO(R) 48 G) KKL (R) 1 H) ADT-39 I) Savitri/Ponmani

Table 3: Comparison of callus induction potential of different genotypes of *Indica* rice

Genotypes	% of SCI	% of WYC
I W PONNI	34	30
C R 1009	0	0
ADT39	0	0
SAVITRI/ PONMANI	0	0
ADT 43	10	8
TKM-9	10	5
BPT 5204	20	15
CO(R) 48	0	0
KKL(R) 1	0	0

SCI = Scutellum Callus Induction, **WYC**- white yellow callus, **CB** -Callus Browning

Zhang et al. 2005). The previous reports in literature showed that variousindica rice varieties had been used for tissue culture (Table 5). However, no work is reported for the indica rice cv. Improved white ponni, KKL (R) 1, TKM-9, Savitri, CO(R) 48, ADT-39 and CR1009. The callus induction was done using MS medium supplemented with 2, 4-D. Different concentration of 2,4-D have been reported to induce callusing in different indica rice varieties (Table. 4). In our studies, we have obtained 8 % of healthy calli at 2.5 mg/l of 2,4-D from rice cv ADT-43 and 20 % of healthy calli at 2mg/l from BPT5204(Table 4). Similar reports were generated by Kartekeyan et al., 2012 and Kumari et al. 2006 on investigation of callus induction from ADT -43 rice genotype at 2.5mg/l 2,4 -D concentration (Table 5). In the present study , it was observed that scutellum derived embryogenic callus was induced in only four indica rice genotypes I W Ponni, ADT 43 BPT5204 and TKM-9 among the nine rice genotypes used (Fig. 1). These results could be supported by Abe and Futsuhara et al. 1986 and Khanna and Rainna et al. 1998 where they reported that the interaction between genotype and medium have significant effects on callus induction. Revathi et al. 2011 also confirmed that callus induction in rice was found highly variable and genotype specific. Carsono and Yoshida et al. 2006 reported that there was genotype × medium × explant interaction for inducing white/ cream/yellow callus with an organized structure (callus type I and II) and for callus browning. In the present study, increasing the 2,4-D concentration above 2mg/l causes callus browning and necrosis of callus, indicating that callus browning is directly proportional to 2.4-D concentration in the medium(Table 4). I W Ponni induces callus highest (34%) among the nine genotypes, but increasing concentration of 2, 4-D in medium, it experienced steep decline in callus induction(Table 4). Contrast to this, callus browning increased with increase in 2, 4-D in same rice genotypes. This illustrates that optimum concentration of 2, 4-D is 2mg/l for efficient scutellum derived callus induction for these rice genotypes. Similar reports were generated by Pandey et al. 1994 who worked with matured dehusked rice seeds using different level of 2, 4-D in nutrient medium concluded that callus induction from seed was best at 2 mg/L. Shanthi et al. 2010 also reported that increasing the 2, 4,-D concentration beyond 15mM per litre, the callus induction frequency also reduced and beyond 25mM per litre there was no callus induction. Using the higher concentration of 2, 4-D more than 10mM per litre was not desirable, because it produced only the necrotic callus. Present study showed that callus browning was high in genotypes ADT-43(19%) and BPT-5204 (30%) as compared to I W Ponni (10%) (Table. 3). According to Prasanna et al. 2010, the formation of callus browning at the stage of callus induction is one of the main reasons for the failure of regeneration in indica rice variety, which is characterized by browning coloration of the callus followed by death, thus preventing further callus proliferation. This confirmed that the frequency of callus induction and callus browning is particularly depends on the interaction between the genotype and the medium used. Karthikeyan et al. 2012 established a reducible and highly efficient protocol for *Agrobacterium* mediated transformation for rice genotype ADT-43 using scutellum derived callus as the tissue material for agro infection that was induced on Linsmaier and Skoog (LS) medium

Table 4: Callus induction from different genotypes of rice on variable 2. 4-D concentraion

Genotype	% of CI and CB	Concentration of 2,4-D				
		2mg/l	2.5mg/l	3mg/l	4mg/l	5mg/l
IW PONNI	CI	34	30	24	8	2
	CB	10	15	18	25	28
CR 1009	CI	0	0	0	0	0
	CB	0	0	0	0	0
ADT39	CI	0	0	0	0	0
	CB	0	0	0	0	0
SAVITRI/ PONMANI	CI	0	0	0	0	0
	CB	0	0	0	0	0
ADT 43	CI	10	8	5	0	0
	CB	15	15	22	25	25
TKM-9	CI	10	8	5	3	0
	CB	19	23	28	34	35
BPT 5204	CI	20	18	10	6	0
	CB	30	34	39	45	47
CO(R)48	CI	0	0	0	0	0
	CB	0	0	0	0	0
KKL(R)1	CI	0	0	0	0	0
	CB	0	0	0	0	0

CI = Callus Induction, **CB** -Callus Browning

supplemented with 2.5mg/l 2, 4-D and 1mg/l thiamine HCL. Kumari et al. 2006 reported BPT-5204 scutellum derived callus induction on MS medium supplemented with 2, 4-D (1mg/L) and benzylaminopurine (BAP) 0.25mg/L. Another report generated by Sridevi et al. 2005 induced scutellum derived callus from rice variety White Ponni using MS mediated supplemented 2,4-D (2mg/l) with combination of 0.5 mg/l BAP, 200mg/l casien hydrolysate, 500mg/l proline. All these reports suggested the optimal concentration of 2, 4-D is required for callus induction in various varieties of *indica* rice and if provided in excess than optimal range would cause necrosis and dead of callus. Furthermore, research about the molecular mechanism underlying the recalcitrant varieties ADT39, SAVITRI, CO(R) 48, CR1009 and KKL(R) 1 is being investigated.

5. CONCLUSION

It could be concluded from the present study that the four genotype of indica rice I W Ponni, ADT-43, BPT-5204 and TMK-9 are potent for callus induction from the scutellum of mature seed among the nine genotypes used in these experiment. These genotypes would be used for further examination of their plant regeneration ability and transformation studies. We believed that these genotypes with good

Table 5. Indica rice genotypes used commercially in tissue culture

Variety	Medium	References
MR219	MS with 4 mg/l 2,4-D	Panjaitan B. et al. 2008
Pusa Basmati1(PB1), Basmati 370	MS with 2.5 mg/l 2,4-D and 0.5 mg/l kinetin	Grewal et al. 2005
Govind, PB1, Jaya	MS with 0.4µm 2,4-D , 0.4 µM kinetin	Verma et al. 2011
PB1, Pusa Sugandhi-4, Pusa Sugandhi -5, White ponni	MS with 2mg /l 2 ,4-D	Aananthi et al. 2010
Rasi, Seshu, Vibhava, Nagarjuna, Jaya	MS with 2mg/l 2,4-D	Visarda et al. 2002
IR 64, CSR10, PB1, Swarna	MS with 3 mg /l 2,4-D and 0.25 mg/l BAP	Sahoo et al. 2011
NDE-624, IR 20, IR36, IR 64, IR72, KHALSA -7, HASAN SARAI (HS), BHOGJEERA -1 , DV -85, IR54, PB1, TN1	MS with 2 mg/l 2 4 -D	Rajam et al. 2000; 2001
ADT 43	Linsmaier and Skoog medium with 2.5 mg/l 2,4 -D	Karthikeyan et al. 2012
White Ponni	MS with 2mg/l 2 4-D and 0.5 BAP	Sridevi G. et al. 2005
BPT 5204	MS with 1 mg/l 2 4-D and 0.25 mg/l BAP	Kumari A et al. 2006

callus induction-related traits would be useful for plant regeneration.

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