IJISET - International Journal of Innovative Science, Engineering & Technology, Vol. 3 Issue 6, June 2016
ISSN (Online) 2348 – 7968 | Impact Factor (2015) - 4.332
www.ijiset.com

Tissue culture of Rice: Problems, Progress and Prospects

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Abstract

Due to growing population, there is an increasing demand of rice production. The rice productivity is affected by various environmental stresses. Further the nutritional improvement of rice can help in decreasing the evil of malnutrition. The use of biotechnological tools is the most workable option to develop such rice varieties. However, the lack of a simple and efficient protocol for embryogenic callus induction and quick plant regeneration in this cereal crop is a major constraint and there is substantial genotype-dependence. Several studies on different rice varieties have been carried out by various workers so as to optimize the conditions to achieve good results. Likely a significant progress has been made in the past few decades. The objective of this article is to review the currently used technique in a full detail. This work gives an in depth idea about the critical requirements in the tissue culture of rice varieties which positively contribute to the biotechnological breeding programs of rice.

Keywords: *Oryza sativa;* embryogenic callus; regeneration; stem nodes; genotype; tissue culture.

Introduction

Rice is a monocot plant belonging to the genus *Oryza* in family Poaceae, cultivated for more than 10,000 years (Sasaki 2001). The genus consists of 26 species (Khush 1997) out of which 24 are wild and only two (*Oryza sativa* L. and *O. glaberrima* Steud) are cultivated. The cultivated rice (*Oryza sativa*) is divided into three subspecies - *indica, javanica* and *japonica* (Datta et al., 2003). In Asia, two main subspecies - *indica* and *japonica* are grown. *Indica* rice comprises 80% of cultivated rice in the world (Ramesh et al., 2009; Tie et al., 2012) and is grown mainly in South and South-East Asian countries (Zhang et al., 2005).

Rice, the staple food of greater than half of the world's population (Hadiarto and Tran 2011) serves more than 90% of the Asian population (Khush and Brar 2001; Ziegler and Barclay 2008) and is the second most widely cultivated cereal in the world, after wheat (Pazuki and Sohani 2013). In Asian countries, Rice provides nearly half of total dietary carbohydrate and supplying 50-80% of the daily calorie intake (Khush 2005). There is an everyday increasing demand of rice production as the rice consumers are increasing at the rate of 1.6-1.8% every year (Karthikeyan et al., 2009; Shobarani et al., 2010). According to Savary et al., (2000), 24–41% of the rice yield was lost annually because of pests, diseases and weeds. In the developing world, the nutritional improvement of rice can also help in decreasing the evil of malnutrition (Bajaj and Mohanty 2005). Hence, the use of biotechnological tools is the novel, powerful and effective way to combat such problems.

Even with a sound methodology on rice tissue culture, *in vitro* regeneration of *indica* rice is still an interesting task and is genotype-dependent (Kumria et al., 2000; Lin and Zhang 2005, Zhang et al., 2005; Ge et al., 2006; Pazuki and Sohani 2013). The genotype and composition of the nutrient media are the two major factors in the tissue culture of rice (Mikami and Kinoshita 1988; Khanna and Raina 1998; Ogawa et al., 1999; Gul et al., 2000; Al-Forkan et al., 2005). In rice, the scutellum is the best explant for callus induction and plant regeneration (Rashid et al., 2001, 2004; Cho et al., 2004; Khaleda and Al-Forkan 2006). Due to a low rate of embryogenic callus induction and plantlet regeneration, the transformation of *indica* rice is still difficult (Lee et al., 2002; Kumar et al., 2005; Nishimura et al., 2006). For carrying out successful genetic transformation in rice, identification and screening of useful cultivars and establishment of efficient regeneration protocols are very essential (Hoque and Mansfield 2004; Dabul 2009; Joyia and Khan 2012). This review will summarize various treatments used to carry out a successful tissue culture of diverse rice varieties.

Tissue culture of rice

There has been a successful tissue culture of rice plants which first initiated from culturing of rice roots (Kawata and Inshihara 1968), seed (Nishi et al., 1968) and embryos (Tamura 1968) [For details see Table 1]. Using exogenous 2, 4-D treatment and yeast extract, callus induction of stem nodes as explants was carried out by Furushashi and Yatazawa (1964). This was further extended by Yamada et al., (1967) using IAA and 2, 4-D with cytokinins. The most effective growth regulator of callus induction in rice is 2, 4-D without cytokinin treatment (Gamborg et al., 1976). On MS media, all parts

formed callus but scutellum and plumule derived callus regenerated rice plants, which were lost by sub culturing it (Kucherenko and Vlasov 1988).

Zafar et al., (1992) reported that using mature embryo and immature inflorescence as explants in Basmati 370 on MS medium with 2, 4-D as growth regulator showed efficient cell lines. Carbohydrate, explant used, plant growth regulators, basal salts of culturing medium, culture condition and most importantly genotype of explants plays an important role in successful tissue culture (Rueb et al., 1994). Zafar et al., (1994) using mature embryo, immature embryo and immature inflorescence in 8 *indica* rice varieties on MS and N6 media with 2, 4-D showed highest regeneration frequency in DM-25, B-70 and B. Kashmir. Japonica rice served as a model plant for standardizing growth regulator concentration and combination which highly vary with the various cultivars of rice (Xie et al., 1995). Kunanuvatchaidach et al., (1995) reported that mature caryopses of Asian *indica* rice when cultured on MS medium with proper nutrients and IAA, NAA, kinetin (kin) and BAP, ethylene combination of growth regulators yielded high frequency of callus induction. They gave mild osmotic stress in R-2 liquid in mature caryopsis of 6 south-east Asian *indica* rice varieties in MS medium with 2, 4-D and showed enhancement in regeneration frequency.

Al-Khayri et al., (1996) reported that 4% sucrose is essential for callus induction and using 0.5 mg/l 2, 4-D, sucrose and sorbitol combination is best for enhancing regeneration. Valdez et a1., (1996) reported that MS medium is best for callogenesis and genotype of rice plant is also responsible for achieving various frequencies of plant regeneration. Marassi et al., (1996) reported that Basmati-370 seeds show highest

frequency of callus induction in MS using 0.5 mg 2, 4-D, 0.1 mg 2iP and regeneration in MS with 1.5 mg BA. Using Aikoku, Senichi and Moritawase, Zhang and Hattori (1996) experimentally proved that a single dominant gene controls the differences between the high and low regeneration ability. Valdez et al., (1997) demonstrated on MS and N6 media using mature embryos of 5 cultivars and 2 lines that CR-1113 and CR-5273 show highest regeneration frequency. Lutts et al., (1999) experimented on 2 *japonica* (I Ilong Poa and Aiwu) and 2 *indica* (IR 2153 and Nano Bolera) varieties of rice using their 3 month old mature embryo giving different concentration of ABA, PEG, IAA, proline, tryptophan treatments and also various doses of NaCl. They reported that ABA and PEG showed no or decrease in regeneration frequency, proline has no effect on regeneration frequency, IAA showed decrease in shoot and increase in root regeneration. NaCl showed decrease in regeneration frequency while as tryptophan showed increase in regeneration frequency.

Abbasi et al., (2000) reported that seeds of Basmati-370, Basmati-385 and KS-282 when grown on MS medium for callusing and for regeneration on LS medium with 2, 4-D, NAA, BAP showed highest regeneration frequency in KS-282 (31.25%) than in Basmati 285 (17.60%) and least in Basmati 370 (6.50%). Rashid et al., (2001) reported that seeds of Super Basmati when cultured on MS and N6 media show good results for callus induction with 2, 4-D. Treatment with different concentration of NAA and BAP enhanced regeneration frequency. Deepti et al., (2001) reported culturing of mature embryos of rice cv. Pusa Basmati l on MS medium. Low concentration of 2, 4-D was proved to be best for callus induction and 0.5 mg/l BA was best for high regeneration

frequency. Sahrawat and Chand (2001) reported that 2.85 µM IAA, 17.77 µM BAP and 3% sucrose proved best for regeneration in coleoptile segments of Indica rice cv. Kasturi. Sahrawat et al., (2001) demonstrated on coleoptiles grown on MS medium that treatment with 2, 4-D, kin, sucrose, tryptophan, IAA and BA, showed enhancement in regeneration frequency to 73.5%. Lee et al., (2002) reported that kin was most important in callus induction and optimum concentration of kin enhanced regeneration frequency by 67-77%. Visarada et al., (2002) in his study proved that among four different media, NBKNB medium is suitable for in vitro culture of recalcitrant *indica* genotypes. Rashid et al., (2003) reported that among 3 varieties of rice (Rachna Basmati, Basmati 2000 and 370), Rachna Basmati showed more response in N6 medium with different hormonal combinations and concentrations. Cho et al., (2004) explained through culturing of scutella of Korean rice that N6 medium was efficient for callus induction and MS for shoot regeneration.

Using the anthers of a commercial hybrid rice line Pakhal 9690, Islam et al., (2004) reported that using N6 as basal medium with 1.0 mg/1 2, 4-D and 2.0 mg/1 α-NAA was found most effective for callus induction (35.5 %). The basal medium with 2.0 mg/l α-NAA and 1.0 mg/1 kin combination showed highest regeneration frequency. Manonmani and Khan (2004) while anther culturing of rice variety proved that the genotype and growth regulators influenced the frequency of callus induction. The use of 2.0 mg/l BAP and GA_3 showed highest regeneration frequency. Pongtongkam et al., (2004) reported that MS medium with 2 mg/l 2, 4-D, l g/l L-proline, 1 g/1 casein hydrolysate and 20 mM L-lysine produced the highest average percentage of callus

formation and basal medium with l mg/l kin, l g/l L-proline, 300 mg/l casein hydrolysate and 20 mM L-lysine showed best regeneration. Bano et al., (2005) proved that MS medium containing 0.5 mg/l BAP and 0.2 mg/l IAA proved best for plant regeneration when seeds of Swat-II were used as explant on MS medium. Grewal et al., (2005) conducted an experiment to demonstrate the promotive effect of maltose, sucrose, proline, cefotaxime and activated charcoal on different rice varieties. It was reported that these nutrients with hormones enhances somatic embryogenesis.

Ge et al., (2006) reported that the callus induction, proliferation and regeneration can be best achieved and enhanced by manipulating growth regulators and nutrients in media. Carsono and Yoshida (2006) demonstrated through genotype x medium x explant interaction that the seeds as explants on MS medium reported high regeneration frequency. Agrawal et al., (2006) using different rice varieties viz., Jaya, Pusa Basmati l, Basmati 370, PR 106 and PR 110 found highest callus induction in MS medium with 2.0 mg/l 2, 4-D and 0.5 mg/l kin. The highest regeneration was found in Jaya variety with BAP (1.0 mg/l), kin (1.0 mg/l), and NAA (0.1 mg/l) as set of hormones. Saqlan Naqvi et al., (2006) reported that Pakhal, Swat-1, JP-5, KS-282 and IR-6 varieties of rice when grown on MS medium with treatment of 2, 4-D, BAP and IAA, the overall regeneration frequency was found to be 82%. KS-282 and Pakhal had high regeneration frequency and low in JP-5. Hoque et al., (2007) reported that seeds of BR 14, BRRF dhan 28, BRRF dhan 29, BRRF dhan 38, BRRF dhan 39 and BRRF dhan 40 when grown on MS medium with 2, 4-D, sucrose and agar, the regeneration frequency lies in between 25.4-54.5%. Ullah et al., (2007) reported that seeds of Basmati-370 and Basmati-385 when cultured

on MS and N6 media and treatment of 2, 4-D and BAP, in case of Basmati-385 highest regeneration frequency was found.

Afolabi et al., (2008) reported that caryopses of Suakoko 8 and NERICA cultivar (FARO-SS) when grown on modified NBm medium with treatment of cytokinin : auxin (1:10), ABA, NAA and BAP, the regeneration frequency is 53 and 42% respectively. Tariq et al., (2008) reported that mature seed scutellum of Super Basmati, Basmati-370, Basmati 371, Fakhre Malakand when cultured on MS and N6 media with different concentration of 2, 4-D, NAA and BAP showed regeneration frequency 61% and 69% for Bas-370 and Bas-371 respectively. Karthikayan et al., (2009) reported that mature seeds of ADT-43 when cultured on LS medium with 2, 4-D, BAP and NAA showed regeneration frequency of 86-74%. Shanthi et al., (2010) reported that embryo of TRY 1, TRY 2, Pokkali, CSR10, W. Ponni, BPT 5204 and IR 29 when cultured on MS medium with 2, 4-D, kin and BAP showed regeneration frequency of 57.25%. Li-na et al., (2010) reported that mature seeds of 9 *japonica*, 9 *indica* and 11 hybrid rice varieties when cultured on M8/MS media along with 2, 4-D, kin and BA showed regeneration frequency of 9.2-59.5%, 3.6-87.5% and 17.2-43.2% respectively. Shahsavarasi et al., (2010) reported that mature seeds of Kusan, Lamsan, Selasi and Siam varieties of rice when grown on MS medium along with 2, 4-D, NAA, kin, BAP showed high regeneration frequency for Lasman and poor for Siam and Kusan. Shahsavari (2011a) using mature seeds of Kusan, Lamsan, Selasi and Siam grown on MSB5 medium with tryptophan and glutamine as special amino acids concluded positive effect of tryptophan on tissue culture of rice. Zuraida et al., (2011) reported that zygotic embryos of MR 219 and MR 232

varieties of rice when grown with maltose, glutamine, asparagine and arginine showed highest regeneration frequency of 87-91%.

Kaswan et al., (2012) reported that when immature/mature seeds of Pusa Basmati 1, HRR 46 and IR 72 varieties of rice were grown in MS basal medium along with NAA showed regeneration frequency of 91.3%, 76.3% and 72.3% respectively. Libin et al., (2012) reported that the seeds of Sarawak Biris variety of rice when grown on medium with 2, 4–D, kin and NAA and showed regeneration frequency of 97%. Ahmed et al., (2013) reported that scutellum of MR 123 and MR 127 varieties of rice when cultured on MS medium with 2.5 mg/l 2, 4-D showed induction frequency of 70 and 76% respectively. Joyia et al., (2013) reported that scutellum of Super Basmati, Basmati 385, Basmati 198, Pak Basmati, Basmati 2000 and Basmati 370 varieties of rice when cultured on modified MS medium with 3 mg/l kin,1 mg/l NAA and 1-5 mg/l 2, 4-D showed enhancement in regeneration frequency.

Ghubeishavi et al., (2014) reported that coleoptiles of Neda and Nemat varieties of rice when given treatment with 2 mg/l kin, 0.5 mg/l NAA and 2 mg/l BAP, 0.5 mg/l kin showed regeneration frequency of 32.30% and 20.95% respectively. Bhuiyan et al., (2014) reported that BRRI dhan32, BRRI52 and FR13A when cultured on MS medium with kin, BA and NAA showed 94% and 87% regeneration frequency for FR13A and BRRI respectively. Poraha et al., (2015) raised the embryos of Jow Haw variety of rice on MS and N6 media with 2,4-D, sucrose and proline. Vennapusa et al., (2015) reported that seeds of AC 39020 genotype when grown on LS/MS/N6 media with BAP, kin, NAA and TDZ, highest regeneration frequency with NAA and BAP was reported.

Conclusions

The different explants used for the rice tissue culture include coleoptile, roots, seed derived scutellum, immature and mature embryos, leaves, stem nodes, inflorescence and anthers. Among these different explants used, seed derived scutellum serve as the best explant for the tissue culture of rice genotypes. From this study we conclude that carbohydrate, explant used, plant growth regulators, basal salts of culturing medium, culture condition and most importantly genotype of explants plays important role in successful tissue culture. MS followed by LS media with some modifications proved best for the embryogenic callus induction in diverse rice genotypes and MS and N6 media proved best for the efficient regeneration. Different carbohydrates (sucrose, glucose, maltose, sorbitol and mannitol) revealed genotype specific results in the tissue culture of rice. Likewise, different gelling agents (agar, agarose, gelrite and phytagel) revealed genotype specific results. Using different concentrations and combination of 2, 4-D, IAA, NAA, kinetin, BAP, ethylene, high frequency of callus induction was observed. The use of tryptophan, proline, casein hydrolysate, lysine, glutamine, asparginine, arginine etc. improved the frequency of callus induction. Similarly, different concentrations and combination of 2, 4-D, IAA, NAA, kinetin, BAP, 2 iP, GA_3, TDZ proved best for the efficient regeneration of rice genotypes.

Acknowledgements

The first author is thankful to Council of Scientific and Industrial Research (CSIR) for providing financial assistance.

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Table 1: Trends in rice (*Oryza sativa* L.) tissue culture.

Variety	Explant	Hormones/ nutrients	Media used	Special treatment (if any)	Results	Reference
-	Stem nodes	2, 4-D	-	-	High frequency of callus induction	Furushashi and Yatazawa (1964)
-	Roots and 3 shoot nodes	High conc. of auxin and cytokinin	-	-	-	Yamada et al., (1967)
Basmati	Mature embryo, immature inflorescence	2, 4- D	MS medium	-	Efficient cell lines of Basmati 370 was established	Zafar et al., (1992)
-	Mature seeds	NAA, kinetin	-	High conc. of agar and gellan gum	-	Tsukahara and Hirosawa (1992)
8 *indica* rice cultivars including DM-25, B-70 and B. Kashmir	Mature embryo, immature embryo, immature inflorescence	Auxin (2, 4-D)	MS and N6 media	-	DM-25, B-70 and B. Kashmir showed high RF*	Zafar et al., (1994)

6 south-east Asian *indica* rice varieties	Mature caryopsis	MS vitamins, 2, 4-D, sucrose, casein hydrolysate and agar	MS medium	Mild osmotic stress in r-2 liquid	Enhanced regeneration	Kunanuvatchaidach et al., (1995)
-	Mature embryo	High conc. of 2, 4-D	LS medium	Antioxidant efficiency was also checked	More cellular growth in absence of 2, 4-D	Dey and Kar (1995)
Alan, Katy and lagrue	Seeds	Diff conc. of kinetin or without kinetin, 2, 4-D	MS medium	-	50 % RF	Al-Khayri and Anderson (1995)
Lagrue, Katy and Alan	-	Sorbitol and sucrose	-	-	Regenerated plants were healthy and fertile	Al-Khayri et al., (1996)
Basmati 370	Seeds	0.5mg 2, 4-D/l, 0.1mg 2iP/l, 1.5mg BA/l	MS medium	-	Callusing was good, plant regeneration	Marassi et al., (1996)
Aikoku, Sen-ichi, Moritaware	Seeds	3 mg 2, 4-D, 5g yeast extract, 30 g sucrose, 11g agar/l	½ conc. MS medium	Callus transferred to ¼ conc. MS medium	Aikoku and sen-ichi 85% regeneration, Moritaware only 2%	Zhang and Hattori (1996)
CR-1113 and CR-5272	Mature embryos	-	MS medium	-	10 to 47% callus frequency, highest regenerated plants	Valdez et al., (1997)
I kong pao, Aiwu, IR 2153 and Nona bokra	Mature embryos	abscisic acid, polyethylene glycol, proline, tryptophan and indoleacetic acid	-	NaCl	NaCl strongly decreased the regeneration frequency but slightly increased the survival of regenerated plantlets	Lutts et al., (1999)
Basmati-370, Basmati-385, KS-282	Seed	2, 4-D, NAA, BAP	MS medium for calluing, LS medium for regeneration	-	RF highest in KS-282 (31.25%), Basmati 285 (17.60%) Basmati 370 (6.50%)	Abbasi et al., (2000)
Pakhal and Basmati	-	-	Modified MS medium i. e. MSK2 and MSK5	-	70% and 58% RF resp.	Gul et al., (2000)
Nipponbare, Koshihikari, Akitakomachi	Embryo	2, 4-D, gelrite	MS and KSP(revised N6) media	Low temp stress	RF high than normal	Tsugawa and Suzuki (2000)
Super basmati	Mature seeds	-	MS and N6 media	-	NAA and BAP enhanced RF	Rashid et al., (2001)

Pusa basmati	Mature embryos	IAA, 6BA, Kin, 30g sucrose/l, 8g agar/l	MS medium	Dark at 26±1˚c	Regeneration best at 0.5mg BA/l	Deepti et al., (2001)
Kasturi	Coleoptile	2, 4-D, kin, 3% sucrose	MS medium	-	Regeneration best using IAA and BAP	Sahwarat and chand (2001)
Japonica	Mature seeds	2, 4-D, sucrose, agar	N6 medium	-	67-77% RF	Lee et al., (2002)
4 genotypes (Lx297, IR64, V1119, IR64-1-1-4)	Scutella	-	N6, MS, and R media	-	Lx297 showed RF=82.50% on MS medium and R media showed 13.89% RF	Khatun et al., (2003)
Quing Livan 1 and IET 13856	Root	2 mg/l BAP and 0.5 mg/l NAA	MS medium	-	Quing Livan 1 showed more callus induction and plantlet regeneration	Mandal, et al., (2003)
Rachna basmati, Basmati 2000, Basmati 370.	Seeds	2, 4-D	N6 medium	-	More callus growth in Rachna basmati	Rashid et al., (2003)
HKR-46 and HKR-126	Seed	2.5 mg/l 2, 4-D, 500 mg, proline, 500 mg casein hydrolysate, 30g sucrose	MS basal medium	Partial desiccation	High frequency plant regeneration	Saharan et al., (2004)
BR22, BRR1 Dhan 29, br 5842-15-4-8 and Moulata.	Root of various ages	2, 4-D	MS medium	-	Younger explants have more RF	Hoque et al., (2004)
Korean rice	Scutella	-	N6 medium	-	Higher regeneration rate and lower rate of browning	Cho et al., (2004)
Jaumala	Caryopses	Auxin (2, 4-D) BA, TDZ	Modified MS medium	-	50% RF in TDZ	Gairi and Rashid (2004)
Xiushii II and XC 95	Mature seeds	2, 4-D, sucrose, agar/agarose	Basal medium N6	Phytagel	RF= 67.5% and 61.2% for Xiushii II and XC 95 resp.	Ali et al., (2004)
Pakhal 9690	Anthers	2, 4-D, α-NAA, IAA, kinetin	N6 medium	Regeneration on agarified MS medium	70% RF	Islam et al., (2004)
7 hybrids	Anthers	BAP, GA3	-	-	Only 3 hybrids show green plant regeneration	Manonmani and Khan (2004)
Kdml 105	Seeds	2, 4-D, proline, casein hydrolysate, lysine	5 diifferent MS medium	Dna fingerprinting	L-lysine cause genetic variation	Pongtongkan et al., (2004)
Jumlimarshi, Tilki, Jethobudo, Manshara,	Mature seeds	-	N6 and MS media	$AgNO_3$	N6 medium supplemented with 2, 4-D, 2.5 mg/l and $AgNO_3$,	Niroula et al., (2005)

Masuli and Pahenle					10 mg/l exhibited better callus induction	
Brri dhan 40 Brri dhan 41 Binnatoa	Embryo	2, 4-D	LS2.5, MS2.5, SRM 1 media	-	74% , 79% and 69% RF resp.	Al-Forkan et al., (2005)
Swat-II	Seeds	Various conc. of auxin and cytokinin	MS medium	Addition of tryptophan	Best regeneration with IAA and BAP	Bano et al., (2005)
Pusa Basmati 1, Basmati 370 and 386	Scutellum	Auxins, sugars, amino acids and growth regulators	-	Supplemented independently with sucrose, activated charcoal, cefotaxime	Highest RF was achieved	Grewal et al., (2005)
Indian and *japonica* rice	Mature seeds, root segments	-	MS and CI media	-	MS medium more suitable for callus induction	Carsono and Yoshida (2006)
Jaya, Pusa basmati1, Basmati 370, PR 106, PR110.		BAP, NAA and kin	MS medium	Dark conditions	RF 57.14%	Agrawal et al., (2006)
MDU-5	Mature seeds	2, 4-D, kin, IAA, BAP	MS media	-	callus induction 98.5%	Zaidi et al., (2006)
Pakhal, Swat-1, JP-5, KS-282, IR-6.	Mature seeds	2, 4-D, BAP, IAA	N6 medium	Mannitol	Mannitol did not supported growth	Saqlan Naqvi et al., (2006)
Six elite Bangladeshi *indica* rice	Seeds	2, 4-D, sucrose, agar	MS medium	-	RF 18.8-45.7%	Hoque et al., (2007)
Basmati-370, Basmati-385.	Seeds	2, 4-D, BAP	MS and N6 media	-	High RF in case of Basmati-385	Ullah et al., (2007)
Suakoko 8 and NERICA cultivar(FARO-SS)	Caryopses	Cytokinin:auxin (1:10) ABA, NAA, BAP	Modified NBm medium	-	RF 53 and 42% resp.	Afolabi et al., (2008)
KDML105	-	Diff conc. of 2, 4-D, 3% sucrose	(MS,Gamborg-B5 (B5), Linsmair and Skoog (LS) and Chu medium (N6))	Dark and light	High RF in MS medium containing 3% sucrose at 25 °C under dark	Summart et al., (2008)
Super basmati, Basmati-370, Basmati 371, Fakhre Malakand	Mature seed scutellum	2, 4-D at diff conc., NAA, BAP	MS and N6 media	-	RF 61% and 69% for Bas-370 and Bas-371 resp.	Tariq et al., (2008)
Basmati -370	Embryo	2,4-D, MET	MS medium	-	85% rooting was observed	Ferdous et al., (2008)

Kajrat-3	Seeds	2, 4-D, kinetin, NAA, proline, Casein hydrolysate	MS medium	-	useful for genetic transformation	Kumar et al., (2008)
HD 297, HD 502, HD 65, ZZ 93.	Mature/immature embryos	Myoinositol 0.1 mg/l, 6-BA 1 mg/l, Kin 2 mg/l and NAA 0.1 mg/l	Modified MS	Addition of increased conc. of sorbitol	RF increases from 27.6 % to 71.8%	Geng et al., (2008)
Brri-28, 29, 30, and 32.	Mature seeds	BAP, IBA	MS medium	-	RF 85.33% in case of BRRI-28	Kabir et al., (2008)
Rasi	Leaf base	2, 4-D, BAP, NAA	MS/LS medium	-	regeneration was effective	Ramesh et al., (2009)
ADT-43	Mature seeds	2, 4-D, BAP, NAA.	MS medium	Thiamine-HCL	RF 74-86%	Karthikayan et al., (2009)
Nepalese	Anthers	2, 4-D, NAA.	Modified N6	Cold treatment	CIM2 was better	Niroula and Bimp (2009)
MR219	Scutellum	2, 4-D, BAP, NAA	Modified MS	-	RF 82.5%	Panjaitan et al., (2009)
9 japonica, 9 *indica* and 11 hybrid rice varieties	Mature seeds	2, 4-D, Kin BA	M8/MS media	-	RF 9.2-59.5%, 3.6-87.5%, 17.2-43.2% resp.	Li-na et al., (2010)
TRY 1, TRY 2, Pokkali, CSR10, W.Ponni, BPT 5204 and IR 29.	Embryo	2, 4-D, kinetin, BAP	MS medium	-	RF 57.25%	Shanthi et al., (2010)
MR232	Mature seeds	2, 4-D, NAA, gelrite	MS medium	ABA	RF 91-97%	Zuraida et al., (2010)
Kusan, Lamsan, Selasi, Siam	Mature seeds	2, 4-D, NAA, kin, BAP	MS medium	-	RF high for Lasman and poor for Siam and Kusan	Shahsavarasi et al., (2010)
Gny-53, Basmati-370, JP-5,	Seeds	2, 4-D, NAA, BAP	MS and N6 media	-	RF - GNY-53= 70.27%, JP5=41.81%, Basmati 370=43.33%.	Hussain et al., (2010)
Several varieties	Anthers	BAP, IAA, NAA.	MS medium	-	RF 0-54.17%	Li et al., (2011)
PAU201	Mature seeds	Sucrose, BAP, Kin, NAA	MS medium	-	RF 42.5%	Wani et al., (2011a)
PR116	Mature seeds	2, 4-D, BAP, NAA	MS medium	-	RF 44.4%	Wani et al., (2011b)
ADS 16, ADS43, Basmati 370, Pusa basmati, Pokkali	Mature seeds	2, 4-D	MS medium	-	RF 58.33- 96.67%	Revathi et al., (2011)
Kusan, Lamsan, Selasi and Siam.	Mature seeds	2, 4-D, NAA, Kin, BAP	MSB5 medium	Tryptophan and gulatime	Positive effect of tryptophan	Shahsavari (2011a)

Kusan, Lamsan, Selasi, Siam.	Mature seeds	2, 4-D, NAA, Kin, BAP, sucrose and gelrite.	MSB5 medium	Sorbitol	Optimum sucrose conc. is 20 g/l for Kusan and Siam and for Selai 10g/l	Shahsavari (2011b).
Govind , Pusa Basmati-1, Jaya	Mature embryo	2, 4-D, kinetin, BAP.	MS medium	-	Regeneration was efficient	Verma et al., (2011)
Basmati-370, DR-82, IR-6.	Mature seeds	BAP, NAA, Kinetin	MS medium	GA_3	RF 40%, 80% and 65% resp.	Rafique et al., (2011)
Super Basmati and IRRI-6	Mature seeds	2,4-D, BAP, NAA	LS/MS media	-	Regeneration was efficient	Afrasiab et al., (2011)
9 varieties	Mature and immature embryos	2, 4-D, NAA, BAP	N6 media	Sorbitol	Highest RF in case of mature embryos.	Noor et al., (2011)
MR 219, MR 232	Zygotic embryos	Maltose, glutamine, asparagine, arginine.	MS medium	ABA	RF 87-91%	Zuraida et al., (2011)
Kdml 105, Supanburi 1, Chainat 1, Pathum thani 1.	Mature seeds	2,4-D, BA, NAA	NN/MS media	-	MS medium was good in terms of response	Rattana et al., (2012)
Super basmati	Root	2, 4-D, NAA, Kin	MS medium	-	1 mg/l NAA, 3 mg/l Kinetin proved best	Joyia and Khan, (2012)
Pusa basmati 1, HRR 46, IR 72	Immature/ mature seeds	NAA, Kin	MS basal medium	Agarose	Immature embryo showed better regeneration	Kaswan et al., (2012)
Sarawak biris	Seeds	2, 4–D, NAA, Kin	MS medium	-	RF 97%	Libin et al., (2012)
MTU 7029	Seeds	0-4mg/l 2, 4-D, 0-1 mg/l kin, Sucrose 0-50g/l, agar 6-16 g/l	MS, N6 & LS	-	30 g/lsucrose, 8 g/l agar were best	Mondal et al., (2013)
Kalijira and Chinigura	Mature seeds	1 mg/l 2, 4-D, 1 mg/l BAP, 1mg/l IBA	MS basal medium	-	Highest RF	Mannan et al., (2013)
PR 118, PR 116,PR121, PR 122 and Kitaake.	Seeds	2,4-D (3mg/l), BAP(0.25mg/l), maltose, phytagel	MS medium	Proline	Better regeneration	Kumar and Ajinder (2013)
Hom kra dang ngah	Mature seeds	0.5 mg/l NAA, 1 mg/l 6-BA, 20 mg/l Kin, 1g/l casein hydrolysate	MS, N6, ARDA media	82 mM sorbitol	RF 75% in ARDA media	Yinxia and Te-chato (2013)
Several varieties	Mature	0.5 g/l NAA,	MS medium	-	Efficient RF	Hoque et al.,

	seeds	0.5 mg/l kin and BA of various conc.				(2013)
MR 123 and MR 127	Mature seeds	2, 4-D	MS medium	500mg/l glutamine and proline	70 % and76% induction frequency resp.	Ahmed et al., (2013)
Basmati rice varieties	Scutellum	3 mg/l kin, 1 mg/l NAA, 1-5 mg/l 2, 4-D	Modified MS medium	-	Efficient RF	Joyia and Khan (2013)
IRRI-6, IRRI-9, KSK-282.	Seeds	Kinetin, NAA, BAP	N6 medium	Proline, glycine and MES	High RF in IRRI-9 and KSK-282	Muhammad et al., (2014)
IR- 64	Scutellum	2, 4-D, BAP, TDZ, NAA	MS medium	-	67.5% RF	Toppo et al., (2014)
Kitaake	Seeds	2,4-D, BAP, proline, phytagel	MS medium	-	RF 82.66%	Sah et al., (2014)
Neda and Nemat varieties	Coleoptile	2 mg/l kin, 0.5 mg/l NAA, 2 mg/l BAP, 0.5 mg/l kinetin	MS basal medium	-	RF 32.30% and 20/95% resp.	Ghubeishavi et al., (2014)
BRRI dhan32, BRRI52, FR13 A	Seeds	2, 4-D, Kin, BA, NAA	MS medium	-	94% and 87% RF for FR 13 A and BRRI resp.	Bhuiyan et al., (2014)
Colombian rice varieties	Seed	1 mg/l NAA, 4 mg/l kin	MS basal medium	-	High RF	Cepeda and Chaparro-giraldo (2014)
Aromatik-1, Baldo, Karadeniz.	Mature embryos	2, 4-D, picloram	MS medium	Dark condition	plant regeneration	Benglioglu et al., (2015)
Jow haw	Embryo	2, 4-D, sucrose, proline.	MS and NB media	-	Growing cell suspension culture	Ranyikar et al., (2015)
AC 39020	Seeds	BAP, Kin, NAA, TDZ	LS/MS/N6 media	Desiccation stress	Increased shoot regeneration frequency	Vennapusa et al., (2015)
21 cultivars	Seeds	2, 4-D, IAA, kinetin and BAP	MS medium	-	Sambha mahsuri showed promising results	Sankepally and Singh (2016)

RF* : regeneration frequency.