Journal of Plant Sciences
2015; 3(5): 248-254
Published online September 6, 2015 (http://www.sciencepublishinggroup.com/j/jps)
doi: 10.11648/j.jps.20150305.11
ISSN: 2331-0723 (Print); ISSN: 2331-0731 (Online)

An Efficient Callus Induction and Regeneration Protocol for a Drought Tolerant Rice *Indica* Genotype AC39020

Amaranatha Reddy Vennapusa[1, 2], Ramu S. Vemanna[2, 3], Rajashekar Reddy B. H.[2], K. C. Babitha[2], K. Kiranmai[1], A. Nareshkumar[1], Chinta Sudhakar[1]

[1]Department of Botany, S.K. University, Anantapur, Andhra Pradesh, India
[2]Department of Crop Physiology, UAS, GKVK, Bangalore, Karnataka, India
[3]Plant Biology Division, the Samuel Roberts Noble Foundation, Ardmore, USA

Email address:

chintasudhakar@yahoo.com (C. Sudhakar), botanyamar@gmail.com (A. R. Vennapusa)

To cite this article:

Amaranatha Reddy Vennapusa, Ramu S. Vemanna, Rajashekar Reddy B. H., K. C. Babitha, K. Kiranmai, A. Nareshkumar, Chinta Sudhakar. An Efficient Callus Induction and Regeneration Protocol for a Drought Tolerant Rice *Indica* Genotype AC39020. *Journal of Plant Sciences*. Vol. 3, No. 5, 2015, pp. 248-254. doi: 10.11648/j.jps.20150305.11

Abstract: Most of the cultivated *indica* rice genotypes are less amenable to genetic modifications due to their poor callus induction and regeneration potential. The prerequisite for genetic enhancement of *indica* rice genotypes by biotechnological approach is to develop an efficient protocol for callus induction and plant regeneration. In the present study, we established an optimized regeneration protocol for rice genotype AC39020, which is moderately drought tolerant with high root growth and biomass. To use this genotype in the crop improvement program the prerequisite is callus induction and regeneration protocol in this *indica* rice genotype. The mature seeds of AC39020 used as explants for callus induction on LS, MS and N6 media with different hormones and amino acid concentrations. LS basal media with 2.5 mgL^{-1}, 2, 4-D and 500 mgL^{-1}glutamine showed 91.3% callus induction frequency. Subsequently the embryogenic callus was cultured on MS media supplemented with BAP, Kinetin, NAA, and TDZ. The MS medium supplementedwith 4mg L^{-1} BAP and 0.5 mgL^{-1} NAA showed 75% regeneration efficiency. Since regeneration in *indica* rice varieties is tedious, far-reaching, highly genotype-specific, we exposed the embryogenic calli for mild desiccation stress for 24 h and 48 h. The desiccation treatment for 48h increased shoot regeneration frequency from 16.7 % to 40.2 % compared to non-desiccated calli. The protocol developed was highly reproducible and this protocol can also be used for further improvement of this rice genotype through genetic modification.

Keywords: Callus, Regeneration, Rice, Hormone, Desiccation Stress

1. Introduction

Rice (*Oryzasativa*L.) has been cultivated for more than 10,000 years. It is one of the most important cereal crops belongs to Poaceae family [1]. More than 50% of the world population consume rice and is increasing 1.8% every year [2]. However, rice production has slowed down and it is estimated that rice production has to be increased 50% by 2025 [3]. In recent years a considerable improvement has been made by exploiting the natural variation through conventional breeding. But traditional breeding efforts alone cannot meet the increasing demand of rice production. From this context tissue culture techniques are being used globally for genetic improvement of rice plants [4]. The biotechnological advances including transformation, *in situ*and *in vitro*hybridization to introgressnew genes from different sources to the cultivated species has been reported. The success of all these methods depends on efficient reproducible regeneration system in any crop [5]. Therefore, the prerequisite and important is the identification of compatible genotypes for callus growth and *in vitro*plant regeneration [6]. It has been reported that callus induction and regeneration potential is not only genotype dependent and also the type of explants and culture medium composition including growth regulators and growth conditions [7].

The agronomically important rice *indica* genotypes are recalcitrant to *in vitro* manipulation mainly due to their poor callus induction and regeneration efficiency. Production of good quantity and quality of callus is prerequisite for potential regeneration of the plant.The callus induction media containing hormones like auxin, 2-4, D and amino acids showed improved callus frequency rates in rice [8]**.** In rice,

the rate of shoot regeneration from callus influenced by the many factors like explants source, genotype, culture conditions, combinations of plant growth regulators [9-11] osmotic pressure [12] and partial desiccation [13].

In rice tissue culture, selection of totipotent explant and combinations of growth hormones and other culture conditions and physical factors like cold, dark, desiccation treatments play a vital role in shoot regeneration [14]. Different explants like mature seed derived callus [15] and immature embryogenic calli [16] basal segments of the stem from the *in vitro* grown rice plants [17]and shoot apical meristem [18], and mesocotyl [19] leaf blade [20], anthers[21] and leaf bases [22] have been reported as potential option for shoot regeneration systems in *Indica* rice genotypes. Plant growth hormones play a crucial role in rice tissue culture and *indica*genotypes exhibit variability for regeneration in terms of media hormonal combination and concentrations. Different combination of BAP and NAA has been reported to induce higher regeneration of shoots from calli of rice cultures [23]. TDZ is a phenyl urea-based compound, exhibits a broader effect than conventional cytokinins over a wide range of species, and it activates the shoot proliferation and adventitious shoot organogenesis [24]. Usage of TDZ in rice shoot regeneration media enhanced multiple shoots [18, 25]. Hormones like kinetin and gibberellin used with combination of other hormones showed improved shoot regeneration in rice [26, 27]. Several reports have shown that partial desiccation treatmentof calli was beneficial for embryogenesis and plant regeneration from embryogenic calli in *indica* rice [28- 30]. The emphasis of the present study is to establish a reproducible plant regeneration system for rice *indica* genotype (AC39020) through mature seed derived embryogenic calli.The AC39020 genotype showed tolerance to drought and superior water mining characters with high root and high biomass. Hence the hypothesis is to improve this genotype by genetic engineering and transform other superior alleles by transgenic approach and subsequently use it for breeding programs. Inthis regard initial effort is to establish the callus induction and regeneration protocol. The LS media showed highest percentage callus induction and subsequently MS media with 4 mgL^{-1} BAP and 48 h of mild desiccation treatment showed efficient regeneration of multiple shoots. The study provided an option to develop transformation protocols in the rice genotype AC39020.

2. Materials and Methods

2.1. Plant Material

The *indica* genotype rice *Oryzasativa* L. genotype AC39020 seeds were used in this study. The seeds were obtained from the Department of crop physiology, University of Agricultural Sciences, Bangalore.

2.2. Seed Sterilization

Seeds were stored at 4°C prior to use, manually dehusked seeds were treated with 70% ethanol for two minutes and washed thrice with sterile distilled water. Further seed sterilization carried out on shaker using 4% sodium hypo chloride contained 2 drops of Tween – 20 for thirty minutes, followed by five rinses in sterile distilled water under laminar air flow cabinet [31].

2.3. Callus Induction

Sterilized seeds were transferred into petri dishes containing callus induction media. The callus induction medium developed for this study denoted as MS media [32], LS media[33] N6 media [34]. Different culture media including MS, LS and N6 medium supplemented with hormones like 2,4-D and kinetin and additionalsupplementation ofamino acids like glutamine, tryptophan and casamino acids were provided (Table 1).

Table 1. *List of media used for Callus induction.*

Media		Growth Regulators (mg L-1)		Amino acids (mg L-1)		
		2,4-D	Kinetin	Glutamine	Tryptophan	Casamino acids
LS media	LS basal	2.5	-	500	-	-
MS media	MS basal	2	-	-	50	1000
N6 media	N6 basal	3	1	-	-	-

Table 2. *List of media used for Shoot regeneration.*

Media		Growth regulators (mg L^{-1})			
		Cytokinin			Auxin
		Kinetin	BAP	TDZ	NAA
MSKN	MS basal	2.5	-	-	0.5
MSBN1	MS basal	-	3	-	1
MSBN2	MS basal	-	4	-	0.5
MST1	MS basal	-	-	2	-
MST2	MS basal	-	-	1	-

After 15 days of incubation under dark 25±1°C, calli initiated from scutella were sub cultured on fresh callus induction medium. The callusinduction frequency on different callus induction media was calculatedby using the following formula [35].

Callus induction frequency = Number of calliproduced from seeds/number of seeds plated × 100

2.4. Shoot Regeneration

To standardize the shoot regeneration media for rice genotype AC39020, we used five different media combinations with different hormones. Twenty five day-old actively growing calli transferred to the five different shoot regener-

ation medium containing different hormonal combination (MSKN1, MSBN1, MSBN2, MSTN1, MSTN2). The combination of hormones used in the media was listed in Table 2. The plant regeneration frequency was calculatedby the following formula [35].

Shoot generation frequency = Number of shoots produced from calli/ Number of calli plated ×100

2.5. Partial Desiccation Treatments

Partial desiccation was carried out by transferring thirty days old creamy white, nodular embryogenic calli to sterile empty petri dishes containing sterile whatman filter paper for desiccation treatment. The Petri dishes were sealed with parafilm and kept at 25±2℃ in the dark for 24 h and 72 h [29]. After desiccation treatment, both desiccated and non-desiccated calli were transferred to shoot regeneration media contains MS basal with different hormonal combination as mentioned in Table 2.

2.6. Root Induction and Plant Hardening

The regenerated plantlets were transferred to culture bottles containing half strength MS basal media supplemented with 0.5 mg L^{-1} IBA for root induction. Rooted plantlets were transferred to pots containing soil rite, the pots were covered with polythene bags for plant hardening. The hardened plants were finally transferred to the soil in the mud pots and were kept in green house and maintained at 30°C with relative humidity 80%.

3. Statistical Analysis

Statistical significance of differences was determined by one-way analysis of variance (ANOVA) followed by Duncan's post-hoc analysis. The data were presented as the mean ± standard deviation means (SDM). Difference was considered significant at $p < 0.05$.

4. Results

4.1. Identification of Rice Genotype

The rice genotype AC39020 was identified as drought tolerant based on physiological screening of more than 200 genotypes. This genotype has 51.83 cm of root length and 42.25 gm of biomass with $\delta^{13}C$ value of 19.136 indicating a better water use efficient rice genotype(Unpublished).

4.2. Callus Induction

Mature seeds used as explants for embryogenic calli production. Significant differences were observed in callus induction frequency as well as quality of the callus grown on different medium (Fig. 1). In general, two types of calli were observed namely, embryogenic that is compact yellowish, and large in size, and non-embryogenic fragile, translucent and slimy (Fig. 2). The highest embryogenic callus induction frequency(91.3%) was observed in LS basal medium supplementation with 2.5 mg L^{-1}, 2,4-D and 500 mg L^{-1} glutamine. The time period for callus induction was taken 3 weeks in LS medium. Whereas MS and N6 media, the time period for callus induction was 3-4 weeks. The MS medium supplemented with 2 mg L^{-1} 2, 4-D, and 50 mg L^{-1} tryptophan and 1 g L^{-1} casamino acids showed significant enhanced callus induction (78.2%) compared to N6 media. Less callus induction (42.5%) was observed on N6 media supplemented with 3 mg L^{-1} 2, 4-D, 1 mg L^{-1} Kinetin (Fig. 3). The calli produced from MS and N6 medium were showed less friable and non-embryogenic. In the present study, callus production and quality was improved significantly on LS media.

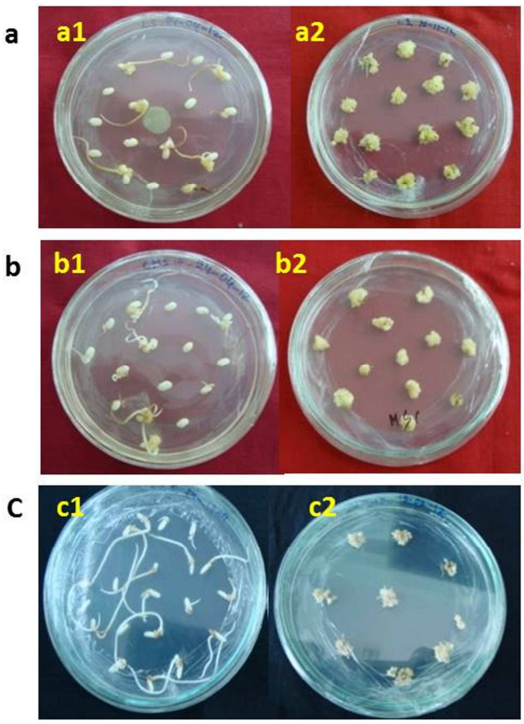

Figure 1. *Effect of different combinations of media on callus inductionof rice genotype AC39020.a) LS media b) MSC media c) N6 media. a1,b1,c1-15 days old calli. a2,b2,c2- 30 days old calli.*

Figure 2. *Effect of callus induction media on quality and embryogenic callusproduction in rice genotype AC39020 a) Non embryogenic calli on N6 media b) Embryogenic calli on MSC media c) Embryogenic calli on LS media.*

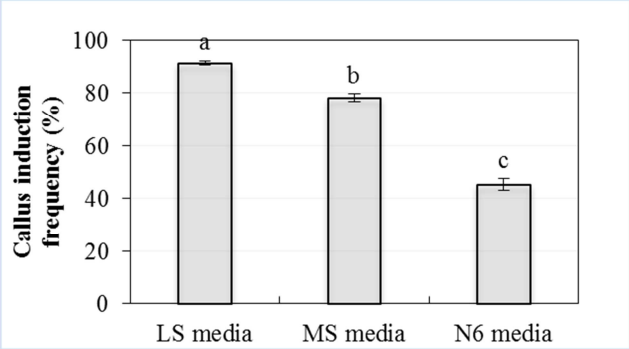

Figure 3. *Callus induction frequency on three different media combinations.*

4.3. Shoot Regeneration

Among the various combinations of hormones, the highest shoot regeneration frequency (75%) was observed on the MS medium supplemented with 4 mg L^{-1} BAP, 0.5 mg L^{-1} NAA (Fig. 4). Both embryogenesis andorganogenesis induced quite earlier compared to other media combinations. Shoot differentiation was detected within 15 - 25 days on the regeneration medium (MSBN2), but 30 - 40 days was taken in other media used in the present study. Another combinationof BAP and NAA (MSBN1) showed less regeneration capacity 56.3% compared MSBN2 media. The calli, which were grown on TDZ containing medium MST1 showed 45.8% and MST2 showed 39.6% regeneration frequency. Usage of TDZ alone using with MS media also showed significant increased regeneration capacity compare to kinetin and NAA combination (MSKN). The less regeneration frequency 27.1% was observed in the medium MSKN.(Fig. 5).

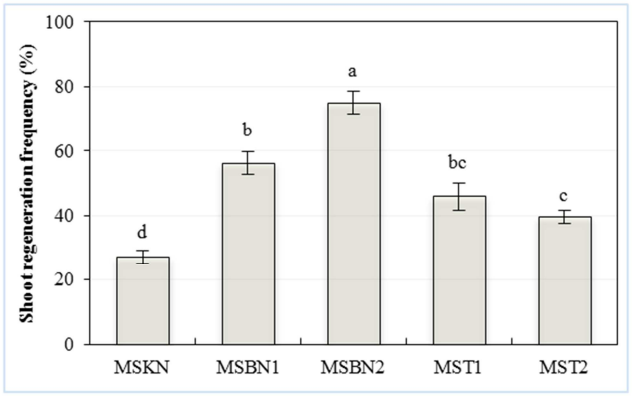

Figure 4. *Effect of different hormones containing media on Shoot regeneration frequency.*

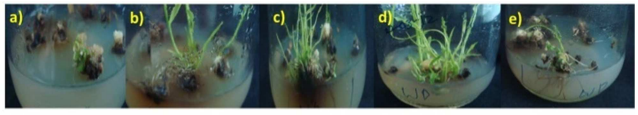

Figure 5. *Effect of different hormonal supplements on shoot regeneration of indica genotype AC39020,a) MSKN b)MSBN1 c)MSBN2 d)MST1e) MST2.*

4.4. Partial Desiccation Treatment

The calli derived from mature seed was subjected to 24 h and 48 h desiccation periods. The partial desiccation of callus enhanced the regeneration frequencies in all the media combinations compared to non-desiccation of calli. The 48 h desiccation treatment for calli showed significantly increased regeneration frequency compared to 24 h and non-desiccation treatments. Upon 48 h of desiccation treatment the regeneration frequency significantly increased up to 70.1% on MSKN media compared to 24 h (36.8%) and 0 h desiccation treatments (29.9%). The similar kind of increased regeneration frequencies was observed with other media combination like MSBN1, 61.8% at 24 h and 79.2% at 48 h but in case of non-desiccated calli comparatively less (59.0%). MSBN2 showed better regeneration frequency 77.1% even under non desiccation treatment, also gradual increase was observed with respect to desiccation treatments at 24 h 79.2% and 48 h 93.8 %.In case of MST1 and MST2 media also showed enhanced regeneration frequency upon desiccation treatment (Fig. 6).

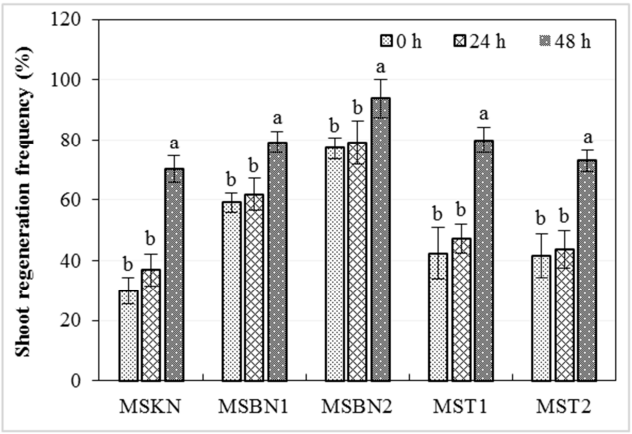

Figure 6. *Effect of partial desiccation treatments on shoot regeneration frequency on different media combinations inindica rice genotype AC39020.*

The callus browning was comparatively high in non-desiccation (0 h desiccation) treatment and regenerated callus was comparatively not so green and healthy as compared to callus regenerated from partially desiccated calli (Figure 7).

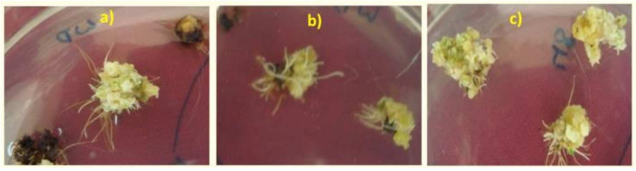

Figure 7. *Partial desiccation effect on browning of callus during shoot regeneration: a) 0 h desiccation b) 24 h desiccation c) 48 h desiccation.*

The time period for regeneration was less in partially desiccation treatment compared to non-desiccation treatment. Even after partial desiccation treatment, the maximum regeneration frequency (93%), greener multiple shoots and less time period for shoot regeneration was observed in media MSBN2 compared to all other media.The regenerated shoots were successfully produced roots on rooting media; the plantlets were hardened and transferred to pots.

5. Discussion

Embryogenic callus induction considered as most critical step in rice tissue culture. Potentiality of callus induction and regeneration frequency of cell depends on in vitro culture conditions like media composition, genotype and source of explants etc. Mature seed derived embryogenic callus culture has been extensively used in rice in vitro culture studies [14]. Hence embryogenic calli source material of mature seeds will be available year around, amenable for transformation and also excellent source for in vitro regeneration and for the development of transgenics. Therefore mature seeds used as explants in the present study.

Callus induction was a critical phase where the regeneration of plants is highly dependent on the quality of callus. In rice invitro culture, the success rate of callus induction and quality of callus depends on nutritional media, growth hormones, and genotype and on the interactions of genotype vs medium [35, 36]. The present investigation showed that both that genotype and media composition and their interaction largely effected on callus induction. The rate of callus induction frequencyin AC39020 genotype varied from 42.1 % to 91.3 %. Maximum calli production (91.3 %) and embryogenic calli were observed in the combination of LS media, which is significantly higher than the combinations of MS media and N6 media. Several studies for rice tissue culture have been reported that plant growth regulator 2, 4-D is the most suitable auxin for callus induction [37, 38]. Also optimal concentration of 2, 4-D, source of explant and genotype influences the callus induction in rice tissue culture [39]. In the present study AC39020 genotype showed highest callus frequency on LS media containing 2.5 mg L^{-1} 2,4-D but using less or more concentration of 2,4-D showed less callus induction.

Rate of regeneration in rice plants was influenced by media composition, explants source and age and culture conditions [40, 6]. A number of reports suggest that genotype and nutrient composition of the medium are the essential factors for efficient rice plant regeneration [12, 41- 43]. To standardize the regeneration medium for rice genotype AC39020, we optimized different hormonal concentration in combinations. We used BAP, Kinetin and TDZ in higher concentrations and lower concentration of auxin (NAA) based on earlier reports in rice. Amongst the media we used in the present study MSBN2 is showed significantly high regeneration frequency and also less time period for regeneration, MSBN2 media contains BAP and NAA also suggesting the stimulatory effect of BAP in combination with NAA reported that facilitate regeneration in rice [23]. Using TDZ alone also showed increased regeneration frequency in MST1 and MST2 compared to Kinetin and NAA combination (MSKN). Recently TDZ have been used in many rice regeneration culture media [18, 25] with different explant sources showed improved regeneration frequencies suggesting TDZ also helps for regeneration in seed derived embryogenic calli as also evidenced from the present study.

Enhanced shoot regeneration is vital for the establishment of a rice tissue culture system. Partial desiccation has been proved as a tool for stimulating embryogenesis and plant regeneration in rice *indica* genotypes [44, 45, 29, 46, 30]. Partial desiccation of the callus and its advantage for regeneration were reported in other species [47]. Under desiccation treatment reduced water availability enhances the uptake of nutrients and also activates genes of late embryogenesis abundant (LEA) protein. Plant LEA proteins have defensive mechanism in embryo tissue during seed maturation in response to osmotic stress. Desiccation may changes in translatable mRNAs and stimulated modification in protein synthesis as reported [13]. Dehydration of calli triggers differences in soluble protein pattern and leads to the maturation of embryo. Altered metabolisms under desiccation treatment stimulate the normal development and maturation of somatic embryos [48]. At cellular level changes upon desiccation leads to high frequency of regeneration and viable plantlets. The improved regeneration of shoots might be because of increased level of abscisic acid in desiccated callus [49] and enhancement of oxygen supply [45].

The partial desiccation treatment for 48 h significantly increased the regeneration frequency in all the combination of media. The increased regeneration frequencies range from the 16.7% to 40.2% was observed on different media combinations. The maximum regeneration frequency in MSBN2 (93.3%) was observed at 48 h of desiccation treatment. The media MSKN, which showed lowest regeneration frequency (28.6%) under non-desiccation also showed improved regeneration frequency (71.5%) at 48 h of desiccation treatment. Compared to 24 h of desiccation treatment 48 h of treatment showed significantly enhanced regeneration in all the combinations of media also been reported by Saharan [29] and Makerly [30] in rice. The present study suggests that exposure of embryogenic calli for 48 h partial desiccation in *Indica* rice genotype AC39020 showed significant enhanced shoot regeneration frequency, reduced the duration for regeneration and also increased multiplication of green shoots. The established media compositions, hormonal concentrations and partial desiccation treatments provide an option to overcome the difficulties in regeneration system of *indica* rice genotype.

6. Conclusions

To conclude, the success of any plant transformation depends on efficient and reproducible plant regeneration system. In this context, we have optimized media composition for efficient callus induction and plant regeneration for *indica* rice genotype AC39020 by using mature seeds as explants. Among different media tested LS media significantly enhanced the callus induction frequency while, plant regeneration frequency was considerably increased in MSBN2 media. Further, a moderate desiccation stress of calli showed substantial improvement in plant regeneration with less browning of calli and multiple green shoots formation. Since, the protocol developed was highly reproducible, this protocol will be very useful in genetic enhancement of the indica

rice genotype through genetic modification.

Acknowledgements

We acknowledge the Department of Crop physiology GKVK, UAS Bangalore for providing the Tissue culture lab facility and UGC BSR, New Delhi for financial support in the form of fellowship to AV.

References

[1] Sasaki, T. (2005) The map based sequence of the rice genome. Nature, 436,793-800.

[2] Christou, P. (1997) Rice transformation: bombardment. Plant MolecularBiology, 35, 197- 203.

[3] Khush, G. S. and Virk, P. S. (2000) Rice breeding: achievements and future strategies. Crop Improvement, 27 (2), 115-144.

[4] Raina, S. K. (1989) Tissue culture in rice improvement: status and potential.Advances in Agronomy, 42, 339-398.

[5] Sikder, M. B. H., Sen, P. K., Mamun, A. A., Ali, M. R. and Rahman, S. M. (2006) In vitro regeneration of aromatic rice (*Oryza sativa* L.). International Journal of Agriculture Biology, 8(6), 759-760.

[6] Hoque, M. E. and Mansfield, J. W. (2004) Effect of genotype and explant age on callus induction and subsequent plant regeneration from root-derived callus of *indica* rice genotypes. Plant Cell Tissue Organ Culture, 78, 217-223.

[7] Rueb, S., Leneman, M., Schilperoort, R. A. and Hensgens, L. A. M. (1994) Efficient plant regeneration through somatic embryogenesis from callus induced on mature rice embryos (*Oryza sativa* L.). Plant Cell, Tissue and Organ Culture, 36, 259-264.

[8] Pravin, V. J., Mahendra, D., Salujat.,Sarawgi, A. K., Saxena, R. A. V. and Girish, C. (2011) Assessment of critical factors influencing callus induction, in vitro regeneration and selection of bombarded *indica* rice genotypes. Journal of Cell & Plant Science, 2 (2), 24-42.

[9] Chu, Q. R. and Croughan, T. P. (1990) Genetics of plant regeneration in immature panicle culture of rice. Crop Science, 30, 1194-1197.

[10] Mandal, N. and Gupta, S. (1995) Effect of and culture medium on androgenic callus formation and green plant regeneration in *indica* rice. Indian Journal of Experimental Biology, 33, 761-765.

[11] Zhu, Y., Ouyang, W., Li, Y. and Chen, Z. (1996) The effects of 2ip and 2, 4-D on rice calli differentiation. Plant Growth Regulators, 19, 19-24.

[12] Jain, R. K. (1997) Effect of some factors on plant regeneration from *indica* rice callus and protoplasts- a review. Indian Journal of Experimental Biology, 35, 323-331.

[13] Rance, I. M., Tian, W., Mathews, H., Dekochko, A., Beachy, R. N. and Fauquet, C. (1994) Partial desiccation of mature embryo-derived calli, a simple treatment that dramatically enhances the regeneration ability of *indica* rice. Plant Cell Reports, 13, 647-651.

[14] Biswas, A. and Mandal, A. B. (2007) Plant regeneration in different genotypes of *indica* rice. Indian Journal of Biotechnology, 6(4), 532-540.

[15] Wani, S. H., Sanghera, G. S. and Gosal, S. S. (2011) An efficient and reproducible method for regeneration of whole plants from mature seeds of a high yielding *indica* rice (*Oryza sativa* L.) Variety PAU 201. New Biotechnology, 28, 418-422.

[16] Hiei, Y. and Komari. T. (2008) Agrobacterium-mediated transformation of rice using immature embryos or calli induced from mature seed. Nature Protocals 3: 824-834.

[17] Joyia, F. A. and Khan, M. S. (2012) Reproducible and expedient rice regeneration system using in vitro grown plants. African Journal of Biotechnology, 11, 138-144.

[18] Yookongkaew, N., Srivatanakul, M and Narangajavana, J. (2007) Development of genotype-independent regeneration system for transformation of rice (*Oryza sativa* ssp. *Indica*). Journal of Plant Research, 120, 237-245.

[19] Puhan, P. and Siddiq, E. A. (2013) Protocol optimization and evaluation of rice varieties response to In vitro regeneration. Advances in Bioscience and Biotechnology, 4, 647-653.

[20] Yan, C. J. and Zhao, Q. H. (1982) Callus induction in plantlet regeneration from leaf blade of *Oryzasativa*L. Subsp*indica*. Plant Science Letter, 29. 175-182.

[21] Genovesi, A. D and Magill, C. W. (1979) Improved rate of callus and plant regeneration from rice anther culture following cold shock. Crop Science, 19, 662–664.

[22] Ramesh, M., Murugiah, V. and Gupta, A. K. (2009) Efficient in vitro plant regeneration via leaf base segments of *indica* rice (*Oryza sativa* L.). Indian Journal of Experimental Biology, 47, 68-74.

[23] Sharma, C., Kaur, M., Kaur, A. andGosal, S.S. (2012) In vitro plant regeneration studies in three *indica* rice varieties. International Journal of Agriculture and Environmental Biotechnology, 5(4), 309-313.

[24] Huetteman, A. and Preece, E. J. (1993) Thiodiazuran: a potent cytokinin for woody plant tissue culture. Plant Cell, Tissue and Organ Culture, 33, 105-119.

[25] Dey, M., Bakshi, S., Galiba, G., Sahoo, L. and Pand, S. K. (2012) Development of a genotype independent and transformation amenable regeneration system from shoot apex in rice (*Oryza sativa* spp. *Indica*) using TDZ. 3 Biotech, 2(3), 233-240.

[26] Rafique, M. Z., Rashid, H., Chaudhary, M. F., Chaudhry, Z. and Cheema, N. M. (2011) Study on callogenesis and organogenesis inlocal cultivars of rice (*Oryza sativa* l.) Pakistan Journal of Botany, 43(1), 191-203.

[27] Alam, M. J., Imran, M., Hassan, L., Rubel, M. H. and Shamsuddoha, M. J. (2012). In vitro Regeneration of High Yielding *Indica* Rice (Oryzasativa L.) Varieties, Journal of Environmental science & Natural Resources, 5, 173-177.

[28] Chand, S. and Sahrawat, A. K. (2000) Stimulatory effect of partial desiccation in plant regeneration in *indica* rice (*Oryza sativa*. L). Journal of plant biochemistry and biotechnology, 10(1), 43-47.

[29] Saharan, V., Yadav, R. C., Yadav, N. R. and Chapagain, B. P. (2004) High frequency plant regeneration from desiccated calli of *indica* rice (*Oryza sativa* L.). African Journal of Biotechnology, 3 (5), 256-259.

[30] Makerlya, H., Rahmata, Z. and Wagirana, A. (2012) Potential use of partial desiccation treatment for regeneration system of malaysian*indica* rice (*Oryza sativa* L.) Jurnalteknologi (sciences & engineering), 59 (1), 97-100.

[31] Wagiran, A. I. I., Zain, C. R. C. M. and Abdullah, R. (2008) Improvement of plant regeneration from embryogenic suspension cell culture of japonica rice. Journal of Biological Sciences, 8, 570-576.

[32] Murashige, T. and Skoog, F (1962) A revised medium for rapid growth and bio assays with tobacco tissue cultures. PhysiologiaPlantarum, 15, 473-497.

[33] Linsmaier, E. M. and Skoog, F. (1965) Organic growth factor requirements of tobacco tissue culture. Physiol. Plant, 18, 100-127.

[34] Chu, C. C., Wang, C. C., Sun, C. S., Hsu, C., Yin, K. C. and Chu, C. Y. (1975) Establishment of an efficient medium for anther culture in rice through comparative experiments on the nitrogen sources. ScientiaSinica, 18, 659-668.

[35] Islam, M. M., Ahmed, M. and Mahaldar, D. (2005) In vitro callus induction and plant regeneration in seed explants of rice (*Oryza sativa* L). Research Journal of Agriculture and Biological Sciences, 1(1), 72-75.

[36] Pandey SK, Ramesh B and Gupta PK (1994) Study on effect on genotype and culture medium on callus formation and plant regeneration in rice (*Oryzasativa* L.). Indian Journal of Genetics. 54: 293-299.

[37] Bajaj, Y. P. S., 1991. Biotechnology in Rice Improvement. In Biotechnology in Agriculture and Forestry 14. Eds. Y. P. S. Bajaj. Rice Spriger-Verlag, pp: 1-18.

[38] Libin, A.; King, P. J. H.; Ong, K. H.; Chubo, J. K. and Sipen P. (2012). Callus induction and plant regeneration of Sarawak rice (*Oryzasativa*L.) variety Biris. African Journal of Agricultural Research, 7(30): 4260-4265.

[39] Mahajan, R., Aslam, L. and Kousar, H. (2013) Effect of growth regulators on in vitro cultures of two basmati rice genotypes: ranbir basmati and basmati 370. International Journal of Pharmaceutical, Chemical and Biological Sciences, 3(4), 1131-1138.

[40] Aditya, T. L., Hoque, M. E. and Khalequzzaman, M.(2004) Response to high frequency callus induction ability from root regions of germinated embryo in *indica* rice. Pakistan Journal of Biological Sciences, 7(5), 861-864.

[41] Khanna, H. K. and Raina, S. K. (1998) Genotype × culture media interaction effects on regeneration response of three *indica* rice cultivars. Plant Cell, Tissue and Organ Culture, 52, 145-153.

[42] Lee K, Jeon H, Kim M (2002). Optimization of a mature embryo-based in vitro culture system for high frequency somatic embryogenic callus induction and plant regeneration from japonica rice cultivars. Plant Cell, Tissue and Organ Culture 71: 237-244.

[43] Khatun, M. M., Ali, M. H. and Desamero, N. V. (2003) Effect of genotype and culture media on callus formation and plant regeneration from mature seed scutella culture in rice. Plant Tissue Culture, 13(2), 99-107.

[44] Tsukuhara, M and Hirosawa, T. (1992) Simple dehydration treatment promotes plantlet regeneration of rice (*Oryzasativa* L.) Callus. Plant Cell Reports, 11 (11), 550-3.

[45] Jain, R. K., Jain, S. and Wu, R. (1996) Stimulatory effect of water stress on plant regeneration in aromatic *indica* rice varieties. Plant cell Reports, 15(6), 449-454.

[46] Haq, I. Zhao, C. X. Mukhtar, Z. Jaleel, C. A. and Azooz, M. M. (2009) Effect of physical desiccation on plant regeneration efficiency in rice (*Oryza sativa* L.). Journal of Plant Physiology, 166, 1569-1575.

[47] Singh, B. (2014) Effect of desiccation and chilling treatment on somatic embryo development and germination in rough lemon (citrus jambhiri lush). British biotechnology journal 4(2): 136-148.

[48] Wenzhong, T., Rance, I., Sivamani, E., Fauquet, C. and Beachy, R. N. (1994) Improvement of plant regeneration frequency in vitro in *indica* rice. Chinese Journal of Genetics, 21, 1-9.

[49] Yang, Y. S., Y. D. Zheng, Y. L. Chen and Y. Y. Jian, 1999. Improvement of plant regeneration from long-term cultured calluses of Taipei 309, a model rice variety in *in vitro* studies. Plant Cell, Tissue and Organ Culture. 57: 199-206.