Journal of
Biomolecular Research & Therapeutics

Sankepally et al., J Biomol Res Ther 2016, 5:1
DOI: 10.4172/2167-7956.1000136

Review Article Open Access

Callus Induction and Regeneration Capabilities of Indica Rice Cultivars to Salt Stress

Samar SR Sankepally[1], Venkateswar R Talluri[2,3], John P Arulmarianathan[3] and Bharat Singh[1*]

[1]*Institute of Biotechnology, Amity University Rajasthan, Jaipur - 303002, India*
[2]*Department of Biotechnology, KL University, Guntur, Andhra Pradesh - 522502, India*
[3]*TNA Innovation Centre, Varsha Bioscience and Technology India Private Limited, Nalgonda, Telangana 508284, India)*

Abstract

The main aim of this study was to test the potentiality of PR-115, PR-116, Sambha Mahsuri, Erramallelu, Cotton Dora Sannalu and Pothana local rice cultivars to NaCl stress. Different concentrations and combinations of growth regulators were supplemented in MS media to achieve the callus induction and regeneration in rice cultivars. Maximum frequency of callus induction (75.54 ± 0.91%) was observed in Sambha mahsuri cultivar with MS+2, 4-D (3 mg/L) supplementation but, the response of 2, 4-D (5.0 mg/L) in callus induction was moderate (70.89 ± 0.45%) in Pothana cultivar of indica rice. The highest frequency of regeneration was achieved (65.45 ± 0.51%) in Sambha mahsuri while lowest in Pothana (42.38 ± 0.73%) cultivar of indica rice at 50 mM NaCl concentration. From the results, it has been established that Sambha Mahsuri was more tolerant to salinity in tissue cultures studies (to 50 mM NaCl concentration) so, this cultivar may be used for better production of rice in saline conditions.

Keywords: NaCl stress; Regeneration frequency; *Oryza sativa*; Sambha mahsuri; Acclimatization

Introduction

Rice is a staple cereal crop and about 70% of the world's poor population depending on rice as their major source of food [1]. During the past few decades, the demand for rice is continuously growing with the increasing population density [2]. The population of the world is increasing rapidly and reduction in rice yield could lead to hunger and famine, especially in developing countries. So, the application of plant tissue culture in combination with conventional breeding systems may help to increase production of rice [3,4]. The detection and screening of useful cultivars for the development of embryogenic calli and subsequent plant regeneration through the *in vitro* culture technique is a vital step in rice genetic transformation [5]. Similarly, the frequencies of callus induction and plant regeneration are affected by several factors such as type of explants and culture conditions [6,7].

Rice is a cereal crop of tremendous economic significance but, rice production is very much suffering from severe loss of yield by abiotic stresses. Soil salinity is one of the major abiotic stresses influencing plant growth adversely and causing significant reductions in crop yield [8,9]. In India, 1710673 hectares of total land area facing various levels of salinity problems due to the NaCl accumulation generated by the using of excessive level of fertilizers and salt is resulting in low crop productivity in rice [10,11]. Plant biotechnologists of the world are putting in their best efforts to develop salt resistant new cultivars of rice for fulfilling the demand of human population [12]. At present plant tissue culture technique used as an important and necessary component of plant biotechnology, which is essential for the genetic manipulation and is an ideal system for assessing the physiological effects of salt at the cellular level of rice crop plants [13,14]. Therefore, de-husked seeds were cultured onto MS medium and screened for salt stress by adding NaCl to obtain resistant embryogenic calli and regenerants to make rice plants tolerant to salt stress.

Materials and Method

Plant material

The mature seeds of two cultivars (PR-115, PR-116) of indica rice were obtained (Aug, 2013) from State Farms Corporation of India (SFCI), Suratgarh, India (authenticated by Neeraj Verma, Director-Incharge, Suratgarh, India) and other 4 indica rice cultivars (Sambha mahsuri, Erramallellu, Cotton dora sannalu, Pothana) (from Agricultural Research Station, Warangal, India). The obtained seeds of these 4 cultivars were authenticated by Dr. C Cheralu, Associate Director, ARS, and Warangal, India. The obtained seeds of these cultivars were stored at room temperature in plant tissue culture laboratory to keep safe from the attack of insects and microbial infections.

Callus induction and regeneration

The mature seeds of six cultivars of indica rice were de-husked and immersed in 75% ethanol for 3 min. After proper washing with distilled water, the de-husked seeds were treated with 1.0% $HgCl_2$ (w/v) for 3 min. After the treatment, these seeds were rinsed 4-5 times with sterilized distilled water to remove the traces of $HgCl_2$ with agitation in the laminar air flow. The sterilized seeds were placed onto the sterilized filter paper on the Petri dish. After the removal of water contents from the upper surface of seeds, the seeds were then placed onto callus induction culture media and incubated in the dark at 25 ± 1°C with 60% relative humidity. For the induction of callus and regeneration of plants, the basal Murashige and Skoog (1962) [15] culture medium was used. The different concentrations of 2, 4-D (1.0–6.0 mg/L) were added into the MS medium for the induction of callus in the six cultivars of indica rice. The other combinations of growth regulators [MS+kinetin (2.5 mg/L); MS+Kinetin+IAA (2.5+3.5 mg/L); MS+IAA+kinetin

***Corresponding author:** Bharat Singh, Institute of Biotechnology, Amity University Rajasthan, Jaipur 303002, India, Tel: +91 1426 212139; Fax: +91 1426 222836; E-mail: bharatsingh217@gmail.com

Received November 11, 2015; **Accepted** December 23, 2015; **Published** January 06, 2016

Citation: Sankepally SSR, Talluri VR, Arulmarianathan JP, Singh B (2016) Callus Induction and Regeneration Capabilities of Indica Rice Cultivars to Salt Stress. J Biomol Res Ther 4: 136. doi:10.4172/2167-7956.1000136

Copyright: © 2016 Sankepally SSR, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

(3.0+2.5 mg/L); BAP+IAA (2.5+2.5 mg/L)] were also screened for the induction and growth of callus in these six experimental cultivars. The embryogenic calli of indica rice cultivars were described as yellowish to granular, compact, white, brownish, greenish-yellow. The regenerated embryogenic calli were selected and used for regeneration of plantlets. Similarly, the plant regeneration was obtained by the supplementation of different combinations of growth regulators in MS culture media (i.e., T1=MS+2.5 mg/L kinetin, T2=MS+3.0 mg/L 2, 4-D+3.0 mg/L BAP, T3=MS+2.5 mg/L kinetin+3.5 mg/L 2, 4-D, T4=MS+2.5 mg/L kinetin+2.5 mg/L BAP, T5=MS+2.5 mg/L kinetin+3.0 mg/L IAA). After evaluating the effects of each of the above mentioned group of growth regulators individually, the combined effects of the media supplements were also assessed in series of the experiments with determining the days of callus induction and regeneration frequency.

Media for the screening of salt tolerance

The developed calli (2, 4-D, 3.0 mg/L) were grown onto MS media with (treated group) and without (control group) NaCl supplement. The concentrations of NaCl were added as 50 mM, 100 mM, 150 mM, 200 mM, 225 mM, and 250 mM in MS media. For each treatment, total fresh weight of 10 calli (2.5-3.0 mm diameter) of 3 replicates were determined and recorded. Similarly, for the screening of salt tolerance in regenerants, the embryogenic calli were transferred onto the MS media, supplemented with different NaCl concentrations (50 mM, 100 mM, 150 mM, 200 mM, 225 mM, 250 mM). At the end of experiment, the callus was taken for growth analysis. Mean fresh weight of calli at different concentrations was analyzed and compared by two-way ANOVA.

Data analysis

The frequency of callus induction and plant regeneration (%) were measured using the formula of following [16]:

$$Callus\ induction\ frequency\ (\%) = \frac{Number\ of\ seeds\ induced\ callus \times 100}{Number\ of\ cultured\ seeds}$$

Small pieces of embryogenic calli (2.5–3.0 mm diameter) were further cultured in 100 ml conical flasks containing regeneration culture medium. Frequency of plant regeneration was determined as follows -

$$Plant\ regeneration\ (\%) = \frac{Number\ of\ regenerated\ calli \times 100}{Number\ of\ calli\ incubated\ for\ regeneration}$$

Statistical analysis

The experiments were arranged in split plot design with 3 replications. Each replication per treatment contained 12 seeds for the induction of callus and six embryogenic calli for the plant regeneration. Data were analyzed using the two-factorial analysis of variance (ANOVA), with plant growth regulator concentration as one treatment and cultivar as the other treatment. Data were analyzed as means of standard error (mean ± SE).

Results and Discussion

Induction of callus and plantlets regeneration

The effects of 2, 4-D on induction of callus in mature de-husked seeds of six indica rice cultivars (PR-115, PR-116, Sambha mahsuri, Erramallelu, Cotton dora sannalu, Pothana) were investigated in present study. The basal MS medium was supplemented with different concentrations of 2, 4-D (1.0–6.0 mg/L) for obtaining the fast induction and growth frequencies of callus. The induction of callus was started after 15–18 days of transfer of the mature seeds to culture tubes. The final data of induction of callus were recorded on 28 day. The maximum frequency (75.54 ± 0.91%) of callus induction was observed in Sambha mahsuri with supplementation of 2, 4-D in MS culture medium (3.0 mg/L) (Figure 1). The effects of several combinations [MS+kinetin (2.5 mg/L); MS+Kinetin+IAA (2.5+3.5 mg/L); MS+IAA+kinetin (3.0+2.5 mg/L); BAP+IAA (2.5+2.5 mg/L)] of other growth regulators were also evaluated for these indica rice cultivars, but the callus induction frequency and growth rate were very low in above mentioned combinations. The colours of calli were white, yellowish-white, yellowish-green, yellowish-brown, light brown and textures of calli observed as compact, friable, and watery (Figure 2). The produced calli of convenient size were transferred onto MS culture media supplemented with different combinations of growth regulators for regeneration of plantlets. The highest frequency of plantlets formation was recorded in Sambha mahsuri, followed by PR-115 and Pothana cultivars of indica rice. The maximum plantlet regeneration frequency (87.34 ± 0.27%) was observed in Sambha mahsuri cultivar when the MS medium supplemented with combination of 2.5 mg/L kinetin and 3.5 mg/L 2, 4-D (Figure 3). The minimum regeneration frequency (20.19 ± 0.17%) was observed in Erramallelu (MS+2.5 mg/L kinetin). After attaining the size of 2-3 inch by the *in vitro* regenerated plantlets, the plantlets were taken out from aseptic culture tubes and transferred to the pots under natural conditions (Figure 4).

The plant tissue culture technique is well recognized as a novel approach to generate genetic variability [17,18] and has been proposed as an important supplementary method for the plants which breeding programmes can be accelerated through the use of new expanded genetic stability and diversity [19,20]. Several tissue culture techniques are being applied for development of new cultivars of rice in different countries of the world [9,12,14]. The various concentrations (1.0-6.0 mg/L) of 2, 4-D were tested for obtaining the fast and high frequency of induction and growth of callus in six cultivars of indica rice. The results revealed that the concentration and type of cultivars had great variability for the early induction and growth of callus. Use of 2, 4-D (3.0 mg/L) was found to be best for fast induction and higher growth of callus in Sambha mahsuri. Rice cultivars display wide range in the capacity of regeneration which depends on their genetic make-up and their interaction with types of culture media [6,21]. The combination of 2.5 mg/L kinetin+3.5 mg/L 2, 4-D was recorded as best combination for enhancement of regeneration frequency in Sambha mahsuri. The combination of MS+2.5 mg/L of kinetin demonstrated lowest regeneration frequency among all the tested combinations of growth regulators in selected cultivars of indica rice. These observed results were agreement with the study of Agrawal et al. [22] in other cultivars of rice.

Effects of NaCl stress on callus growth and plant regeneration

The effects of NaCl stress on growth of calli in six cultivars of indica rice were studied by using different concentrations of NaCl and it resulted to decrease in yield of fresh weight of calli compared to control. For the determination of growth of callus, the initial inoculants were used as 100 mg (fresh weight) in control as well as treated groups. In this course of study, the mean fresh weight (Figure 5) of calli was highest in PR-116, when the calli exposed to 50 mM NaCl, and least growth observed at the exposure of 250 mM NaCl. In case of Sambha mahsuri, the growth of calli was observed highest in the control but the growth was not highest after exposing the calli with 50 mM NaCl concentration. In the span of 28 days of culture, the mean fresh weight of calli of control group was higher than the group whose calli were exposed with different concentrations of NaCl (50 mM, 100 mM, 150 mM, 200 mM, 225 mM, and 250 mM). Comparative study of control

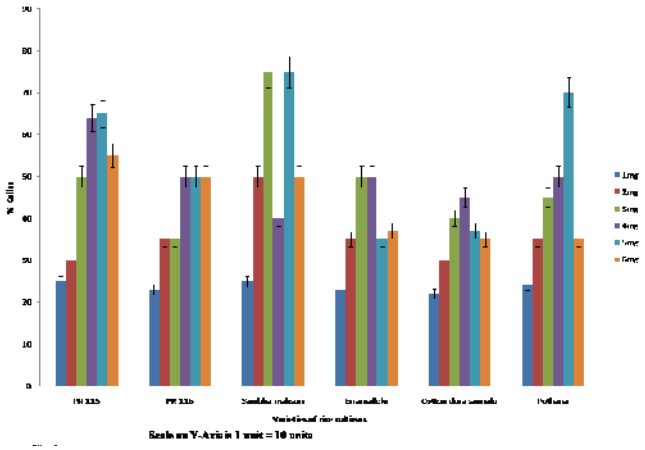

Figure 1: Effects of 2, 4-D on callus induction frequency (%) in selected cultivars of indica rice. The maximum callus induction frequency (75.54 ± 0.91%) in Sambha mausuri cultivar with 2, 4-D (3.0 mg/L).

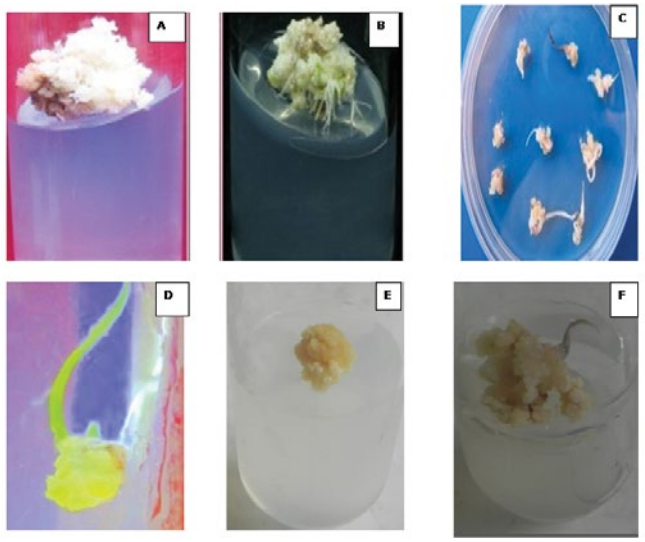

Figure 2: Induction of callus without any stress in indica rice cultivars: A) PR-115; B) PR-116; C) Sambha mahsuri; D) Erramallelu; E) Cotton dora sannalu; F) Pothana.

and treated group revealed that higher concentration exposure (250 mM) of NaCl restricted the growth of calli, because at this concentration no growth was observed in rice cultivars, except Sambha mahsuri (Figure 6). The colour of calli was converted into dark brown or black after exposing to 100-250 mM NaCl in case of Erramallelu. Similarly, the treatment of NaCl stress was also reduced the growth of calli of PR-115 and PR-116 at above 50 mM NaCl (Figure 6).

The regeneration frequency of plants was determined on the basis of development of plantlets from the individual embryogenic callus. This experiment was designed to test the inherent potentiality of embryogenic calli to regenerate plantlets on the medium which was prepared adding NaCl. The frequency of regeneration was highest (65.45 ± 0.51%) in Sambha mahsuri while lowest in Pothana (42.38 ± 0.73%) cultivar of indica rice at 50 mM NaCl (Figure 7). There was normal plant regeneration frequency observed in salt free culture medium but increased NaCl concentration decreased plant regeneration frequency in all the selected experimental cultivars of

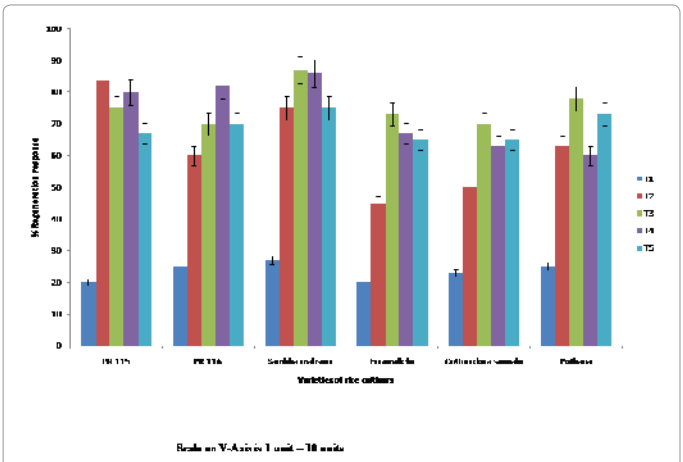

Figure 3: Frequency of regeneration (%) in selected cultivars of indica rice with different concentration of growth regulators (mg/L) (control group). T1=MS+2.5 mg/L Kin; T2=MS+3.0 mg/L 2, 4-D+3.0 mg/L BAP; T3=MS+2.5 mg/L Kin+3.5 mg/L 2, 4-D; T4=MS+2.5 mg/L Kin+2.5 mg/L BAP; T5=MS+2.5 mg/L Kin+3.0 mg/L IAA. The maximum regeneration frequency (87.34 ± 0.27%) in Sambha mahsuri with kinetin (2.5 mg/L) and 2, 4-D (3.5 mg/L).

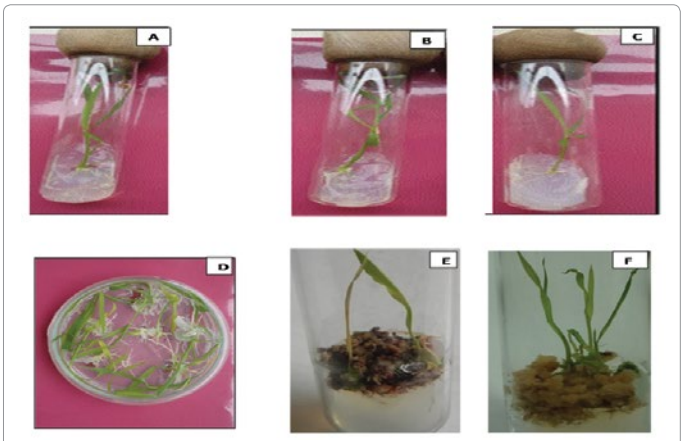

Figure 4: Regenerated plantlets in selected cultivars of indica rice (without stress): A) PR-115; B) PR-116; C) Sambha mahsuri; D) Erramallelu; E) Cotton dora sannalu; F) Pothana.

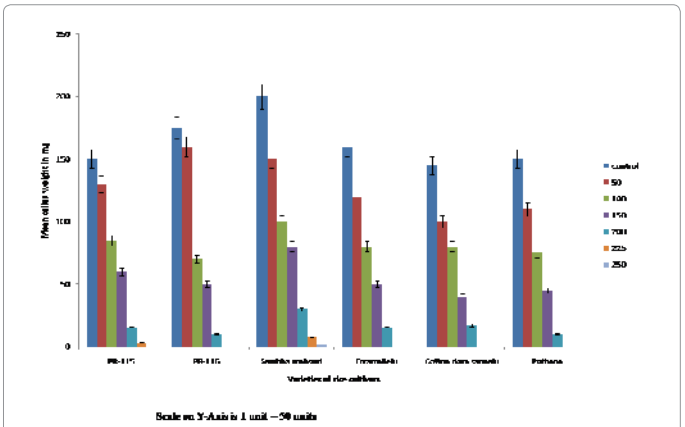

Figure 5: Callus fresh weight (mean ± SE) in experimental cultivars of indica rice with different concentrations of NaCl stress. Maximum callus growth in Sambha mahsuri at 50 mM while moderated to mild growth in other rice cultivars. No growth of callus at 250 mM in rice cultivars except Sambha mahsuri (minimum callus growth).

indica rice. No regeneration was observed at the treatment of 200 and 225 mM NaCl in the cultivars of indica rice except Sambha mahsuri, which responded very poor regeneration growth (5.16 ± 0.59%) (Figure 7). The observed results revealed that the addition of above 50 mM NaCl in the regeneration medium could not lead to the regeneration of plantlets in all selected cultivars of indica rice. Even at 50 mM NaCl concentration, the poor root system was observed in PR-115 and Cotton dora sannalu but, the Sambha mahsuri responded more optimistically in relation the regeneration of roots (Figure 8).

Salinity is one of the major abiotic stresses that adversely affect the overall physiological activities and cause the death of plants [23]. Production potentialities of certain agronomic crops are reported to be reduced in saline soils [24]. Worldwide more than 30 million hectares of irrigated land have already been damaged by increasing salinity [25]. The regeneration frequency was higher in Sambha mahsuri while lower in other rice cultivar in 50 mM NaCl supplemented MS medium. There was normal plant regeneration frequency observed in salt free culture medium but by increasing the NaCl concentration in medium, plant regeneration frequency of experimental cultivars of indica rice was reduced. No callus induction and plant regeneration was recorded at the treatment of 200 and 225 mM NaCl in the cultivars of indica rice but very poor response observed in Sambha mahsuri.

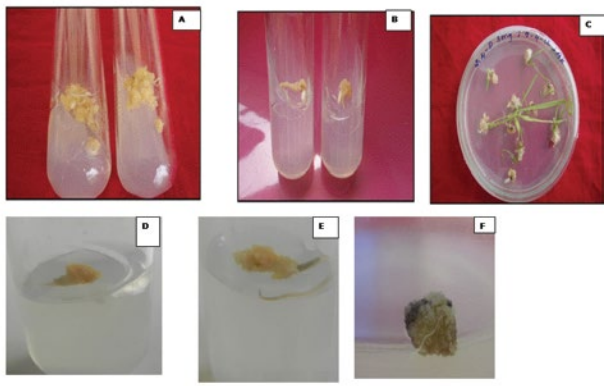

Figure 6: Morphological characters of induced callus under NaCl stress: A) PR-115; B) PR-116; C) Sambha mahsuri; D) Erramallelu; E) Cotton dora sannalu; F) Pothana.

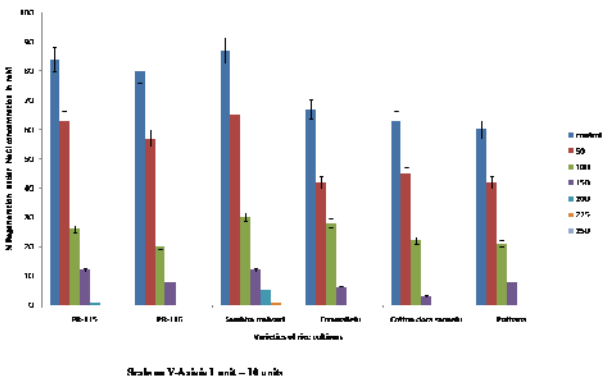

Figure 7: Regeneration frequency (%) of different rice cultivars with different concentrations of NaCl (mM). Except Sambha mahsuri, no regeneration in other rice cultivars at 225–250 mM NaCl concentration.

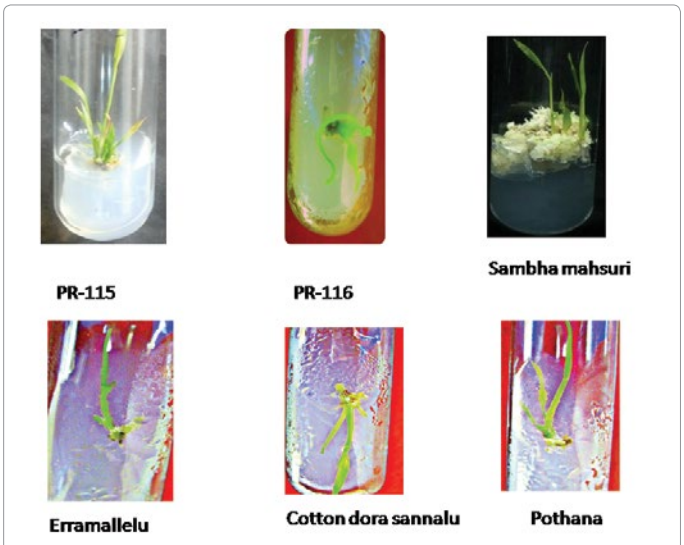

Figure 8: Morphological appearance of under stress of NaCl at 50 mM concentration.

Conclusion

Salinity area is increasing every year in India and demand for food grain increasing day by day. Therefore, it is an urgent need of modern times to develop new rice cultivars of indica rice that could grow stronger under saline conditions if the countries are to sustain production of rice. Hence, we conducted this study to screen the callus induction and regeneration potentialities of experimental cultivars under NaCl stress conditions. As per our observed results, the Sambha mahsuri calli showed poor growth response but showed high regeneration frequency in saline conditions.

Acknowledgement

Authors are grateful to CII (confederation of Indian industry), DST (Department of Science and Technology, Ministry of Science and Technology, New Delhi, India) for providing the Prime Minister's Fellowship (S Reddy). Similarly, Shri Neeraj Verma, Director In-charge (State Farms Corporation of India Limited) for authenticating and providing 2 indica rice cultivars and Dr. C Cheralu, Associate Director Agricultural Research Station, Warangal, India for authenticating and providing 4 indica rice cultivars for our present study.

References

1. Molina J, Sikora M, Garud N, Flowers JM, Rubinstein S, et al. (2011) Molecular evidence for a single evolutionary origin of domesticated rice. Proc Natl Acad Sci USA 108: 8351-8356.
2. Ramesh M, Murugiah V, Gupta AK (2009) Efficient in vitro plant regeneration via leaf base segment of indica rice (Oryza sativa L.). Indian J. Exp. Biol 47: 68-74.
3. Khush GS (2005) What it will take to feed 5.0 billion rice consumers in 2030. Plant Mol. Biol 59: 1-6.
4. Krishnan SR, Priya AM, Ramesh M (2013) Rapid regeneration and ploidy stability of 'cv IR36' indica rice (Oryza sativa L.) confers efficient protocol for in vitro callus, organogenesis and Agrobacterium tumefaciens-mediated transformation. Bot. Studies 54: 47.
5. Hoque ME, Ali MS, Karim NH (2007) Embryogenic callus induction and regeneration of elite Bangladeshi indica rice cultivars. Plant Tiss. Cult. Biotechnol 17: 65-70.
6. 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 Tiss. Org. Cult 71: 237-244.

7. Rafique MZ, Rashid H, Chaudhary MF, Chaudhary Z, Cheema NM (2011) Study on callogenesis and organogenesis in local cultivars of rice (Oryza sativa L.). Pakistan J. Bot 43: 191-203.
8. Rajkumar R (2013) A study on effect of salt stress in the seed germination and biochemical parameters of rice (Oryza sativa L.) under in vitro conditions. Asian J. Plant Sci. Res 3:20-25.
9. Roly ZY, Islam MM, Shaekh MPE, Arman MSI, Shahik SM, et al. (2014) In vitro callus induction and regeneration potentiality of aromatic rice (Oryza sativa L.) cultivars in different growth regulators. Int J Appl Sci Biotechnol 2: 160-167.
10. Theerakulpisut P, Bunnag S, Kong-ngern K (2005) Genetic diversity, salinity tolerance, physiological responses to NaCl to six rice (Oryza sativa L.) cultivars. Asian J. Plant Sci 4: 562-573.
11. Sah SK, Kaur A, Sandhu JS (2014) High frequency embryogenic callus induction and whole plant regeneration in Japonica rice cv. Kitaeke. J. Rice Res 2: 125.
12. Zahid MN, Hasan M, Adil M, Hossain MM, Mian MAK (2014) In vitro screening for salt tolerance in aromatic rice genotypes. Open Sci. J. Biosci. Bioeng 1: 28-32.
13. Blumwald EA, Grover A (2006) Salt tolerance in plant biotechnology: current and future uses of genetically modified crops. John Wiley & Sons Ltd, UK.
14. Hasan M, Sarker RH (2013) In vitro selection for NaCl salt tolerance in aromatic rice genotypes. Indian J Agric Sci 83: 109-114.
15. Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassay with tobacco tissue cultures. Physiol Planta 15: 473-497.
16. Mannan MA, Sarker TC, Akhter MT, Kabir AH, Alam MF (2013) Indirect plant regeneration in aromatic rice (Oryza sativa L.) var. 'Kalijira' and 'Chinigura'. Acta Agric Slov 101: 231-238.
17. Larkin PJ, Scrowcroft WR (1981) Somaclonal variation a novel source of variability from cell culture for plant improvement. Theor Appl Genet 60: 197-214.
18. Negrao S, Almadanim MC, Pires IS, Abreu IA, Maroco J, et al. (2013) New allelic variants found in key rice salt-tolerance genes: an association study. Plant Biotechnol J 11: 87-100.
19. Ray DK, Mueller ND, West PC, Foley JA (2013) Yield trends are insufficient to double global crop production by 2050. PLoS One 8: e66428.
20. Jeong JS, Kim YS, Redillas MCFR, Jang G, Jung H, et al. (2013) OsNAC5 over expression enlarges root diameter in rice plants leading to enhanced drought tolerance and increased grain yield in the field. Plant Biotechnol. J 11: 101-114.
21. Lin YJ, Zhang Q (2005) Optimizing the tissue culture conditions for highly efficiency transformation of indica rice. Plant Cell Rep 23: 540-547.
22. Agrawal PK, Kohli A, Twyman RM, Christou P (2005) Transformation of plants with multiple cassettes generates simple transgene integration patterns and high expression levels. Mol Breed 16: 247-260.
23. Raychaudhary A, Basu S, Sarkar SN, Sengupta DN (2008) Comparative physiological and molecular responses of a common aromatic indica rice cultivar to high salinity with non-aromatic indica rice cultivars. Plant Cell Rep 27: 1395-1410.
24. Rani CR, Reema C, Alka S, Singh PK (2012) Salt tolerance of Sorghum bicolor cultivars during germination and seedling growth. Res J Recent Sci 1: 1-10.
25. Xiong L, Zhu JK (2001) Abiotic stress signal transduction in plants: molecular and genetic perspectives. Physiol Planta 112: 152-166.