Pranjali Anand et al. / IJIDD / Vol 5 / Issue 2/ 2015 / 77-81.

International Journal of

Innovative Drug Discovery

e ISSN 2249 - 7609
Print ISSN 2249 - 7617

www.ijidd.com

STUDIES ON OPTIMIZATION OF PROTOCOL FOR SOMATIC EMBRYOGENESIS AND REGENERATION OF RICE (APMS – 6B)

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ABSTRACT

Establishment of an efficient tissue culture *in – vitro* protocol is an essential prerequisite in harnessing the advantage of cell and tissue culture for genetic improvement. The application of advanced tissue culture techniques may lead to new avenues in crop improvement. Rice is an important cereal crop and is the primary source of food. Present study was aimed to develop an efficient and reproducible regeneration system through somatic embryogenesis from scutellar embryo derived calli of rice i.e. APMS – 6B. Calli were maintained and after some weeks by subculturing they were transferred onto modified MS and N6 media and supplemented with different concentration of BAP and NAA. Shoot regeneration percent for somatic embryogenesis was successfully obtained and transplantation was performed.

KEY WORDS: Somatic embryogenesis, Plant regeneration, Callus, Shoot regeneration.

INTRODUCTION

The global population is steadily growing while the amount of arable land is steadily decreasing. Thus, it is essential that sustainable strategies be implemented to use agricultural resources efficiently to yield an abundant healthy diet[1].Rice is the target crop for many improvement programmes because it is the staple diet for nearly two billion people worldwide and the major food for over half of those living in Asia [2]. It is feared that world population would be around 10 billion by 2050. Thus more food will be required to feed the human population. This will be in the backdrop of diminishing cultivated land. Studies are underway to increase yield as well as quality of rice. The available cultivable land is being utilized for non-agricultural purposes [3, 4].

Attacks by pests and insects are responsible for decrease in production. Thus there is a constant need to improve crops to overcome all these hazards [5]. Somatic embryogenesis in rice have been reported from culture of leaf tissue, root tissue, inflorescence and protoplast [6]. Organogenic capacity of callus tissues depends upon the plant species, type of explant from which the callus was derived, age of callus tissue and composition of the nutritional medium. Another important factor is nature and level of various growth regulators [7]. Plant cells are unique in that they retain totipotency and developmental plasticity in the differentiated state and have the ability to dedifferentiate, proliferate, and subsequently regenerate into mature plants under appropriate culture conditions in a hormone-dependent manner [8].

Tissue Culture now is a common way to propagate crop plants of commercial importance. The original expectation was that all plants regenerated from cell or tissue culture has a genetic constitution identical to that of the original one [9]. The term ‘somaclonal variation’ refers to tissue culture induced stable genetic, epigenetic or phenotypic variation in clonally propagated plant populations.

These somaclonal variations generated in vitro have been efficiently exploited in developing new varieties with superior agronomic traits in diverse species [10]. Plant stem cells naturally present in the root and shoot apex of intact plants are considered ‘pluripotent’, since they are able to form cell and tissue types present in either root or shoot tissue.

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Factors influencing in vitro adaptability and regeneration are varied, ranging from genotype, origin of explant, culture conditions, and hormonal effects. Establishment of stable, efficient in vitro regeneration systems in economically important crops is a prerequisite for biotechnology and molecular breeding applications [11]. Genetic changes frequently associated with in vitro regenerated plants lead to stable, lasting modifications to the genome that are inherited in subsequent generations. Some of these molecular changes are associated with phenotypic differences and hence referred to as somaclonal variations. This is an *in-vitro* induced variation method has been exploited as a technology to develop new cultivars with improved and desirable agronomic traits such as yield, early maturity, and resistance to biotic and abiotic stresses The somaclonal variation method has been employed successfully for desirable traits such as herbicide tolerance , drought and abiotic stress tolerance and disease resistance. This technology is particularly relevant in asexually propagated plants and self pollinated crops with a narrow genetic base. Mutant selection, anther and pollen culture, and somatic hybridization are techniques that may be useful in rice improvement. The feasibility of these approaches is solely dependent upon the availability of a tissue culture system for the regeneration of a particular cultivar. Systems for in vitro regeneration of a number of rice cultivars have been established [12].

The totipotent character of plant cells allow that any differentiated cells that retains its nucleus has the ability to regenerate an entire new plant by organogenesis or somatic embryogenesis (SE). SE is the developmental process by which bipolar structures that resemble zygotic embryos are developed from haploid or diploid somatic cell through an orderly embryological stage without gametes fusion.Two types of somatic embryogenesis are recognized: direct somatic embryogenesis (DSE) and indirect somatic embryogenesis (ISE). DSE is characterized by the induction of somatic embryos directly from pro-embryogenic cells from leaves, stem, microspores or protoplasts without the proliferation of calli, whereas in ISE somatic embryos are developed from friable embryogenic calli [13] . Somatic embryogenesis is a unique process in plants and it is of remarkable interest for biotechnological applications such as clonal propagation, artificial seeds and genetic engineering. Precisely, when somatic embryogenesis is integrated with conventional breeding programs and molecular and cell biological techniques, it provides a valuable tool to enhanced genetic improvement of crop species [14, 15].

MATERIALS AND METHODS

Explants Collection-

Explant material for this research work were rice seeds variety APMS -6B obtained from DRR (ICAR) Hyderabad (A.P.). Rice caryopses containing scutellar region of embryo, were isolate by removing lemma and palea from the seeds.

Surface Sterilization of Seeds

Rice caryopses were sterilized using the caryopses were sterilized using 70% alcohol for 3 minute, followed by shaking in 30% Clorox containing 2-3 drop of Tween-20 on an orbital shaker, at 120 rpm for 20 minute. Finally, the explants were rinsed with sterile double distilled water for 6 times and cultured onto the medium with the different treatments tested in the study.

Preparation of Media

Two basic media used in this study, first one was half MS (Murashige and Skoog, 1962) supplemented with 500 mgL^{-1} (w/v) of glutamine, 100 mgL^{-1} (w/v) of proline. Second one was N6 medium supplemented with 500 mgL-1(w/v) L – glutamine. Both the media were solidified with 0.2% (w/v) of agar. The pH of the media was adjusted to 5.8.

Callus Induction Media

Different concentrations of 2,4-D [0.1, 1.5, 2.5, 3.5 and 5 mgL^{-1} (w/v)] were used as the treatments for embryogenic callus induction. These cultures were then kept at 25 ± 2ºC in the growth room (incubation room) in a dark condition for one week and followed by transferring the cultures under 16 hours lighting, provided by fluorescent bulbs with 15.75 $\mu molm^{-2}s^{-1}$ light intensity until the eighth week of culture.

Somatic Embryo Germination Media

MS medium containing different concentrations of BAP (0, 1, 2, 3, 4and 5 mgL^{-1}), in combination with different concentrations of NAA (0, 0.5, 1.0, 1.5, 2.5 and 4.0 mgL^{-1}) were used as treatments for the germination of somatic embryos. The cultures were kept at 25 ± 2ºC in the growth culture, with 16 hours of light, provided by fluorescent bulbs and a light intensity of 16.75 $\mu molm^{-2}s^{-1}$ for eight weeks.

Calculation

During data collection callus induction frequency was recorded considering that each callus piece originated from a single seed. Regenerated plantlets were counted based on the number of callus-producing plantlets. The frequency of callus induction and plant that of regeneration were calculated as follows:

Callus induction frequency (%) =

$$\frac{\text{Number of seeds produced calli}}{\text{Number of seeds inoculated}} \times 100$$

The percent of explants which responded to form embryogenic callus or plant regeneration frequency % was calculated as :

Plant regeneration frequency (%) =

$$\frac{\text{Number of regenerated calli}}{\text{Number of calli incubated}} \times 100$$

Figure 1. Seed inoculation in MS media

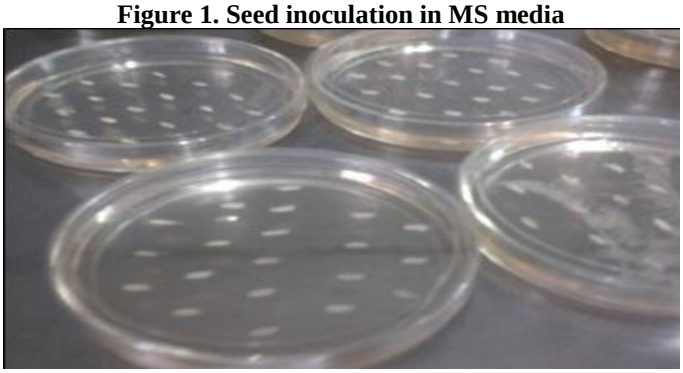

Figure 2. Callus formation by 2, 4-D

Figure 3. Shoot induction by differ. Conc. Of BAP and NAA

Figure 4. Transplantaion

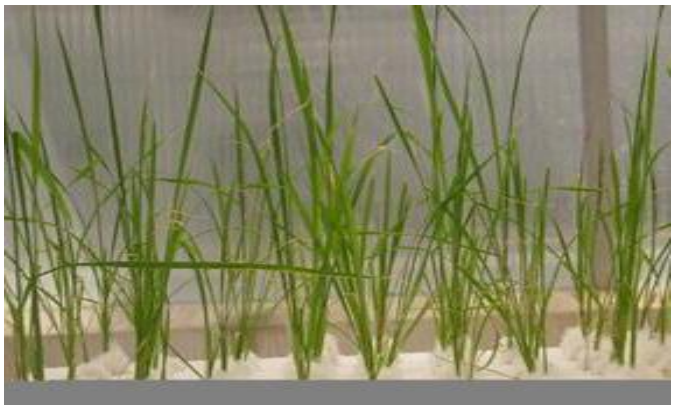

Table 1. Callus induction percent of rice for Somatic Embryogenesis

S.No.	Conc. Of 2,4-D (mgL^{-1})	Callus Induction Frequency % from rice
1	0	No Callus
2	1.0	76 ± 35
3	1.5	80 ± 40
4	2.5	88 ± 45
5	3.5	95 ± 30
6	5.0	86 ± 45

Table 2. Effect of PGRs in rice

S.No.	Conc. Of NAA (mgL^{-1})	Shoot Induction %	No. of Shoots
1	0	31.33	2.6 ± 0.48
2	0.5	25.65	2.5 ± 0.64
3	1.0	33.45	3.0 ± 0.54
4	1.5	41.60	3.5 ± 0.64
5	2.5	45.60	4.0 ± 0.59
6	4.0	48.55	4.5 ± 0.60

Table 3. Effect of PGRs in rice

S.No.	Conc. Of BAP (mgL^{-1})	Shoot Induction %	No. of Shoots
1	0	30.33	2.0 ± 0.87
2	1	23.45	1.8 ± 0.48
3	2	31.85	2.2 ± 0.16
4	3	40.68	3.0 ± 0.18
5	4	38.67	2.5 ± 0.64
6	5	35.45	2.4 ± 0.35

Table 4. Effect of BAP + NAA

S.No.	BAP + NAA (mgL^{-1})	Shoot Induction %	No. Of Shoots
1	1 + 0.5	26.85	2.1 0.63
2	2 + 1.0	29.65	2.5 0.83
3	3 + 1.5	39.60	3.5 0.54
4	4 + 2.0	35.45	3.2 0.45
5	5 + 4.0	30.40	3.0 0.54

RESULTS AND DISCUSSIONS

According to the observation, the callus started to grow from scutellar embryo of rice, after three days of culture. Then embryo derived callus subsequently started to enlarge and some yellowish to greenish nodules grew around the explants after ten days of culture. After 2 months of culture, calli almost covered the explants surface. Figure showing the embryos formed during the experiments from calli.Seed inoculation, callus formation, shoot induction and transplantaion are shown in figure 1,2,3,4.

The somatic embryos were then transferred onto the MS medium containing different concentrations of plant growth regulators. For callus induction MS medium supplemented with different concentrations of 2,4-D(0.1.0, 1.5, 2.5, 3.5 and 5 mgL^{-1} (w/v) was used in which 3.5 5 mgL^{-1} 2,4 -D showed high callus induction percentage. Our result were in agreement with the result of Panjaitan *et al.*, 2009, Revathi and Arumugam, 2011, Verma *et al*., 2011, Islam *et al*., 2009. Who found about similar results in rice [16-19].

Table -1 shows that the media containing different 2,4-D concentrations on the percentage of explants forming callus after the eighth week of culture. The presence of different 2,4-D concentrations in the media tested gave significant response for 95% callus formation wherever the absence of 2,4-D (control) inside the media did not produce any callus. The result showed that the addition of 2,4-D upto 3.5 mgL^{-1} in modification of media showed an increment on the callus formation frequency but increased concentration of 2,4 –D more than 3.5 mgL^{-1} decreased the callus formation percentage.

MS medium supplemented with 0.8% agar , 70g/L sucrose , 4g/L casein , 1mg/L BAP and 3mg/L NAA was used for derived calli. It was noted that the plant regeneration ability of plated calli depends on the variety and the callus inducing media. It can be observed from table-2 and table-3 that 3mgL^{-1} BAP concentration showed good results and 4 mgL^{-1} of NAA concentration show good results concentration of BAP and NAA for plantlet regeneration was used to obtain more good results.

MS medium supplements with conc. of 0 , 1, 2, 3, 4 and 5 mgL^{-1} was used whereas NAA was used in conc. of 0, 0.5, 1.0, 1.5, 2.0 and 3.0 mgL^{-1}. Combination of BAP and NAA(table-4) was used as (1.0 BAP + 0.5 NAA), (2.0 BAP +1.0 NAA), (3.0 BAP + 1.5NAA), (4.0 BAP + 2.5 NAA) mgL^{-1} and (5.0 BAP + 4.0 NAA).

Result showed that combination of 3 BAP + 1.5 NAA mgL^{-1} showed highest results further combination increased cause the decrement of percent of shoot induction.

CONCLUSION

Somatic embryogenesis is an efficient plant regeneration system and it is a potentially useful tool for genetic transformation .This study was taken out to obtain somatic embryogenesis from callus of scutellar embryo of rice APMS – 6B .This work reported a successful rice high frequency regeneration protocol from scutellar embryo through somatic embryogenesis.2,4-D played most dominant role in somatic embryogenesis because its role is in cell devision and to increase the rate of cell division and its attributes to increased amount of callus.

As demonstrated in India, for rice somatic embryogenesis can be used to increase rice production with such consequent positive benefits as generating rural employment, land savings and women's empowerment. Experts have predicted that demand for hybrid rice technology in the India will increase as a result of the country's high labour-to-land ratio and the high proportion of irrigated rice land. India government launched a national hybrid rice programme that aims at utilizing hybrid rice technology as a new approach to increasing national rice production and the productivity and competitiveness of Philippine farmers over the short term, and for attaining national rice self-sufficiency and food security over the long term. Regeneration of rice with somatic embryogenesis is the only process of plant biotechnology, which is being utilized for a large production of fully resistance from abiotic, biotic stress and completly disease free rice plant. The chief advantage of somatic embroygenesis for invitro regeneration of rice plant is the extremely high multiplication rates, e.g., 10^6 plants year from a single explants, it can be carried out throughout the year independent of seasons .Therefore, this technique is highly suited for rapid multiplication of rare genotypes, and of plants having low multiplication rates.

ACKNOWLEDGEMENT

Authors greatefully acknowledge the Director of D.R.R. Indian Agriculture Research Institute, Hyderabad (A.P.) for providing plant tissue culture facilities and for his constant encouragement.

REFERENCES

1. Tu JM, Zhang GA, Xu CG, He YQ, Zhang QF, Khush GS, Datta SK. Field performance of transgenic elite commerical hybrid rice expressing Bacillus thuringiensis δ– endotoxin. *Nat Biotech*, 18, 2000, 1101–1104.
2. Heyser JW, Dykes TA, Demott KJ, Nabors MW. High frequency, long-term regeneration of rice from callus culture. *Plant Sci*, 29, 1983, 175-182.
3. Oard JH, Rutger JN.Callus induction and plant regneration in elite U.S. rice lines. *Crop Sci*, 28, 1988, 565-567.
4. Raghava R, Nabors MW. Cytokinin mediated longterm, high-frequency plant regeneration in rice tissue cultures. *Z. Pflanzenphysiol. Bd*, 113, 1984, 315-323.
5. Siriwardana S, Nabors M. Tryptophan enhancement of somatic embryogenesis in rice. *Plant Physiol*, 73, 1983, 141-146.
6. Wernicke W, Brettell R, Wakizuka T, Potrykus I. Adventitious embryoid and root formation from rice leaves. *Z. Pflanzenphysiol*, 103, 1981, 361-365.
7. Abe T , Futsuhara Y. Efficient plant regeneration by somatic embryogenesis from root callus tissue of rice (*Oryza sativa* L.). *J. Plant Physiol*, 121, 1985, 111-118.
8. Chen T, Lau L , Chen S. Somatic embryogenesis and plant regeneration from cultured young inflorescences of *Oryza sativa* L. *Plant Cell, Tissue and Organ Culture*, 4, 1985, 51-54.
9. Yamada Y, Yang ZQ, Tang DT. Plant regeneration from protoplast derived callus of rice. *Plant Cell Report*, 5, 1986, 85-88.
10. Rueb S, Leneman M, Schilperoort RA, Hensgens LAM. Efficient plant regeneration through somatic embryogenesis from callus induced on mature rice embryos (*Oryza sativa* L.). *Plant Cell, Tissue and organ Culture*, 36(2), 1994, 259-264.
11. De Block M, De Brouwer D, Tenning P. Transformation of Brassica napus and Brassica oleracea using Agrobacterium tumefaciens and the expression of the *bar* and *neo genes* in the transgenic plants. *Plant Physiol*, 96, 1989, 694-701.
12. Hagio T, Blowers AD, Earle ED, Berlin WG. Stable transformation of sorghum cell cultures after bombardment with DNA-coated Micro projectiles. *Plant Cell Rep*, 10, 1991, 260-264.
13. Hebert D, Kikkert JR, Smith FD, Reisch BI, Berlin WG. Optimization of biolistic transformation of embryogenic grape cell suspensions. *Plant Cell Rep*, 12, 1993, 585-589.
14. Koprek T, Hansch R, Nerlich A, Mendel RR , Schulze J. Fertile transgenic barley of different cultivars obtained by adjustment if bombardment conditions to tissue response. *Plant Science*, 119, 1996, 79-91.
15. McCabe DE, Swain WF, Martinel BJ, Christou P.Stable transformation of soybean *Glycine max*) by particle acceleration. *Bio/Technology*, 6, 1988, 923-926.
16. Panjaitan SB, Abdullah SNA, Aziz MA, Omar O.Somatic Embryogenesis from Scutellar Embryo of *Oryza sativa* L. var. MR219 Pertanika. *J. Trop. Agric. Sci*, 32(2), 2009, 185-194.
17. Verma D, Joshi R, Shukla A and Kumar P.Protocol for invitro somatic embryogenesis and regeneration of rice (*Oryza* sativa L.). *Indian journal of experiment Biology*, 49, 2011, 958-963.
18. Islam MM, Washed SA, Khan SAKU. Studies on Callus Induction and Regeneration from Dehusked Rice (*Oryza sativa* L.) Seeds. *Plant Tissue Cult*, 14(2), 2004, 155-160.
19. Revathi S, Pillai A. Invitro callus induction in rice, (*Oryza Sativa* L.) *Research in plant Biology*, 1(5), 2011, 13-15.