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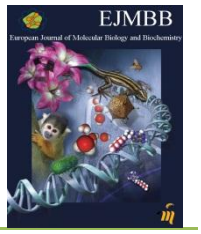

STUDIES ON OPTIMIZATION OF PROTOCOL FOR SOMATIC EMBRYOGENESIS AND REGENERATION OF RICE (APMS – 6B)

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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.

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 has 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 soma clonal variations generated in vitro have been efficiently exploited in developing new varieties with superior agronomic traits in diverse species

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[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. 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 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 (%)

$$= \frac{\text{Number of grains producing calli}}{\text{Number of grains plated}} \times 100$$

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

Plant regeneration frequency (%)

= Number of grains producing calli × 100
Number of grains plated

RESULTS AND DISCUSSION

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. The embryos formed during the experiments from calli are shown in figure.

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 was 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, 17, 18, 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 te plant regeneration ability of plated calli depends on the variety and the callus inducing media. It can be observed from table-2 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 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.

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 Transplantation 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 Transplantation 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

Fig 1. Seed inoculation in MS mediu

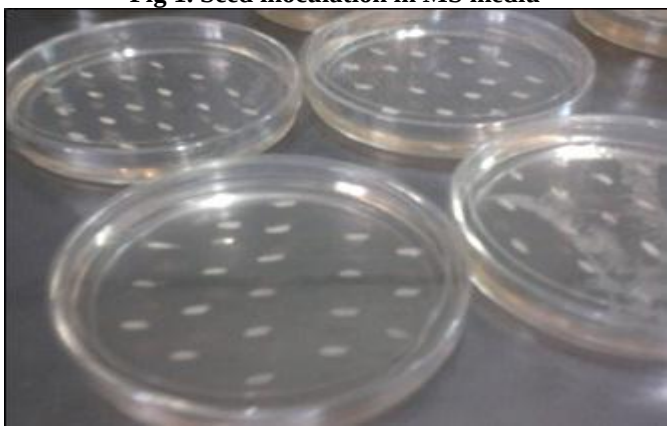


Fig 2. Callus formation by 2, 4-D

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

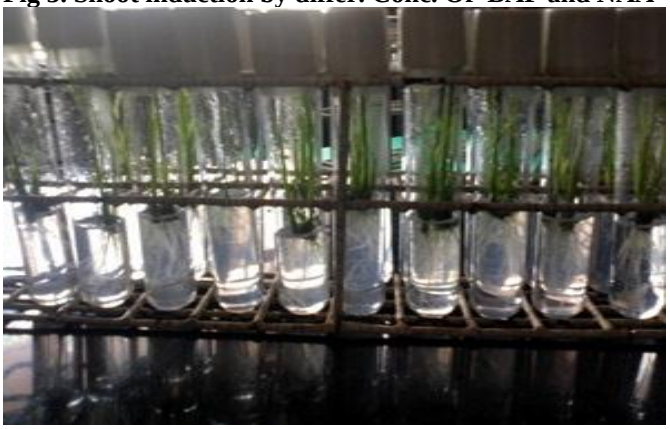
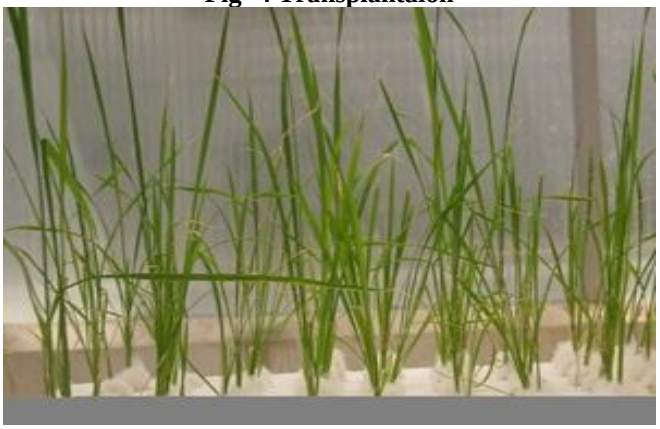


Fig -4 Transplantaion



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 and BPT – 5204.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 division and to increase the rate of cell division and its attributes to increased amount of callus.

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