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ORIGINAL RESEARCH ARTICLE

Optimization of *in vitro* regeneration protocol for a popular Indica rice (*Oryza sativa* L. cv Swarna)

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Abstract: Though regeneration system in rice has been very well established compare to other crop plants, the fact remains that, most of the *indica* rice varieties are still recalcitrant for regeneration and genetic transformation. Therefore, refinement of tissue culture protocol for generation of embryogenic calli and regeneration of the fertile plants from a single cell should be a pre requisite event for development of transgenic plants. Here, in this study we reported high frequency robust regeneration protocols for a popular *Indica* cultivar Swarna.Mature seeds were used as initial material as explants. Highest callus induction % was observed in MSCIMP medium containing 2.0 mg^{-1} 2,4, D + 0.5 mg^{-1} Kn as phytohormonal combinations. In addition, maximum regeneration was observed in 2.0 mg^{-1} KN + 0.5 mg^{-1} NAA. Regenerated plants were shifted to rooting medium followed by polyhouse for hardening. The callus induction and regeneration reported in this study were well suited for transformation agronomical important genes or functional genomics studies.

Key words: *Oryza sativa*; Somatic embryogenesis; Swarna; Variety

Introduction

More than half of the world's population depends on rice for carbohydrate source and rice is cultivated worldwide under diverse agro-climatic conditions around 160 Mha with production capacity of 745 million tons (FAOSTAT). The majority of the rice production comes from developing countries in Asia, Africa, and Latin America. About 96.24% of total rice production in the world is contributed by developing countries that are grown by small and marginal farmers with minimum inputs and infrastructure. In general, rice is a water loving crop, it grows in all ecologies such as irrigated, upland, lowland and submerged conditions with frequent irrigation facilities.

Growth and production of rice are limited by a variety of abiotic and biotic stresses, of which drought and salinity stress are major abiotic constraints that limit substantial rice productivity across the world (Zhou *et al.,* 2007; Shobbar *et al.,* 2010). Genetic engineering has become a viable alternative to overcome these problems associated with narrow genetic base and sexual incompatibility of classical breeding to develop the resistant/tolerant lines. tissue culture is the pre requisite event for generation of transgenic plants. though regeneration in rice is well established the fact remains that, most of the indica cultivars are recalcitrant in nature. Therefore, refinement of tissue culture protocol for generation of embryogenic calli and regeneration of the fertile plants from a single cell should be a pre requisite event for development of transgenic plants (Toki *et al.,* 2006).

Materials and Methods

Plant material and culture conditions

Seeds of Swarna were collected from Indian Institute of Rice Research, Hyderabad. MSB_5 medium with appropriate carbon source and gelling agent were used for all the experiments. pH of the medium was adjusted to 5.8±0.1 prior to the addition of gelling agents and autoclaved at 121°C for 15 min at 15 psi pressure. The cultures were maintained at 25±2°C under a 16/8 h light/dark photo period supplemented with cool white fluorescent lamps at an intensity of 85µ mol m^{-2} s^{-1}.

Seed surface sterilization

Dehusked healthy, uniform and good quality seeds of Swarna were initially washed 2-3 times with sterile distilled water for removing dust and other visible floating particles. Seeds were allowed for fungicide treatment (1% (w/v) Bavistin) about 3 min and then they were surface sterilized with 70 % (v/v) ethanol for 1 min followed by 0.1% (w/v) Mercuric chloride ($HgCl_2$) for 4 min. Mercuric chloride was decanted within the laminar air flow chamber and the seeds were thoroughly washed with sterile distilled water for 4-5 times to remove

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any residual disinfectant. Sterilized seeds were blot dried on sterile filter papers and inoculated on MSB_5 basal medium supplemented with various concentrations and combinations of hormones, gelling agents and carbon sources. About 20 seeds were inoculated per each petri plate.

Optimization of media composition for callus induction

For callus induction, various concentrations and combination of hormones, carbon sources and solidifying agents were tried along with MSB_5 basal medium. Different concentrations of 2, 4-D (1.0, 2.0, 3.0 mg^{-l}) along with kinetin (0.5, 1.0 mg^{-l}) was tried for callus induction. A total of 10 different types of media were tried with varying concentrations and combinations of hormones (MSCI-1 to MSCI-6), carbon sources (MSCIM and MSCIS) and gelling agents (MSCIM-agar and MSCIM-phytagel) for callus induction. The composition of all the 10 types of media has listed in Table 1. Based on the callus induction %, fresh weight of calli and morphology of calli, best media was selected. In all the media, irrespective of the carbon source, gelling agent and hormonal combination, 0.1 g^{-l} of myo-inositol, 0.5 g^{-l} casein enzymatic hydrolysate and 0.6 g^{-l} L-proline were included. The pH of the media was adjusted to 5.8 before addition of the solidifying agents and it was sterilized by autoclaving at 121ºC, 15 psi pressure for 15min. Callus induction frequency was recorded after 15 days of inoculation. All the cultures were incubated at 25±2ºC under stable 16/8 h light/dark photoperiod. Frequency of callus induction was calculated based on the following formula and amount of calli in terms of fresh weight were also recorded.

$$\text{Callus induction \%} = \frac{\text{No. of seeds with callus}}{\text{Total no. of seeds inoculated}} \times 100\%$$

After 15- 18 day based on their texture and colour of the calli they were selected and fragmentized into small pieces using sterile scalpel and transferred to the fresh callus induction media for a period of 7-10 days to get more embryogenic calli.

Table 1: Composition of different types of media for callus induction of Swarna

Media	Composition
MSCI-1	MSB_5 + 1.0 mg^{-l} 2, 4, D + 0.5 mg^{-l} KN + 0.1 g^{-l} myo-inositol + 0.5 mg^{-l} casein enzymatic hydrolysate + 0.6 g^{-l} L-proline + 30 g^{-l} sucrose + 8 g^{-l} agar
MSCI-2	MSB_5 + 1.0 mg^{-l} 2, 4, D + 1.0 mg^{-l} KN + 0.1 g^{-l} myo-inositol + 0.5 g^{-l} casein enzymatic hydrolysate + 0.6 g^{-l} L-proline + 30 g^{-l} sucrose + 8 g^{-l} agar
MSCI-3	MSB_5 + 2.0 mg^{-l} 2, 4, D + 0.5 mg^{-l} KN + 0.1 g^{-l} myo-inositol + 0.5 g^{-l} casein enzymatic hydrolysate + 0.6 g^{-l} L-proline + 30 g^{-l} sucrose + 8 g^{-l} agar
MSCI-4	MSB_5 + 2.0 mg^{-l} 2, 4, D + 1.0 mg^{-l} KN + 0.1 g^{-l} myo-inositol + 0.5 g^{-l} casein enzymatic hydrolysate + 0.6 g^{-l} L-proline + 30 g^{-l} sucrose + 8 g^{-l} agar
MSCI-5	MSB_5 + 3.0 mg^{-l} 2, 4, D + 0.5 mg^{-l} KN + 0.1 g^{-l} myo-inositol + 0.5 g^{-l} casein enzymatic hydrolysate + 0.6 g^{-l} L-proline + 30 g^{-l} sucrose + 8 g^{-l} agar
MSCI-6	MSB_5 + 3.0 mg^{-l} 2, 4, D + 1.0 mg^{-l} KN + 0.1 g^{-l} myo-inositol + 0.5 g^{-l} casein enzymatic hydrolysate + 0.6 g^{-l} L-proline + 30 g^{-l} sucrose + 8 g^{-l} agar
MSCIM	MSB_5 + 2.0 mg^{-l} 2, 4, D + 0.5 mg^{-l} KN + 0.1 g^{-l} myo-inositol + 0.5 g^{-l} casein enzymatic hydrolysate + 0.6 g^{-l} L-proline + 30 g^{-l} maltose + 8 g^{-l} agar
MSCIS	MSB_5 + 2.0 mg^{-l} 2, 4, D + 0.5 mg^{-l} KN + 0.1 g^{-l} myo-inositol + 0.5 g^{-l} casein enzymatic hydrolysate + 0.6 g^{-l} L-proline + 30 g^{-l} sucrose + 8 g^{-l} agar
MSCIM-Phytagel	MSB_5 + 2.0 mg^{-l} 2, 4, D + 0.5 mg^{-l} KN + 0.1 g^{-l} myo-inositol + 0.5 g^{-l} casein enzymatic hydrolysate + 0.6 g^{-l} L-proline + 30 g^{-l} maltose + 4 g^{-l} phytagel
MSCIM-Agar	MSB_5 + 2.0 mg^{-l} 2, 4, D + 0.5 mg^{-l} KN + 0.1 g^{-l} myo-inositol + 0.5 g^{-l} casein enzymatic hydrolysate, 0.6 g^{-l} L-proline + 30 g^{-l} maltose + 8 g^{-l} agar
MSCIMP	MSB_5 + 2.0 mg^{-l} 2, 4, D + 0.5 mg^{-l} KN + 0.1 g^{-l} myo-inositol + 0.5 g^{-l} casein enzymatic hydrolysate, 0.6 g^{-l} L-proline + 30 g^{-l} maltose + 4 g^{-l} phytagel

***In vitro* regeneration of plantlets from calli**

To obtain high efficient *in vitro* regeneration of Swarna high proliferating 4 to 5 week old embryogenic calli were taken. A total of six different media combinations tried for regeneration and the composition of media listed in Table 2. Various concentrations of Kn (1.0, 2.0 and 3.0 mg^{-l}) along with different concentrations of NAA (0.5 and 1.0 mg^{-l}) were tried for regeneration efficiency in this study. Sucrose (30 g^{-l}) and phytagel (5 g^{-l}) used as the carbon source and solidifying agent respectively. In addition to the phytohormones, 0.1 g^{-l} of myo-inositol and 2.0 g^{-l} casein enzymatic hydrolysate also included. The pH of the media adjusted to 5.8 before addition of the solidifying agents and sterilized by autoclaving at 121ºC, 15 psi pressure for 15 min. Calli on regeneration media initially kept under dark conditions about one week at 25°C±2 and then transferred to 16/8 h light/dark photoperiod. After shoot buds initiation, they

shifted to MSRM-7 medium for shoot elongation. The regeneration efficiency calculated based on the following formula.

$$\text{Regn. efficiency} = \frac{\text{No. of calli producing regenerants}}{\text{Total no. of calli cultured on regeneration medium}} \times 100\%$$

Table 2: Composition of different type's media for regeneration of Swarna.

Media	Composition
MSRM-1	MSB_5 + 1.0 mg^{-1} KN + 0.5 mg^{-1} NAA + 0.1 g^{-1} of myo-inositol and 2.0 g^{-1} casein enzymatic hydrolysate + 30 g^{-1} sucrose + 5 g^{-1} phytagel
MSRM-2	MSB_5 + 1.0 mg^{-1} KN + 1.0 mg^{-1} NAA + 0.1 g^{-1} of myo-inositol and 2.0 g^{-1} casein enzymatic hydrolysate + 30 g^{-1} sucrose + 5 g^{-1} phytagel
MSRM-3	MSB_5 + 2.0 mg^{-1} KN + 0.5 mg^{-1} NAA + 0.1 g^{-1} of myo-inositol and 2.0 g^{-1} casein enzymatic hydrolysate + 30 g^{-1} sucrose + 5 g^{-1} phytagel
MSRM-4	MSB_5 + 2.0 mg^{-1} KN + 1.0 mg^{-1} NAA + 0.1 g^{-1} of myo-inositol and 2.0 g^{-1} casein enzymatic hydrolysate + 30 g^{-1} sucrose + 5 g^{-1} phytagel
MSRM-5	MSB_5 + 3.0 mg^{-1} KN + 0.5 mg^{-1} NAA + 0.1 g^{-1} of myo-inositol and 2.0 g^{-1} casein enzymatic hydrolysate + 30 g^{-1} sucrose + 5 g^{-1} phytagel
MSRM-6	MSB_5 + 3.0 mg^{-1} KN + 1.0 mg^{-1} NAA + 0.1 g^{-1} of myo-inositol and 2.0 g^{-1} casein enzymatic hydrolysate + 30 g^{-1} sucrose + 5 g^{-1} phytagel
MSRM-7	MSB_5 + 2.0 mg^{-1} KN + 0.5 mg^{-1} NAA + 0.1 g^{-1} of myo-inositol and 2.0 g^{-1} casein enzymatic hydrolysate + 30 g^{-1} sucrose + 4 g^{-1} phytagel

Rooting

The actively growing elongated shoots of approximately 2-3cm in length were kept for rooting on root induction medium containing half strength MSB_5 medium and cultured for two weeks.

Acclimatization

Well rooted shoots were carefully taken out from the culture bottles and washed thoroughly under running tap water to remove any traces of agar and transferred into small nursery covers containing sterilized 1:1 ratio of puddled black soil and sand. These bags were then kept under poly house to minimize transpiration losses. After 7-10 days they were gradually exposed to the open environment. Finally primary hardened plantlets were transferred to earthen or plastic pots (25 litre) containing 1:1 ratio of puddle soil and sand without disturbing their roots and allowed to grown till their maturity in transgenic glass house under controlled conditions.

Statistical analysis

Observations recorded for effect of hormones on % of callus induction, effect of carbon source on % of callus induction, effect of carbon source on fresh weight of callus, effect of gelling agent on % of callus induction, effect of gelling agent on fresh weight of callus and effect of hormones on % of regeneration. All the experiments were repeated thrice with three replicates per each treatment. The data were analyzed by analysis of variance (ANOVA) and means were compared by Duncan's Multiple Range Test (DMRT) at $P \leq 0.05$ using SPSS 10.0.

Results and Discussion

MS medium is widely used medium and forms the basis for many other media formulations. Along with the MS medium, various other factors such as carbon source, growth regulators, organic supplements and gelling agents play critical role in callus induction and plant regeneration, especially in *indica* rice varieties. In fact, it is always difficult to define a universal culture medium for any given genotype/variety, a little refinement of the media composition by alteration of the carbon source, growth regulators and addition of amino acids attempted to produce embryogenic calli and eventually embryogenesis was tested in a popular *indica* rice variety called Swarna.

Effect of hormones on callus induction

Dehusked, surface sterilized Swarna seeds were transferred to callus induction medium (MSCI-1 to MSCI-6). Within 48-72 h, callus induction was observed from scutellar portion of embryo on all the treatments. Rigorous proliferation was observed after 72 h. However, significant variation was observed among the treatments (Table 3). Among all the treatments MSCI-3 observed to be good and resulted in 71.7% of embryogenic calli compared to other treatments. Hence this, MSCI-3 treatment has been used for callus induction medium in all subsequent experiments. Exogenously applied 2, 4, D is responsible for higher levels of IAA through reprogramming of cells to achieve an embryogenic state from a somatic state of a particular cell (Merkle *et al.,* 1995). Among the exogenously applied hormones, auxins played an active role in the conversion of somatic cells to embryogenic cells and 2, 4, D is the best growth regulators for achieving this (Cooke *et al.,* 1993; Khalequzzaman *et al.,* 2005; Sridevi *et al.,* 2005; Tyagi *et al.,* 2007; Hoque *et al.,* 2013). However, in this study, a cytokine, Kn was used along with the auxin 2, 4, D which resulted in generation of higher embryogenic calli compared to cultures grown on 2, 4, D alone. A significant amount of embryogenic calli was noted in 2, 4, D (2.0 mg^{-1}) and Kn (0.5 mg^{-1}) compared to cultures grown on other combinations of phytohormones. Further, Yellowish, compact, friable calli were observed in this combination of media and calli exhibiting these type of morphology often considered as embryogenic calli. Similar observations were also noted by Biswas and Mandal (2007) in indica and japonica cultivars. Concentrations of 2, 4, D and Kn has significant effect on callus induction percentage. For instance,

increase in the concentration of 2, 4, D and Kn has resulted in significant decrease of percent callus induction in both the cultivars tested. Similar observations were noted by Aananthi *et al.,* (2010); Sahoo *et al.,* (2011). The highest percentage of callus induction in Swarna have noted with the concentrations of 2.0 mg^{-1} 2, 4, D and 0.5 mg^{-1} Kn.

Table 3: Effect of hormones on % of callus induction

Media	Hormonal combinations	% of Callus induction
MSCI-1	MSB_5+1.0 mg^{-1} 2, 4, D+0.5 mg^{-1} KN	57.0±1.2e
MSCI-2	MSB_5+1.0 mg^{-1} 2, 4, D+1.0 mg^{-1} KN	54.7±0.7f
MSCI-3	MSB_5+2.0 mg^{-1} 2, 4, D+0.5 mg^{-1} KN	71.7±0.3a
MSCI-4	MSB_5+2.0 mg^{-1} 2, 4, D+1.0 mg^{-1} KN	68.0±0.5b
MSCI-5	MSB_5+3.0 mg^{-1} 2, 4, D+0.5 mg^{-1} KN	63.3±1.2c
MSCI-6	MSB5+3.0 mg^{-1} 2, 4, D+1.0 mg^{-1} KN	61.7±0.7d

Values represent mean ± SE of three replicates (n = 100). Means in a column followed by same alphabet are not significantly different at P<0.05

Table 4: Effect of hormones on % of regeneration

Media	Hormonal combinations	% of Regeneration
MSRM-1	MSB_5+1.0 mg^{-1} KN+0.5 mg^{-1} NAA	24.3±2.0e
MSRM-2	MSB_5+1.0 mg^{-1} KN+1.0 mg^{-1} NAA	28.0±2.1d
MSRM-3	MSB_5+2.0 mg^{-1} KN+0.5 mg^{-1} NAA	47.7±1.4a
MSRM-4	MSB_5+2.0 mg^{-1} KN+1.0 mg^{-1} NAA	44.7±2.6a
MSRM-5	MSB_5+3.0 mg^{-1} KN+0.5 mg^{-1} NAA	39.3±1.7b
MSRM-6	MSB_5+3.0 mg^{-1} KN+1.0 mg^{-1} NAA	34.3±1.4c

Values represent mean±SE of three replicates (n=100). Means in a column followed by same alphabet are not significantly different at P<0.05

Effect of carbon source on callus induction

Effect of various types of carbon sources also studied on callus induction of Swarna. Here, in this study we compared two most predominantly used carbon sources like maltose (30 g^{-1}) and sucrose (30 g^{-1}) separately. Generally, carbon source act as energy source for cellular development and growth. Maltose act as a better carbon source compared to sucrose for callus induction in this study. Striking differences were observed not only in terms of percentage of the calli but also in terms of fresh weight of the calli in both varieties. Similar results were also observed by Kumria *et al.,* (2001); Kumria and Rajam (2002); Kumar *et al.,* (2005), Zaidi *et al.,* (2006). For instance, Significant differences were observed in terms of percent callus induction between sucrose and maltose containing medium (Fig. 1). ~4% more callus induction observed in Swarna. Similarly, significant increase in fresh weight of calli was also noted in both the varieties (Fig. 2). Approximately 300 mg of more fresh weight of calli recorded in Swarna respectively on maltose medium compare to sucrose. Moreover, calli grown on maltose containing medium observed to be more nodular and yellowish which is a characteristic feature of embryogenic calli.

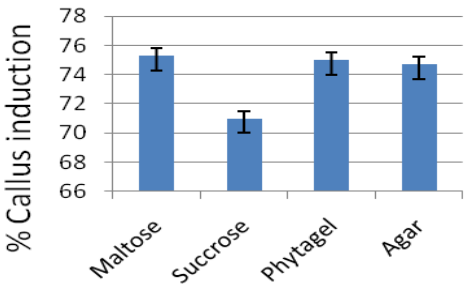

Figure 1: Effect of carbon source and gelling agent on % of callus induction

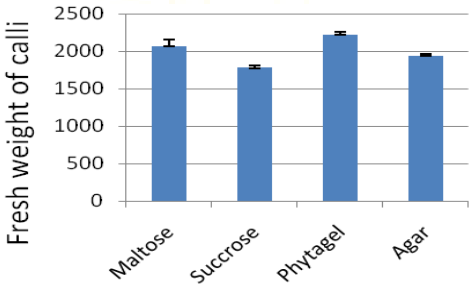

Figure 2: Effect of carbon source and gelling agent on fresh weight of calli

Effect of gelling agent

Effect of different gelling agents like plant tissue culture grade agar and phytagel on callus induction of Swarna was also studied. The role of gelling agents like agar and phytagel has profound influence on callus induction as well as regeneration as these gelling agents not only solidifies the medium, but also maintains the moisture levels of the medium, thereby influencing the cultures. Phytagel is a compound produced by *Pseudomonas elodea* which is having a smaller amount of free minerals compared to agar (Pasqualetto *et al.,* 1986, Huang *et al.,* 1995). Whereas, agar is the most frequently used gelling agent produced by red algae, contains impurities like agropectins with sulphate side chain groups generally used at 0.8 to 1.0% (W/V) concentrations (Ali *et al.,* 2004; Khaleda and Al-Forkan, 2006). To find the effect of different gelling agents, 0.8% of plant tissue culture grade agar and 0.4% of phytagel was used as gelling agent in MSCI-3 containing 3% (w/v) maltose as carbon source. Cultures grown on phytagel observed to produce more quantity of calli in terms of fresh weight. In fact, 30% more callus fresh weight observed in Swarna compared to calli grown on plant tissue culture grade agar containing medium

(Fig. 2). However, variation was not observed in terms of % of callus induction (Fig. 1). Further, calli grown on medium containing phytagel observed to be compact, globular and embryogenic compared to calli grown on plant tissue culture grade agar. Other factors which have profound effect on callus induction as well as regeneration besides the gelling agents, carbon source and phytohormones are organic supplements like L-Proline and casein enzymatic hydrolysate. Addition of L-proline in the callus induction medium observed to enhanced in development of embryogenic calli. Similar observations also reported by Moura *et al.,* (1997); Rattana *et al.,* (2012); Pawar *et al.,* (2015) in rice. L-proline an amino acid which was reported to play a prominent role in initiation and maintenance of embryogenic calli in rice through osmotic adjustments in cytoplasm and vacuoles in the presence of phytohormones (Toki *et al.,* 2006; Sivakumar *et al.,* 2000). Simialr to L-Proline, Casein enzymatic hydrolysate is more effective organic compound which contains calcium, micronutrients besides the all essential amino acids and greatly helps in regeneration of shoots from calli (Hiei *et al.,*1994; Toki, 1997). Based on the results MSCIMP medium (MSCI 3+4 g^{-1} phytagel+maltose 30 g^{-1}) has been considered as the best suitable combination for callus induction and it termed as MSCIMP here after.

***In vitro* regeneration**

To identify a suitable basal medium for plant regeneration, 25-30 day aged actively proliferating calli developed on MSCIMP medium were transferred to six different types of regeneration medium having different concentrations and combinations of phytohormones (Table 4). Among them, calli kept on MSRM-3 media resulted more number of green shoot buds which were further transformed into complete plantlets within two to three weeks' time. Generally, combination of cytokinin and auxin were used for achieving regeneration from obtained calli under callus induction medium. Particularly the concentration of cytokinin, in the regeneration medium has been described as more critical for efficient regeneration of calli (Rueb *et al.,* 1994). Based on the earlier reports to obtain higher regeneration frequency of Swarna, we have tried with a hormonal combination of NAA and Kn with varying concentrations (MSRM1 to MSRM6). Highest regeneration frequency was observed in MSRM3 (NAA 0.5 mg^{-1} and Kn 2.0 mg^{-1}). Similar observations were noted in other indica rice varieties by Sharan *et al.,* (2004); Rao *et al.,* (2009); Sahoo *et al.,* (2011). On contrary to these reports, Tariq *et al.,* (2008); Aananthi *et al.,* (2010); Sankepally and Singh (2016) reported good regeneration with combination of BAP+NAA over Kn+NAA. However, in this study Kn+NAA combinations were used, as BAP often results in more multiple shoots from a single clone i.e more plants from single event, instead of more events. With this phenomenon we cautiously used Kn for somatic embryo regeneration than BAP along with NAA combination. The developed shoot buds were transferred to MSRM-7 media for further shoot development. Table 4.6 shows the effect of different growth regulators on regeneration. Apart from these phytohormonal combinations, higher percentage of phytagel i.e (0.5% W/V) was used in regeneration medium for somatic embryo initiation and germination. Higher concentrations of phytagel creates the moisture stress, thereby mimics the natural germination conditions at the time of regeneration (Ali *et al.,* 2004; Juturu *et al.,* 2015). Apart from the phytagel, sorbitol (20% (w/v)) was also used in regeneration medium. The schematic pictures showing different stages of callus induction and regeneration of Swarna variety is depicted in Fig. 3.

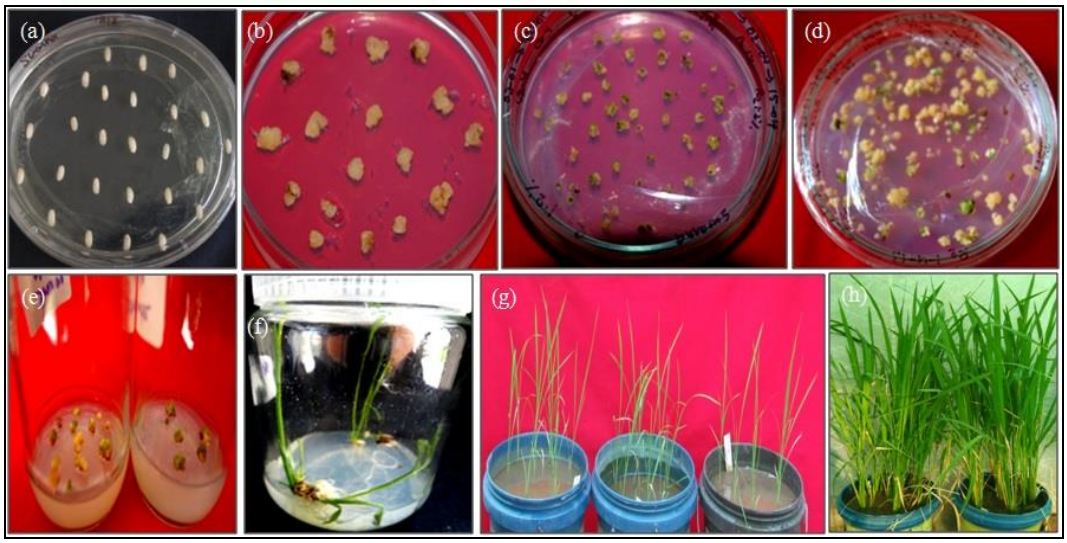

Figure 3: Representation of different stages of callus induction and regeneration of Swarna **(a)** seeds inoculated for callus induction **(b)** calli after 3 weeks on callus induction **(c, d & e)** regenerating calli **(f)** shoots regenerating after 3-4 weeks with roots **(g)** hardening of regenerated plants **(h)** mature plants.

Conclusions

Optimization of callus induction and regeneration of popular Indica cultivar Swarna will fecilitate the efficient transformation of agronomical important genes either with *Agrobacterium* or biolistic transformation. Finally, we have optimized a robust plant regeneration protocol which may be useful for increasing the transformation efficiency.

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References

1. Aananthi N, C. R Anandakumar, R. Ushakumari, P. Shanthi, "Regeneration study of some indica rice cultivars followed by *Agrobacterium* mediated transformation of highly regenerable cultivar Pusa Basmati", Electronic Journal of Plant Breed (2010): 41249-1256.

2. Ali S, Q.Z Xue, X.Y Zhang, "Assessment of Various Factors Involved in the Tissue Culture System of Rice", Rice Sci, 11(2004): 345-349.

3. Biswas A, A.B Mandal, "Plant regeneration in different genotypes of indica rice", Indian J of Biotech, 6 (2007): 532-540

4. Cooke T.J, R.H Racusen, J.D Cohen, 'The role of auxin in plant embryogenesis", Plant Cell, 5 (2007): 1494-1495

5. Hiei Y, S. ohta, T. komari, T. kumashiro, "Efficient transformation of rice (Oryza sativus L) mediated by *Agrobacterium* and sequence analysis of the boundaries of the T-DNA", Plant J, 6 (1994): 271-282

6. Hoque K.M.A, Z.A Azdi, S.H Prodhan, "Development of Callus Initiation and Regeneration System of Different Indigenous indica Rice Varieties', J Biol, 1 (2013): 46-51

7. Huang Li-Chung, C. Kohashi, R. Vangundy, T. Murashige, "Effects of Common Components on Hardness of Culture Media Prepared with Gelrite", In Vitro Cell Dev Biol Plant, 31 (1995): 84-89

8. Juturu V.N, G.K Mekala, P.B Kirti, "Current status of tissue culture and genetic transformation research in cotton (Gossypium spp)", P lant Cell Tissue Organ Cult, 120 (2015):813-839

9. Khaleda L, M. Al-Forkan, "Genotypic variability in callus induction and plant regeneration through somatic embryogenesis of five deep water rice (*Oryza sativa* L) cultivars of Bangladesh", Afr J Biotech, 6 (2006): 1435-1440

10. Khalequzzaman M, N. Haq, M. Hoque, T. Aditya, "Regeneration efficiency and genotypic effect of 15 indica type Bangladeshi rice (*Oryza sativa* L) Landraces", Plant Tissue Cult & Biotech, 15 (2005): 33-42

11. Kumar K.K, S. Maruthasalam, M. Loganathan, D. Sudhakar, P. Balasubramanian, "An improved *Agrobacterium*-mediated transformation protocol for recalcitrant elite indica rice cultivars", Plant Mol Biol Rep, 23 (2005): 67-73

12. Kumria R, M.V Rajam, "Alteration in polyamine titres during *Agrobacterium*-mediated transformation of indica rice with ornithine decarboxylase gene affects plant regeneration potential", Plant Sci, 162 (2002): 769-777

13. Kumria R, B. Waie, M.V Rajam, "Plant regeneration from transformed embryogenic callus of an elite indica rice via *Agrobacterium*", Plant Cell Tiss Organ Cult, 67 (2001): 63-71

14. Merkle S.A, W.A Parrott, B.S Finn, "Morphogenenic aspects of somatic embryogenesis, In: Thrope TA (ed) In vitro embryogenesis in plants", Kulwer Dordrecht, (1995) pp 155-203

15. Moura D.S, F.J ZapataArias, A. Ando, A.T Neto, "Plant regeneration from protoplasts isolated from primary calli using mature embryos of two Brazilian rice cultivars", Euphytica, 94 (1997): 01-05

16. Pasqualetto P.L, R.H Zimmerman, I. Fordham, "Gelling agent and growth regulator effects on shoot vitrification of phytagel", Apple in vitro J Am Soc Hort Sci, 111(1986): 976-980

17. Pawar B, P. Kale, J. Bahurupe, A. Jadhav, A. Kale, S. Pawar, "Proline and glutamine improve in vitro callus induction and subsequent shooting in rice", Rice Sci, 22 (2015): 283-289

18. Rao M.V.R, K.S Behra, N. Baisakh, S.K Datta, G.J.N Rao, "Transgenic indica rice cultivar 'Swarna' expressing a potato chymotrypsin inhibitor pin2 gene show enhanced levels of resistance to yellow stem borer", Plant Cell Tissue Organ Cult, 99 (2009): 277-285

19. Rattana K, Threerakulpisut, S. Bunnag, "The effect of plant growth regulators and organic suppliments on callus induction and plant regeneration in rice (*Oryza sativa* L)", Asian J Plant Sci, 11 (2012): 182-189

20. Rueb S, M. Leneman, R.A Schilperoort, L.A.M Hensgens, "Efficient plant regeneration through somatic embryogenesis from callus induced on mature rice embryos (*Oryza sativa* L)", Plant Cell Tiss Org Cult, 36 (1994): 259-264

21. Saharan V, R.C Yadav, N.R Yadav, B.P Chapagain, "High frequency plant regeneration from desiccated calli of indica rice (*Oryza sativa* L)", Afr J Biotechnol, 3 (2004): 256-259

22. Sahoo K. K, A.K Tripathi, A. Pareek, S.K Sopory, S.L SinglaPareek, "An improved protocol for efficient transformation and regeneration of diverse indica rice cultivars" Plant Methods, 7 (2011): 49

23. Sankepally S.S.R, B. Singh, "Optimization of regeneration using differential growth regulators in indica rice cultivars", 3 Biotech, 6 (2016): 19, DOI 101007/s13205-015-0343-0

24. Shobbar M.S, V. Niknam, Z.S Shobbar, H. Ebrahimzadeh, "Effect of salt and drought stresses on some physiological traits of three rice genotypes differing in salt tolerance", JUST, 36 (2010): 1-9

25. Sivakumar P, P. Sharmila, P. Pardhasaradhi, "Proline alleviates salt-stress-induced enhancement in ribulose-1 5-bisphosphate oxygenase activity", Biochem Biophys Res Commun, 279 (2000): 512-515

26. Sridevi G, M. Dhandapani, K. Veluthambi, "*Agrobacterium* mediated transformation of white ponni a non-basmati variety of indica rice (*Oryza sativa* L)", Curr Sci, 88 (2005): 128-132

27. Tariq M, G. Ali, F. Hadi, S. Ahmad, N. Ali, A.A Shah, "Callus induction and in vitro plant regeneration of rice (*Oryza sativa* L) under various conditions", Pak J Biol Sci, 11(2008): 255-259

28. Toki S, "Rapid and efficient *Agrobacterium*-mediated transformation in rice", Plant Mol Biol Rep, 15 (1997): 16-21

29. Toki S, N. Hara, K. Ono, H. Onodera, A. Tagiri, S. Oka, H. Tanaka, "Early infection of scutellum tissue with *Agrobacterium* allows high-speed transformation of rice", The Plant J, 47 (2006): 969–976

30. Tyagi H, S. Rajasubramanium, I. Dasgupta, "Regeneration and *Agrobacterium* mediated transformation of popular indica rice variety ADT39", Curr Sci, 93 (2007): 678-683

31. Zaidi M.A, M. Narayanan, R. Sardana, I. Taga, S. Postel, R. Johns, M. McNulty, Y. Mottiar, J. Mao, E. Loit, I. Altosaar, "Optimizing tissue culture media for efficient transformation of different indica rice genotypes", Agronomy Res, 4 (2006): 563-575

32. Zhou J, X. Wang, Y. Jiao, Y. Qin, X. Liu, K. He, C.H Chen, L. Ma, J. Wang, L. Xiong, Q. Zhang, L. Fan, X.W Deng, "Global genome expression analysis of rice in response to drought and high-salinity stresses in shoot flag leaf and panicle", Plant Mol Biol, 63 (2007): 591-608.

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