



Research Article

Callus induction and regeneration in high-altitude Himalayan rice genotype SR4 via seed explant

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ABSTRACT

SR4 genotype of rice is high altitude Himalayan rice prone to various abiotic stresses such as cold stress and therefore gives a poor yield. An efficient protocol for callusing and regeneration via direct and indirect means was established using mature seeds as an explant which can be utilized for molecular studies for genetic advancement of Himalayan rice genotype SR4 through transformation. Highest frequency (96.6%) of callus induction was obtained on MS media 3.0 mg/L 2, 4-D. While maximum regeneration frequency (100%), number of shoots with maximum length 9.14 ± 0.204 (cm) from callus was recovered from MS media amended with 5.0 mg/L BAP in combination with 0.5 mg/L NAA with highest number of shoots having an average shoot length 9.14 ± 0.204 (cm) after four weeks of culture. Direct multiple shoot regeneration from seed explants was obtained using various concentrations of TDZ and BAP with highest regeneration frequency was observed on MS media fortified with 6 mg/L of TDZ with maximum number of shoots. The shoots developed roots on MS media supplemented with 0.6 mg/L IBA.

1. Introduction

Rice (*Oryza sativa* L.) being a staple food crop of the world nourishes over 2.7 billion people throughout the planet. Like other tropical or subtropical crops it is susceptible to cold temperatures, with continuous stress culminating in chilling damage [25]. Environmental stress, on the other hand, has an impact on rice quality and productivity [30,52]. The rising demand necessitates a significant improvement in productivity to bridge the requirement and productivity gap [38]. Six Asian countries use over 89 percent of world rice produce viz., Bangladesh, Japan, China, Vietnam, Indonesia and India [36]. Rice is presently planted in over 100 nations, with a combined harvested area of around 158 million ha and about 470 million tonnes of processed rice generated [5]. To sustain the predicted 8.6 billion world population until 2030, yearly rice output of 756 million metric tonnes (MMT) is necessary to ensure food supply [10]. As a result, in order to secure the food supply of the world's fast rising population, productivity of rice should be boosted to 852 million tonnes until 2035 [8]. To address this gap in productivity and consumption, biotechnological techniques especially genetic

engineering can be used along with traditional breeding technologies. This combination of technologies can lead to creation of novel rice types having superior quality and high crop produce as well as stress tolerance to both abiotic and biotic stress [19,42]. For the efficient application of biotechnology in enhancement of rice yield, successful *in vitro* plant regeneration is critical [4]. The *in vitro* growth process is a crucial step in genetic transformation and provides substrate options that might be receivers of foreign DNA [16]. So we can say that tissue culture is the main technique involved in augmenting traditional breeding efforts and agricultural enhancement via biotechnology.

In addition, plant regeneration has also been utilized to improve the desired plant's quality and massive output [18]. Callus formation occurs once explants are subjected to numerous growth stimuli, resulting in the creation of an undifferentiated clump of cells. The callus could subsequently be employed to rejuvenate complete plants or implemented successfully for the synthesis of key metabolites in suspension cultures [14]. The formation of callus as well as its eventual regeneration is indeed the primary phases in agricultural plant manipulation by biotechnological techniques [44]. Genetic transformation dependent on

Abbreviations: MS, Murashige and Skoog; 2,4-D, 2,4-Dichlorophenoxyacetic acid; NAA, Naphthalene acetic acid; BAP, 6-Benzylaminopurine; TDZ, Thidiazuron; IBA, Indole butyric acid; Kn, Kinetin; PGR, Plant growth regulators.

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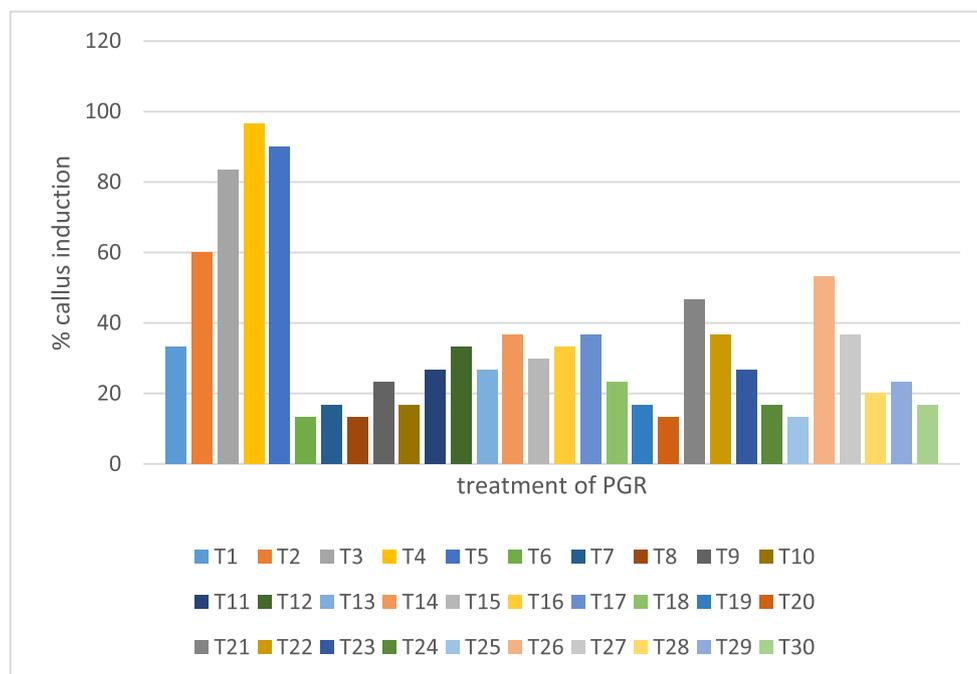


Fig. 1. Callus induction frequency in response to different combinations of 2,4-D and NAA concentrations. 2,4-D:NAA; T1(1.5:0); T2(2:0); T3(2.5:0); T4(3:0); T5(3.5:0); T6(1.5:2.5); T7(1.5:3); T8(1.5:3.5); T9(1.5:4); T10(1.5:4.5); T11(2:2.5); T12(2:3); T13(2:3.5); T14(2:4); T15(2:4.5); T16(2.5:2.5); T17(2.5:3); T18(2.5:3.5); T19(2.5:4); T20(2.5:4.5); T21(3:2.5); T22(3:3); T23(3:3.5); T24(3:4); T25(3:4.5); T26(3.5:2.5); T27(3.5:3); T28(3.5:3.5); T29(3.5:4); T30(3.5:4.5).

tissue culture has now been actively used in various crops to create modified variants [19]. However, the absence of an excellent *in vitro* propagation technique for regenerating plants continues to be a significant hurdle to genetic alteration of a vast variety of plant organisms [7]. It should be emphasized that several agronomically desirable rice varieties are resistant to *in vitro* modification due to poor callus formation and regenerative capabilities [34]. Because of their low regenerative capacity, many indica rice cultivars, the globes widely farmed rice kinds, stay less receptive to genetic modification [23]. The existence of an effective *in vitro* propagation procedure is a must for successful transformation and rejuvenation of this rice variety also [51]. Keeping in view the importance and requirement of *in-vitro* regeneration protocol for the rice variety SR4 present study was undertaken. We report the ascertainment of effective indirect and direct plant regeneration protocol from seed explants of Himalayan indica rice variety SR4, which is stress prone, however it can be used for molecular studies and as biotechnological tool for improving the variety against various stresses and to increase productivity.

2. Material and methods

2.1. Plant material

A high-altitude Himalayan rice genotype SR-4 has been selected for the present study. The seeds were collected from Division of Plant Biotechnology, SKUAST-K, Shalimar.

2.2. Media preparation and sterilization

Media used for the present study is MS media supplemented with 30 g/l sucrose as source of carbon. Media was augmented with different concentrations and combinations of phytohormones. pH of media was adjusted between 5.6 and 5.8 and agar 8 g/litre was used as solidifying agent. The media was dispensed in culture vials and these culture vials containing 20–25 ml of media were autoclaved at 15 psi and 121 °C for 20 min for sterilization. The media was solidified in the vials before inoculation.

2.3. Sterilization of seeds

Seeds were dehusked manually and properly washed under running tap water after immersion in surfactant Labolene for 10 min. This was followed by immersion of seeds in 70% ethanol for one minute. After a wash by autoclaved distilled water seeds were surface sterilized using 0.1% HgCl₂ for 7 min under Laminar Flow Cabinet. The seeds were then rinsed with autoclaved double distilled water three times. The seeds were then dried on sterile filter paper before inoculation. Inoculation and incubation.

2.4. Inoculation and incubation

Seeds were inoculated on sterilized media supplemented with various concentrations of growth hormones. The seed was horizontally inoculated on to culture medium containing different concentrations of plant hormones under aseptic environment. The plant hormones used in this study were 2, 4-D, NAA, BAP, IBA, Kn, TDZ. Inoculation for all concentration and combinations was done in replica. The cultures were incubated at 25 ± 2 °C under 16/8 h (light/ dark) photoperiod.

2.5. Callus induction and regeneration

For callus induction firstly different concentrations of 2, 4-D (1.5, 2, 2.5, 3, 3.5 mg/L) were used and then different concentrations of NAA (2.5, 3, 3.5, 4, 4.5 mg/L) were also added to the optimized concentrations of 2, 4-D. Development of callus was analyzed from the first week of inoculation of seeds up to three weeks. After three weeks of inoculation callus induction frequency was recorded using the following equation:

$$\text{Callus induction frequency} = \frac{\text{number of seeds with callus}}{\text{number of seeds inoculated}} \times 100$$

For callus maintenance, the callus was subcultured on fresh media which showed best results for callus induction (MS + 3.0 mg/L 2,4-D) after three weeks. The callus was then transferred on to plant regeneration media after 15 days for shoot initiation. Plant generation medium

Table 1

Regeneration frequency of callus in response to different concentrations of PGRs on MS media.

Treatment	Plant growth regulator mg/L			Regeneration frequency%	Number of shoots	Shoot length (cm)
	BAP	NAA	Kn			
RM0	0	0	0	100	1 ± 0	9.5 ± 0.74
RM1	5.0	0.5	0	100	23 ± 1.88	9.14 ± 0.64
RM2	4.0	0.5	0	80	13.8 ± 1.54	8.51 ± 0.63
RM3	3.0	0.5	0	73.33	9.4 ± 1.89	7.52 ± 0.83
RM4	5.0	1.0	0	93.33	16.1 ± 3.38	9.66 ± 0.73
RM5	4.0	1.0	0	80	7.7 ± 1.33	7.74 ± 0.56
RM6	3.0	1.0	0	66.66	4.3 ± 1.49	6.59 ± 0.40
RM7	0	0.5	5.0	93.33	10 ± 1.05	5.99 ± 0.72
RM8	0	0.5	4.0	80	6.4 ± 1.83	5.14 ± 0.55
RM9	0	0.5	3.0	73.33	3.1 ± 1.19	5.54 ± 0.70
RM10	0	1.0	5.0	73.33	2.6 ± 1.17	7 ± 0.84
RM11	0	1.0	4.0	86.66	12 ± 1.15	7.3 ± 0.64
RM12	0	1.0	3.0	53.33	2.6 ± 0.96	5.24 ± 0.57

RM denotes regeneration media. Values are the means of ten replicates ± SD. the data was analysed by one way ANOVA, values are significant at $p \leq 0.05$. BAP: benzylaminopurine, NAA: naphthaleneacetic acid, Kn: kinetin. Highest regeneration frequency is represented by bold letters.

consisted of MS media supplemented with growth hormones and sucrose as a source of carbon. Growth hormones used for regeneration were NAA, BAP, Kn in different concentrations and combinations. The regeneration frequency was calculated on the basis of callus responding to shoot regeneration by the total callus transferred on regeneration media. Multiple shoot induction ability was calculated by scoring the mean number of shoots induced from responding callus and the mean shoot length.

2.6. Direct regeneration

For direct regeneration MS media was supplemented with different concentrations of BAP and TDZ ranging from 2 to 6 mg/L. The shoot produced per explant was calculated after four weeks.

2.7. Rooting

The isolated shoots produced by both methods *i.e.*, indirect and direct were transferred to MS basal media for elongation and then were placed on rooting media consisting of 0.6 mg/L IBA.

2.8. Acclimatisation

In vitro regenerated plants with healthy roots were transferred to pots containing soil for acclimatization. The roots were washed off the media to avoid fungal attack.

2.9. Statistical analysis

The callus induction frequency and plant regeneration were calculated for ten replicates. Statistical analysis one way ANOVA was performed using OPSTAT.

3. Results

3.1. Callus induction

In the present study seeds were inoculated on MS media supplemented with different concentrations of 2,4-D and later a combination of 2,4-D and NAA was tried for callus induction. The callus induction from seed explants was clearly visible after 15 days of inoculation on MS media supplemented with different concentrations of 2,4-D and combination of 2,4-D and NAA. Callus induction frequency and response of explants on different concentrations of phytohormones was recorded on weekly basis. It was observed that explants inoculated on MS media supplemented with 3.0 mg/L of 2,4-D showed high callus induction frequency (96%) followed by MS media supplemented with 2,4-D 3.5 mg/L which showed 90% callus induction (Figs. 1, 5a,b). This study reported that increase in concentration of 2,4-D increased callus induction frequency being maximum at 3.0 mg/L and then decreased at 3.5 mg/L of 2,4-D. However MS media supplemented with the combination of 2,4-D and NAA also showed callus induction. In case of MS

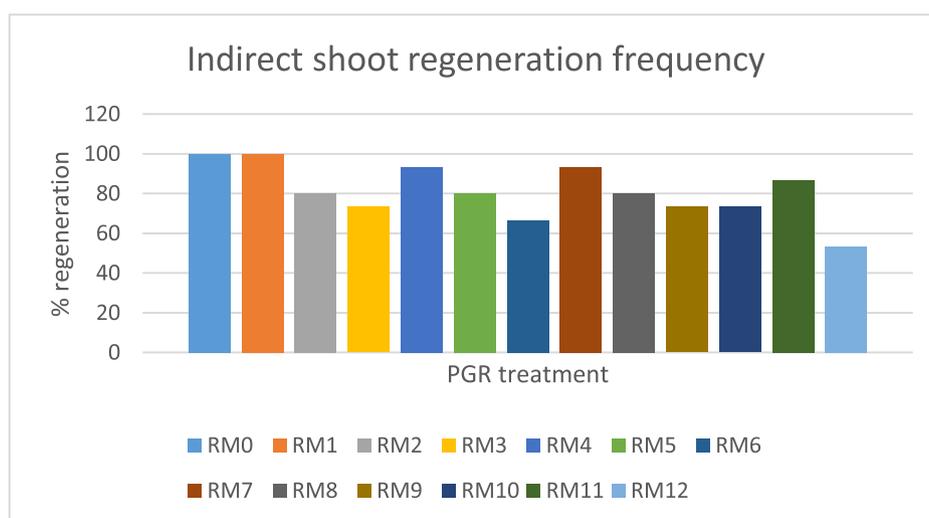


Fig. 2. Shoot regeneration frequency from callus in response to various concentrations and combinations of PGRs NAA:BAP:Kn; RM0(0:0:0) RM1(0.5:5:0); RM2(0.5:4:0); RM3(0.5:3:0); RM4(1:5:0); RM5(1:4:0); RM6(1:3:0); RM7(0.5:0:5); RM8(0.5:0:4); RM9(0.5:0:3); RM10(1:0:5); RM11(1:0:4); RM12(1:0:3).

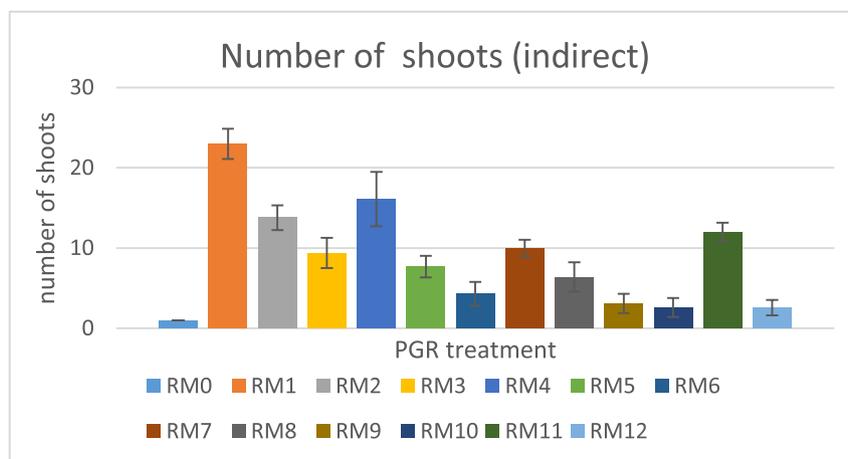


Fig. 3. Number of shoots obtained in response to various concentrations and combinations of PGRs NAA:BAP:Kn; RM0(0:0:0) RM1(0.5:5:0); RM2(0.5:4:0); RM3(0.5:3:0); RM4(1:5:0); RM5(1:4:0); RM6(1:3:0); RM7(0.5:0:5); RM8(0.5:0:4); RM9(0.5:0:3); RM10(1:0:5); RM11(1:0:4); RM12(1:0:3).

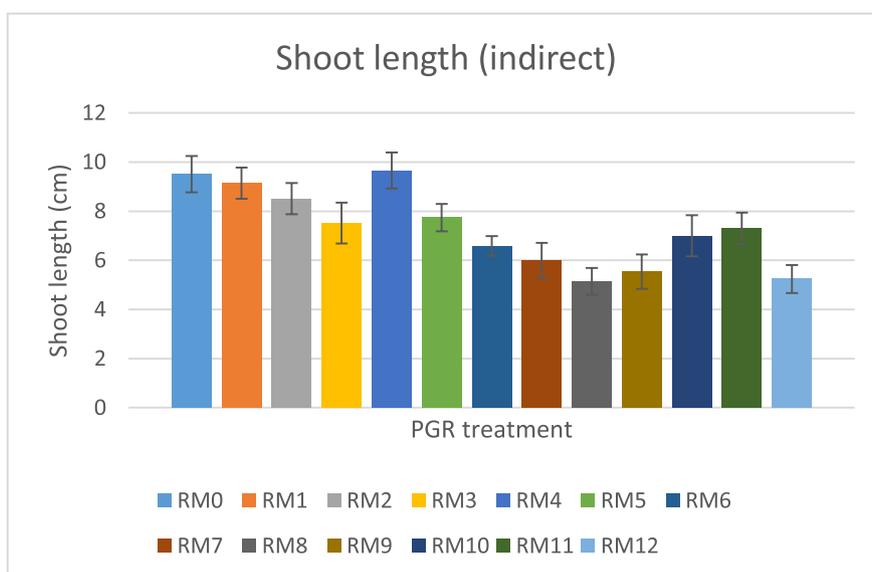


Fig. 4. Shoot length obtained in response to various concentrations and combinations of PGRs NAA:BAP:Kn; RM0(0:0:0) RM1(0.5:5:0); RM2(0.5:4:0); RM3(0.5:3:0); RM4(1:5:0); RM5(1:4:0); RM6(1:3:0); RM7(0.5:0:5); RM8(0.5:0:4); RM9(0.5:0:3); RM10(1:0:5); RM11(1:0:4); RM12(1:0:3).

media with combination of phytohormones (2,4-D and NAA), highest callus induction frequency (53.33%) was reported in MS media supplemented with combination of 2.5 mg/L of 2,4-D and 3.5 mg/L of NAA. These findings suggested that combination of phytohormones did not improve callusing frequency. The absolute media with highest callusing frequency (96%) exhibiting friable and embryogenic type II callus was MS media supplemented with 3.0 mg/L of 2,4-D. Three weeks after induction, callus was sub cultured in fresh media having same composition as media that showed highest callus induction frequency *i.e.* MS + 2,4-D (3.0 mg/L).

3.2. Indirect shoot regeneration and plantlet formation

Embryogenic callus obtained from MS media supplemented with 3.0 mg/L of 2,4-D was transferred to shoot induction media. The shoot induction media consisted of MS media supplemented with different concentrations and combinations of plant growth hormones including NAA + BAP and NAA + Kn. Shoot regeneration frequency (%) and number of shoots were recorded after four weeks as shown in Table 1. Our results suggest that increase in concentration of NAA decreased the

shoot regeneration frequency however increase in concentration of either BAP or Kn increased regeneration frequency. Highest shoot regeneration frequency was reported in MS media supplemented with 5 mg/L of BAP + 0.5 mg/L of NAA (RM1) depicting 100% regeneration frequency and high shoot number (23 ± 1.88) as well as shoot length (9.14 ± 0.64 cm) (fig. 8f) which was followed by 5 mg/L BAP + 1 mg/L NAA (fig. 8 g) and 0.5 mg/L NAA + 5 mg/L Kn Table 1 showing 93% regeneration frequency but a smaller number of shoots (16.1 ± 3.38 and 10 ± 1.05 respectively) (Figs. 2–4, Table 1).

3.3. Direct regeneration

In present study both BAP and TDZ has been used for inducing direct multiple shoot regeneration from seed explants on MS media. The media was supplement with different concentrations of BAP (2, 4, 6 mg/L) and TDZ (2, 4, 6 mg/L). Even though multiple shoot induction was observed in both BAP and TDZ supplemented medium but at equimolar concentrations, effect of TDZ was more prominent than BAP. Multiple shoot regeneration was observed within three weeks. Our findings depicted that addition of TDZ did increase the multiple shoot regeneration



Fig. 5. Establishment of tissue culture process for Himalayan indica rice variety SR4. a) Embryogenic calli induction from seed explants on MS media supplemented with 3.0 mg/L 2,4-D after two weeks of inoculation b) Callus maturation and enlargement after four weeks of incubation c) embryogenic calli showing shoot regeneration on regeneration media as indicated by green spots (black arrow) d, e) embryogenic calli showing different stages of regenerated shoot; multiple shoot regeneration on MS media supplement with f) 0.5 mg/L NAA and 5 mg/L BAP g) 1.0 mg/L NAA and 5 mg/L BAP h) 0.5 mg/L NAA and 4.0 mg/L BAP i) 1.0 NAA and 4.0 mg/L Kn j) 0.5 mg/L NAA and 5.0 mg/L Kn k) *in vitro* regenerated plants showing rooting on MS media supplemented with 0.6 mg/L of IBA l) *in vitro* regenerated plantlets hardening in pots.

percentage which increased with increase in concentration of TDZ (Table 2). It was observed that MS media supplemented with 6 mg/L of TDZ (RM3) showed highest regeneration frequency of 80% with an average number of 20 ± 0.66 shoots Table 2 followed by TDZ 4 mg/L (RM2) which showed 70% regeneration frequency with 15.3 ± 1.15 average shoot number (fig. 9c). 60% regeneration frequency was observed in MS media supplemented with 4 mg/L (RM5) and 6 mg/L (RM6) of BAP with 7 ± 1.15 and 10 ± 1.49 average shoot number respectively (Table 2, Fig. 6).

3.4. Root induction

To induce root formation the *in vitro* raised shoots using both the methods *i.e.* direct and indirect regeneration, were first transferred to MS media for elongation to remove the effect of the shoot induction media. Later on after two weeks the shoots were transferred to root induction media supplemented with IBA 0.6 mg/L. The roots produced were thick and fibrous and more than 98% shoots produced roots. *In vitro* regenerated plantlets were then transferred to *ex vitro* conditions for acclimatization and 96% hardening was achieved.

Table 2

Direct multiple shoot regeneration in response to different concentrations of PGRs in MS media.

Treatment	Plant growth regulator mg/L		Regeneration frequency	Number of shoots	Shoot length (cm)
	TDZ	BAP			
RM0	0	0	100	1 ± 0	9.5 ± 0.74
RM1	2	0	50	3.2 ± 0.91	5.07 ± 0.96
RM2	4	0	70	15.3 ± 1.15	8.6 ± 0.94
RM3	6	0	80	20 ± 0.66	6.59 ± 1.43
RM4	0	2	50	3.8 ± 0.91	6.68 ± 0.75
RM5	0	4	60	7 ± 1.15	7.75 ± 0.70
RM6	0	6	60	10 ± 1.49	8.53 ± 1.14

RM denotes regeneration media. Values are the means of ten replicates ± SD. The data was analysed by one way ANOVA and is significant at $p \leq 0.05$. TDZ: thidiazuron, BAP: benzylaminopurine. Bold letters depict highest regeneration frequency.

4. Discussion

A significant proportion of regenerable embryogenic callus is required for genetic modification in the direction of varietal development [24]. The purpose of present study was to examine and enhance the embryogenic capability of callus in order to increase regeneration efficacy of plant. Earlier studies revealed that callus formation is primarily dependent on exogenous supply of auxin as well as the kind and

quantity of auxin [40]. Auxins and cytokinins could function via synergistic, additive, or antagonistic pathways based on tissue culture inoculation circumstances that favour developmental choice for callus formation and production of shoot [12]. Auxin, particularly 2,4-D in concentrations ranging from 1 to 3 mg/L, is required for the production of embryogenic callus from embryo of cereals [35]. Endress [13] have proposed that 2,4-D causes hypermethylation of DNA throughout the pre-embryonic stage, hence keeping the cell in a heavily mitotic state [13]. Several investigations have found that the addition of 2,4-D which is an auxin had been a critical element in effective rice callus initiation [22,28,32].

Earlier many workers [3,29,31,50] have also obtained maximum callus induction for indica rice species on MS medium with 2–3 mg/L 2,4-D, so our studies are in agreement with these findings. In grass tissue culture, the auxin 2,4-D has been utilised in different proportion such as in *Panicum maximum* 10 mg/L, in *Paspalum scrobiculatum* 20 mg/L and in switch grass 5 mg/L [17]. According to Bronsema et al., [9] in order to surpass the threshold for a shift from germination to callus initiation a minimum 2.0 mg/L 2,4-D is necessary [9] which is also true to present study as callus induction was observed above the reported threshold level of 2.0 mg/L.

In our study decrease in callus production was observed in MS media supplemented with different combinations of 2,4-D and NAA which is consistent with previous studies that found combining 2,4-D and NAA reduced the incidence of callus initiation [38]. A study by Manivannan et al. [35], on maize have showed that type I and type II callus cultures could be initiated by premature embryos from the surfaces of their



Fig. 6. Multiple shoot proliferation from seed explants of *Oryza sativa* ssp. indica variety SR4 at different concentrations of BAP and TDZ a) shoot induction in MS basal media; shoot regeneration in MS media supplemented with b) 6.0 mg/L TDZ c) 4.0 mg/L TDZ d) 2.0 mg/L TDZ e) 6.0 mg/L BAP f) 4.0 mg/L BAP g) 2.0 mg/L BAP h, i) rooting in MS media supplemented with 0.6 mg/L IBA.

scutella. Type I was organogenic and compacted and it was easy to generate out of an underdeveloped embryo. While as type II was friable and embryogenic, highly regenerative and it was induced at a less rate compared to type I [35] in our study we have obtained type II callus. Furthermore, we utilized developed seeds as a source of explant since they are accessible all year and are receptive to transformation by callus induction.

According to Lee and Huang [27], the equilibrium of cytokinin and auxin is having a significant effect in the commencement of rejuvenation of stimulated callus [27] and are believed to impact plant cell cycle and ability of morphogenesis [21]. Apart from the established role of BAP in inducing shoot multiplication [1,20,47] many workers have reported the shoot induction on auxin and cytokinin combination also [4,12,38]. As in present study highest regeneration frequency has been obtained on BAP (5.0 mg/L) and NAA (0.5 mg/L) combination, earlier some workers have also reported shoot induction on same combination [33,45]. A study by Pilahome et al., [41] has reported highest indirect shoot regeneration rate in rice on MS media supplemented with BAP (2.0 mg/L) and NAA(0.5 mg/L) [41] however Alam et al. [4] has reported highest indirect shoot regeneration frequency on MS media supplemented with Kn 6.0 mg/L + NAA 0.5 mg/L [4] while in our study best shoot regeneration frequency was obtained on MS media supplemented with combination of BAP (5 mg/L) and NAA (0.5 mg/L) which was followed by combination of NAA (0.5 mg/L) + Kn (5 mg/L) and BAP (5 mg/L) + NAA (1.0 mg/L). Mostafiz and Wagiran [38] have shown that increased levels of BAP and Kn accelerate early multiplication of cells and perform a vital function in somatic embryogenesis and regeneration of plantlet from callus [38]. In rice indirect shoot proliferation from callus obtained from scutellum explants was demonstrated on MS basal medium treated with Kn 1.0 mg/L and NAA 1.0 mg/L [34] while [7] obtained shoot regeneration from callus derived from seed explants using MS media supplemented with Kn 2.0 mg/L and NAA 1.0 mg/L [7]. The auxins and cytokinins included in regenerative medium have been shown to increase regenerative frequencies of rice in genotypes which likely do not respond to tissue culture [26].

Cytokinins, in particular, play an important function in plant growth, like regulating shoot growth and proliferation and promoting cell multiplication and enlargement [37]. A phenylurea-based chemical, Thidiazuron, has been demonstrated to have significant cytokinin action in generating effective shoot proliferation in a variety of species of plants [43,46]. BAP which is an adenine and purine based cytokinin has been found to promote the growth of shoots in a variety of essential species of plants [6]. Unlike BAP, TDZ is resilient to cytokinin disintegrating enzymes [11]. Once apices of shoot were inoculated in medium treated with TDZ 4 mg/L, the highest shoot multiplication rate (10.3 shoots every explant) was seen [11]. This is consistent with findings of this experiment where highest shoot regeneration rate with highest average shoot number was observed in medium supplemented with 4 and 6 mg/L TDZ. High percent of direct multiple shoot regeneration was reported in MS media supplement with 4 mg/L TDZ which was followed by 6 mg/L BAP in rice using basal portion of shoot as explant in the previous study [42]. Our research is in accordance with the findings of this publication. In our study highest regeneration rate was observed in medium supplemented with TDZ as compared to BAP. It was observed that on solid MS medium enriched with BAP 50 µM, a higher proportion of direct shoot proliferation might be produced from germinated seed [41]. MS basal medium treated with TDZ 1.5 mg/L appeared to be significantly advantageous for seed germination and the growth of numerous shoots in direct regeneration [50].

IBA and IAA are natural plant growth hormones from the auxin group that perform an important part in root formation in both *in vitro* and *ex vitro* cultivated plants [48,49]. Many workers have obtained successful rooting of medicinal and other plant species using IBA either in isolation [2,4,15] or in combination with some other auxin [1,20,39]. In rice Several researchers have observed root formation from *in vitro* generated shoots on MS basal media without PGRs [11,12,38], however

in our study root formation was not seen on MS media without PGR while the addition of auxin IBA (0.6 mg/L) resulted in thick fibrous roots, our finding is consistent with the previous report where no rooting from *in vitro* generated rice shoots was observed on MS media without PGR [50]. Our study is in accordance with previous investigations where highest root induction rate in rice was observed in MS medium treated with 0.6 mg/L IBA [4].

5. Conclusion

In conclusion, we developed a rapid and an efficient regeneration system from seed explants via both indirect and direct morphogenesis for Himalayan rice genotype SR4. In the preliminary study it was reported that the increase in concentration of 2,4-D increases the callus induction frequency of SR4 being maximum upto 3.0 mg/L then showed decrease after further increase in 2,4-D concentration. The combination of auxins 2,4-D and NAA also showed the callus induction but with lower frequency than that of 2,4-D alone. Friable and embryogenic callus suitable for shoot regeneration was obtained. Cytokinin and auxin used in combination is best suited for shoot regeneration. The optimum concentrations of BAP and NAA are required for multiple shoot regeneration from callus. Combination of NAA and Kn was also utilized for shoot regeneration from callus however combination of NAA and BAP at particular concentration was most suitable showing high regeneration frequency and number of shoots. For direct multiple shoot regeneration optimum concentration of TDZ was found to be most suitable. This study will pave a way for molecular studies and utilizing biotechnology for improvement of SR4 genotype against various stresses via transformation of genes responsible for tolerance to various stress conditions and hence enhancing the productivity.

Contributions

ANK and AMH designed the experiment. WN and RL did the experimental work and wrote the manuscript. ANK and AMH did the critical revisions. All authors read the manuscript before submission.

Declaration of Competing Interest

The authors declare that they have no conflict of interest associated with publication of this article.

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