

Full Length Research Paper

# High frequency plant regeneration from desiccated calli of *indica* rice (*Oryza Sativa* L.)

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An efficient and reproducible protocol is required to achieve high frequency transformation from transformed calli. We report here high frequency plant regeneration from mature seed derived embryogenic calli of two recalcitrant *indica* rice cultivars HKR-46 and HKR-126 after partial desiccation treatment. Embryogenic and nodular callus was initiated on MS basal medium supplemented with 2.5 mg l<sup>-1</sup> 2,4-D, 500 mg l<sup>-1</sup> proline, 500 mg l<sup>-1</sup> casein hydrolysate, 30 g l<sup>-1</sup> sucrose and 2.5 g l<sup>-1</sup> gelrite. Several media with different combinations of growth regulators were tried. Maximum shoot regeneration frequency (63%) was observed in partially desiccated calli for 48 h in cv. HKR 46 and 82.1 per cent in cv. HKR-126 on the MS modified medium supplemented with 2 mg l<sup>-1</sup> kinetin + 0.5 mg l<sup>-1</sup> NAA + 30 g l<sup>-1</sup> sucrose + 6 g l<sup>-1</sup> gelrite followed by in the medium supplemented with 1 mg l<sup>-1</sup> 2ip + 30 g l<sup>-1</sup> sucrose + 6 g l<sup>-1</sup> gelrite (61% in cv. HKR-46 and 79.2 % in cv. HKR-126). Highly significant regeneration differences were observed in partially desiccated calli (48 h) in comparison to non-dehydrated (0 h desiccation) calli. Shoot regeneration frequency increased from 1.2 to 5.6 fold after 48 h of desiccation in both the cultivars on different regeneration media. Shoot regeneration frequency declined at 72 h desiccation treatment as compared to 48 h treatment. Well-developed plantlets were hardened and transferred to the green house.

**Key words:** Plant regeneration, *indica* rice, mature embryo, partial desiccation.

## INTRODUCTION

Production of callus and its subsequent regeneration are the prime steps in crop plant to be manipulated by biotechnological means. Different tissues have been used in rice as explants (Bhaskaran and Smith, 1988). However, embryos are easily amenable to *in vitro* techniques due to high totipotency of calli produced from them (Maggioni et al., 1989). Regeneration from callus was achieved long back in *japonica* varieties (Nishi et al., 1973). The potential for callus formation and regeneration have been reported to be varietal characteristic and efficient regeneration in *indica* rice is still poses a major

problem for genetic manipulation through innovative approaches (Toki, 1997). Strategies to improve plant regeneration frequency in cereals, including rice, have been steadily evolving during the last decade (Kyojuka et al., 1988; Datta et al., 1992; Raman et al., 1999).

While it has been possible to obtain high plant regeneration frequencies in *japonica* rice varieties, the success for reproducible fertile plant regeneration has been limited in *indica* rice varieties, especially those belonging to group 1 (Kyojuka et al., 1988; Raman et al., 1994). As a result progress towards the transfer of useful genes in to *indica* rice has been slow. Many factors have been examined to improve the frequency of plant regeneration in rice. Different reports have shown that many factors affect plant regeneration frequency in rice: genotype, development stage of callus in the explant, and hormonal composition of medium (Jain, 1997; Kyazuka et al., 1998). Partial desiccation treatments have been reported to be beneficial for embryogenesis and plant regeneration in several plant species. Tsukahara and Hirokawa (1992) have reported that dehydration for 24 h

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**Abbreviations;** 2,4-D: 2,4-Dichlorophenoxy acetic acid, 2-iP: 2-isopentenyl adenine, MS: Murashige and Skoog medium, TDZ: Thidiazuran, NAA: Nephthalene acetic acid, Kinetin: 6 Furfuryl amino purine, Z: Zeatin

of cell suspension derived calli of *japonica* rice increased shoot regeneration from 5 to 47 per cent. Jain et al. (1996) have reported three fold increase in shoot regeneration frequency following partial desiccation for 24 h of suspension cells in *indica* rice. Chand and Sahrawat (2000) carried out partial desiccation of embryogenic calli prior to transfer to regeneration medium and observed increased regeneration frequency of desiccation treatment to callus cultures of cv. safari-17 and cv. kasturi. In this paper we report high frequency plant regeneration from mature seed derived calli of two recalcitrant *indica* rice varieties after desiccation treatment.

## MATERIALS AND METHODS

Mature seeds of *indica* rice cvs. HKR-46 and HKR-126 were collected from CCS Haryana Agricultural University, Rice Research Station, Kaul, Haryana (India). These are dwarf and high yielding varieties. Dehusked seeds were washed in 70 per cent (w/v) ethanol for 60 sec and then rinsed with sterilized water to remove traces of ethanol. Sterilization of seeds was carried out on Shaker using a solution of sodium hypochloride (with 2% active chloride) and a drop of tween-20. After 40 min the solution was removed and seeds were thoroughly washed 5-6 times with sterilized water.

### *In vitro* culture

For callus induction, dehusked seeds of two cvs. HKR-46 and HKR-126 were surface sterilized as described earlier and transferred in the petri dishes containing MS basal medium (Murasnige and Skoog, 1962) supplemented with 2.5 mg l<sup>-1</sup> 2,4-D, 500 mg l<sup>-1</sup> proline, 500 mg l<sup>-1</sup> casein hydrolysate, 30 g l<sup>-1</sup> sucrose and 2.5 g l<sup>-1</sup> gelrite. After 3 weeks of incubation under dark at 25±1°C, calli initiated from acutella were sub cultured on fresh callus inducing medium.

### Partial desiccation

Desire extent of desiccation was obtained by transferring 3 weeks old calli to sterile empty petri dishes containing two sterile whatman-1 filter papers. The petri dishes were sealed with parafilm and kept at 25±1°C in dark for 48 and 72 h to obtain the desiccation of calli. After desiccation treatment, the partially desiccated calli were transferred to various regeneration media (Table 1). The regenerated plants were taken out from the petri dishes and transferred to Magenta boxes or culture bottles for shoot elongation on the MS medium. After 3-4 weeks, the plantlets were transferred to tubes containing water for hardening and incubated at 25±1°C in the culture room for a week. The plants were finally transferred to the pots.

## RESULTS AND DISCUSSION

### Callus induction

Callus formation invariably developed from the scutellar region of the seeds and was visible within 7-10 days. The mean callus induction frequency was 60.5 per cent in cv. HKR-46, whereas in HKR-126 it was 83.5 per cent. The 3-4 old weeks old calli of two cultivars used for regeneration.

**Table 1.** List of media used for plant regeneration.

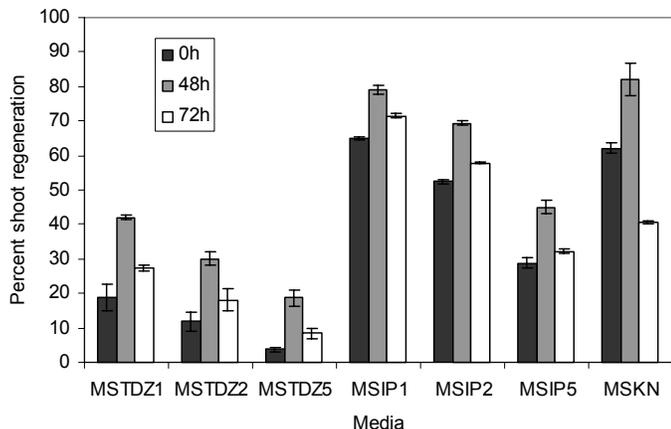
Medium	Growth regulators			
	Cytokinins (mg l <sup>-1</sup> )			Auxins (mg l <sup>-1</sup> )
	Kin	2-iP	TDZ	NAA
MSIP <sub>1</sub>	-	1.0	-	-
MSIP <sub>2</sub>	-	2.0	-	-
MSIP <sub>5</sub>	-	5.0	-	-
MSTDZ <sub>1</sub>	-	-	1.0	-
MSTDZ <sub>2</sub>	-	-	2.0	-
MSTDZ <sub>5</sub>	-	-	5.0	-
MSKN	2.0	-	-	0.5

MS: basal+Proline 500 mg l<sup>-1</sup>+CH 500 mg l<sup>-1</sup>+Sucrose 30 g l<sup>-1</sup> + gelrite 6 g l<sup>-1</sup>, pH 5.8.

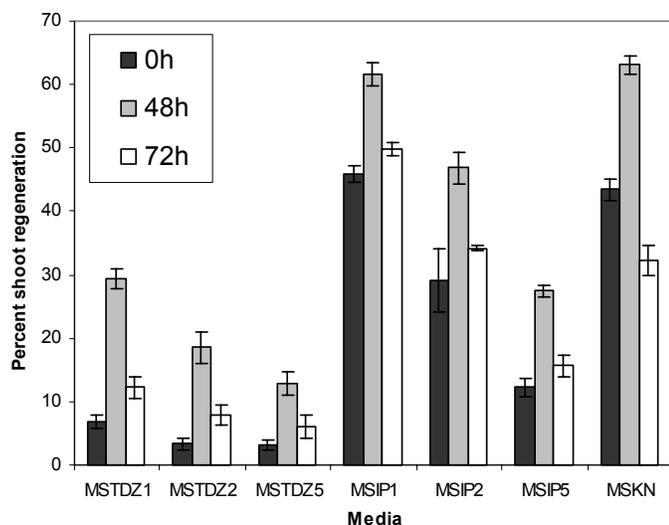
### Plant regeneration

Plantlet/shoots regeneration started within one week of transfer of partially dehydrated calli to MSTDZ<sub>1</sub>, MSTDZ<sub>2</sub>, MSTDZ<sub>5</sub>, MSIP<sub>1</sub>, MSIP<sub>2</sub>, MSIP<sub>5</sub> and MSKN media (Table 1). Within 4 weeks, calli were entirely covered with green shoot buds. During sub culture the shoot buds further elongated and multiplied vigorously. Shoots multiplication rates was comparatively low in 0 h desiccation (without desiccation) treatment and regenerated plantlets were comparatively not so green and healthy as compared to plantlets regenerated from partially desiccation calli. The regeneration frequency in cv. HKR-46 was 63.7 and 61.6 per cent on MSKN and MSIP<sub>1</sub> media, respectively. In cv. HKR-126 maximum regeneration frequency was observed when calli were desiccated for 48 h (82.0 and 79.2 per cent on MSKN and MSIP<sub>1</sub> media, respectively). For both the cultivars maximum average number, 8-10 plantlets per callus were observed in partially desiccation calli. In 0 h desiccation 1-6 plantlets per callus were recorded (data not shown). In both cultivars the regeneration frequency and the average number of plantlets decreased in 72 h desiccation treatment. An increase of 1.2 to 5.6 fold in shoot regeneration frequency was obtained in both the cultivars with 48 h desiccation treatment, as compared to 0 h desiccation (Figures 1 and 2). The plants were hardened and transferred to the pot for further growth. These plants were found fully fertile as compared to normal plants.

Availability of an efficient regeneration system is a prerequisite for under taking any transformation study. Till date, most of the regeneration studies in rice have been done in *japonica* rice. *Indica* rice varieties including HKR-46 and HKR-126 are considered recalcitrant to tissue culture manipulation and number of research groups are still investigating the optimum media requirement and other culture condition for efficient plant regeneration (Khanna and Raina, 1997).



**Figure 1.** Effect of partial desiccation (0, 48, 72 h) on shoot regeneration from matured seeds derived calli of *indica* rice cultivars HKR-126 cultured on different regeneration media. Values are the mean of  $6 \pm SE$ .



**Figure 2.** Effect of partial desiccation (0, 48, 72 h) on shoot regeneration from matured seeds derived calli of *indica* rice cultivars HKR-46 cultured on different regeneration media. Values are the mean of  $6 \pm SE$ .

Induction of embryogenic calli in rice is considered most critical step. Several different media including MS medium have been used for rice tissue culture. Mostly 2, 4-D has been used as the only growth regulator in callus induction media (Katiyar et al., 1999; Zhenyu et al., 1999). Use of casein hydrolysate was found to be beneficial for generation of embryogenic calli in *japonica* (Hiei et al., 1994; Toki 1997) as well as in *Indica* rice varieties (Zhang et al., 1996). The use of proline in the medium has been reported to be effective for the initiation

and maintenance of embryogenic calli (Datta et al., 1992; Kishor et al., 1999) in present investigation, MS basal medium containing 2, 4-D ( $2.5 \text{ mg l}^{-1}$ ), casein hydrolysate ( $300 \text{ mg l}^{-1}$ ) proline ( $500 \text{ mg l}^{-1}$ ) and sucrose ( $30 \text{ g l}^{-1}$ ) have been successfully used for induction of embryogenic calli from mature seed acutella of two rice cultures. Partial desiccation has been found promotive to plant regeneration (Diah and Bhalla, 2000; Chand and Sahrawat, 2001). Partial desiccation treatment (48 h) gave maximum shoot regeneration, 82 per cent in cv. HKR-126 and 63.1 per cent in cv. HKR-46.

The results of this study showed that in both *indica* rice cultivars maximum average number of plantlets (8 to 10 per callus) were observed in 48 h desiccation. Shoot regeneration frequency was also higher by 1.2 to 5.6 fold in both cultivars in 48 h desiccation whereas in 72 h desiccation treatment regeneration frequency declined. These findings were in conformation with the result reported by Diah and Bhalla (2000) and Chand and Sahrawat (2001).

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