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SHOOT REGENERATION IN RECALCITRANT *INDICA* RICE THROUGH PARTIAL DESICCATION OF CALLUS

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ABSTRACT

***Indica* rice cultivars are recalcitrant to *in vitro* manipulations, such as shoot regeneration. The present study was undertaken to induce shoot regeneration through partial desiccation of callus induced from mature seeds of PR 121 *indica* rice cultivar. Callus was exposed to four partial desiccation treatments i.e. 30 min, 60 min, 90 min and 120 min. The partially desiccation effect was examined in terms of shoot regeneration percentage upon transfer of the desiccated callus to regeneration media. The non-desiccated callus did not show shoot regeneration, whereas 31.50±0.05 percent regeneration was recorded in callus partially desiccated for 60 min. The partial desiccation is likely to be beneficial for inducing shoot regeneration in recalcitrant *indica* rice cultivars.**

Keywords: *Indica* rice, Partial desiccation, PR 121, Shoot regeneration

Shoot regeneration is a major constraint for *in vitro* manipulation in *indica* rice. Various explants, such as coleoptile, roots, seed derived scutellum, immature and mature embryos, leaves, stem nodes, inflorescence and anthers have been used to induce callus (Bhaskaran and Smith, 1988) but shoot regeneration has been negligible (Kyozuka *et al.,*1988). The main reason for trivial regeneration in *indica* rice is because of browning or necrosis of callus during sub-culturing phases (Chu and Croughan, 1990; Khan *et al.,* 2019). Several factors, such as genotype, development stage of callus, hormonal composition of medium (Jain, 1997), concentration of gelling agent, specific growth regulators (auxin:cytokinin ratio), amino acids and polyamines (Chand and Saharawat.,1999; Feng *et al*., 2011; Sahoo *et al*., 2011;) have been examined for promoting shoot regeneration but with limited success.

Another important factor i.e. partial desiccation of callus, has been reported to be beneficial for inducing shoot regeneration in several plant species including rice (Zuraida *et al.,* 2010; Ming *et al*., 2019). Desiccation removes excess water, osmolytes, and moisture content from callus thus inducing embryogenesis (Repalli *et al.,* 2017). Further desiccation also leads to increase in absisic acid concentration of callus (Rengel, 1986) that results into better shoot regeneration. In literature, it is reported that *indica* rice is recalcitrant to tissue culture manipulation (Ge *et al.,* 2006), therefore we undertook this study to obtain shoot regeneration in *indica* rice cultivar PR 121 through partial desiccation of callus.

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MATERIALS AND METHODS

Plant material

Mature seeds of *indica* rice cultivar PR 121 were used as explants for establishing rice callus cultures. PR 121 is a medium maturing rice variety released for general cultivation in low land irrigated ecosystem of Punjab in the year 2013. The seeds of PR 121 were obtained from Department of Plant Breeding & Genetics, Punjab Agricultural University, Ludhiana.

Surface sterilization of seeds

The seeds were dehusked and treated with 1 percent bavistin for 45 minutes to 1 hour to avoid any fungal contamination. Seeds were then rinsed 3 times with autoclaved double distilled water in order to remove any disinfectant. Treatment with 0.1 percent mercuric chloride solution for 10 minutes was given to avoid any bacterial contamination and again seeds were rinsed with sterile double distilled water. Sterilized seeds were then dried on Whatmann filter paper in laminar air flow cabinet (Klenzaids, Mumbai) and used for callus induction.

Callus induction

Callus was induced on MS media supplemented with 2,4-D (1.5mg/l), kinetin (0.5mg/l), proline (600mg/l), sucrose (35g/l) and clarigel (5g/l) by placing the seed on the medium with scutellar end just outside the media. The embryogenic callus was transferred to pre-regeneration medium i.e. MS supplemented with maltose (35g/l), NAA (naphthalene acetic acid) [1.0mg/l], kinetin (2.0mg/l), sorbitol (20g/l), agar (9gl) for 15 days under dark conditions.

Partial desiccation of callus

The embryogenic calli was partially desiccated by placing on sterile filter paper (Fig.1) in 90mm petridishes in laminar air flow cabinet for 30 min, 60 min, 90 min and 120 min according to method given by Repalli *et al*., (2017).

Shoot regeneration

Desiccated calli were shifted to shoot regeneration medium supplemented with 6-Benzyl amino purine (1.5mg/l), kinetin (1mg/l), maltose (35g/l), clarigel (5g/l). The cultures were incubated at 25±2°C under light for shoot regeneration. The shoot regeneration percentage was calculated after 3 weeks. The regeneration percentage was calculated as: Number of callus regenerated/Total number of callus cultured on regeneration medium

Statistical analysis

The results were analyzed using Tukey's post hoc test to determine the significant difference between the partial desiccation treatments and control (Abdi and Williams, 2010).

RESULTS AND DISCUSSION

Callus induction initiated after four weeks on callus induction medium in dark. The shoot regeneration was not observed upon transfer of callus directly to shoot regeneration medium suggesting recalcitrance of *indica* rice cultivar PR 121 for shoot regeneration. Subsequently, the partially desiccated embryogenic callus initiated shoot formation after 3 weeks of transfer to shoot regeneration medium (Fig 1). Partial desiccation of callus for 30 min resulted into 29.75±1.02 percent shoot regeneration. The desiccation of callus for 60 min led to 31.50±0.05 percent shoot regeneration. The desiccation for 90 min resulted 16.50±1.15 percent shoot regeneration and desiccation for 120 min showed shoot regeneration of 7.50±0.00 percent. The possible explanation for low shoot regeneration after 120 min is browning of callus that caused damage to callus cells prior to their transfer to regeneration media (Makerly *et al*., 2012). The callus desiccation for 60 min resulted in significantly better shoot regeneration as compared to other partial desiccation treatments (Fig 2). The shoot regeneration enhanced 5-fold in callus partially desiccated for 60 minutes as compared to callus desiccated for 120 min. Similarly, Siddique *et al.,* (2014) reported that desiccation of *indica* rice callus enhanced

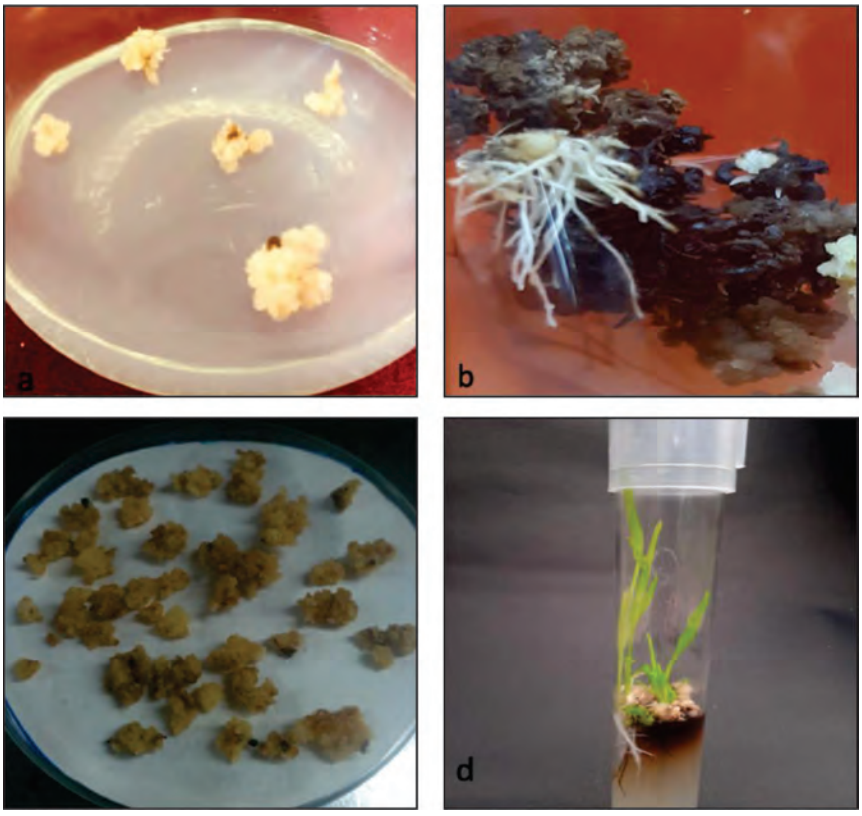

Fig. 1. a) Non-desiccated callus transferred on shoot regeneration media b) Non-desiccated callus on shoot regeneration media after 3 weeks c) Partial desiccation of callus for 60 min d) Shoot regeneration from partially desiccated callus after 3 weeks

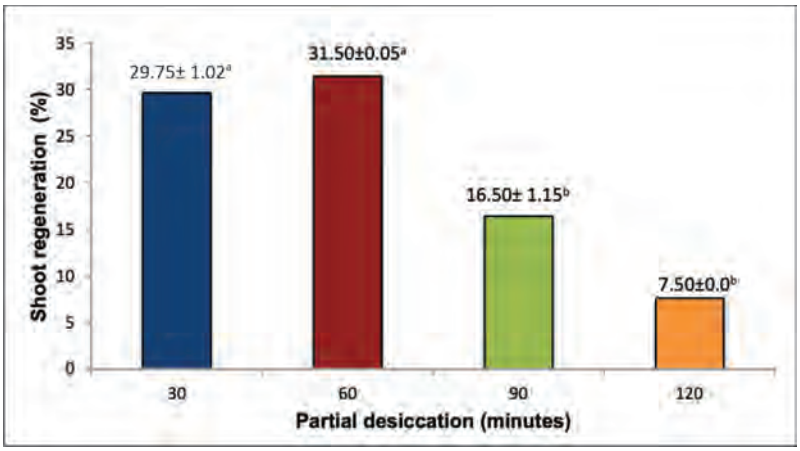

Fig. 2. Shoot regeneration after partial desiccation. The shoot regeneration percentage marked with the same alphabet indicates that they do not differ significantly ($p \leq 0.05$) in Tukey's-test.

shoot regeneration upto 4-fold. Our study shows that partial desiccation of callus induces shoot regeneration in *indica* rice which is recalcitrant to tissue culture manipulation. Thus, we suggest inclusion of partial desiccation step in shoot regeneration protocol of *indica* rice genotypes for better results.

Authors' contribution

Conceptualization and designing of research work (JSS); Execution of field/lab experiments and data collection (MS); Analysis of data (MS); Interpretation of data and preparation of manuscript (JSS, MS).

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