HOSTED BY
ELSEVIER

Available online at www.sciencedirect.com

ScienceDirect

Rice Science, 2015, 22(6): 283–289

Proline and Glutamine Improve *in vitro* Callus Induction and Subsequent Shooting in Rice

Bhausaheb PAWAR, Prashant KALE, Jyoti BAHURUPE, Ashok JADHAV, Anil KALE, Sharad PAWAR
(*State Level Biotechnology Centre, Mahatma Phule Agricultural University, Rahuri 413722, India*)

Abstract: This study was conducted to evaluate the effects of proline and glutamine on *in vitro* callus induction and subsequent regeneration and to develop a reproducible and highly efficient plant regeneration protocol in four rice genotypes, viz. Pawana, Jaya, Indrayani and Ambemohar. Considerable variation in response to plant growth regulators and amino acid supplements used was observed in all the four genotypes. Medium supplemented with proline and glutamine was shown to be superior to medium without proline and glutamine. The best callusing from mature embryo was observed on Murashige and Skoog (MS) medium supplemented with 2.0 mg/L 2,4-dichlorophenoxyacetic acid (2,4-D), 500 mg/L proline and 500 mg/L glutamine. Shoot induction was higher in the callus obtained from medium supplemented with 500 mg/L proline and 500 mg/L glutamine. The highest shoot regeneration frequency (83.2%) was observed on MS medium with 2.0 mg/L benzylaminopurine, 0.5 mg/L 1-naphthaleneacetic acid, 500 mg/L proline, and 500 mg/L glutamine in the callus obtained from MS medium supplemented with 2.0 mg/L 2,4-D, 500 mg/L proline and 500 mg/L glutamine. Among the four genotypes, Pawana has the highest regeneration efficiency (83.2%), whereas the regeneration efficiency of the rest three rice genotypes was in the range of 32.0% to 72.3%. This optimized regeneration protocol can be efficiently used for *Agrobacterium* mediated genetic transformation in rice.
Key words: callus induction; glutamine; proline; rice; *Agrobacterium* mediated genetic transformation; 2,4-dichlorophenoxyacetic acid; Murashige and Skoog medium

Rice (*Oryza sativa* L.) is an economically important staple food for more than 80% of people in Asia and the most extensive cultivated cereal crop in the world. Its growth and productivity are adversely affected by various abiotic and biotic stresses, which prevents crop from reaching its full genetic potential and limits crop yield worldwide. With constant increase in global population, it became essential to develop new rice genotype tolerant to abiotic and biotic stress with high yield potential to fulfill the increasing demand for food. Genetic manipulation contributes to the agronomic improvement of rice by conquering some of the limitations in traditional breeding methods. There is a great potential for genetic manipulation in rice to enhance productivity by increasing the resistance to pest and disease and the tolerance to environmental stress.

Development of a reliable and efficient regeneration system, including callus induction and differentiation as well as plant regeneration, is pre-requisite for a tissue culture-based transformation system for developing transgenic rice genotypes with useful genes. *In vitro* regeneration in rice from various explants, such as mature seed (Ge et al, 2006), immature seed (Lee and Huang, 2013), leaf (Karthikeyan et al, 2011), shoot apex (Dey et al, 2012), and root (Hoque and Mansfield, 2004), has been reported previously. However, highly genotype specific morphogenetic response is a major limitation with rice tissue culture. Therefore, optimization of regeneration protocol for desired genotype is essential.

In vitro regeneration depends on the composition and concentration of the basal salt, growth regulators and the organic components (Ge et al, 2006). Proline and glutamine are supplemented in the culture medium as an organic nitrogen source. Inclusion of

Received: 16 February 2015; **Accepted:** 29 June 2015
Corresponding author: Bhausaheb PAWAR (bhau.raje@gmail.com)

Peer review under responsibility of China National Rice Research Institute
http://dx.doi.org/10.1016/j.rsci.2015.11.001

proline and glutamine into callus medium has been shown to promote callusing in rice (Chowdhry et al, 1993; Ge et al, 2006; Shahsavari, 2011). Quality of calli might be a key factor for the success of regeneration and transformation, and selecting the most suitable medium to improve the quality of calli might be a key step for the success of transformation (Lin and Zhang, 2005). In view of these factors, the present investigation has been undertaken to evaluate the effects of proline and glutamine on callus induction and subsequent regeneration to develop an efficient plant regeneration protocol using rice mature embryo explant in four rice genotypes.

MATERIALS AND METHODS

Rice materials

Mature seeds of four indica rice genotypes, namely Ambemohar, Indrayani, Jaya and Pawana, were obtained from the Agriculture Research Station, Radhanagari, India. Manually dehusked seeds were surface sterilized with 70% ethanol for 30 s, followed by 0.1% mercuric chloride ($HgCl_2$) solution for 6 min. The seeds were further rinsed five times with sterile distilled water.

Callus culture and plant regeneration

For callus induction, sterilized seeds were cultured on Murashige and Skoog (MS) medium (Murashige and Skoog, 1962) supplemented with 3% sucrose, 0.8% agar and different concentrations of 2,4-dichlorophenoxy acetic acid (2,4-D) (Table 1). All cultures were maintained at (28 ± 2) °C under a 16 h photoperiod provided by cool white fluorescent lamps. Additionally, to investigate the effects of proline and glutamine on callus induction, various combinations of proline and glutamine were used (Table 1). Thirty days after incubation on callus induction medium, calli were transferred on MS medium supplemented with different concentrations and combinations of benzylaminopurine (BAP), kinetin, thidiazuron (TDZ), 1-naphthaleneacetic acid (NAA), proline and glutamine (Table 1). During the course of culture, the explants were sub-cultured after every 15 d to fresh callus induction medium or shooting medium of the same composition. The shoots (5–7 cm) were transferred to MS medium devoid of growth regulator for root formation. Plantlets with well-developed roots were transferred to plastic pots containing perlite. Those were initially covered with plastic bags for 5–7 d, and kept at polycarbonated polyhouse. Plants were irrigated with 1/2 MS solution for 14 d and finally transferred to pot containing soil and cow dunk (4:1), and those plants were irrigated with water at regular intervals.

Statistical analysis

The effects of genotype and hormone treatment on callus induction were tested by using two-way analysis of variance (ANOVA) in the INDOSTAT program. Further effects of genotype, callusing medium and shooting medium on *in vitro* regeneration were tested by using three-way ANOVA. All treatments of regeneration experiments had three replicates with 40 explants in each replication.

RESULTS

Optimization of 2,4-D concentration for callus induction

Callus induction of rice is known to depend on 2,4-D in the induction medium. Therefore, MS medium supplemented with five different concentrations of

Table 1. Media tested for callus induction and shoot regeneration in rice.

Medium number	Callus induction				Medium number	Shoot regeneration						
	MS	2,4-D (mg/L)	Proline (mg/L)	Glutamine (mg/L)		MS	Kinetin (mg/L)	BAP (mg/L)	TDZ (mg/L)	NAA (mg/L)	Proline (mg/L)	Glutamine (mg/L)
C_1	1	0.5	0	0	S_1	1	2.0	0	0	0	0	0
C_2	1	1.0	0	0	S_2	1	0	2.0	0	0	0	0
C_3	1	1.5	0	0	S_3	1	0	0	2.0	0	0	0
C_4	1	2.0	0	0	S_4	1	2.0	0	0	0.5	0	0
C_5	1	2.5	0	0	S_5	1	0	2.0	0	0.5	0	0
C_6	1	2.0	500	0	S_6	1	0	0	2.0	0.5	0	0
C_7	1	2.0	0	500	S_7	1	2.0	0	0	0.5	500	500
C_8	1	2.0	500	500	S_8	1	0	2.0	0	0.5	500	500
					S_9	1	0	0	2.0	0.5	500	500

MS, Murashige and Skoog; 2,4-D, 2,4-dichlorophenoxy acetic acid; BAP, Benzylaminopurine; TDZ, Thidiazuron; NAA, 1-naphthaleneacetic acid.