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Regenerating Plants from Cryopreserved Adventitious Buds of Haploids in Rice*

Zhang Zhihong, Hu Zhangli

College of Life Sciences, Wuhan University, Wuhan 430072, China

Abstract **A large number of adventitious buds were induced from *in vitro* cultured young inflorescences of haploids of rice. Having been subcultured on solidified subculture media at 26 C for 7 days, the adventitious buds were loaded into 1.8 mL plastic cryotubes with cryoprotectant and kept on ice for 45-60 min. After cooled at a rate of 1.0 C/min down to −40 C, the samples were kept in liquid nitrogen. The adventitious buds which have been cryopreserved for about 30 days were thawed rapidly in 38-40 C water and then plated on solidified MS medium containing 3 % sucrose, 0.5 mg/L NAA and 2.0 mg/L kinetin. After plated, 23%-32 % of adventitious buds resumed growth and 15%-22 % regenerated plantlets. The results of this work indicated that the adventitious buds derived from *in vitro* cultured young inflorescences is a critical factor for the success and subculturing adventitious buds on MS medium containing 3% sucrose and 4% sorbitol or 20% potato extract is essential to the procedure. The effective cryoprotectant is 10 % DMSO(dimethyl sulfoximide)+0.5 mol/L sorbitol.**

Key words *Oriza sativa*, **haploid, adventitious bud, cryopreservation**

0 Introduction

The development of cryopreservation has offered a new way of long-term preservation of plant germplasms. Since Nag and Street (1973)[1] won success in cryopreserving cultured cells of carrot, more than one hundred kinds of plant materials so far have been successfully cryopreserved (Brian 1990[2], Kartha 1985[3]). The haploid of rice (*Oriza sativa* L.) is one of most important materials in rice genetic study and breeding, and is generally preserved by traditional asexual reproduction. Due to the non-stability of its cultured cells or calli in chromosome ploidy (Tsay *et al* 1986[4]), it is not suitable for it to be cryopreserved by cultured cells or calli. The effective shoot apex cryopreservation of potato, peanut and wasabi and winter bud cryopreservation of pear, mulberry and apple (Fukai *et al* 1991[5], Kartha 1985[3], Matsumoto *et al* 1994[6]) have showed that the meristem of shoot apex or bud has highly genetic stability and strong morphogenetic potential, which has appeared promising for the long-term storage of haploid of rice. Maybe, the small size and the physiological fragility of shoot apex have retarded the progress of the shoot apex cryopreservation in rice. In present study, we tried to cryopreserve the adventitious buds derived from *in vitro* cultured young inflorescences of rice haploids, so as to construct a useful procedure for long-term storage of rice haploids.

1 Material and methods

1.1 Material

The haploid plants derived from the cultured anthers of rice variety Nongkeng 58 were used as material in this study.

1.2 Methods

1.2.1 Induction of adventitious buds from *in vit-*

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ro cultured young inflorescences

Referring to the methods by Ling *et al* (1985)[7] and Shu *et al* (1985)[8], we excised the young inflorescences (3-10 mm in length) at the stage from second branch primordia differentiation to pistil and stamen formation, and inoculated them on solidified induction medium at 26 ℃ in light to induce adventitious buds. Induction medium: MS +3% sucrose +2 mg/L NAA +2 mg/L kinetin.

1.2.2 Subculture of adventitious buds

The adventitious buds about 3 mm in size were isolated from *in vitro* cultured young inflorescences and transferred to solidified subculture media to be subcultured for 7 days before they was to be cryopreserved.

Subculture media:

Ⅰ. MS +3 % sucrose +4 % sorbitol

Ⅱ. MS +3 % sucrose +20 % potato extract

Ⅲ. MS +3 % sucrose +30 % potato extract

1.2.3 Cryopreservation of adventitious buds

The subcultured adventitious buds were loaded into 1.8 mL plastic cryotubes with pre-cooled cryoprotectants enough to cover the samples. Kept on ice for 45-60 min.

Cryoprotectants:

Ⅰ. 10 % DMSO(dimethyl sulfoximide)+0.5 mol/L sorbitol

Ⅱ. 10 % DMSO + 0.5 mol/L mannitol

Ⅲ. 10 % DMSO + 0.5 mol/L glycerine

Freezing procedure: The samples which have been kept on ice were freezed at a rate of 1.0℃/min down to −40 ℃. After kept in −40 ℃ for 1 h, plunged into liquid nitrogen for preservation.

1.2.4 Thaw and reculture of adventitious buds cryopreserved in liquid nitrogen

Took out the adventitions buds which have been cryopreserved for about 30 d in liquid nitrogen. After thawed rapidly in 38-40℃water and washed 3 times with liquid medium of MS containing 3 % sucrose, the buds were plated on solidified regeneration medium for 7-10 d to resume growth in dark and then to regenerate into plants in light at 26 ℃.

Regeneration medium: MS +3 % sucrose + 0.5 mg/L NAA +2 mg/L kinetin

2 Results and analyses

2.1 Induction of adventitious buds

A large number of adventitious buds were formed by organ-genetic process from the *in vitro* cultured young inflorescences affer inocularion for 15-20 d. On the average, there were 7.3 adventitious buds a inflorescence, with a range of 4-13. When the adventitious buds were transferred to regeneration medium to be cultured for 10-15 d, they rapidly developed into plants. The chromosome numbers of cells in random eight plants from the regenerated plants were all 12 when tested with their root tips after they were transplanted into the pots outside. This results indicated that the adventitious buds have powerful morphogenetic potential and is highly stable in chromosome ploidy.

2.2 Effects of subculture

After plated on regeneration media, 23%-32% of cryopreserved adventitious buds resumed growth, and 15%-22 % regenerated into plants if the adventitious buds were subcultured on subculture media prior to cryopreservation. Comparatively, only 8% of cryopreserved adventitious buds which were not subcultured prior to cryopreservation resumed growth, and no one regenerated into plant. Comparing of effects of different subculture media revealed that subculture medium Ⅱ was the best, 32% of cryopreserved adventitious buds resumed growth and 22% regenerated into plants (see Table 1).

Table 1 Effects of different subculture media*

No. of media	No. of buds cryopreserved	growth-resumed buds		plant-regenerating buds	
		No.	%	No.	%
I	132	37	28**	22	17
II	78	25	32**	17	22
III	60	14	23**	9	15
not being subcultured	50	4	8	0	0

* Cryoprotectant I (10 % DMSO + 0.5 mol/L sorbitol) was used in this experiment

* * The data were sigmficantly different from that when not being subcultured

2.3 Effects of different cryoprotectants

The optimal cryoprotectant was the number I. With use of it, 28 % of adventitious buds survived the cryopreservation, and 17 % still kept the potential of regenerating plant. The effects of Cryoprotectant Ⅱ、Ⅲ were greatly inferior to that of cryoprotectant I (see Table 2).

Table 2 Effects of different cryoprotectants*

No. of cryoprotectants	No. of buds cryopreserved	growth-resumed buds		plant-regenerating buds	
		No.	%	No.	%
I	132	37	28	22	17
II	50	9	18	5	10
III	50	6	12	3	6

* Subculture medium used in this experiment was number I (MS +3 % sucrose +4 % sorbitol)

3 Discussion

Selecting the adventitious buds induced from *in vitro* cultured young inflorescences of haploids of rice as material for the cryopreservation is a major factor which facilitated the success of the present study. The adventitious buds were derived from somatic cells by organ-genetic process (Ling *et al* 1985)[7], and have highly genetic stability and powerful morphogenetic potential just as reported in the successfully cryopreserved shoot apice in many other plants(Brian 1990[2],Kartha 1985)[3]. Therefore, the adventitious buds are the ideal materials of cryopreservation for the plants such as the major food crops rice, wheat and maize *et al* whose shoot apex cryopreservation is difficult, or for the plants which do not produce seeds and the cultured cells or calli of which are not stable in chromosome ploidy,for example, the haploids.

Subculturing adventitious buds on MS medium containing 3% sucrose and 4% sorbitol or 20% potato extract is essential to the procedure,which makes them to survive cryopreservation. After the adventitious buds is subcultured, the water contained in the tissue of them is reduced, their cytoplasm of cells become rich, and their tissue structure is thicker, which all give the adventitious buds the potential of surviving freezing process. This mechanism is similar to that in the winter bud cryopreservation of pear、mulberry and apple (Brian 1990[2],Kartha 1985)[3].

The effects of three subculture media used in the study are different. Subculture medium I (MS +3 % sucrose +4 % sorbitol) is the better, which is made up easily, and have stable functions. Subculture medium Ⅱ (MS +3 % sucrose + 20 % potato extract) has the best effect, however, the consist of the potato extract is complex, and its functions will be affected by the physiological conditions of potato to be used.

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