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Studies on Developing a High Regeneration from Seed Derived Calli of Rice (*Oryza sativa* L.) C.v. Super Basmati

Hamid Rashid, Mehreen Saleem, Zubeda Chaudhry, [1]S. Tallat Gilani and [2]Asfari Sharif Qureshi
Agricultural Biotechnology Programme, Institute of Agricultural Biotech and Genetic Resources (IABGR), National Agricultural Research Centre, Park Road, Islamabad, Pakistan
[1]Pesticide Research Institute, SARC, Karachi, Pakistan
[2]Department of Biological Sciences, Q.A.U, Islamabad, Pakistan

Abstract: Super Basmati is a commercial cultivar and is subjected to various stresses. This study was conducted to obtain high frequency regeneration from Super Basmati which is a pre-requisite for transformation protocol. Callus induction was obtained from N_6 media with 2 mg l^{-1} 2,4-D. The frequency of callus induction was 83.3% with N_6 media and 75.05% on MS media. Regeneration of calli was the best on MS media with NAA 1 mg l^{-1} and BAP 5 mg l^{-1} i.e. 81.6%.

Key words: Super Basmati, N_6, callus induction, regeneration

INTRODUCTION

Super Basmati is a major commercial variety grown in rice region of Pakistan. It's yield is affected by many diseases like others Basmati cultivars and bacterial blight is one of them. Hence there is a need to improve this commercial cultivar by biotechnological applications. Although there are many reports available on callus induction, regeneration and transgenic plant production in other Basmati cultivars like 6129, Basmati 370 and Basmati 385[1] but no report is available for Super Basmati. Super Basmati is also recalcitrant to the tissue cultures techniques. Earlier, Rashid *et al.*[2] reported a low regeneration frequency in Super Basmati, but still there is a need to develop an efficient regeneration system, as a pre-requisite for the transformation protocol.

The main objective of present study is to develop a reproducible regeneration system which is essential for establishing *Agrobacterium* mediated transformation in this cultivar.

MATERIALS AND METHODS

Mature seeds of Super Basmati were sterilized by protocol described by Rashid *et al.*[1]. Poisoned seeds of Super Basmati were dehusked. Seeds were surface sterilized by 70% ethanol for one minute followed by 50% Chlorox (*Sodium hypochlorite*) for 20 min. Seeds were continuously shaked during treatment with Chlorox and subsequently rinsed with autoclaved distilled water for 3-4 times after a regular interval of 5 min.

The sterilized seeds were aseptically inoculated on N_6 media[3] with 30 g l^{-1} sugar, 2,4-D 2 mg l^{-1} and 6 g l^{-1} of agar which was used as solidifying agent. The pH of the media was adjusted to 5.8 before autoclaving at 121 °C for 20 min. These inoculated seeds were placed in the growth room for three weeks for callus induction. The callus contained both embryogenic (white to light yellowish in color, compact and friable) and non-embryogenic (mucilaginous and smooth) part. The calli were carefully dissected and only embryogenic part was selected for further experiments and non-embryogenic part was discarded. Three weeks old scutellum derived calli were transferred to maintenance media. Maintenance media contained N_6 salts and vitamins supplemented with 2 mg l^{-1} of 2, 4-D, 30 g l^{-1} sugar and agar 6 g l^{-1} as a solidifying agent. This process was carried out in the inoculation chamber. Callus was placed on maintenance media for about two weeks.

Media used for regeneration contained basic MS salts and vitamins[4] 3% sucrose and 0.4% gelrite. Regeneration media contained different combinations of BAP and NAA as growth regulators. Maintained calli were placed on regeneration media and were placed in the growth room (light and dark period at 23±2°C with 16/8 h photoperiod with light intensity of 16 m.mol.m.s). After shoot regeneration, plantlets were transferred to MS media without growth regulators for root initiation. In order to determine regeneration frequency, 40-60 cultures were raised for each treatment and each experiment was

Corresponding Author: Hamid Rashid, Agricultural Biotechnology Programme, National Agricultural Research Center, Park Road, Islamabad, Pakistan

performed thrice. Subculture period was maintained at 2-3 weeks intervals. Observations were taken every week on the basis of %age of regeneration for the development of shoot/culture and shoot development.

RESULTS AND DISCUSSION

N_6 and MS media were used for callus induction and callus growth. Sterilized seeds were placed on these media for three weeks. Both media showed callus induction and growth with 2 mg l^{-1} 2, 4-D. Our results were similar to Azria *et al.*[5] that callus induction on N_6 and MS media required 0.5-2.0 mg l^{-1} 2,4-D. With MS media callus induction frequency was 70% while on N_6 media callus induction and growth frequency was observed as 83.3%. Our results are contradictory to Rashid *et al.*[2] which reported that callus induction frequency of Super Basmati on MS media was 23.9% and on N_6 47.7%. Comparing callus induction and growth frequency N_6 media proved to be better (Table 1 and 2). N_6 media showed better growth than MS as reported by Rashid *et al.*[1]. It is perhaps due to the reason that N_6 media contained more nitrogen than the MS media.

Further these calli were placed on N_6 media with 2 mg l^{-1} 2,4-D for growth and proliferation. After two weeks, it was observed that 76% calli showed growth and increased 2-3 times in size (Table 3).

Maintained calli were transferred to basic MS media for regeneration. In addition to basic MS salts and vitamins different concentrations of NAA and BAP were used i.e., NAA 1 mg l^{-1} and BAP 1,2 and 5 mg l^{-1} (Table 4). Both NAA and BAP ever required for shoot initiation[5]. Pons *et al.*[6] reported that BA yielded more shoots than kinetin in all varieties while in case of using auxin NAA and IAA, it depends on the varieties. Callus regenerated on all the concentrations of growth hormones, but the highest frequency 81.60% was observed on NAA 1 mg l^{-1} and BAP 5 mg l^{-1}. Our results are different from Rashid *et al.*[2] in which regeneration frequency was 45.3%. There are some albinos plants also produced which are showed in the parenthesis.

In addition to growth hormones use of sorbitol as the osmoticum was also proved necessary for the growth of rice. Gelrite was used as gelling agent for the regeneration media. Water stress conditions enhance the regeneration frequency. On Media which lack casine hydrolysate and sorbitol there was proliferation and growth of calli without plant formation and media which contain sorbitol and casine hydrolysate has 81.60% regeneration frequency (Table 5). High osmolarity also stimulates the regeneration frequency[7]. For this purpose high concentration of sorbitol was used.

Table 1: Callus induction and callus growth frequency of *Oryza sativa* (cv. Super Basmati) on MS media supplemented with 2,4-D 2 mg l^{-1}, 3% sugar and 0.6% agar

Media used for inoculation	Number of seeds	Callus induction	Callus growth	Frequency (%)
1	120	100.0	90	75.0
2	120	100.0	95	79.2
3	120	95.0	87	72.5
4	120	92.0	86	71.6
5	120	105.0	92	77.0
Average	120	98.4	90	75.2

Table 2: Callus induction and callus growth frequency of *Oryza sativa* (cv. Super Basmati) on N_6 media supplemented with 2,4-D 2 mg l^{-1}, 3% sugar and 0.6% agar

Experiment	Number of seeds	Callus induction	Callus growth	Frequency (%)
1	120	105.0	100.0	83.3
2	120	105.0	102.0	85.0
3	120	100.0	95.0	79.2
4	120	104.0	100.0	83.3
5	120	109.0	101.0	84.1
Average	120	104.6	99.6	82.9

Table 3: Maintenance of embryogenic calli of Super Basmati on N_6 media supplemented with 2 mg l^{-1} 2,4-D

Number of calli	Browning	Growth and proliferation	Age of Growth (%)
25	2	18	72
25	1	19	76
25	4	18	72
25	2	21	84

*Number of calli shown in column I represent the repeat of the experiment

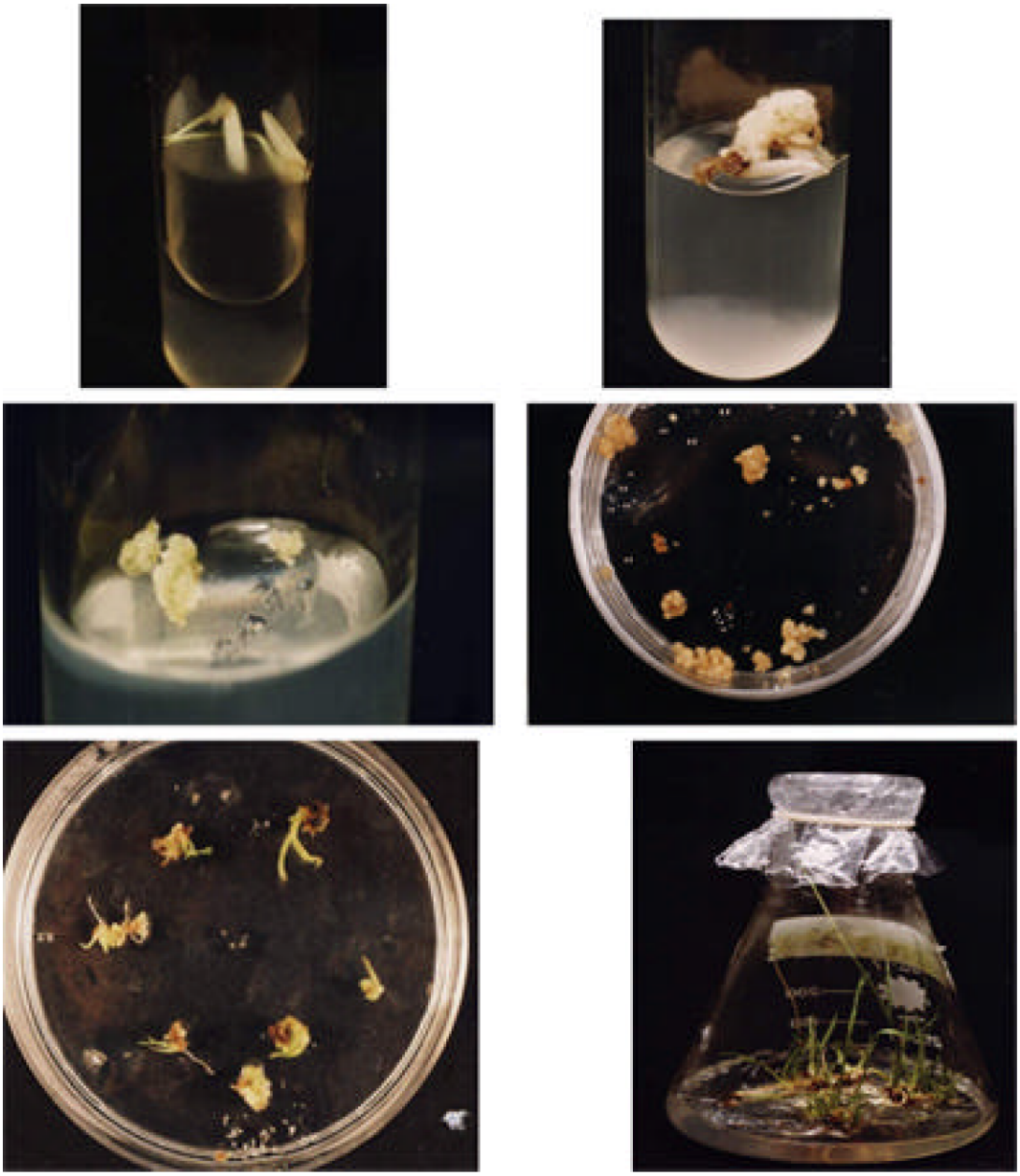
Table 4: Regeneration frequency of Basmati rice with different combination of auxin and cytokinins

Media used	Number of calli	Browning	Differentiation	Number of green spots	Plantlet regenerated	Frequency (%)
RM1	40	0	28	12	6	15.00
RM2	60	8	48	42	32	53.30
RM3	60	0	52	52	49	81.60

RM1=MS + Sucrose + Casine hydrolysate + Sorbitol + NAA 1 mg l^{-1} + BAP 1 mg l^{-1}
RM2=MS + Sucrose + Casine hydrolysate + Sorbitol + NAA 1 mg l^{-1} + BAP 2 mg l^{-1}
RM3=MS + Sucrose + Casine hydrolysate + Sorbitol + NAA1 mg l^{-1}+ BAP 5 mg l^{-1}

Table 5: Regeneration frequency of Super Basmati on MS media with NAA 1 mg l^{-1} and BAP 5 mg l^{-1} without addition of caseine hydrolysate and sorbitol

No. of experiment	Number of calli	Browning	Differenti-ation	Number of green spots	Plantlet formed	Regenerate frequency (%)
1	60	4.0	30.0	0.00	0	0
2	60	8.0	37.0	0.00	0	0
3	60	8.0	42.0	2.00	0	0
Average	60	5.3	36.3	0.66	0	0



Regeneration from scutellum derived calli.
(A) Arising of calli from scutellum
(B) Callus induction and growth
(C) Three week old calli on matainace media
(D) Calli became green on regeneration media
(E) Calli showing regeneration on MS media with NAA 1 mg L^{-1} and BAP 5 mg L^{-1}
(F) Regenerated plants from scutellum derived calli

We can conclude from the present study that now it is possible to obtained high regeneration frequency i.e. upto 80% from Super Basmati, which is shown more than other varieties. This high regeneration frequency will lead to improve the variety by genetic transformation technology.

It is concluded from the present study that a high frequency of regeneration (80%) has been achieved. This study is different from the previous ones because in this study higher frequency regeneration system is developed in super Basmati which is commercial cultivar.

REFERENCES

1. Rashid, H., S. Yokoi, K. Toriyama and K. Hinata, 1996. Transgenic plant production mediated by *Agrobacterium* in *indica* rice. Plant Cell Report, 15: 727-730.
2. Rashid, H., S.Y.A. Bokhari and A. Quraishi, 2001. Callus induction, regeneration and hygromycin selection of rice (Super Basmati). Online J. Biol. Sci., 1: 1145-1146.
3. Chu, C.C., C.S. Wang, C.C. Sun, C. Hsu, K.C. Yin and C.Y. Chu, 1975. Establishment of an efficient medium for anther culture of rice through comparative experiments on the nitrogen sources. Sci. Sinica, 18: 659-668.
4. Murashige, T. and F. Skoog, 1962. A revised medium for rapid growth and bioassy with tobacco tissue culture. Physiol. Plant, 15: 473-497.
5. Azria, D. and P.L. Bhalla, 2000. Plant regeneration from mature embryo derived calli of Australian rice (*Oryza sativa* L.) varieties. Aus. J. Agri., 51: 305-312.
6. Pons, M.J., V. Marfa, E. Mele and J. Massager, 2000. Regeneration and genetic transformation of Spanish rice cultivars using mature embryo. Euphytica, 114: 117-122.
7. Higuchi, N. and E. Maeda, 1991. Effect of pre treatment with excess sucrose or mannitol on plant regeneration from rice callus cultures. Jpn. J. Crop Sci., 60: 122-129.