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Research Article

EFFECT OF GROWTH REGULATORS ON *IN VITRO* CULTURES OF TWO BASMATI RICE GENOTYPES: RANBIR BASMATI AND BASMATI 370

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ABSTRACT

Basmati rice is preferred for its characteristic aroma and high export value in an international market. The techniques of tissue culture are employed to generate variations for improving the cultivars. In the present study a two stage sterilization treatment was found to be the most effective leading to maximum number of uncontaminated explants in two rice genotypes. Different combination of growth hormones in MS medium was used for shoot proliferation and multiplication. Maximum number of shoots in both Ranbir basmati and Basmati 370 were obtained in MS medium supplemented with 0.5 mg/l of Kinetin and 0.5mg/l BAP. Effective rooting of plantlets was observed on MS media supplemented with 0.2mg/l NAA with 93.48% in Ranbir Basmati and 90.21% in Basmati 370. An optimum concentration of 2.0mg/l of 2,4-D was found to be effective for callus induction. Best combination of plant growth regulators that resulted in plantlet regeneration from callus was 2 mg/l 2,4-D , 0.5 mg/l Kn and 2 mg/l NAA in both the Ranbir basmati and Basmati 370 leading to 84.67% and 83.33% shoots respectively.

Keywords: *Oryza sativa,* Callus, Basmati, Growth regulators, Genotypes.

INTRODUCTION

Rice (*Oryza sativa*) is the most important crop for one-third of the world's population, providing 35% of calorie intake with *indica* cultivars growing mostly in developing countries[1]. Rice is the staple food of many countries so, with the growing demand it is very important to ensure the constant availability of more rice at less cost. However, conventional propagation methods have several disadvantages such as the unavailability of large-scale true-to-type planting materials and vulnerability to environmental changes. Thus using biotechnological approaches the regeneration methods can be enhanced along with the variability.

Basmati rice is a small but an important export commodity of India due to its aroma and excellent cooking qualities[2]. Its production is limited by various stresses. Thus there is a need to improve the existing germplasm of Basmati cultivars by introducing variability into plants that could be utilized for crop improvement which further also depends on their regeneration capacity[3]. Thus callus production and its regeneration are the prime steps to manipulate a crop by biotechnological means. Present study was, therefore, carried out to develop a high frequency callus induction and regeneration system in two commercial varieties of basmati rice i.e. Ranbir basmati and Basmati 370 so as in order to investigate the effect of growth regulators on them.

MATERIAL AND METHODS

The plant material used in present study were the rice seeds of Ranbir Basmati (Ran Bas) and Basmati 370 (Bas 370) which were brought from SKUAST Jammu and were used for all the experiments (Fig 1).

Sterilization and establishment of aseptic cultures

The seeds of both varieties Ranbir Basmati and Basmati 370 were dehusked manually. The dehusked seeds were washed with detergent Tween 20, rinsed thrice with tap water and finally with distilled water. Then under aseptic

conditions in laminar flow cabinet the seeds were treated with 70% alcohol for 30 sec and with mercuric chloride (0.1% and 0.2%) for 2-3 mins. The seeds were placed on basal MS[4] nutritive media with 3% sucrose and 0.8% agar for their initial establishment and to monitor the percentage of contamination.

Shoot Proliferation

After 15 days of culturing, the explants that formed shoots were taken out under aseptic conditions and were inoculated into fresh MS medium with different concentrations and combinations of Benzyl amino purine (BAP) (0.5mg/l and 1.0mg/l) and Kinetin (Kn) (0.5mg/l and 1.0mg/l). Data were recorded after 10 days of culturing. Every possible care was taken to prevent any further contamination.

Rooting and hardening

Rootable shoots were excised from lavishly multiplying shoot clusters of both basmati varieties (Ran Bas and Bas 370) and were then transferred singly to culture tubes. Rooting medium was MS supplemented with different concentrations of NAA (Napthalene acetic acid) (0.1mg/l, 0.2mg/l and 0.3mg/l). The percentage of root induction was recorded after 30 days of culture. After the roots were well developed, the rooted plants were taken out of culture tubes, washed gently to remove agar and then transferred to the pots with a mixture of sand and soil in the ratio of 2:1. The plantlets in the pots were covered with jars to maintain the humidity. After 2 weeks, the jars were removed and the established plants were then transferred to soil in the field conditions and their survival rate was observed.

Callus induction

Dehusked seeds of Ranbir basmati and Basmati 370 were surface sterilized with 0.2% mercuric chloride for 2 mins and cultured on MS media supplemented with different concentrations of 2,4-D (2,4-dichlorophenoxy acetic acid). The cultures were kept at 25 ± 3°C in diffused light for initial 15 days and then were transferred to 2000 lux intensity for 16 hour photoperiod. Seeds were screened regularly for contamination and callus induction. Healthy callus (Granular, light yellow in color) was subcultured on fresh callus proliferating media for another three weeks. The callus induction frequency was recorded considering that each callus piece originated from a single seed.

Shoot regeneration

After 30 days the callus formed was transferred to fresh MS media containing kinetin and NAA keeping the concentration of 2,4-D constant (2.0 mg/l). Regenerated plantlets were counted based on the number of callus-producing plantlets.

Data collection and analysis

The average number and length of shoots per explant and the total number of shoots produced were calculated and used for evaluation of the different treatments. The shoots and roots were removed from the cultures to measure the number and length of shoots and roots. The statistical analysis is based on mean values and standard error.

RESULTS AND DISCUSSION

Shoot establishment

The surface sterilization treatment given to explants (seeds) in RB and Bas 370 using single sterilant for different time periods was not applicable, since it could not completely control the contamination. A two stage sterilization process which included the combination of sterilants i.e.70% alcohol (for 30 sec) and $HgCl_2$ (0.2% for 3 minutes) was found to most effective leading to maximum uncontaminated explants. All explants produced shoots within 30 days of their inoculation on MS medium.

Shoot multiplication

The different plant growth regulators are widely used for shoot induction and multiplication in tissue culture studies. The effect of BAP and Kinetin on shoot multiplication in both the rice varieties i.e. Ranbir basmati and Basmati 370 was studied and the results are presented in Table 1a and1b respectively.

The maximum number of shoots were established in MS media supplemented with (0.5mg/l) BAP and (0.5mg/l) Kinetin in both varieties as shown in table 1a and 1b . However in Ranbir Basmati much difference in number of shoots was not observed on MS media supplemented with either BAP (0.5 mg/l) or Kinetin (0.5 mg/l) as they were 16 and 15 shoots respectively (Table 1b) . Shoots were longer and dark green in colour (Fig 2). The results revealed that the length of shoots and the number of shoots increased with increasing concentration of BAP and Kinetin . The maximum shoot length (8.1±0.94) and the maximum number of shoots i.e. 18 were observed in Ranbir basmati (Table 1a), where as in Basmati 370 the maximum shoot length was (7.31±.0.4549) and maximum

number of shoots were 17 (Table 1b, Fig 3). This combination of BAP and Kinetin in concentration of 0.5 mg/l each was found to be the best as compared to other concentrations of BAP and Kinetin either used singly or in combination. This combination of BAP and Kn resulted in multiple shoot formation in Ranbir basmati but not in Basmati 370 (Fig 2). The results were at par with the earlier studies where cytokinins induced multiple shoot formation and increased shoot length [5,6].

***In vitro* Rooting and Hardening**

The shoots obtained in Ranbir basmati and Basmati 370 were transferred to MS medium containing different concentrations of NAA. Root initiation took place after 10-15 days of inoculation and well developed root system was attained in 3 weeks in both the varieties. The maximum percentage of root regeneration was 93.48% in Ranbir basmati and 90.21% in Basmati 370 on MS medium containing 0.2 mg/l NAA whereas basal MS media has 35.45% and 33.24% roots in Ranbir basmati and Basmati 370 respectively (Table 2, Fig 4).

Data analysis showed that the addition of NAA had a significant effect on the number and length of roots (Table 2). Similarly, the studies of Gautam *et al.*[7] on *in vitro* regeneration of *Matthiola incana* showed that the addition of NAA at concentration of 1.0mg/l and 4.0mg/l) induced high rooting in the explants.

Callus Induction

2, 4-D is the most suitable auxin for callus induction in rice tissue culture and the optimum concentration of 2, 4-D in callus induction depends on the explant source and rice genotypes [8,9]. In the present study, the effectiveness of different concentrations of 2, 4-D alone were evaluated for callus induction from dehusked seed of Ranbir basmati and Basmati 370. The callus initiation started 15 days after inoculation of dehusked seeds of both Ranbir basmati and Basmati 370 in culture tubes. The data on callus induction was recorded after three weeks of inoculation. MS medium supplemented with 0.5 mg/l of 2, 4-D produced lowest percentage (50%) of callus in both the basmati rice varieties whereas high frequency of callus induction occurred in MS media containing 2, 4-D at a concentration of 2.0 mg/l. Though difference was found between callus of both the varieties but Ranbir basmati had more potential towards callus formation than Basmati-370. The observed difference was not only in callus induction frequency but also in the quality of callus produced. Callus produced by Ranbir basmati was more embryogenic. Significant difference between two varieties for callogenesis under the same nutritional condition indicated that the callus induction quality is genotype dependent. These findings are in accordance with the reports of Khanna and Raina [10].

Shoot Regeneration and Multiplication

It was observed that the plant regeneration ability of plated calli depends on the variety and the callus inducing media. Ranbir basmati regenerated maximum number of plants. The current study showed that the best combination of plant growth regulator that caused plant regeneration from callus was 2,4-D (2.0 mg/l), Kn (0.5 mg/l) and NAA (2.0 mg/l) in both the varieties of rice i.e Ranbir basmati and Basmati 370. However, highest percentage of shoot regeneration was observed in Ranbir basmati (84.61%) and in Basmati 370, it was 83.33% (Table 3a and 3b, Fig 6). The plant regeneration was only 50% in both the varieties when only 2,4-D (2.0mg/l) was added as growth regulator to MS media. All calli were white in colour and their texture was friable. Libin *et al.* [11] also observed the production of desired calli on MS medium supplemented with 2.0 mg/l 2, 4-D in Biris rice with the frequency as high as 97%. Same results were observed by Azria and Bhalla [12] of getting plantlets from callus, induced from embryos of mature seeds of four Australian varieties of rice, when the MS medium was supplemented with 2.0 mg/l of 2,4-D.

Thus in the present study out of two different basmati rice genotypes, Ranbir basmati gave better response as compared to Basmati 370 under *in vitro* conditions. 2,4-D along with Kn and NAA gave high frequency of shoot regeneration from callus. Thus the calli formed from dehusked seeds in MS medium supplemented with 2.0 mg/l 2,4-D in both Ranbir basmati and Basmati 370 can be used for cell suspension and also in creating variations and transformation studies in both Ranbir basmati and Basmati 370.

Table 1 a: Effect of different concentration of BAP and Kinetin on shoot regeneration in Ranbir basmati

Medium MS + Growth regulators(mg/l)	No. of seeds inoculated	No. of Shoots regenerated	Number of shoots (after 12 days) (mean±S.E)	Length in (cm) after 12 days (mean±S.E)
MS+BAP (0.5) +Kinetin (0.0)	25	16	2.12 ± 0.54	4.14±0.5876
MS+BAP (0.0) +Kinetin (0.5)	25	15	2.37 ±0.88	5.01±0.6303
MS+BAP (0.5) +Kinetin (0.5)	25	20	3.5 ± 0.90	8.1±0.9476
MS+BAP (1.0) +Kinetin (0.0)	25	14	2.38 ± 0.89	6.35±0.4381
MS+BAP (0.0) +Kinetin (1.0)	25	12	2.27 ± 0.79	5.08±0.6501
MS+BAP (1.0) +Kinetin (1.0)	25	10	2.14 ± 0.63	4.96±0.6163

Table 1b: Effect of different concentrations of BAP and Kinetin on shoot regeneration in Basmati 370

MS + Growth regulators (mg/l)	No. of seeds inoculated	No. of Shoots regenerated	Number of shoots (after 12 days) (mean±S.E)	Length in (cm) after 12 days (mean±S.E)
MS+BAP(0.5)	25	14	1.99 ± 0.41	3.71±0.3405
MS+Kinetin(0.5)	25	12	2.12 ± 0.65	4.49±0.4479
MS+BAP(0.5) + Kinetin(0.5)	25	18	2.98 ± 0.89	7.31±0.4549
MS+BAP(1.0) + Kinetin(0.0)	25	13	2.72 ± 0.72	5.32±0.5446
MS+BAP(0.0) + Kinetin(1.0)	25	12	2.25 ± 0.70	4.27±0.3890
MS+BAP (1.0) + Kinetin(1.0)	25	10	2.10 ± 0.59	3.51±0.2896

Table 2: Effect of different concentrations of NAA on root initiation in Ranbir basmati and Basmati 370

Medium MS+Growth regulator NAA(mg/lt)	Ranbir basmati		Basmati 370	
	%age rooting	Type of roots	%age rooting	Type of roots
MS+NAA(0.0)	35.45	Thin, fragile roots with fewer root hairs	33.24	Small, thread like, with lesser root hairs
MS+NAA(0.1)	73.61	Thin, fragile roots with few root hairs	67.23	Small, thread like, with less root hairs
MS+NAA(0.2)	93.48	Long,well developed roots with numerous root hairs	90.21	Long,well Developed roots with numerous root Hairs
MS+NAA(0.3)	81.01	Thin,fragile roots with few root hairs	78.23	Small, thread like with lesser root hairs

Total number of shoots for each treatment were 20.

Table 3a: Effect of NAA and Kinetin on plantlet regeneration from callus in Ranbir basmati

Combinations (mg/l)	Total Seeds inoculated	Number of calli regenerated	Plantlet regeneration	Percent Regeneration	Type of shoots
MS+2,4-D (2.0)	15	6	3	50.0	Callus Proliferation without shoot regeneration
MS+2,4-D (2.0) + Kinetin (0.5)	15	8	5	62.50	2 long Shoots + numerous short shoots
MS+2,4-D (2.0) +NAA(2.0)	15	11	8	72.62	6 long shoots + numerous short shoots
MS+2,4-D(2.0)+Kinetin (0.5)+ NAA (2.0)	15	13	11	84.61	10 long shoots + numerous short shoots

Table 3b: Effect of NAA and Kinetin on plantlet regeneration from callus in Basmati 370

Combinations (mg/l)	Total seeds inoculated	Number of calli regenerated	Plantlet regeneration	Percent Regeneration	Type of Shoots
MS+2,4-D (2.0)	15	4	2	50.0	Callus proliferation, no shoot regeneration
MS+2,4-D (2.0)+ Kinetin (0.5)	15	7	5	71.42	2 long shoots + numerous short shoots
MS+2,4-D (2.0) + NAA (2.0)	15	9	7	77.77	4 long shoots + numerous short shoots
MS+2,4-D (2.0) +Kinetin (0.5) + NAA(2.0)	15	12	10	83.33	8 long shoots + numerous short shoots

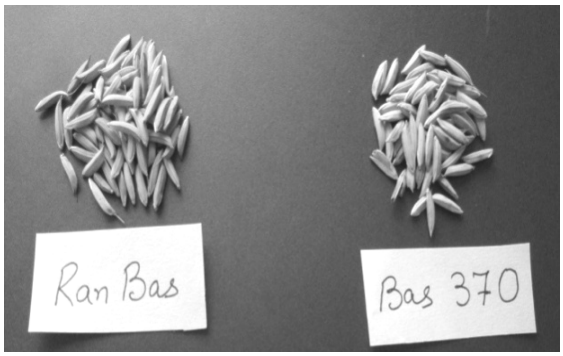

Fig. 1: Seeds of Ranbir Basmati and Basmati 370

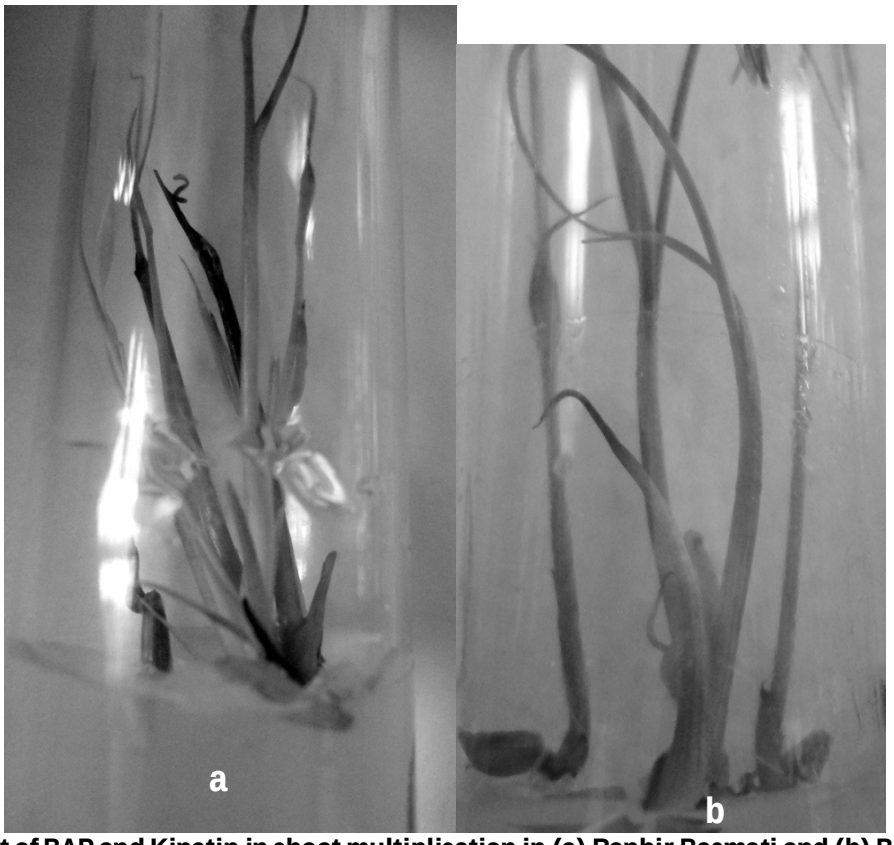

Fig. 2: Effect of BAP and Kinetin in shoot multiplication in (a) Ranbir Basmati and (b) Basmati 370

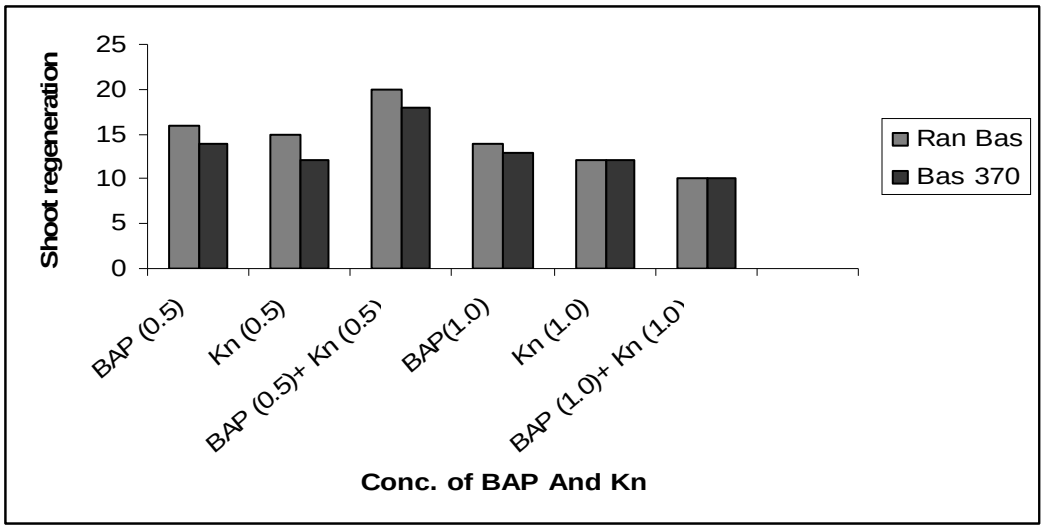

Fig. 3: Comparison of Shoot Regeneration in Ranbir Basmati and Basmati 370

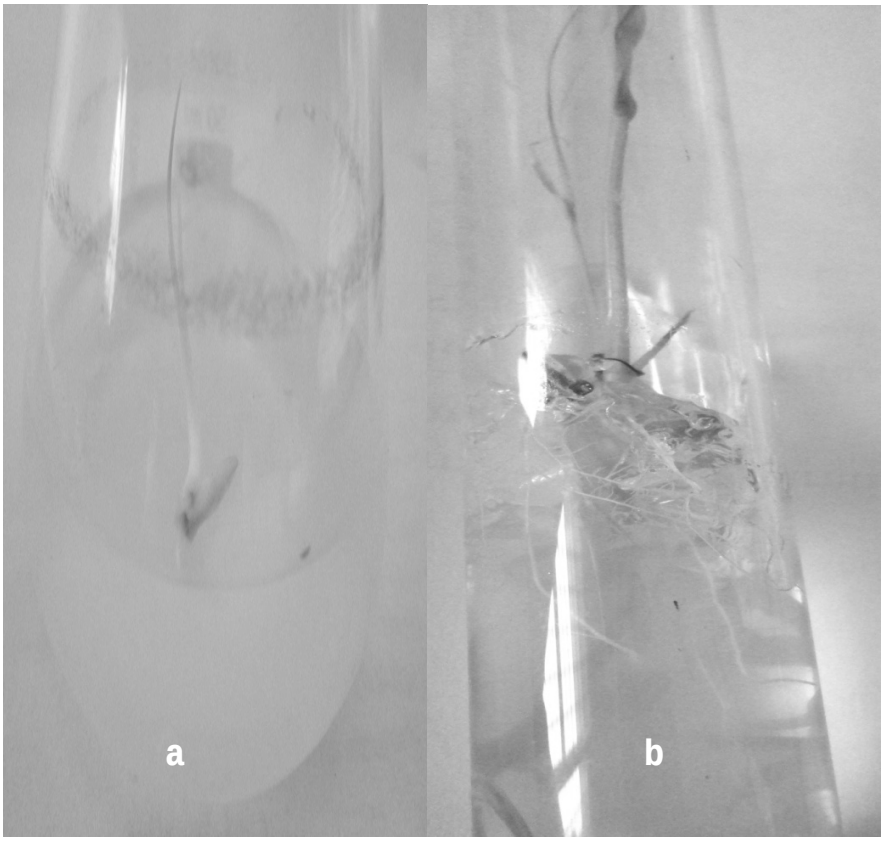

Fig. 4: Effect of NAA on root induction (a) MS without NAA (B) MS with NAA

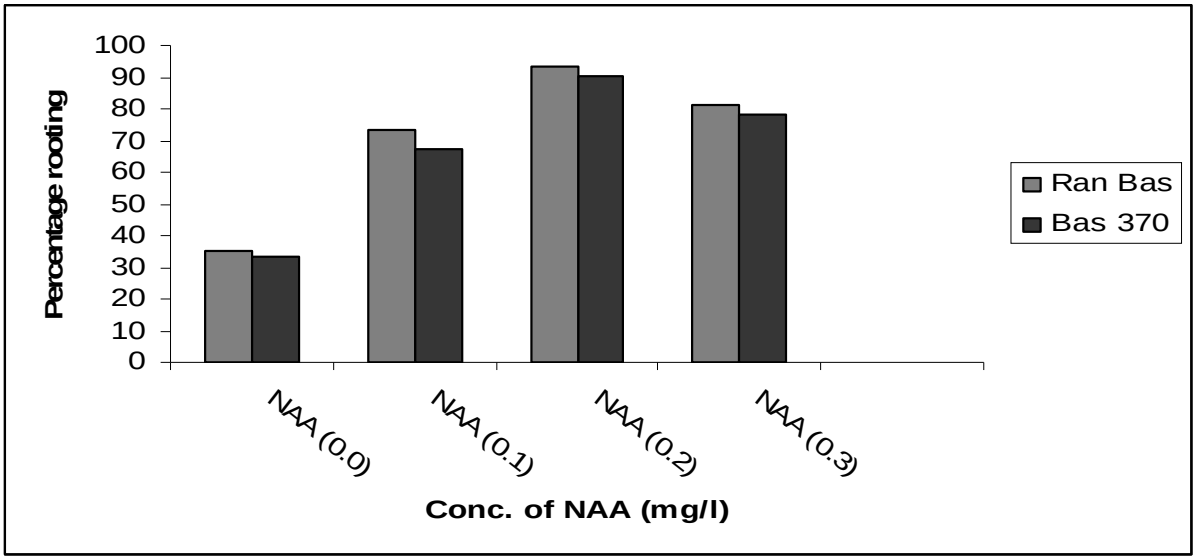

Fig. 5: Percentage rooting in Ranbir basmati and Basmati 370 at different concentrations of NAA

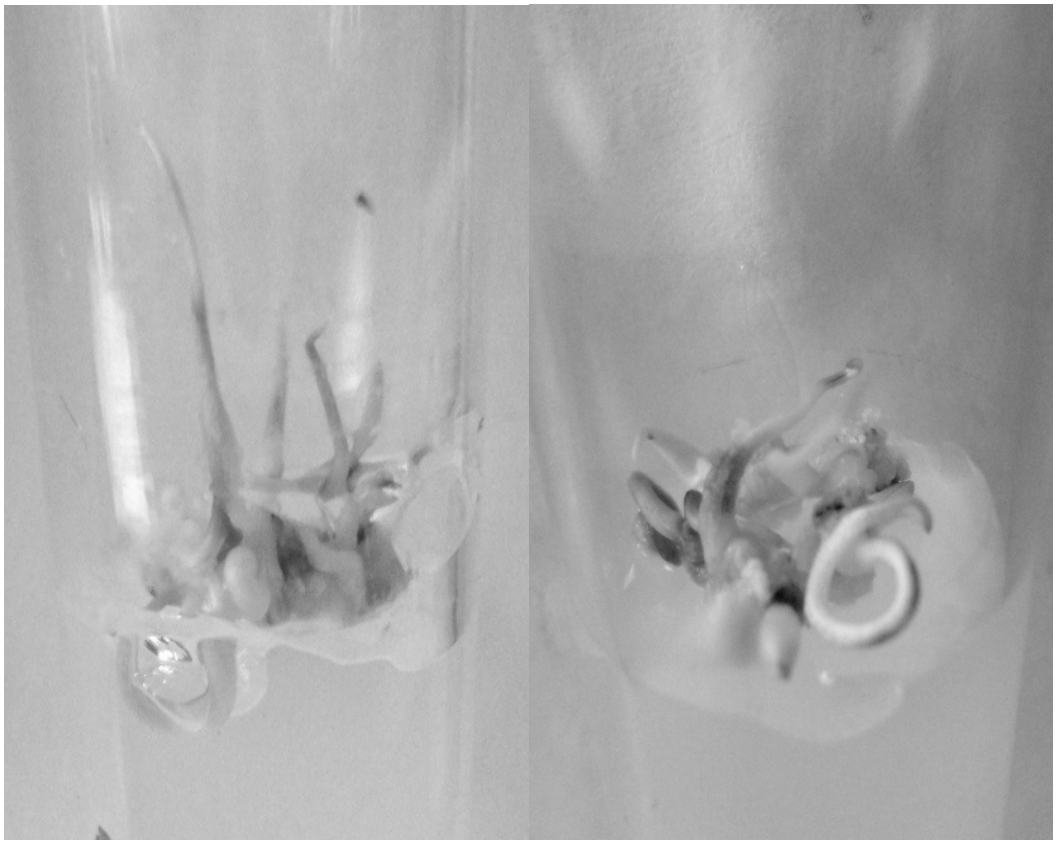

Fig. 6: Shoot regeneration from callus in (a) Ranbir Basmati and (b) Basmati 370

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