DOI : 10.58993/ijh/2023.80.1.3

Indian J. Hortic. 80(1), March 2023: 10-16

Index based selection of treatments and genotypes for the *in-vitro* shoot regeneration in banana

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ABSTRACT

The in vitro shoot regeneration studies for four banana cultivars (Poovan, Karpuravalli, Rasthali and Monthan) were conducted via shoot tip culture using three plant bioregulators (PBRs) viz., 6-Benzylaminopurine (BAP), Kinetin (KIN) and 1-naphthaleneacetic acid (NAA) in different combinations and concentrations. Shoot regeneration revealed the significant effects of various PBRs combinations. This study utilized index-based selection to select the best treatments and cultivars for shoot regeneration in bananas. Based on Smith's selection index score, the PBRs combination T6 (BAP@5.0mg/l+NAA@0.5mg/l) ranked first (0.24) and was selected as the best PBRs combination for shoot regeneration. Among the cultivars, 'Rasthali' (-0.76) ranked first as the best genotype for shoot regeneration in bananas. However, the combined effect of hormonal combination (T6) and cultivar 'Poovan' (2.61) ranked first. These results also showed that the response of ABB type (Karpuravalli and Monthan) was poorer than that of the AAB group (Poovan and Rasthali), indicating that the in vitro regeneration was much influenced by the genotype of plant species and the type of explants material as well as the type of PBR combinations. Overall, the cultivar-specific regeneration protocol proved essential for better in vitro regeneration of bananas.

Keywords: *Musa* spp., Plant growth hormones, Shoot tip cultures, Cultivars, Selection index.

INTRODUCTION

Banana (*Musa sp*.) is the world's fourth most important crop after three important kinds of cereals *viz*., rice, wheat and maize in gross production value (Khatun *et al*., 6). *In vitro* propagation is preferred as a substitute in crops like banana to overcome the issues related to conventional vegetative propagation, and also to satisfy the need for food production (Madhulatha *et al*., 7). There are many cultivars of banana, of that, Poovan, Rasthali, Karpuravalli, and Monthan are extensively cultivated and most preferred by the farmers of the cauvery delta region of Karaikal. In the traditional method of banana propagation, each mother plant can supply only 1 to 2 suckers. Thus, planting materials are in great demand during the planting season of banana cultivation. Moreover, suckers obtained from infested areas, are important sources of inoculum for several diseases. Hence, it is important to obtain suckers free from diseases.

Several protocols are available for *in vitro* propagation of banana. While establishment of cultures, this propagation type faces some inherent problems, such as frequent contaminations, browning of cultures, poor rate of multiplication, etc. affecting the rate of multiplication adversely. Further, it is genotypic dependent; the past evidence showed that the rate of multiplication would be less if there was 'B' genomes in banana cultivar group (Hirimburegama and Gamage, 3). The banana cultivars *viz*., 'Poovan' (AAB), 'Rasthali' (AAB), 'Karpuravalli' (ABB) and 'Monthan' (ABB), has 'B' genome, hence needs a potent regeneration protocol for its efficient micropropagation. In tissue culture, PGRs play a significant role in influencing the efficient regeneration protocol and multiplication rate. The effect of cytokinin on enhancing the shoot formation has been accounted for different cultivars (Aman *et al*., 2; Iqbal *et al*., 4; Kale, 5). Shoot elongation and proliferation rates are not only determined by cytokinin but also by the genotypes of given plant species (Ngomuo *et al*., 9). So, the present study investigates the effects of various combinations of PBRs on *in vitro* shoot regeneration of different local banana cultivars.

MATERIALS AND METHODS

The *in vitro* shoot regeneration experiment was performed in the Plant Tissue Culture laboratory of the Department of Plant Breeding and Genetics, PJN College of Agriculture and Research Institute, Karaikal, U.T. of Puducherry during the year 2018-2019 sponsored by National Horticultural Mission, Department of Agriculture and Farmers Welfare, Govt. of Puducherry. Four popular banana cultivars *viz*., 'Poovan', 'Karpuravalli', 'Rasthali' and 'Monthan'

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were selected as the genotype for study. The different PBRs combinations used for shoot regeneration are given in Table 1. For all the treatments, the readymade dehydrated MS (Murashige and Skoog) media (Hi media) were used.

The sword suckers of these four banana cultivars were collected from healthy banana plants from the farmer's field in and around Karaikal region as explant for this study. After collection, the explants are well cleaned in the running tap water. The washed explants were trimmed to about 10 cm, and then surface sterilized with ethanol (70%) followed by $HgCl_2$ (0.1%) followed by washing with double distilled water two to three times. Finally the explants that were trimmed about 1-2 cm were inoculated in bottles containing suitable media. Later on the explants produced well-developed shoots from the sucker (Fig. 1) and then these shoots were cross sectioned, and cultured on multiplication media (same as in initiation media). Five parameters were recorded after shoot regeneration *viz*., days to shoot initiation, shooting per cent, shoots per culture, shoot length (cm) and leaves per shoot. The inoculated culture bottles were maintained at 26±2°C temperature, 55-60% relative humidity (RH) with a photoperiodic cycle of 16 h of white fluorescent light (2000 lux). The data were statistically analyzed in a Completely Randomized Design. The variances and means for different parameters were analyzed by Tukey's HSD (honestly significant difference) test using STAR (Statistical Tool for Agricultural Research) software version 2.0. Smith's selection index score was calculated using the software PB (Plant Breeding) tools version 1.4 for better selection on shoot regeneration in banana.

RESULTS AND DISCUSSION

The results had shown a significant variation among different cultivars and treatments for all the characters under study. For days to shoot initiation, the cultivar 'Rasthali' responded earlier for shoot initiation (19.88 days), whereas 'Monthan' responded late for shoot initiation (25.37 days) (Table 3). Among the treatments, T_6 took the minimum duration to shoot initiation (15.92 days) (Table 2). Similar results were obtained by Paulos *et al.* (10) for days to shoot initiation for the same PBRs combination in cultivar 'Grand Naine'. The combined effects (Table 4) have shown the minimum days to shoot initiation in case of T_6 for both 'Karpuravalli' and 'Rasthali'; T_3 and T_4 for 'Poovan'and T_4 for 'Monthan'. Aman *et al*. (2) reported the shoot initiation in banana cv. 'Rajapuri Bale' with 6-Benzylaminopurine@4.0mg/l, whereas Ahmed *et al*. (1) found a similar observation in banana cv. 'Grand Naine'.

Table 1. Treatments used in shoot regeneration of different banana cultivars.

Treatments	Plant bioregulator
T_1	BAP@1.0mg/l (Control)
T_2	BAP@3.0mg/l
T_3	BAP@5.0mg/l
T_4	BAP@7.0mg/l
T_5	BAP@3.0mg/l+NAA@0.5mg/l
T_6	BAP@5.0mg/l+NAA@0.5mg/l
T_7	BAP@7.0mg/l+NAA@0.5mg/l
T_8	KIN@2.0mg/l+NAA@0.5mg/l
T_9	KIN@4.0mg/l+NAA@0.5mg/l
T_{10}	KIN@6.0mg/l+NAA@0.5mg/l

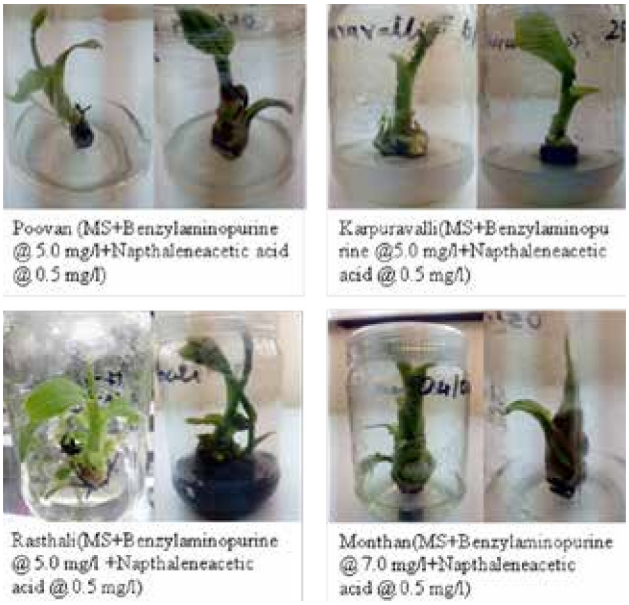

Fig. 1. Shoot growth on different cultivars of banana

For shooting per cent, the maximum regeneration (78.89%) was recorded in cultivar 'Rasthali' , while it was minimum in 'Monthan' (72.63%) (Table 3). Of the treatments (Table 2), T_7 treatment showed the highest shooting (97.69%). These results are similar to the findings of Khatun *et al.* (6), who recorded the best response with the use of benzylaminopurine@5.0mg/l. On contrary, MS medium with the least benzylaminopurine concentration was found to give better shooting per cent in banana cvs. 'Basrai', 'Ardhapuri', 'Srimanti' and 'Grand Naine' (Kale *et al.,* 5). These differences in the results might be due to the differences in the genotypes used.

The interaction of cultivar and treatment effects (Table 4), 'Rasthali' and 'Poovan' exhibited 100 per

Table 2. Effect of different treatments on shoot regeneration of banana.

Treatment	Days to shoot initiation	Shooting per cent	Shoots/ explant	Shoot length (cm)	Leaves/ shoot	Smith index
T_1	30.00[a]	38.68[f]	1.00[b]	2.27[e]	2.00[c]	-2.33
T_2	25.58[b]	39.44[f]	1.08[b]	3.59[d]	2.17[c]	-2.09
T_3	18.58[cd]	91.39[ab]	1.75[ab]	4.69[c]	2.17[c]	-0.08
T_4	18.92[c]	82.52[cd]	1.58[ab]	4.90[c]	2.33[bc]	-0.4
T_5	25.58[b]	83.34[cd]	2.42[a]	3.22[d]	2.17[c]	-0.48
T_6	15.92[d]	93.80[ab]	1.83[ab]	6.36[b]	3.42[a]	0.24
T_7	18.29[cd]	97.69[a]	1.75[ab]	7.69[a]	3.00[ab]	0.14
T_8	25.83[b]	73.33[e]	1.17[b]	3.34[d]	2.33[bc]	-0.86
T_9	20.50[c]	87.09[bc]	1.50[b]	4.73[c]	2.00[c]	-0.29
T_{10}	24.75[b]	75.56[de]	1.33[b]	5.04[c]	2.17[c]	-0.77

Mean performance having same letters are on par at 5% level of probability. (T_1-BAP@1.0mg/l, T_2- BAP@3.0mg/l, T_3- BAP@5.0mg/l, T_4- BAP@7.0mg/l, T_5- BAP@3.0mg/l+NAA@0.5mg/l, T_6- BAP@5.0mg/l+NAA@0.5mg/l, T_7- BAP@7.0mg/l+NAA@0.5mg/l, T_8- KIN@2.0mg/l+NAA@0.5mg/l, T_9- KIN@4.0mg/l+NAA@0.5mg/l, T_{10}- KIN@6.0mg/l+NAA@0.5mg/l)

Table 3. Effect of cultivars on shoot regeneration of banana.

Cultivar	Days to shoot initiation	Shooting per cent	Shoots/ explant	Shoot length (cm)	Leaves/ shoot	Smith index
Poovan	22.23[b]	78.11[a]	1.57[a]	4.63[a]	2.43[a]	-0.82
Karpuravalli	22.10[b]	75.50[ab]	1.57[a]	4.63[a]	2.43[a]	-0.91
Rasthali	19.88[c]	78.89[a]	1.40[a]	4.68[a]	2.33[a]	-0.76
Monthan	25.37[a]	72.63[b]	1.63[a]	4.39[a]	2.30[a]	-1.08

Mean performance having same letters are on par at 5% level of probability.

cent shooting. These results are at par with Ahmed *et al*. (1), wherein MS medium with BAP and NAA showed the maximum (100%) responses on shooting per cent in banana cv. 'Grand Naine'. The cultivar group AAB, 'Poovan' (78.11%) and 'Rasthali' (78.89%) tended to give the better shooting per cent than that of ABB group, 'Karpuravalli' (75.50%) and 'Monthan' (72.63%) (Table 3). Similarly, Hirimburegama and Gamage (3) also reported that the multiplication rate was adversely affected by more 'B' genome in the group than 'A' genome.

For shoots per explant, cultivars failed to exert any significant effect. The treatment T_5 had shown the maximum shoots per explant (2.42). Similar findings were reported by Khatun *et al*. (6) and Suseno (14) in banana cv. 'Sabri' and 'Raja Bulu' respectively with MS medium supplemented with 6-benzylaminopurine@3.0mg/l. Further, Iqbal *et al*. (4) also recorded 3.0 shoots per explants on the MS medium supplemented benzylaminopurine@3.0mg/l and indoleacetic acid @ 1.0mg/l. The combined effects (Table 4) exhibited the maximum number of shoots per explant (3.67) with the treatment combination of T_5 for the cultivars 'Poovan' and 'Karpuravalli'; T_4 and T_9 for the cultivar 'Rasthali' (2.33), and T_6 for the cultivar 'Monthan' (3.33). Udaya *et al*. (15) also found almost the similar results in which MS medium supplemented with 6-Benzylaminopurine @ 6.0 mg/l achieved multiple shoot formation (2.93) in banana cv. 'Monthan'.

Similar to shoots per explant, shoot length also showed non-significant variation due to the cultivars. Among the treatments (Table 2), T_7 had the highest shoot length (7.69 cm) followed by T_6 (6.36 cm). The combined effects (Table 4) exhibited the longest shoot length with T_7 (8.57 cm in each) for the cultivar 'Poovan' and 'Rasthali'. These results correspond with the findings of Ngomuo *et al*. (8) who recorded the highest shoot length with 6-Benzylaminopurine @ 6.0 mg/l in banana var. Yangambi. In this experiment, the highest shoot length (6.60 cm) was achieved in T_6 for 'Monthan' (Table 4). This investigation disagrees with the results of Udaya *et al*. (15), where they found the shoot length ranging from 4.5 to 5.5 cm with the treatments combination of 6-Benzylaminopurine @ 6.0 mg/l and naphthaleneacetic acid @ 0.5 mg/l in 'Monthan'. These differences in the results might be due to differences in treatment concentrations used.

Table 4. Combined effects of cultivars and treatments on shoot regeneration of banana.

Cultivar	Treatment	Days to shoot initiation	Shooting per cent	Shoots/ explant	Shoot length (cm)	Leaves/ shoot	Smith index
Poovan	T_1	30.00^{a}	35.55^{c}	1.00^{b}	2.00^{e}	2.00^{a}	-2.54
	T_2	25.00^{abc}	40.47^{c}	1.00^{b}	3.37^{de}	2.33^{a}	-1.59
	T_3	14.67^{e}	94.44^{a}	2.33^{ab}	4.33^{cd}	2.33^{a}	1.41
	T_4	14.67^{e}	84.07^{ab}	1.00^{b}	5.20^{bc}	2.33^{a}	0.37
	T_5	29.00^{a}	91.90^{ab}	3.67^{a}	3.17^{de}	2.00^{a}	0.75
	T_6	15.67^{de}	100.00^{a}	1.33^{b}	6.70^{b}	3.67^{a}	2.61
	T_7	21.67^{bcd}	98.33^{a}	1.33^{b}	8.57^{a}	3.33^{a}	1.91
	T_8	27.00^{ab}	76.67^{b}	1.33^{b}	3.17^{de}	2.33^{a}	-0.57
	T_9	19.00^{cde}	83.01^{ab}	1.33^{b}	4.37^{cd}	2.00^{a}	-0.19
	T_{10}	25.67^{ab}	76.67^{b}	1.33^{b}	5.47^{bc}	2.00^{a}	-0.71
Karpuravalli	T_1	30.00^{a}	37.78^{d}	1.00^{b}	2.00^{d}	2.00^{a}	-2.48
	T_2	23.00^{bc}	40.47^{d}	1.00^{b}	3.37^{cd}	2.33^{a}	-1.46
	T_3	18.00^{cd}	93.33^{a}	2.33^{ab}	4.33^{bc}	2.33^{a}	1.16
	T_4	18.67^{cd}	81.90^{abc}	1.00^{b}	5.20^{b}	2.33^{a}	0.05
	T_5	25.67^{ab}	81.11^{abc}	3.67^{a}	3.17^{cd}	2.00^{a}	0.68
	T_6	12.67^{e}	91.11^{ab}	1.33^{b}	7.33^{a}	3.67^{a}	2.57
	T_7	20.00^{cd}	95.77^{a}	1.33^{b}	5.87^{ab}	3.33^{a}	1.95
	T_8	29.67^{a}	72.22^{c}	1.33^{b}	3.17^{cd}	2.33^{a}	-0.86
	T_9	17.33^{de}	86.35^{abc}	1.33^{b}	4.37^{bc}	2.00^{a}	-0.01
	T_{10}	26.00^{ab}	75.00^{bc}	1.33^{b}	5.47^{b}	2.00^{a}	-0.78
Rasthali	T_1	30.33^{a}	40.47^{d}	1.00^{b}	2.00^{f}	2.00^{a}	-2.43
	T_2	28.33^{ab}	46.67^{d}	1.33^{ab}	3.43^{de}	2.00^{a}	-1.83
	T_3	14.67^{d}	96.67^{ab}	1.00^{b}	4.50^{bcd}	2.33^{a}	0.27
	T_4	25.33^{abc}	83.01^{bc}	2.33^{a}	5.40^{b}	3.33^{a}	0.48
	T_5	19.00^{cd}	81.11^{bc}	1.33^{ab}	3.07^{ef}	2.00^{a}	0.07
	T_6	12.00^{d}	94.44^{ab}	1.33^{ab}	5.43^{b}	3.33^{a}	2.23
	T_7	12.17^{d}	100.00^{a}	1.00^{b}	8.57^{a}	2.00^{a}	1.58
	T_8	19.00^{cd}	72.22^{c}	1.00^{b}	4.00^{cde}	2.33^{a}	-0.31
	T_9	17.67^{cd}	96.00^{ab}	2.33^{a}	5.30^{bc}	2.67^{a}	0.93
	T_{10}	20.33^{bcd}	78.33^{c}	1.33^{ab}	5.13^{bc}	2.33^{a}	0.05
Monthan	T_1	29.67^{a}	33.33^{e}	1.00^{b}	3.10^{e}	2.00^{a}	-2.50
	T_2	26.00^{ab}	37.78^{e}	1.00^{b}	4.20^{cd}	2.00^{a}	-2.06
	T_3	27.00^{ab}	81.11^{bc}	1.33^{b}	5.60^{b}	2.00^{a}	-0.67
	T_4	17.00^{d}	81.11^{bc}	2.00^{ab}	3.80^{de}	2.33^{a}	0.66
	T_5	28.67^{a}	79.26^{cd}	1.00^{b}	3.47^{de}	2.33^{a}	-0.79
	T_6	23.33^{bc}	89.63^{ab}	3.33^{a}	6.60^{a}	3.00^{a}	2.27
	T_7	19.33^{cd}	96.67^{a}	3.00^{a}	5.07^{bc}	2.67^{a}	2.22
	T_8	27.67^{a}	72.22^{d}	1.00^{b}	3.03^{e}	2.33^{a}	-0.94
	T_9	28.00^{a}	83.01^{bc}	1.00^{b}	4.90^{bc}	2.00^{a}	-0.94
	T_{10}	27.00^{ab}	72.22^{d}	1.33^{b}	4.10^{cd}	2.33^{a}	-0.62

Mean performance having same letters are on par at 5% level of probability. (T_1-BAP@1.0mg/l, T_2- BAP@3.0mg/l, T_3- BAP@5.0mg/l, T_4- BAP@7.0mg/l, T_5- BAP@3.0mg/l+NAA@0.5mg/l, T_6- BAP@5.0mg/l+NAA@0.5mg/l, T_7- BAP@7.0mg/l+NAA@0.5mg/l, T_8- KIN@2.0mg/l+NAA@0.5mg/l, T_9- KIN@4.0mg/l+NAA@0.5mg/l, T_{10}- KIN@6.0mg/l+NAA@0.5mg/l)

The effect of treatments alone exhibited a significant variation for leaves per shoot. Among the hormonal combinations (Table-2), T_6 showed the maximum number of leaves per shoot (3.42) followed by T_7 (3.00). Similar observations were reported by Paulos *et al.* (10), wherein 3.4 leaves per shoot were recorded with 6-benzylaminopurine @ 5.0 mg/l in banana cv. 'Grand Naine'. Prabhuling *et al*. (12) achieved the maximum number of leaves per shoot with 6-Benzylaminopurine @ 4.0 mg/l and naphthaleneacetic acid @ 1.0 mg/l in banana cv. 'Ney Poovan, as also observed in the present study.

The selection index helps us in computing these traits to choose a genotype. Smith (13) was first proposed the use of a selection index for simultaneous manipulation of several characters under selection. The use of a base index was suggested by Pesek and Baker (11), in which the weightage of the traits is given only for their economic values. For the first time, this index is being used in a tissue culture experiment to identify the best treatment. Index-based selection is the need of the hour even in tissue culture experiments, because here the parameters for regeneration can be weighted and based on that, best treatment may be selected, which will be the real best treatment, rather than judging best treatment on one individual character. Hence, this study differs from other routine studies by utilizing index based selection towards the different treatments and cultivars on shoot regeneration in banana. The economic weightage *viz*., -10, 10, 10, 8.5 and 7 was given manually for days to shoot initiation, shooting per cent, shoots per explant, shoot length and leaves per shoot respectively based on the importance of characters towards the shoot regeneration. The results of the present investigation comprising 10 treatments and four cultivars for all five characters are presented in the Table-5, 6 and 7. The calculated index score for different treatments ranged between -2.33 (T_1) to 0.24 (T_6).

The treatment T_6 showed the highest index value of 0.24 and was ranked first. The top 50 per cent of treatments were selected based on index scores (Table 5). The calculated index score for different cultivars ranged between -1.08 (Monthan) to -0.76 (Rasthali). Among the cultivars, 'Rasthali' ranked first, and had the highest Smith index value (-0.76). Top 50 per cent cultivar selection based on Smith's index, revealed that the cultivars 'Rasthali' and 'Poovan' were the best genotypes for shoot regeneration for all the characters studied (Table 6). The calculated index score for combined effects of treatments and

Table 5. The best treatments selected based on Smith's Selection Index (top 50 per cent).

Treatment	Days to shoot initiation	Shooting per cent	Shoots/ explant	Shoot length (cm)	Leaves/ shoot	Smith index
T_6	15.92	97.69	1.83	6.36	3.42	0.24
T_7	18.29	93.80	1.75	7.69	3.00	0.14
T_3	18.58	91.39	1.75	4.69	2.17	-0.08
T_9	20.50	87.09	1.50	4.73	2.00	-0.29
T_4	18.92	82.52	1.58	4.90	2.33	-0.4
MSI	19.63	89.30	1.80	36.91	2.51	NA
MAI	22.39	76.28	1.54	33.90	2.37	NA
SD	2.76	-13.01	-0.26	-3.00	-0.13	NA

MSI- Mean of selected individuals, MAI- Mean of all individuals and SD- Selection differential. (T_3- BAP@5.0mg/l, T_4- BAP@7.0mg/l, T_6- BAP@5.0mg/l+NAA@0.5mg/l, T_7- BAP@7.0mg/l+NAA@0.5mg/l, T_9- KIN@4.0mg/l+NAA@0.5mg/l)

Table 6. The best cultivars selected based on Smith's Selection Index (top 50 per cent).

Cultivars	Days to shoot initiation	Shooting per cent	Shoots/ explant	Shoot length (cm)	Leaves/ shoot	Smith index
Rasthali	19.88	78.89	1.63	4.38	2.33	-0.76
Poovan	22.23	78.11	1.57	4.63	2.43	-0.82
MSI	21.05	78.5	1.48	4.50	2.38	NA
MAI	22.39	76.28	1.54	4.50	2.37	NA
SD	1.34	-2.21	0.05	0.002	-0.007	NA

MSI- Mean of selected individuals, MAI- Mean of all individuals and SD- Selection differential.

Table 7. Selection on combined effects of cultivar and treatment based on Smith's Selection Index (top 10 per cent).

Cultivar	Treatment	Days to shoot initiation	Shooting per cent	Shoots/ explant	Shoot length (cm)	Leaves/ shoot	Smith index
Poovan	T_6	15.67	100	1.33	6.70	3.67	2.61
Karpuravalli	T_6	12.67	91.11	1.33	7.33	3.67	2.57
Monthan	T_6	23.33	89.63	3.33	6.60	3.00	2.27
Rasthali	T_6	12.00	94.44	1.33	5.43	3.33	2.23
MSI		15.92	93.80	1.83	6.36	3.42	NA
MAI		22.40	76.29	1.54	4.58	2.37	NA
SD		-6.48	17.51	0.29	1.77	1.04	NA

MSI- Mean of selected individuals, MAI- Mean of all individuals and SD- Selection differential. (T_6- BAP@5.0mg/l+NAA@0.5mg/l)

cultivars ranged between -2.54 to 2.61. Based on the combined effects (Table 7), treatment T_6 with the cultivar 'Poovan' ranked first and has recorded the highest index value (2.61).

Based on the index score, top most ranked treatment combination was T_6 (6-benzylaminopurine @ 5.0 mg/l + napthaleneacetic acid @ 0.5 mg/l). Hence this treatment can be used for effective micropropagation with minimum time interval. Among the four cultivars chosen from the Cauvery delta region, the cultivar 'Rasthali' ranked first and hence, this cultivar may be utilized for rapid *in vitro* propagation method.

Based on the characters under study, the response of ABB type (Cultivars Karpuravalli and Monthan) was poorer than that of AAB group (Cultivars Poovan and Rasthali) for shoot regeneration from the shoot tip explant because of the presence of more number of B genome in the cultivars 'Karpuravalli' and 'Monthan'. Hence, the *in vitro* shoot regeneration was much influenced by the genotype of plant species as well as the type of PBR combinations, and their concentrations.

AUTHORS' CONTRIBUTION

Conceptualization of research (KV, NS, UD, VV); Designing of the experiments (KV, NS, UD, VV); Contribution of experimental materials (KV, NS, UD); Execution of field/lab experiments and data collection (GD); Analysis of data and interpretation (GD); Preparation of the manuscript (GD, KV, NS, UD).

DECLARATION

The authors declare no conflict of interest.

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Received : December, 2022; Revised : February, 2023; Accepted : February, 2023