Effect of Paclobutrazol Drenching on Growth of Micropropagated and Seedling Plantlets of 12 Citrus Cultivars: Principal Component Analysis

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Paclobutrazol was applied to soil (0,5,25,50 and 100 mg/plant) in potted plants of 12 citrus cultivars propagated by tissue culture and seedlings. Required quantity of active ingredient of paclobutrazol was applied in 200 ml of water around the base in polythene bags and control plants were given 200 ml of water only on 4 month old seedings. Six random plants were selected for morphological observations at the age of one year. Results indicated that all citrus types were sensitive to paclobutrazol. Micropropagated plants seems less responsive to paclobutrazol than seedlings which might be due to increase in secondary roots of seedlings at early stage than micropropagated plantlets, which helped plants in absorption of paclobutrazol. Principal component analysis indicated that the cultivars, JL, AL and P registered very high positive loading in PC1 which indicated that response of paclobutrazol was least on these species where as distinct response on growth reduction was observed in *Citrus indica*, KM, SAT, SOB, SLS and CV. All the variables have very high positive loading except per cent of thick roots, which registered negative loading indicative of one of the most important parameters to be used. It was observed that application of paclobutrazol significantly affect the root morphology particularly root thickness.

Key words : Citrus, Micropropagation, Paclobutrazol, Principal Component Analysis

Citrus is a major fruit crop of north-eastern hill region of India. There is huge demand for planting material. Non availability of scientifically propagated planting material from elite clones for plantation are the main constraints in citrus cultivation. In recent years, tissue culture techniques (micropropagation) are increasingly used for rapid clonal propagation of several economic plants, restoration of vigor and yield and preservation of germplasm. However, information on performance of tissue culture plants are lacking. Besides, bioregulant like paclobutrazol (PCB) play a very important role in fruit production. Its application has been consistently documented as an effective retardant of vegetative growth in citrus (Monelise, 1986; Yelenosky *et al.*, 1995; Minger and Sanyu, 1996 and Matta and Tominga, 1998). Therefore, the present experiments were planned and the effect of various treatments were investigated.

Materials and Methods

A large number of micropropagated and seedling plants of 12 citrus cultivars were transplanted in polythene bag containing 1:1 soil and FYM mixture after 40 days of rooting/germination. Weeding and spraying of insecticides were done at regular intervals to protect the plants from the insect pests. The experiment was conducted at ICAR Research Complex for NEH Region, Umiam, Meghalaya, on 4-month old plant of 12 citrus cultivars kept in open sky after hardening. The experiment consisted of 5 concentrations of paclobutrazol (0, 5, 25, 50 and 100 mg/plant) as soil drench. The required quantity of active ingredient PCB was dissolved in water and 100ml of water was applied around the stem base in each polythene bag as soil drenching and control plants were given an application of 100ml water only. The experiment was laid out in factorial design replicated 3 times. Six plants were randomly selected for morphological observations at the age of one year. Plant characters were recorded for shoot length, root length, number of leaves, number of tap roots, number of secondary roots, shot weight, root weight and plant weight. The data were subjected to principal component analysis (Adams and Wiersma, 1978).

Twelve important citrus cultivars, mostly indigenous to NEH region, were selected for this study, including *Citrus volkameriana* as control.

Results and Discussion

A perusal of the data presented in Table 1 indicates

S.No.	Cultivars (Species)	Abbreviations
i.	Satkara (*Citrus macroptera* Mont.)	SAT
ii.	Khasi papeda (*Citrus latipes* Tanaka)	LAT
iii.	Sweet lime (*Citrus limettioides* Tanaka)	SLS
iv.	Soh Bitara (*Citrus sinensis* Osbeck)	SOB
v.	Indian wild orange (*Citrus indica* Tanaka)	I
vi.	Ada Jamir (*Citrus assamensis* Dutta & Bhatnagar)	ADA
vii.	Khasi mandarin (*Citrus reticulata* Blanco)	KM
viii.	Soh myndong (*Citrus jambhiri* Lush)	JL
ix.	Jaintia lemon (*Citrus limon* Burm)	JL
x.	Pummelo (*Citrus grandis* Osbeck)	P
xi.	Assam lemon (*Citrus limon* Burm)	AL
xii.	Volkamer Lemon (*Citrus volkameriana* Pasq.)	CV

marked variation in growth of planting material due to paclobutrazol drenching. Plant height was significantly reduced by paclobutrazol (PCB). Highest per cent of thick roots were observed with 100mg paclobutrazol drenching. The increasing concentration of paclobutrazol significantly influenced all the characters except root length, number of secondary roots and total dry weight of plant. In general, response of PCB was more pronounced on seedling than micropropagated plants. This may be because at initial stage, growth of seedlings was more than micropropagated plants. Seedling had more number of secondary roots at the time of application, which may absorb more PCB. However, at the age of one year, maximum secondary roots were observed in micropropagated plantlets.

Principal Component Analysis (PCA)

The data pertaining to principal component analysis is presented in Table 2. All the cultivars showed very high positive loading in PC1 and PC2. JL and P registered very high postive loading in PC1, which indicated that response of paclobutrazol was least on these species where as distinct response on growth reduction was observed in *C. indica*, KM, SAT, SOB, SLS and CV.

Data pertaining to PCA presented in Table 3 indicates that all the variables have very high positive loading for PC1 except per cent of thick roots which registered negative loading in PC1 indicative of one of the most important parameters to be used. It was observed that application of paclobutrazol significantly affect the root morphology particularly root thickness. PC1 contributes 52 per cent variance towards the total variability by recording higher loading. Leaf area recorded negative loading in PC2 which showed that this is also an important variable. Number of secondary roots and length of internode showed negative loading in PC3 which

Table 1. Interaction between paclobutrazol drenching and method of propagation

S.No.	Paclobutrazol	Method of propagation	Plant height (cm)	No. of leaves	Root length (cm)	Stem diameter (mm)	length of internode (mm)	Leaf area (cm^2)	No. of secondary roots	%of thick roots	Plant dry wt. (g)			
											stem	leaves	roots	total
1	0	S	22.54	23.9	23.14	3.69	8.71	19.94	66.39	0.00	1.06	1.42	1.29	3.77
		M	27.76	27.17	25.13	3.80	8.81	20.60	79.14	0.00	1.23	1.55	1.51	4.29
2	5mg	S	21.72	22.77	22.20	3.66	8.26	19.36	64.39	64.39	17.02	0.93	1.24	3.25
		M	26.27	26.16	24.46	3.74	8.36	19.81	76.29	15.39	1.04	1.34	1.26	3.64
3	25mg	S	17.89	20.39	20.32	3.31	6.83	16.86	53.16	38.32	0.75	0.91	0.82	2.48
		M	21.63	23.14	22.39	3.42	7.16	17.29	57.16	35.49	0.84	1.01	0.92	2.77
4	50mg	S	14.76	17.47	18.28	2.96	5.96	14.64	45.53	52.23	0.58	0.74	0.66	1.98
		M	18.25	20.32	20.95	3.09	6.29	15.45	48.24	49.06	0.67	0.83	0.75	3.00
5	100mg	S	9.97	13.8	14.24	2.38	4.19	9.59	30.22	63.37	0.34	0.54	0.50	1.38
		M	14.01	17.49	17.37	2.51	4.52	10.76	32.99	54.48	0.42	0.62	0.59	1.63
	SEm ±		0.18	0.15	1.70	0.17	0.07	0.11	2.95	4.73	0.01	0.012	0.011	0.16
	C.D. (P=0.05)		0.49	0.41	NS	0.47	0.19	0..30	NS	13.10	0.03	0.033	0.03	NS

S= Seedling, M=Micropropagation

Table 2. Principal component scores for citrus growth as influenced by paclobutrazol drenching

Sl.No.	Cultivars	PC1	PC2	PC3	PC4	PC5	PC6
1	SAT	27.11	58.91	1.89	-12.15	-2.815	2.76
2	LAT	45.81	60.25	3.70	5.67	-1.57	4.03
3	SLS	34.91	67.82	14.67	-1.90	-1.37	1.01
4.	SOB	31.19	82.02	7.08	1.21	-7.65	4.19
5.	I	28.68	42.72	4.76	0.45	-4.88	3.74
6.	ADA	41.38	59.92	22.83	-9.30	-3.45	4.40
7.	KM	24.97	68.45	0.39	-7.14	-3.38	2.36
8.	SM	47.77	70.06	3.18	-4.87	2.30	4.62
9.	JL	67.35	63.91	5.83	-1.90	-4.35	2.10
10	P	54.69	57.64	5.65	-4.42	-5.54	3.75
11	CV	39.78	60.09	9.13	2.83	-2.82	1.25
12	AL	57.88	63.48	2.80	-11.14	-5.28	2.45

Table 3. Latent vectors and Latent roots for different variables under influence of paclobutrazol drenching

Sl.No.	Variables	Principal Component					
		PC1	PC2	PC3	PC4	PC5	PC6
1	Plant height	0.45	0.15	0.05	0.40	0.34	-0.40
2	No. of leaves	0.15	0.18	-0.10	0.37	0.52	0.41
3	Root length	0.17	0.19	0.13	0.61	-0.72	0.14
4.	Stem diameter	0.05	0.01	0.03	0.02	0.05	0.07
5.	Length of internode	0.17	0.02	-0.07	0.17	0.15	-0.3
6.	Leaf area	0.25	-0.04	0.93	-0.20	-0.07	0.04
7.	No. of secondary roots	0.66	0.41	-0.27	-0.51	-0.02	0.04
8	% of thick roots	-0.46	0.86	0.15	-0.05	0.08	-0.11
9	Stem dry wt.	0.03	0.02	0.01	-0.00	0.05	0.21
10	Leaves dry wt.	0.02	0.02	0.01	-0.01	0.05	0.17
11	Roots dry wt.	0.02	0.02	0.01	0.01	0.02	0.21
12.	Plant dry wt.	0.08	0.06	0.01	0.00	0.11	0.62
	Latent roots	178.37	86.12	39.51	31.79	6.36	1.52
	Percentage variance	51.76	24.99	11.47	9.23	1.85	0.44
	Cumulative variance	51.76	76.76	88.22	97.45	99.30	99.74

contributed 12 per cent variance. It can be concluded that per cent thick root is the most important variable to be taken into account followed by leaf area, number of secondary roots and internodal length.

It is evident from Table 4 that 50 and 100 mg/l paclobutrazol registered negative loading in PC1 indicating that these are the best treatment to reduce the overall growth of citrus. However, 100mg/l paclobutrazol drenching showed complete suppression of growth. The PCA presented in Table 5 shows that among all the variables, per cent of thick root registered negative loading in PC1 which indicate the importance of treatment. PC1 contributes 99 per cent variance towards total variability which again confirms the importance of this PC.

The principal component scores using correlation matrix for different cultivars propagated by tissue culture and seedling and influenced by paclobutrazol drenching

Table 4. Principal component scores for paclobutrazol drenching

Sl. No.	Paclobutrazol levels	PC1	PC2	PC3	PC4
1.	Control	49.45	70.46	-9.66	5.39
2.	05mg/plant	34.41	76.87	-11.91	4.93
3.	25mg/plant	8.28	75.80	-10.98	6.15
4.	50mg/plant	-8.48	76.56	-8.38	5.17
5.	100mg/plant	-33.68	71.25	-11.41	5.20

Table 5. Latent vectors and latent roots for variables under influence of paclobutrazol levels

	Variables	Principal Component			
		PC1	PC2	PC3	PC4
1.	Plant Height	0.16	0.17	-0.15	0.27
2.	No. of leaves	0.12	0.11	-0.13	0.59
3.	Root length	0.09	0.38	0.88	-0.13
4.	Stem Diameter	0.02	0.04	-0.00	0.09
5.	Length of internode	-0.05	0.07	0.02	0.1
6.	Leaf area	0.12	0.27	0.15	0.63
7.	No. of secondary roots	0.51	0.66	-0.37	-0.37
8.	% of thick roots	-0.82	0.54	-0.16	-0.01
9.	Stem dry wt.	0.01	0.00	0.00	0.04
10.	Leaves dry wt.	0.01	-0.01	-0.01	-0.04
11.	Roots dry wt.	0.01	-0.02	-0.01	0.03
12	Plant dry wt.	0.03	-0.05	-.0.04	-0.06
	Latent Roots	1101.09	9.48	2.06	0.22
	Percentage variance	98.94	0.85	0.19	0.02
	Cumulative variance	98.94	99.79	99.98	100.00

are presented in Table 6. All the cultivars recorded highest loading for factor 2 followed by factor 1. The latent vectors presented in Table 7 indicate that the first three principal component cumulatively contributed 87.17%, 28.11% and 10.38% respectively for factor 1, factor 2 and factor 3.

Table 6. Principal component scores for interaction between cultivar and methods of propagation under influence of paclobutrazol drenching

Sl. No.	Cultivars	Method of propagation	PC1	PC2	PC3	PC4
1.	SAT	S	25.59	55.79	-5.53	-14.54
		M	31.70	59.65	-2.98	-11.44
2.	LAT	S	43.47	59.42	6.05	3.13
		M	49.99	59.55	4.05	2.46
3.	SLS	S	35.88	50.13	9.25	-9.79
		M	32.71	84.44	13.78	-7.65
4.	SOB	S	28.36	80.94	6.66	-1.58
		M	36.89	81.25	2.74	-5.32
5.	I	S	24.36	42.03	5.39	-1.97
		M	34.58	42.00	2.22'	-2.56
6	ADA	S	39.80	59.58	16.79	-18.57
		M	39.34	58.65	16.63	-18.29
7.	KM	S	15.71	63.40	0.84	-5.09
		M	37.52	71.40	-9.14	-8.82
8.	SM	S	45.11	67.66	-0.14	-8.82
		M	53.30	70.41	0.89	-4.95
9.	JL	S	66.25	61.79	4.46	-5.39
		M	70.76	63.21	5.35	-4.24
10	P	S	53.42	55.60	3.18	-7.42
		M	57.60	56.84	3.64	-6.86
11.	CV	S	36.95	58.63	9.23	-2.07
		M	44.24	60.29	8.12	-1.92
12.	AL	S	55.21	59.81	-2.12	-14.66
		M	63.61	63.58	-0.95	-10.03

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Table 7. Latent vectors and latent roots for variables under influence of cultivar and method of propagation

Sl. No.	Variables	Principal Component PC1	PC2	PC3	PC4
1.	Plant height	0.43	0.18	0.24	0.36
2.	No. of leaves	0.16	0.21	0.05	0.37
3.	Root length	0.16	0.18	0.33	0.49
4.	Stem diameter	0.04	0.00	0.04	0.01
5.	Length of internode	0.15	0.01	0.02	0.18
6.	Leaf area	0.22	-0.02	0.79	-0.54
7.	No. of secondary roots	0.69	0.36	-0.44	-0.37
8.	% of thick roots	-0.45	0.87	0.07	-0.13
9.	Stem dry wt.	0.02	0.01	0.01	0.00
10.	Leaves dry wt.	0.02	0.02	0.01	-0.01
11.	Roots dry wt.	0.02	0.02	0.01	0.01
12.	Plant dry wt.	0.07	0.05	0.02	0.00
	Latent Roots	192.44	110.00	40.63	33.94
	Percentage variance	49.17	28.11	10.38	8.67
	Cumulative variance	49.17	77.28	87.67	96.34

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