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EFFECT OF PACLOBUTRAZOL DRENCHING ON GROWTH OF MICROPROPAGATED AND SEEDLING PLANTLETS OF 12 CITRUS CULTIVARS

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Paclobutrazol (0, 5, 25, 50 and 100 mg/plant) was applied to soil in potted plants of 12 citrus cultivars propagated by tissue culture and seedlings. Required quantity of active ingredient of paclobutrazol was applied in 100 ml of water around the base in polythene bags and control plants were given 100 ml of water. The treatments were applied on 4 month old seedling. Six randomly selected plantlets were used for recording morphological observations at the age of one year. Results indicated that all citrus types were sensitive to paclobutrazol. Overall growth of seedlings was checked by paclobutrazol application. Treated plants produced shorter internode, shorter/thicker roots and reduced plant height and dry weight. Micropropagated plants seem less responsive to paclobutrazol than seedlings this might result in an increase in secondary roots of seedlings at early stage than micropropagated plantlets, which helped plants in absorption of paclobutrazol.

Key words: herbicides, plant phisiology

Efeito da rega de paclobutrazol no crescimento de plântulas micropropagadas e mudas seminais de doze cultivares de Citros. Paclobutrazol (0, 5, 25, 50 e 100 mg/planta) foi aplicado no solo em plantas envazadas de 12 cultivares de Citrus propagadas por cultura de tecido e mudas seminais. A quantidade do ingrediente ativo de paclobutrazol foi diluido em 100ml de água e aplicado ao redor da base em sacos de polietileno e as plantas testemunhas receberam 100ml de água. Os tratamentos foram aplicados em mudas com 4 meses de idade. Seis mudas selecionadas ao acaso foram usadas para anotações das observações morfológicas na idade de 1 ano. Os resultados indicaram que todos os tipos de Citros foram sensíveis ao paclobutrazol. O crescimento das mudas foi paralisado pela aplicação do paclobutrazol. As plantas tratadas apresentaram internódios mais curtos, raízes mais curtas e mais espessas, o peso seco e altura das plantas foram reduzidos. As plantas micropropagadas parecem ser menos sensíveis ao paclobutrazol do que as mudas seminais, podendo resultar num incremento de raízes secundárias das plântulas num estágio mais cedo do que as mudas micropropagadas, o que contribuiu na absorção do paclobutrazol pelas plantas.

Palavras-chave: herbicida, fisiologia vegetal

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Introduction

Citrus is a major fruit crop of North Eastern Hills (NEH)Region of India. There is huge demand of planting material. Non availability of scientifically propagated planting material from elite clones for plantations 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 vigour and yield cleaning of plant materials and preservation of germplasm. However information on performance of tissue culture plants are lacking. Besides, bioregulants 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 (Hazarika *et al.*2000*;* Hazarika *et al.*2001*:* Monelise, 1986; Yelenosky et al., 1995; Miner and Sanyu, 1996 and Matt and Timings, 1998) Therefore, the present experiment were planned and the effect of various treatment were investigated.

Materials and Methods

A large number of plantlets obtained through micropropagation and through seeds of 12 citrus cultivars of same age group were transplanted in polythene bag containing 1:1 soil and FYM mixture after 40days of rooting/germination. Weeding and spraying of insecticides were done at regular interval to protect the plantlets from insects and pests. The experiment was conducted at the Biotechnology laboratory of ICAR Research Complex for NEH Region, Umiam, Meghalaya, using 4-month-old plantlets of 12 citrus cultivars, which were kept in open sky after hardening.The experiment consisted of 5 levels of paclobutrazol (0, 5, 25, 50 and 100 mg/plant) as soil drench. The required quantity of PCB active ingredient was dissolved in water and 100ml of the PCB solution was applied around the stem base in each polythene bag as soil drenching. Control plants were given an application of 100ml water.The experiments were laid out in factorial design replicated 3 times. Six randomly plantlets were selected for morphological observations at the age of one year. Plant characters were recorded for shoot and root length, number of leaves, taproots and secondary roots and shoot, root and plant weight. The data were subjected to statistical analysis as described earlier (Parthasarathy and Nagaraju, 1998). The data were analysed by pooled analysis and presented in the tables.

Twelve important citrus cultivars mostly indigenous to NEH Region were selected for this study, including *C.volkameriana* as control (Table 1).

Results and Discussion

The data presented in Table 2 indicate marked variation in growth of plant parts in all the materials due to paclobutrazol drenching. Plant height was significantly reduced by paclobutrazol (PCB). Decrease in plant height, number of leaves, root length, stem diameter, length of internode, leaf area, number of secondary roots and plant dry weight were recorded with increasing concentrations of PCB. However the thick root percentage increased with increasing concentration of PCB. Highest thick roots (68.93 %) were observed with 100mg paclobutrazol drenching.

Mean growth parameters of planting material as influenced by paclobutrazol drenching are given in Table 3. Highly significant differences among the genotypes were observed for all the traits indicating a wide range of variation among the genotypes. Among the cultivars maximum thick roots percentage was observed in SOB (55.13) followed by KM (46.27) and SLS (43.46). It indicates that these cultivars are more responsive to paclobutrazol.

Interaction between cultivars and paclobutrazol drenching planting materials indicated that there were significant variations for all characters studied. PCB significantly influenced all the characters. At higher (100 mg/plant) concentration, number of secondary roots was significantly reduced in the all cultivars.

Table 1. Cultivars selected for the study.

Sl.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 & Bhattacharya)	ADA
vii.	Khasi mandarin (*Citrus reticulata* Blanco)	KM
viii.	Soh myndong (*Citrus Jambhiri* Lush)	SM
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

Table 2. Mean effect of paclobutrazol drenching on growth of planting material (Pooled analysis).

Sl. No.	Paclobu - trazol (mg/plant)	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	C	25.15	25.54	24.14	3.74	8.76	20.27	72.76	0.00	1.14	1.48	1.4	4.02
2	5 mg	23.99	24.47	23.33	3.70	8.31	19.59	70.34	16.21	0.98	1.29	1.17	3.44
3	25 mg	19.77	21.76	21.35	3.36	6.99	17.08	55.51	36.31	0.79	0.96	0.87	2.62
4	50 mg	16.51	18.90	19.62	3.03	6.13	15.05	46.88	50.64	0.62	0.78	0.70	2.1
5	100 mg	11.99	15.65	15.80	2.45	4.35	10.17	31.61	68.93	0.38	0.58	0.54	1.5
	SEm ±	0.13	0.109	1.21	0.012	0.05	0.07	2.08	3.35	0.007	0.008	0.007	0.11
	C.D. (P=0.05)	0.36	0.3	3.35	0.033	0.138	0.19	5.76	5.28	0.02	0.022	0.02	0.30

Table 3. Mean growth parameters of planting material as influence by paclobutrazol drenching (Pooled analysis).

Sl. No.	Cultivars	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	SAT	8.88	15.71	11.88	2.51	3.75	10.09	49.70	37.64	0.38	0.58	0.44	1.40
2	LAT	24.34	26.25	25.64	3.98	10.18	12.97	52.72	29.53	1.40	1.09	1.52	4.01
3	SLS	19.77	20.87	21.49	2.06	6.97	21.55	49.35	43.46	0.40	0.82	0.72	1.94
4	SOB	17.61	23.09	29.46	2.71	5.18	12.08	54.67	55.13	0.82	1.17	1.28	3.27
5	I	11.68	16.91	18.71	2.37	3.75	11.06	37.33	22.53	0.14	0.31	0.21	0.66
6	ADA	16.87	17.60	19.67	3.68	3.92	32.52	52.57	34.72	1.07	1.32	1.07	3.46
7	KM	11.46	18.90	16.52	2.01	3.72	6.75	50.20	46.27	0.21	0.53	0.35	1.09
8	SM	23.93	27.05	18.64	3.65	8.02	15.01	62.90	37.60	1.53	1.97	1.51	5.01
9	JL	32.25	25.37	27.29	4.43	10.22	21.29	72.31	23.26	1.03	1.34	1.15	3.52
10	P	22.68	20.96	25.55	3.79	9.47	18.80	62.86	23.76	0.92	1.17	1.51	3.60
11	CV	21.50	22.28	24.12	3.30	8.43	16.93	48.86	33.31	0.33	0.59	0.46	1.38
12	AL	22.81	20.17	20.39	3.58	9.32	18.09	71.60	27.24	1.19	1.32	1.02	3.53
	SEm ±	0.19	0.17	1.87	0.019	0.07	0.11	3.23	5.19	0.011	0.014	0.01	0.17
	C.D (P=0.05)	0.53	0.47	5.18	0.053	0.19	0.30	8.95	14.37	0.03	0.038	0.03	0.47

Mean growth of seedling and micropropagated plantlets of citrus under influence of paclobutrazol drenching presented in Table 4 indicated significant differences in all characters studied except root length and thick root percentage. It is clear from the table that seedlings are more sensitive to paclobutrazol than micropropagated ones.

Data pertaining to interaction between paclobutrazol drenching and method of propagation are given in Table 5. The results showed that increasing concentration of paclobutrazol significantly influenced all the characters except root length, number of secondary roots and plant total dry weight. At higher concentrations seedling height was reduced more than micropropagated ones.

Interactions between cultivars and method of propagation were also significant except for root length and total plant weight (Table 6). The results indicated that paclobutrazol significantly decrease plant height, number of leaves, leaf area, number of secondary roots and dry weight of stem, leaves and roots with increasing concentration in all cultivars. However the response was more pronounced on seedling than micropropagated ones. Paclobutrazol induced inhibition of secondary roots in all cultivars.

In general there has been a significantly decrease in all characters studied with increasing concentrations of paclobutrazol (PCB) except thickroots. Paclobutrazol appeared to have interactive effects on growth of citrus

Table 4. Mean growth of seedlings and micropropagated plantlets of citrus under influence of paclobutrazol Drenching (Pooled analsis).

Sl. No.	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	Seedling	17.38	19.67	20.84	3.20	6.79	16.08	52.08	34.19	0.73	0.97	0.87	2.57
2	Micropro-pagated	21.59	22.86	22.06	3.31	7.03	16.78	58.77	34.89	0.84	1.07	1.00	2.91
	SEm ±	0.08	0.06	0.76	0.007	0.03	0.05	1.32	2.12	0.004	0.005	0.005	0.07
	C.D. (P= 0.05)	0.22	0.17	NS	0.019	0.08	0.138	3.65	5.87	0.011	0.014	0.014	0.19

Table 5. Interaction between paclobutrazol drenching and method of propagation.

Sl. No.	Paclobu-trazol	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	C	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	17.02	0.93	1.24	1.08	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

plants. PCB affected vegetative growth of plantlets differently at two propagation regimes. Development of new and thicker roots near the soil surface and reduction in secondary fibrous roots as a result of soil drenching was common at higher concentration of PCB. These results show that paclobutrazol reduced the vegetative growth in citrus plantlets. Similar results were also recorded by some earlier workers (Vu and Yelenosky, 1992; Yelenosky et al., 1995; Minger and Sanyu, 1996; Okuda et al., 1994 and Matta and Tominga, 1998). Retardation of shoot growth involves a reduction in gibberellin level, since vegetative growth inhibition by paclobutrazol can be completely countered by GA application (Greene and Murray, 1983). Leaf number and leaf area per seedling was reduced by paclobutrazol (Singh, et al., 1999).

There were major differences in cultivar response to paclobutrazol. These results are in agreement with the finding of Yelenosky et al. (1995) and Singh et al. (1999).

Total plant dry weight; dry weight leaves dry weight and root dry stem weight was reduced by paclobutrazol. These results are in conformity with the finding of Mehouachi et al., (1996), indicating that PCB inhibited dry weight of citrus rootstocks. PCB is also known to alter the source-sink relations in plant and directly or indirectly reallocates carbohydrates resources (Anon, 1984)

Plant growth retardants generally induce a shortening of the internodes of higher plants *in vivo* and some have additional effect such as reduction in leaf size and thickening of roots. All the citrus cultivars were sensitive to PCB, which caused a proliferation of shorter/thicker roots. Similar types of observation on variations in roots morphology at high level of PCB drenching were made by Steffens et al. (1995) And Vu and Yelenosky, 1992.

In general, responses to PCB were more pronounced

Table 6. Interaction between cultivar and method of propagation under influence of paclobutrazol drenching.

Sl. No.	Cultivar	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	SAT	S	7.03	13.81	9.09	2.31	3.32	9.33	47.87	37.61	0.31	0.48	0.36	1.15
		M	10.75	17.61	14.66	2.71	4.17	10.87	51.53	37.67	0.45	0.68	0.52	1.65
2	LAT	S	24.01	25.29	25.35	3.89	9.68	12.72	49.73	31.07	1.37	1.06	1.49	3.92
		M	24.67	27.21	25.94	4.07	10.68	13.23	55.71	27.98	1.43	1.13	1.54	4.1
3	SLS	S	15.27	16.23	20.05	3.04	6.94	21.33	47.29	26.98	0.28	0.72	0.56	1.56
		M	24.26	25.51	22.95	3.08	6.99	21.77	51.41	59.95	0.52	0.91	0.89	2.3
4	SOB	S	13.57	21.61	20.73	2.66	5.07	11.68	51.27	56.53	0.71	1.01	1.23	2.95
		M	21.66	24.56	24.2	2.76	5.29	12.49	58.07	53.73	0.93	1.24	1.33	3.5
5	I	S	10.41	15.97	18.11	2.34	3.73	10.98	32.8	25.07	0.13	0.30	0.18	0.61
		M	12.94	17.85	19.31	2.39	3.77	11.15	41.87	20.00	0.15	0.33	0.23	0.71
6	ADA	S	16.38	16.87	19.1	3.64	3.92	32.08	51.07	36.27	1.03	1.28	1.02	3.33
		M	17.36	18.33	20.24	3.71	3.92	32.97	54.07	33.16	1.10	1.36	1.12	3.58
7	KM	S	10.13	17.73	15.25	1.97	3.68	6.65	37.07	48.00	0.19	0.51	0.32	1.02
		M	12.79	20.07	17.79	2.04	3.74	6.85	63.33	44.53	0.22	0.56	0.38	1.16
8	SM	S	20.73	24.73	17.09	3.61	7.95	14.74	60.6	38.55	1.49	1.92	1.38	4.79
		M	27.13	29.37	20.19	3.68	8.09	15.27	65.2	36.65	1.57	2.02	1.65	5.24
9	JL	S	30.94	24.53	26.2	4.36	10.19	20.77	70.83	23.76	0.96	1.30	1.10	3.36
		M	33.55	26.21	28.39	4.51	10.25	21.81	73.79	22.75	1.09	1.39	1.21	3.58
10	P	S	21.51	19.57	23.15	3.76	9.43	18.15	61.25	24.35	0.89	1.15	1.47	3.51
		M	23.86	22.35	23.95	3.82	9.52	19.45	64.47	23.17	0.95	1.19	1.55	3.69
11	CV	S	19.82	21.5	23.44	3.27	8.4	16.65	45.77	34.34	0.29	0.55	0.41	1.25
		M	23.19	23.07	24.81	3.33	8.46	17.21	51.96	32.29	0.36	0.62	0.52	1.5
12	AL	S	18.75	18.18	18.47	3.53	9.18	17.85	69.4	27.74	1.10	1.24	0.91	3.25
		M	26.87	22.16	22.31	3.63	9.47	18.33	73.8	26.74	1.28	1.40	1.12	3.8
	SEm ±		0.27	0.23	2.64	0.27	0.11	0.16	4.57	7.34	0.015	0.019	0.017	0.25
	C.D. (P= 0.05)		0.75	0.64	NS	0.75	0.30	0.44	12.66	20.33	0.041	0.053	0.047	NS

on seedling than micropropagated one. This may be attributed that at initial stage growth of seedling was more than micropropagated plants. Seedling had more number of secondary roots at the time of application of PCB, which may have increased the absorption of more PCB than plants with less secondary roots (micropropagated). However, at the age of one year maximum secondary roots were observed in micropropagated plantlets. That may be the reason for the better response of PCB on seedling than micropropagated one.

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