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THE EFFECT OF GROWTH REGULATORS ON THE ROOTING OF STEM CUTTINGS OF *CITRUS*, RELATED GENERA AND INTERGENERIC SOMATIC HYBRIDS

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Abstract. **Semi-hardwood cuttings of 12 citrus genotypes, including genera closely related to *Citrus* and somatic hybrid plants produced by protoplast fusion, were treated with root-inducing hormones. The cuttings were rooted under intermittent mist in a greenhouse. The treatments included 1000 and 3000 ppm NAA, 1000 and 3000 ppm IBA, Rootone powder (a commercial formulation containing 2000 ppm NAA and 1000 ppm IBA), and the control. Evaluations after 6 weeks revealed that stem cuttings of the different clones responded significantly in root production to NAA and IBA treatments. The lowest concentration of NAA (1000 ppm) and the highest concentration of IBA (3000 ppm) yielded the maximum rooting percentage (75%) across all selections. Cuttings treated with NAA or IBA, both at 1000 and 3000 ppm, produced greater numbers of roots that were longer and thicker than those of the control. The effect of varying concentrations of NAA and IBA on root production of the cuttings was not uniform for all of the experimental clones. Cuttings of Swingle citrumelo, Flying Dragon, Cleopatra mandarin, Cleopatra + Flying dragon (4X-somatic hybrid), Cleopatra + Swingle citrumelo (4X-somatic hybrid), and Hamlin sweet orange treated with the different growth regulator treatments produced better quality root systems compared with those of the untreated control.**

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Large numbers of clonal rootstocks are needed to produce the commercial trees propagated by citrus nuserymen each year. Propagation of clonal material can be by nucellar seed, by micropropagation in tissue culture (Grosser and Chandler, 1986), or by the use of "own-rooted citrus" via cuttings (Halma, 1931). All commercially important rootstocks are seed propagated, and therefore rootstock improvement efforts have been limited to germplasm that exhibits a high degree of polyembryony. Alternatives to seed propagation would greatly expand the genetic diversity available for rootstock improvement. Extended juvenility in *Citrus* has also impeded the testing of new citrus hybrids for rootstock improvement. Alternative methods of propagation would circumvent the need to wait for promising hybrids (including new somatic hybrids) to produce seed in order to obtain enough uniform plants for thorough evaluation. Cutting propagation may therefore be useful in commercial propagation, and in the production of plants for experimental horticultural, physiological, or pathological studies.

The propagation of rootstocks and scion cultivars by rooting shoot cuttings can be a valuable tool for nurserymen. However, it can be an expensive operation, so a high

Table 1. Clonal sources of stem cuttings used to evaluate adventive rooting potential.

Plant source	Scientific name	Reference
Hamlin sweet orange (2X)	*C. sinensis* (L.) Osbeck	Swingle and Reece (1967)
(4X) Hamlin (H0-17)	*C. sinensis* (L.) Osbeck	
Hamlin (embryogenic suspension) + *S. disticha* (nonembryogenic callus) (4X-somatic hybrid)	*C. sinensis* (L.) Osbeck + *Severinia disticha* Blanco	Grosser et al. (1988a)
S. disticha (2X)	*Severinia disticha* Blanco	Swingle and Reece (1967)
Hamlin (embryogenic suspension) + Flying Dragon (leaf) (4X-somatic hybrid)	*C. sinensis* (L.) Osbeck + *P. trifoliata* (L.) Raf.	Grosser et al. (1988b)
Swingle citrumelo (2X-sexual hybrid)	*C. paradisi* x *P. trifoliata*	Swingle and Reece (1967)
Carrizo citrange (2X-sexual hybrid)	*C. sinensis* x *P. trifoliata*	Swingle and Reece (1967)
Flying Dragon (2X)	*P. trifoliata*	Swingle and Reece (1967)
Argentinian *Poncirus trifoliata* (2X)	*P. trifoliata*	Swingle and Reece (1967)
Cleopatra mandarin (2X)	*C. reticulata* Blanco	Swingle and Reece (1967)
Cleopatra (embryogenic suspension) + Swingle citrumelo (leaf) (4X-somatic hybrid)	*C. reticulata* Blanco + *C. paradisi* x *trifoliata*	Grosser (1990)
Cleopatra (embryogenic suspension) + Flying Dragon (leaf) (4X-somatic hybrid)	*C. reticulata* Blanco + *P. trifoliata* (L.) Raf.	Grosser (1990)

Table 2. The effect of growth regulators on the percentage of rooted stem cuttings of citrus clonal materials (including 4 somatic hybrids).

	Plant source												
Treatment	Hamlin sweet orange	Hamlin (HO-17)	Hamlin + *S. disticha* somatic hybrid	*Severinia disticha*	Hamlin + Flying Dragon somatic hybrid	Swingle citrumelo	Carrizo citrange	Flying Dragon	Argentinian *Poncirus trifoliata*	Cleopatra mandarin	Cleoptra + Swingle somatic hybrid	Cleopatra + Flying Dragon somatic hybrid	Mean
Control	66.67	0	0	0	33.33	100	33.33	83.33	16.67	100	33.33	50	43.06
Rootone	66.67	0	0	16.67	50	100	66.67	83.33	16.67	83.33	83.33	66.67	52.78
NAA (1000 ppm)	100	66.67	33.33	50	50	100	83.33	83.33	66.67	66.67	100	100	75.00
NAA (3000 ppm)	66.67	50	66.67	33.33	83.33	100	66.67	66.67	33.33	33.33	66.67	100	63.89
IBA (1000 ppm)	83.33	16.67	16.67	66.67	33.33	100	66.67	83.33	50	83.33	66.67	66.67	61.11
IBA (3000 pm)	66.67	33.33	33.33	66.67	66.67	100	50	100	83.33	100	100	100	75.00

percentage of rooted cuttings with good quality root systems is required (Meredith et al., 1970). Cuttings of certain *Citrus* genotypes are very difficult to root, even within cultivars of the same species, i.e. *Citrus sinensis* (Young and Sauls, 1979), and improved methods are needed.

The formation of roots on cuttings of *Citrus* species and related genera is improved by treatment with root-inducing hormones. The effect of hormones, however, is not uniform with all *Citrus* (Platt and Opitz, 1973). Thus, the major objective of the present study was to evaluate the effect of certain growth regulators on the rooting of stem cuttings of citrus clones. Moreover, several of the experimental genotypes were produced from somatic hybridization using protoplast fusion techniques (Grosser and Gmitter, 1990). Cutting propagation preserves the characteristics of the parent plant, and true-to-type plants may be produced more quickly.

Materials and Methods

Cuttings of the 12 different juvenile clones (Table 1) were placed under intermittent mist for 10 sec every 5 min during daylight only. Semi-hardwood cuttings 15-cm long were collected during the last week of Sep. from the terminal growth of the experimental seedlings. Basal leaves were removed and stem cuttings with 3 terminal leaves were prepared. Cuttings were dipped for 5 min in a 50% alcohol solution as a control treatment, NAA at 2 concentrations (1000 and 3000 ppm), IBA (1000 and 3000 ppm), and Rootone® powder (a commercial plant hormone with fungicide containing 2000 ppm NAA and 1000 ppm IBA). Cuttings were then placed in a 15-cm deep tree tray containing Metro-Mix 500® (a commercial growing medium mixture of peat moss, vermiculite, bark ash, and washed granite sand). The 6 treatments were arranged in a completely randomized design with 6 replications per treatment. Thus, 432 stem cuttings were included in this investigation (12 clones, 6 treatments, and 6 replicates). Cuttings were harvested 6 weeks after planting and evaluated for percent cuttings rooted, number of roots per cutting, and average of length and diameter of roots per cutting.

Results and Discussion

The effect of growth regulators on the formation of roots on cuttings of the 12 citrus clones was studied by determining rooting percentage and the number, length, and diameter of roots per cutting (Tables 2-5). It was noted generally that the stem cuttings of the different clones responded significantly to NAA and IBA treatments at 2 concentrations. The lowest concentration of NAA (1000 ppm) and the highest concentration of IBA (3000 ppm) yielded the maximum rooting percentage across all clones (75%) (Table 2). Concerning the effect of different treatments on the number, length, and diameter of roots per cutting, data presented in Tables 3 to 5 revealed that the root production of cuttings of the experimental clones treated with NAA or IBA at the 2 concentrations (1000 and 3000 ppm) was statistically greater than those of the control. Whereas, no significant differences were found in the root production between cuttings treated with Rootone and those of the control.

The results show that both auxins NAA and IBA can increase the percentage of cuttings that form roots and

Table 3. The effect of growth regulators on the number of roots per cutting of citrus clonal materials (including 4 somatic hybrids).

	Plant source												
Treatment	Hamlin sweet orange	Hamlin (HO-17)	Hamlin + *S. disticha* somatic hybrid	*Severinia disticha*	Hamlin + Flying Dragon somatic hybrid	Swingle citrumelo	Carrizo citrange	Flying Dragon	Argentinian *Poncirus trifoliata*	Cleopatra mandarin	Cleoptra + Swingle somatic hybrid	Cleopatra + Flying Dragon somatic hybrid	Mean
Control	1.50 a	0 b	0 a	0 b	0.50 b	3.83 c	0.67 b	1.17 b	0.17 b	3.67 ab	0.83 c	1.50 c	1.15 d
Rootone	2.17 a	0 b	0 a	0.67 ab	1.00 ab	5.83 b	1.50 b	1.50 b	0.17 b	4.33 ab	2.17 abc	2.00 bc	1.78 cd
NAA (1000 ppm)	4.17 a	1.17 a	0.83 a	1.83 ab	1.83 ab	6.67 ab	2.50 ab	3.33 ab	3.17 a	2.00 b	3.67 ab	5.17 ab	3.03 b
NAA (3000 ppm)	3.00 a	0.83 ab	1.33 a	0.83 ab	3.83 a	7.83 ab	4.83 a	4.17 ab	1.83 ab	1.33 b	1.83 bc	5.17 ab	3.07 b
IBA (1000 ppm)	2.83 a	0.17 b	1.00 a	2.17 ab	0.83 b	6.67 ab	1.33 b	3.33 ab	1.33 ab	4.17 ab	2.83 abc	2.00 bc	2.39 bc
IBA (3000 ppm)	4.00 a	0.33 ab	0.83 a	3.00 a	3.00 ab	8.67 a	2.33 ab	5.50 a	4.00 a	7.00 a	4.67 a	7.00 a	4.19 a

Means not sharing the same letter within columns are significantly different (P < 0.05), Duncan's multiple range test (SAS Institute, 1985).

Table 4. The effect of growth regulators on the length of roots (cm) per cutting of citrus clonal materials (including 4 somatic hybrids).

	Plant source												
Treatment	Hamlin sweet orange	Hamlin (HO-17)	Hamlin + *S. disticha* somatic hybrid	*Severinia disticha*	Hamlin + Flying Dragon somatic hybrid	Swingle citrumelo	Carrizo citrange	Flying Dragon	Argentinian *Poncirus trifoliata*	Cleopatra mandarin	Cleopatra + Swingle somatic hybrid	Cleopatra + Flying Dragon somatic hybrid	Mean
Control	6.78 a	0 a	0 b	2.08 b	33.70 a	4.73 ab	3.63 b	0.62 b	25.03 ab	3.10 b	3.68 b	6.95 c	
Rootone	9.70 a	0 a	0 b	4.87 ab	3.22 b	47.25 a	11.77 ab	3.88 b	0.65 b	34.00 ab	14.88 ab	6.28 b	11.38 bc
NAA (1000 pm)	18.65 a	2.95 a	3.75 b	6.87 ab	5.45 b	51.28 a	24.78 ab	11.15 ab	19.88 a	19.23 b	21.82 ab	13.70 ab	16.63 b
NAA (3000 ppm)	9.78 a	2.87 a	13.22 a	4.92 ab	17.13 a	49.65 a	35.57 a	8.08 ab	5.50 b	12.62 b	5.67 ab	17.05 ab	15.17 b
IBA (1000 ppm)	11.87 a	0.27 a	1.00 b	14.30 ab	1.92 b	55.85 a	8.75 b	13.55 ab	4.23 b	32.03 ab	18.12 ab	8.45 b	14.19 b
IBA (3000 ppm)	29.30 a	1.07 a	4.42 ab	17.67 a	7.58 b	56.28 a	17.08 ab	16.08 a	13.33 ab	64.13 a	25.10 a	26.93 a	23.25 a

Means not sharing the same letter within columns are significantly different (P < 0.05), Duncan's multiple range test (SAS Institute, 1985).

Table 5. The effect of growth regulators on the diameter of roots (mm) per cutting of citrus clonal materials (including 4 somatic hybrids).

	Plant source												
Treatment	Hamlin sweet orange	Hamlin (HO-17)	Hamlin + *S. disticha* somatic hybrid	*Severinia disticha*	Hamlin + Flying Dragon somatic hybrid	Swingle citrumelo	Carrizo citrange	Flying Dragon	*Poncirus trifoliata*	Argentinian Cleopatra mandarin	Cleopatra + Swingle somatic hybrid	Cleopatra + Flying Dragon somatic hybrid	Mean
Control	0.82 a	0 a	0 b	0 a	0.35 b	1.47 a	0.40 a	0.38 b	0.07 b	1.23 a	0.42 b	0.25 b	0.47 b
Rootone	0.77 a	0 a	0 b	0.30 a	0.30 b	1.52 a	0.65 a	0.52 ab	0.13 ab	0.85 ab	1.02 ab	0.40 ab	0.56 ab
NAA (1000 ppm)	1.23 a	0.70 a	0.40 ab	0.49 a	0.50 ab	1.32 ab	0.88 a	0.73 ab	0.47 ab	0.58 b	1.42 a	0.67 a	0.75 a
NAA (3000 ppm)	0.90 a	0.82 a	0.77 a	0.34 a	0.95 a	1.37 ab	0.88 a	0.43 ab	0.27 ab	0.38 b	0.90 ab	0.65 a	0.74 a
IBA (1000 ppm)	0.79 a	0.25 a	0.13 b	0.56 a	0.17 b	1.22 b	0.67 a	0.70 ab	0.37 ab	0.92 ab	0.82 ab	0.55 ab	0.61 ab
IBA (3000 ppm)	0.90 a	0.48 a	0.40 ab	0.40 a	0.62 ab	1.35 ab	0.58 a	0.85 a	0.55 a	0.93 ab	1.12 ab	0.77 a	0.71 a

Means not sharing the same letter within columns are significantly different (P < 0.05), Duncan's multiple range test (SAS Institute, 1985).

Table 6. *Summation* of all treatments on rooting %, number of roots, length of roots (cm), and diameter of roots (mm) per stem cutting of experimental clones.

Plant source	Rooting %	Number of roots per cutting	Length of roots per cutting (cm)	Diameter of roots per cutting (mm)
Swingle citrumelo	100	6.58 a	49.00 a	0.86 ab
Flying Dragon	83.33	3.17 bcd	9.40 cdef	0.38 ef
Cleopatra + Flying Dragon	80.56	3.81 b	12.68 cde	0.70 bcd
Cleopatra mandarin	77.78	3.75 bc	31.18 b	0.77 abc
Hamlin sweet orange	75.00	2.94 bcde	14.35 cde	0.57 cde
Cleopatra + Swingle	61.11	2.19 defg	17.11 c	0.71 bcd
Hamlin + Flying Dragon	52.78	1.83 efg	6.23 ef	1.01 a
Argentinian *Poncirus trifoliata*	44.44	1.78 fg	7.40 def	0.76 abc
Severinia disticha	38.89	1.42 gh	8.10 def	0.43 ef
(4X) Hamlin (HO-17)	27.78	0.42 h	1.19 f	0.27 f
Hamlin + *S. disticha*	25.00	0.67 h	3.73 f	0.45 def

Means not sharing the same letter within columns are significantly different (P < 0.05), Duncan's multiple range test (SAS Institute, 1985).

significantly increase the number and quality of roots produced per cutting. There is considerable variability in the rooting response of the different citrus clones to treatment with these hormones. These findings were in agreement with those obtained by many investigators, including Biale and Halma (1938), Erickson and Bitters (1953), Kossuth et al. (1981), and Ferguson et al. (1985).

In addition, a summation of the data across all treatments/clone revealed that root production of the cuttings was not uniform with all the experimental clones (Table 6). This could be due to genotypic differences and/or to differences in the age of the juvenile source plants. The present results showed that growth regulator treatments in general caused an increase in the percentage of cuttings initiating roots and in the number, length, and diameter of roots per cutting on Swingle citrumelo, Flying Dragon, Cleopatra mandarin, Cleopatra + Swingle (4X-somatic hybrid), Cleopatra + Flying Dragon (4X-somatic hybrid), and 'Hamlin' sweet orange over those of the other experimental clones. All cuttings of Swingle and Cleopatra rooted without treatment; but, the number and size of roots were considerably greater on the treated cuttings.

These results were in agreement with those obtained by Cooper and Knowlton (1940), Erickson and Bitters (1953), Platt and Opitz (1973), and Ferguson et al. (1985). They observed that some citrus varieties and species of genera closely related to citrus rooted more efficiently when growth regulators were used.

In conclusion, the results suggest that for a wide range of citrus genotypes, growth regulator enhanced rooting of cuttings can be a quick and efficient means of propagating healthy true-to-type plants.

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NITROGEN RATE EFFECT ON GROWTH OF CONTAINERIZED CITRUS NURSERY PLANTS

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Abstract. **Container solution levels of nitrogen required to optimize growth of containerized citrus nursery plants, while minimizing nitrogen loss through leaching, were determined. Carrizo citrange [*Citrus sinensis* (L.) Osb. × *Poncirus trifoliata* (L.) Raf.] and Cleopatra mandarin [*Citrus reticulata* Blanco] seedlings, budded with 'Hamlin' orange [*Citrus sinensis* (L.) Osb.], were grown in a greenhouse in 2-liter plastic containers in washed sand media. In experiments 1 and 2, the plants received one liter daily of either 0, 12.5, 25, 50, 100, or 200 ppm of N as NH_4NO_3 dissolved in a complete nutrient solution. In experiment 3, they received either 0, 3.13, 6.25, 12.5, or 50 ppm N as NH_4NO_3. Shoot length and leaf area of the second and third scion flush, total shoot length, and total leaf area increased as nitrogen levels increased up to a critical level but leveled off above the critical level. The critical container solution nitrogen level was between 15 and 20 ppm. Root, shoot and total dry weights increased as nitrogen levels increased up to this critical level with no further increases at higher nitrogen levels. Results from this study suggest that nitrogen fertilization levels lower than those normally applied in commercial citrus nurseries may be adequate for optimal growth of container-grown citrus nursery plants.**

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Improving the utilization efficiency of applied fertilizers is one way of reducing production costs while minimizing potential ground water contamination. Nitrogen is the most important nutrient in most fertilization programs and it can be especially critical in nursery situations where high plant densities are used. The potential for loss through leaching and runoff in nurseries is high. Container grown Alberta spruce, fertigated at 800 pounds per acre per year of 20-4-8 soluble fertilizer, lost over 34% of the applied nitrogen fertilizer in the leachate and runoff water (16). Containerized plant nurseries have extremely high plant densities that require large amounts of fertilizer per unit area. In Florida, the current recommendation for citrus field nurseries is 500-1000 pounds of nitrogen per acre per year (18) but surveys have found that much higher levels are used (3,5,6).

In 1987-1989, approximately 196,500 acres of citrus were planted in Florida (9) and the production of container grown citrus nursery trees has increased dramatically in recent years to meet the demand. A 1989 survey among citrus nurserymen in Florida revealed a wide variation in nitrogen fertilizer usage, suggesting that there are no established nitrogen fertilizer requirements for container-grown citrus plants (19). In a survey of Florida nurseries, Bridges and Youtsey (3) found that nitrogen fertilizer rates of 800-1000 pounds per acre per year were common and appeared to be excessive. In more recent studies on citrus nurseries, Castle and Ferguson (5) and Castle and Rouse (6) found fertilizer rates had increased substantially since 1976 and rates of 2000 pounds per acre per year were common. These findings suggest that nitrogen fertilizer is often applied at rates in excess of that needed to optimize growth.