ROOTING RESPONSE AND SUBSEQUENT GROWTH OF SELECTED CITRUS SP. TO APPLICATION OF DIFFERENT HORMONES

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ABSTRACT

The study analyzed the rooting responses of selected citrus species using stem cuttings. Different rooting hormones, including IBA, IAA, NAA, Hormex, and Saredex, were applied to cuttings at the recommended rate. Six morphological characteristics were measured and detected after 60 days. Interaction effects between citrus species and rooting hormones in all parameters were not significant. Still, hormones and citrus mean had significant effects in all parameters except for days to shoot, number of shoots, number of leaves, and number of roots. The rooting performance of citrus species improved when NAA, IAA, IBA, and Hormex hormones were used. The results of the study clearly showed that the use of any rooting hormones, especially Naphthalene Acetic Acid (NAA), could enhance the rooting ability of selected citrus species (Calamansi, Calamandarin, and Dalandan) as early as 60 days. Additionally, the application of nitrogenous fertilizer could improve the growth of potted, rooted citrus cuttings in nursery operations.

Keywords: Rooting response, citrus, different hormones

INTRODUCTION

Citrus fruits have a nutritive value that provides strength and serves as a medicine for certain illnesses, making them an essential part of our daily diet. Citrus fruits come in various shapes and are rich in vitamin C, a vital nutrient needed by the human body; their taste is usually sweet but occasionally sour. In Mediterranean regions, citrus species such as orange, mandarin, and lemon trees have been cultivated for centuries. These trees thrive in good soil and climatic conditions, which can achieve a higher level of fruit quality than in other regions.

Cutting is often the preferred method of propagation in horticulture since it is cheap, fast, and modest technology, and no skill is needed, unlike in grafting, budding, or micro-propagation. For citrus, like many fruit crops, this method is necessary not only to maintain the desirable characteristics of the mother plant (Hartmann & Kester, 1983) but also to provide them with desirable quantities of true-to-type rootstocks. This fact may be of great importance since rootstocks are known to affect many scion characteristics such as vigor, yield, and fruit quality and, disease resistance, conditioning, therefore, the success of citrus industries (Espinoza-Nunez et al., 2011). Furthermore, it is reported that cutting tends to decrease the juvenile stage of plants and reduce the time of nursery development (Spiegel-Roy & Goldschmidt, 1996). According to de Oliveira (2017), rootstock through cuttings can produce the same quality, uniform in size, and good characteristics as the mother plants, and production is faster and more rapid to meet the demand. Like tissue culture plantlet, tissue culture production protocol is not easy, and warrants for is it takes time to develop protocols and sufficient size to warrant in variety of trial testing.

Nitrogen is the most important nutrient in most fertilization and can be critical in nursery situations where high densities are used. According to Razaq et al. (2017), the application of fertilizer is a significant factor in plant growth, development, and crop yield, as well as in root morphology. It is a vital factor for assessing the effects of applied elements in promoting plant growth and early flowering in crops.

Therefore, an alternative method for propagating citrus should be explored. One method is the use of stem cuttings through the rooting of terminal stems. The hormone that aids the growth of adventitious roots is called auxin. However, analytical forms of auxins, such as Indole Butyric Acid (IBA), Indole Acetic Acid, Naphthalene Acetic Acid (NAA), Hormex, and Saredex, are commercially available. This study, therefore, aimed to determine the most appropriate rooting hormone solutions for propagating stem cuttings of citrus and the growth performance of citrus cuttings applied with nitrogen fertilizer.

A study of stem cutting of citrus species was conducted at the University

of Southern Mindanao, Kabacan, Cotabato. The research aimed to evaluate the effects of different rooting hormone applications on the root ability of citrus cuttings. The research consisted of two studies: the first focused on using different rooting hormones to enhance callus and rooting formation in citrus, and the second examined the application of nitrogen fertilizer to different rooted citrus species' cuttings under potting conditions.

Experimental Design and Treatments

A Double Factor Completely Randomized Design (CRD) was employed with six treatments, each replicated three times. Sixty (60) sample cuttings were used per treatment. Semi-hardwood stem cuttings of citrus species served as Factor A, while different rooting hormones served as Factor B. The following treatments were used:

Factor A (Species)	Factor B (Hormones)
S_1 - Calamansi	T1 –Control (Water only)
S_2 - Calamandarin	T2 – IBA (1000ppm)
S_3 - Dalandan	T3 – IAA (1000ppm)
	T4 – NAA (1000ppm)
	T5-Hormex (pure soln)
	T6 –Saradex (pure soln)

Preparation of the Experimental Area

The experimental setup is situated in a partially shaded area, not directly exposed to sunlight, to prevent stress on the experimental cuttings, as shown in Plate 1.

Rooting Media Preparation

River sand and coir dust were used as the rooting media. The sand and coir dust were thoroughly sieved. The sand was mixed with coconut coir dust with a ratio of 1:1 before placing them in the polyethylene bag measuring 4 x 6 x 0.02 cm. The river sand and coir dust were disinfected by pouring with fungicide. One thousand eighty (1,080) polyethylene bags filled with ¾ mixtures of river sand and coir dust were used. The polyethylene bags were arranged according to the design and layout of the experiment. The chamber was covered with a thick sheet of polyethylene suspended on a wire frame to maintain moisture from 80 to 90 percent. When covered with a clear plastic bag, stem cutting about 3 to 4 inches would root.

Selection and Preparation of Citrus Stem Cuttings

A proper and careful selection of experimental plants was made to obtain the experimental samples. The mother plant, where the

Plate 1. *Overview of the experimental area of the study on the rooting response of selected citrus sp. to the application of different hormones*

Plate 2. *(A) Deleting of citrus cuttings and (B) soaking of cuttings to fungicide.*

Sample stem cuttings were taken from healthy plants that were already bearing fruit and free from any disease infestation. Healthy stem cuttings were selected and then carefully cut or harvested early in the morning using sharp pruners. The leaves were cut in half to reduce transpiration. These cuttings were transported and handled properly from the source to the experimental area and planted within the same day. The de-leafing of citrus cuttings and soaking in fungicide are shown in Plate 2.

Preparation of Rooting Hormone Solutions

The different rooting hormone solutions used as treatments were prepared following the recommended rate. One thousand parts per million (1000 ppm) of each hormone, namely Indole Butyric Acid (IBA), Indole Acetic Acid (IAA), and Naphthalene Acetic Acid (NAA) were measured, weighed, and

diluted to one liter of water. Hormex and Saredex used the pure solution for each hormone.

Treatment of Stem Cuttings with Rooting Hormone Solution

Plate 3 shows the basal portions of the prepared sample stem cuttings, which were dipped for 10 minutes in separate plastic containers containing different rooting hormones. At least 2.5 cm of the stem cuttings were depth in the different solutions based on the specific treatments.

Plate 3. *Soaking of citrus cuttings to different rooting hormones*

Plate 4. *Planting of citrus cuttings into a prepared rooting medium composed of coconut coir dust and river sand.*

Planting of Citrus Cuttings

The treated cuttings were planted into prepared potting media by inserting the basal portion of the stem cuttings. Sixty (60) sample stem cuttings per treatment were planted at least 5 cm of the stem cuttings inserted into the soil media, as shown in Plate 4.

Care and Maintenance

Experimental plants need proper care and maintenance. The chamber was maintained at a humidity level of 80% to 90% to ensure sufficient moisture. Depending on the weather and soil moisture conditions, 250 ml of water was added as needed. Regular weeding was performed to minimize competition for nutrients, water, and sunlight.

Data Gathered

Number of days to shoot formation. This was determined by counting the number of days from the time of planting to the emergence of the first shoot in 10 sample plants.

Number of shoots. The number of shoots was gathered from the 10 sample plants upon termination of the study or 60 days after treatment. The number of shoots per sample cutting was counted individually.

Length of shoot. The length (cm) of newly developed shoots was measured from the 10 sample plants using a ruler. The measurement was done upon termination of the study or 60 days after treatment.

Number of leaves. This was determined by counting and recording every noticeable leaf on each of the 10 sample plants, including the new leaves just beginning to emerge. The count was taken upon the termination of the study.

Number of roots. The number of roots was determined by carefully uprooting the rooted cuttings from the 10 sample plants upon termination of the study or 60 days after treatment. The roots were then washed and counted. The number of newly developed primary roots per sample cutting was counted individually.

Length of roots. The length (cm) of newly developed primary roots was measured from 10 sample plants using a ruler after uprooting the cuttings and washing the roots. Measurements were taken upon termination of the study or 60 days after treatment.

Percentage rooting. The percentage rooting was determined by counting the number of rooted cuttings from the 10 sample plants upon termination of the study or 60 days after treatment of the cuttings.

Percentage survival. The percentage (%) survival was gathered from the 10 sample plants upon termination of the study or 60 days after treatment of the cuttings. The percentage was computed using the formula:

$$Percentage\ (\%)Survival = \frac{\text{Number of rooted cuttings}}{\text{Total number of cuttings}} x\ 100$$

Additional observations were also noted and recorded in addition to the above data and parameters. Photo documentation was done.

A number of Rotted citrus cutting. The number of rooted cuttings was observed at 30, 45, and 60 days after treatment. Three (3) sample cuttings were uprooted. (destructed) to get the number of rooted cuttings

Temperature and Relative Humidity. The temperature and relative humidity readings were taken over three months using a hygrometer. Three separate recordings were taken every day.

Statistical Analysis

The various data collected were subjected to statistical analysis using Tukey's HSD (Honest Significant Difference) test. Analysis of Variance (ANOVA) was used to test the significance of differences among treatments at a 0.5% level.

Study 2. *Growth Response of Potted Rooted Stem Cuttings of Citrus Species to Application of Nitrogenous Fertilizer*

Experimental Design and Treatments

The study was carried out using a Randomized Completely Block Design (RCBD) with three treatments replicated five times. Fifty samples of each citrus species from Study 1 were used per treatment. There was a total of 150 sample plants in this study. The following treatments were used:

T1- Rooted Dalandan Stem Cuttings
T2-Rooted Calamansi Stem Cuttings
T3- Rooted Calamandarin Stem Cutting

Preparation of the Experimental Area

The experimental area was located at the University of Southern Mindanao Agricultural Research Center (USMARC), Kabacan, Cotabato, and the study was conducted from December 2018 to April 2019. The setup was situated in a closed and partially shaded area to prevent cuttings from stress and to protect against stray animals and human beings that might distract the setup.

Potted Rooted Citrus Cuttings

Two-month-old rooted citrus species were asexually propagated through stem cuttings and planted in a 3" x 3" x 6" polyethylene bag using a 2:1 ratio of topsoil and coir dust as the soil medium. The plants were then disinfected with a fungicide. Only one seedling per pot of the same age was transplanted and treated with 0.5 grams of nitrogen fertilizer in each sample plant.

Fertilizer Application

Urea fertilizer was applied to each pot (seedling) at 0.5 grams per seedling per treatment per application to enhance the growth of the 2-month-old rooted citrus cuttings. Fertiliser application was conducted two weeks or 15 days after transplanting, at a frequency of one application per week for four weeks, using the side-dress method. Two (2) weeks after the last application of urea fertilizer, the study was terminated.

Care and Maintenance

Watering and weeding were employed to avoid nutrient competition and possible disturbance to the rooting performance of the potted cuttings. The cuttings inside the chamber were regularly monitored, and watering was done as needed, depending on the moisture condition, or 250 ml of water was added per seedling. If diseases or insect pests occurred, fungicides and insecticides were applied at the recommended rates stipulated on the labels.

Data Gathered

Number of shoots. The number of newly developed shoots was determined upon termination of the study or 1.5 months after transplanting the rooted citrus species from the 10 sample plants. The number of shoots was counted individually per sample plant.

Length of shoot. The length (cm) of shoots was measured from the 10 sample plants using a ruler. This was done upon termination of the study or 1.5 months after transplanting.

Stem diameter. Data were gathered by measuring the main stem base diameter (cm) on each of the 10 sample plants, at least 1 cm above the soil surface, using a digital vernier caliper as the measuring device. Collection was done 1.5 months after transplanting or upon termination.

Plant height was measured from 10 sample plants by recording the height in centimeters (cm) from the base to the shoot tip of each plant.

Gathering was performed 1.5 months after transplanting or upon termination, using a ruler, and the initial plant height was recorded.

Number of leaves. This was determined by counting and recording every noticeable leaf on each of the 10 sample plants, including the new leaves just beginning to emerge. The count was taken upon termination of the study.

Percentage survival. The percentage (%) survival was determined upon termination of the study or 1.5 months after transplanting the 10 sample citrus cuttings. The percentage was computed using the formula:

Other observations were also noted and recorded in addition to the above data and parameters. Photo documentation was also done as necessary.

Statistical Analysis

The various data collected were analyzed using Statistical Tools for Agricultural Research (Turkeys). Analysis of Variance (ANOVA) was used to test the significance of differences among treatments at a 0.5% level.

RESULTS AND DISCUSSION

Study 1. *Rooting and Subsequent Growth of Selected Citrus Species Stem Cuttings to the Application of Different Hormones*

Number of Days to Shoot Emergence Statistical analysis revealed that the number of days to shoot emergence was not significantly affected by selected citrus species and rooting hormones, as presented in Table 1. Regardless of the different rooting hormones, no significant differences were found. The cuttings treated with NAA formed earlier shoots at 19.17 days, and the untreated cuttings produced shoots at 23.63 days. The citrus species were significantly different from each other in terms of days to shoot emergence. Calamansi cutting formed shoots as early as 16.35 days, followed by Dalandan with a mean of 21.28 days, and the latest formed shoots were found in Calamandarin with a mean of 26.07 days.

Citrus species can begin forming shoots quite early with the application of hormones. Research studies conducted by Dipa (2010) in Lanzone's cuttings showed that early shoot development occurred in hardwood cuttings treated with 400 and 500 ppm ANAA, respectively, compared to semi-hardwood cuttings. In this study, it was observed that cuttings treated with hormones formed shoots earlier than expected, indicating that active buds were present on the cuttings, and their growth may have been stimulated or encouraged.

Table 1. *Number of days to shoot emergence of selected citrus species stems cuttings applied with different rooting hormones 60 days after planting. USM, Kabacan, Cotabato, 2019.*

Hormones	Citrus Cutting			B-Mean^{ns}
	Calamansi	Calamandarin	Dalandan	
Control	17	28.1	25.8	23.63
IBA	18.5	23.4	19.1	20.33
IAA	16.6	27	22	21.87
NAA	15.3	24.7	17.5	19.17
Hormex	13.9	25.8	23.7	21.13
Saredex	16.8	27.4	19.6	21.27
A – Mean 1/	16.35^{a}	26.07^{c}	21.28b	21.23
C.V. - %	19.48			

1/ Means of citrus species with the same letter superscript are not significantly different at a 5% level of significant (Tukey-test) ns - not significant

Table 2. *Numbers of shoots of selected citrus species stem cuttings applied with different rooting hormones 60 days after planting. USM, Kabacan, Cotabato, 2019.*

Hormones	Citrus Cutting			B-Mean^{ns}
	Calamansi	Calamandarin	Dalandan	
Control	2.21	1.33	1.35	1.63
IBA	2.09	1.25	2.10	1.81
IAA	2.43	1.30	1.53	1.75
NAA	1.73	1.10	2.67	1.83
Hormex	2.44	1.43	1.77	1.88
Saredex	1.71	1.97	1.57	1.75
A- Means	2.10	1.40	1.83	1.78
C.V. - %	27.14			

ns- not significant

According to Sun and Bassuk (1993), Blythe 2004 and Ghazijahani (2017), the application of synthetic auxin to stem cuttings at high concentrations can inhibit bud development, sometimes to the point at which no shoot growth will take place. Cline (1991) Takahashi et al. (1991) found that hormones, like

auxin, gibberellins, and cytokinins, play a vital role in the control of growth not only within the plant as a whole but, apparently, also within individual organs.

Number of Shoots. There was no significant effect between different citrus species cutting and different rooting hormones on the number of shoots developed per cutting shown in Table 2. Regardless of the selected citrus sp. cuttings and different rooting hormone treatments, the number of shoots developed per cutting mean ranged from 1.63 to 1.88 shoots. Results showed that Calamansi produced the greatest number of shoots, with a mean of 2.10 shoots, and the least was found in Calamandarin, with a mean of 1.40 shoots. However, analysis of variance was not significant between citrus species.

Length of Shoot. There was no significant interaction effect between citrus species and rooting hormones on the length of shoots, as shown in Table 3. The application of rooting hormones showed a significant difference in terms of shoot length. Indole Acetic Acid (IAA) produced the longest shoots, with a length of 3.32 cm, which was significantly different from the control. The shortest shoot length of 2.37 cm was found in untreated cuttings, but IAA was comparable to the other rooting hormones (IBA, NAA, Hormex, and Saredex). Additionally, NAA, IBA, Hormex, and Saredex were comparable to untreated cuttings. Moreover, selected citrus species have significant differences between treatments mean. Calamansi and Dalandan had no significant difference in terms of shoot length but were significantly longer than Calamandarin.

Mancera (2013) reported that in calamansi cuttings, the application of 200 ppm ANAA promoted the development of more leaves, and hardwood cutting developed longer shoots. Seran and Umadevi (2011) noted that lemon stem cuttings had higher shoot lengths (5.73 cm), rooting percentages (73.33%), and survival rates (90.0%) when treated with 3000 ppm IBA under a sand medium.

Table 3. *Length (cm) of shoots of selected citrus species stem cuttings applied with different rooting hormones 60 days after planting. USM, Kabacan, Cotabato, 2019.*

	Citrus Cuttings			
	Calamansi	Calamandarin	Dalandan	1/
Control	3.28	1.5	2.34	2.37
IBA	3.07	2.41	3.32	2.93ab
IAA	3.44	3.06	3.46	3.32[a]
NAA	3.23	2.57	3.45	3.08ab
Hormex	2.9	2.52	3.39	2.94ab
Saredex	3.31	1.95	2.89	2.72ab
A-Mean	3.21[a]	2.34[b]	3.14[a]	2.89
C.V. – 1%	19.19			

[1/]Means of rooting hormones with the same letter superscript are not significantly different at a 5% level of significant (Tukey-test)
[2/] Means of citrus species with the same letter superscript are not significantly different at a 5% level of significant
(Tukey's-test)

Number of Leaves. The data presented in Table 4 (Plates 5, 6, 7) show no significant interaction effects between selected citrus species and rooting hormones on the number of leaves. Different rooting hormones did not significantly affect the number of leaves, with mean ranges of 5.25 to 6.76. However, selected citrus species were significantly influenced by rooting hormones. Calamansi formed the most leaves, with 8.19, which was significantly different from Dalandan, which formed 5.65 leaves, and Calamandarin, with 4.99 leaves. However, Dalandan and Calamandarin were comparable to each other.

As cited by Cordon (2006), research studies conducted by Dipa (2010) on Lanzones cuttings showed early shoot development in hardwood cuttings compared to semi-hardwood cuttings treated with 400 and 500 ppm ANAA, respectively. In this study, it was observed that cuttings treated with hormones formed shoots earlier. It was assumed that active buds were present on the said cuttings, and their growth may have been stimulated or encouraged.

Cline (1991), Blythe et al. (2004), and Ghazijahani et al. (2017) found that hormones, such as auxin, gibberellins, and cytokinin, play a vital role in controlling growth not only within the plant as a whole but also within individual organs.

Table 4. *Number of leaves of selected citrus sp. stem cuttings as applied with different rooting hormones 60 days after planting. USM, Kabacan, Cotabato, 2019.*

Citrus Cutting				
Hormones	Calamansi	Calamandarin	Dalandan	B-Mean[ns]
Control	8.44	3.72	3.6	5.25
IBA	8.86	4.75	6.37	6.66
IAA	9.77	5.08	5.23	6.69
NAA	6.86	5.07	8.2	6.71
Hormex	8.78	5.79	5.7	6.76
Saredex	6.44	5.55	4.8	5.6
C.V. - %	26.87			

[1/] Means of citrus species with the same letter superscript are not significantly different at a 5% level of significant
(Tukey's-test) ns – not significant

Number of Roots. The data in Table 5 (Plates 5, 6, 7) showed that there were no significant interaction effects between the selected citrus species and different hormones on the number of roots. The results revealed that different rooting hormones affect the number of roots in stem cuttings of the selected citrus species. After

After 60 days of treatment, the number of roots produced ranged from 1.57 to 2.93 roots. IBA was significantly different from the control but comparable to IAA and NAA. Hormones like IAA, NAA, and Hormex were comparable to each other, and IAA, Hormex, and Saredex were not significantly different from each other. Additionally, it was observed that there was no significant difference in the number of roots in the selected citrus species. The number of roots produced ranged from 2.03 to 2.40.

Based on the data presented, the application of hormones can improve the number of roots, and prolonging the period of data collection can also enhance the number of roots. The rooting of cuttings is not solely due to hormone treatment, but rather, the interaction of factors in the stem and environmental conditions may also be considered. According to Mancera (2013), 200 ppm ANAA is sufficient to initiate the development of roots in calamansi cuttings 90 days after hormone treatment. The possibility of a higher amount of endogenous auxin accumulated in cuttings and its effective response to synthetic hormone treatment favors the development of roots. While Hartmann and Kester (1983) reminded us that auxin treatment is not a guarantee for root formation, they further emphasized that the physiological as well as environmental conditions of the stocks play an important role in the process. Bhatt and Tomar (2011) reported that the maximum root formation, root length, root diameter, and shoot sprout were recorded in citrus cuttings at a concentration of 500 ppm IBA.

Table 5. *Number of roots of selected citrus species stems cuttings as applied with different rooting hormones 60 days after planting. USM, Kabacan, Cotabato, 2019.*

	Citrus Cutting			
Hormones	Calamansi	Calamandarin	Dalandan	1/
Control	1.57	1.29	1.86	1.57[d]
IBA	3.17	2.94	2.67	2.93[a]
IAA	2.32	2.47	2.53	2.44abc
NAA	2.00	3.26	3.23	2.83ab
Hormex	1.61	2.18	2.40	2.06bcd
Saredex	1.5	2.06	1.70	2.26cd
A-Means	2.03	2.37	2.40	2.26
C.V. - %	25.03			

[1/] Means with the same letter superscript are not significantly different at a 5% level of significant (Tukey-test) ns – not significant

Length (cm) of Roots per Cutting. The analysis of variance revealed no significant interaction effects between different citrus species stem cuttings and hormones 60 days after treatment, as shown in Table 6, Plate 9. There was a significant effect on treatment means on the length of roots. NAA hormones had the longest root length of 4.51 cm, which was significantly different from the control, which had the shortest root length of 2.33 cm. However, all rooting hormones were comparable, except for Saredex, IAA, and IBA, which were similar to the control. On the other hand, the length of roots of selected citrus species had a significant difference in the mean. Calamansi had the longest root length of 4.84 cm, which was significantly different from Calamandarin and Dalandan; however, the last two citrus species were comparable. Moreover, Dalandan produced the shortest root length.

As cited by Marcera (2013), 200 ppm ANAA is sufficient to initiate root growth in calamansi cuttings 90 days after hormone treatment. The possibility of higher quantities of endogenous auxin accumulated from cuttings, along with their effective response to synthetic hormone treatment, favored root development. This encouraged the development of more leaves and longer shoots from hardwood cuttings. While Hartmann et al. (1997) recall that treatment with auxin is not a guarantee of root formation, they also stress that physiological and environmental conditions play a significant role in the process.

Table 6. *Length (cm) of roots of selected citrus species stem cuttings as applied with different rooting hormones 60 days after planting. USM, Kabacan, Cotabato, 2019.*

Citrus Cutting				
Hormones	Calamansi	Calamandarin	Dalandan	1/
Control	2.13	2.64	2.22	2.33[b]
IBA	4.34	4.32	3.13	3.93ab
IAA	5.82	2.75	2.82	3.80ab
NAA	5.55	3.81	4.16	4.51ab
Hormex	6.58	3.72	2.56	4.29[a]
Saredex	4.6	2.91	3.02	3.51ab
A-Mean 2/	4.84[a]	3.36[b]	2.99[b]	3.73
C.V. - %	32.16			

1/ Means with the same letter superscript are not significantly different at a 5% level of significance (Tukey test)

2/ Means of citrus species with the same letter superscript are not significantly different at a 5% level of significant (Tukeys-test).

Percentage Rooting. There was no significant interaction effect between the two factors on the rooting percentage of selected citrus species cuttings applied with different rooting hormones, as shown in Table 7.

Analysis of variance revealed significant effects among treatment means on the rooting percentage. Naphthalene Acetic Acid (NAA) was significantly different from untreated cuttings but comparable to all rooting hormones applied. Indole Butyric Acid (IBA), Indole Acetic Acid (IAA), Hormex, and Saredex had no significant differences and were comparable to the control. There were significant effects of citrus species on percentage rooting. The results implied that Dalandan cuttings had better performance in rooting percentage than Calamansi and Calamandarin cuttings, but both Calamandarin and Calamansi were comparable.

Table 7. *Percentage (%) rooting of selected citrus species stem cutting as applied with different rooting hormones 60 days after planting. USM, Kabacan, Cotabato, 2019.*

	Citrus Cutting			
Hormones	Calamansi	Calamandarin	Dalandan	B-Mean [1/]
Control	20.0	15.0	43.3	26.11[b]
IBA	60.0	46.7	53.3	53.33ab
IAA	53.3	40.0	46.7	46.67ab
NAA	53.3	33.3	90.0	58.7[a]
Hormex	30.0	33.3	56.7	40.00ab
Saredex	33.3	30.0	46.7	36.67ab
A-Mean [2/]	41.65[b]	33.05[b]	56.12[a]	43.61
C.V. - %	45.19			

[1/] Means of rooting hormones with the same letter superscript are not significantly different at a 1% level of significant (Tukey-test)
[2/] Means of selected citrus species with the same letter superscript are not significantly different at a 1% level of significant (Tukey-test)

Maximum rooting and shoot growth characters have been recorded below the IBA concentration of 500 ppm (Singh et al., 2015). Bhusal (2001) showed that the ability to root varied between species and varieties (0-100 %).

There was 100% rooting of rough lemon, while Tengu and Kuno satsuma mandarin had 0%. Root initiation is temperature-driven, but subsequent root growth is strongly dependent on the available carbohydrates present in the cuttings. Control of temperature was a must and a very important factor in the rooting of cuttings since cuttings with leaves should be handled carefully to prevent desiccation and the roots under conditions that avoid excessive water loss from the leaves. Temperature was maintained during rooting between 23 °C and 27 °C at the base of the cutting and 30 °C to 32 °C at ambient, without detriment to rooting (Lesturgez et al., 2004).

Percentage Survival. The percentage survival of selected citrus species cuttings applied with different rooting hormones 60 days after

planting is presented in Table 8. Formation of buds, leaves, shoots, and roots were considered viable cuttings.

There were no interaction effects between selected citrus species and rooting hormones. However, analysis of variance revealed significant effects among treatment means. Rooting hormones showed a 95.56% survival rate for cuttings treated with IAA, which was comparable to cuttings treated with IBA, IAA, NAA, and Hormex, with survival rates ranging from 92.22% to 93.33% and significantly different from the untreated control with a survival rate of 82.21%. Moreover, Saredex's had a 90% survival rate, comparable to the control. In terms of selected citrus species, analysis of variance showed significantly different results, revealing that Calamansi and Dalandan had comparable survival rates of 97.22%, which was significantly different from Calamandarin with a 78.89% survival rate.

Table 8. *Percentage (%) survival of different Citrus sp. stem cutting as applied with different rooting hormones 60 days after planting. USM, Kabacan, Cotabato, 2019.*

Citrus Cutting				
Hormones	Calamansi	Calamandarin	Dalandan	B-Mean 1/
Control	83.30	76.70	86.7	82.21[b]
IBA	100.00	76.70	100.0	92.22ab
IAA	100.00	90.00	96.7	95.56[a]
NAA	100.00	80.00	100.0	93.33[a]
Hormex	100.00	80.00	100.0	93.33[a]
Saredex	100.00	70.00	100.0	90.00ab
A-Mean 2/	97.22[a]	78.89[b]	97.22[a]	91.11
C.V. - %	6.88			

1/ Means of rooting hormones with the same letter superscript are not significantly different at a 5% level of significant (Tukey-test)

2/ Means of citrus species with the same letter superscript are not significantly different at a 5% level of significant (Tukey-test)

The results indicated that citrus species cuttings treated with rooting hormones exhibited better root development, resulting in a higher percentage of survival. In Singh's (2018) study on citrus production through cuttings, he observed that cuttings treated with a high IBA concentration had the best rooting and survival percentage performance. Furthermore, the mist house growing condition was found to be effective in increasing the cuttings' success rate.

Other Observations

Number of Rooted Citrus Cuttings. The number of rooted cuttings was observed at 30, 45, and 60 days after treatment. After 30, 45, and 60 days, three sample cuttings were destructively sampled to determine the number of rooted cuttings, as shown in Table 9.

Table 9. *Number of rooted citrus species as applied with different rooting Hormones at 30, 45, and 45 days after planting. USM, Kabacan, Cotabato, 2019.*

Citrus	Days			Total Rooted Cuttings
	30	45	60	
Calamansi				
Control	0	1	1	2
IBA	2	3	2	7
IAA	1	3	3	7
NAA	1	3	4	8
Hormex	0	1	2	3
Saredex	0	1	2	3
Calamandarin				
Control	0	0	2	2
IBA	0	2	1	3
IAA	1	3	3	7
NAA	0	1	1	2
Hormex	0	1	2	3
Saredex	0	3	4	7
Dalandan				
Control	0	2	4	6
IBA	0	3	3	6
IAA	1	3	2	6
NAA	1	3	4	8
Hormex	0	3	4	7
Saredex	0	3	3	6

At 30 DAP, after uprooting, Calamansi treated with IBA, IAA, and NAA had the presence of roots with 2, 1, and 1, respectively. In contrast, cuttings treated with Hormex, Saredex, and control did not produce roots. Dalandan treated with IAA and NAA produced only one rooted cutting, while Calamandarin treated with IAA had the presence of roots. After 45

DAP, three samples were uprooted from all treatments. Cuttings treated with IBA, IAA, and NAA produced roots in all three samples, while Hormex, Saredex, and control produced only one rooted cutting. The least number of rooted cuttings were found in Calamandarin treated with NAA and Hormex, while cuttings treated with IAA had the greatest number of rooted cuttings. Moreover, Dalandan cuttings produced rooted cuttings in all treatments. Upon termination of the study, all selected citrus species formed roots in all hormone treatments. Dalandan had the highest number of rooted cuttings, followed by Calamansi and Calamandarin, with 14 and 12 rooted cuttings, respectively.

Temperature and Relative Humidity. The data on temperature and relative humidity is presented in Table 9, Plate 17. The temperature ranged from 32.1 °C in the morning to 38.2 °C during noon and 33.3 °C in the afternoon, while the relative humidity ranged from 96.3% to 97%.

In asexual propagation by cuttings, the primary challenge is preventing the cuttings from decaying and drying until the missing organ is regenerated, resulting in new individual plants. The essential environmental requirements include a proper temperature (not exceeding 27°c), a very humid atmosphere (85 to 100% relative humidity), and ample light, as well as a clean, moist, well-aerated, and well-drained rooting medium.

A temperature that is too high for normal growth causes physiological disorders by inhibiting or inactivating certain enzyme systems and possibly accelerating other systems. It also causes protein denaturation and coagulation, disruption of the cell membrane, and eventual cell death. Damage by high temperature is increased several-fold by a lack of moisture and intense light (Bautista et al., 1983).

Table 10. *Average Temperature and Relative Humidity of Experimental Area, USMARC, Kabacan, Cotabato*

Month	Temperature (°C)			Relative Humidity (%)		
	9am	12nn	3pm	9am	12nn	3pm
Jan	30.2	35.2	32.0	96.0	97.0	96.0
Feb	33.6	40.6	35.5	97.0	97.0	97.0
Mar	32.7	39.2	32.8	96.0	97.0	96.0
Average	32.2	38.3	33.4	96.3	97.0	96.0

Study 2. *Subsequent Growth of Potted Citrus Cuttings as Applied with Nitrogenous Fertilizer*

Number of Shoots. Table 11, column 1, presents the data on the number of shoots of rooted and selected potted citrus species as influenced by nitrogenous fertilizer 45 days after transplanting. The data showed no significant difference among treatment means. Number of shoots ranged from 2.58 to 3.03 shoots. The highest number of shoots was found in Calamandarin, followed by

Dalandan, and the lowest in Calamansi.

Length of Shoot. Analysis of variance revealed no significant difference in shoot length (cm) of potted citrus species as influenced by nitrogen fertilizer 45 days after transplanting. As shown in Table 11, column 2, the numerically longest shoot length was found in calamansi, with a mean of 7.63 cm, followed by Calamandarin and Dalandan, with means of 6.71 and 6.52 cm, respectively.

Table 11. *Number of shoots and length of shoots of potted citrus cuttings applied with nitrogen fertilizer (45 days) after transplant. USM, Kabacan, Cotabato, 2019*

Citrus	Number of Shootns	Length of Shoot(cm)^{ns}
Dalandan	2.89	6.52
Calamansi	2.58	7.63
Calamandarin	3.03	6.71
CV	20.70%	23.70%

ns – not significant

Table12. *Stem diameter of rooted citrus cutting applied with nitrogen fertilizer (15, 30 and 45 days). USM, Kabacan, Cotabato, 2019*

Citrus	Nu	member of Leaves	
	15	30	45
Dalandan	2.79	3.00	4.0b
Calamansi	2.57	2.65	5.1a
Calmandarin	2.57	2.65	5.3a
			1/
Tukeys test	Ns	ns	
CV%	15.87	12.80	5.88

1/ Means with the same letter superscript are not significantly different at a 5% level of significant (Tukey-test) ns –not significant

Stem Diameter (mm). The stem diameter of potted rooted citrus species applied with nitrogenous fertilizer showed no significant difference from each species at 15 and 30 days but at 45 days. A significant difference in treatment mean was noted at 45 days, as shown in Table 12, Plate 15. At 15 DAPS, it was observed that the broadest stem diameter was found in Dalandan, while Calamandarin and Calamansi had the same stem diameter. At 30 DAPS, Dalandan still had the broadest stem diameter, and the least was found in both Calamandarin and Calamansi. At 45 DAPS, Dalandan was significantly different from Calamansi and Calamandarin, while Calamandarin and Calamansi were not significant from each other. A wider stem diameter was observed from Calamandarin with a mean of 5.3 mm, while the narrowest was observed in Dalandan with a mean of 4.0 mm. In most instances, as cited

by Bell and Robson (1999) and Liu et al. (2008), the application of nitrogen fertilizer resulted in increased vigor in the growth and development of plants. Ahmed et al. (2018) and Bracke et al. (2019) said that in cutting propagation, nutrients can be leached out from both plants and potting medium. The combination of ammonium sulfate and chicken dung, according to Naik et al. (2018), Pereira et al. (2017), and Yildiz (2018), had significantly affected the stem diameter of guava seedlings, as reported.

Plant Height (cm). As shown in Table 13, Plate 16, the data on plant height at 15, 30, and 45 days after transplanting of rooted selected citrus species. Analysis of variance revealed that there was no significant difference among citrus species. At 15 DAPS, Dalandan obtained the highest plant height and the shortest found in Calamandarin. The plant heights ranged from 12.23 cm to 14.5 cm. After 30 DAPS, it was observed that Dalandan had the highest plant height, followed by calamansi and Calamandarin. At 45 DAPS, it was observed that Dalandan had the highest plant height, followed by calamansi and Calamandarin, ranging from 16.4 cm to 18.8 cm in height.

Number of Leaves. A significant effect on the number of leaves of potted, rooted citrus cuttings, as influenced by the application of nitrogen fertilizer 15 days after transplanting, was found in Table 14. Dalandan had a significant difference from Calamansi but not from Calamandarin, whereas Calamansi had no significant difference from Calamandarin. After 30 days after planting (DAP), it was observed that the greatest number of leaves was found in Dalandan, followed by Calamandarin, and the least in Calamansi. The study observed that plants developed a greater number of leaves when applied with a nitrogen fertilizer at 45 days, ranging from 12.8 to 15.7 leaves.

Table 13. *Plant (cm) height of potted rooted citrus cuttings applied with nitrogen fertilizer (15, 30 and 45 days). USM, Kabacan, Cotabato, 2019.*

Citrus	Plant Height		
	15	30	45
Dalandan	14.52	17.11	18.8
Calamansi	13.80	14.85	16.7
Calmandarin	12.23	14.24	16.4
Tukeys test	Ns	ns	ns
CV%	11.45	12.09	12.18

ns – not significant

Table 14. *A number of leaves of potted-rooted selected citrus seedlings were applied with nitrogen fertilizer (15, 30, and 45 days). USM, Kabacan, Cotabato, 2019*

Citrus	N	number of Leaves	
	15	30	45
Dalandan	9.67[a]	10.60	12.9
Calamansi	6.84[b]	8.40	12.8
Calmandarin	8.26ab	9.05	15.7
	1/		
Tukeys test		ns	ns
CV%	18.15	24.42	20.91

1/ Means with the same letter superscript are not significantly different at a 5% level of significant (Tukey-test) ns –not significant

This implied that the use of nitrogen can support the vegetative growth and development of juvenile plants. The results showed that the taller the plant, the more leaves developed, and this further promoted growth because the leaves photosynthesized and produced food to support further seedlings. Thus, it also had a bigger stem diameter. Villasurda (1989) observed a significant increase in the number of leaves of guava nine months after fertilizer application, and the continued growth of plants in the nursery necessitates the addition of supplementary minerals, especially nitrogen.

Percentage Survival. Table 15 presents the data on the percentage survival of potted, rooted citrus cuttings, as influenced by nitrogenous fertilizer, 45 days after transplanting. Analysis of variance revealed significant differences among the mean percentage survival of the citrus species. Potted citrus cuttings planted in Dalandan obtained a percentage survival of 96%, followed by Calamandarin with 66%, and the lowest was found in Calamansi with 48%.

This implied that the application of nitrogenous fertilizer enhanced plant growth. Pangilanmen (2004) observed that mortality was numerically higher during the hardening-off period for leaf bud cutting than terminal and basal Pomelo cuttings.

Table 15. *Survived Potted Citrus Seedlings applied with Nitrogen Fertilizer (45 days)*

Citrus	Percentage survival [1/]
Dalandan	96a
Calamansi	48b
Calamandarin	66b
CV-%	18.35

[1/] Means with the same letter superscript are not significantly different at a 5% level of significance (Tukey test)

SUMMARY, CONCLUSION AND RECOMMENDATION

The study aimed to evaluate the effects of different rooting hormones applied to selected citrus species and determine which hormones can enhance the rooting of citrus species, as well as improve the growth performance of potted, rooted citrus species when combined with nitrogen fertilizer.

The results of the study are summarized as follows: There were no significant interaction effects between the selected citrus species and different rooting hormones in any of the parameters at 60 days after treatment. The different hormones had a significant effect on the number of roots, root length, rooting percentage, percentage survival, and shoot length. In contrast, they had no significant effect on the number of days to shoot, shoot length, and number of leaves. Citrus cuttings had a significant effect on rooting percentage, root length, number of leaves, days to shoot, and shoot length, while no significant effect was observed on the number of roots and number of shoots.

All rooting hormones improved the rooting performance of selected citrus species. Naphthalene Acetic Acid performed better in terms of rooting percentage, length of roots, and number of days to shoot emergence. Indole Acetic Acid also performed better in percentage survival and length of shoots, while IBA performed better in a number of roots. Hormex performed better in a number of shoots and a number of leaves.

The application of nitrogenous fertilizer improved subsequent growths of potted citrus cuttings.

It can be concluded that the use of rooting hormones can enhance the rooting ability of selected citrus species as early as 60 days, and the application of nitrogenous fertilizer can improve the growth of potted, rooted citrus cuttings in any nursery operation.

Based on the study's results, rooting of selected citrus species (Calamansi, Calamandarin, and Dalandan) cuttings is possible by treating them with any rooting hormone, especially Naphthalene Acetic Acid (NAA), at the recommended rate. The researcher recommends conducting a similar study using different potting media and varying levels of the same rooting hormones,

as well as considering different soaking times. More experimental samples should be used, and the experimental period should be prolonged to achieve better results and facilitate a comparative study on the field growth of rooted citrus species cuttings.

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