Nepalese Horticulture 16 : 81-90, 2022
ISSN : 2092-1122 | Print : 2542-2936 (Online)
DOI: https://doi.org/10.3126/nh.v16i1.44968

Research Article

OPEN ACCESS

Vegetative Propagation of Eureka Seedless Lemon (*Citrus limon* L. *Cv Eureka Seedless*) Using Different Types of Stem Cutting and Concentrations of Indole-3-Butyric Acid in Winter

Pushpa Gnawali[1], Maya Gurung[1], Sudip Kadel[1], Samriddha Bhantana[1], Narendra Bahadur Chand[1], Rukmagat Pathak[1] and Puspa Raj Poudel[1*]

Tribhuvan University, Institute of Agriculture and Animal science, Paklihawa campus, Siddharthanagar-1, Rupandehi, Lumbini, Nepal

[*]Corresponding Author's Email: poudelpuspa@gmail.com

[*]Orcid ID: https://orcid.org/0000-0001-7694-4137

Received on: 3 December, 2021 **Revised on:** 15 March, 2022 **Accepeted on:** 7 May, 2022

Abstract

Citrus can be propagated successfully through stem cutting. However, success of the propagation depends on various internal as well as external factors. The 2*7 factorial Randomized Complete Block Design (RCBD) experiment was conducted at the Institute of Agriculture and Animal Sciences, Paklihawa Campus, Rupandehi, Nepal from 22 November 2019 to 5 March 2020 to assess the interaction effect of types of stem cuttings and indole 3-butyric acid (IBA) concentrations on the vegetative propagation of Eureka seedless lemon in winter. Stem cutting types had two levels: hardwood and semi-hardwood cutting and IBA concentrations had seven different level i.e control, 500 ppm, 1000 ppm, 2000 ppm, 3000 ppm, 4000 ppm, and 5000 ppm respectively forming overall 14 treatments and three replications. Statistically, significant interaction effects were seen in root parameters. The highest total number of the root (18.00) were found in hardwood cuttings treated with 4000 ppm and lowest total number of roots (1.66) was found in semi-hardwood cuttings treated with 0 ppm of IBA. A statistically significant interaction effect was not found in most of the shoot parameters. However, maximum sprouting (93.33%), the average number of shoots (4.67), average length of the shoot (7.86) and minimum days for first sprouting (30.67 days) were found in hard wood cuttings treated with 4000 ppm IBA. Considering different root and shoot parameters, hardwood cuttings treated with 4000 ppm performed best for root and shoot development. Thus, hardwood cuttings treated with 4000 ppm IBA can be appropriate method for easy and early propagation of eureka seedless lemon.

Keywords: Hardwood, Parameters, Semi-hardwood, Sprouting, Treatment

Introduction

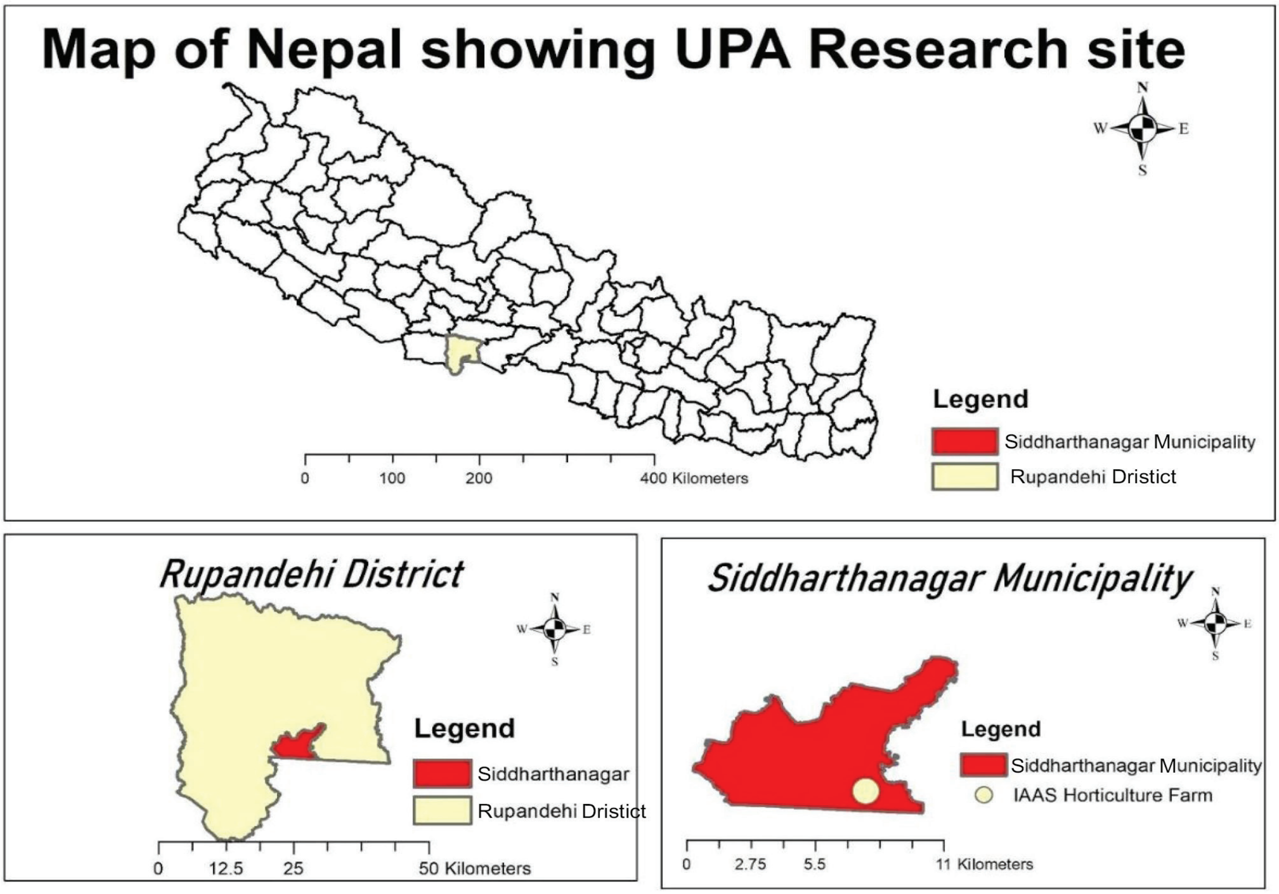
Lemon (*Citrus limon* L.), one of the most important fruit plants of *Citrus spp*. belonging to the family Rutaceae, is originated in India (Spiegel-Roy & Goldschmidt, 1996). Lemon is a fair source of vitamin C, B, A, and minerals K, Ca, Fe, Mg, Na, S, and P thus has medicinal properties like antimicrobial, appetizer, stomachic, anti-helminthic, constipation, abdominal pain, antioxidant, and antiseptic, hypotensive, anti-allergic, anti-inflammatory, antiviral, anticancer, and anti-carcinogenic (Mante, 2019). Lemon-flavored drinks and candies are sold in the market hence having great socio-economic importance (Liu et al., 2012). Eureka is the standard commercial lemon variety with its consistent flavor, firm texture, high oil content, and nearly year-round growing season (Traub & Robinson, 1937). Eureka Seedless lemons are large, oblong-shaped with a pronounced blossom-end knob (mammilla), have vibrant yellow skin with sunken oil glands, and a medium-thick white pith (Ford, 1942).

For commercial plantation of citrus, seedlings are not advised because these seeds do not produce true fruits (Bose et al., 1986). Thus vegetative propagation methods like cuttings, budding, grafting, layering, etc. should be practiced to produce true-to-type plants having desirable characteristics as the mother plant. Stem cutting is a fast and efficient method for propagating citrus species (Hartmann & Kester, 1975). Many internal and external factors such as age, vigor, growth regulator, environment, nutrient and genetic make-up related to the genotype determine the propagation ability of the cuttings (Abou Rawash et al., 1998). Among these factors, the plant growth regulator is an important factor to determine plant growth and development (Mapelli & Kinet, 1992). Auxin enhances the histological features like the formation of callus tissue and the differentiation of vascular tissues (Mitra & Bose, 1954). The effect of Indole 3-butyric acid (IBA) has been proved as the best root-promoting substance in lemon and lime cultivars (Khursheed & Abdul, 2003).

The current study was carried out to determine the effect of different concentrations of IBA on rooting and shooting potential of hardwood and semi-hardwood stem cuttings of seedless Eureka lemon and standardizing the concentration of IBA and type of cutting ensures higher success rate in rooting.

Materials and Methods:

Experimental site: The experiment was conducted at the Institute of Agriculture and Animal Sciences, Paklihawa Campus, Rupandehi, Nepal from 22 November 2019 to 5 March 2020.



Plant materials: Eureka seedless lemon plant of age nine years, raised by seedling and grown under normal management conditions in the Institute of Agriculture and Animal Sciences, Paklihawa Campus Farm was selected as a source mother plant for taking hardwood and semi-hardwood cuttings for the experimental purpose.

Preparation of hormonal solutions: A total of 50 mg IBA (manufactured by: Consolidated Chemicals and Solvent Pvt. Ltd) was dissolved in a few drops of 1N sodium hydroxide (NaOH) solution and the final concentration was made up by adding the distilled water. The subsequent concentrations 1000 ppm, 2000 ppm, 3000 ppm, 4000 ppm, and 5000 ppm were prepared similarly from the stock solution of IBA.

Preparation of media for propagation: Soil, sand, and farmyard manure were taken in the ratio of 1:1:1 and mixed, then solar sterilized by covering media with transparent polyethylene cover under direct sunlight for seven days.

Filling of media in the polybag: Media was filled in the nursery polybags up to 4/5 part and kept in the replication plot in such a way that there were nine nursery black poly bags of breadth and height of 7 cm and 17 cm respectively per treatment.

Preparation of cuttings: Dormant branches were selected for taking the hardwood cuttings. At the same time, semi-hardwood cuttings were taken from a partly mature and partly immature branch from the physiological point of view. A total of 189 hardwoods and 189 semi-hardwood cuttings of about 5 to 15 mm thickness and 15 cm length with 6 buds were selected for the experiment. Then, leaves were removed from the cuttings taken. The cuttings were cut sharply from the top leaving 1 cm above the node and in a slanting position in the basal portion about 1 cm below the node with the help of secateurs sterilized with 75 % ethanol.

Method of IBA treatment and planting of the cutting: The quick dip method was used for the IBA treatment purposes which include dipping the basal portion of cuttings in the hormonal solution for 10 seconds. The basal portion of the stem cuttings was treated with respective IBA concentrations as per the treatment and was planted.

Experimental design: The experiment was conducted in a 2*7 factorial randomized complete block design with 14 treatments and 3 replications.

Factor details: Factor a: two different cuttings (hardwood cutting and semi-hardwood cutting)

Factor b: Different concentrations of IBA (0 ppm, 500 ppm, 1000 ppm, 2000 ppm, 3000 ppm, 4000 ppm, 5000 ppm)

Sampling technique and sample size: Simple random sampling was used for the sampling process and two cuttings were selected from each treatment as a sample cutting.

Observations recorded: The average number of shoot, the average length of the longest shoot, the average number of leaves in the shoot, average length of the leaf, average width of the leaf, sprouting percentage, days required for the first bud sprouting, the average number of the adventitious primary root, average number of the adventitious secondary root, total number of roots and the average length of the longest root were measured at:

$$\text{The average number of shoots} = \frac{\text{Total number of shoots from the 2 sample cuttings}}{2}$$

$$\text{The average length of the longest shoot} = \frac{\text{Total Length of the longest shoots from the sample cuttings}}{2}$$

$$\text{The average number of leaves in the shoot} = \frac{\text{Total number of leaves in the shoot in the sample}}{2}$$

$$\text{The average length of the leaf} = \frac{\text{Total Length of the largest leaves in 2 sample cuttings}}{2}$$

$$\text{The average width of the leaf} = \frac{\text{Total width of the largestleaves in the sample cuttings}}{2}$$

$$\text{Sprouting percentage} = \frac{\text{Total bud sprouted *100\%}}{\text{Total number of buds in a cutting}}$$

$$\text{The average number of adventitious primary root} = \frac{\text{Total number of adventitious primary root}}{2}$$

$$\text{The average number of adventitious secondary root} = \frac{\text{Total number of adventitious secondary root}}{2}$$

$$\text{Total number of roots} = \frac{\text{Total adventitious primary root + Total adventitious secondary root.}}{2}$$

$$\text{The average length of the longest root} = \frac{\text{Length of the longest root in the sample cuttings}}{2}$$

Data collection method: From the initial sprouting until the completion of the sprouting, data on days needed for first sprouting were gathered by carefully observing each cutting daily. Counting the number of sprouted nodes among the six nodes that remained above ground in each sample cutting determined the sprouting percentage. Counting the total number of sprouts in each sample cutting provided data on the number of shoots. After selecting the longest sprouts from all sprouts on the sample cutting, the length of the longest shoot was measured on a centimeter-scale. The number of leaves was determined by counting the total number of leaves on the sample cutting's longest shoot. After selecting a well-developed leaf on each sample cutting, the length and width of the leaf were measured on a centimeter-scale. The total number of roots, primary roots, and secondary roots were counted after removing the soil slowly and making a vertical cut on the polybag from one side carefully without injuring the total roots. After picking the longest root among all, the length of the roots was measured on a centimeter scale.

Data Analysis: The collected data were processed using R studio software R-3.4.1, processed data were analyzed using a two-way ANOVA (Analysis of Variance) table, and the mean between treatments was differentiated by DMRT (Duncan's Multiple Range Test) at 5% level.

Results:

Days to first sprouting: At a given level of significance, data on the influence of different concentrations of IBA on days to first sprouting of semi-hard and hardwood cuttings of lemon revealed that type of cutting and different concentrations of IBA both have a significant effect on days to first sprouting (Table 1). hardwood cuttings sprouted in the shortest amount of time (42.8 days), while semi-hardwood cuttings sprouted in 49.92 days. In 4000 ppm IBA treated hardwood cuttings, the shortest mean number of days to initial sprouting (33.16 days) was reported.

The interaction of auxins and types of cuttings was also significant. In an overall experiment, hard-wood cuttings treated with 4000 ppm IBA sprouted in the shortest amount of time (30.67 days), while semi-hardwood cuttings treated with 0 ppm IBA sprouted in the longest amount of time (61 days).

Percentage of sprouting: The percentage of sprouting was significantly affected by different IBA concentrations, as shown in table 1. The cuttings treated with 4000 ppm IBA had the highest mean percent sprouting at 86.67 percent. The cuttings treated with 3000 ppm IBA sprouted at a rate of 66.67 percent. The control group had the lowest mean sprouting rate of 26.6 percent. The influence of cutting type on sprouting percentage was similarly considerable, with hardwood cutting sprouting 58.09 percent and semi-hardwood cutting sprouting 48.57 percent.

There was no significant association between IBA concentration and cutting type. However, hardwood cutting treated with 4000 ppm IBA had the highest mean sprouting percentage of 93.33 percent, whereas semi-hardwood cutting treated with oppm percent had the lowest mean sprouting percentage. Souidan et al. (1995) Hore and Sen (1992) also got more sprouting success of cuttings by treating with IBA 4000 ppm.

Number of shoots: The data on the number of sprouts are presented in table 1. Highly significant differences were observed among various concentrations of IBA for sprouting. It is revealed from the results that maximum sprouting percentage (4.33 %) was observed by treating cuttings with 4000 ppm of IBA solution and minimum sprouting (5.0 %) was recorded in control. There was also significant variation among the sprouting of different types of cutting. In terms of cutting type, the maximum mean shoot number was 2.86 in hardwood cutting. In the interaction of IBA and cutting type, a significant difference was not found among treatments. The result was in line with Singh et al. (2014), Sharma and Aier (1989), Patel et al. (2017), and Kareem et al. (2016).

Length of largest shoot: In semi-hard and hardwood cuttings of lemon, different doses of IBA had a substantial effect on the length of the largest shoot. The cuttings treated with 4000 ppm IBA had a maximum mean length of 7.18 cm. The control group had the shortest mean length of the greatest sprout. The effect of IBA on different types of cuttings is seen in table 1, with mean lengths of 7.09 cm and 6.01 cm in hard and semi-hardwood cuttings, respectively. On the length of the shoot, the interaction effect of IBA concentration and cutting type was not significant. The results confirm with Kumar et al. (2015).

Number of leaves in largest sprout: Table 2 shows that the influence of IBA concentration on the number of leaves in the largest sprout had a significant effect in both hardwood and semi-hardwood lemon cuttings. Cuttings treated with 4000 ppm IBA had the highest mean number of leaves (10) followed by cuttings treated with 3000 ppm IBA which had the lowest mean number of leaves (1.67). IBA had no effect on the types of cutting, with mean leaves of 6.71 and 6.80 in hard and semi-hard wood cuttings, respectively. The interaction between IBA concentrations and cutting techniques was not significant.

Number of leaves in largest sprout: The data shown in table 2 indicates that the effect of IBA concentration on the number of leaves in the largest sprout had a significant effect on hardwood and semi-hardwood cutting of lemon. The maximum mean number of leaves (10) was recorded in cuttings treated with 4000 ppm IBA followed by (9) in 3000 ppm IBA treated cuttings and the minimum mean number of leaves (1.67) was recorded in control. The effect of IBA on types of cuttings was non-significant with a mean of 6.71 and 6.80 leaves in hard and semi-hardwood cuttings respectively. The interaction of IBA concentrations and types of cuttings was also non-significant.

Table 1. Effect of stem cuttings and IBA concentrations on days required for first sprouting, sprouting percentage, the average number of shoots and an average length of longest shoot

Treatment details	Days of first sprouting	Sprouting %	Average number of shoot	Average length of longest shoot(cm)
Cutting Types (Factor[a])				
Semi-Hardwood	49.92[a]	48.57[b]	2.48[b]	6.01[b]
Hardwood	42.8[b]	58.09[a]	2.86[a]	7.09[a]
sem(±)	1.136	2.5	0.09	0.22
LSD	3.30	13.96	0.28369	0.66
F test	**	***	*	**
IBA concentration (Factor[b])				
0 ppm	55.5[a]	26.67[e]	1.50[d]	4.88[b]
500 ppm	50.16[b]	40[e]	1.83[d]	6.58[a]
1000 ppm	46.50[b]	46.67[cd]	2.50[c]	6.93[a]
2000 ppm	44.39[b]	60[bc]	2.83b[c]	7.10[a]
3000 ppm	45.33[b]	66.67[b]	3.16[b]	7.13[a]
4000 ppm	33.16[c]	86.67[a]	4.33[a]	7.18[a]
5000 ppm	47.67[b]	46.67[cd]	2.5[c]	6.03[ab]
sem(±)	2.12	4.8	0.18	0.42
LSD	6.17	7.46	0.53	1.24
F test(α=0.05)	***	*	***	**
Grand Mean	46.10	53.33	2.67	6.55
CV%	11.29	22.06613	16.77	16.04

CV – coefficient of variation, LSD- least significant difference (* for 5% ** for 1% *** for 0.1% - level of significance) means are separated by DMRT, and means followed by the same letter(s) are non-significantly different among each other.

Length of the largest leaf: The effect of IBA concentrations on the maximum leaf length (given in table 2) was significant. The longest mean length of the leaf was reported in cuttings treated with 4000 ppm IBA (6.23 cm), followed by 5.56 cm in 3000 ppm IBA treated cuttings (5.56 cm), and 0.98 cm in control cuttings. The influence of cutting type on leaf length was similarly significant, with hardwood and semi-hardwood cuttings having mean lengths of 5.77 cm and 2.61 cm, respectively. The interaction of IBA concentration and cutting type was also significant, with hardwood cuttings treated with 4000 ppm IBA reaching a maximum length of 8.80 cm.

Width of the largest leaf: In table no. 2, data on the effect of various auxin (IBA) concentrations on the width of a leaf of semi-hard and hardwood cuttings of lemon show that IBA concentrations have a significant impact on the width of the leaf. In 4000 ppm IBA treated cuttings, the highest mean width of a leaf (2.90 cm) was recorded. While cuttings treated with 0 ppm IBA, or control, had the smallest leaf diameter (0.42 cm). The difference in leaf breadth between different cutting types (semi-hard and soft-wood) was not significant. The relationship between auxins concentrations and cutting types was not significant.

Table 2: Effects of stem cuttings and IBA concentrations on average length, width, and number of leaf

Treatment details	Average width of Leaf (cm)	Average number of Leaves	Average length of leaf (cm)
Types of stem cuttings			
Semi-Hardwood	1.73	6.14	2.61[b]
Hardwood	1.87	6.85	5.77[a]
sem(±)	0.06	0.27	0.12
LSD	NS	NS	0.35
F test	NS	NS	***
IBA concentration			
0 ppm	0.42[e]	1.50[e]	0.98[f]
500 ppm	0.81[d]	4.83[d]	4.67[c]
1000 ppm	1.91[c]	6.33[c]	3.95[d]
2000 ppm	2.40[b]	8.16[b]	5.10[bc]
3000 ppm	2.55[b]	9.00[ab]	5.56[b]
4000 ppm	2.90[a]	10.00[a]	6.23[a]
5000 ppm	1.58[c]	5.67[cd]	2.88[e]
sem(±)	0.11	0.50	0.22
LSD	0.34	1.47	0.66
F test	**	***	***
Grand Mean	1.8	6.5	4.19
CV%	15.83	19.15	13.26

CV – coefficient of variation, LSD- least significant difference (* for 5% ** for 1% *** for 0.1% - level of significance) means are separated by DMRT and means followed by the same letter(s) are non-significantly different from each other.

Number of total roots: The root's mean total number varies significantly depending on the IBA concentration. The highest mean number of total roots was observed in cuttings treated with 4000 ppm IBA, followed by 12 in 3000 ppm IBA (shown in table 3), while the lowest number of total roots was found in control.

At a 5% level of significance, the influence of cutting types on the number of total roots was significant. Patel et al. (2017) found that hardwood cuttings had the considerably largest mean number of total roots at 10.85. On the total number of roots, the interaction effect of IBA concentration and cutting type was extremely significant. In hardwood cuttings treated with 4000 ppm IBA, the greatest mean number of total roots was determined to be 18 but the number of roots decreased at 5000 ppm IBA. These results are similar to the results reported by Hore and Sen (1992) and Souidan et al. (1995).

Number of primary adventitious root: There were significant differences in the mean number of primary adventitious roots per plant across different IBA concentrations, different methods of cutting and the relationship between types of cutting and IBA concentrations. In hardwood cuttings (given in table 3), there were considerably more adventitious roots (4.19) than in semi-hardwood cuttings (2.23). The highest number of primary roots per plant (4.67) was found in cuttings treated with 4000 ppm IBA concentration, whereas the lowest number of roots (1.17) was found in control.

Number of secondary adventitious root: There was a significant difference in mean no.of lateral roots per plant among different concentrations of IBA, different types of cutting and interaction between types of cutting and IBA concentrations. The significantly maximum number of secondary roots per plant (8) was recorded in those cuttings treated with 4000 ppm of IBA concentration and the minimum number of roots (1.33) was recorded in control (Shown in table 3). The maximum mean number of the secondary roots was 6.67 in treated hardwood cutting and a minimum number of 2.52 in semi-hardwood cutting. In interaction, a significantly high mean number of secondary roots 11.67 was found in hard-wood cuttings treated with 4000 ppm IBA.

Length of the longest root: Root length was significantly affected by different IBA concentrations, different types of cutting and the relationship between types of cutting and IBA concentrations. Cuttings treated with 4000 ppm IBA had the longest root length (7.71 cm) of all the IBA treatments. The control group had a minimum root length of 3.67 cm. In hardwood, the maximum root length was 7.55 cm, and in semi-hardwood, the minimum root length was 3.73 cm (shown in table 3). Because hardwood contains more starch, it creates a favorable environment for root initiation, resulting in an increase in root length.

The difference in root length between hardwood cuttings treated with 4000 ppm IBA and control was statistically significant, with a maximum root length of 10.36 cm in hardwood cuttings treated with 4000 ppm IBA and a minimum in control. It's possible because IBA promotes root length by altering the synthesis of enzymes involved in cell expansion. These findings are in line with those of Kumar and Singh (2020), Ozcan et al. (1993), Mohamed-Yasseen and Splittstoesser (1990), Kareem et al. (2016), Al-Jabbari et al. (2020).

Table 3: Effects of stem cutting types and IBA concentrations on root parameters

Treatment details	Average number of adventitious primary root	Average number of adventitious secondary root	Number of total root	Average length of the longest root (cm)
Types of stem cuttings (Factor a)				
Semi-hardwood	2.23[b]	2.52[b]	4.76[b]	3.73 [b]
Hardwood	4.19[a]	6.67[a]	10.85[a]	7.55 [a]
sem (±)	0.17	0.14	0.22	0.165
LSD	0.49	0.42	0.66	0.47
F test	**	***	***	***
IBA concentration (Factor b)				
0 ppm	1.17[c]	1.33[e]	2.5[e]	3.67[e]
500 ppm	2.83[b]	2.50[d]	5.33[d]	4.75[d]
1000 ppm	3.00[b]	3.50[c]	6.5[cd]	5.00[cd]
2000 ppm	4.00[a]	4.67[b]	8.67[c]	5.91[bc]
3000 ppm	4.33[a]	7.67[a]	12[b]	6.61[b]
4000 ppm	4.66[a]	8.00[a]	12.66[a]	7.71[a]
5000 ppm	2.50 [b]	4.50[b]	7.00[c]	5.87[bc]
sem ±	0.31	0.27	0.42	0.308
LSD	0.92	0.78	1.24	1.26
F test	**	***	***	***
Grand Mean	3.21	4.59	7.80	7.80
CV%	24.29	14.43	13.42	13.36

CV – coefficient of variation, LSD- least significant difference (* for 5% ** for 1% *** for 0.1% - level of significance) means are separated by DMRT and means followed by same letter(s) are non-significantly different among each other .

Discussion:

Effect of rooting hormone (IBA) on root and shoot development: Auxin, a rooting hormone is essential for adventitious root formation on stems (Singh et al., 2018). Auxins, either administered or endogenous, are required for the division of the first root initial cells (Blakesley, 1994; Jarvis, 1986). The rooting hormone IBA is mostly utilized in commercial propagation (Kotis et al., 2009). Auxin stimulates the linear growth of the shoot due to cell elongation, resulting in maximal length. As IBA concentrations rise, root and shoot development rises as well, up to a point (Hartmann et al., 2011). This could be due to the effect of exogenous IBA on endogenous hormonal balance, differential auxin mobilization within cuttings, hydrolysis of reserve food materials, protein synthesis, enzyme activity, membrane permeability metabolism at the rooting zone for root regeneration (Ghosh & Basu, 1974) and other processes such as callus tissue formation and vascular tissue differentiation (Mitra & Bose, 1954).

The physiological explanation for IBA's rooting stimulatory effect could be related to the abundance of co-factor in nature but a restricted amount of auxin, resulting in external application of auxin considerably increasing rooting (Hartmann et al., 2011). Thus in our research an increase in IBA concentration upto 4000 ppm improved the root and shoot performance accordingly. However, after a certain threshold (4000 ppm IBA) or at 5000 ppm IBA, the sprouting percentage was slowed due to the toxicity caused by a higher IBA content (Blythe et al., 2007). Singh and Singh (1973) also reported harmful effects of IBA at greater concentrations. According to Hartmann et al. (2011) auxin concentrations that are significantly greater than those observed in plant tissues may hinder root initiation and development.

Al-Jabbari et al. (2020) investigated the influence of different concentrations of Indole butyric acid (IBA (0.00, 1000, 2000, 4000, 6000 mg / L)) on pomegranate root performance. For shoot numbers, leaf numbers, and root length, 4000 ppm IBA treatment was shown to be the best. The investigation was also carried out by Patel et al. (2017) to investigate the influence of various amounts of IBA and NAA on the rooting of hardwood and semi-hardwood cuttings of Fig. In hardwood cuttings, IBA 4000 ppm produced the best rooting results, followed by IBA 2000 ppm.

Effect of cutting types on root and shoot development:

The development of root and shoot parameters was greatest in the hardwood cuttings possibly due to more efficient use of stored supplies in hardwood cuttings compared to semi-hardwood cuttings. Cuttings with higher amounts of sugar, total carbohydrate, and peroxidase enzyme activity, as well as low nitrogen, are ideal for rooting (Bhardwaj & Mishra, 2005). The hardwood cuttings showed the best root and shoot development, according to Malakar et al. (2019). The largest rooting percentage of hardwood (73.24 percent) was treated with 4000 ppm IBA, according to Patel et al. (2017).

Conclusion:

The hardwood cuttings of eureka lemon treated with 4000 ppm IBA can be useful to propagate eureka seedless lemon. Hardwood cuttings coupled with a higher dose of IBA increase the development of root and shoot parameters of the lemon resulting in better survivability of the rooted cuttings. However, an increase in the concentration of IBA should be limited as higher concentration causes cell death due to toxicity developed by higher concentration. Optimizing the concentration of IBA and appropriate types of cutting should be selected for successful propagation.

Further similar research can be conducted in different seasons by adopting other methods of vegetative propagation. Effects of different media, growing conditions, and combinations of different rooting hormones can also be studied. Hence, further research in this area improves the vegetative propagation of eureka seedless lemon.

Acknowledgement:

We must appreciate the support received from the lab assistant Mr. Rupesh Singh for field preparation.

Declaration of Conflict of Interest And Ethical Approval:

All authors mentioned in the manuscript have agreed on authorship, read and approved the manuscript and given consent for submission and subsequent publication. There is no conflict of interest.

References:

Abou Rawash, M., El-Wakeel, H., Nabila, K., & Mohamed, E. (1998). Studies on the vegetative propagation of some citrus rootstocks. *Annals of agricultural science-cairo-*, 43, 523-538.

Al-Jabbari, K., Pakyurek, M., & Yavic, A. (2020). Comparison of rooting situations for salakhani and zivzik pomegranates under different iba doses. *Applied ecology and environmental research, 18*(1), 201-217.

Bhardwaj, D. R., & Mishra, V. K. (2005). Vegetative propagation of Ulmus villosa: effects of plant growth regulators, collection time, type of donor and position of shoot on adventitious root formation in stem cuttings. *New Forests, 29*(2), 105-116. https://doi.org/10.1007/s11056-005-0240-1

Blakesley, D. (1994). Auxin metabolism and adventitious root initiation. In *Biology of adventitious root formation* (pp. 143-154). Springer. https://doi.org/http://dx.doi.org/10.1007/978-1-4757-9492-2_11

Blythe, E. K., Sibley, J. L., Tilt, K. M., & Ruter, J. M. (2007). Methods of auxin application in cutting propagation: A review of 70 years of scientific discovery and commercial practice. *Journal of Environmental Horticulture, 25*(3), 166-185. https://doi.org/http://dx.doi.org/10.1007/978-1-4757-9492-2_11

Bose, T., Mitra, S. K., & Sadhu, M. (1986). Propagation of tropical and subtropical horticultural crops. *1, 662*. (Kolkata Naya Udyog)

Ford, E. S. (1942). Anatomy and histology of the Eureka lemon. *Botanical Gazette, 104*(2), 288-305.

Ghosh, S., & Basu, R. (1974). Metabolic changes during the regeneration of roots on cuttin gs. *Indian journal of experimental biology*.

Hartmann, H., Kester, D., & Davies Jr, F. (2011). Plant Propagation Principles and Practices. *Prentice Hall Upper Suddle River, New Jersey*, 7458, 770.

Hartmann, H. T., & Kester, D. E. (1975). *Plant propagation: principles and practices*. Prentice-Hall.

Hore, J., & Sen, S. (1992). Role of non-auxinic compounds and IBA on adventitious root formation in air-layers of cashew nut. *Cashew, 6*, 11-15. https://doi.org/https://doi.org/10.1051/fruits/2010026

Jarvis, B. (1986). Endogenous control of adventitious rooting in non-woody cuttings. In *New root formation in plants and cuttings* (pp. 191-222). Springer. https://doi.org/https://doi.org/10.1007/978-94-009-4358-2

Kareem, A., Manan, A., Saeed, S., Rehman, S. U., Shahzad, U., & Nafees, M. (2016). Effect of different concentrations of IBA on rooting of Guava Psidium guava L. in low tunnel under shady situation. *Journal of Agriculture and Environment for International Development (JAEID), 110*(2), 197-203. https://doi.org/https://doi.org/10.12895/jaeid.2016110.432

Khursheed, M. Q., & Abdul, K. S. (2003). Effect of different auxins and application's methods on rooting ability of olive. *Journal of Dohuk University, 10*(2), 161-166.

Kotis, M., Yupsanis, T., Syros, T., & Economou, A. (2009). Peroxidase, acid phosphatase, RNase and DNase activity and isoform patterns during in vitro rooting of Petunia× hybrida microshoots. *Biologia Plantarum, 53*(3), 530. https://doi.org/http://dx.doi.org/10.1007/s10535-009-0096-x

Kumar, R., & Singh, J. (2020). Influence of IBA and PHB on regeneration of Kagzi lime (Citrus aurantifolia Swingle) through stem cutting. IJCS, 8(1), 1952-1958. https://doi.org/https://doi.org/10.22271/chemi.2020.v8.i1ac.8550

Kumar, V., Singh, M., Kumar, M., Prakash, S., Kumar, A., Rao, S., & Malik, S. (2015). Effect of different doses of Iba and Rooting Media on Rooting of Stem Cutting of Lemon (Citrus Limon Burm) cv. Pant Lemon-1. *Journal of Plant Development Sciences, 7*(7), 587-591.

Liu, Y., Heying, E., & Tanumihardjo, S. A. (2012). History, global distribution, and nutritional importance of citrus fruits. *Comprehensive reviews in Food Science and Food safety, 11*(6), 530-545. https://doi.org/10.1111/j.1541-4337.2012.00201.x

Malakar, A., Prakasha, D., Kulapati, H., Reddi, S. G., Gollagi, S., Anand, N., & Satheesh, P. (2019). Effect of Growing Media and Plant Growth Regulators on Rooting of Different Types of Stem Cuttings in Acid-Lime Cv. Kagzi. *Int. J. Curr. Microbiol. App. Sci, 8*(10), 2589-2605. https://doi.org/http://dx.doi.org/10.20546/ijcmas.2019.810.300

Mante, R. (2019). *Evaluation of Some Nutraceutical Properties of Lesser Known Functional Foods in Ghana* University of Ghana].

Mapelli, S., & Kinet, J. (1992). Plant growth regulator and graft control of axillary bud formation and development in the TO-2 mutant tomato. *Plant Growth Regulation, 11*(4), 385-390. https://doi.org/https://doi.org/10.1007/BF00130646

Mitra, G., & Bose, N. (1954). Rooting and histological responses of detached leaves to B-Indolebutyric acid with special reference to Boerhavia diffusa Linn. *Phytomorphology, 7*(3/4), 370-381.

Mohamed-Yasseen, Y., & Splittstoesser, W. (1990). The relationship of several enzymes with IAA and phenols on flower induction in endive. *Plant Growth Regulator Society of America Quarterly, 18*(3), 133-139.

Ozcan, M., Tuzcus, O., Kaplankiran, M., & Yesiloglu, T. (1993). The effect of plant growth regulators and different propagation times on the percentage rooting of semi hardwood cuttings of some citrus root stocks. *Hort. Abst, 63*(12), 1209.

Patel, H. R., Patel, M., & Singh, S. (2017). Effect of different levels of IBA and NAA on rooting of hardwood and semi hardwood cutting in fig. *Int. J Agri. Sci. Res, 7*(4), 519-523.

Sharma, S., & Aier, N. (1989). Seasonal rooting behaviour of cuttings of plum cultivars as influenced by IBA treatments. *Scientia Horticulturae, 40*(4), 297-303. https://doi.org/https://doi.org/10.1016/0304-4238(89)90103-9

Singh, K., Choudhary, T., & Kumar, A. (2014). Effect of various concentrations of IBA and NAA on the rooting of stem cuttings of Mulberry (Morus alba L.) under Mist House Condition in Garhwal Hill Region. *Indian Journal of Hill Farming, 27*(1), 125-131.

Singh, K. K., Krishan, C., & Singh, K. (2018). Propagation of citrus species through cutting: A review. *Journal of Medicinal Plants Studies, 6*(1), 167-172.

Singh, R., & Singh, R. (1973). Effects of IBA, Potting Media and Maturity of Wood in Propagation of Sweetlime and Lemon By Cuttings. *Indian Journal of Horticulture, 30*(3 and 4), 505-510.

Souidan, A., Zayed, M., & Dessouky, M. (1995). A Study on Improving the Rooting of Ficus Elastica var. Decora Stem Cuttings I-The Effect of Some Auxinic Treatments. *ANNALS OF AGRICULTURAL SCIENCE-CAIRO*-, 40, 821-830.

Spiegel-Roy, P., & Goldschmidt, E. E. (1996). *The biology of citrus*. Cambridge University Press.

Traub, H. P., & Robinson, T. R. (1937). *Improvement of subtropical fruit crops: citrus* (Vol. 1). In US Department of agricutures. Yearbook of Agriculture.