A Study on the Rooting Responses of Stem Cuttings of *Hibiscus rosa-sinensis* L.

Dissertation submitted to the University of Kerala
in partial fulfillment of the requirement
of the award of the degree

Bachelor of Science
in
Botany

by

Arya -24519101022,

Devika Murali – 24519101023,

Divya Nair -24519101024,

Gowri Narayan– 24519101025,

Jahana Fathima A. – 24519101026

Post Graduate Department of Botany & Biotechnology
Bishop Moore College
Mavelikara

2022

Post Graduate Department of Botany & Biotechnology

Bishop Moore College

Mavelikara

CERTIFICATE

This is to certify that the work presented in this dissertation entitled "A Study on the Rooting Responses of Stem Cuttings of *Hibiscus rosa-sinensis* L." is based on the original research work done by Arya -24519101022, Devika Murali – 24519101023, Divya Nair -24519101024, Gowri Narayan– 24519101025, Jahana Fathima A. – 24519101026 under my supervision and guidance and no part of the work has been included in any other project or thesis for the award of any degree or diploma.

Dr. Dinesh Raj R.
Head of the Department

Dr. A. SIVAPRASAD
Supervising Teacher

DECLARATION

This is to declare that the dissertation work entitled **"A Study on the Rooting Responses of Stem Cuttings of *Hibiscus rosa-sinensis* L."** is a record of the original research work done by me under the guidance of Dr. A. Sivaprasad, Assistant Professor, Post Graduate Department of Botany & Biotechnology, Bishop Moore College, Mavelikara, during the period of 2021-22.

Arya -24519101022,
Devika Murali – 24519101023,
Divya Nair -24519101024,
Gowri Narayan– 24519101025,
Jahana Fathima A. – 24519101026

ACKNOWLEDGEMENT

Firstly, I thank "God Almighty" for the immense blessings showered on me during the whole tenure.

I am taking this opportunity to express my deep gratitude to my supervising teacher Dr. A. Sivaprasad whose whole hearted effort to complete the dissertation and without his inspiration and encouragement the work will be in vain.

I am grateful to Dr. Dinesh Raj R., Head, Dept. of Botany for providing facilities during the tenure of the work.

I thank all my teachers, my fellow workers and others students for their help and suggestions during the study.

Last but not least I thank my parents and relatives for their inspiration throughout.

CONTENTS

ABSTRACT

Hibiscus rosa-sinensis L. is a native plant of tropical and south eastern Asia. The common propagation method of this plant is using stem cuttings. For evaluating the rooting potential and bud development, plant growth regulators viz. IAA and IBA were used along with a natural growth promoting substance, Aloe vera gel. Three concentrations 100ppm, 250ppm and 500ppm of these growth regulators were prepared. Uniform sized stem cuttings were collected ad treated with these growth regulators for 24 hours and planted in polythene bags filled with planting medium and incubated in mist chamber for 14 days. It was observed that the rooting efficiency was high in IBA. The results showed that IBA 500ppm was with maximum root growth followed by IBA 250ppm. Bud proliferation showed a reverse effect with the maximum reduction in bud growth was observed in higher concentrations of both IAA and IBA. An enhanced proliferation of buds was observed in cuttings treated with Aloe vera gel.

INTRODUCTION

Plant propagation is defined as multiplication or reproduction of plants. Various techniques of propagation have been developed with the objective to have uniformity in crops, early bearing, increased propagation, resistance against pests and diseases, and introduce certain characteristics in new generations.

Plants can be propagated by sexual and asexual means. Sexual means induces propagation by seeds, while asexual propagation is based on the utilization of vegetative parts of plants for raising new ones. Vegetative parts of plants like shoots, leaves, root, stem, buds and underground parts are used in different ways for propagation of plants. The most common asexual propagation methods include cutting, layering, grafting and budding, which need specialized skill and are done differently in different plants.

TYPES OF PROPAGATION

1. ***Sexual propagation***: Propagation or multiplication of plants by seeds is known as 'sexual propagation'. Seeds are formed as a result of successful fertilization and combination of parental gametes.

2. ***Asexual propagation***: It is also called 'vegetative propagation'. A wide variety of crop plants are now propagated by asexual means. It involves reproduction from vegetative parts of plants like leaf, stem, root or their modified forms so that the new individual is exactly like the parent plant. Merits of asexual propagation are:

■ Many fruits and ornamental plants that do not produce seeds are multiplied by this method.

■ Plants propagated by asexual propagation are true-to-type genetically.

■ By top working, old and economically low produce fruit plants can be converted into superior ones.

Different types of cuttings

Cuttings are usually classified into following groups according to the particular part of the plant used.

Root cuttings**:** propagation by means of root cutting is simple. Plants like breadfruit, teak, curry leaf etc. can be easily grown by root cuttings.

Stem cuttings: it is the most widely used method. Jasmine, rose, grapes etc. are examples.

Leaf cuttings: certain plants with thick and fleshy leaves can produce plantlets on their leaves. More plantlets can be produced from a single parent plant. (Example: Begonia)

Stem cutting It is the cheapest and most commonly used method of plant propagation. Stem cuttings of shrubs are easier to root than that of trees. The success of this method depends on the site of cutting, age of parent plant, humidity etc. At least one node must be present in each cutting. When cuttings are planted in soil roots and shoots grow from lower and upper end respectively. The four main types of cuttings are:

● Softwood cutting: It is also known as green wood cutting. They are taken from woody plants before lignification, when tissues are relatively soft. It is easy and rooting is quick (Example: Jasmine, plum etc.).

● Semi-hardwood cutting: Semi-hardwood cuttings are produced when the stems are partly, but not fully mature. At this point, the wood is relatively firm but still flexible enough to bend easily and break with a snap. Semi-hardwood cuttings are usually taken between late summer and early fall. Cut off the semi-hardwood stem squarely below a node. The length of the cutting is between 7–

15 cm long with two or more nodes. Remove the lower leaves and any buds growing at the tip. (Example: *Hibiscus, Duranta* etc.).

● Hardwood cutting: 15 to 25cm long with 2 to 3 nodes. They are taken from the material of previous summer's growth. Basal end just below a node, top end 2 to 3cm above last node. (Example: Grapes, Bougainvillea etc.).

● Herbaceous cutting: they are made from herbaceous succulent plants. These plants will not develop woody tissues. It roots in a short period (Example: *Coleus*, Geranium).

PLANT GROWTH REGULATORS

Plant growth regulators are organic compounds which modify or regulate physiological processes in an appreciable measure in plants when used in small concentrations. Plant hormones are also regulators but are produced by the plants in very low concentration and these hormones move from the site of production to the site of action. There are five groups of plant-growth-regulating compounds: auxin, gibberellin (GA), cytokinin, ethylene, and abscisic acid (ABA). For the most part, each group contains both naturally occurring hormones and synthetic substances.

Auxins - Auxins are a powerful growth hormone produced naturally by plants. They are found in shoot and root tips and promote cell division, stem and root growth. They can also drastically affect plant orientation by promoting cell division to one side of the plant in response to sunlight and gravity. Auxins positively influence the plant length and increase the distance between nodes. When an auxin is applied to a cut stem, the stem will initiate roots at the cut.

Gibberellins- Gibberellins are the plant growth regulators involved in regulating the growth and influencing different developmental processes which include stem elongation, germination, flowering, enzyme induction, etc. The Gibberellins overcome the genetic limitations in different dwarf varieties. There

are more than 70 gibberellins isolated. They are GA1, GA2, GA3 and so on. GA3 Gibberellic acid is the most widely studied plant growth regulator.

Cytokinins- Cytokinins are a group of plant growth regulators which are primarily involved in performing cell division in plant roots, shoot system. This hormone helps in promoting the cell's growth, development, differentiation, affecting apical dominance and delay in leaf senescence. This is commonly used by farmers to increase the production of crops

Abscisic acid-. Abscisic acid is the growth inhibitor hormone in plants. It is synthesized within the stem, leaves, fruits, and seeds of the plant. It acts as an antagonist to Gibberellic acid. It is also referred to as the stress hormone because it helps by increasing the tolerance of plants to different kinds of stress.

Ethylene- Ethylene is unique in that it is found only in the gaseous form. It induces ripening, causes leaves to droop (epinasty) and drop (abscission), and promotes senescence. Plants often increase ethylene production in response to stress, and ethylene often is found in high concentrations within cells at the end of a plant's life. The increased ethylene in leaf tissue in the fall is part of the reason leaves fall off trees. Ethylene also is used to ripen fruit.

Role of plant growth regulators in rooting

The early characterization of auxins as "root forming hormones of plants" established a long-standing link between this class of small molecules and root development (Thimann and Went 1934). Auxins facilitate the initiation of roots. These hormones induce both growth of root branching (lateral root initiation) and roots that are pre-existing. They also have a role to play in the formation of the adventitious root. With all the more native auxins being moved down the roots from the stems, the complete development of roots is triggered.

Success of rooting of cuttings depends on the physiological stage of the mother plant (Day and Loveys, 1998), time which cutting was taken (Darwesh, 2000) and the type of PGRs used (Rowzack, 2001).

Replacement of synthetic PGRs with natural alternatives are becoming popular because of high cost of synthetic PGRs, risk of toxicity in plants, human and animals due to the application of overdoses (Cutler and Schneider, 1990). Alternative natural plant extracts rich in plant hormones and natural antioxidants can be used to improve, induce and stimulate growth of another plant species.

IAA (Indole acetic acid) is considered as the major endogenous auxin that influence the root induction but the exogenous supply of IBA (Indole butyric acid) showed a promotive effect in many plants. (Jarvis B.C., 1986; Kumar and Singh 2012).

Among many of the natural alternatives, the extract from the leaves of *Aloe vera* is also being used. *Aloe vera* is an important medicinal plant which belongs to the family Liliaceae. It is a shrubby succulent herb grows in many countries around the world (Pandey and Singh, 2016). Its large leaves consist of three layers with inner clear gel, middle layer of latex and outer thick layer. Inner clear gel consists of 99% of water and some glucomannans, amino acids, sterols and vitamins. Middle parenchymatic cells contain liquid of yellow latex of a bitter sap, which is rich in essential amino acids, mono and polysaccharides, lignin, macronutrients, micronutrients, vitamins, gibberellins and salicylic acid (Surjushe.*et al.,* 2008).

At present, many farmers and villagers use fresh *Aloe vera* gel for inducing rooting of stem cuttings and air layering of plants. Rooting of cuttings may be facilitated due to its antibacterial properties and/or its composition which includes root inducing substances like growth hormones.

The present study focuses to study the effect of different growth promoting factors in root induction of stem cuttings in *Hibiscus rosa-sinensis*.

Description of the plant selected for the present study

Hibiscus rosa-sinensis belongs to the family Malvaceae, is native to tropical and southeastern Asia, this plant is commonly found throughout the tropics and as a house plant throughout the world and are grown as ornamental varieties. Many species of Hibiscus are grown for their showy flowers.

This plant is popular landscape shrub, creates a bold effect with its bed-textured, glossy dark green leaves and with 4- 6 inch wide and up to 8 inch long, showy flowers, produced throughout the year and grows up to 7-12 feet. Flowers usually have five petals, five stigmatic lobes, a five celled ovary, five teeth on the calyx and with numerous stamens.

Hibiscus is packed with flavonoids, phenolic compounds, anthocyanins, fatty acids and other pigments that work wonders for hair. It is particularly known for its hair growth-enhancing abilities, and is commonly used as hair oils, shampoos, conditioners, and even hair masks.

Hibiscus also has medicinal properties and takes part as a primary ingredient in many herbal teas. Essential oil of this plant has antifungal activity and one of its constituents was found to be active against human cancer cell lines in several stages of cellular division. The leaves are useful inhaling of ulcers and promoting hair growth activity.

It can be easily propagated by stem cuttings. Propagation by cuttings is usually used in households and commercially.

Figure 1: Habit of *Hibiscus rosa-sinensis* L.

Objectives of the present study

The following are the objectives of the present study

1. To study the rooting efficiency of selected auxins viz IAA and IBA and its action in different concentrations.
2. To compare the effect of these plant growth regulators with a commonly used natural growth promotor *Aloe vera* gel

REVIEW OF LITERATURE

Stem cutting is one of the common methods of propagation in *Hibiscus rosa-sinensis.* There are many differences between plant species and between the plant growth regulators in rooting potential of cuttings and it is very difficult to forecast easy and hard to rooting of cuttings in the plants.

Works of Mukhopadhyay and Bose (1972) revealed that retention of four leaves developed per cent rooting and many number of roots (22.00) per cuttings of *Hibiscus rosa-sinensis* L. with IBA 3000 ppm as compared to control. Bhattacharjee and Balkrishna (1986) conducted experiment on the standardization of propagation of hibiscus (*Hibiscus rosa-sinensis* L.) and found that fifteen-centimeter tip cutting with four leaves of hibiscus treated with 4000 ppm IBA and planted in sand under mist give best performance in rooting and survival of rooted cuttings.

Gupta (1989) conducted an experiment to investigate rooting potentiality of semi-hard wood cuttings of Snow Flake cultivar of *Hibiscus rosa-sinensis* L. under intermittent mist with the application of auxins and concluded that IBA at 4000 ppm induced higher percentage of rooting (80), greater number of roots (18.25) per rooted cutting and also produced longer roots (73.23 mm).

Shadparvar, (2010) reported that increased the root length as compared to the controls in *Hibiscus rosa-sinensis*, which were treated for 12 hours by different kinds of plant growth regulators, like IAA, ,2,4-D, IBA etc. with water as control. The results showed that the effect differs from different kinds of plant growth regulators on taking root of *Hibiscus rosa-sinensis*, and even different densities of the same plant growth regulators has different influence too. There into, using 400 mg/L of IAA,2 mg/L of 2,4-D,400 mg/L of IBA was the most effective in producing roots.

Supplemental irradiance to the stock plant of *Hibiscus rosa-sinensis* during winter and treated with IBA Rooting was enhanced reported by Hunter-Cario.

(2007). There is another experiment conducted by Bhattacharjee and Balakrishna (1986) that fifteen-centimeter tip cuttings with four leaves treated with IBA and planted in sand under mist give best performance in rooting and survival of rooted cuttings.

Ahir and Parmar (2007) studied on the rooting in *Hibiscus rosa-sinensis* L. Hawaii by air Layering with the aid of IBA, NAA and different coloured polythene found that IBA at 3000 mgl-1 with black polythene wrappers was the most effective in early root initiation and increasing the rooting percentage, increasing the sprouting of rooted layers.

Ali Mohammadi Torkashv and Vahid Shadparvar (2012) have conducted an experiment on rooting in Hibiscus by IBA and different rooting substrates and obtain highest number. of roots in sand-perlite substrate with concentration of 4000 ppm IBA.

Savaliya Rohitkumar Rameshbhai (2016) studied the efficiency of IBA on different types of cutting for rooting in Hibiscus (*Hibiscus rosa-sinensis* L.) cv. local found that maximum percentage of rooting by hardwood cutting treated with IBA 1000 mg/l. Chowdhuri et al., (2017) studied on the effect of different growth regulators on propagation of China rose (*Hibiscus rosa sinensis* L.) in subtropical zone of West Bengal found that NAA at all concentrations (1000-3000 ppm) may take for rooting of China rose, but higher doses is more beneficial in this aspect during rainy season in subtropical zone, the second best growth regulator is IBA at 3000 ppm. They also observed that semi hardwood cutting is better than tip cutting for propagation of China rose.

In conclusion all the above investigations showed that stem cutting is the most important method of vegetative propagation. The cuttings treated with different plant growth regulators showed a varied effect. The survey also showed that there is little work carried out using other organic supplements that are used as promotors of root growth from stem cuttings

. MATERIALS AND METHODS

Plant Material

China rose (*Hibiscus rosa-sinensis* L.) is a very important ornamental flowering shrubs growing wide range of climate all over the world. Propagation by cuttings is usually used in households and commercially. In the present study, stem cuttings of *Hibiscus rosa-sinensis* were taken from plants growing in the homesteads of the students. Stems with uniform thickness is selected and cut to the length of approximately 30 centimeters and were used as the planting materials.

Figure 2: Stem cuttings of *Hibiscus rosa-sinensis* L.

Plant Growth Regulators

The synthetic plant growth regulators used in this study is Indole Acetic acid (IAA) and Indole butyric acid (IBA). 500mg of research grade chemicals were weighed out using electronic balance and were transferred to a conical flask and it is dissolved in 1000ml distilled water and were used as the stock solution with the concentration 500ppm. From this, 200ml of 250ppm and 100ppm solutions were prepared by making proper dilutions from the stock solutions and stored in conical flasks.

Freshly collected leaves of *Aloe vera* plant were brought to the laboratory. The fleshy leaves were pressed using a clean spatula to ooze out the gel and is collected in a clean dry beaker. This clear gel is used for treating the stem cuttings.

Planting Medium

The Potting mixture with garden soil: coco peat: farmyard manure mixed in the ratio 1:1:1 is prepared and were filled in the polythene bags of size 6 x 3 inches.

Procedure

The selected stems with pencil thickness are pruned to remove the leaves and the basal region of the stem is provided with a slant cut. The prepared concentrations of the growth regulators, viz. 100, 250, 500 ppm of IAA and IBA, were taken in beakers. Freshly collected juice of *Aloe vera* taken in the beaker is used for dipping the stem cuttings. The stem cuttings were inserted into the respective beakers and kept in the solution for 24 hours. After 24 hours the stem cuttings were taken out and allowed to air dry for a while and then planted in the polythene covers filled with potting mixture. A control set is maintained by dipping the stem cuttings in distilled water. The treated stem cuttings and the control set were kept in the mist chamber and mist irrigation was provided twice a day for 14 days. The growth of the cuttings was monitored every day and the moisture content of the soil is also checked to prevent the plant from drying out. Five replications were maintained for each treatment and also for the control set. The whole set of experiment is maintained in green house of Bishop Moore College, Mavelikkara.

Figure 3: Stem cuttings of *Hibiscus rosa-sinensis* L. treated with different concentrations of IAA, IBA and Aloe vera gel along with control plants.

After 14 days of the plant growth, the following observations were measured. The number of buds developed on each cutting is calculated and noted down. Then each cutting is removed from the polythene bag without damaging the roots. Length of the longest root of each cutting is measured with a scale and noted down.

RESULTS AND DISCUSSION

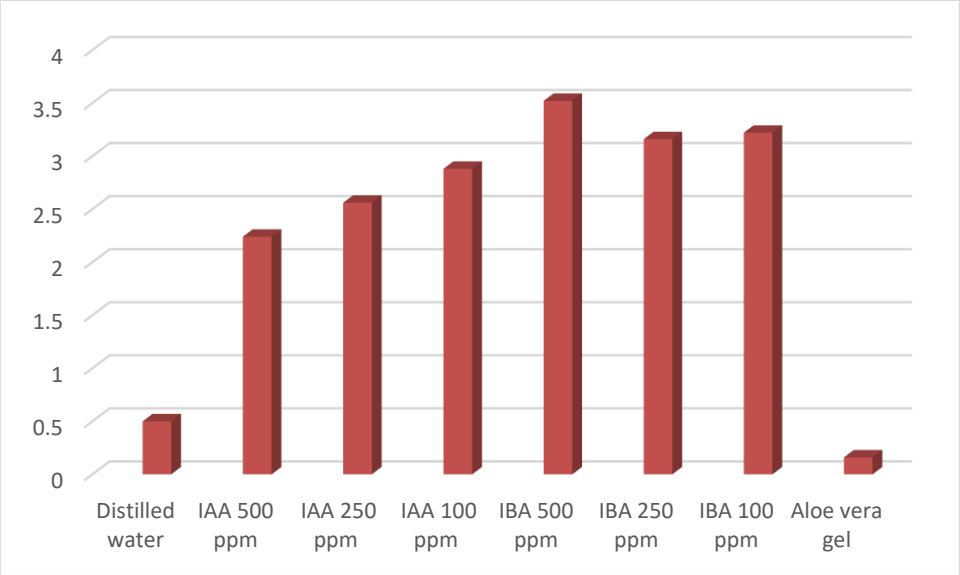
The present study focused to analyze the rooting efficiency in concentrations such as 100ppm, 250ppm, and 500ppm of selected auxins viz IAA and IBA and to compare the effect of these plant growth regulators with a commonly used natural growth promotor Aloe vera gel. The experiment was suspended after the 14^{th} day and the following observations were taken from the present study. The data presented is the mean value of five replications.

Rooting Efficiency and Maximum Root Length

After 14 days, the potting mixture is washed off from the stem cuttings and were carefully uprooted for assessing the rooting efficiency and the length of the maximum grown root was measured. The data is presented in table 1.

Treatment		**Rooting Efficiency**	**Maximum Root length (cm)**
Distilled water		*	0.5
IAA	500 ppm	**	2.24
	250 ppm	**	2.56
	100 ppm	**	2.88
IBA	500 ppm	***	3.52
	250 ppm	***	3.16
	100 ppm	***	3.22
Aloe vera gel		*	0.16

Visual grading was done to assess the rooting efficiency and it was observed that the maximum efficiency was in IBA. When compared to the auxins, Aloe vera gel showed a very low efficiency. Maximum root length was calculated by measuring the longest root and the mean value was calculated and presented in the table 1 and graph 1.

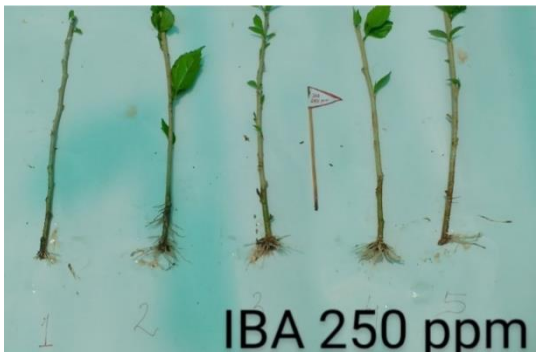


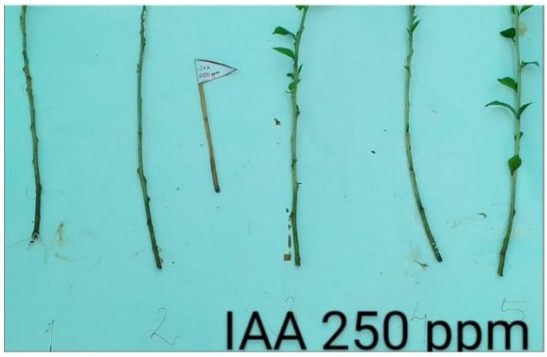
Graph 1: Effect of plant growth regulators on Root length of *Hibiscus rosa-sinensis* L.

IBA showed a better response in Maximum root length when compared to the others and the highest root length was observed in IBA 500. Root length was lowest in plants treated with Aloe vera gel.


IBA 500ppm


IAA 500 ppm


IBA 250 ppm


IAA 250 ppm

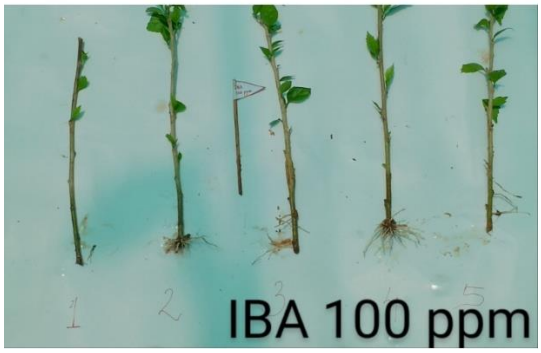


Plate 1: Effect of plant growth regulators on Rooting efficiency and bud proliferation in *Hibiscus rosa-sinensis* L.

Axillary bud Proliferation

To assess the growth and root establishment, axillary bud proliferation was also measured by counting the buds emerged from each plant and the mean value is presented in the table 2 and graph 2.

Treatment		**Mean No. of buds**
Distilled water		9.6
IAA	500 ppm	6.0
	250 ppm	5.8
	100 ppm	8.8
IBA	500 ppm	2.0
	250 ppm	5.0
	100 ppm	7.6
Aloe vera gel		6.4

Table 2: Effect of plant growth regulators on bud proliferation in *Hibiscus rosa-sinensis* L.

In this study, it was observed that the maximum number of bud proliferation was observed in the control plants. Very low rate of proliferation was observed in the stem cuttings treated with IBA. Of these the lowest value was in IBA 500. On comparison of the auxins used in both the cases, cuttings treated with 100 ppm showed a better result than the higher concentrations. Higher number of bud proliferation was also observed in the plants treated with *Aloe vera* gel when compared to the higher concentrations of auxins.

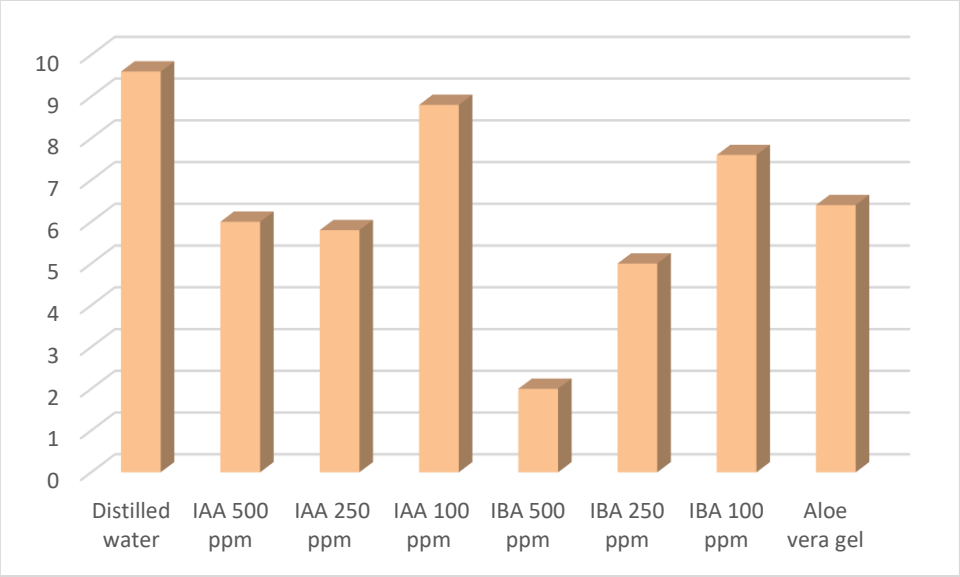


Table 2: Effect of plant growth regulators on bud proliferation in *Hibiscus rosa-sinensis* L.

Auxins are a group of hormones that can induce root formation. Evidence from many studies highlights the central role of auxins in orchestrating the final root architecture. Defining the role of auxins as a component of endogenous developmental programs as well as in mediating environmental stimuli to shape the final root architecture is reported by many scientists (Mirihagalla, & Fernando, Menaka. 2020; Chatse, and Kedar, 2021).

In roots, the most well-characterized auxin-associated events are the dose-dependent increase in the length of root hairs, the bimodal effect of auxin concentration on primary root length and the dose-dependent increase in number of lateral root primordia (Overvoorde *et.al*., 2010).

Even though the IAA is considered as the major rooting hormone, the present study showed a better response in the plants treated with IBA. The different responses to different types of auxins might be explained by the higher stability of IBA in the rooting medium, and IBA is less sensitive than IAA to non-biological degradation (Epstein and Ludwig-Muller, 1993).

Endogenous cytokinins are transported to the axillary buds and promote their outgrowth. Nordstrom *et al.*, (2004). suggest that auxin play an important role by downregulating the synthesis of cytokinins, restricting its availability to axillary buds, thereby repressing their growth. This may be the reason of the reduced bud proliferation in the higher concentrations of auxins.

Aloe vera as a bio stimulant, was used to enhance growth and yield in many plants (Padmaja *et al.*, 2007). Since Aloe vera gel extract is rich in essential amino acids, mono-and polysaccharides, lignin, macronutrients, micronutrients, vitamins, gibberellins and salicylic acid this can be used as a bio stimulant (Surjushe, 2008).

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