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Review of Related Literature: *Influence of Bottom Heat and Naphthalene Acetic Acid Application on the Regeneration of Eugenia (Eugenia Myrtifolia L.) Stem*

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ABSTRACT— One of those ornamental plants was Eugenia *myrtifolia* L. from Myrtaceae family. There was several factors that determine success of plant propagation by cuttings including plant factors (species/varieties, type of cuttings, physiological maturity of the plant and cuttings), environmental factors (rooting medium temperature, medium moisture content, relative humidity and light), treatment of cuttings (root inducer application, cutting storage etc.), rooting medium temperature, type of cuttings, and treatment of cuttings with root inducer influenced the regeneration of Eugenia stem cuttings. Due to limited available information regarding the rooting of Eugenia, stem cuttings the study was conducted to generate information that could be used in designing cutting propagation protocol for Eugenia. Hence, this study on *Journal of the Austrian Society of Agriculture Economics (JASAE), Vol 17, Issue 02.*

KEYWORDS: NAA, bottom heat, type of cuttings, *Eugenia Myrtifolia*

1. REVIEW OF RELATED LITERATURE

1.1 Botanical Description of Eugenia

Eugenia myrtifolia was a worldwide plant, although highly unevenly distributed in tropical and subtropical regions. It was a versatile evergreen shrub or tree, which if left unclipped can reach over 30 feet high and when its branches were occasionally trimmed, it produces glossy foliage [1]. All species was woody evergreen trees and shrubs with very attractive glossy foliage, in another country like India, the roots and leaves were traditionally used as medicine [2] for the treatment of hypoglycemic, antibacterial, anti-HIV activity, and anti-diarrhea effects [3], [4]. [5] reported that its leaves and bark have anti-inflammatory properties.

1.2 Methods of Eugenia Propagation

Unlike other ornamental plants which regularly produce seeds throughout the growing season and hence was mass-produced sexually, Eugenia was not. This species can be easily propagated through seeds but because only a few plants bear fruits, and if able to bear fruits, its fruits contain only a few viable seeds [6] thus sexual propagation was not commonly practiced. Furthermore, since the plant was continuously trimmed throughout the growth period as topiary, its vegetative growth dominates making the plant less capable of producing seeds. Eugenia was therefore commonly propagated by stem cuttings although being one of those considered difficult-to-root species, Eugenia stem cuttings take a longer time (2 to 3 months) to be rooted than other plant species [7].

1.3 Influence of Bottom Heat in Stem Cutting Propagation

The application of bottom heat has been long recognized as beneficial in stimulating root development in cuttings. [8] stressed that the effectiveness of bottom heat in improving rooting success was related to the warmer temperature of the rooting medium that causes the greater activity of the basal portion of cutting. It was further claimed by [9] that bottom heat application can keep the top of the cutting dormant promoting a more active metabolic process at the basal area of the cuttings. Furthermore, [10] revealed that bottom heat treatment of the rooting medium improved rooting efficiency in cuttings even in extremely difficult to root species like rhododendron. Rooting medium temperature was more critical than air temperature for rooting [11]. Optimum root medium temperature was generally reported to be in the range of 22 to 24 ^{0}C (72 to 75 ^{0}F). Rooting medium temperatures can be reduced to as low as 15 ^{0}C (60 ^{0}F) after the roots had formed. Air temperatures do affect rooting and should be maintained at 18 to 24 ^{0}C (65 to 75 ^{0}F) [10]. Maintaining air temperature lower than the medium temperature retards shoot growth and promotes root development [12]. [13] reported that high air temperature caused profuse callusing of the base of on Salix tetrasperma cuttings that resulted in poor rooting percentages and reduced cutting survival. In the temperate region, bottom heat was reported to have both beneficial and detrimental effects on rooting depending on the species [14]. [15] reported that the rooting performance of six evergreen taxa *(Hick's yew, Hetz juniper, Savin juniper, Ramlosa juniper, Tamarix juniper, and Arborvitea)* was significantly higher at bottom heat temperature at 12±2 0C than that at 21± 2 ^{0}C. Moreover, he explained that high bottom heat temperature at 21±2 ^{0}C caused more death of cuttings due to rotting of the cutting base. Contrary, [16] claimed that there was an increase in rooting and reduction in rotting of rhododendron (evergreen species) cuttings when basal rooting temperature increased from 15 ^{0}C to 25 ^{0}C. In the tropical region, [17] reported that at an optimum temperature (25 ^{0}C and 28 ^{0}C) of rooting media improved the root weight of *Pinus patula and Pinus ellioti* + *Pinus caribaea* stem cuttings and even increased during the warmest months of the year but the rooting percentage decline as the rooting medium temperature was increased to more than 30^{0}C.

1.4 Type of Cuttings

The most suitable type of cutting for use in propagation depends on several factors. Cuttings was sometimes prepared with shoot or leaves and sometimes was leaf-less. Leafy cuttings occupy more bench space and thus reduce the number of cuttings that can be rooted in the propagation chamber than leafless cuttings [18]. Therefore, the leaf surface area was frequently reduced before placing inside the chamber to maximize the number of cuttings. Despite the benefits of leaf maximizing the capacity of rooting chambers, results of most investigations on the role of the leaf in the regeneration process of cuttings suggest that the supply of assimilates produced during propagation was essential for adventitious root production [11], [19- 22]. For example, [23] reported that the rooting percentage of *Cotinus coggygria* cv. Royal purple cuttings were enhanced when lateral shoot and full leaf area were retained (80%) than when only 22%removal of lateral shoots was retained. Moreover, [24] found that leaves retained in cuttings carry out photosynthesis which produced carbohydrates needed for root formation and growth in particular when the rooting process lasts several weeks. Thus, the removal of leaves which limits photosynthesis of pea and Acer *rubrum* cuttings [25], [26] prevented rooting of their cutting [27]. In contrast, [28], [29] reported a negative effect of retaining more leaves in avocado cutting on rooting which was attributed to the high level of *Mn* which activates IAA oxidase and hence reduced level of IAA in the cutting base.

1.5 Influence of Naphthalene Acetic Acid (auxin) in Cuttings

NAA a synthetic auxin and like other auxins was toxic to plants at high concentrations but at suitable concentrations promote rooting of cuttings and was commonly used in vegetative propagation by cuttings. It was considered more effective in inducing root initiation than the naturally occurring Indole acetic acid (IAA) but was more toxic than IBA and at a high concentration which may cause inhibition of rooting and root growth or both [30]. Among the effects of NAA, [11] revealed that NAA was more effective than IBA in stimulating the rooting of Douglas fir. Furthermore, [31] revealed that compound NAA promotes faster IAA accumulation in cuttings and was more resistant to degradation by plant enzymes and so was more widely used in agriculture for various purposes including an ingredient in many commercial rooting hormones. The effect of NAA in the regeneration of Eugenia stem cuttings still requires further evaluation because except for the study of [32] there was only a few studies conducted using NAA in treating Eugenia cuttings. Meanwhile, the effectiveness of NAA in rooting cuttings of other plant species was reported to vary with concentrations. [33] recommended concentrations ranging from 20-100 ppm for softwood cuttings while [34] reported that NAA at 100 ppm improved rooting of *Dracontomelon dao*. Furthermore, [35] claimed that in Acacia *auriculiformis* leaf cuttings, the rooting was improved with the application of 250 ppm. Likewise, in black pepper (*Piper nigrum* L.), [36] obtained better rooting with early sprouting, increased the number, length, and diameter of shoots per cutting, number of leaves per shoot, and number of primary and secondary roots following application with 250 ppm NAA. On the other hand, [37] achieved best rooting (90%) in Jasminum *grandifolium* cuttings by application of 500 ppm of NAA. Besides, the Application of NAA at the 500-1000 ppm NAA promotes best rooting in Sarpgandha (*Rauwolfia serpentine*). [38] claimed that application of 2000 ppm NAA, gave higher survival (78%) in white lauan stem cuttings. [39] found the application of 2,000 ppm NAA gave a higher percentage of rooting (72.45%) of *Clerodendrum splendens*. [40] reported that NAA at 3,000 ppm gave a better rooting percentage in J. auriculatum cuttings. In contrast, [41] did not successfully regenerate cypress stem cuttings even at higher rates (1,000 ppm and 2,400 ppm) NAA concentrations. [42], [22], [43], [44] attributed the inhibitory effect of high NAA concentrations to rooting of cuttings to the accumulation of ethylene at the cutting base.

1.6 Auxin Mechanism in Adventitious Root Initiation

The role of plant hormones in the regeneration of cutting was deliberately one of the most important factors in horticultural production practices. Although naturally occurring auxin was present in plants and promotes rooting, it was only true to many easy to- root-species like Forysthia, Ribes, Salix, Philadelphus [45] while cuttings from others do not root easily [46- 49]. However, the amount of naturally occurring auxin (IAA) varies from every species due to the presence or absence of rooting co-factors or auxin synergists [11], [50]. The purpose of treating cutting with growth regulators was to increase the number of cuttings to form roots, hasten the process of root initiation, and increase the uniformity and number of cuttings [30], [11]. The formation of pre-root initials in stem cutting was the influence of native auxins present in the plant, which altogether leads to the synthesis of RNA involved in the initiation of root primordia [51]. [32] emphasized that compounds generally considered auxins were synthesized by the plant and have the ability to induce cell elongation in stems, branches, and roots. In another view, [52] pointed out that auxins were not synthesized in all cells even if, cells retain the potential ability to do so, but require specific conditions for auxin synthesis to occur. In fact, because of this condition, auxins have to be translocated toward those sites where they were needed. Translocation was driven throughout the plant body, primarily from peaks of shoots and to peaks of roots (basipetal). [53] revealed that auxin movement was regulated in the plant via "auxin poll" (consist of the conjugates of sugars, amino acids, and peptides), through transport delivery from the phloem by an influx and efflux carriers. At the root tip, the auxin flow reverses toward the shoot [54].

Adventitious root formation of cuttings in response to auxin application was investigated by [11] where they found that root formation and development involved two initiation stages namely: the auxin-active stage and auxin-inactive stage; and the root elongation and growth stage. Their findings also revealed that the second stage (root elongation and growth stage from which the root tip grows) had no response to exogenous application of auxin. [53] described that at the root tip, an auxin gradient already exists which controls proper development, maintenance of the root meristem, and cell identity within the root system. At this stage, auxin controls not only initiating root hair development but regulates root elongation. On the other hand, the auxin-active stage might be influenced by wounding. Wounding stimulates the natural accumulation of auxins and carbohydrates in the wounded area and increased the respiration rate in the new "sink area" [11]. [55] reported that wounding was required to achieve root. [56] revealed that wounding-related compounds play a main role in the dedifferentiation phase because of greater contact and absorption of the growth regulators by the tissues at the base of the cuttings. [11] reported that auxins conjugate with amino acids that play also an important role in the sequence of root development since amino acid resembles Indole-acetyl aspartic acid which was a conjugate of IAA which increases rapidly during the first day of root induction/initiation and which decline at latter part of root development. Moreover, failure of exogenously applied auxin to promote rooting of cuttings was attributed due to the lack of necessary enzymes to synthesize the root-inducing auxin-phenol conjugate; lack of enzyme activators; the presence of enzyme inhibitors; lack of substrate phenolics, and physical separation of enzyme reactants due to cellular compartmentalization [48]. The rooting performance of cuttings treated with auxin was affected by pH. [57] revealed that the addition of IAA induced root hairs of the lettuce only at pH 6.0, and that auxin inhibitors prevented root hair formation at pH 4.0. [58] found that auxin was rapidly taken up in cells by pH trapping during transport.

1.7 Methods of Rooting Hormone Application

Most plant propagators used different methods of applying rooting hormones to cuttings [48]. These include the quick dip method, dilute solution soaking method, and powder talc application. The quick dip method was done through the basal ends of the cutting and dipped at 5-15 seconds in concentrated solution 500 to 10,000 ppm (0.05 to 0 1.0 percent) of the growth hormone dissolved in alcoholic solution (50:50 ethyl alcohol – distilled water). The dilute solution soaking method was done through the basal part-2.5 cm (1 in) of the cutting and soaked in a dilute solution of the material for up to 24 hours just the cuttings was inserted into the rooting medium. The concentrations used vary from 20 ppm for easily rooted species to about 200 ppm for the more difficult species. Powder talc application was in the form of a powder containing the auxins [30]. The length of time that growth hormones remain in contact with the base of the cutting can also affect the profound effect on rooting [30]. [11] reported that longer dilution soaks increased root number, but inhibit shoots break. On the other hand, [48] stated that many propagators prepare the quick dip because of consistency of results and ease of application. Also, he further emphasized that greater rooting and more consistent rooting responses have been reported with quip dip due to more uniform coverage and reduce the environmental influence on chemical uptake.

1.8 Influence of Environment in Adventitious Rooting in Cuttings

1.8.1 Relative Humidity

In propagating stems cuttings, especially evergreen, high RH (85%) must be maintained. The most and efficient method used to maintain humidity was to build an improvised propagation chamber with a plastic film cover. The cover keeps the humidity level high for the cuttings and does not allow the soil to dry out quickly. To maintain high RH, mist should be applied often enough to prevent leaves of cuttings from wilting and long enough for the water to evenly coat the leaf surface but does not drip off [59].

1.8.2 Light

High light was not needed for rooting. Shade was used to lower light levels and thereby reduce transpiration. Not enough light, though, reduces rooting, so supplemental light may be necessary [10]. [59] pointed out that light was the driving energy source for photosynthesis and carbohydrate accumulation in plants but vegetative cuttings require a minimum quantity of light at approximately 50 % of shading to provide the energy for root initiation and development. Light intensities below this minimum result in little or no root development, leading to delayed or failure of rooting. Conversely, too much light can bleach leaves and reduce root formation due to excessive stress on the cuttings.

1.8.3 Transpiration effects of leaves of cuttings

When cutting was removed from the parent plant, they continue to transpire due to the absence of the root system. Cuttings was poorly equipped to obtain water before the advent of the intermittent mist system, frequent wetting of leaves, reducing the foliage of cutting or leaf area, shading, and syringing to maintain high humidity [30]. As revealed by [48] that the water status of cutting was a balance between transpiration losses and uptake of water. Water absorption through the leaves was not a major contributor to water balance in most species. Rather, cutting the base and any foliage immersed in the propagation media was the main entry points for water. [60] emphasized that water uptake of cuttings was directly proportional to the volumetric water content of the propagation media, with wetter water uptake. Excess water reduces media aeration and can lead to anaerobic conditions and the death of cuttings; therefore, adequate drainage must be provided [51]. [60] stated that water uptake in cuttings declines after they was initially inserted into propagation media. He further stated that wounding the base of cutting increases the contact area between the cutting base and propagation medium that initiate improves water uptake of cuttings. [32] claimed that the water loss from cutting was the difference between vapor pressure between the cutting leaf and surrounding air of the mist bed.

1.9 Rooting Medium

A suitable rooting medium was required for the successful propagation of plants by stem cutting as the kind of rooting medium influences the growth and development of adventitious roots. [61] pointed out that sand, as a medium for cutting was the most important material but should be clean, coarse-grained, and free from salts or other harmful substances. [51] reported that sand alone was already a satisfactory rooting medium for better rooting of cuttings of evergreen species. [62] pointed out that for successful rooting of cuttings and faster root proliferation, the rooting medium must be well-drained but should have sufficient moisture. [48] however, emphasized that there was no universal ideal rooting mix for cutting as the appropriate propagation medium depends on the species, cutting type, season, and propagation system. They emphasized, however, that a good rooting medium should be able to hold the cutting in place during the rooting period, provide moisture for cutting; permits exchange of air at the base of the cutting; and create a dark or opaque environment by reducing light penetration to the cutting base. Additionally, [10] pointed out the importance of depth of staking of cutting into the medium and recommended avoiding deep staking to avoid the water-saturated zone.

1.10 Characteristics of Stock Plant

The characteristic of the stock plant by which the cuttings was collected was essential in the success of root initiation of cuttings taken from them. To have a successful rooting, cutting should be taken from nutritionally healthy stock plants, preferably from the upper plant part to have an active nutrients accumulation of cuttings brought about by sufficient nutrients stability assimilated within the stock plants [48], [63]. [64] also pointed out that stock plants and other sources from which cutting was obtained should be free of diseases and insect pests and be at a proper physiological state so that cuttings root successfully. On the other hand, plants that have been fertilized heavily with nitrogen may not root well [63]. Too much N favors excessive growth such that carbohydrates (food) produced in the leaves during photosynthesis was utilized as fast as they were made to supply shoot growth rather than to fuel and trigger the root initiation process of the cuttings [10]. [63] reported that early in the morning is the best time to take cuttings because the plant was fully turgid and stock plants was not under moisture stress. [11] reported that cuttings of cacao and pea had reduced rooting when cuttings were taken from the stock plant having water deficit because of high endogenous abscisic acid and ethylene levels in cuttings. The age of the plant and position of the vegetative part from where the cutting was collected could also influence the rooting efficiency of cutting. [48, 30] revealed that stem cuttings taken from young plants root much more readily than those taken from older trees because of the juvenility factor. [11, 63] also emphasized that many of the cuttings will root much better if the cuttings were taken from the lower branches of the plant (lateral shoots) and branches near the base of plants that had been cut back severely. [32] revealed that the enzymes responsible for the synthesis of auxins from all plant tissue was most active in young tissues such as shoot apical meristems and growing leaves.

1.11 Care and Management of Cuttings

Care and maintenance of cutting was very important in the success of rooting especially, to a hard-to- root plant-like Eugenia. According to [65], evergreen stem cuttings should be rooted in the greenhouses, propagation boxes, or chambers because they were the dormant type of species. High light ws not needed in early propagation, and in fact, it can stress cuttings and such structures should always be shaded to reduce light irradiance [24]. [48] emphasized the provision of adequate temperature and drainage for successful rooting of cuttings of evergreen species. Weed in rooting medium can be also a serious problem during rooting. [48] revealed that weeds should be removed to prevent them from competing with cuttings nutrients and space and to prevent the entry of pests and diseases inside the chamber.

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