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Influence of alpha naphthalene acetic acid (ANAA) on rooting and growth of Mulberry (*Morus alba* L.) cuttings

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Abstract

The study determined the influence of varying concentrations of ANAA (1500 ppm, 2000 ppm, 2500 ppm) on the rooting of stem cuttings and growth of four (4) mulberry varieties: Alf 23, Alf 47, Alf 55, and Alfonso. Ten cuttings of each variety soaked in distilled water (control) and three concentrations of ANAA were incubated and planted in polyethylene plastic bags. Data gathering was done 90 days after planting. All data were statistically analyzed. The varieties varied significantly based on the length of root and shoot, and root-to- shoot ratio in length. The different concentrations of ANAA also varied significantly on the fresh weight of root and shoot, and length of root. On the interaction effect of variety and concentration of ANAA, only the root-to-shoot ratio in weight was affected. Alf 55 produced the longest shoot (76.01 cm) while Alfonso produced the longest root (28.30 cm) and recorded the highest root-to-shoot ratio in length (0.41). Cuttings soaked in 2000ppm ANAA were the heaviest (8.61 g) and with the longest root (28.0 cm). Cuttings soaked in 2500 ppm produced the heaviest shoot. Cuttings of Alf 47 soaked in 2000 ppm ANAA recorded the highest root-to-shoot ratio in weight (0.22 g). The 2000 ppm concentration of ANAA enhanced the rooting of mulberry cuttings, and thus could be used in massive sapling production, particularly of low-rooting but high-yielding varieties.

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Introduction

Mulberry (*Morus specie*) is a deciduous woody perennial tree that belongs to the family Moraceae with leaf productivity for almost 20 years after planting (Sudhakar *et al*., 2018). It is a very important plant in rearing the silkworm (*Bombyx mori* L.). The leaves are the only source of food because of the morin content to spin cocoons which are the raw materials in the production of raw silk through the process of reeling. Mulberry is a commercially propagated plant through stem cuttings (Ravindran & Rajanna, 2005; Sudhakar *et al*., 2018) called vegetative propagation (Hawramee *et al*., 2019). This is the best method to multiply valuable trees (Corpuz, 2013), where the parent materials are produced faster and the features of the propagated plants are the same to the stock plant (Yogananda Murthy *et al*., 2012). A variety intended for the establishment of a mulberry farm should have a rooting percentage of 70% and above 60 days from planting the cuttings (Dandin & Kumar, 1989). If a variety has high-quality mulberry leaves, but, with a low rooting percentage and survival rate of production, the application of hormone auxins such as Indole Butyric Acid (IBA) and Alpha Naphthalene Acetic Acid (ANAA) in stem cuttings is involved in the development of the leaf, fruit, shoot, and, the lateral root of a plant (Bertoni, 2011; Singh *et al*., 2014; Sokhuma *et al*., 2018). The flow of carbohydrates to the leaves and the upper stem and acceleration of their transport to the rooting zone and in the clonally propagated plant, as well as their adventitious root formation is a crucial physiological process (Husen *et al*., 2017). This is a function of auxin which is recommended in the propagation of shrubs through stem cuttings (Veloza, 2014) and trees (Gehlot *et al*., 2014; Ou *et al*., 2015) that will enhance their adventitious root formation (Husen, 2007 and 2008) and stimulate the ethylene synthesis which rooting of cuttings favours (Patel & Patel, 2018). Kroin (2006) found out that auxins are useful to induce new roots when applied to plants as dry-dip rooting hormone powders and rooting solutions. Meanwhile, previous studies on the application of auxin in stem cuttings as ANAA in the different dosages had already been exploited and showed variations in rooting ability (Yan, 2014; Hossain & Urbi, 2016). The need for synthetic hormones such as auxins, particularly ANAA can help to enhance rooting (Chowdary & Bindroo, 2013), and may consequently affect overall growth of mulberry.

Alf 23, Alf 47, Alf 55, and Alfonso are among the evolved mulberry varieties at the DMMMSU-Sericulture Research and Development Institute, Philippines that were identified as having low rooting traits but with high-quality leaf yield. This study aimed to determine the influence of the different concentrations of Alpha Naphthalene Acetic Acid (ANAA) on the rooting and growth of cuttings of the three varieties of mulberry hybrids along with the check variety, Alfonso.

Materials and methods

Research Design

Alf 23, Alf 47, Alf 55, and Alfonso were used as treatments. Three ANAA dipping concentrations viz**;** C_1, 1500ppm ANAA solution, C_2 - 2000ppm ANAA solution and C_3, 2500ppm ANAA solution along with the control treatment, C_o, control (1 liter distilled water) no ANAA).

Preparation of planting media

Each polyethylene plastic bag having a size of 10' x 10' x 17' was filled with five (5) kg of the thoroughly mixed one- part garden soil and one- part sand. A total of 48 polyethylene plastic bags were filled up.

Preparation of mulberry cuttings

Branches of the three varieties of mulberry hybrids and the check variety were pruned, then branches were cut into pieces with three viable buds, 10 – 15cm long at an angle of 45^o using sharp pruning shear without split or bark peeling.

Bundling of cuttings

One hundred twenty cuttings per variety were counted and bundled with plastic twine. Each bundle was properly labeled to avoid mixing with the other varieties.

Preparation of the control and the different ANAA solutions

The control treatment was one liter distilled water. Concentration 1 (C1= 1500ppm ANAA) was a mixture of 998.5 ml distilled water + 1.5 ml ANAA; Concentration 2 (C2= 2000ppm ANAA) contained 998.0 ml distilled water + 2.0 ml ANAA); and Concentration 3 (C3 = 2500ppm ANAA) contained 997.5 ml distilled water + 2.5 ml ANAA. Each mixture was placed in a plastic basin enough to accommodate the cuttings to be dipped in the solution, and mixed thoroughly using a stirring rod.

Dipping of cuttings

After each preparation, the basal portion of thirty cuttings each of Alf 23, Alf 47, Alf 55, and Alfonso were soaked simultaneously in each plastic basin of one (1) liter distilled water and prepared ANAA solutions (1500ppm ANAA, 2000ppm ANAA and 2500ppm ANAA solution), for 24 hours.

Incubation of cuttings of the different mulberry varieties

After 24 hours of dipping, cuttings were taken out from the dipping basin at one time and were incubated for five days in an upside-down position and covered with wet cloth and sprinkled with water every day to prevent dehydration of cuttings during incubation.

Planting of cuttings and maintenance of the different mulberry varieties

Immediately after incubation, 10 cuttings were planted in a slanting position with 2 nodes buried in each polyethylene plastic bag. Forty-eight (48) polyethylene plastic bags were planted and arranged in Factorial-Randomized Complete Block Design with three replications. Immediately after planting, watering was done with an equal volume of water for each pot. Newly-planted cuttings were watered every other day with an equal volume of water per pot to sustain the newly planted cuttings. One month after planting, urea fertilizer was applied through the drill method at a rate of 5 g/pot. Weeds growing within the pots were removed through hand pulling.

Data gathering

Survival percentage (%)

Ninety (90) days after planting, the number of cuttings that survived was counted. Destructive sampling was done on 10 sample plants/treatment 90 days after planting on the following data:

Weight of the roots (g)

All the roots from each sapling were detached from the point of growth with the aid of a sharp scissor and weighed in an electronic weighing scale.

Length of root (cm)

The longest root was measured with a meter stick.

Length of shoot (cm)

The shoot was cut from the point of growth and was measured with a meter stick starting from the basal portion of the shoot to the tip of the largest glossy leaf.

Weight of the shoot (g)

The shoot was also weighed in an electronic weighing scale. Shoot and roots were placed in a brown paper bag separately, labeled and oven-dried in a hot air oven at 80^{o}C for 48 hours. The dry weight of leaf samples was recorded accordingly. The following data were computed based on the formula:

$$\text{Root-shoot ratio (in weight)} = \frac{\text{weight of root}}{\text{weight of shoot}}$$

$$\text{Root–shoot ratio (in length)} - \frac{\text{length of root}}{\text{length of shoot}}$$

Analysis of Data

Analysis of variance in Randomized Complete Block Design, Factorial (2 factors) were used to analyze the data on Statistical Tool for Agricultural Research (STAR) statistical software. Further test among the treatment means were determined through the Honest Significant Difference (HSD).

Results and discussion

Survival percentage

The variety of mulberry, concentration of ANAA and the interaction effect had not influenced the survival percentage of saplings 90 days after planting (Table 1).

The genetic constitution of a variety determines the survival percentage (Madhurithinnaluri *et al.*, 2015). The result of the present study implies that variety or genotype has no significant effect on the said parameter. It further implies that they survive comparably having a mean survival percentage ranged of 90.50 – 93.75% which is still ideal for a variety indicating they have a good survival performance wherein replanting could be minimized during the establishment period of a mulberry farm of the different varieties. The ability of a mulberry plant to survive is important since mulberry is propagated through cuttings. Likewise, survival of cuttings dipped in different concentrations of ANAA were not influenced which corroborates the findings of Gonzales, (2018) on lubeg marcots treated with different concentrations of ANAA. The results of the present study revealed that different concentrations of ANAA had no significant effect on the survival percentage of saplings propagated through cuttings. This implies that mulberry cuttings treated with no ANAA could still survive as in treated cuttings with ANAA (Table 1). Moreover, the survival percentage of the cuttings of different varieties dipped in different concentrations of ANAA revealed no significant differences. Result implies that cuttings of the different varieties dipped in different concentrations of ANAA survive comparably well with no ANAA application (Table 1).

Table 1. Mean survival percentage (%) of saplings grown from cuttings of different varieties of mulberry soaked in different concentrations of ANAA.

Concentration	Variety				Mean
	V_1	V_2	V_3	V_4	
C_0 - 1 l distilled water	91.67	93.89	90.56	96.11	93.06
C_1 - 1500ppm ANAA	93.89	92.22	90.56	89.45	91.53
C_2 - 2000ppm ANAA	96.11	96.67	91.11	94.45	94.59
C_3 - 2500ppm ANAA	93.33	91.67	93.89	90.00	92.22
Mean	93.75	93.61	91.53	90.50	

Legend: V_1 - Alf 23 V_2 - Alf 47 V_3 – Alf 55 V_4 - Alfonso (check variety)

Fresh weight of roots

Root system of a plant determines the maximum utilization of nutrients from the soil for growth and development. Roots serve as structural anchorage (Haling *et al.*, 2013), absorbs water and nutrients during plant growth and development (Guan *et al.*, 2019). Root weight is related to the root volume of a plant (Yogananda Murthy *et al.*, 2012). In the present study, results revealed that fresh weight of roots (Table 2) and weight of shoot (Table 3) did not vary significantly among the varieties. The result implies that the different varieties had produced root and shoot with comparable fresh weights. Lu (2002) stressed that genotype determines the success of rooting and play a significant role. Further, this is due to cuttings potential to root is a juvenile character that if matured enough, it decreases, that results in reduced capacity to induce rooting in matured shoot cuttings (Kibbler *et al.*, 2004). The fresh weight of roots produced varied significantly among the different concentrations of ANAA. Result implies that auxin promotes rooting of stem cuttings and varies with its nature and concentration (Hartman *et al.*, 2011). Cuttings dipped in 1500, 2000, and, 2500ppm ANAA produced roots comparably well having means of 8.17, 8.61, and 8.49 g respectively significantly heavier than the cuttings dipped in pure distilled water. Result implies that the cuttings dipped in the different concentrations of ANAA produces heavier roots than the untreated cuttings. The rooting of the treated cuttings is stimulated by the auxin in the hormone ANAA and has already been reported by many researchers (Rout, 2006). This reflects the result of (Yan *et al.*, 2014) where the different concentrations of ANAA influenced the rooting characteristics which enhances the physiological functions in the cuttings (Iqbal *et al.*, 1999), as cited by Pallavi *et al.* (2018) and rooting was accelerated (Zheng, 2020). Roots are a form through the increasing sugar availability wherein ANAA promotes the hydrolysis of starch into sugar, and short and thick roots production is also induced (Altman & Wareing, 1975), as cited by Zheng (2020). The formation of active roots was indirectly influenced by auxins through enhancing the speed of transformation of rooting primordia and movement of sugars to the base of cuttings. It is also attributed to the applied auxin which increases the endogenous auxin that accumulated in the portion of the cuttings that acts as a metabolizing agent that induces a signal for rooting (Table 2). The interaction effect of variety and concentration of ANAA has not significantly affected the fresh weight of roots which implies that the fresh weight of roots was not influenced by the interaction effect of the variety and concentration of ANAA (Table 2).

Table 2. Mean fresh weight of roots (g) of cuttings of different varieties of mulberry soaked in different concentrations of ANAA.

Concentration	Variety				Mean2/
	V_1	V_2	V_3	V_4	
C_0 - 1 l distilled water	5.57	8.35	6.17	7.12	6.80b
C_1 - 1500ppm ANAA	8.15	7.97	6.95	9.61	8.17a
C_2 - 2000ppm ANAA	8.34	8.12	8.74	9.22	8.61a
C_3 - 2500ppm ANAA	8.20	8.17	8.25	9.35	8.49a
Mean	7.57	8.15	7.53	8.83	

2/Concentration means followed by a common letter are not significantly different at .05 levels (HSD).

Fresh weight of shoot

The different concentrations of ANAA significantly affected the shoot weight of cuttings produced (Table 3). The result implies that ANAA has significantly influenced the weight of shoot. Cuttings dipped in 2500ppm ANAA produced significantly heavier shoots which implies that the said concentration of ANAA enhances the growth of shoot of cuttings for propagation. Plant growth hormones enhance the overall growth of cuttings through the effects on cell elongation and cell division that produces a heavier shoot weight.

Table 3. Mean fresh weight of shoot (g) of cuttings of different varieties of mulberry soaked in different concentrations of ANAA.

Concentration	Variety				Mean2/
	V_1	V_2	V_3	V_4	
C_0 - 1 l distilled water	35.76	37.01	39.51	35.90	37.05b
C_1 - 1500ppm ANAA	40.04	37.17	43.47	40.12	40.20ab
C_2-2000ppm ANAA	36.26	35.50	46.30	39.19	39.31ab
C_3 - 2500ppm ANAA	40.89	45.88	43.43	41.53	42.93a
Mean3/	38.24	38.89	43.18	39.18	

2/Concentration means followed by a common letter are not significantly different at .05 levels (HSD).

Root length

Variations were observed among the varieties pointing to the significant effect based on the performance of the mulberry varieties on root length (Table 4). Alfonso has produced significantly the longest root (28.30cm) comparable with Alf 23 and Alf 55 having a respective means of 26.11 and 26.04cm. Meanwhile, Alf 47 has produced significantly shorter root compared to Alfonso having a mean of 24.38cm. However, it was comparable to Alf 23 and Alf 47. Long root penetrates deeper into the soil, thus a deep root system can have a complete utilization of the stored water resources and assist the plant in making efficient use of soil water and also increase water efficiency (Quisenberry *et al.*, 1981). Root length is positively correlated with moisture retention of detached leaves, showing a direct relationship of the said parameter with drought resistance in mulberry, thus the said parameters are important in the evaluation of a mulberry for drought resistance (Susheelamma and Jolly, 1985).

Table 4. Mean length of root (cm) of cuttings of different varieties of mulberry soaked in different concentrations of ANAA.

Concentration	Variety				Mean2/
	V_1	V_2	V_3	V_4	
C_0 - 1 l distilled water	23.60	22.46	23.57	26.43	24.02b
C_1 - 1500ppm ANAA	25.38	24.62	25.81	29.17	26.25ab
C_2 - 2000ppm ANAA	29.38	24.63	28.39	29.62	28.00a
C_3 - 2500ppm ANAA	26.08	25.81	26.40	28.00	26.57ab
Mean3/	26.11ab	24.38b	26.04ab	28.30a	

2/Concentration means followed by a common letter are not significantly different at .05 level (HSD).

3/Variety means followed by a common letter are not significantly different at .05 level (HSD).

The different concentrations of ANAA had significantly affected the length of root, indicating a significant influence on the said parameter (Table 4). Cuttings soaked in 2000ppm ANAA produced significantly the longest root (28.00cm), significantly longer than in no ANAA treatment (24.02cm) which performed comparably well with lower concentration (1500ppm ANAA) having a mean of 26.25cm and higher concentration of ANAA (2500ppm ANAA) having a mean of 26.57cm. The shortest root was recorded in untreated cuttings (24.02cm). The result implies that treatment of cuttings with 2000ppm ANAA auxin concentration enhances the length of root which corroborates with the findings of Singh *et al*., (2014). There was an increase in root length observed in cuttings dipped in 2000ppm ANAA from that of the control treatment which might be due to the early formation of roots and more utilization of reserved food materials of the treated cuttings (Ghatnatti, 1997). Root length increase is also due to the treatment of auxin and auxin-like compounds which are inducing root length. ANAA is one of the effective inducers of rooting in plants (Geetha & Murugan, 2017). According to Ghatnatti (1997), maximum root length was produced due to the action of auxin which aids in the hydrolysis and translocation of carbohydrates towards the cuttings base that leads to cell division and cell elongation.

Length of shoot

Variations were observed among the varieties on their performance to grow which implies that variety plays a significant role with regards to the length of shoot (Table 5). Alf 55 has produced significantly the longest shoot (76.01cm), but comparable with Alf 47 (73.75cm). Meanwhile, Alf 47 was comparable with Alfonso which was comparable with Alf 23 (67.64cm). Length of shoot is one of the component traits of leaf yield of a mulberry plant which is regarded as growth rate. On the other hand, variations were not observed among the concentrations of ANAA based on the length of shoot. This implies that ANAA has not enhanced the development of shoots of saplings.

Table 5. Mean length of shoot (cm) of cuttings of different varieties of mulberry soaked in different concentrations of ANAA.

Concentration	Variety				Mean[2/]
	V_1	V_2	V_3	V_4	
C_0 - 1 l mineral water	67.30	71.58	74.30	70.89	71.02
C_1 - 1500ppm ANAA	70.05	76.72	74.36	72.51	73.41
C_2 - 2000ppm ANAA	66.45	75.89	79.55	71.97	73.47
C_3 - 2500ppm ANAA	66.75	70.79	75.83	66.28	69.91
Mean[3/]	67.64c	3.75ab	76.01a	70.41bc	

[3/]Variety means followed by a common letter are not significantly different at .05 levels (HSD

Further, the concentration of ANAA and the interaction effect of variety and concentration of ANAA had no significant effect on the length of shoot since no significant differences were observed.

Root-to-shoot ratio (in weight)

No significant variations were observed among the varieties on the root-to-shoot ratio in weight (Table 6). The result implies that the different varieties performed comparably well. Each of the varieties recorded a ratio of 0.20. Likewise, the different concentrations of ANAA did not influence the root-to-shoot ratio in weight. ANAA concentrations of 1500ppm, 2000ppm, and 2500ppm registered a root-to-shoot ratio of 0.25, 0.21, and 0.20 respectively, while in no ANAA, a ratio of 0.16 was registered.

The interaction effect of variety and concentrations of ANAA influenced the said parameter (Table 6). Moreover, cuttings of Alf 47, Alf 55 and Alfonso soaked in 1500ppm ANAA solution recorded a mean ratio of 0.20 in both Alf 47 and Alf 55, and 0.21 was recorded in Alfonso while cuttings of Alf 55 and Alfonso were also comparable. Meanwhile, cuttings of Alf 23 soaked in no ANAA recorded the lowest ratio of 0.14 which was comparable with the cuttings of Alf 47 (0.15) and Alfonso (0.17). The result implies that if cuttings of Alf 23 are not treated in ANAA solution, rooting is not enhanced (Table 6).

Table 6. Mean root to shoot ratio in weight of cuttings of different varieties of mulberry soaked in different concentrations of ANAA[1/].

Concentration	Variety				Mean[2/]
	V_1	V_2	V_3	V_4	
C_0 - 1 l distilled water	0.14d	0.15d	0.18bc	0.17cd	0.16
C_1 - 1500ppm ANAA	0.17cd	0.20ab	0.20ab	0.21ab	0.25
C_2 - 2000ppm ANAA	0.21ab	0.22a	0.21ab	0.21ab	0.21
C_3 - 2500ppm ANAA	0.20ab	0.21ab	0.20ab	0.21ab	0.20
Mean[3/]	0.18	0.20	0.20	0.20	

[1/]Interaction means followed by a common letter are not significantly different at .05 levels (HSD).

Root-to-shoot ratio (in length)

Alfonso produced root and shoot with a computed ratio of (0.41) comparable with Alf 23 (0.38). Meanwhile, Alf 47 (0.34) performed significantly the lowest which performed comparably with Alf 55 (0.35). Results imply significant variation among varieties (Fotadar *et al.*, 1989) as cited by (Yogananda Murthy *et al.*, 2012). These are due to the genetically controlled variation in the relative concentrations of auxin in the cuttings which the various growth hormones auxin and caline influence the adventitious root formation in cuttings.

On the other hand, the different concentrations of ANAA and the effect between variety and concentrations of ANAA did not influence the root-to-shoot ratio in length (Table 7).

Table 7. Mean root-to-shoot ratio in the length of cuttings of different varieties of mulberry in different concentrations of ANAA.

Concentration	Variety				Mean[2/]
	V_1	V_2	V_3	V_4	
C_0 - 1 l distilled water	0.36	0.32	0.33	0.38	0.35
C_1 - 1500ppm ANAA	0.37	0.33	0.33	0.37	0.37
C_2 - 2000ppm ANAA	0.33	0.35	0.36	0.42	0.37
C_3 - 2500ppm ANAA	0.40	0.37	0.36	0.43	0.39
Mean[3/]	0.38ab	0.34c	0.35bc	0.41a	

[3/]Variety means followed by a common letter are not significantly different at .05 levels (HSD).

Conclusion and recommendation

The different varieties of mulberry varied on their root and shoot length, root to shoot ratio in length. Mulberry cuttings treated with Alpha Naphthalene Acetic Acid concentration at 2000ppm recorded the highest increase in the weight of root and length of root compared to the untreated cuttings, since longer roots absorb water and nutrient deeper in the soil, thus the saplings grow more vigorously. Cuttings dipped in 2500ppm ANAA produced the heaviest shoot. The ANAA concentration of 1500ppm recorded the highest root to shoot ratio in weight compared to that of no ANAA.

The highest root to shoot ratio in weight was recorded in the cuttings of Alf 47 treated with 2000ppm ANAA. Alfonso produced the longest root while Alf 55 produced the longest shoot which could be exploited for future breeding program on drought resistance. The concentration of ANAA at 2000ppm enhances rooting of mulberry cuttings which could be used in massive sapling production of low-rooting but high-yielding varieties. Further, researches are needed to enhance the rooting and growth performance of other mulberry varieties through treatment of cuttings exploiting other rooting hormones and different planting media for higher survival and quality of mulberry saplings. ANAA solution of 2000ppm is recommended for enhanced rooting of mulberry cuttings.

Conflict of interest statement

The authors declare no conflict of interest.

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