__Short Research Article__

Possible effect on the recovery of grafted mango seedlings using naphthaleneacetic acid

Abstract

The application of naphthalene acetic acid (NAA) of different concentration (25, 50, and 75ppm) was applied to grafted mango seedlings to identify its effect on the callus formation, callus diameter, shoot emergence, and plant survival. NAA effectively reduced the number of days to callus formation, particularly using 75ppm. Lower than this concentration exhibited a comparable callus formation same as the untreated seedlings. The callus diameter, shoot emergence, and plant survival showed no significant effect by the application of NAA even how high or low the concentration. It is suggested that low and higher than the concentrations used and young scions may be used for further investigation. Likewise, another plant growth regulators should be used to hasten the callus development and higher rate of plant recovery in grafted mango seedlings.

Keywords: callus development, grafting, NAA, plant growth regulator

Introduction

Asexual propagation is a method of plant propagation to multiply the number of fruit trees, especially those with long seedlings and vegetative stages. At the same time, this method of plant propagation will shorten the waiting time before it flowers and bears fruits. Moreover, the mango is a fruit tree with a long duration of vegetative phase. Hence, various types of asexual propagation are available to shorten the waiting time, and grafting is just among them. Grafting is the union of two healthy plant parts, rootstock, and scion. Rootstock is the root system, while scion is the shoot system.

On the other hand, plant growth regulators can be used to facilitate the faster growth of plants. Exogenous plant hormones~~, such as fruit crops,~~ are commonly used in horticulture. ~~Some~~One of them is ~~the~~ naphthalene acetic acid (NAA). It was reported that it could stimulate cell elongation and shoot growth (Majda and Robert, 2018; Camponi and Nick, 2005; Iersel, 1998). Moreover, it can hasten the formation of callus in plants (Ikeuchi et al., 2013). Therefore, if NAA is used directly in grafting, this may provide faster callus formation in grafted mango seedlings~~, which provides additional information for plant propagators~~. Thus, the study was conducted to identify the effect of varying concentrations of NAA on grafted mango seedlings.

Materials and methods

Selection of rootstock seedlings and scion. A pencil size in diameter and 8-10 months old healthy rootstock seedlings were used. A pencil size with 6-8 inches long healthy scion was used for grafting. The moist newspaper was used to wrap the harvested scions (well-formed buds) from the identified bearing mother tree and placed them in an ice box to avoid desiccation.

Grafting process. Leaves were removed from the scion.(Generally leaves are removed from the scion stick, before wrapping and placing in ice box?) Then both sides of the base were cut perpendicular to form a wedge-shaped tip. Thenthe main stem of the root stock was cut at 8-10 cm height andtwo-centimeter slit was done in the middle of the rootstock using a grafting knife. A grafting tape was used to wrap the union of the scion and rootstock to ensure a good grip. A shaded area was used to observe the performance of all grafted mango seedlings.

Preparation and application of NAA.A commercially available NAA was used to apply in the grafted seedlings. The bottle has a net content of 250 ml per bottle with 0.10% or 1000 ppm. $C_1V_1= C_2V_2$ was used to come up 25, 50, and 75 ppm; it was mixed in a liter of distilled water. Prior to grafting, scions were separately dipped in NAA depending on the concentration for 1 minute.

Fertilizer application and irrigation. At 40 days after grafting (DAG), 5g of urea per seedling was applied. This was applied 5 cm away from the base of the plant and then watered immediately. Manual irrigation of the seedlings using a garden hose was done three times a week.

Plant survival. At 60 DAG, the percent survival of grafted mango was recorded.

Callus diameter. A vernier caliper was used to measure the callus diameter. Initial measurement was done just after grafting. Afterward, a follow-up measurement was done at 30, 60, and 90 DAG.

Days to the emergence of shoots and number of leaves per plant. The emergence of shoots from grafting was counted. Leaf count per seedling was done at 30, 60, and 90 DAG.

Statistical analysis

The grafted mango seedlings were arranged in Randomly Complete Block Design with three replications. Analysis was done using the SPSS program(SPSS for Windows Version 17.0, Released 2008, SPSS Inc., Chicago, Illinois, USA). The data was presented by mean standard errors (SE). Tukey's Honestly Significance Difference (HSD) test was used to compare the means.

Results and discussion

Days to callus development

Auxin induced callus formation (Ikeuchi et al., 2013),and NAA application exhibited significant variation.~~At~~Treatment with75 ppm NAA applicationwas ~~showed~~ the earliest to form callus in grafted mango, whereas lower than this concentration ~~was~~ resulted ~~to~~inlater formation of callus (Table 1).The other factors affecting the callus werealso considered and it was reported that the appearance of the callus is affected by complex factors (Mayerni et al., 2020).

The callus development in untreated seedlings may be due to wound-induced callus formation since the plants have natural auxin, which ~~thus, it has~~ enough ~~amount~~ to form callus. Therefore, a lower concentration of NAA may be needed to hasten the callus formation. However, in the previous report, the lower concentration of another source of auxin depicted the same time of callus formation as untreated (Tahir et al., 2015). In contrast, the percentage of callus formation increased between 2 and 4 mg L^{-1}, but further increase resulted in a decrease (Rahayu et al., 2016). Moreover, a previous study reported that NAA effectively forms callus early than without application (Libunao et al., 2013). Yet, a lower level of NAA is recommended for future investigation for mango. However, it was previously reported that NAA decreased callus formation, which influenced the success rate in grapevine (Kose and Guleryuz, 2006).

Table 1. The days to callus formation, callus diameter, day to emergence of shoots, and plant survival of grafted mango seedlings applied with different concentrations of NAA.

ANAA Concentration (ppm)	Days to callus development	Callus diameter		Days to emergence of shoots	Plant survival
		30 DAG	60 DAG		
0	35.67 ± 0.33 a	0.34 ± 0.13 a	0.85 ± 0.23 a	17.83 ± 1.26 a	55.67 ± 8.09 a
25	34.00 ± 1.15 a	0.35 ± 0.13 a	0.70 ± 0.22 a	18.73 ± 1.68 a	60.00 ± 4.04 a
50	35.67 ± 1.86 a	0.25 ± 0.05 a	0.64 ± 0.17 a	14.70 ± 1.47 a	53.33 ± 10.04 a
75	26.00 ± 2.08 b	0.27 ± 0.07 a	0.71 ± 0.18 a	15.10 ± 2.45 a	44.33 ± 4.33 a

Values are means ± standard error (SE); Means followed by different lowercase letters[(a-b)] in a column are significantly different at $p \leq 0.05$ by using Tukey's Honestly Significant Difference (HSD) test.
DAG – days after grafting

Callus diameter

NAA, as a source of auxin, varying concentrations did not produce a significant variation in callus diameter (Table 1). Generally, as the grafted mango seedlings matured, the callus diameter became thicker. The callus diameter decreased as the concentration increased. A 25 ppm NAA produced a higher diameter, but untreated plants had the highest at 90 DAG.

Days to emergence of shoots

The days to the emergence of shoots were not significantly affected by the application of NAA (Table 1). The earliest emergence of shoots was observed from plants applied with 25ppm, followed by the other concentrations, and untreated had the latest.

It was reported that auxin is involved in bud dormancy (Pan et al., 2021; Qiu et al., 2019); hence, if there is a higher concentration of NAA, it will inhibit the shoot initiation, as observed in the study. Contrary, shoot induction was observed between 50 and 500 mg L-1 (Shekhawat and Manokari, 2016). Therefore, a more prolonged immersion of the scion in NAA may be needed, same as the previous study. Also, higher than 100 ppm is suggested for future study. However, no or low level of NAA promoted the formation of shoots as previously reported (Ahmad and Spoor, 1999).

Plant survival

On the other hand, the plant survival of grafted mango seedlings was comparable with the treated seedlings (Table 1). Numerically, plant survival was higher in 25ppm, followed by untreated seedlings. The lowest was recorded in high concentration (75ppm) of NAA despite that the days to shoot emergence was almost 2 to 3 days earlier than 0 and 25ppm.

Conclusion

Irrespective of the concentration of NAA, it did not contribute to producing early callus formation and increased callus diameter. Moreover, a further increase in NAA concentration did not affect the emergence of shoots; hence, NAA exhibited an inhibitory effect. Further investigations on the lower level and longer immersion time in NAA than the study is needed. Moreover, the younger age of the scion may be considered for another study. Another plant growth regulators and concentrations should be used that suits for the grafted mango seedlings.

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