

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 concentrations (25, 50, and 75ppm) was applied to grafted mango seedlings to identify its effect on callus formation, callus diameter, shoot emergence, and plant survival. NAA effectively reduced the number of days to callus formation, mainly using 75ppm. Lower than this concentration exhibited a comparable callus formation, the same as the untreated seedlings. The callus diameter, shoot emergence, and plant survival showed no significant effect by applying NAA, even with how high or low the concentration was. Low and higher than the concentrations used, young scions may be used for further investigation. Likewise, other plant growth regulators should be used to hasten the callus development and higher plant recovery rate 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 are commonly used in horticulture. One of them is 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 plant calli formation (Ikeuchi et al., 2013). Therefore, if NAA is used directly in grafting, this may provide faster callus formation in grafted mango seedlings. 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 leaves are removed from the scion stick before wrapping and placing it in ice box. The moist newspaper was used to wrap the harvested scions (well-formed buds) from the identified bearing mother tree and place them in an ice box to avoid desiccation.

Grafting process. Both sides of the base of the scion were cut perpendicular to form a wedge-shaped tip. The main stem of the rootstock was cut at 8-10 cm height, and a two-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. Before 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.

Days to callus development and callus diameter. The union part of the grafted seedlings was observed to determine the callus formation or development by counting the days from grafting. Measurement of the callus diameter was done using a vernier caliper. 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. The emergence of shoots from grafting was counted. Leaf count per seedling was done at 30, 60, and 90 DAG.

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

Statistical analysis

The grafted mango seedlings were arranged in a 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. Treatment with 75 ppm NAA application was the earliest to form callus in grafted mango, whereas lower than this concentration resulted in the later formation of callus

(Table 1). The other factors affecting the callus were also 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, enough to form a callus, and auxin is efficient callus inducer (Ikeuchi et al., 2013). 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 no application (Libunao et al., 2013). Nevertheless, a lower level of NAA is recommended for future investigation of 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 the 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 the 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

As a source of auxin, the varying concentrations of NAA 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. For example, a 25 ppm NAA produced a higher diameter, but untreated plants had the highest at 90 DAG.

Days to the 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 25 ppm, 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 **as compared with the 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 **at 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 early emergence and higher plant survival. However, days to development were significantly affected by the NAA application. Results showed that 75 ppm had the earliest callus development. Higher than this concentration of NAA may need a future investigation. In terms of days to emergence of shoots, numerically, 75 ppm had the earliest, and it is recommended to have a further study of NAA higher than the mentioned concentration.

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