

Effects of Different Concentrations of IBA and NAA on Rooting of Pomegranate (*Punica granatum* L.) Cuttings.

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ABSTRACT:- This investigation was conducted to evaluate the effects of a range of indole-3-butyric acid (IBA) and naphthalene acetic acid (NAA) concentrations on rooting of pomegranate cuttings. Concentrations were, IBA (200,400,600) and (2000,4000,6000), NAA (200,400,600) and (2000,4000,6000). Two methods of dipping, quick dip and slow dip. Hard wood cuttings were used. The best result of length of roots was with 4000ppm IBA and 2000ppm IBA. IBA4000ppm gave the best result on number of cuttings. The larger number of leaves was obtained by the control and 200ppm IBA. Length of roots with 4000ppm IBA. The better result in number of roots was obtained by the concentration 6000ppm IBA.

Key Words: IBA, NAA, Wood Cutting. Pomegranate, Punice.

I. INTRODUCTION:

Pomegranate (*Punica granatum*. L.) :

Pomegranate is a dicotyledonous plant which belongs to the family Punicaceae. It is a Native to Iran and Himalayas of northern India (Ibrar ,2012).

Pomegranate is one of the deciduous plants. The tree tolerates cold when dormant, and it is resistant to temperatures less than 10⁰F(-22⁰), but sensitive to frost before complete dormancy. Good fruit quality of pomegranate are produced in areas with cool winter and hot, dry summers(Sharma,etal.2009). It is grown in well-drained soil. In addition, it is tolerant to drought, salt, iron chlorosis and limestone(Ibrar,2012).

Karimi (2011) reported that pomegranate gardens are found in arid and semi-arid areas with low quality soil and water. Rootstock is tolerant to soil borne diseases and withstand chlorosis induced by calcareous soils (Karimi,2011).

Pomegranate can be propagated by cuttings, commonly taken from suckers at the base of the stem(Ibrar ,2012).

Vegetative propagation is done to achieve the best desirable fruit qualities and true to type plants,(Sharma *et al*,2009).

The pomegranate plant is a shrub or small tree, about 8 meters high, most of them are small, deciduous trees with oblong leaves and short stems. The trunk is covered by a red brown bark which becomes gray by time.

While pomegranate trees may live up to 200 years, some pomegranate trees starts fruiting in the second year but normal production begins from 3 to 5 years(Glozer and Ferguson, 2011).

The formation of adventitious roots is genetic controlled and regulated by two factors, exogenous and endogenous (Druge and kadner,2008, Pop et al,2011).

The cutting forms new adventitious root system (Hartman *et al*, 2011). Ciampi and Gellini(1958) and Ciampi (1963) wrote about anatomy of rooting and relation between anatomical structure of stem and rooting ability in a wide range of plant species.Plant cutting removed from the stock plant normally passes through various anatomical changes accompanied by modification in metabolic activity and gene expression.

The cutting is exposed to physiological stress after severance from the mother plant. The cutting obtains little amount of water and insufficient nutrient absorption until root development is achieved(Druge and kadner, 2008, Pop et al 2011).

Okoro and Grace(1978) reported that cuttings of varieties with high native cytokinin levels have been more difficult to root than those with low cytokinin. Cytokinin and gibberellins play a role in inhibiting adventitious root formation. Hartman and Kester (1983) noted that high concentrations of gibberellins prevent adventitious root formation in cuttings.

Callus is a patchy mass of parenchyma cells in different phases of lignification that mostly grow at the basal end of cuttings placed under environmental conditions suitable for rooting (Hartman *et al*, 2011). Callus develops from cells at the base of the cutting, primarily from the vascular cambium, however cells of cortex and pith may also contribute to its formation (Hartman *et al*, 2011).

In many types, rooting success has been related to endogenous auxin concentrations. Generally, auxin doses were greater in peach trees with a pillar (upright branches) than a standard (spreading branches) growth (Workoski and Takeda, 2007).

Hard wood, (sometimes called dormant or leafless), stem cuttings are taken from the previous season's growth. Leaves can be removed, without peeling the bark from the cutting. Cuttings are prepared in late fall, winter, or early spring. The cuttings must be healthy and free from disease or injury with at least two nodes when collected (Pijut, *et al*. 2011).

The rooting medium has the function of holding the cutting in place during the growth and development, permit exchange of air at the base of the cutting and create dark environment by reducing light penetration to cutting base (Hartman *et al*, 2011).

II. MATERIALS AND METHODS:

Site of the Experiment:

This experiment was conducted at a nursery belonging to the Department of Horticulture, College of Agricultural Studies, Sudan University of Science Technology at Shambat (latitude 15-39N longitude 32-39E). The experiment was conducted to study the effect of indole 3- butyric acid (IBA) and naphthalene acetic acid (NAA) at different concentrations, two methods of soaking and one type of stem cuttings (hard wood cutting).

3.3 Experimental Materials:

The cuttings were taken from Shambat Research Station Field and the Nursery of the College. Branches growing vigorously, medium to large in size, 8-years old trees and with spreading branches were used in the experiment.

Preparation of Cuttings:

Pomegranate stem cuttings were taken from mature wood of the previous season's growth and one type of stem cuttings was used. At least five nodes were included in each cutting, length of cuttings, 20cm. Thickness of cuttings range from thick, 1.5cm, medium, 0.7mm and thin, 0.3mm.

Soaking Methods:

Slow -dip method (lower concentrations):

The basal part (2.5cm) of the cutting was soaked in dilute solution of IBA or NAA for 24 hours just before the cuttings were inserted into the rooting medium. Concentrations used for soaking the cuttings were: IBA, 200PPM, 400PPM, 600PPM and NNA: 200PPM, 400PPM and 600PPM).

Cuttings were placed under shade in the nursery.

This slow method is a cumbersome technique that is not commercially popular. Equipments are needed for soaking cuttings and long time, 24 and 48 hours is required.

Quick-dip method (concentrated solution):

In the quick-dip method, concentrations of solution were as follows: IBA: 2000PPM, 4000PPM, 6000PPM) and NNA: 2000PPM, 4000PPM, 6000PPM. Duration time of soaking or dipping was ten seconds.

The basal portion of the cutting, 0.5-1cm, was dipped in the solution for short time, 10 seconds.

Preparation of IBA and NAA:

The concentrations were prepared as follows:

1MG/L ----- 1ppm

200ppm = 0.2GM/L.

2000ppm ----- 2000MG/L ===== 2GM/L.

4000ppm ----- 4000MG/L ----- 4GM/L.

These solutions were prepared at the tissue culture laboratory, Sudan University of Science and Technology, Shambat.

The material was dissolved in sodium hydroxide (NaOH) and made to volume (1000ml) with potable water. 2mg IBA was weighed using precision balance and this furnished 200ppm solution.

In the second method the same steps for the preparation of concentrations of indol-3-butyric acid (IBA) and naphthalene acetic acid (NAA) were followed.

Table 1: Type of hormones, concentrations, Time of dip and type of cuttings.

| Concentration ppm | type of growth hormones | Type of cuttings | Dip period hours |
|-------------------|-------------------------|------------------|------------------|
| 200PPM | IBA | hard woodcutting | 24 |
| 400PPM | IBA | hard woodcutting | 24 |
| 600PPM | IBA | hardwood cutting | 24 |
| 200PPM | NAA | hardwood cutting | 24 |
| 400PPM | NAA | hardwood cutting | 24 |
| 600PPM | NAA | hard woodcutting | 24 |

Table (2): Quick Soaking:

| Concentration ppm | Type of growth hormone | Type of cutting | Dip period second |
|-------------------|------------------------|-------------------|-------------------|
| 2000 | IBA | hard woodcutting | 10 |
| 4000 | IBA | hard wood cutting | 10 |
| 6000 | IBA | hard wood cutting | 10 |
| 2000 | NAA | hardwood cutting | 10 |
| 4000 | NAA | hard wood cutting | 10 |
| 6000 | NAA | hard wood cutting | 10 |

Duration of Experiments:

The duration of the experiments was 12weeks. The cuttings were placed under a lath house with 50% shade.

Plastic Bags:

Plastic bags with dimension of about 50-40-30cm with a number of drainage holes at the bottom were used. They were filled with a soil mixture of sand and silt(1:1).Each plastic bag contained 15 cuttings kept under the lath house under a covered frame in the nursery.

Irrigation:

Water was applied by putting a hose under the bags, the cutting absorb water through the holes at the bottom of the bag, The water was applied every other day.The pesticide(Apron star) was added with irrigation water to reduce any infection by pathogens.

Weeding:

Shallow weeding was done when required by hand.

Parameters:

Experiment parameters measured included the number of leaves, number of branches, the number of roots and root length, after three months.

Experimental design and statistical analysis:

The experimental units were in complete randomized block design. The data were analyzed using GenStat(computer program) versions 4 and the means were separated using Duncan^s multiple Range Test(DMRT) at p<0.05(Gomez and Gomez 1984).

Results and discussion:

Table 1: Effects of low concentrations and slow soaking of IBA and NAA on number of branches;-

depicted in table 1 best result for was obtained by followed by the 400ppm, then Comparison NAA, in low slow soaking, has negative

| Concentrations | length of branches: | Time of soaking |
|----------------|---------------------|-----------------|
| 1-IBA200ppm | 48.77abc | 24 hour |
| 2-IBA400ppm | 66.17ab | 24 hour |
| 3-IBA600ppm | 63.00ab | 24hour |
| 4-NAA200ppm | 2.00c | 24 hour |
| 5-NAA400ppm | 9.50c | 24 hour |
| 6-NAA600ppm | 18.00bc | 24 hour |
| 7-CONTROL | 77.50a | |
| CV% | 62.9 | |

The results revealed that the number of leaves the control low concentration 600ppm IBA. between IBA and concentrations and showed that NAA

effects on number of leaves This maybe due to an inhibitive effect of this growth regulator on pomegranate branching. IBA has neither promoting nor inhibitive role.

Table (2) Effect of high concentrations of IBA and NAA, quick dipping on length of branches:

| Concentrations | length of branches: | Time of dipping |
|----------------|---------------------|-----------------|
| 4-IBA2000ppm | 73.00a | 10sec |
| 5-IBA4000ppm | 73.07a | 10sec |
| 6-IBA6000ppm | 64.60ab | 10sec |
| 10-NAA2000ppm | 28.50abc | 10 sec |
| 11-NAA4000ppm | 35.33abc | 10sec |
| 12-NAA6000ppm | 5.00c | 10sec |
| 13-CONTROL | 77.50a | |
| CV% | 62.9 | |

AS shown in table 2, the results reflect that the concentrations of IBA,2000 ppm and 4000ppm gave the best result in length of branches compared with NAA, while the control was superior to all of them. This result showed an inhibitive effect of NAA on length of branches while IBA had no effect. According to Donald(1976) and Hartman(2011), high concentrations of IBA (2500 and 6000) with quick soaking of apple cuttings is preferred. They also found that NAA had an inhibitive effect. These results are in conformity with our findings.

Table 3:Effects of low concentrations and slow dipping on number of rooted cuttings:

| Low concretions | Number of rooted cuttings | Time of soaking |
|-----------------|---------------------------|-----------------|
| 1-IBA200ppm | 1.66bcd | 24 hour |
| 2-IBA400ppm | 2.33abcd | 24 hour |
| 3-IBA600ppm | 4.00ab | 24hour |
| 4-NAA200ppm | 0.77d | 24 hour |
| 5-NAA400ppm | 0.33d | 24 hour |
| 6-NAA600ppm | 1.66bcd | 24 hour |
| 7-CONTROL | 2.66abcd | |
| CV% | 53.9 | |

The results presented in table 3 show that the concentration 600ppm of IBA gave the highest number of rooted cuttings compared with other concentrations. NAA also had an inhibitive effect on number of rooted cuttings. So IBA is preferred than NAA for rooting of pomegranate cuttings.

Table 4: Effects of high hormone concentrations and quick dipping on number of rooted cuttings.

| Concentrations | Number of rooted cuttings | Time of soaking |
|----------------|---------------------------|-----------------|
| 4-IBA2000ppm | 2.66abcd | 10sec |
| 5-IBA4000ppm | 4.33a | 10sec |
| 6-IBA6000ppm | 3.66ab | 10sec |
| 10-NAA2000ppm | 1.66bcd | 10 sec |
| 11-NAA4000ppm | 3.33abc | 10sec |
| 12-NAA6000ppm | 1.00cd | |
| 13-CONTROL | 2.66abcd | |
| CV% | 53.9 | |

As depicted in Table 4, the number of rooted cuttings was higher due to the treatment with 4000ppm IBA. This finding is in conformity with Goshetal(1988) who reported that good rooting in both semi hard wood cuttings and hard wood cuttings of pomegranate was due to treatment with IBA, 5000 ppm. It is also evident from the results that IBA is much more effective to stimulate rooting in the two groups of cuttings of pomegranate as compared to NAA. Purohit and Shekharappa(1985) reported that treatments with high concentrations and quick dipping on hardwood cuttings of pomegranate were more effective in inducing rooting. This is in agreement

with our results. It is also in conformity with Hartman(2011) who said that IBA is the best auxin for general use because it is non-toxic to plants over a wide range of concentrations and it is effective in promoting rooting of a large number of plant species.

Table 5: Effects of low concentrations and slow dipping on number of branches:

| Concentrations | Number of branches | Time of soaking |
|----------------|--------------------|-----------------|
| 1-IBA200ppm | 4.00cde | 24 hour |
| 2-IBA400ppm | 4.00cde | 24 hour |
| 3-IBA600ppm | 7.00bc | 24hour |
| 4-NAA200ppm | 0.33e | 24 hour |
| 5-NAA400ppm | 0.66e | 24 hour |
| 6-NAA600ppm | 3.33cde | 24 hour |
| 13-CONTROL | 6.33bcd | |
| CV% | 48.5 | |

As shown in table 5, there were highly significant differences between low concentration of IBA, slow dipping. and NAA, same concentration and dipping. The highest number of branches was obtained by 600ppm IBA followed by both 400ppm and 200ppm IBA. The lowest number of branches was obtained by 200ppm NAA followed by 400ppm NAA. These results indicated that 600ppm IBA had promotive effect on number of branches of pomegranate cuttings while lower concentrations of IBA and all concentrations of NAA had inhibitive effect compared to the control.

Auxin is required for initiation of adventitious roots on stems but divisions of the first root initial cells are dependent upon either applied or endogenous auxin(Hartmanetal,2011). IBA is superior than NAA and the high concentrations are best when compared to low concentrations

Table 6: Effectsof high concentrations and quick dipping on number of branches:

| Concentrations | Number of branches | Tim of soaking |
|----------------------|--------------------|----------------|
| 4-IBA2000ppm | 7.33bc | 10sec |
| 5-IBA4000ppm | 12.00a | 10sec |
| 6-IBA6000ppm | 10.33ab | 10sec |
| 10-NAA2000ppm | 2.33de | 10 sec |
| 11-NAA4000ppm | 4.33cde | 10sec |
| 12-NAA6000ppm | 0.66e | 10sec |
| 13-CONTROL | 6.33bcd | |
| CV% | 48.5 | |

As depicted in Table 6, IBA 4000ppm recorded the highest number of bbranches then 6000ppm and 2000ppm, all higher than the control and than all concentrations of NAA which recorded the lowest numbers than the control. This indicates the inhibitive effect of NAA on number of branches of pomegranate cuttings.

Table(7): Effect of low concentrations of hormones and slow dipping on number of leaves:

| Concentrations | Number of leaves | Time of soaking |
|----------------|------------------|-----------------|
| 1-IBA200ppm | 42.11 a | 24 hour |
| 2-IBA400ppm | 29.25abc | 24 hour |

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| | | |
|-------------|-----------|---------|
| 3-IBA600ppm | 25.07abcd | 24hour |
| 4-NAA200ppm | 3.00d | 24 hour |
| 5-NAA400ppm | 1.07d | 24 hour |
| C-NAA600ppm | 19.00abcd | 24 hour |
| 7-CONTROL | 42.77a | |
| CV% | 56.6 | |

The results depicted in Table 7 revealed highly significant differences between IBA and NAA treatments except 600ppm. The best result was due to treatment with 200 ppm IBA and the control.

Table 8: Effects of high concentrations of hormones on number of leaves:

| Concretions | Number of leaves | Time of soaking |
|--------------|------------------|-----------------|
| 1-IBA2000ppm | 27.94abc | 10sec |
| 2-IBA4000ppm | 36.60ab | 10sec |
| 3-IBA6000ppm | 35.77ab | 10sec |
| 6-NAA2000ppm | 13.17bcd | 10 sec |
| 5-NAA4000ppm | 13.15bcd | 10sec |
| 6-NAA6000ppm | 7.33cd | 10sec |
| 7-CONTROL | 42.77a | |
| CV% | 56.6 | |

The control recorded the best result followed by IBA4000ppm and 6000ppm, without significant difference between them (table 8). This revealed that IBA had no augmenting effect on number of leaves, in contrast to Singh and Singh (2002) findings who reported that application of IBA played some role in augmenting the number of leaves per cutting. All IBA concentrations and the control were significantly different from NAA treatments.

Table 9: Effects of low hormone concentrations and slow dipping on length of roots;

| Concretion | Length of root | Time of soaking |
|-------------|----------------|-----------------|
| 1-IBA200ppm | 8.74ab | 24 hour |
| 2-IBA400ppm | 5.92b | 24 hour |
| 3-IBA600ppm | 10.51ab | 24hour |
| 4-NAA200ppm | 0.13b | 24 hour |
| 5-NAA400ppm | 0.66b | 24 hour |
| 6-NAA600ppm | 1.33b | 24 hour |
| 13-CONTROL | 9.83ab | |
| CV% | 91.6 | |

As depicted in table 9 the best result was obtained by the control followed by treatment with IBA600ppm. This indicates that IBA had no significant difference from the control while NAA had a significant inhibitive effect on length of roots in pomegranate cuttings.

Table 10: Effects of high concentrations of hormones and quick dipping on length of roots:

| Concentrations | Length of root | Time of soaking quick |
|----------------|----------------|-----------------------|
| 1-IBA2000ppm | 7.62ab | 10sec |
| 2-IBA4000ppm | 19.20a | 10sec |
| 3-IBA6000ppm | 18.21a | 10sec |
| 4-NAA2000ppm | 1.52b | 10 sec |
| 5-NAA4000ppm | 4.42b | 10sec |
| 6-NAA6000ppm | 0.32b | 10sec |
| 7-CONTROL | 9.83ab | |
| CV% | 91.6 | |

The results shown in table 10 indicates that there were significant differences in root length due to the treatments 4000 ppm and 6000 ppm IBA which gave the highest root length, while the 4000 NAA and 200 NAA recorded the lowest root length. This might be explained by the analogy of Pearse(1948) who noted that the auxin IBA generally has distinct advantage over NAA as it is slowly destroyed by the auxin destroying enzyme linked system. Likewise, Atman *et al* (2022) found that the highest rooting percentage in hardwood cuttings was obtained by 3000⁰⁰mg IBA concentration. Conversely, NAA has an inhibitive effect.

Table 11: Effects of low concentrations of hormones and slow dipping on number of roots;

| Concentrations | Length of root | Time of soaking |
|----------------|----------------|-----------------|
| 1-IBA200ppm | 4.67cde | 24 hour |
| 2-IBA400ppm | 10.88cde | 24 hour |
| 3-IBA600ppm | 14.66bcd | 24hour |
| 4-NAA200ppm | 0.22e | 24 hour |
| 5-NAA400ppm | 3.11de | 24 hour |
| 6-NAA600ppm | 3.89de | 24 hour |
| 13-CONTROL | 14.11bcd | |
| CV% | 59.8 | |

The results shown in table 11 indicates that 600ppm IBA gave the highest length of roots followed by the control and 400ppm IBA. These results are significantly different from all concentrations of NAA which recorded the lowest length of roots.

Table (12): Effects of high concentrations of hormones and dipping on length of roots :

| Concentrations | Length of root | Time of soaking |
|----------------|----------------|-----------------|
| 1-IBA2000ppm | 15.88bc | 10sec |
| 2-IBA4000ppm | 23.99ab | 10sec |
| 3-IBA6000ppm | 31.11a | 10sec |
| 4-NAA2000ppm | 2.44e | 10 sec |
| 5-NAA4000ppm | 6.22cde | 10sec |
| 6-NAA6000ppm | 0.66e | 10sec |
| 7-CONTROL | 14.11bcd | |
| CV% | 59.8 | |

As presented in table12, there were highly significant differences among the treatments, 6000 IBA gave the highest number of roots. This result is in agreement with Kumar(1995) who reported that the highest rooting of lemon cuttings were by treatment with 2000ppm IBA in combination with 200ppm p-hydroxybenzioc acid. These results are also in agreement with Panda *et al*(1990) who found that highest ratio of rooting and length of

roots of pomegranate cuttings were obtained by treatments by high concentrations of IBA. This is also in agreement with the findings of Bharad and Bhogave(2015).

V. CONCLUSION:

Treatment of pomegranate cuttings with high concentrations of IBA promote formation of rooted cuttings, number and length of roots, number of branches and number of leaves. On the other hand all concentrations of NAA were inhibitive to all these parameters. So it is recommended to use the growth regulator IBA rather than NAA in pomegranate cuttings root formation.

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