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***Corresponding Author**

Muhammad Abuzar Jaffar

E-mail

abuzar_jaffar@yahoo.com

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Effect of Naphthalene Acetic Acid on Sprouting and Rooting of Stem Cuttings of Grapes

Safeer Ahmed¹, Muhammad Abuzar Jaffar^{2*}, Nadir Ali¹, Sher Ahemd³, Muhammad Ramzan², Qeasar Habib²

¹ Department of Horticulture, Agriculture College, Quetta, Pakistan

² Faculty of Agriculture, Lasbela University of Agriculture, Water and Marine Science, Uthal, Balochistan, Pakistan

³ Balochistan Agriculture Research and Development Center, Quetta, Pakistan

Abstract

A field trial was laid to investigate the effect of naphthalene acetic acid (NAA) on sprouting and rooting of stem cuttings of grapes during 2015 at the experimental area of the Horticulture Department, Sindh Agriculture University, Tando Jam, Pakistan. The field trial was laid out in a three replicated completely randomized design (CRD) factorial. The stem cuttings of two varieties of grapes (Thompson seedless and Crimson seedless) about 6 inches in length with 3-4 buds were obtained from three-year-old plants. The basal ends of the cuttings were dipped in NAA solutions viz. 1000, 2000 and 3000 mg L⁻¹ plus control treatment by the quick dip method for 10 seconds before planting them in the growth medium. The results revealed that all the traits related to sprouting and rooting potential of stem cuttings of grapes were significantly influenced by various NAA concentrations. However, the interaction between varieties and NAA concentrations was observed at par for all sprouting and rooting-related parameters. The maximum mean number of sprouts cutting⁻¹ (6.33), leaves cutting⁻¹ (20.50), roots cutting⁻¹ (95.83) and fresh weight of roots (13.43 g) was achieved in response to NAA₁ (1000 mg L⁻¹). However, there was no significant effect of NAA on sprouting and rooting percentages. In varietal comparison, Crimson seedless produced significantly higher number of leaves (18.91) and number of roots cutting⁻¹ (82.25).



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Introduction

The grapevine (*Vitis vinifera* L.) belongs to the *Vitaceae* family, which includes about 60 species of wild *Vitis* distributed in Asia, North America, and Europe under subtropical, Mediterranean and temperate continental climate [1, 2]. The *Vitis* species have acquired an important economic interest over time; some other *spp.* such as North American *V. rupestris*, *V. riparia* and *V. berlandieri* are also used as breeding rootstocks due to their resistance against pathogens of the vine, such as phylloxera, and oidium and mold [3, 4]. The grape is used as fresh and dry minor fruit in Pakistan and among fruits it is ranked 10th. In Pakistan, the total area under grape cultivation is 15,300 hectares with an annual production of 64,400 tons ha⁻¹ [5]. Bangladesh is the only main importer of grapes from Pakistan with almost 96% share of quantity and value [6]. In Pakistan, its cultivation is restricted to upland areas of Baluchistan and northern hilly tracts of Punjab and Khyber Pakhtunkhwa. The province of Baluchistan contributes 98% to the national grape production in Pakistan [7]. A number of grape varieties are grown in upland areas of Baluchistan province. The most famous varieties, Haita, Kishmishi, Shundokhani, Sahibi, and Shekhali are commonly grown in Quetta, Pishin, Killa Abdullah, Mastung, Kalat, Loralai and Zhob districts [8].

In 2011, Food and Agriculture Organization (FAO) [9] reported that 75.866 square km of the world is dedicated to grapes. Around 2% of the world grapes are used, as dried fruit, 27% as fresh fruit and 71% grape production is used for wine production. Spain is the largest producer of grapes for wine making with the planted area of 11750 km². There are no reliable statistics that break down the production of grape per variety. It is believed that the most widely planted variety is Sultana, also known as Thompson seedless, with at least (3.56123 hectares). Kale [10] reported that sixteen byproducts are made from grapes, like raisin, vinegar, wine, juices, pickles, chocolates, tartaric acid, squash, syrup, jam, jelly, livestock feed, tannin, etc. However, looking at the world scenario of different byproducts, it was necessary to consider the establishment of projects for the production of other value-added products

from grapes. Grapes comprise multipart compounds such as sugars in the form of glucose and fructose, acids like tartaric, malic, and amino acids, proteins, anthocyanin, and flavonol, but apart from these, grapes contain an abundant amount of water [11]. Grapes are rich in vitamin A, B6, and C, as well as minerals, such as, phosphorus, magnesium, potassium, calcium, iron, and selenium, which are essential for human health. Grapes contain flavonoids, which are powerful antioxidants that slow the aging process by reducing damage effects caused by free radicals [12, 13].

Auxin is one of the most important hormones that is used on the stem cuttings for accelerating the formation of adventitious roots [14]. Auxin has an effect on root formation and increases the percentage of rooting on cuttings. Plants produced natural auxin in shoots and young leaves, but synthetic auxin should be used for successful rooting to prevent the cuttings death [15]. Synthetic rooting hormones are usually applied in liquid form on the basal side of cuttings. The immersion method is often preferred by commercial propagators for the sake of easy application of auxin, time-saving, fast application and to obtain good results. Cutting of certain species, which are difficult for rooting are soaked for a longer time [16]. The naphthalene acetic acid (NAA) is a synthetic form of auxin. It can be used to facilitate rooting in vegetative cuttings, such as root cuttings, leaf cuttings, and stem cuttings [17]. NAA increases the formation of cellulose fibers in plants when applied with gibberellic acid. NAA is also used to prevent premature drop and thinning of fruits. It is useful after flowering. Higher quantity may cause inhibition of plant growth and development. NAA can be used on different plants including apples, olives, oranges, and various other hanging fruits. To get the best results of NAA, it should be applied in the concentrations ranging from 20 to 100 mg L⁻¹ [18]. Rosier et al. [19] in his study reported that the response of NAA in enhancing adventitious root formation is better than compared to Indole-3-butyric acid in Fraser Fir stem cuttings. In this study, we investigate the effect of NAA on the sprouting and rooting of stem cuttings of two varieties of grapes (Thompson and crimson seedless).

Materials and Methods

Grape stem cuttings

The hardwood cuttings of two different varieties of grapes (Thompson seedless and crimson seedless) were obtained from Agriculture Research Institute (ARI), Quetta, Baluchistan, Pakistan. The cuttings were taken from healthy plants which were same in age and size. The cuttings were about 6 inches long with 3-4 buds.

Experiment conditions

The study was conducted at Horticulture Garden, Sindh Agriculture University, Tandojam, Pakistan during March 2015. Twelve basal end cuttings up to 2.5 cm were dipped for 10 seconds in different concentrations of NAA, *i.e.*, 1000 (NAA₁), 2000 (NAA₂) and 3000 (NAA₃) mg L⁻¹ and cuttings which were dipped in distilled water without NAA were used as a control. NAA is insoluble in water, therefore, the solution was first dissolved in a desired quantity of ethyl alcohol and then diluted with distilled water up to 1000 ml. After that, cuttings were put in growth medium at field conditions for 75 days to collect data of different parameters. The growing medium sandy soil was filled in perforated polyethylene bags of size 9 × 4 inches. The experiment was laid out according to complete randomized design (CRD) factorial. Every treatment was comprised of three replicates in this way each variety was comprised of 48 cuttings.

Growth parameters

The following different growth parameters were studied during this experiment:

Number of sprouted cuttings and sprouting percentage

The number of sprouted cuttings was counted in each treatment after 15 days of planting date up to termination of the experiment and an average sprout number was calculated. The sprouting percentage was calculated by counting the number of sprouts cuttings remained alive after 75 days.

Number of leaves cutting⁻¹

Number of leaves cutting⁻¹ were counted in each treatment at 15 days interval from the planting date

up to termination of the experiment and average leaf number was then calculated.

Mortality of cuttings (%)

A cutting was considered as dead, which was totally dry and had no sprouted buds and roots. Data regarding mortality (%) of cuttings were taken at the termination of an experiment right before pulling out of the cuttings from the soil.

Number of roots cutting⁻¹ and rooting percentage

The number of roots was counted after pulling out the cuttings from the soil and then the average was calculated. The cuttings were pulled out from the soil, then rooting percentage was calculated by counting the number of rooted cuttings remained alive after 75 days.

Fresh weight of roots (g)

Fresh weight of clean roots was weighed by physical balance after pulling out the cutting from the soil. The roots were washed with tap water and blot dried before weighing.

Statistical analysis

The obtained data were statistically analyzed by using two-way ANOVA and means were further compared through LSD using SPSS ver. 19.0 statistical software (SPSS, Chicago, IL).

Results

Number of sprouts cutting⁻¹

Data regarding number of sprouts cutting⁻¹ (Table 1) was significantly ($p < 0.05$) influenced by applying NAA at various levels. However, there was no significant ($p < 0.05$) difference between both varieties. The result further revealed that maximum number of sprouts cutting⁻¹ (6.33) were recorded in NAA₁ (1000 mg L⁻¹) treatment and minimum number (3.83) was recorded in the control treatment. In varietal comparison, Crimson seedless produced more sprouts (4.91) as compared to Thompson seedless (4.83), but the difference was nonsignificant. The Thompson seedless produced maximum number of sprouts (6.67) in NAA₁ treatment, followed by at the same concentration in Crimson seedless (6.00) and minimum number of sprouts (3.66) were recorded by Crimson seedless in control treatment.

Table 1 Effect of different naphthalene acetic acid (NAA) levels on the number of sprouts cutting⁻¹ of stem cuttings of grapes.

NAA levels (mg L ⁻¹)	Varieties		Mean
	Thompson seedless	Crimson seedless	
Control	4.00	3.66	3.83 B
1000 (NAA ₁)	6.67	6.00	6.33 A
2000 (NAA ₂)	4.66	4.66	4.67 B
3000 (NAA ₃)	4.00	5.33	4.67 B
Mean	4.83	4.91	

Value with different letters are significantly different from one another at $p = 0.05$.

Table 2 Effect of different naphthalene acetic acid (NAA) levels on the sprouting percentage of stem cuttings of grapes.

NAA levels (mg L ⁻¹)	Varieties		Mean
	Thompson seedless	Crimson seedless	
Control	83.33	91.67	87.50
1000 (NAA ₁)	91.67	100.00	95.83
2000 (NAA ₂)	91.67	75.00	83.33
3000 (NAA ₃)	66.67	91.67	79.16
Mean	83.33	89.58	

Table 3 Effect of different naphthalene acetic acid (NAA) levels on the number of leaf cuttings⁻¹ of stem cuttings of grapes.

NAA levels (mg L ⁻¹)	Varieties		Mean
	Thompson seedless	Crimson seedless	
Control	10.33	13.00	11.67 B
1000 (NAA ₁)	18.00	23.00	20.50 A
2000 (NAA ₂)	13.33	20.33	16.83 A
3000 (NAA ₃)	12.66	19.33	16.00 AB
Mean	13.58 B	18.92 A	

Value with different letters are significantly different from one another at $p = 0.05$.

Sprouting (%)

The sprouting percentage showed non-significant ($p < 0.05$) difference in various levels of NAA (Table 2). On the basis of overall mean, there was also no significant ($p < 0.05$) difference among the varieties.

Number of leaves cutting⁻¹

The data regarding number of leaves showed significant ($p < 0.05$) differences on the basis of NAA levels. The result also showed a significant difference between both varieties ($p < 0.05$) as shown in Table 3. Comparing different NAA levels, the maximum number of leaves (20.50) were recorded in NAA₁ (1000 mg L⁻¹) treatment and the minimum number of leaves (11.67) were observed in the control treatment. Among the varieties, Crimson seedless produced a significantly higher number of leaves (18.92) compared to Thompson seedless (13.58). Crimson seedless produced maximum number of leaves (23.00) in NAA₁ (1000 mg L⁻¹) treatment, followed by Crimson seedless at NAA₂ (2000 mg L⁻¹). Minimum number of leaves were produced by Thompson seedless variety in the control treatment

(10.33). The maximum number of leaves by Thompson seedless variety were found at NAA₁ (1000 mg L⁻¹) treatment. In general, the response of Crimson seedless was significantly better than Thompson seedless.

Mortality of cuttings (%)

The results of mortality (%) of cuttings are shown in Table 4. There was no significant ($p < 0.05$) difference found among various levels of NAA compared to control and also between both varieties.

Number of roots cutting⁻¹

The statistical analysis of the data showed significant ($p < 0.05$) differences of number of roots cutting⁻¹ among various concentrations of NAA and between both varieties (Table 5). The maximum number of roots cuttings⁻¹ (95.83) were recorded in NAA₁ (1000 mg L⁻¹) treatment, and minimum roots cuttings⁻¹ (43.67) were observed in control. For varieties, Crimson seedless showed the significantly higher number of roots cuttings⁻¹ (82.25) compared to Thompson seedless (51.25). Overall, maximum

Table 4 Effect of different naphthalene acetic acid (NAA) levels on mortality percentage of stem cuttings of grapes.

NAA levels (mg L ⁻¹)	Varieties		Mean
	Thompson seedless	Crimson seedless	
Control	16.67	8.33	12.50
1000 (NAA ₁)	8.33	0.00	4.17
2000 (NAA ₂)	8.33	8.33	8.33
3000 (NAA ₃)	16.67	8.33	12.50
Mean	12.50	6.25	

Value with different letters are significantly different from one another at $p = 0.05$.

Table 5 Effect of different naphthalene acetic acid (NAA) levels on the number of roots cutting⁻¹ of stem cuttings of grapes.

NAA levels (mg L ⁻¹)	Varieties		Mean
	Thompson seedless	Crimson seedless	
Control	34.00	53.33	43.67 B
1000 (NAA ₁)	77.00	114.67	95.83 A
2000 (NAA ₂)	51.00	84.67	67.83 AB
3000 (NAA ₃)	43.00	76.33	59.67 B
Mean	51.25 B	82.25 A	

Value with different letters are significantly different from one another at $p = 0.05$.

number of roots cuttings⁻¹ were noted in Crimson seedless variety (114.7) in NAA₁ (1000 mg L⁻¹) treatment followed by Thompson seedless variety (77.0) at the same concentration of NAA, while minimum number of number of roots cuttings⁻¹ (34.0) were noted in Crimson seedless. Crimson seedless showed better performance regarding roots production compared to Thompson seedless.

Rooting (%)

The results of rooting (%) of cuttings are shown in Table 6. There was no significant ($p < 0.05$) difference was found among various levels of NAA compared to control and also between both varieties.

Fresh weight of roots (g)

The results showed that the fresh weight of roots (g), presented in Table 7, was significantly ($p < 0.05$) influenced by the application of NAA at various levels. However, there was no significant ($p < 0.05$) difference between two varieties. The maximum fresh weight of roots (13.43) was obtained in NAA₁ (1000 mg L⁻¹) treatment, which was statistically similar to the fresh weight of roots (10.90) in NAA₂ (2000 mg L⁻¹) treatment. Minimum fresh weight (6.02) was observed in the control treatment. Comparison between both varieties of grapes showed that the maximum fresh weight of roots (14.53) was achieved by Crimson seedless in NAA₁ (1000 mg L⁻¹) treatment followed by Thompson seedless at the same concentration of NAA, and minimum fresh

weight of roots (5.10) was observed by Thompson seedless in the control treatment. In general, the Crimson seedless showed better performance and produced more fresh weight of roots as compared to Thompson seedless.

Discussion

Vegetative propagation is widely used in horticulture and forestry for the purpose to multiply the number of plants from healthy plants of natural populations [20]. Adventitious root formation is an important step in asexual propagation, so plant growth regulators are used for root initiation from stem cuttings, mostly auxin is used for root initiation which plays important role in the differentiation phase [21]. Commercially, NAA is used for root formation in cuttings, which is a synthetic form of auxin [17]. In this study, the vegetative growth was affected by applying various levels of NAA like data regarding number of sprouts cutting⁻¹ was notably affected by using different levels of NAA. The results of different treatments depicted significant dominance of NAA₁ (1000 mg L⁻¹) over the rest of the treatments and control was observed at the lowest. The high doses of NAA (NAA₂ 2000 mg L⁻¹, NAA₃ 3000 mg L⁻¹) were achieved least numbers as compared to NAA₁ (1000 mg L⁻¹). It was noticed that lower doses gave the better number of sprouts in grapes than higher doses of NAA. Tiwari and Das [22] also observed that the reduction in the applied concentration of NAA (1000

Table 6 Effect of different naphthalene acetic acid (NAA) levels on the rooting percentage of stem cuttings of grapes.

NAA levels (mg L ⁻¹)	Varieties		Mean
	Thompson seedless	Crimson seedless	
Control	83.33	83.33	83.33
1000 (NAA ₁)	91.67	100.00	95.83
2000 (NAA ₂)	91.67	91.67	91.61
3000 (NAA ₃)	83.33	83.33	83.33
Mean	87.50	89.58	

Value with different letters are significantly different from one another at $p = 0.05$.

Table 7 Effect of different naphthalene acetic acid (NAA) levels on the fresh weight of roots (g) of stem cuttings of grapes.

NAA levels (mg L ⁻¹)	Varieties		Mean
	Thompson seedless	Crimson seedless	
Control	5.10	6.93	6.02 C
1000 (NAA ₁)	12.23	14.53	13.43 A
2000 (NAA ₂)	10.27	11.63	10.90 AB
3000 (NAA ₃)	9.03	9.20	9.17 BC
Mean	9.16	10.62	

Value with different letters are significantly different from one another at $p = 0.05$.

mg L⁻¹) gave better results for sprouting. Similarly, Wahab [23] observed better sprouting in guava cuttings when treated with NAA (2000 ppm). Also, Imran [24] observed a maximum number of sprouts in rose cuttings at 500 mg L⁻¹ of NAA, followed by at 1000 mg L⁻¹, and concluded that indole butyric acid (IBA) affects the number of sprouts buds along with planting time. These findings were in accordance with the present study.

Results regarding sprouting percentage were non-significant. The maximum mean value of this factor was observed in NAA₁ 1000 mg L⁻¹ followed by control. Higher levels of NAA, 2000 mg L⁻¹ and 3000 mg L⁻¹ produced minimum sprouting percentage. Similar results were found in different studies, like Agile et al. [25] found differences in between NAA and coconut water treatments, the emergence of shoots from NAA and coconut water was 100% in Guava compared to IBA and IAA, which gave 50% and 75% emergence of shoots, respectively. However, auxins being root promoting hormone had no direct impact on sprouting percentage of buds [26]. Sprouting is mainly attributed to the stored carbohydrate in the cuttings [27]. In fact, sprouting depends on physical status and food reserves of cuttings as the cuttings utilize the reserved food when the temperature rises and start sprouting earlier than callus formation [28]. Tehseen et al. [29] reported that paclobutrazol (1000 ppm) was considered effective as a chemical to give maximum sprouting

percentage. Taleb et al. [30] noticed cuttings treated with 1000 ppm of NAA gave the best results but do not differ significantly from high concentrations of NAA, it is, therefore, preferable to use the minimum concentration of the auxin to get good results.

The mean value for the number of leaves cutting⁻¹ depicted significant superiority of NAA₁ 1000 mg L⁻¹. The other treatment expressed frequent overlapping so that the superiority of one treatment over other could hardly be established. Imran [24] observed that IBA treatments gave the maximum number of leaves in rose cuttings at 1000 mg L⁻¹, followed by at 500 mg L⁻¹. Similarly, Khan et al. [31] reported that the maximum number of leaves was achieved per cutting by using minimum concentration of hormone. Also, Shahba and Alshmmmary [32] observed maximum number of leaves per cutting soaked for 12 and 24 hours with IBA at 500 ppm. Singh et al. [33] observed a maximum number of leaves on new growth from 50 cm long cuttings of bougainvillea in response to the treatment where IBA was used at 5000 ppm. NAA₁ 1000 mg L⁻¹ significantly affected the number of roots cutting⁻¹, followed by NAA₁ 2000 mg L⁻¹. Paul and Aditi [34] proved that the dose of NAA (1000 ppm) and IBA (1000 ppm) are statistically more important among all other concentrations of IBA and NAA. Auxins such as NAA or IBA at a concentration of 1000 ppm are more efficient and improve the roots. On the other hand, Alizadeh and Grigorian [35] indicated that the

increase in the concentration of the hormone NAA 1000-2000 mg L⁻¹ increased the number of roots in hardwood cuttings of almond, peach hybrid, then less rooting was observed at concentrations of 3000 mg L⁻¹. Imran [24] observed a maximum number of roots in rose cuttings at 1000 mg L⁻¹ of IBA. Ali and Muhammad [36] reported that IBA at 1000 ppm achieved the highest number of roots in rose cuttings. Rehman et al. [37] found a maximum number of roots in softwood cutting treated with IBA at 1000 ppm. These reported results were in accordance with the findings of this study.

Sulaiman et al. [38] indicated that the medial cutting parts with 500 and 1000 ppm of IBA performed better in terms of root percentage (100%) in *Citrus medica*. L. In the present study, nonsignificant effect on rooting was observed at all levels of NAA levels. Gupta et al. [39] examined those bougainvillea cuttings with 1000 ppm IBA gave highest rooting percentage with more roots in the dipping method. Paul and Aditi [34] found the highest percentage of rooting air layering when treated with 1000 ppm NAA, followed by layers treated with IBA 1000 ppm. Munir et al. [40] recorded the highest rooting percentage of explants of grapes in MS medium supplemented with 1 mg L⁻¹ NAA. Shahba and Alshmary [32] also observed a better response to the fresh weight of root cuttings soaked for 12 hours and 24 hours with 500 ppm IBA. In the present study, the response of 1000 mg L⁻¹ NAA was maximum compared to control and other NAA treatments. Paul and Aditi [34] found the highest fresh weight of roots in water apple per layer while applying 1000 ppm NAA. Similarly, Galavi et al. [41] found that maximum fresh weight was obtained in hardwood stem cuttings of grapes while using 4000 mg L⁻¹ of IBA in sand planting bed. These previous results are in accordance with the findings of the present study.

Conclusions

The sprouting and rooting of stem cuttings of grapes showed a better response at different NAA levels compared to untreated cuttings. The most appropriate level of NAA on the basis of results of sprouting and rooting was noted at NAA₁ (1000 mg L⁻¹). On the

basis of varietal comparison, Crimson seedless showed better performance and gave good results compared to Thompson seedless. These results showed that the use of NAA at the concentration of 1000 mg L⁻¹ is highly effective in the propagation of sprouting and rooting of stem cuttings of grapes.

Conflict of interest

The authors declare that they have no conflict of interest.

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