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Indirect shoot regeneration from callus of the shoot tip explants of *Gerbera jamesonii* Bolus cv. "Festival"

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Abstract

The study was conducted in the Tissue Culture Laboratory, Fadak Agricultural Company, Basrah, Iraq. Mother plants of the "Festival" gerbera cultivar were used for micropropagation by taking the shoot tip explants. The concentration of 2 mgL^{-1} benzyl adenine (BA) resulted in recording the highest percentage of response to callus induction, reaching 90%, which was significantly superior to the other two treatments, 1 and 1.5mgL^{-1} BA. The 1 mgL^{-1} BA treatment was significantly superior in response to indirect shoot generation when compared with the 1.5 and 2 mgL^{-1} BA treatments. The 1 mgL^{-1} BA treatment recorded the highest percentage of response to shoot growth induced by callus, which reached 100%. The treatment of callus grown in MS medium supplied with BA concentration of 1 mgL^{-1} recorded the highest average number of shoots, which reached 10 shoots per 50 mg of callus. The MS medium treatment supplied with a concentration of 0.5 mgL^{-1} of naphthalene acetic acid (NAA) was significantly superior to the other two treatments of 1 and 1.5 mgL^{-1} NAA in the percentage of shoot response to root formation. The concentration of 0.5mgL^{-1} NAA recorded the highest response to root formation, which reached 100%. The treatment of the MS medium supplied with a concentration of 0.5 mgL^{-1} NAA was significantly superior to the treatments of 1 and 1.5 mgL^{-1} NAA in the average number of main roots formed on the shoots. This treatment recorded the highest average of main roots, amounting to 3.2 main roots $shoot^{-1}$.

Keywords: *Callus, cultivar, indirect shoot, micropropagation, proliferation, MS salts*.

I. INTRODUCTION

Gerbera is one of the perennial herbaceous plants that belong to the *Gerbera* genus and the Asteraceae family. The common name for this plant in English is African daisy or Barbarian Daisy (Singh et al., 2014; Singh et al., 2017). The original homeland of this plant is South Africa. The flowers of this plant have special specifications for cut flowers, including the length and diameter of the floral stem. The flower petals of this plant have attractive colors, including yellow, pink, orange, red, etc. (Parthasarathy and Nagaraju, 1999). The gerbera plant is a herbaceous plant with beautiful flowers that is suitable for garden landscaping and engineering (Kanwar and Kumar, 2008). The flowers of this plant are also considered among the beautiful cut flowers, which rank fourth after the flowers of the rose, the daisy, and the tulip, and they have a high economic return due to the high prices of their bouquets (Teeri et al., 2006). Their flowers are distinguished by their high quality, bright colors, the long period of their survival after picking, and their durability (Chung et al., 2016). The common method of plant propagation is the vegetative method to obtain plants similar and identical to the mother plants. The method of propagation by suckers and division is used as a common vegetative propagation method for this plant, but it is not preferred by farmers due to the small number of suckers obtained from the mother plants. The other method that is used in propagating gerberas is the sexual method through seeds, which is an easy method, but it is not good because of obtaining plants that are not heterozygous as a result of cross-pollination, which results in variation in genetic characteristics, especially in the case of the desire to propagate gerbera cultivars of good quality and high commercially desirable

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(Shalahi et al., 2013). All the previous obstacles that affected the propagation of the gerbera plant made many scientists and producers resort to the tissue culture technique as one of the advanced and modern methods of propagation to confront the problems and obstacles in the traditional propagation methods. The plant tissue culture technique is characterized by high specifications that producers and specialists in ornamental plant cultivation desire, including obtaining large numbers of plants identical to the parent plants, True to type plants, and commercial cultivars with good and desirable specifications that are to be propagated without being restricted by the growing season and the cultivated area. Also, plants propagated using the plant tissue culture technique are free of viral and fungal diseases, pathogens, and insect infestations because they are grown and propagated in a sterile agricultural environment free of pathogens and insect infestations. Gerbera plants have been propagated in vitro culture using multiple explants, including leaf petioles; shoot tips, flower buds, and leaves (Murashige et al., 1974; Jerzy and Lubomski, 1991; Orlikowska et al., 1999; Aswath et al., 2003; Son et al., 2011). The most important components of the nutrient medium in plant tissue culture are growth regulators, as auxins and gibberellins play a fundamental and important role in the propagation of plants in vitro culture in general and on the gerbera plant in particular, as they work to activate and stimulate cell division, cell expansion, and differentiation, and thus obtain new growth in large numbers that can They are multiplied and rooted to obtain true to type plants (Puglisi, 2002; Rout and Jain, 2004; Williams, 2011). Because the gerbera plant is a cut flower plant, it has good specifications and high economic returns on the one hand. To overcome the problems of propagating this plant through traditional propagation methods and to obtain plants in large numbers that are identical to the mother plant and free of pathogens, viruses, and insect infestations without being restricted by the growing season and the need for large agricultural areas, this study was conducted.

II. Materials and Methods

The study was conducted in the Plant Tissue Culture Laboratory, Fadak Private Agricultural Company, Basrah, Iraq. Gerbera seeds of the Festival cultivar were obtained from one of the Baghdad nurseries to use in tissue propagation by using shoot tips as explants.

Preparing the nutrient medium

The ready-made MS medium (Murashige and Skoog, 1962) is produced by Caisson Lab, an American Company, which is added in an amount of 4.43gL^{-1}. Then, organic components were added to the MS medium, consisting of 30 gL^{-1} of sucrose, the vitamins thymine, pyridoxine, glycine, and nicotine amide at a concentration of 1 mgL^{-1} each of them, myoinositol at 100 mgL^{-1}, adenine sulfate at a concentration of 80 mgL^{-1}, and acidic orthophosphate at a concentration of 200 mgL^{-1}, and polyvinylpyrrolodine at a concentration of 1 gL^{-1}. After that, growth regulators represented by naphthalene acetic acid at a concentration of 0.1 mgL^{-1}, gibberellin GA_3 at a concentration of 0.5 mgL^{-1}, and benzyl adenine at different concentrations (1, 1.5, and 2 mgL^{-1}) were added to the medium prepared for callus induction and indirect shoot proliferation. As for the nutrient medium, the same components of the MS medium were added to the rooting medium, except for growth regulators, which were added at a fixed concentration of benzyl adenine (0.1 mgL^{-1}) and different concentrations of naphthalene acetic acid (0.5, 1, and 1.5 mgL^{-1}). Then the pH of the nutrient medium was adjusted to 5.7. Then agar was added to the MS medium to solidify the nutrient medium. The prepared nutrient medium is placed in culture containers and then sterilized using an autoclave for twenty minutes at a pressure of one bar and a temperature of 121ºC. Then, the cultivation containers containing the sterilized MS medium are kept in the growth room until they are used in culturing.

Sterilization of explants

The shoot tips were removed from the mother plants and then washed with running water to get rid of the dust and dirt stuck on the surfaces of the explants. Then the explants were washed with water and liquid soap, then with distilled water several times. After that, the explants were placed with an antioxidant solution consisting of 100 mgL^{-1} of ascorbic acid and 150 mgL^{-1} of citric acid for an hour.

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Then the explants were transferred to sterile glass containers containing 70% ethyl alcohol to sterilize them for two minutes. Then they were washed with sterile distilled water three times. The shoot tips were then immersed in a solution of Elsa fungicide at a concentration of 500mgL^{-1} for a quarter of an hour with continuous stirring. Then it was washed with sterile distilled water. It was then kept in sterile glass containers until used for tissue culture, which should not be a long preservation period, i.e. not more than 1-2 hours.

The shoot tips were cultured in sterile MS media inside the laminar air-flow cabinet under sterile conditions. Then, after culturing, the containers were transferred to the growth room chamber at a temperature of 27°C±2, a lighting intensity of 1000 lux, and a lighting duration of 16 hours day^{-1}.

Studied indicators

1. Percentage callus response

2. Percentage response to the shoot proliferation from the induced callus

3. The number of shoots produced from indirect shoot proliferation

4. Percentage response to main root formation

5. Number of main roots

Experimental design and statistical analysis

The simple study experiments were designed according to a randomized complete design. Each treatment was repeated ten times. The data were analyzed statistically according to the analysis of variance using the ready-made statistical program SPSS, version 14. The averages of the treatments were compared using the least significant difference at the 1% probability level, based on Al-Rawi and Khalafallah (2000).

III. Results and Discussion

Data from Figure (1) indicate significant differences between the benzyl adenine treatments in the percentage response of shoot tips to callus induction of gerbera plants grown in vitro culture. The concentration of 2 mgL^{-1} benzyl adenine resulted in recording the highest response to callus induction, reaching 90%, which in turn was significantly superior to the other two treatments, 1 and 1.5 mgL^{-1}. The 1 mgL^{-1} treatment recorded a response rate to callus induction of 60%, which in turn was significantly superior to the 1.5 mgL^{-1} treatment. The lowest response rate of young leaves to callus induction was recorded by 1 mgL^{-1} benzyl adenine, which amounted to 40%. The induction of callus from plant tissues is affected by several factors, including the components of the nutrient medium, the type and age of the explant used for propagation, and plant growth regulators. Cytokinins, including benzyl adenine, activate cell division in plants (Wiesman, 1989).

The data from Figure (2) indicate significant differences between the benzyl adenine treatments in response to the regeneration of shoots from calluses induced from the tips of the shoots of the Gerbera plant via in vitro culture. The 1 mgL^{-1} benzyl adenine treatment was significantly superior in response to shoot regeneration when compared with the 1.5 and 2 mgL^{-1} benzyl adenine treatments. The treatment with 1 mgL^{-1} benzyl adenine recorded the highest percentage of callus-induced response to the regeneration of shoots, which reached 100%. The response rate of callus induced from the tips of the shoots to the regeneration of shoots decreased to 80% when treated with 1.5 mgL^{-1} benzyl adenine. As for the induced callus, which was grown on MS medium containing a concentration of 2 mgL^{-1} benzyl adenine, it recorded a 70% response to shoot regeneration (Figure 2). The response to shoot regeneration is related to growth promoters, most notably cytokinins, including benzyl adenine, which

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work to break the apical dominance and encourage the growth of lateral shoots and the formation and emergence of adventitious buds on the callus that differentiate into shoots (Tobert et al., 1998).

Figure (3) shows that there are highly significant differences between the two concentrations of 1 and 1.5 mgL^{-1} of benzyl adenine in the emergence of shoots from the induced callus. The treatment of callus grown in MS medium prepared with benzyl adenine at a concentration of 1 mgL^{-1} recorded the highest average number of shoots, which reached 10 shoots per 50 mg of callus. The induced callus that was grown on MS medium and prepared with benzyl adenine at a concentration of 1.5mgL^{-1} recorded the formation of 5 shoots for every 50 mg of callus. The 2 mgL^{-1} treatment recorded the lowest average number of shoots, reaching 3 shoots per 50 mg of callus.

It is clear from the data in Figure (4) that the concentration of naphthalene acetic acid significantly affected the response of shoots to root formation via in vitro culture. The MS medium treatment prepared with a concentration of 0.5 mgL^{-1} of naphthalene acetic acid was significantly superior to the other two treatments of 1 and 1.5 mgL^{-1} of naphthalene acetic acid in the percentage of response of shoots to root formation. The concentration of 0.5 mgL^{-1} naphthalene acetic acid recorded the highest response to root formation on shoots, which reached 100%. The treatment of the medium prepared with a concentration of 1.5 mgL^{-1} of naphthalene acetic acid recorded the lowest percentage of response to rooting, amounting to 40%.

The data from Figure (5) indicate that there are significant differences between the treatments in the number of main roots formed on the shoots resulting from the induced callus of the gerbera plant via in vitro culture. The treatment of the medium prepared with a concentration of 0.5 mgL^{-1} of naphthalene acetic acid was significantly superior to the treatments of 1 and 1.5 mgL^{-1} of naphthalene acetic acid in the average number of main roots formed on the shoots. This treatment recorded the highest average of main roots formed, amounting to 3.2 main roots shoot-1. The treatment of the MS medium prepared with naphthalene acetic acid at a concentration of 1.5 mg L^{-1} recorded the lowest average number of main roots, reaching 1.5 main roots $shoot^{-1}$.

Auxins, including naphthalene acetic acid, have an essential role in the formation and emergence of root principles by stimulating cell division and the formation of new cells on the vegetative shoots that differentiate into root cells. This depends on the type of auxin, its concentration, the type of plant part to be rooted, the components of the nutrient medium, and the conditions surrounding it (Ibrahim and Abdul-Hameed, 2001).

IV. Conclusion

The concentration of 2 mgL^{-1} benzyl adenine (BA) recorded the highest percentage of response to callus induction. The 1 mgL^{-1} BA treatment recorded the highest percentage of response to shoot induced by callus (100%), and the number of shoots. The MS medium treatment supplied with a concentration of 0.5 mgL^{-1} of naphthalene acetic acid (NAA) was recorded as the highest percentage of shoots and the number of main roots formed on the shoots.

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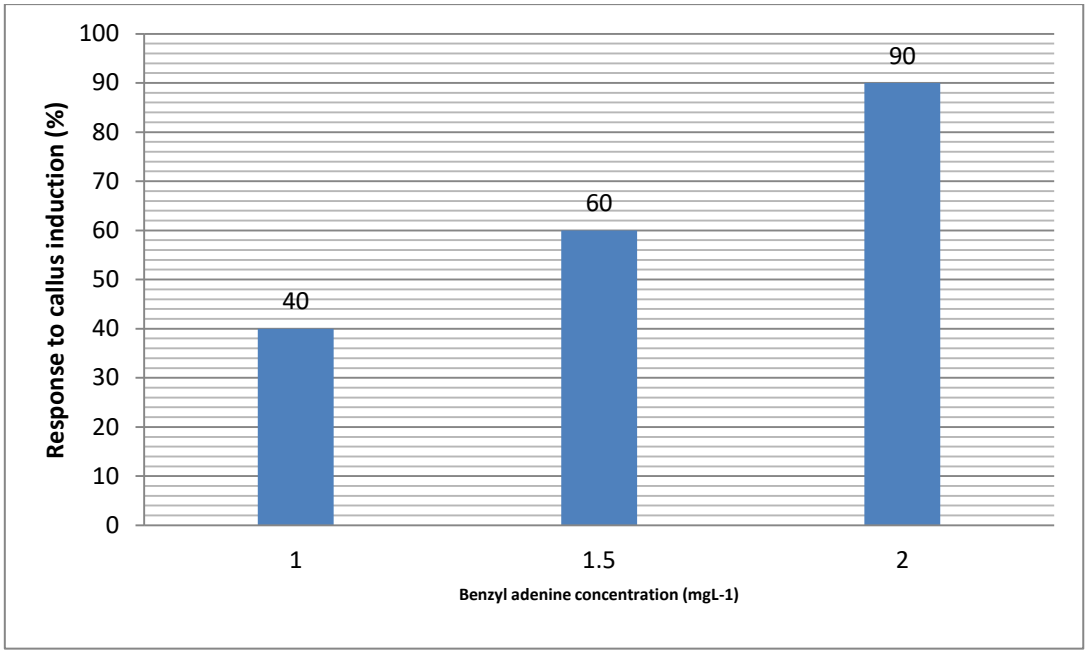

Figure 1: The effect of different concentrations of benzyl adenine on the response to callus induction from the shoot tips of the Gerbera plant, cultivar "Festival". (The least significant difference at the 1% probability level = 4.40).

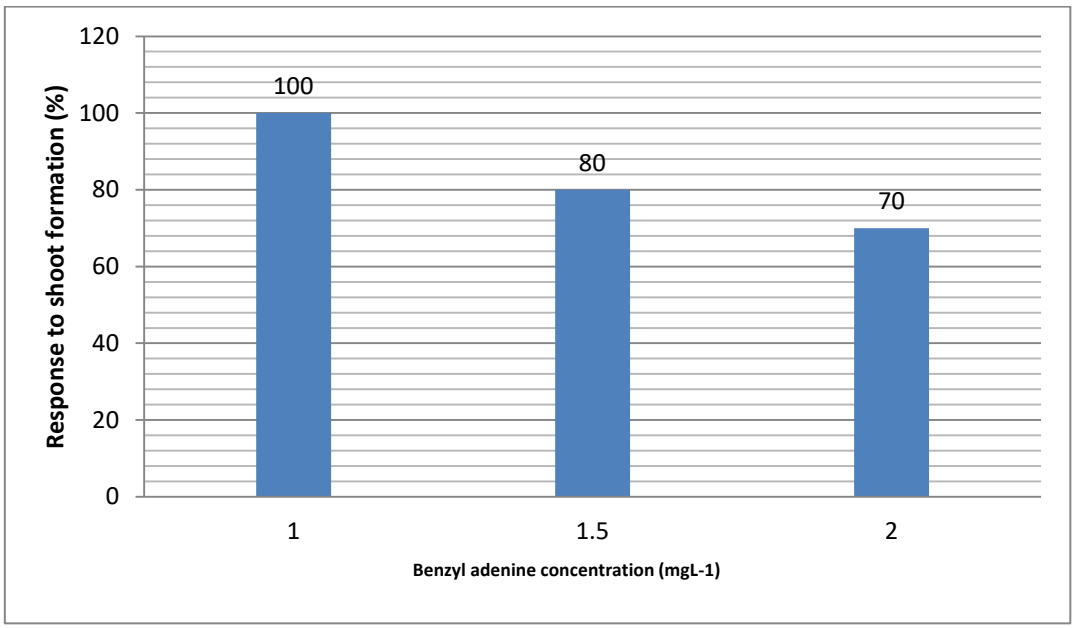

Figure 2: The effect of different concentrations of benzyl adenine on the response to the regeneration of shoots from the induced callus of the Gerbera

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plant, cultivar "Festival". (The least significant difference at the 1% probability level = 4.20).

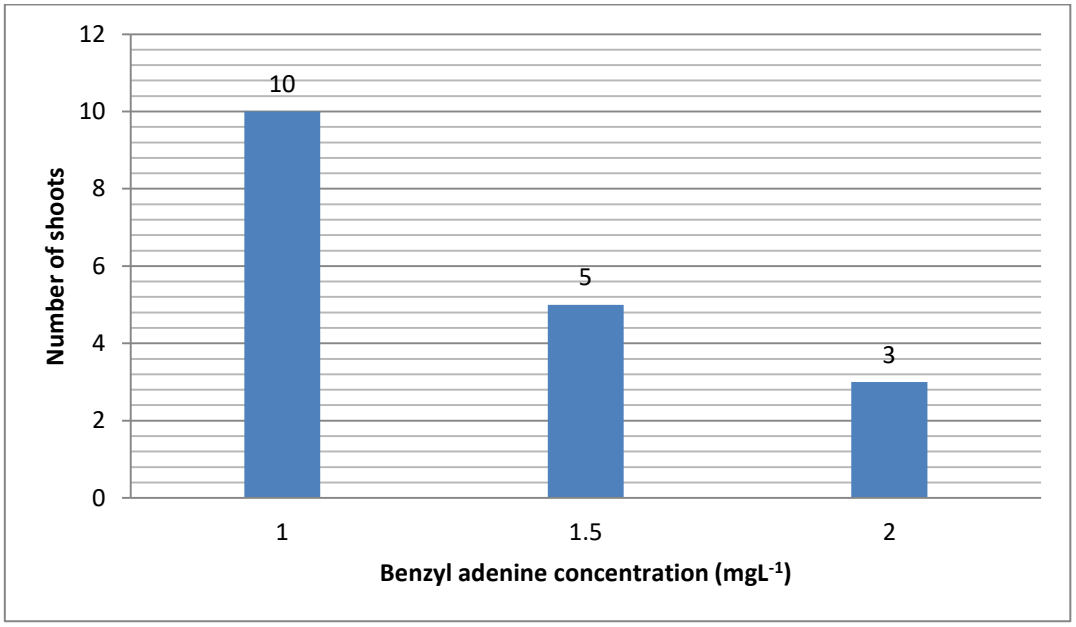

Figure 3: The effect of different concentrations of benzyl adenine on the number of shoots formed from induced calli of the Gerbera plant, cultivar "Festival". (The least significant difference at the 1% probability level = 2.10).

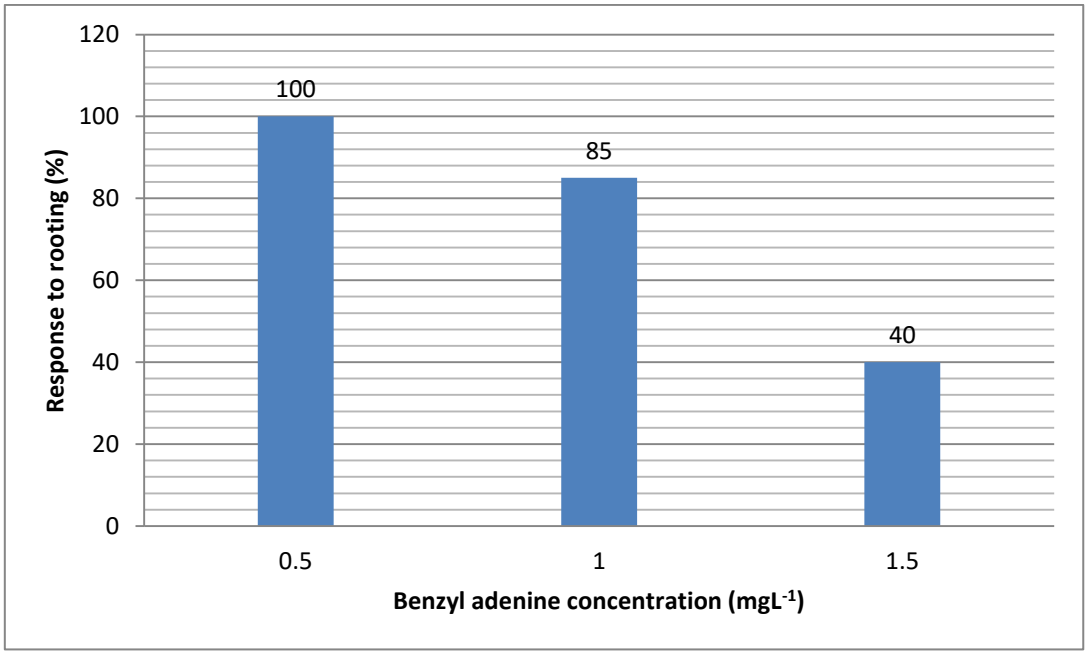

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Figure 4: The effect of different concentrations of naphthalene acetic acid on the response to rooting of shoots of the Gerbera plant, cultivar "Festival".
(The least significant difference at the 1% probability level = 6.20).

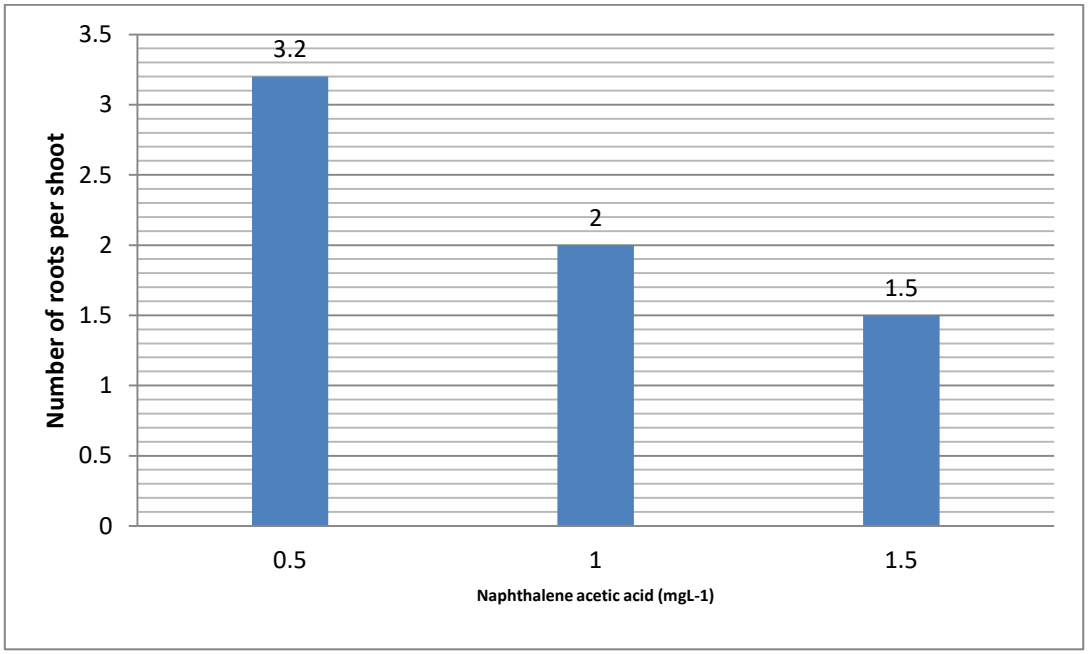

Figure 5: The effect of different concentrations of naphthalene acetic acid on the number of main roots formed on the shoots of the "Festival" gerbera plant. (The least significant difference is at the 1% probability level = 0.21).