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Effects of GA_3 Concentrations and Mechanical Scarification on Germination and Seedling Growth Performance of Sour Orange (*Citrus aurantium* L.) Rootstock

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ABSTRACT

The current experiment was conducted in Agriculture Faculty Lab (pro trays), at Nangarhar University, Afghanistan. As the germination of the sour orange seed is very poor and needs a long time for their competent germination, it was aimed to enhance germination and produce standard seedlings for proper growth and development. The experiment was laid out in a completely randomized design (CRD) having six treatments, T_1 (50 ppm + scarification), T_2 (100 ppm + scarification), T_3 (150 ppm + scarification), T_4 (200 ppm + scarification), T_5 (250 ppm + scarification) and T_6 (Control) with three replications. The scarification was done manually for seed coat removal after the extraction of seeds from fruits. Analysis of variance revealed to be significant for most of the characters under study. The study revealed that among all treatments, T_5 (250 ppm + scarification) was the best treatment for growth and root parameters, followed by T_4 (200 ppm + scarification) while the lowest of growth and root parameters were registered in T_6 (control). This investigation confirmed that high concentration of GA_3 significantly affected the seed germination of sour orange.

Keywords: Gibberellic Acid, Mechanical Scarification, Sour Orange

INTRODUCTION

Citrus is a genus of flowering trees having numerous species belonging to the family Rutaceae with chromosome number ($2n = 2x = 18$) experiencing more diversity in the species. Citrus is one of the most extensively cultivated fruit crops in the world and a substantial part of the human diet among fruit crops (Liu et al., 2012). Cultivated species of citrus fruit crops are the large group, containing approximately 16 to 156 species around the world, and have spread throughout the globe (Ollitrault & Navaro, 2012). In addition, citrus have relative complexity in their broad morphological characters, which caused difficulty in their identification. Sour orange (Citrus aurantium L.) is considered one of the most popular rootstocks for different citrus species especially for sweet oranges in Eastern Zone of Afghanistan. This rootstock can significantly influence scion growth and development, fruit size, fruit skin, fruit quality, fruit acidity, and resistance to biotic and abiotic stresses. Rootstock is one of the important and essential components of budded /grafted fruit plants, which determine the success, or failure of the established orchards, apart from the influence on the plant vigour, size, and longevity of the tree, it helps in the early production of quality fruits (Yadav et al., 2022).

To make the scion adapted to wide range of biotic and abiotic conditions; selection of the competent rootstock is the most crucial method to overcome the adverse effects of climate, soil, and resistance to certain diseases and pests. However, potent struggles have always been made to increase environmental adaptability, resistance to biotic stress (Gummosis / Phytophthora foot rot, quick decline or Citrus tristeza) and abiotic stress (Drought, Cold, Heat, Salinity and Light), enhanced nutrient uptake by using the appropriate scion and rootstock combinations in citrus fruit crops propagation (Sharaf *et al*., 2016).

The plants of this rootstock are medium in size, early maturing, and prolific with quality produce and make a good union with sweet oranges. It is widely used across the globe and show resistance to gummosis caused by *Phytophthora*, while susceptible against Citrus Tristeza Virus (CTV).

Citrus seeds of different rootstocks are observed to have poor germination, poor seedling uniformity, and uneven growth performance and take a long time for budding/grafting stages. Thus, it is highly required to enhance germination, uniformity, and rapid establishment for budding or grafting, by choosing suitable rootstock (Castle, 1982). GA3 is considered an important germination promoter, which increase, seed germination and proper root development (Zanotti & Barros, 2014). Gibberellin hormone is one of the most popular phytohormones that plays an important role in multifunction, initial plant development, and promote germination. In recent years, gibberellic acid has been used widely as a synthetic hormone or plant growth regulator to promote and stimulate seed germination and seedling stem elongation (Khatana, 2010; Stejskalová & Kupka, 2015). Gibberellin enhances seed germination by the mean of Alpha- amylase enzyme break down to the starch and glucose into smaller sugar contents to promote embryo growth until it becomes autotrophic (Vieira, 2002). Gibberellic acid encourages the growth of seeds that increases the growth rates in sweet orange (Hoda, 2010). This rootstock is commonly obtained through seed but seed germination and proper development is not up to the mark. Keeping these points in mind, an investigation has been conducted to study the effects of GA3 concentrations and mechanical scarification on germination and seedling growth performance of sour orange (Citrus aurantium L.) rootstock.

MATERIALS AND METHODS

This experiment was laid out in February 2022 in the Agriculture Faculty Lab (pro trays), Nangarhar University; Afghanistan. Geographically, the research farm is situated between 34^0 28ʹ 38.40ʺ North latitude and 70^0 22ʹ 0.1.41ʺ East longitude. It is situated at about 600 meters above the mean sea level. The full-ripened and colored fruits were brought from the sour orange orchards of the University campus, then disease-free, uniform-sized fruits were selected, and seeds were extracted from fruits and cleaned with the help of tap water. After extraction, seeds were spread out on cotton cloth to dry and remove excessive water and moisture. The study was conducted in a completely randomized design having six treatments T_1 (50 ppm + scarification), T_2 (100 ppm + scarification), T_3 (150 ppm + scarification), T_4 (200 ppm + scarification), T_5 (250 ppm + scarification) and T_6 (Control = distilled water + no scarification), with three replications. Gibberellic acid (Duchefa biochemical, assay > 90%) was weighed (50, 100,150,200, and 250) mg separately by helping electronic balance (jewelry scale, model 1020), then dissolved every required amount of GA_3 powder separately in 10 ml of 99% pure ethyl alcohol in different beakers and distilled water was added to make up the volume equal to (1000) ml or 1 liter to obtain desired concentrations of (50, 100, 150, 200 and 250 ppm GA_3 solution and then seeds were kept for 10 minutes (Quick Deeping method) in every solution.

All prepared seeds were subjected to mechanical scarification and 100 seeds were used for each treatment and then were kept in GA_3 solutions for 10 minutes. The final count of germination percentage was recorded on 20^{th} day of germination test. Five competitive plants were chosen randomly and tagged to keep a record of other observations. To determine the mean values of the parameters, the data collected for various plant properties were averaged out independently. Data related to different parameters e.i. initial emergences, germination (%), seedling height (cm), stem or diameter (mm), length of root (cm), and number of roots/plant, were recorded.

The data of the present experiment was subjected to statistical analysis in completely randomized Design (CRD). With one-way, ANOVA and then the treatment means were compared by using Critical Differences test at $P \leq 0.05$.

RESULTS

Germination percentage and Initial emergence

The attentive study of the results depicted in (Table 1) divulged that the combined effect of GA_3 and mechanical scarification enhanced days taken for initial germination, and germination percentage. The highest germination percentage (96.67 %) was recorded in T_5 followed by T_4 at the tune of (86.67 %) which was statistically at par with T_3 having germination percentage (83.33 %) while the lowest germination percentage (63.33 %) was recorded in control. Maximum number of days for initial emergence (33.33) was observed in T_6 (control), followed by T_1 with (29.37) days to initial emergence while minimum number of days to initial emergence (26.86) were recorded in T_5.

Wingspan

The result in (Table 1) clearly shown that the concentration of GA_3 had significant effect on wingspan of new seedlings of sour orange. The wingspan (4.90 cm) was recorded in T_5 which was significantly higher than rest of the treatments, followed by T_4 (4.33 cm) which was statistically at par with rest of treatments rather than control and the minimum wingspan (3.43 cm) was recorded in control.

Stem diameter

The data in (Table 1) revealed that the different concentration of GA_3 had significant effect on the stem diameter of sour orange. The stem diameter (1.77 mm) was obtained in T_5 which was significantly superior than the rest of the treatments, followed by T_4 with stem diameter (1.53 mm) which was statistically similar with T_3 having diameter (1.51 mm). While the lowest stem diameter (1.32 mm) was obtained from untreated seed (control) which was statistically on par with the T_1 and T_2 (1.33 mm,1.45 mm) respectively.

Table 1. Impact of different GA_3 concentrations and scarification on germination percentage, days taken for initial germination, wingspan, and diameter on sour orange rootstock

Treatments	Germination (%)	Initial germination	Wingspan (cm)	Diameter (mm)
T_1	73.33	29.37	4.00	1.33
T_2	76.67	29.03	4.28	1.45
T_3	83.33	28.35	4.27	1.51
T_4	86.67	28.08	4.33	1.53
T_5	96.67	26.86	4.90	1.77
T_6	63.33	33.33	3.43	1.32
SEm±	**1.80**	**0.12**	**0.08**	**0.03**
C.D. at 5%	**9.61**	**0.64**	**0.44**	**0.14**

Seedling Height

The data presented in (Table 2) revealed that, the application of GA_3 increased seedling height significantly. The results showed that the tallest plant height (4.99 cm) was observed in T_5, which was statistically at par with T_3 and T_4 (4.9 cm, 4.57 cm) respectively. The shortest seedling height (3.88 cm) was found in T_6 (control) which was statistically the same as the remaining treatments.

Root Number

Data recorded in (Table 2) indicated that different concentrations of GA_3 have influenced root numbers. The maximum root number (7.07) was observed in T_5 which was statistically at par with T_4 (6.23), followed by T_3, T_2 and T_1 (5.97, 5.83 and 5.67) respectively. While the lowest root number (4.23) was obtained in untreated seed (control).

Root Length

The results related to root length were shown in (Table 2) indicating that A_3 concentrations highly promoted root length. The highest root length (8.00 cm) was observed in T_5, followed by T_4 with root length (6.20 cm) which was statistically at par with T_3, $T_{2,}$ and T_1 (6.13 cm, 5.73 cm, and 5.50 cm) respectively. While the lowest root length (4.67 cm) was recorded in control, which was statistically on par with T_1.

Table 2. Impact of different GA_3 concentrations and scarification on seedling growth, root number, and root length of sour orange rootstock

Treatments	Seedling height(cm)	Root number	Root length(cm)
T_1	4.31	5.67	5.50
T_2	4.23	5.83	5.73
T_3	4.90	5.97	6.13
T_4	4.57	6.23	6.20
T_5	4.99	7.07	8.00
T_6	3.88	4.23	4.67
SEm±	**0.09**	**0.18**	**0.18**
C.D. at 5%	**0.49**	**0.95**	**0.98**

DISCUSSIONS

The attentive study of data from the (Table 1) displayed that different GA_3 concentrations and scarification significantly improved germination (%), initial germination emergence, wingspan (cm) and diameter (mm) in T_5 over the control. Gibberellic acid is one of the eco-friendly plant growth regulator on the embryo, leading to synthesis of hydrolytic enzymes e.i. amylase that hydrolyze food for embryo growth and enhance their germination (Leite *et al*., 2003). The results are in agreement with the previous findings of (Kalalbandi *et al*., 2003; Pawar *et al*., 2010) The accelerated and enhanced germination in sour orange under different GA_3 concentrations and scarification may be due to increase in assimilation of water and increase in enzymes activity. (Meena *et al*., 2003) also reported similar results and found that treated seed with the GA_3 @ 100 ppm encouraged early and highest germination. Another study reported by (Çalişkan *et al*., 2012) and (Patel *et al*., 2016) stated that the seed were subjected to 500 and 1000 ppm concentration of GA_3 achieved the maximum (100%) germination percentage and significantly decreased the time for initial emergence. Oure results are in close conformity with the earlier researchers who reported that the application of GA_3 increased the seed germination and stimulated the seedling growth (Mtambalika *et al*., 2014). Our findings are similar with the earlier study conducted by (Kumari *et al*., 2007) who reported that the maximum germination percentage of aonla seeds (75.50 %) was recorded when seeds were treated with GA_3 500 ppm over other treatments under Haryana conditions.

Our findings are also similar with reported literature of the fruit crops like Rangpur lime GA_3 application promoted and enhanced the morphological parameters including leaves number and stem diameter (Dala *et al*., 2002). As well as (Qadri *et al*., 2021) also reported that application of GA_3 @ 400 ppm increased growth parameters in rough lemon.

It is evident from the (Table 1) that GA_3 concentrations have significantly affected the seedling height, which may be due to the gibberellins that stimulate the plant's physiological activities and stimulate the rapid formation of new cells that are required for the better growth of seedlings (Sharma, 2012 & Sharma, 2016) therefore increase in plant height of seedling might be due to the increased in meristems tissues by the GA_3 applications. This investigation is confirmed by other researchers who stated that the shoot length, root length, fresh and dry weights of guava CV. SR-4 seedlings were highest when treated with 250 ppm GA_3 solution (Jholgiker *et al*., 2017). Our results are similar to the findings of (Qadri *et al*., 2021), who observed the maximum stem diameter (4.6 mm) with the application of GA_3 @ 400 ppm and matching findings reported by another researcher who stated that the application of GA_3 promoted vegetative growth (Hoda *et al*, 2010).

The result reported in (Table. 2) indicated that maximum root number and root length were observed in T_5, which was superior to other treatments, and the minimum was observed in control. It might be due the application of GA_3, which caused vigorous root growth, hence, resulted in more roots per seedling. The GA_3 also accelerates the assimilation and translocation of auxins, which imparts better root growth, and vegetative characteristics of the plant as reported by (Pandey *et al*., 2011). These results are in close agreement with (Patel *et al*., 2017) in mango and also these results closely match with (Dilip *et al*., 2017) in Rangpur lime; (Jain *et al*., 2017) in Custard apple; (Parab *et al*., 2017) in Papaya; Anjanawe *et al*., 2013) in Papaya.

The increase in the length of roots may be due to the early formation of roots by GA_3. Our results are in harmony with other researchers who stated that the maximum root length (8.82 cm) was obtained in T_3 @ 400 ppm GA_3 concentration. Similar results are also reported by (Dilip *et al*., 2017) in Rangpur lime; (Singh *et al*., 2017) in Kagzi lime; (Patil *et al*., 2012) in Rangpur lime and they also reported regarding the influence of GA_3, (Brijwal *et al*., 2013; Shah *et al*., 2013; Jholgiker *et al*., 2017).

CONCLUSION

It is known from the results of the present investigation that application of 250 ppm GA_3 with the mechanical scarification of sour orange seeds observed to be the best treatment for enhancing the growth and root parameters as well as rapid seedling establishment for early budding or grafting. However, using the intact seed should be avoided, because it reduces water absorption for proper imbibition and release some acids that inhibit germination. Availability of new technologies for plant improvement will make many plant hormone options available in the future. It is expected that growers will produce more seedling in short duration with access of plant hormone for citrus seeds. Highest benefit cost ratio was also observed in (1:6.124). Future scope the seed treated with GA_3 250ppm identified in this investigation can be recommended commercially for overall growth of seedlings and Cost benefit ratio for sour orange rootstock.

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