Impact of different growing media and Gibberellic acid (GA_3) concentrations on rough lemon (Citrus jambhiri) seed germination and its growth attributes

Rashad Qadri[1*], Shahzad Hussain[1], Muhammad Tahir Akram[2], Muhammad Azam Khan[2], Muhammad Mumtaz Khan[3], Khalid Hussain[1], Muhammad Ahsan Khatana[1], Shahid Nadeem[4], Umais Asghar Khan[1]

[1]*Institute of Horticultural Sciences, University of Agriculture Faisalabad, Pakistan.*
[2]*Department of Horticulture, PMAS-Arid Agriculture University, Rawalpindi, Pakistan.*
[3]*Department of Plant Sciences, College of Agricultural and Marine Sciences, Sultan Qaboos University, P.O. Box 34, Al-Khod 123, Muscat, Sultanate of Oman.*
[4]*Horticultural Research Institute, National Agricultural Research Centre, Islamabad.*

**Corresponding author's e-mail: waseemrana_83@yahoo.com; tahiruaf786@gmail.com*

Abstract

Citrus is among one of the most important and widely grown fruit crops that is cultivated worldwide. The rootstocks of citrus have a significant impact on the yield and quality of fruits. Therefore, before selecting a proper rootstock, a number of its attributes and characteristics are evaluated such as, vigor, yield, rooting ability. Gibberellic acid (GA_3) is an environment-friendly growth regulator that is being used in multiple fruits crops to enhance phenotypic traits. In this study, an experiment was conducted to explore the influence of various GA_3 concentrations and different growth media to check their influence on germination percentage and growth attributes of rough lemon. The experiment was laid as a completely randomized design (CRD) under two factorial arrangements. The results revealed that seeds treated with GA_3 @ 400 ppm concentration and grown in S5 media (sand, silt and leaf manure) enhanced maximum seed germination (%), plant height (cm), stem diameter (mm), number of leaves, number of roots, root length (cm) and seedling fresh weight (g). Over all among GA_3 concentrations, GA_3 @ 400 ppm concentration was most predominated followed by GA_3 @ 200 ppm, Similarly among growing media, (S5) the combination of sand, silt and leaf manure improved seed germination and its growth parameters.

Keywords: Citrus, Gibberellic acid (GA_3), Rootstock, Substrate

Introduction

Citrus belongs to the family "Rutaceae" are one of the most commonly consumed fruits in the world[1] [2]. It includes oranges, tangerines, limes, lemons, grapefruit and citron [3] [4]. These fruits are enriched with various minerals, vitamins, fibers, and phytochemicals that have a significant effect on human health and pharmacological properties[5]. The rootstock has a pronounced impact on scion growth and development, and it also influences nutrition, yield and quality of the fruit [6] and tolerance to stress either environmental or caused by living organisms [7]. Therefore, the selection of rootstock plays a vital role in the establishment of citrus orchards [8].

In Pakistan, normally propagation of citrus is mostly conducted on the rough lemon seeds, however, its propagation through seeds is slow and irregular that might be due to the existence of growth inhibitors and seed coat resistance to radical protrusion [9]. The growth of citrus seedlings is affected by several factors such as cultivation measures, different rooting medium growth regulators, and their combinations [10]. Therefore, the rooting medium should be in such a composition that meets the chemical and physical needs of crops to maximize their growth and development [11][12], as the substrates with proper aeration, moisture and nutrients help to form excessive roots that are essential for the production of quality plants [13] [14] [15]. Besides, a good substrate must be easy to supply, process and inexpensive. It can also significantly play a vital role in canopy development and other processes such as photosynthesis [16].

Gibberellin is a multifunctional plant hormone that plays a key role in initial plant development. In recent years, gibberellic acid has been used extensively to promote seed germination and seedling elongation [17] [18]. Seed germination is supported by gibberellins that through alpha-amylase breaks down starch and sugars to promote embryo growth until it becomes autotrophic [19]. It encourages the growth of numerous seeds in different ways and increases the growth rates in sweet orange [20]. Keeping these points in view, an investigation was conducted to evaluate the effects of different media and gibberellin concentrations on the germination of rough lemon seeds.

Materials and Methods

This experiment was carried out in a screen house located at Institute of Horticultural Sciences, University Of Agriculture, Faisalabad. Seeds of fresh citrus fruits species, rough lemon (*Citrus jambhiri*) were obtained from the experimental fruit garden (Square # 9), University of Agriculture Faisalabad, Pakistan. The seed surface was disinfected with 10% sodium hypochlorite solution for 10 minutes and washed thoroughly with distilled water. Before use, the seeds were held in sterile distilled water with Thiram (Agrichem Ltd.). The Citrus seeds were treated with different concentrations (0, 100, 200, and 400 ppm) of Gibberellic acid (GA_3) and are planted in seven substrates as mentioned S1 (baggasse and sand), S2 (baggase and silt), S3 (Sand and Silt), S4 (Sand and button mushroom compost), S5 (sand, silt and leaf manure), S6 (peat moss and baggase) and S7 (silt and button mushroom compost) were filled in plastic bags of size 7x10" for seeding having equal ratio. The depth of the seed was twice the width of the seed and regularly moistened with deionized water.

Data collection and statistical analysis

Data regarding different parameters like days of the first emergence, mortality (%), seedling height (cm), stem diameter (mm), no. of leaves/plant, root length (cm), no. of roots/plants, root weight (g), seedling vigor, media pH, moisture (%) and electrical conductivity analysis of media for nitrogen, phosphorus, potassium, organic matter (%), saturation (%) was recorded. This experiment has 28 treatments, each repeated 3 times and three bags in each replication Completely randomized design (CRD) with the factorial arrangement was used in this research trial and analysis of variance (ANOVA) was used for data analysis while LSD test was used for comparison of differences between treatment means.

Results

Days to the first emergence

The combinations of growing media and growth regulators enhanced the germination rate. The results depicted that different concentrations of GA_3 and growing media have a significant effect on the days of the first emergence (Table 1). The results revealed that the untreated seeds (control) took the maximum number of days to first emergence (3.8), whereas, the minimum number of days for first leaf emergence (2.6) was @ 400 concentration of GA_3.

Mortality (%)

The results in table 2 showed that comparison of 7 growing media and GA_3 concentrations had a significant effect on mortality (%). High mortality percentage (35.6%) was recorded in the control treatment while among different substrates, maximum mortality (36.9%) was observed in S1 (bagasse and sand). Likewise, the interaction between growing media and GA_3 concentrations was highly significant and maximum mortality (49.3%) was observed in S1 (bagasse and sand) media at 0 ppm GA_3 concentrations. While the minimum mortality (19.8 %) was observed @ 400 ppm of GA_3, while in rooting substrates lowest mortality (16.7) was in S5 (Sand, Silt and Leaf Manure).

Seedling height (cm)

Table 3 results revealed that seedling height (cm) was significantly affected in response to the application of different media and GA_3 concentrations. GA_3 @ 400 ppm gave a higher seedling height (34.1 cm) as compared to other concentrations. Among growing media maximum seedling height (36.0 cm) was observed in S5 (sand, silt and leaf manure). Likewise, seeds treated with a similar concentration of GA_3 and grown in S5 (sand, silt and leaf manure) produced the highest seedling height (43.3 cm).

Stem diameter (mm)

The impact of different substrates and GA_3 concentrations on the stem diameter of rough lemon is shown in table 4. The results indicated that GA_3 has a significant impact on stem diameter growth and GA_3 application @ 400 ppm gave maximum stem diameter (4.6 mm) while in comparison to growing media maximum stem diameter (5.7 mm) was recorded in S5 (sand, silt and leaf manure). The interaction among growing media and GA_3 concentrations depicted that maximum stem diameter (6.8cm) was obtained in S5 (sand, silt and leaf Manure) media with 400 ppm GA_3 concentrations.

Number of leaves per plant

The results showed that GA_3 concentrations had a significant effect on the number of leaves as well (Table 5). The highest number of leaves (6.2) was recorded @ 400 ppm, while in growing substrates the highest number of leaves (7.9) were observed in S5 media (sand, silt and leaf manure) at par with S7 (Sand, Silt and Button Mushroom Compost) media (7.5). The interaction of different media and GA_3 concentrations was also significant and the highest number of leaves (8.6) were observed GA_3 in S5 media (sand, silt and leaf manure).

Root length (cm)

The results regarding root length were shown in Table 6. The results regarding root length showed that GA_3 was highly effective in promoting root length and maximum root length (8.9 cm) was observed @ 400 ppm concentration followed by 200 ppm (6.2 cm). While among growing media, the highest roots (9.6) were developed in S5 (sand, silt and leaf manure) at par with S_7 (Sand, Silt and Button Mushroom Compost). The interaction of different media and GA_3 concentrations was also significant and maximum root length (14 cm) @ 400 ppm in S5 (sand, silt and leaf manure) media.

Number of Roots/plants

The use of gibberellins is considered to be the most important growth regulator affecting the development of root traits. The GA_3 concentrations and growing substrates showed a significant impact on the number of roots (Table 7). The results depicted that GA_3 @ 400 ppm was more influential as compared to other concentrations and gave the maximum number of roots (4.6). While in growing media the highest number of roots (5.7) was recorded in S5 (sand, silt and leaf manure).

Root weight (g)

The application of GA_3 in the combination of different growing media had a significant effect on the root weight (Table 8). The results revealed that maximum results root weight (25.8 g) was observed in GA_3 @ 400 ppm concentration. While in growing media the maximum root weight (30.4 g) was observed in S5 (sand, silt and leaf manure) media. The interaction between different growing media and GA_3 concentrations was also significant. In interaction, the maximum root weight (34g) was observed in S5 media (sand, silt and leaf manure) @ 400 ppm GA_3 concentration.

Discussion

The use of growing media and gibberellin treatments in raising rough lemon seeds has shown a significant effect on seed growth and germination. The early germination in the rough lemon seed may be due to the beneficial effects of the mixed media as they have better water retention capacity, porosity, soil aeration and availability of nutrients, especially nitrogen and trace elements due to the presence of leaf manure. Gibberellic acid as a plant growth regulator acts on the embryo, leading to the synthesis of hydrolytic enzymes like amylase and protease that hydrolyzed food to use it for embryo growth, and enhanced its germination [21]. Meena et al. (2003) also reported similar results and found that pre-sowing treatment of seeds with GA_3 @ 100 ppm promoted early and highest germination that might be due to the influence of GA_3 that plays a key role in the germination [22, 23]. The combined application of GA_3 with rooting substrates may promote seed germination as it has sufficient nutrients for enhancing plant growth [24].

The effect of growth regulators on seed germination might be due to the increase of transcription and translation during protein synthesis. Protein and lipid storage are mobilized by specific enzymes that hydrolyzed storage molecules to provide the necessary structural components for the growth and development of the embryo. Our findings are in harmony with other researchers who stated that the application of organic media increases seeds germination success and enhances seedling growth earlier [25] [26].

In our findings, the seedling height, stem diameter and the number of leaves in rough lemon seedlings were increased significantly in GA_3 with the combination of sand, silt and leaf manure media that might be due to gibberellins that activates the plant physiological processes and promotes the rapid formation of new cells that are required for the better growth and seedling survival [27]. These results are consistent with the findings of reported literature that among fruit crops such as mango, and rangpur lime, GA_3 can provide the highest germination rate and increase seedling morphological parameters including plant height and the number of leaves [28].

Therefore, the increase in plant height was due to the use of gibberellin application that increases the size of the meristem and significantly stems diameter and the number of leaves. Similar results were reported by other researchers who stated that the use of gibberellin promoted vegetative growth and total leaf carbohydrates as both are positively correlated [20]. The translocation of sucrose promotes seedling growth and growth rate due to the accumulation of soluble sugars, starch as well as cell wall polysaccharides as GA_3 accelerates the synthesis and metabolism of sugar and increases the carbon supply in the buds [29]. In addition, gibberellin transfers the assimilates to the stem, which may promote the growth of the shoot. Moreover, when gibberellin was applied, the soluble sugars in the stems and roots and starch content become higher and the excellent seedling vigor may be due to growth media that maintains sufficient aeration and moisture content for better growth of seedlings, especially for root anchorage and development [26].

Conclusion

The results concluded that $GA_3$and growing substrates have a significant impact on rough lemon seed germination and on its growth parametrs. The combined application of growing media (sand, silt and leaf manure) along with GA_3 @ 400 ppm has the highest capability to promote seed germination and plant morphological attributes. Among GA_3 concentrations, 400 ppm was an optimum dose that may enhance seed growth and development and can be used to promote seed growth.

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Table 1. Impact of different substrates and GA_3 concentrations on day of first emergence of rough lemon (*Citrus jambhiri*) seed

	Gibberellic acid levels (ppm)

Sowing Media	0	100	200	400	Mean
S_1 = Baggasse and Sand	3.3	5.0	4.3	3.3	4.5 **A**
S_2 = Baggase and Silt	4.6	4.3	4.0	3.6	4.1 **AB**
S_3 = Sand and Silt	3.6	3.6	3.0	3.0	3.3 **C**
S_4 = Sand and Button Compost	3.3	3.0	2.6	2.6	2.9 **CD**
S_5 = Sand, Silt and Leaf Manure	3.2	2.6	1.6	1.6	2.2 **DE**
S_6 = Peat Moss and Baggase	4.0	3.6	3.3	3.0	3.5 **BC**
S_7= Sand, Silt and Button Mushroom Compost	3.0	2.3	1.6	1.3	2.0 **E**
Mean	3.8 **A**	3.5 **A**	2. 9 B	2.6 **B**	
LSD value for GA = 0.67	**LSD value for SM=1.03**				

***Means with same letters do not differ significantly at p 0.05 by LSD test**

Table 2. Impact of different substrates and GA_3 concentrations on mortality (%) of rough lemon (*Citrus jambhiri*) seed

Sowing Media	Gibberellic acid levels (ppm)				
	0	100	200	400	Mean
S_1 = Baggasse and Sand	49.3	43.3	30.3	24.6	36.9 **A**
S_2 = Baggase and Silt	44.3	39.6	32.0	27.6	35.9 **A**
S_3 = Sand and Silt	39.6	36	28.3	25.0	32.2 **B**
S_4 = Sand and Button Compost	34.0	32.6	23.3	23.3	28.3 **C**
S_5 = Sand, Silt and Leaf Manure	24.0	21.3	12	9.6	16.7 **E**
S_6 = Peat Moss and Baggase	31.6	28.3	17.6	16.0	23.4 **D**
S_7= Sand, Silt and Button Mushroom Compost	26.6	22.6	14.0	12.3	18.9 **E**
Mean	35.6 **A**	32.0 **B**	22.5 **C**	19.8 **D**	
LSD value for GA = 2.32	**LSD value for SM=3.5**				

***Means with same letters do not differ significantly at p 0.05 by LSD test**

Table 3. Impact of different substrates and GA_3 concentrations on plant height of rough lemon (*Citrus jambhiri*) seed

	Gibberellic acid levels (ppm)

Sowing Media	0	100	200	400	Mean
S_1 = Baggasse and Sand	13.3	14.6	25.0	25.7	19.6 **F**
S_2 = Baggase and Silt	15.6	16.3	24.0	28.0	21.0 **E**
S_3 = Sand and Silt	19.3	20.0	28.0	30.7	24.5 **D**
S_4 = Sand and Button Compost	22.0	23.0	38.0	37.3	30.0 **B**
S_5 = Sand, Silt and Leaf Manure	28.3	31.0	41.6	43.3	36.0 **A**
S_6 = Peat Moss and Baggase	23.3	24.3	32.6	33.0	28.3 **C**
S_7= Sand, Silt and Button Mushroom Compost	29.7	30.3	40.0	41.0	35.2 **A**
Mean	21.6 **D**	22.8 **C**	32.7**B**	34.1 **A**	
LSD value for GA = 1.2	**LSD value for SM=1.9**				

***Means with same letters do not differ significantly at p 0.05 by LSD test**

Table 4. Impact of different substrates and GA_3 concentrations on stem diameter of rough lemon (*Citrus jambhiri*) seeds

Sowing Media	Gibberellic acid levels (ppm)				
	0	**100**	**200**	**400**	**Mean**
S_1 = Baggasse and Sand	3.3	3.6	4.2	4.5	3.9 **D**
S_2 = Baggase and Silt	4.3	4.6	5.1	5.5	4.9 **C**
S_3 = Sand and Silt	4.0	5.3	5.7	6.2	5.3 **BC**
S_4 = Sand and Button Compost	5.5	5.83	6.1	6.5	6.0 **B**
S_5 = Sand, Silt and Leaf Manure	7.0	7.6	8.3	8.6	7.9 **A**
S_6 = Peat Moss and Baggase	5.5	5.8	6.4	6.5	6.1 **B**
S_7= Sand, Silt and Button Mushroom Compost	6.6	7.0	8.0	8.3	7.5 **A**
Mean	5.1 **C**	5.7 **BC**	6.2 **AB**	6.6 **A**	
LSD value for GA = 0.7	**LSD value for SM=1.2**				

***Means with same letters do not differ significantly at p 0.05 by LSD test**

Table 5. Impact of different substrates and GA_3 concentrations on number of leaves of rough lemon (*Citrus jambhiri*) seeds

Sowing Media	**Gibberellic acid levels (ppm)**				
	0	**100**	**200**	**400**	**Mean**
S_1 = Baggasse and Sand	3.3	3.6	3.6	4.0	3.6 **D**
S_2 = Baggase and Silt	3.3	4.0	4.6	5.0	4.2 **CD**
S_3 = Sand and Silt	3.3	5.0	5.3	6.0	4.9**BC**
S_4 = Sand and Button Compost	4.0	5	5.6	6.0	5.1 **B**
S_5 = Sand, Silt and Leaf Manure	7	7.7	8.3	8.6	7.9 **A**
S_6 = Peat Moss and Baggase	5	5.7	5.6	6.0	5.5 **B**
S_7= Sand, Silt and Button Mushroom Compost	6.6	7.3	8.0	8.3	7.5 **A**
Mean	4.6 **C**	5.4 **B**	5.9 **AB**	6.2 **A**	
LSD value for GA = 0.67	**LSD value for SM=1.03**				

***Means with same letters do not differ significantly at p 0.05 by LSD test**

Table 6. Impact of different substrates and GA_3 concentrations on root length (cm) of rough lemon (*Citrus jambhiri*) seeds

Sowing Media	**Gibberellic acid levels (ppm)**				
	0	**100**	**200**	**400**	**Mean**
S_1 = Baggasse and Sand	3.6	3.6	4.0	5.0	4.0 **C**
S_2 = Baggase and Silt	4.0	4.6	5.0	6.0	4.9 **C**
S_3 = Sand and Silt	5.0	5.3	6.0	7.3	5.9 **B**
S_4 = Sand and Button Compost	5.0	5.6	6.0	8.0	6.1 **B**
S_5 = Sand, Silt and Leaf Manure	7.6	8.3	8.67	14.0	9.6 **A**
S_6 = Peat Moss and Baggase	5.6	5.6	6.0	10.0	6.8 **B**
S_7= Sand, Silt and Button Mushroom Compost	7.3	8.0	8.3	12.3	9.0 **A**
Mean	5.4 **C**	5.9 **BC**	6.2 **B**	8.9 **A**	
LSD value for GA = 0.98	**LSD value for SM=1.5**				

***Means with same letters do not differ significantly at p 0.05 by LSD test**

Table 7. Impact of different substrates and GA_3 concentrations on number of roots of rough lemon (*Citrus jambhiri*) seeds

Sowing Media	Gibberellic acid levels (ppm)				
	0	**100**	**200**	**400**	**Mean**
S_1 = Baggasse and Sand	1.5	1.7	2.0	3.1	2.0 **E**
S_2 = Baggase and Silt	1.7	2.0	2.5	3.5	2.4 **DE**
S_3 = Sand and Silt	2.0	2.5	2.9	3.3	2.8 **D**
S_4 = Sand and Button Compost	2.7	3.0	3.6	4.5	3.4 **C**
S_5 = Sand, Silt and Leaf Manure	4.8	5.3	5.8	6.8	5.7 **A**
S_6 = Peat Moss and Baggase	2.6	3.1	3.6	4.6	3.5 **C**
S_7= Sand, Silt and Button Mushroom Compost	3.6	4.1	4.6	5.6	4.5 **B**
Mean	2.7 **D**	3.1 **C**	3.6 **B**	4.6 **A**	
LSD value for GA = 0.2	**LSD value for SM=0.41**				

***Means with same letters do not differ significantly at p 0.05 by LSD test**

Table 8. Impact of different substrates and GA_3 concentrations on root weight (g) of rough lemon (*Citrus jambhiri* L.)

Sowing Media	Gibberellic acid levels (ppm)				
	0	**100**	**200**	**400**	**Mean**
S_1 = Baggasse and Sand	11.0	13.3	15.0	16.3	13.9 **F**
S_2 = Baggase and Silt	13.0	15.6	17.6	19.6	16.5 **E**
S_3 = Sand and Silt	19.0	21.0	23.0	25.0	22 **D**
S_4 = Sand and Button Compost	22.0	24.0	26.0	28.0	25.0 **C**
S_5 = Sand, Silt and Leaf Manure	27.0	29.0	31.6	34.0	30.4 **A**
S_6 = Peat Moss and Baggase	23.0	25.0	26.6	28.0	25.6 **C**
S_7= Sand, Silt and Button Mushroom Compost	24.3	26.3	28	30.0	27.1 **B**
Mean	19.9 **D**	22.0 **C**	24.0 **B**	25.8 **A**	
LSD value for GA = 0.4	**LSD value for SM=0.8**				

***Means with same letters do not differ significantly at p 0.05 by LSD test**