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EFFECT OF PRESOAKING TREATMENTS OF GROWTH PROMOTERS ON ROUGH LEMON (CITRUS JAMBHIRI LUSH.)

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An experiment was conducted in 2022 at the Department of Fruit Sciences, College of Horticulture, Bengaluru, Karnataka, India to study the presoaking impact of various plant growth promoters on rough lemon (*Citrus jambhiri* Lush.) seed germination and growth parameters. The experiment employed a Completely Randomized Design with eleven treatments, each replicated three times. Before sowing, rough lemon seeds were soaked for 30 minutes in different solutions, including GA₃, KNO₃, thiourea, humic acid, vermiwash and distilled water (control) and one treatment of overnight soaking. The highest seed germination percentage (93.33%) and survivability (92.67%) were observed in seeds soaked overnight in 500 ppm GA₃ (T₁₀). After 90 days after sowing, the maximum seedling height (9.73 cm), seedling girth (1.95 mm), number of leaves (10.33), root length (11.73 cm), fresh weight (0.41 g) and dry weight (0.09 g) were recorded for seeds soaked overnight in 500 ppm GA₃ (T₁₀), followed by the 500 ppm GA₃ treatment (T₂).

Key words : Rough lemon, Citrus jambhiri, Gibberellic acid, Germination percentage, Growth parameters.

Introduction

The genus Citrus, belonging to the Rutaceae family, encompasses around 140 genera and 1300 species distributed worldwide. Citrus fruits such as oranges, lemons, grapefruits and limes are renowned for their pleasant aroma attributed to the terpenes in their rind. These long-lived perennial crops are cultivated in over 100 countries (Saunt, 1990). Sour orange was traditionally the primary rootstock due to its resilience in various soil conditions and resistance to foot rot and cold. However, the emergence of the Citrus tristeza virus (CTV) led to a shift towards rough lemon rootstock, demonstrating CTV tolerance (Savita et al., 2018). Indigenous to Northeastern India, the rough lemon, commonly referred to as Jattikhatti, is believed to be a natural hybrid owing to its elevated polyembryony in contrast to other lemon species. In the Punjab region and adjacent areas, the rough lemon

serves as a pivotal rootstock for a variety of citrus fruits such as lemons, oranges, mandarins and grapefruits. Flourishing in warm and humid climates characterized by deep sandy soils, this citrus species exhibits resistance to Citrus Tristeza Virus (CTV). This rootstock boosts the plant's ability to withstand different viruses and results in the production of sizable fruits in grafted scion cultivars, as reported by Altaf *et al.* (2008). It thrives in sandy, well-drained soil and demonstrates tolerance to alkalinity, along with a moderate ability to tolerate salinity.

Rough lemon rootstock is typically propagated through seed germination, which is essential for large-scale production. However, the seeds must be sown immediately after extraction due to their recalcitrant nature. Seed germination is a critical stage in nursery production but can be slow, with variation in the time it takes for seeds to germinate. This delay may result from inhibitors in the seed coat or imbalances in growth promoters and inhibitors. This can lead to time-consuming and costly production processes. To improve seed germination and seedling growth, various plant growth regulators and chemicals have been studied. Plant hormones play a crucial role in regulating cell division, growth and differentiation (Hooley, 1994). Gibberellin, for instance, enhances cell wall plasticity and water entry into cells, promoting elongation (Arteca, 1996). Humic acid acts as an organic fertilizer, improving nutrient absorption by plants (Khazaie et al., 2009). Potassium nitrate is a commonly used chemical for seed treatment to promote germination in different crops (Rajamanickam et al., 2004). Vermiwash is a liquid produced as water passes through a worm-processed column and it is enriched with enzymes and secretions from earthworms, known to enhance the growth and yield of crops. Seed treatment is vital to achieve uniformity in seedling growth and reduce irregularities in germination, a common issue in citrus rootstock seeds. This study aims to enhance seed germination and seedling survivability using various plant growth regulators and chemicals.

Materials and Methods

The study, entitled 'Effect of presoaking treatments of growth promoters on rough lemon' was conducted in the 2022 period under the agro-climatic conditions of Bengaluru, Karnataka, India. The research was carried out at the Department of Fruit Science, College of Horticulture, Bengaluru, University of Horticultural Sciences, Bagalkot. For this study, uniform, fully matured and true-to-type fruits from Rough Lemon trees were carefully collected. The seeds were extracted, cleaned, and subsequently sown in polythene bags measuring 25 x 15 cm. To ensure proper drainage, the bags were punctured, and they were filled with a garden mixture prepared by combining two parts of soil, one part of fine sand, and one part of well-rotted FYM. In this study, 30 normal and uniform-sized rough lemon seeds were selected for each treatment. To prepare the soaking solutions, the desired concentrations were created from stock solutions using distilled water. Then seeds were soaked in the prepared solution for half an hour except T_{10} , which was soaked overnight. The treatments included: T₁- GA₃ @ 250 ppm, T₂- GA₃ @ 500 ppm, T₃- KNO_3 @ 1%, T_4 - Thiourea @ 1%, T_5 - Ethrel @ 100 ppm, T₆- Humic acid @ 1%, T₇- Humic acid @ 3%, T₈-Vermiwash @ 1:1 ratio (Vermiwash: water), T_o-Vermiwash@ 2:1 ratio (Vermiwash: water), T₁₀- GA3 overnight soaking @ 500 ppmand T₁₁- control (Water soaking). Each treatment, except for GA₃ at 500 ppm overnight soaking, involved soaking the seeds for 30 minutes. The control seeds were soaked in distilled water, then removed and rinsed with distilled water. These pretreated seeds were sown in polythene bags at 10:00 AM, properly labelled and arranged according to the experimental design. The experiment followed a Completely Randomized Design with three replications. Cultural practices such as regular watering, weeding and plant protection measures, including insecticide spraying against caterpillars and leaf miners, were implemented.

Results and Discussion

The information gathered in the current study regarding seed germination, vegetative growth and root development of rough lemon underwent statistical analysis.

Seed germination and survivability percentage

The growth regulators and biostimulants enhanced the germination rate in the present study. From Table 1, the maximum seed germination (93.33%) and survivability (92.67%) were observed in the GA₃ at 500 ppm overnight soaked seeds (T_{10}), which are statisticallyon par with treatment T_1 and T_9 . Whereas the lowest seed germination was observed in the 100 ppm etherl (T_5) and survivability in control (63.67%). The increased and improved germination is likely attributed to GA₃'s activation of hydrolytic enzymes, such as alpha-amylase and other

Table 1 : Impact of different growth promoters on the germination percentage of rough lemon.

Treatment	Germination percentage (%) 45 DAS	Survival percentage (%) 90 DAS
$T_1 - GA_3 @ 250 ppm$	86.67 (68.83) *	86.00(68.22)*
T_2 - GA ₃ @ 500 ppm	83.33 (66.12)	91.33(72.95)
T ₃ - KNO ₃ @ 1%	76.67 (61.20)	80.67 (63.97)
T ₄ - Thiourea @ 1%	70.00 (56.98)	73.33 (59.07)
T_5 - Ethrel @ 100 ppm	66.67 (54.76)	66.33 (54.58)
T_6 - Humic acid @ 1%	73.33 (58.98)	65.33 (53.94)
T ₇ - Humic acid @ 3%	76.67 (61.20)	77.33 (61.57)
T ₈ - Vermiwash @ 1:1	70.00 (56.98)	72.67 (58.47)
T ₉ - Vermiwash@ 2:1	83.33(66.12)	86.67 (68.83)
T ₁₀ - GA ₃ overnight soaking @ 500 ppm	93.33 (77.69)	92.67 (77.08)
T ₁₁ - Control	70.00 (56.98)	63.67 (52.92)
C. D. 5 % level	12.23	8.60
S.Em±	4.14	2.92

* The values are angular transformed.

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Treatment	Height of seedling (cm)			Stem girth (mm)		
	30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS
T ₁ - GA ₃ @ 250 ppm	4.23	7.07	9.37	2.00	5.33	8.00
$T_2 - GA_3 @ 500 ppm$	4.50	7.43	9.00	2.00	5.67	8.33
T ₃ -KNO ₃ @ 1%	3.90	7.27	8.03	2.33	5.00	8.33
T ₄ - Thiourea @ 1%	3.57	6.97	8.37	2.00	5.67	7.67
T ₅ -Ethrel @ 100 ppm	3.70	6.77	7.93	2.33	5.33	8.00
T ₆ - Humic acid @ 1%	3.77	6.33	8.10	2.33	5.33	7.00
T ₇ - Humic acid @ 3%	3.78	7.33	8.23	2.33	6.00	7.67
T ₈ - Vermiwash @ 1:1	3.73	7.23	8.20	2.33	5.33	6.67
T ₉ - Vermiwash@ 2:1	4.09	6.60	7.90	2.67	5.67	8.33
T_{10} - GA ₃ overnight soaking @ 500 ppm	4.43	7.73	9.73	2.33	6.67	10.33
T ₁₁ - Control	3.23	6.30	7.97	2.67	4.67	6.67
C. D. 5 % level	0.68	0.77	0.68	N/A	0.89	0.98
S.Em±	0.23	0.26	0.23	0.28	0.30	0.33

Table 2: Impact of different growth substances on the seedling height (cm) and stem girth (cm) of rough lemon.

hydrolases, within the endosperm. Amylase plays a key role in converting starch into essential sugars that the growing embryo uses for its growth. Additionally, GA₃ overcomes various forms of dormancy, including photo dormancy, thermos-dormancy, dormancy linked to incomplete embryo development, mechanical barriers, and the presence of germination inhibitors (Diaz and Martin, 1971). Similar germination rates were observed by Sharma and Dhaliwal (2013) and Choudhary *et al.* (2023) in rough lemon and Arghistani *et al.* (2020) also reported comparable findings in a hydroponic system.

Height of seedling

Plant height measurements were taken at 30-day intervals revealing significant differences. At 30 DAS, the tallest seedlings (4.50 cm) were found in the 500 ppm GA₃ treatment, statistically similar to T_9 and T_{10} . At 60 DAS (7.73 cm) and 90 DAS (9.73 cm), the maximum plant heights were seen in seeds soaked overnight in 500 ppm GA₃. In contrast, the control consistently displayed the shortest seedling heights at each interval (3.23 cm, 6.30 cm, 7.90 cm). The improved seedling height with GA₃ treatment is likely a result of heightened osmotic nutrient uptake induced by this hormone, leading to cell elongation and subsequent growth in seedling height.

In addition to enhancing germination, GA_3 treatment promotes subsequent seedling growth by initiating sugar hydrolysis within the seed, which is then used for the synthesis of auxin and proteins. Auxin is vital for the growth of young seedlings, while proteins serve as a nutritional source during seedling development and later growth stages. The increased plant height with GA_3 may be attributed to cell division and elongation resulting in greater internodal length (Stowe and Yamaki, 1957). The outcomes align closely with the research by Singh and Chahal (2021) on rough lemon after 90 DAS and Dilip *et al.* (2017) in different citrus species.

Girth of stem

Table 2 displays the effect of growth substances on rough lemon stem diameter—no significant difference at 30 DAS. However, at 60 and 90 DAS, growth substances significantly impacted stem diameter. The maximum stem girth (1.41 mm and 1.95 mm) was from seeds soaked in 500 ppm GA₃ overnight. T₃ and control had the minimum girths. The increased stem girth in GA₃ pre-soaked seedlings is likely due to internode elongation, increased cell size, rapid cell division, and potential cambium stimulationand similar results seen by Sharma and Dhaliwal (2013).

Number of leaves

The data in Table 3 shows no significant difference at 30 DAS. However, at 60 and 90 DAS, the highest leaf count (6.67 and 10.33) was in seeds soaked overnight in 500 ppm GA₃, while the lowest was in the control group. The increase in leaf count seems associated with plant height. The positive effect of GA₃ is likely due to its capacity to accelerate cell multiplication and promote cell elongation after germination, as theorized by Salisbury and Ross (1988). This result agrees with the findings of Choudhary *et al.* (2023) andYadav *et al.* (2022) in Jattikhatti and Joshi *et al.* (2015) in acid lime.

Root length

The data in Table 3 demonstrate that the longest taproot was observed with the treatment involving overnight soaking in 500 ppm GA_3 (11.73 cm), which is statistically similar to the 500 ppm GA_3 treatment.

Treatment	Number of leaves			Root length (cm)	Fresh weight (g)	Dry weight (g)
	30 DAS	60 DAS	90 DAS	45 DAS	45 DAS	45 DAS
T ₁ - GA ₃ @ 250 ppm	2.00	5.33	8.00	9.37	0.33	0.08
$T_2 - GA_3 @ 500 ppm$	2.00	5.67	8.33	11.47	0.41	0.09
T ₃ -KNO ₃ @ 1%	2.33	5.00	8.33	8.47	0.28	0.06
T ₄ - Thiourea @ 1%	2.00	5.67	7.67	5.80	0.34	0.06
T_5 -Ethrel @ 100 ppm	2.33	5.33	8.00	9.10	0.24	0.06
T ₆ - Humic acid @ 1%	2.33	5.33	7.00	8.27	0.18	0.06
T ₇ - Humic acid @ 3%	2.33	6.00	7.67	9.60	0.30	0.08
T ₈ -Vermiwash @ 1:1	2.33	5.33	6.67	7.63	0.32	0.08
T ₉ - Vermiwash@ 2:1	2.67	5.67	8.33	8.73	0.32	0.08
T_{10} - GA ₃ overnight soaking @ 500 ppm	2.33	6.67	10.33	11.73	0.36	0.09
T ₁₁ - Control	2.67	4.67	6.67	7.47	0.27	0.05
C. D. 5 % level	N/A	0.89	0.98	1.04	0.03	0.01
S.Em±	0.28	0.30	0.33	0.35	0.01	0.00

 Table 3 : Impact of different growth substances on the number of leaves, root length (cm), fresh weight (g) and dry weight (g) of rough lemon.

Conversely, the shortest taproot was observed in the control group (7.47 cm GA_3 's influence on taproot length is likely due to its apical dominance restoration, promoting root initiation, nutrient uptake, and root cell elongation. Enhanced root growth is linked to improved metabolic activity and increased sugar and starch utilization (Kour *et al.*, 2021). These results are in line with Kour *et al.* (2021) and Verma *et al.* (2005).

Fresh weight and dry weight

The highest fresh weight (0.41 g) was achieved with the 500 ppm GA₃ treatment, significantly outperforming all other treatments, with T_{10} (0.36 g) as the closest contender (Table 3). The maximum dry weight was obtained with T_2 and T_{10} (0.09 g each), followed by T_2 , T_7 , T_8 and T_9 (0.08 g each). In contrast, the control group exhibited the lowest fresh weight (0.27 g) and dry weight (0.05 g). This can be attributed to improved water and nutrient transport, promoting increased photosynthetic production and translocation to various plant parts, leading to enhanced seedling growth and increased fresh and dry weight (Shenmugavelu, 1966). The heightened seedling length and greater fresh weight, resulting in increased shoot dry weight, may also be due to improved nutrient mobilization with GA, application (Yadav et al., 2022). These findings align with the results of Dilip et al. (2017) in Rangpur lime and Yadav et al. (2022) in Jattikatti.

Conclusion

In conclusion, the experiment demonstrates that the application of 500 ppm GA_3 , especially when seeds are

soaked overnight (T_{10}), significantly enhances rough lemon seed germination and growth parameters. This finding can be valuable for optimizing rough lemon cultivation practices and achieving more robust and healthy seedlings. Further research may explore the longterm effects of GA₃ on fruit yield and quality to provide a comprehensive understanding of its potential in commercial citrus farming.

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