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Studies on the effect of plant growth regulators on seed germination and seedling growth of acid lime (*Citrus aurantifolia* Swingle)

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Abstract
The present investigation entitled "Studies on the Effect of Plant Growth Regulators on Seed Germination and Seedling Growth of Acid Lime (*Citrus aurantifolia* Swingle)" was carried out during July, 2024 to December, 2024 at the Horticulture Farm, Rajasthan College of Agriculture, MPUAT, Udaipur. The study aimed to evaluate the influence of various plant growth regulators (PGRs) on seed germination behavior, seedling vigour and physiological performance of acid lime, a species known for slow and erratic germination due to its recalcitrant seed nature. The experiment comprised thirteen treatments including combinations of gibberellic acid (GA3), 6-benzyl adenine (BA) and Brassinolide, laid out in a Completely Randomized Design with three replications. Observations were recorded on germination parameters, seedling growth parameters at 60, 90 and 120 days after sowing (DAS) and physiological traits such as chlorophyll content, vigor index and survival percentage. Among the treatments, T_{13} (GA3 100 ppm + BA 75 ppm + Brassinolide 4 ppm) significantly outperformed all others, recording the minimum days to germination initiation (5.40 days), 50 percent germination (10.00 days) and 100 percent germination (16.30 days), along with the highest germination percentage (88.00%). It also resulted in superior seedling height (15.60 cm), number of leaves (14.00), tap root length (12.00 cm), shoot and root fresh weights (10.00g and 6.40g, respectively), shoot and root dry weights (2.60 g and 1.66 g, respectively), root shoot ratio (0.64) and vigour index I and II (2419.20 and 375.17 g).
Furthermore, the highest chlorophyll content (1.88 mg g-1) and survival percentage (85.40%) were also recorded in this treatment.

Keywords: Acid lime, PGRs pre-soaking, germination, nursery seedlings

Introduction

Acid lime (*Citrus aurantifolia* Swingle) is an important fruit crop belonging to the family Rutaceae with a chromosome number of 2n = 18. It is an evergreen, multipurpose citrus species widely cultivated in India, especially due to its adaptability to tropical and subtropical climates. India is the largest producer of acid lime, with an area of approximately 317 thousand hectares and production reaching 3.7 million metric tonnes (Anonymous, 2020) [1]. The fruit is popularly known by various names such as Kagzi lime, Pati lime and Mexican lime and is grown extensively in states like Andhra Pradesh, Maharashtra, Gujarat, Tamil Nadu and Madhya Pradesh.

Acid lime is prized not only for its refreshing flavour and juice quality but also for its medicinal and nutritional properties. The juice contains 6.3-6.6% citric acid and is a rich source of vitamin C and antioxidants. Every 100 g of juice provides 80% water, 22.86 mg vitamin C and essential minerals like potassium, calcium and iron. Its multipurpose utility in preparing beverages, candies, pickles, marmalades and cosmetics enhances its market demand. Despite this significance, the production and propagation of acid lime face major bottlenecks, primarily due to problems in seed germination and seedling establishment.

Propagation of acid lime is commonly carried out through seeds because of the high percentage of nucellar embryony (39-60%), which ensures true-to-type seedlings. However, the seeds are recalcitrant in nature, meaning they are highly perishable and sensitive to drying and chilling. These seeds lose viability quickly and require immediate sowing. If stored beyond their critical viability period, seed germination drops drastically. Moreover, germination is slow and erratic, often taking up to three weeks and is generally accompanied by poor seedling vigour and high mortality in the nursery stage (Cheema *et al*., 1954 [3]; Gupta, 1989) [5]. These issues are further complicated during the monsoon due to fungal infections and low seedling survival rates.

Low germination percentage, short seed storage life, and weak seedling performance make acid lime cultivation challenging, particularly for nurserymen who need healthy, vigorous seedlings in a shorter period. In Kagzi lime, germination rates have been reported as low as 27% to 58%, and the growth of seedlings in both nurseries and field conditions remains sluggish (Naik, 1949) [10]. To address these challenges, various seed treatments and the use of plant growth regulators (PGRs) have been explored. Plant growth regulators are naturally occurring or synthetic organic compounds that influence physiological processes in plants at very low concentrations. These include gibberellins, cytokinins, auxins, ethylene and brassinosteroids. PGRs regulate key aspects such as seed germination, root and shoot development, enzyme activation and stress resistance. In seed technology, they are increasingly used to overcome seed dormancy, enhance germination and improve seedling establishment. Among them, gibberellic acid (GA3) is widely used in horticulture. It promotes seed germination by stimulating the production of enzymes like amylase that break down stored food reserves, enabling embryo growth. GA_3 also enhances stem elongation, root growth and breaks dormancy in various plant species (Kohl and Kofranek, 1957) [6]. Cytokinin, such as benzyl adenine (BA), stimulate cell division, delay leaf senescence and encourage shoot development and nutrient mobilization (Shudo, 1994) [14]. They are especially useful in improving the growth of seedlings by enhancing chlorophyll production and shoot multiplication. Brassinosteroids, a newer group of PGRs, are plant steroid hormones that play a vital role in seed germination, embryo development and stress tolerance (De Jesus *et al*., 2024) [4].

Materials and methods

The present investigation entitled "Studies on the Effect of Plant Growth Regulators on Seed Germination and Seedling Growth of Acid Lime (*Citrus aurantifolia* Swingle)" was undertaken during July, 2024 to December, 2024 at the Horticulture Farm, Rajasthan College of Agriculture, MPUAT, Udaipur. The experiment included thirteen treatments: T_1 (Control), T_2 (Gibberellic Acid @ 50 ppm), T_3 (Gibberellic Acid @ 100 ppm), T_4 (6-Benzyl Adenine @ 50 ppm), T5 (6-Benzyl Adenine @ 75 ppm), T_6 (Brassinolide @ 2 ppm), T_7 (Brassinolide @ 4 ppm), T_8 (Gibberellic Acid @ 50 ppm + 6-Benzyl Adenine @ 50 ppm), T_9 (Gibberellic Acid @ 100 ppm + 6-Benzyl Adenine @ 75 ppm), T_{10} (Gibberellic Acid @ 50 ppm + Brassinolide @ 2 ppm), T_{11} (Gibberellic Acid @ 100 ppm + Brassinolide @ 4 ppm), T_{12} (Gibberellic Acid @ 50 ppm + 6-Benzyl Adenine @ 50 ppm + Brassinolide @ 2 ppm) and T_{13} (Gibberellic Acid @ 100 ppm + 6-Benzyl Adenine @ 75 ppm + Brassinolide @ 4 ppm). The study was carried out in Completely Randomized Design (CRD) with three replications.

The experiment was conducted at field of Horticulture Farm, Department of Horticulture, Rajasthan College of Agriculture, Maharana Pratap University of Agriculture and Technology, Udaipur which is situated at South Eastern part of Rajasthan. This region falls under Agro-Climatic Zone IV A *i.e*., sub-humid southern plain and aravalli hills of Rajasthan at altitude of 582.17 meter above mean sea level, 24°35' N latitude and 74°42' E longitude.

Climatic and weather conditions

This region has a typical sub-tropical climate, characterized by mild winters and summers. The average rainfall of this tract ranges from 760 to 900 mm per year. More than 90 percent of rainfall was received during mid-June to September with scanty showers during winter months.

Collection of seed

The fruits of Acid lime were bought fresh from the local market of Udaipur mandi as the source of seeds. The fruits of uniform size that were fresh and fully matured had their seeds properly removed. The extracted seeds were washed 2-3 times in clean water. The cleaned seeds was floating test by immersing in water. The heavy seeds which sink in water were selected for the experiment.

Filling of polybags

Polybags of 9" × 4" size was taken for each treatment in each replication. Four to six small holes were made on each bag, for proper drainage after that bags were filled as per treatments with soil.

Sowing of seeds

Treated seed of acid lime were sown in polybags filled with soil. One seed per poly bag was sown at 2-
2.5 cm depth. Afterwards sowing, immediately irrigated the polybags and light irrigation every day unless seedling emergence does not occur. It was light irrigated twice a day.

$$\text{Germination percentage (\%)} = \frac{\text{Number of seeds germinated}}{\text{Number of seed sown}} \times 100$$

$$\text{Root shoot ratio} = \frac{\text{Dry weight of the root}}{\text{Dry weight of the shoot}}$$

Vigour index-I = Height of seedling (cm) x Germination percentage (%)

Vigour index-II = Dry weight of seedling (g) x Germination percentage (%)

$$\text{Survival percentage (\%)} = \frac{\text{No. of survived seedling}}{\text{Total no. of seedlings}} \times 100$$

Results and Discussion

Germination Parameters

However, significantly the minimum days for initiation of germination of acid lime (5.40 days), days to 50% germination (10.00 days), days to 100% germination (16.30

days) and germination percent (88.00) were recorded in treatment T_{13} (GA_3 100 ppm + 6-BA 75 ppm + Brassinolide 4 ppm) while maximum days required for initiation of germination (12.00 days), days to 50% germination (19.50 days), days to 100% germination (30.00 days) and germination percent (58.00%) were recorded in treatment T_1 (control). The promoting of germination may be due to the antagonistic effect of different plant growth substances against the influence of inhibitors as reported by Brian and Hemming (1958) [2] and Wareing *et al*., (1968) [16] and endogenous gibberellin increased by soaking. These results are in accordance with the results obtained by Shinde *et al*., (2008) [13] in rangpur lime.

Seedlings height (cm)

The maximum seedlings height (6.67, 12.08 and 15.60 cm) was observed at 60, 90 and 120 days after sowing in treatment T_{13} (GA_3 100 ppm +6-BA 75 ppm + Brassinolide 4 ppm), while the minimum seedling height was observed (3.41, 6.28 and 7.35 cm) in treatment T_1 (control) at 60, 90 and 120 days after sowing. GA3 promotes elongation by stimulating cell division and loosening of cell walls, while BA enhances shoot meristem activity. These findings are in line with the reports of Richardson and Wilkinson (1984) [12], who demonstrated that these PGRs are instrumental in stem elongation and apical growth.

Number of leaves

The maximum number of leaves per seedling (8.37, 11.96 and 14.00) was observed in sowing in treatment T_{13} (GA_3 100 ppm + 6-BA 75 ppm + Brassinolide 4 ppm) at 60, 90 and 120 days after sowing. Whereas, minimum number of leaves per seedling (5.72, 7.23 and 8.10) was observed in treatment T_1 (control) at 60, 90 and 120 days after sowing. The increase in leaf number due to the cytokinin activity of BA, which promotes the initiation of leaf primordia and enhances chloroplast development, leading to increased leaf production. GA_3 also promotes overall vegetative growth, contributing indirectly to leaf formation. These results are in accordance with Mohammed *et al*., (2020) [9], who reported similar increases in leaf number due to BA application in citrus seedlings.

Fresh and dry weight of root and shoot (g)

The maximum fresh weight and dry weight of shoot (10.00 and 2.60 g) respectively, as well as the maximum fresh weight and dry weight of root (6.40 and 1.66 g) respectively, were recorded under treatment T_{13} (GA_3 100 ppm +6-BA 75 ppm + Brassinolide 4 ppm). The minimum values for both shoot and root weights were recorded in treatment T_1 (Control). Improved photosynthetic activity, better nutrient absorption and enhanced assimilate partitioning due to the combined effect of GA_3, BA, and Brassinolide. These results are in agreement with Mohammed *et al*., (2020) [9], who reported significant increases in root and shoot biomass with PGR treatments in citrus seedlings.

Root shoot ratio

The maximum root shoot ratio (0.64) was recorded under treatment T_{13} (GA_3 100 ppm + 6-BA 75 ppm + Brassinolide 4 ppm), while the minimum (0.55) was observed in treatment T_4 (BA 50 ppm). The balanced growth between root and shoot systems, as the combined effect of GA_3, BA and Brassinolide promoted both root elongation and shoot development simultaneously. This balance indicates better nutrient uptake and seedling vigour. These results are supported by Yadav *et al*., (2012) [17], who reported an improved root-shoot ratio under PGR treatments in citrus crops.

Tap root length (cm)

The highest tap root length per seedling (12.00 cm) was observed under treatment T_{13} (GA_3 100 ppm + 6-BA 75 ppm + Brassinolide 4 ppm) at 120 days after sowing whereas, the lowest tap root length was recorded treatment T_1 (control). The increase in root length due to the combined effect of GA_3 and Brassinolide, which promote cell elongation in roots by enhancing auxin sensitivity and stimulating root meristem activity. Brassinolide also improves water and nutrient uptake, contributing to better root development. Similar results were reported by Vishwakarma and Sharma (2020) [15], who found that combined PGR application enhanced root growth in citrus.

Seedling vigour index (g)

The highest vigour index-I (2419.20) and vigour index-II (375.17) were recorded under treatment T_{13} (GA_3 100 ppm + 6-BA 75 ppm + Brassinolide 4 ppm), while the lowest values (942.50 and 102.08) were observed in treatment T_1 (Control). The improved germination percentage, seedling height and biomass accumulation, resulting from enhanced metabolic activity and nutrient translocation due to the combined effect of GA_3, BA and Brassinolide. These findings are supported by Yadav *et al*., (2012) [17], who reported higher seedling vigour with PGR application in citrus.

Total Chlorophyll Content (mg g^{-1})

The highest chlorophyll content (1.88 mg g^{-1}) was recorded under treatment T_{13} (GA_3 100 ppm + 6-BA 75 ppm + Brassinolide 4 ppm), while the lowest (1.00 mg g^{-1}) was observed in treatment T_1 (Control). The role of Brassinolide and BA in stimulating chlorophyll biosynthesis and reducing chlorophyll degradation, thereby enhancing photosynthetic efficiency. Improved nutrient uptake and metabolic activity may also have contributed to higher pigment accumulation. Similar findings were reported by Kurdi and Al-Zebari (2022) [7], who observed increased chlorophyll levels under plant growth regulator treatments.

Survival percentage (%)

The highest survival percentage (85.40%) was recorded under treatment T_{13} (GA_3 100 ppm + 6-BA 75 ppm + Brassinolide 4 ppm), while the lowest (52.20%) was observed in treatment T_1 (Control). The improved survival percentage due to better root development, increased chlorophyll content and enhanced stress tolerance caused by the synergistic action of GA_3, BA and Brassinolide. These plant growth regulators help in strengthening the root system and maintaining better plant water relations, resulting in higher seedling establishment and survival. Similar results were reported by Lunagariya *et al*., (2022) [8] in acid lime.

Experimental plot

Table 1: Studies on the Effect of Plant Growth Regulators on seed germination of Acid lime

Treatments	Initiation of germination (Days)	50 percent germination (Days)	100 percent germination (Days)	Germination percent (%)
T_1	12.00	19.50	30.00	58.00
T_2	10.50	17.00	25.70	65.00
T_3	9.80	16.00	24.20	72.00
T_4	11.20	18.50	27.00	61.00
T_5	10.70	17.20	25.00	67.00
T_6	10.30	16.80	23.30	66.00
T_7	9.60	15.50	22.00	70.00
T_8	8.80	14.80	21.30	74.00
T_9	7.90	13.20	19.80	78.00
T_{10}	8.50	14.50	20.50	73.00
T_{11}	7.30	12.50	18.50	80.00
T_{12}	6.10	11.30	17.80	83.00
T_{13}	5.40	10.00	16.30	88.09
SE(m)±	0.11	0.17	0.36	1.01
CD at 5%	0.32	0.51	1.07	2.97

Table 2: Studies on the Effect of Plant Growth Regulators on seedling height (cm), number of leaves and total chlorophyll content (mg g-1) of acid lime.

Treatments	Seedling height (cm)			No. of leaves			Chlorophyll content (mg g-1)		
	60 DAS	90 DAS	120 DAS	60 DAS	90 DAS	120 DAS	‘a’	‘b’	Total
T_1	3.41	6.28	7.35	5.72	7.23	8.10	1.10	0.30	1.40
T_2	3.91	7.17	8.46	6.12	8.96	9.01	1.13	0.33	1.46
T_3	4.40	8.03	9.54	6.52	8.66	9.9	1.17	0.36	1.53
T_4	3.75	6.89	8.11	5.99	7.73	8.73	1.12	0.31	1.43
T_5	4.10	7.52	8.90	6.28	8.26	9.38	1.14	0.34	1.48
T_6	4.00	7.34	8.68	6.19	8.11	9.21	1.13	0.33	1.46
T_7	4.30	7.88	9.35	6.43	8.53	9.74	1.16	0.35	1.51
T_8	4.60	8.43	10.04	6.67	9.0	10.3	1.18	0.36	1.54
T_9	5.20	9.50	11.38	7.18	9.86	11.39	1.24	0.4	1.64
T_{10}	4.55	8.35	9.94	6.63	8.94	10.22	1.18	0.36	1.54
T_{11}	5.30	9.68	11.60	7.26	10.0	11.56	1.23	0.39	1.62
T_{12}	5.75	10.48	12.60	7.63	10.65	12.4	1.27	0.42	1.69
T_{13}	6.67	12.08	15.60	8.37	11.96	14.0	1.42	0.46	1.88
SE(m)±	0.07	0.15	0.15	0.11	0.13	0.15	0.01	0.01	0.02
CD at 5%	0.20	0.44	0.44	0.33	0.39	0.43	0.04	0.01	0.06

Table 3: Studies on the Effect of Plant Growth Regulators on growth and survival of Acid lime at 120 DAS

Treatments	Fresh weight (g)		Dry weight (g)		Root shoot ratio	Tap root length (cm)	Vigor index (g)		Survival percentage (%)
	Shoot	Root	Shoot	Root			I	II	
T_1	4.30	1.11	2.50	0.65	0.58	6.00	775.80	102.08	52.20
T_2	5.01	1.30	2.89	0.75	0.58	6.91	999.65	133.26	59.20
T_3	5.77	1.50	3.30	0.85	0.57	7.76	1244.16	168.95	67.00
T_4	4.78	1.24	2.65	0.68	0.55	6.39	884.50	117.12	55.50
T_5	5.30	1.37	3.07	0.79	0.58	7.18	1082.86	144.72	61.60
T_6	5.15	1.33	3.00	0.78	0.58	7.03	1031.88	139.70	60.70
T_7	5.64	1.46	3.23	0.84	0.57	7.59	1184.80	161.00	65.10
T_8	6.11	1.58	3.5	0.91	0.57	8.06	1342.00	184.26	69.60
T_9	7.13	1.85	4.26	1.10	0.6	9.44	1620.96	230.11	74.10
T_{10}	6.03	1.56	3.45	0.89	0.57	7.95	1310.37	178.61	68.60
T_{11}	7.32	1.90	4.23	1.10	0.58	9.17	1657.60	240.27	76.00
T_{12}	8.17	2.12	4.71	1.22	0.58	9.90	1863.30	277.22	79.70
T_{13}	10.00	2.60	6.40	1.66	0.64	12.00	2419.20	375.17	85.40
SE(m)±	0.09	0.02	0.06	0.01	0.01	0.14	19.62	2.78	0.01
CD at 5%	0.26	0.05	0.18	0.03	0.02	0.41	57.36	8.45	0.03

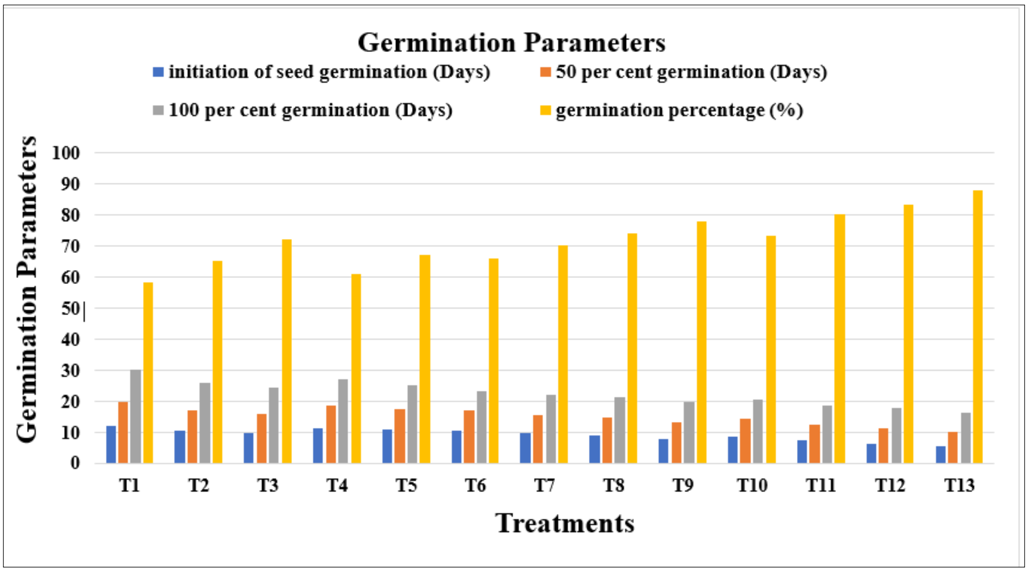

Fig 1: Effect of Plant Growth Regulators on Germination Parameters of Acid lime

Conclusion

Based on the results of research trial, it can be concluded that the treatment (T_{13}) Gibberellic Acid @ 100 ppm + 6-Benzyl Adenine @ 75 ppm + Brassinolide @ 4 ppm proved to be the most effective with minimum days to initiation of germination, 50 percent germination, 100 percent germination, highest germination percentage, maximum seedling height, number of leaves, vigour index-I and II, survival percentage, fresh weight of shoot and roots, dry weight of shoot and roots, length of tap root, root shoot ratio and total chlorophyll content in leaves.

Therefore, the present study will be helpful to the farmers for the cultivation of these important fruit crops as well as the commercialization of acid lime nurseries.

Disclaimer (artificial intelligence)

Author (s) hereby declare that NO generative AI technologies such as large language models (Chat GPT, COPILOT, etc) and tect-to-image generators have been used during writing or editing of this manuscript.

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