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Effect of Gibberellic Acid on Germination and Vigour of Kagzi Lime Seedlings

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Authors' contributions

This work was carried out in collaboration among all authors. The research was conceptualized by authors TRA, SK and AC. The work was carried out by author AC under the supervision of authors TRA and SJ. Data curation and statistical analysis were carried out by authors AC, DP and SJ assisted author AC in preparing this manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To investigate the effect of storage treatments, gibberellic acid (GA_3) and duration of soaking on seed germination and seedling growth in Kagzi lime.
Study Design: The experiment was laid out in a Completely Randomized Design (CRD) with factorial concept and three repetitions.
Place and Duration of Study: The experiment was conducted during July to November 2017 at Department of Fruit Science, Navsari Agricultural University, Navsari, Gujarat, India.

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Methodology: The experiment consisted of sixteen treatments which were repeated thrice. It comprised of three factors and their respective levels. Factor S_1 indicated freshly extracted kagzi lime seeds and S_2 comprised of seeds stored for 15 days. The different concentrations of gibberellic acid were G_1 - 200 ppm; G_2 - 300 ppm; G_3 - 400 ppm and G_4 - 500 ppm. Whereas, duration D_1 stood for 12 hours and D_2 for 24 hours.
Results: The interaction between storage treatments, pre-sowing treatments and duration of soaking was found significant for all parameters included in the study except collar diameter and sturdiness quotient. Soaking freshly extracted Kagzi lime seeds in an aqueous solution of 500 ppm GA_3 for 12 hours resulted in the minimum days for 50% germination (27.89), Seedling Vigour Index (3654.83) and survival percentage (84.70). The same treatment when extended for 24 hours recorded the highest germination percentage (95.68) and number of leaves (28.76). Soaking freshly extracted seeds in 400 ppm GA_3 solution for 12 hours registered the maximum shoot length (21.63 cm). Kagzi lime seedlings raised from freshly extracted seeds resulted in higher collar diameter (2.04 mm) and sturdiness quotient (8.70). Further, a soaking duration of 12 hours proved better over 24 hours for collar diameter (2.04 mm) and sturdiness quotient (8.69). Between the different concentrations of GA_3, 400 ppm gave better results for collar diameter (2.09 mm) and 200 ppm for sturdiness quotient (8.65).
Conclusion: Soaking freshly extracted kagzi lime seeds in an aqueous solution of 500 ppm GA_3 for 12 hrs proved to be the best treatment combination for inducing early germination, higher Seedling Vigour Index and survival percentage. Nurserymen can employ these findings for early germination and vigorous seedlings in Kagzi lime.

Keywords: Kagzi lime; seeds; germination; shoot growth; GA_3; 12 hours.

1. INTRODUCTION

Kagzi lime is used in almost every home of the Indian sub-continent to flavour food or in the preparation of pickles. India occupies the fifth position among major lime and lemon producing countries in the world and is the largest producer of acid lime [1]. In India, lime and lemon cover an area of 259 thousand hectares with a production of 2789 thousand MT [2]. Gujarat produced about 605 thousand MT of citrus fruits from an area of 46 thousand hectares with a productivity of 13 MT/ha [3]. In Gujarat, Kagzi lime is the only citrus fruit grown on commercial scale particularly in the Northern part of the state.

Kagzi lime is still propagated by seeds, throughout the country as this is the easiest and cheapest method of propagating this crop. Sexually raised plants are long lived, have extensive root system and bear a heavy crop. It is observed that citrus seeds lose their viability very soon. Nurserymen and growers often face problems like lower seed germination and high mortality of seedlings at the nursery stage. The seed coat in kagzi lime reportedly acts a barrier because it interferes in early germination of seeds due to presence of certain inhibitory substances. Further raising optimum sized seedlings is also a time consuming job. In order to get plants ready within the shortest possible time, growth must be accelerated for which, certain treatments *viz;* chilling or soaking seeds or spraying plants with certain growth regulators *etc* is advised.

Now-a-days plant growth regulators are widely used in increasing the germination percentage of seeds and improving the subsequent growth of seedlings. Gibberellic acid is widely employed to improve seed germination, stimulate the growth of various plant parts and enhance the rate of elongation of young seedlings. However, reports differ in the range of GA_3 concentration (50-700 ppm) and the duration of soaking (6-40 hrs) which promote germination. Kagzi lime seeds are recalcitrant in nature. The recalcitrant seeds impose serious storage problems due to their desiccation and chilling sensitivity. That soaking seeds in water for 12-36 hrs induces early germination, increases germination percentage and promotes growth has been demonstrated in kagzi lime [4], mango [1], jackfruit [5], aonla [6] and tamarind [7]. An experiment was therefore framed to study the effect of storage treatments, gibberellic acid and duration of soaking on seed germination and seedling growth in Kagzi lime.

2. MATERIALS AND METHODS

2.1 Experimental Design and Treatments Preparation

This investigation was carried out in a net house at Regional Horticultural Research Station,

Navsari Agricultural University, Navsari, Gujarat. Fully mature and healthy fruits of acid lime cv. Kagzi lime were collected for this study. Seeds were extracted carefully, washed in running water and dried under shade for 1 hour. Before drying of seeds, they were dipped in water to remove the dead floating seeds. The treatment details are as under-listed.

Factor I: Seed Storage (S)

S_1: Freshly extracted seeds
S_2: Seeds stored for 15 days

Factor II: Gibberellic acid (GA_3)

G_1: Soaking in 200 g/L GA_3
G_2: Soaking in 300 g/L GA_3
G_3: Soaking in 400 g/L GA_3
G_4: Soaking in 500 g/L GA_3

Factor III: Duration of Soaking (D)

D1: 12 hours
D2: 24 hours

Seeds were stored in an aluminium foil for 15 days at room temperature (29 ± 2^0 C). Gibberellic acid solution was prepared by dissolving 200, 300, 400 and 500 mg GA_3 in 1 litre of water, respectively. Treated citrus seeds were sown in polythene bags which were properly filled with red soil, sand and vermicompost, labelled with tags and placed in net house at proper spacing. Seeds were irrigated immediately after sowing using a rose-can and subsequently seedlings were watered as and when required.

2.2 Observations Recorded

Observations were recorded from five randomly selected and labelled saplings in each treatment in a repetition. The data obtained from all plots per repetition under each treatment were averaged and reported. Fifty percent (50%) germination was calculated as to when 50 seedlings out of 100 germinated in each repetition. Germination percentage was recorded after 20, 30 and 40 days of sowing. Shoot length was measured from the soil surface to the growing tip and was recorded after 60, 90 and 120 days of sowing. Collar diameter of plants was recorded with the help of vernier callipers at a height of 3 cm from the base. The total number of leaves per plant was recorded at 30 days interval up to 4 months after sowing. The Seedling Vigour Index (SVI) was calculated after 120 days of sowing by multiplying germination percentage with the summation of shoot length and root length. The sturdiness quotient was calculated after 120 days of sowing by dividing the shoot length and collar diameter. Similarly, the survival percentage was calculated after 120 days of sowing.

2.3 Data Analysis

The experimental data were subjected to the statistical analysis by using variance technique as described by Panse and Sukhatme [8]. The method of analysis of variance for completely randomized design with Factorial concept (FCRD) was used. The treatment differences were tested by F-test of significance based on null hypothesis. The appropriate standard error of mean (S.EM ±) was calculated in each treatment and critical difference (CD) at 5 % level of probability was worked out to compare the treatment means, where the treatment effects were significant.

3. RESULTS AND DISCUSSION

3.1 Days taken for 50% Germination

A perusal of Table 1 indicated that interaction effect of storage treatments, pre-sowing treatments of GA_3 and duration of soaking was found significant with respect to days taken for 50 % germination. Freshly extracted kagzi lime seeds when soaked for 12 hours in an aqueous solution of GA_3 500 ppm ($S_1G_4D_1$) gave minimum days for germination (27.89). This might be attributed to the synergistic effects of these combined inputs on the stimulation of combined growth through cell division and expansion, improved physico-chemical properties of protoplasm, respiration, nucleic acid metabolism *etc*. These results are in conformity with earlier findings in kagzi lime [9]. It is believed that GA_3 increases *de novo* synthesis of hydrolyzing enzymes particularly amylase and protease. The hydrolyzed food was subsequently utilized for growth of embryo which in turn enhanced germination [10]. Similar results were reported in papaya [11] and in jackfruit [12].

3.2 Seedling Vigour Index

It is clear from the data (Table 1) that interaction effect of storage treatments, pre-soaking treatments of GA_3 and duration of soaking was found significant with respect to Seedling Vigour Index. Freshly extracted kagzi lime seeds treated with GA_3 500 ppm and soaked for 12 hours ($S_1G_4D_1$) showed maximum seedling vigour

index (3654.83). The reason might be attributed to the increased dry matter production in the concerned treatments [13]. The hike in vigour might be due to the direct influence on the extensive growth of seedlings probably by increasing mobilization of reserve foods to growing apices [14].

3.3 Germination Percentage

It is evident from the data that interaction effect of storage treatments, pre-sowing treatments of GA_3 and duration of soaking was found significant with respect to germination percentage and is presented at 20, 30 and 40 DAS in (Table 2). Freshly extracted kagzi lime seeds treated with GA_3 500 ppm and soaked for 24 hours ($S_1G_4D_2$) showed highest germination percentages (56.63, 76.12 and 95.68). Soaking kagzi lime seeds for 24 hours may have softened the hard seed coat and leached out some of the water soluble inhibitors resulting in higher germination percentage [15]. These results are in conformity with the findings in karonda [16], tamarind [7] and pummelo [17]. Whereas, GA_3 might have acted on the embryo and caused *de novo* synthesis of hydrolyzing enzymes particularly amylase and protease and this hydrolyzed food may have been utilized for growth of embryo and thereby improved the germination [10].

3.4 Shoot Length

Data indicates that interaction effect of storage treatments, pre-sowing treatments of GA_3 and duration of soaking was found significant with respect to shoot length (Table 3). Freshly extracted kagzi lime seeds treated with

Table 1. Interaction between storage treatments, pre-sowing treatments and duration of soaking on days taken for 50% germination and Seedling Vigour Index in kagzi lime seeds

	Days taken for 50% germination			Seedling Vigour Index		
	D_1	D_2	Mean	D_1	D_2	Mean
S_1G_1	39.33	34.17	36.75	2339.67	2528.25	2433.96
S_1G_2	31.49	33.42	32.46	2723.42	2678.39	2700.90
S_1G_3	28.33	30.22	29.52	3073.04	2731.84	2902.90
S_1G_4	27.89	29.03	28.46	3654.83	2677.73	3166.28
S_2G_1	33.67	35.62	34.65	2566.30	2604.60	2585.45
S_2G_2	33.22	36.34	34.78	2617.75	2513.22	2565.49
S_2G_3	30.49	32.39	31.44	2688.47	2536.51	2612.50
S_2G_4	29.65	30.93	30.29	2669.16	2593.51	2631.34
Mean	31.82	32.76	32.29	2791.58	2608.01	2699.79
SEM.±	0.78			107.72		
C.D. at 5 %	2.28			310.31		
CV %	4.29			6.91		

Table 2. Interaction between storage treatments, pre-sowing treatments and duration of soaking on germination percentage of kagzi lime seeds

	Seed germination (%) 20 DAS			Seed germination (%) 30 DAS			Seed germination (%) 40 DAS		
	D_1	D_2	Mean	D_1	D_2	Mean	D_1	D_2	Mean
S_1G_1	29.04	34.09	31.56	37.35	44.29	40.82	57.56	67.26	62.41
S_1G_2	39.82	40.99	40.40	52.78	53.82	53.30	72.02	75.19	73.60
S_1G_3	48.19	52.20	50.19	64.51	69.76	67.14	79.70	86.61	83.15
S_1G_4	48.73	56.63	52.68	64.33	76.12	70.22	82.23	95.68	88.96
S_2G_1	37.92	29.89	33.91	50.62	39.52	45.07	76.35	60.26	68.31
S_2G_2	34.35	38.05	36.20	44.69	50.14	47.42	65.27	71.26	68.27
S_2G_3	42.35	46.79	44.57	55.56	61.97	58.76	71.00	78.05	74.53
S_2G_4	44.78	48.79	46.79	58.44	63.63	61.04	72.64	80.24	76.44
Mean	40.65	43.43	42.04	53.53	57.41	55.47	72.10	76.82	74.46
SEM.±	1.71			2.23			2.85		
C.D at 5%	4.93			6.43			8.21		
CV %	7.05			6.98			6.63		

Table 3. Interaction between storage treatments, pre-sowing treatments and duration of soaking on shoot length (cm) of kagzi lime seeds

	Shoot length (cm) at 60 DAS			Shoot length (cm) at 90 DAS			Shoot length (cm) at 120 DAS		
	D_1	D_2	Mean	D_1	D_2	Mean	D_1	D_2	Mean
S_1G_1	2.40	2.93	2.66	6.62	8.40	7.51	12.56	15.91	14.24
S_1G_2	3.45	3.22	3.34	8.97	8.68	8.83	17.38	16.51	16.94
S_1G_3	4.40	4.04	4.22	11.21	9.44	10.33	21.63	18.71	20.17
S_1G_4	4.29	3.93	4.11	10.12	9.33	9.73	19.94	18.27	19.10
S_2G_1	3.06	2.69	2.87	8.66	7.61	8.13	16.36	14.41	15.39
S_2G_2	3.11	2.96	3.04	8.33	7.72	8.03	15.92	14.91	15.41
S_2G_3	4.07	3.64	3.86	9.68	8.38	9.03	19.15	16.56	17.86
S_2G_4	3.89	3.57	3.73	9.21	8.18	8.69	18.09	16.18	17.14
Mean	3.58	3.37	3.48	9.10	8.47	8.78	17.63	16.43	17.03
SEM.±	0.10			0.35			0.65		
C.D at 5%	0.33			1.02			1.89		
CV %	5.17			6.97			6.66		

Table 4. Effect of storage treatments, pre-sowing treatments and duration of soaking on collar diameter (mm) of kagzi lime seeds

Treatments	Collar diameter (mm)		
	60 DAS	90 DAS	120 DAS
S - Seed storage			
S_1 : Freshly extracted seeds	1.13	1.82	2.04
S_2 : Seeds stored for 15 days	1.10	1.80	2.01
S.Em.±	0.01	0.01	0.01
C.D. at 5 %	0.02	0.02	0.03
G - Levels of Gibberellic acid (GA_3)			
G_1: 200 ppm	0.93	1.68	1.96
G_2: 300 ppm	1.05	1.77	2.01
G_3: 400 ppm	1.29	1.94	2.09
G_4: 500 ppm	1.17	1.85	2.05
S.Em.±	0.01	0.01	0.01
C.D. at 5 %	0.02	0.02	0.04
D - Duration of soaking			
D_1: 12 hours	1.13	1.82	2.04
D_2: 24 hours	1.10	1.80	2.01
S.Em.±	0.01	0.01	0.01
C.D. at 5 %	0.02	0.02	0.03

GA_3 400 ppm and soaked for 12 hours ($S_1G_3D_1$) showed highest shoot length (4.40, 11.21 and 21.63 cm). This increase in seedlings height with GA_3 treatment may be due to the fact that GA_3 increased osmotic uptake of nutrients, causing cell elongation and thus increasing the shoot length [18]. Similar findings were reported in jackfruit [5] and tamarind [7].

3.5 Collar Diameter

The interaction effect between different pre-sowing treatments and duration of soaking was found non-significant with respect to collar diameter at 60, 90 and 120 days after sowing. However, there was a significant influence of individual factors on collar diameter (Table 4). Seedlings raised from freshly extracted seeds had higher collar diameter (2.04 mm) than seedlings raised from seeds stored for 15 days (2.01 mm) at 120 days after sowing. Similarly, seeds soaked for duration of 12 hours had higher collar diameter (2.04 mm) at 120 days after sowing as compared to seeds soaked for 24 hours (2.01 mm). Amongst GA_3 treatments, kagzi lime seeds treated with GA_3 400 ppm had the highest collar diameter (2.09 mm). This could be due the fact that gibberellic acid promoted cell

division and cell elongation in the collar region [19]. Akin results were reported in papaya [20], jackfruit [5] and in tamarind [7].

3.6 Number of Leaves

It is evident from the data that interaction effect of storage treatments, pre-sowing treatments and duration of soaking was found significant with respect to number of leaves (Table 5). Freshly extracted kagzi lime seeds treated with GA_3 500 ppm and soaked for 24 hours ($S_1G_4D_2$) showed maximum number of leaves (10.89, 19.38 and 28.76) after 60, 90 and 120 days of sowing, respectively. This can be attributed to the movement of GA_3 to the shoot apex which promoted cell division and cell growth apparently leading to increased development of young leaves [21]. This is in line with results in papaya [20,11].

3.7 Survival Percentage

The interaction between storage treatments, pre-sowing treatments and duration of soaking was found significant for survival percentage (Table 6). Freshly extracted seeds treated with GA_3 500 ppm and soaked for 12 hours ($S_1G_4D_1$) showed highest survival percentage (84.70) at 120 days of sowing. The higher survival percentage under GA_3 treatment might be due to early germination of kagzi lime seeds which helped in successful acclimatization and establishment. Higher germination percentage and higher seedling vigour index under GA_3 treatment may also have played a part in increasing the survival percentage [22]. Similar results were obtained in aonla [6,23]. While, soaking kagzi lime seeds for 12 hours may have accelerated the hydrolysis of complex sugar into simple sugars which are better utilized in the synthesis of auxins and proteins. It is a well known fact that proteins are utilized in the production of new tissues and that auxins promote growth. Similar results were put forth in jackfruit [24] and in papaya [25].

3.8 Sturdiness Quotient

Sturdiness quotient is a non-destructive index which compares height with root collar diameter. It determines the ability of seedlings to withstand wind, drought and frost [26]. Seedlings with a small quotient have better chances of survival under windy conditions. Pre-sowing treatments have a significant influence on the sturdiness quotient. The interaction effect of storage treatments, different pre-sowing treatments of GA_3 and duration of soaking was found non-significant with respect to sturdiness quotient at 120 days after sowing. Nevertheless, individual factors had a significant influence on sturdiness quotient (Table 7). Freshly extracted seeds (8.70) had lower sturdiness quotient as compared to seeds stored for 15 days (8.76). Soaking seeds for 12 hours (8.69) resulted in lower sturdiness quotient as compared to soaking for 24 hours (8.77). Additionally, seeds soaked in aqueous solution of 200 pm GA_3 exhibited the lowest sturdiness quotient (8.65) though the observed value did not differ significantly.

Table 5. Interaction between storage treatments, pre-soaking treatments and duration of soaking on number of leaves of kagzi lime seeds

	Number of leaves at 60 DAS			Number of leaves at 90 DAS			Number of leaves at 120 DAS		
	D_1	D_2	Mean	D_1	D_2	Mean	D_1	D_2	Mean
S_1G_1	4.84	5.51	5.18	10.11	10.87	10.49	14.61	16.09	15.35
S_1G_2	7.00	7.22	7.11	12.62	13.02	12.82	19.00	19.51	19.25
S_1G_3	8.60	9.65	9.12	14.11	16.18	15.15	22.06	24.73	23.39
S_1G_4	8.44	10.89	9.66	13.93	19.38	16.66	21.94	28.76	25.35
S_2G_1	6.68	5.05	5.86	12.62	10.49	11.55	18.70	15.01	16.85
S_2G_2	5.76	6.57	6.17	11.11	12.49	11.80	16.49	18.58	17.53
S_2G_3	7.31	8.28	7.80	12.55	13.92	13.23	19.40	21.61	20.51
S_2G_4	7.92	8.47	8.20	13.23	13.70	12.46	20.51	21.54	21.03
Mean	7.07	7.71	7.39	12.54	13.76	13.15	19.09	20.73	19.91
SEM.±	0.36			0.64			0.91		
C.D at 5%	1.02			1.85			2.61		
CV	8.32			8.47			7.89		

Table 6. Interaction effect of storage treatments, pre-soaking treatments and duration of soaking on survival percentage of kagzi lime seeds

	Survival percentage at 120 DAS		
	D_1	**D_2**	**Mean**
S_1G_1	60.87	71.63	66.25
S_1G_2	72.63	72.89	72.76
S_1G_3	77.71	73.99	75.85
S_1G_4	84.70	73.77	79.24
S_2G_1	73.18	66.36	69.77
S_2G_2	70.31	65.87	68.09
S_2G_3	73.33	66.60	69.97
S_2G_4	75.54	67.81	71.67
Mean	73.53	69.87	71.10
S.Em.±	2.53		
C.D. at 5%	7.26		
CV %	6.10		

Table 7. Effect of storage treatments, pre-sowing treatments and duration of soaking on sturdiness quotient of kagzi lime seeds

Treatments	**Sturdiness quotient**
S - Seed storage	
S_1 : Freshly extracted seeds	8.70
S_2 : Seeds stored for 15 days	8.76
S.Em.±	0.02
C.D. at 5%	0.07
G - Levels of Gibberellic acid (GA_3)	
G_1: 200 ppm	8.65
G_2: 300 ppm	8.70
G_3: 400 ppm	8.81
G_4: 500 ppm	8.76
S.Em.±	0.03
C.D. at 5%	0.09
D - Duration of soaking	
D_1: 12 hours	8.69
D_2: 24 hours	8.77
S.EM.±	0.02
C.D. at 5%	0.07

4. CONCLUSION

Based on the above investigation, it can well be concluded that soaking freshly extracted kagzi lime seeds in an aqueous solution of 500 ppm GA_3 for 12 hrs emerged as the best treatment for ensuring early germination, higher seedling vigour index and survival percentage. Further, the maximum germination percentage and number of leaves were recorded when freshly extracted kagzi lime seeds were soaked in 500 ppm GA_3 for 24 hrs. Thus, nurserymen can raise healthy kagzi lime rootstocks in a shorter duration by soaking freshly extracted kagzi lime seeds in an aqueous solution of 500 ppm GA_3 for a duration of 12/24 hrs prior to sowing.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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