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Promoting seedling growth in kagzi lime through pre-sowing treatments

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Abstract
An experiment was conducted to study the effect of gibberellic acid on seedling growth in Kagzi lime at Regional Horticultural Research Station, Navsari Agricultural University, Navsari during the year 2016-17. The experiment was laid out in a Completely Randomized Design with factorial concept with sixteen treatments and three repetitions. Freshly extracted kagzi lime seeds (S_1) and those stored for 15 days (S_2) were soaked for 12 hours (D_1) and 24 hours (D_2) in aqueous solution of gibberellic acid at different concentrations i.e. 200 ppm (G_1), 300 ppm (G_2), 400 ppm (G_3) and 500 ppm (G_4). Freshly extracted kagzi lime seeds had significantly higher leaf area, total chlorophyll content, tap root length, tap root diameter, shoot dry weight and total dry weight. For all above mentioned characters, a soaking duration of 12 hours proved significantly better over 24 hours. GA_3 at 500 ppm recorded significantly the highest leaf area, total chlorophyll content and tap root diameter. Further, for tap root length, shoot dry weight and total dry weight, significantly highest values were recorded under 400 ppm GA_3 treatment. Shoot root dry weight ratio was significantly lower in case of freshly extracted seeds, GA_3 at 200 ppm and a soaking duration of 12 hours when taken as separate factors. The interaction effect between storage treatments, different concentrations of GA_3 and soaking duration was found non-significant for all parameters chosen in this study.

Keywords: Kagzi lime, GA_3, leaf area, chlorophyll content, tap root length

Introduction
Citrus fruits have a prominent place among popular and extensively grown tropical and sub-tropical fruits. Among them, kagzi lime (*Citrus aurantifolia* Swingle) is an important citrus crop which is grown on commercial scale in India. It is a member of the family *Rutaceae* and originated in the tropical and sub tropical regions of South East Asia, particularly India and China. In India, kagzi lime covers an area of 259 thousand hectares with a production of 2789 thousand MT, being the sixth largest producer of citrus in the world contributing 4.8 per cent share in production (Anon., 2017) [3]. Gujarat is the second largest producer of kagzi lime in the country contributing about 16% to the total production. It produces about 605 thousand MT of kagzi lime from an area of 46 thousand hectares with a productivity of 13 MT/ha. Kagzi lime is grown in almost all districts of Gujarat (Anon., 2018) [2].

Kagzi lime is used for table purpose in daily life of Indians for flavouring vegetable curries, salad, fish and meat. It is also used in the preparation of refreshing cold drinks especially to beat the summer heat. Kagzi lime is commercially propagated by seeds as it is the easiest and cheapest method of propagating this crop. Kagzi lime propagation is hampered by high mortality at the nursery stage moreover; seeds lose their viability very soon. Nurserymen and growers thus face problems like lower seed germination and poor vigour of seedling. The growth of kagzi lime seedlings is also very slow and therefore to raise plants within the shortest possible time, growth has to be accelerated for which pre sowing treatments can be employed.

Plant growth regulators are often employed to increase the germination percentage of seeds and improve the subsequent growth of seedlings. In this regard, seed treatment with GA_3 has given remarkable results in mango (Patel *et al.,* 2016) [18], papaya (Mishra *et al.,* 2017) [12] and kagzi lime (Dilip *et al.,* 2017) [7]. Nevertheless, there is wide variation in the range of GA_3 concentration (50-700 ppm) and the duration of soaking (6-40 hrs) which promote germination.

It was therefore felt necessary to study the effect of storage treatment, gibberellic acid and duration of soaking on seedling growth of kagzi lime.

Material and Methods

This experiment was conducted at Regional Horticultural Research Station, Navsari Agricultural University, Navsari, Gujarat during 2016-17 in a net house and evaluated in completely randomized design based on factorial concept. Seeds were extracted from fully mature and healthy fruits of acid lime cv. Kagzi lime, washed in running water and dried under shade for about an hour. Treatment details were as under

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)
D_1: 12 hours
D_2: 24 hours

Seeds were stored in an aluminium foil for 15 days. 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, labelled with tags and placed at proper spacing. Seeds were irrigated immediately after sowing using a rose-can and subsequently watered as and when required. Observations were recorded from five randomly selected and labelled seedlings in each treatment in a repetition. The data obtained from all plots per repetition under each treatment were averaged and reported. Leaf area was calculated using a leaf area meter and expressed in centimetre square. It was recorded at an interval of 30 days up to 4 months after sowing. Total Chlorophyll Content was estimated using chlorophyll measuring meter and expressed as SPAD value. Tap root length (cm) was measured using a measuring tape and tap root diameter (cm) was measured using a vernier callipers. The average dry weight of seedling from each treatment was recorded after oven drying for 24 hours and thereafter, the shoot root dry weight ratio (g/g) was calculated from the formula

$$\text{Root shoot dry weight ration} = \frac{\text{Dry shoot weight (g)}}{\text{Dry root weight (g)}}$$

All parameters except leaf area were calculated after 120 days of sowing.

Results and Discussion

Leaf area

The data regarding leaf area of kagzi lime seedlings presented in Table 1 revealed significant differences due to storage conditions, GA_3 treatment and soaking duration. Leaf area was higher at 60, 90 and 120 days (6.37, 8.04 and 9.57 cm^2) in freshly extracted seeds as compared to stored seeds. The treatment comprising of 500 ppm GA_3 at 60, 90 and 120 days after sowing resulted in maximum leaf area (7.06, 8.31 and 10.17 cm^2), respectively. It was at par with the treatment comprising GA_3 at 400 ppm (6.54, 8.07 and 9.92 cm^2) at 60, 90 and 120 days after sowing. This can be very well attributed to the higher number of leaves under these treatments. Further, it is a well-known fact that gibberellic acid promotes cell division, elongation and expansion which may have increased leaf initiation and leaf growth (Taiz and Zeiger, 2002) [21]. These results are in conformity with those of Rahemi and Baninasab (2000) [19] in pistachio and Joshi *et al.* (2015) [10] in acid lime. Further, leaf area was higher at 60, 90 and 120 days (6.35, 8.05 and 9.57 cm^2) in 12 hours soaking treatment. The interaction effect of seed storage, GA_3 treatments and duration of soaking was found non- significant with respect to leaf area.

Total chlorophyll content

The total chlorophyll content in kagzi lime seedlings was significantly influenced by storage conditions, imposition of GA_3 treatments and duration of soaking (Table 1). The maximum total chlorophyll content was observed at 120 days (78.52) in freshly extracted seeds, when treated with GA_3 500 ppm (81.38) and under 12 hours soaking (78.47) treatment. The interaction effect of storage conditions, different treatments of GA_3 and duration of soaking was found non-significant with respect to total chlorophyll content.

Table 1: Effect of pre-sowing treatments on leaf area and total chlorophyll content of kagzi lime seedlings

Treatments	Leaf area (cm^2)			Total chlorophyll content (SPAD value)
	60 DAS	90 DAS	120 DAS	120 DAS
S - Seed Storage				
S_1 : Freshly extracted seeds	6.37	8.04	9.57	78.52
S_2 : Seeds stored for 15 days	6.21	7.86	9.36	74.29
S. Em.±	0.05	0.06	0.07	1.43
C.D. at 5%	0.13	0.18	0.21	4.11
G - GA_3 Levels				
G_1: 200 ppm	5.51	7.59	8.64	71.40
G_2: 300 ppm	6.03	7.83	9.14	74.77
G_3: 400 ppm	6.54	8.07	9.92	78.07
G_4: 500 ppm	7.06	8.31	10.17	81.38
S.Em.±	0.06	0.09	0.10	2.02
C.D. at 5%	0.18	0.26	0.29	5.81
D – Duration of Soaking				
D_1: 12 hours	6.35	8.05	9.57	78.47
D_2: 24 hours	6.22	7.85	9.36	74.34
S.Em.±	0.05	0.06	0.07	1.43
C.D. at 5%	0.13	0.18	0.21	4.11

Interaction Effect				
S x G				
S. Em.±	0.09	0.13	0.15	2.85
C.D. at 5%	NS	NS	NS	NS
S x D				
S. Em.±	0.06	0.09	0.11	2.02
C.D. at 5%	NS	NS	NS	NS
G x D				
S. Em.±	0.09	0.13	0.15	2.85
C.D. at 5%	NS	NS	NS	NS
S x G x D				
S. Em.±	0.13	0.18	0.21	4.04
C.D. at 5%	NS	NS	NS	NS
CV%	3.51	3.88	3.98	9.15

Tap root length

A perusal of Table 2 indicated significant differences in tap root length due to storage conditions, different GA_3 treatments and duration of soaking. After 120 days of sowing tap root length was higher in freshly extracted kagzi lime seeds (23.02 cm) as compared to stored seeds. Among GA_3 treatments, application at 400 ppm recorded maximum tap length (24.88 cm) which was at par with GA_3 at 500 ppm (23.47 cm). GA_3 treatment may have resulted into increased production of photosynthates and their translocation through phloem to the root zone could be responsible for the increase in tap root length. Gibberellic acid also induces cell division and elongation of existing cells which in turn may have contributed to increased root length. These results are in close agreement with Shaban (2010) [20] in mango, Brijwal and Kumar (2013) [6] in guava, Vasantha *et al.* (2014) [22] in tamarind and Megha Shukla *et al.* (2012) [11] in kagzi lime. Twelve hours soaking treatment resulted in longer tap roots (23.02 cm). The interaction effect of storage treatments, different GA_3 treatments and duration of soaking was found non-significant with respect to tap root length.

Tap root diameter

Significant differences were observed in tap root diameter due to pre-sowing treatments (Table 2). Tap root diameter after 120 days was higher in freshly extracted seeds (1.71 cm). As far as GA_3 treatments were concerned, the maximum tap root diameter (1.75 cm) was recorded when kagzi lime seeds were treated with GA_3 500 ppm and it was at par with GA_3 at 400 ppm (1.72 cm). The significant increase in tap root diameter can also be explained based on the reasons provided under tap root length. Higher tap root diameter (1.71 cm) was noticed when kagzi lime seeds were soaked for 12 hours. The interaction effect of these three factors was found non-significant with respect to tap root diameter.

Table 2: Effect of pre-sowing treatments on tap root length, tap root diameter, shoot dry weight, total dry weight and shoot root dry weight of kagzi lime seedlings

Treatments	Tap root length (cm)	Tap root diameter (cm)	Shoot dry weight (g)	Total dry weight (g)	Shoot root dry weight ratio (g/g)
S - Seed storage					
S_1 : Freshly extracted seeds	23.02	1.71	3.52	7.39	3.82
S_2 : Seeds stored for 15 days	22.52	1.70	3.40	7.22	3.87
S.Em.±	0.17	0.01	0.02	0.04	0.02
C.D. at 5%	0.49	0.02	0.06	0.11	0.05
G - Levels of GA_3					
G_1: 200 ppm	20.66	1.66	2.83	6.56	3.73
G_2: 300 ppm	22.07	1.69	3.25	7.06	3.81
G_3: 400 ppm	24.88	1.72	4.09	7.97	3.88
G_4: 500 ppm	23.47	1.75	3.67	7.63	3.96
S.Em.±	0.24	0.01	0.03	0.05	0.03
C.D. at 5%	0.69	0.02	0.08	0.15	0.07
D - Duration of Soaking					
D_1: 12 hours	23.02	1.71	3.50	7.37	3.82
D_2: 24 hours	22.52	1.70	3.42	7.24	3.87
S.Em.±	0.17	0.01	0.02	0.04	0.02
C.D. at 5%	0.49	0.02	0.06	0.11	0.05
Interaction Effect					
S x G					
S.Em.±	0.34	0.01	0.04	0.07	0.04
C.D. at 5%	NS	NS	NS	NS	NS
S x D					
S.Em.±	0.24	0.01	0.03	0.05	0.03
C.D. at 5%	NS	NS	NS	NS	NS
G x D					
S.Em.±	0.34	0.01	0.04	0.07	0.04
C.D. at 5%	NS	NS	NS	NS	NS
S x G x D					
S.Em.±	0.48	0.02	0.05	0.11	0.05
C.D. at 5%	NS	NS	NS	NS	NS
CV%	3.66	1.70	2.75	2.51	2.31

Shoot dry weight

Data presented in Table 2 indicates that shoot dry weight at 120 days was significantly influenced by different treatments. Shoot dry weight was higher in case of freshly extracted seeds (3.52 g). Similarly, seeds were soaked for 12 hours had higher shoot dry weight (3.50 g). Amongst GA_3 treatments, application at 400 ppm (4.09 g) gave the maximum shoot dry weight which was at par with GA_3 at 500 ppm (3.67 g). Application of GA_3 treatments recorded significantly higher shoot length, number of leaves and leaf area which may have contributed to an increase in shoot dry weight (Pampanna and Sulikeri, 1999) [14]. These results corroborate the findings of Pandey (1992) [15] in citrus and Anjanaw *et al.* (2013) [1] in papaya. The interaction effect of all the factors was found non- significant with respect to shoot dry weight.

Total dry weight

It is evident from Table 2 that total dry weight of kagzi lime seedlings was significant affected by storage conditions, treatments of GA_3 and duration of soaking. At 120 days, higher total dry weight was observed in freshly extracted kagzi lime seeds (7.39 g). Further, seeds treated GA_3 at 400 ppm had the maximum total dry weight (7.97g). Whereas, soaking seeds for 12 hours (7.37 g) recorded higher total dry weight. The increased total dry weight may be due to higher shoot length, root length, number of leaves and leaf area under these treatments. All of these may have led to the overall assimilation and redistribution of photosynthates within the plant, thereby promoting growth and development (Brian and Hemming, 1955) [5]. Identical results were obtained by Padma Lay *et al.* (2013) [13] in papaya and Gurung *et al.* (2014) [8] in passion fruit.The interaction effect of these three factors were found non- significant with respect to total dry weight.

Shoot root dry weight ratio

The shoot root dry weight ratio is an effective and safe index to evaluate seedling quality, as described by Parviainem (1981) [17]. It is an important measure for seedling survival but requires destructive sampling. Lower values of shoot root dry weight ratio indicate a healthy seedling with better chances of survival (Jaenicke, 1999) [9]. The data regarding root shoot dry weight ratio of kagzi lime seedlings presented in Table 2 revealed significant differences due to storage conditions. Lower shoot root dry weight ratio was observed in freshly extracted kagzi lime seeds (3.82) and with seeds soaked in GA_3 at 200 ppm (3.73) at 120 days. It was at par with the treatment comprising GA_3 at 300 ppm (3.81). Twelve hours soaking treatment reported lower root shoot dry weight ratio (3.82). With regard to statistical analysis, the interaction between storage treatments, different GA_3 treatments and duration of soaking was found non-significant for shoot root dry weight ratio.

Conclusion

Based on the current investigation, it can be deduced that in case of kagzi lime, seedlings raised from freshly extracted seeds were superior over stored seeds and a soaking duration of 12 hours was superior over 24 hours for all parameters considered in this study. Between different levels of GA_3, 400 ppm emerged as the best treatment for ensuring better seedling growth with regard to leaf area, total chlorophyll content and tap root diameter. Hence, nurserymen can employ any of these findings to promote vegetative growth in kagzi lime seedlings.

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