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Effect of growing media and GA_3 on seed germination, growth and survival of Acid lime (*Citrus aurantifolia* Swingle) var. Kagzi seedling

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Abstract
A field experiment was carried out at Fruit Research Station Imalia, Department of Horticulture, College of Agriculture, JNKVV Jabalpur to assess the effect of growing media and GA_3 on seed germination, growth and survival of Acid lime (*Citrus aurantifolia* Swingle) var. Kagzi. The treatments comprised five levels of seeds soaking in GA_3 solutions and four different growing media were arranged thrice in Randomised Block Design. It was observed that the seed soaked with 150 ppm concentration of GA_3 and growing media comprising soil + slurry enrich with Azotobacter significantly improve the parameters with regard to day taken to initiate germination, to attained 50% germination, germination at 30 days after sowing, height of shoot, stem girth, number of leaves, total length of seedling, fresh weight of shoots, dry weight of shoots, fresh weight of roots, dry weight of roots, length of taproot, number of secondary roots, seedling vigour index I, seedling vigour index II, survival percentage of seedling. The higher value of physiological parameter were also recorded with seed soaked with 150 ppm concentration of GA_3 and growing media comprising soil + slurry enrich with Azotobacter individually.

Keywords: Citrus, GA_3, growing media, azotobacter, PSB

Introduction
Citrus is one of the largest and most important fruits of tropical and subtropical regions. It is a native to India and South eastern China. It occupies 3rd ranks after mango and banana in India. Citrus is a member of the family Rutaceae, subfamily Aurantioidae. Small-fruited kagzi limes are classified botanically under *Citrus aurantifolia.* Kagzi lime is also called as acid lime. It occupies the highest commercial importance of all the available types of limes and lemons. Kagzi limes are mostly used as fresh fruits for the table purpose, manufacture of beverages, industrial and medicinal purpose. It is the rich source of vitamin C and also contains vitamin B, pectin, minerals and other nutritive substance which are required for human health. Lime juice is used for scurvy diseases. They also have laxative effect on the digestive system. The fruit have long post harvest life and can withstand rough handling during marketing.
Lime is usually propagated by seed while seed germination is slow and erratic. The possible reasons of slow germination are presence of growth inhibitors and physical resistance of seed coat to radical protrusion (Elza 1949) [6]. There is considerable evidence that gibberellins may promote the germination of various seeds in different ways. The growing media is one of the important environmental factors, which plays an important role in growth and survival of seedlings. Growing media must retain moisture, nutrients and provide support to seedling. Organic matters are required for successful seedling production of fruit and vegetables. Citrus thrives best in a soil with a pH slightly below the neutral point. Vigorous growth is needed to face the seasonal hazards and this is entirely based on chemical and physical characteristics of the media. Optimum water holding capacity, electrical conductivity, better aeration, and organic matter of media may help in better seedling stand and plant growth of citrus seedling. Producing plants from seeds is most important propagation method. Seedling propagation involves careful management of seeds, seed storage period, germination conditions and knowledge of requirements of seed for germination as well as the overall growth.

Material and Methods

The present investigation was carried out in the fruit research station Imalia, department of horticulture, college of agriculture, JNKVV Jabalpur (M.P.). There were twenty treatment combinations comprising five levels of seed priming with GA_3 (G_1: distil water, G_2: $GA_3$50 ppm seed soaking, G_3: $GA_3$100 ppm seed soaking, G_4: $GA_3$150 ppm seed soaking and G_5: $GA_3$200 ppm seed soaking) and four growing media i.e. (M_1: Soil+ slurry, M_2: soil+ slurry enrich with PSB, M_3: soil+ slurry enrich with AZO and M_4: soil+ slurry enrich with PSB and AZO). These treatments were arranged in a randomized block design with three replications. These poly bags after seed sowing were placed in polyhouse, watered regularly with the help of watering rose can to keep medium moist and observations were recorded as per study schedule. Five plants in each treatment were selected at random for periodical biometric observations on height of seedling was measured with the help of meter scale from ground level to growing tip, number of leaves per seedling were counted every month up to 150 days, diameter of stem was measured with the help of digital verniear calliper, fresh and dry weight of seedling was measured by electronic balance and average weight calculated, length of longest tap root was measured from the point of initiation of roots to the tip of the root with the help of a meter scale, after washing the soil ball total number of secondary roots were counted, diameter of tap root was measured near the point of initiation of root with the help of verniear calliper.

Results and Discussion

Seed Germinability

It was observed the seed soaking in 150 ppm solution GA3 resulted early germination (6.09 DAS) and 10.32 days to taken attain 50% germination with 82.42% germination at 30 days after sowing. The increase in germination may be due to involvement of GA3 in the activities of cycological enzyme along with increase in cell wall plasticity and better water observation. These findings are also supported by Dhankhar and Singh (1996) [5].

As regards the growing media minimum days (6.44) taken to initiation of germination and to attain 50% germanibility with 79.00% of seed germination at 30 days after sowing. This is due to that physical and nutritional condition of media which initiated the early germination, the finding is similar to that of Vasu *et al*. (2010) [14] who reported that 10 g inoculation of Azotobacter showed 100% germination and also reduce the average time to taken to start germination.

The interaction effect of seed growing media and seed soaking with GA3 did not showed significant effect of seed germinability. However, the minimum days (5.86) taken to start germination and to attain 50% germination in 9.17 days alongwith 85.67% germination after at 30 days after sowing with M_3 G_3 comprising soil + slurry enrich with Azotobacter and seed soaked in 150 GA_3 concentration. The increase in germination percentage due to involvement of GA_3 in the activation of cytological enzymes along with increase in cell wall plasticity and better water absorption (Dhanker and Singh, 1996) [5].

Growth Parameters

As regards, the growth of seedling at 150 days after sowing were recorded and data revealed that the maximum value of height of shoot (27.71 cm), number of leaves (24.47), girth of stem (3.18 mm), length of seedling (51.93 cm), shoot fresh (4.03g) and dry (1.51g) weight, root fresh (1.57g) and dry (0.54 g) weight, seedling vigour index -1 (3922.41 cm) and seedling rigour-II (162.38 g) as well as higher percentage of 82.84 seedling survival were recorded when seed soaked before sowing in concentration of 150 ppm GA3. It was due to additional GA3, activated ∝ - amylase which digested the available carbohydrate into simple sugar so that energy and nutrition were easily available to faster growing seedlings. Increase in plant height due to GA3 has also been reported by Shant and Rao (1973) [12]. Gibberellins are well known for inter nodal cell elongation, thereby increase in seedling length and other parameters. This finding is supported by Wanyama *et al.* (2006) [15].

The growing media had given significant effect of growth and survivability of seedlings. The maximum value of seedling height (27.71cm), number of leaves (24.47), girth of stem (3.18), length of seedlings (51.93cm) number of roots (45.12), fresh and dry weight of shoots (4.03 & 1.51g), fresh and dry weight of roots (1.57 & l.57g), seedling vigrour index -1 (3922.41g), seedling vigrour index -11 (162.38g) with 82.84% seedling survival was recorded with growing media of soil +slurry enrich with Azotobacter. The probable reason may be that the media created sufficient porous space, to let the excess water drain away and pertaining adequate aeration for the better seedling growth. The improvement in vegetative character might be due to the ability of Azotobacter to fix atmospheric nitrogen which may share its role in increasing the percentage of mineral nutrient in soil. The media comprising soil+ slurry enrich with Azotobacter improve soil texture, structure, water holding capacity, activity of useful micro flora and fauna, maintained soil temperature and improve soil health and nutrient status of media for better root growth. These findings supported by the findings of Hartmann and Kester (1997) [7]

Application of soil + slurry + Azotobacter + 150 ppm GA_3 had given significantly maximum height of shoot (31.49 cm), number of leaves per seedling (26.53), girth of stem (3.52 mm), total length of seedling (57.86 cm), fresh weight of shoots (4.77g), dry weight of shoots (1.87g), seedling vigour index I (4996.93cm) and seedling vigour index II (215.93g), survival percentage of seedling (88.72) after 150 days of sowing. The increase in the shoot growth parameters due to application of soil + slurry + Azotobacter + 150 ppm GA_3 could be attributed to the conducive effect of his medium mixture on water holding capacity, porosity, soil aeration and supplying substantial amount of nutrient specially nitrogen and micro nutrients for good root and shoot growth over control (Chopde *et al*., 1999) [3]. Increase in number of leaves might be mainly due to corresponding increase in plant height (Govind and Chandra, 1993). This treatment also has higher leaf chlorophyll content which might certainly improved the photosynthetic rate, dry matter production and their by more fresh and dry weight of shoot. The increase in height of seedling with inoculation of *Azotobacter* may be due to fact that it stimulates nutrient uptake especially nitrogen which has role in the assimilation of numerous amino acids that are subsequently incorporated in proteins and nucleic acid, which provides framework for chloroplast, mitochondria and other structures in which the most of the biochemical reactions occurs (Awasthi *et al*., 1996) [2]. The leaf size and chlorophyll content were maximum in *Azotobacter* treatment, it may be because of synthesis of chlorophyll and the higher absorption of nutrients especially nitrogen as a result of inoculation with *Azotobacter* (Joolka *et al*., 2004) [8].

Root parameters

The growth of roots at 150 days after sowing were recorded and data revealed that the higher number of roots (45.12) and maximum weight of roots (fresh 1.57g) and (dry 0.54 g) were recorded when seed soaked before sowing in concentration of 150 ppm GA_3. Similarly, growing media had given significant effect of growth of seedlings root. The maximum number of roots (44.63) and fresh and dry weight of roots (1.57 and l.57g), was recorded with growing media of soil + slurry enrich with Azotobacter.

The length of longest tap roots, number of secondary roots, fresh weight of roots, dry weight of roots and increased significantly due to application of soil + slurry + Azotobacter + GA_3. Likewise, at 150 day after sowing the length of number of roots (48.68), fresh weight of roots (1.87g), dry weight of roots (0.61g) were recorded maximum with treatment comprising soil + slurry enrich with Azotobacter and seed sown after soaking in 150ppm concentrations of GA_3. The beneficial effect on root growth parameters due to application of the medium treatment consisting of soil + slurry + Azotobacter + GA_3 might be due to improved soil texture, structure, porosity, water holding capacity, activity of useful soil micro fauna and flora, maintained soil temperature and improved soil health and nutrient status of medium (Hartmann and Kester, 1997) [7].

Physiological Parameter

The effect of GA3 had significant effect on physiological parameters and higher value of LA1 (0.65), LAD (6135.02), LTR (45.24) and EI (0.40) were recorded with seed soaked in 150 ppm concentration of GA3. This was higher ascribed to higher magnitude increase in parameter associated with leaf area. The findings are supported by Throne (1996) and Munde and Gajbhiye (2010) [9]. The growing media significantly influenced the physiological parameters and significantly higher value of LAI (0.74), LAD (6982.25), LTR (38.44) was recorded with soil +slurry enrich with Azotobacter and PSB. The present investigation showed that the application of growing media had higher magnitude for LAD over remaining treatments which was attributed to increase in LA and LAI, influenced by treatment. This also suggested the role of nitrogen enhancing persistence and longevity of LA which is key factor in terms of photosynthesis productivity of the plants, that assimilates higher amount of photosynthets production and if the mobilization is proper to the sink it will enhance the economic productivity. These findings are supported by findings of Throne (1996), Dasappa (1990) [4], Roy *et al.* (2011) [11] and Peng *et al.* (2013) [10].

Table 1: Effect of growing media and GA_3 on seed germination and growth parameters of acid lime seedlings

Treatments	Days taken to start germination	Days taken to 50% germination	Germination at 30DAS (%)	at 150 day after sowing				
				Height of shoot (cm)	Number of leaves per seedling	Girth of stem (mm)	Length of seedling (cm)	Roots / seedling
G_0 -Water	7.09	12.56	67.42	19.27	18.30	2.45	37.89	36.92
G_1 -50ppm	6.89	11.50	69.33	23.34	20.71	2.61	44.96	41.47
G_2 -100 ppm	6.74	10.64	71.67	24.18	22.48	2.92	47.57	43.99
G_3 -150 ppm	6.09	10.32	82.42	27.71	24.47	3.18	51.93	45.12
G_4-200 ppm	6.51	11.09	70.83	24.01	21.87	2.81	48.38	44.53
S.Em±	0.09	0.18	2.13	0.65	0.33	0.09	1.01	5.21
CD at 5%	0.28	0.50	6.11	1.86	0.95	0.24	2.89	1.49
M_1 Soil+ slurry	6.99	12.08	66.27	20.40	19.65	2.45	41.18	40.43
M_2 Soil+ slurry enrich with PSB	6.69	11.53	71.00	22.56	20.79	2.61	44.01	41.52
M_3 Soil+ slurry enrich with AZO	6.44	10.54	79.00	27.70	23.39	3.18	51.76	44.63
M_4 Soil+ slurry enrich with PSB &AZO	6.55	10.75	73.40	24.15	22.55	2.81	46.87	43.05
S.Em±	0.08	0.16	1.91	0.58	0.29	0.08	0.90	0.47
CD at 5%	0.25	0.49	5.46	1.66	0.85	0.22	2.58	1.33
M_1G_0	7.53	13.08	58.67	17.77	16.87	2.18	34.39	35.54
M_1G_1	7.13	12.25	64.00	19.20	19.52	2.39	40.12	40.98
M_1G_2	7.08	11.67	66.67	19.95	20.33	2.74	41.50	40.90
M_1G_3	6.25	11.75	74.67	22.64	23.17	2.91	45.65	42.14
M_1G_4	6.93	11.64	67.33	22.43	18.37	2.29	44.24	42.56
M_2G_0	7.05	12.50	65.67	18.57	18.00	2.58	36.02	36.50
M_2G_1	6.83	12.08	69.33	20.13	19.92	2.62	41.20	41.77
M_2G_2	7.00	11.38	68.00	23.64	22.33	2.94	45.53	42.88
M_2G_3	5.97	10.92	84.00	26.47	22.37	3.01	50.90	43.00
M_2G_4	6.58	10.73	68.00	23.99	21.38	2.95	46.49	43.45
M_3G_0	6.75	12.50	76.00	21.79	19.43	2.61	42.05	38.11
M_3G_1	7.00	10.08	77.33	27.49	22.85	2.78	49.23	42.44
M_3G_2	6.38	9.21	77.33	30.10	23.97	3.14	56.26	47.56
M_3G_3	5.87	9.17	85.67	31.49	26.53	3.52	57.86	48.68
M_3G_4	6.21	11.75	78.67	27.65	24.17	3.16	53.43	46.33
M_4G_0	7.05	12.17	69.33	18.95	18.91	2.42	39.11	37.51
M_4G_1	6.58	11.58	68.33	26.52	20.53	2.64	49.27	40.70
M_4G_2	6.50	10.28	68.33	23.04	23.27	2.85	45.52	44.62
M_4G_3	6.27	9.45	85.33	30.25	26.47	3.30	54.90	46.67
M_4G_4	6.33	10.25	69.33	21.97	23.57	2.84	45.51	45.76
S.Em±	1.19	0.35	4.27	1.30	0.66	0.17	2.029	1.04
CD at 5%	NS	1.01	NS	3.719	NS	NS	NS	NS

Table 2: Effect of growing media and GA_3 on physiological and seedling survival of acid lime seedlings

Treatments	at 150 day after sowing										
	LAI	LAD	LTR	EI	Weight of shoots		Weight of roots		Seedling Vigour Index I (cm)	Seedling Vigour index II (g)	Seedling Survival (%)
					Fresh (g)	Dry (g)	Fresh (g)	Dry (g)			
G_0 -Water	0.50	4667.22	48.23	0.17	2.12	0.70	1.05	0.28	2561.10	67.00	75.95
G_1 -50ppm	0.57	5474.32	43.36	0.19	2.54	0.96	1.18	0.35	3068.06	90.18	76.95
G_2 -100 ppm	0.64	6017.32	39.73	0.21	3.43	1.32	1.39	0.42	3590.08	129.75	77.89
G_3 -150 ppm	0.65	6135.02	45.24	0.40	4.03	1.51	1.57	0.54	3922.41	162.38	82.84
G_4-200 ppm	0.60	5692.90	34.19	0.33	2.65	1.22	1.24	0.38	3712.86	132.19	79.96
S.Em±	0.01	98.30	0.90	0.08	0.09	0.07	0.06	0.03	119.15	9.35	3.06
CD at 5%	0.02	281.43	2.58	NS	0.28	0.20	0.18	0.09	341.10	18.18	NS
M_1 Soil+ slurry	0.44	4063.01	54.10	0.14	1.88	0.92	0.97	0.34	2794.00	96.15	74.80
M_2 Soil+ slurry enrich with PSB	0.52	4859.04	55.63	0.25	2.88	1.06	1.17	0.36	3245.61	112.70	76.78
M_3 Soil+ slurry enrich with AZO	0.68	6485.12	31.43	0.35	3.71	1.36	1.59	0.50	4032.37	145.81	83.21
M_4 Soil+ slurry enrich with PSB &AZO	0.74	6982.25	38.44	0.30	3.36	1.18	1.44	0.38	3411.63	110.56	79.81
S.Em±	0.01	87.92	0.81	0.08	0.19	0.06	0.56	0.03	106.57	12.69	2.73
CD at 5%	0.02	251.72	2.30	NS	0.55	0.18	0.16	0.08	305.09	NS	NS
M_1G_0	0.38	3319.47	60.52	0.11	1.37	0.51	0.62	0.25	1932.25	57.51	70.90
M_1G_1	0.43	4035.40	57.33	0.08	1.52	0.75	0.90	0.31	2497.68	81.28	74.47
M_1G_2	0.41	3839.23	55.82	0.09	1.62	1.14	1.16	0.31	3059.75	109.65	75.37
M_1G_3	0.49	4610.63	57.66	0.18	3.40	1.21	1.26	0.47	3300.20	112.24	77.23
M_1G_4	0.48	4510.30	39.18	0.23	1.51	0.97	0.89	0.37	3180.14	120.07	76.02
M_2G_0	0.43	4066.07	51.26	0.14	1.54	0.67	1.07	0.25	2489.11	68.25	74.17
M_2G_1	0.47	4451.73	41.37	0.16	2.40	0.88	1.19	0.32	2915.84	84.83	74.75
M_2G_2	0.48	4499.81	39.91	0.25	3.72	1.24	1.27	0.37	3250.07	111.23	76.57
M_2G_3	0.52	4933.33	52.17	0.28	3.81	1.36	1.33	0.51	3653.55	170.70	80.00
M_2G_4	0.67	6344.23	38.42	0.41	2.91	1.17	0.98	0.33	3922.47	128.47	78.39
M_3G_0	0.59	5547.80	36.86	0.24	3.03	0.83	1.29	0.34	3196.28	71.84	80.02
M_3G_1	0.60	5914.00	34.56	0.29	3.23	1.21	1.46	0.45	3758.38	125.90	79.58
M_3G_2	0.74	6982.93	26.24	0.27	4.34	1.60	1.65	0.57	4090.28	162.56	82.62
M_3G_3	0.79	7425.30	33.72	0.53	4.77	1.87	1.87	0.61	4996.93	215.93	88.72
M_3G_4	0.70	6555.57	25.79	0.40	3.16	1.30	1.69	0.52	4119.99	152.80	85.11
M_4G_0	0.61	5735.53	44.28	0.19	2.55	0.80	1.23	0.28	2629.79	70.39	78.73
M_4G_1	0.79	7496.13	40.18	0.21	3.02	1.01	1.20	0.31	3100.33	68.73	78.97
M_4G_2	0.93	8747.30	36.96	0.23	4.05	1.29	1.51	0.43	3960.23	135.57	76.98
M_4G_3	0.80	7570.80	37.40	0.60	4.15	1.58	1.82	0.58	3738.96	150.68	85.39
M_4G_4	0.57	5361.50	33.86	0.26	3.01	1.22	1.41	0.30	3628.86	127.43	78.96
S.Em±	0.02	196.60	1.80	0.17	0.19	0.14	0.13	0.07	238.30	12.70	6.11
CD at 5%	0.06	562.86	5.15	NS	0.554	NS	NS	NS	NS	NS	NS

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