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Effect of Growing Media and GA_3 on Seed Germination and Seedling Growth of Acid Lime (*Citrus aurantifolia* Swingle) Cv. Vikram

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A B S T R A C T

The experiment consist of six growing media viz. (M_1) soil (M_2) soil + sand (1:1), (M_3) soil + sand + vermicompost (1:1:1), (M_4) soil + vermicompost (1:1), (M_5) soil + sand + azotobacter (1:1+5g/ kg AZO), (M_6) soil + sand + vermicompost + azotobacter (1:1:1+5g/kg AZO) and three concentration of gibberellic acid i.e. (G_1) water soaking, (G_2) GA_3 50 ppm, (G_3) GA_3 100ppm having 18 treatment combinations. Among the growing media, soil + sand + azotobacter (1:1+5g/ kg AZO) and among the seed treatment, GA_3 (50ppm) were proved most promising as compare to others. Among the various treatment combinations, the M_5G_1 treatment combination (soil + sand + azotobacter; 1:1+5g/ kg AZO + 50ppm GA_3) was proved most superior over rest of the treatment combinations with respect to early seed germination (9.33 days), early 50% seeds germination (19.66 days) maximum percentage of germination at 30 days(76.68 %), growth parameters like seedling height (5.96, 7.22, 8.36 cm), number of leaves per seedling (7.33, 9.66, 11.66), girth of stem (1.50, 1.85, 2.74 mm) and survival percentage (76.66%) at 30, 60, 90 and 120 DAS respectively.

Introduction

Citrus fruits are one of the most delicious fruits belonging to the family Rutaceae. Acid lime (*Citrus aurantifolia* Swingle) is grown commercially worldwide in tropical, sub-tropical and some temperate regions. India is the largest producer of acid lime in the world. It is successfully grown in state of Andhra Pradesh, Maharashtra, Tamilnadu, and Madhya Pradesh. It is a good source of vitamin C and has good antioxidant properties. Acid 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). The growth of citrus seedlings is influenced by many factors such as cultural practices, different media combinations, application of bio fertilizers, fertilizers and the use of growth regulators. Citrus rootstocks are propagated from seeds. Citrus seeds are easily obtainable, relatively inexpensive, plentiful, and grow true to type. Therefore, an attempt has been made to utilize the effect of different

medium combination with or without *Azotobacter* for growth and development of acid lime seedlings.

Materials and Methods

The experiment was carried out at Fruit Research Station, Imalia, Department of Horticulture, College of Agriculture, JNKVV, Jabalpur (M.P.) during September 2018 to January 2019. The experiment comprised of six growing media viz (M_1) soil (M_2) soil + sand (1:1), (M_3) soil + sand + vermicompost (1:1:1), (M_4) soil + vermicompost (1:1), (M_5) soil + sand + azotobacter (1:1+5g/ kg AZO), (M_6) soil + sand + vermicompost + azotobacter) (1:1:1+5g/kg AZO) and three concentration of gibberellic acid i.e. (G_1) water soaking, (G_2) GA_3 50 ppm, (G_3) GA_3 100ppm having 18 treatment combinations. The experiment was laid out in poly bags in factorial randomized block design with three replications. Observations were recorded using standard procedure and statistically analysed.

Germination percentage

The germination in each treatment was recorded at 30 days after sowing. Number of seedlings were counted and expressed as germination percentage.

$$\text{Germination percent} = \frac{\text{Total no. of seeds germinate}}{\text{Total no. of seeds sown}} \times 100$$

Number of days taken for 50 per cent germination

The number of days taken to reach 50 per cent of germination was recorded in each treatment from the germination percentage calculated for each observation.

Growth parameter-The fallowing observations were recorded at 120 days after sowing. Randomly selected five plants were tagged for following observations.

Height of seedling (cm) – Height was measured from ground level to the tip of opened leaf.

Girth of stem (mm) – The girth of stem was measured with the help of digital verneer calipers just above the ground surface and the average was calculated.

No. of branches per plant - The total number of branches per plant was counted and the average was calculated.

Number of leaves per seedling – The total number of leaves per seedling was counted and the average was calculated. Matured leaves were taken into account.

Survival percentage of seedlings

The survival percentage of each treatment was recorded at 120 days after seed sowing. The survival percentage was calculated by using formula as given below:

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

Results and Discussion

Days taken to first seed germination, 50 percent seeds germination and germination Percent at 30 days (Table 1)

The combinations of growing media soil + sand + azotobacter (1:1+ 5g/kg) enhance germination rate and took minimum days for first seed germination (11.33), fifty percent seeds germination (20.55) and maximum germination percent (74.80) at 30 days. Early germination, fifty percent germination and final germination percent might be attributed to the conducive effect of this medium

mixture on water holding capacity, porosity, soil aeration and supplying substantial amount of nutrients specially nitrogen and micro nutrients as well as improve the microbial activities which might be helping in better germination. Availability of nutrients in the soil also responsible for enhance germination. Monika *et al.,* (2010) also reported that the application of biofertilizer increase germination percentage in Lens culinaris Medic. These findings are in conformity with the findings of Sinish *et al.,* (2005) who found the influence of organic matter inoculated with biofertilizer on the induced earlier germination with better germination percentage.

The seed soaking in the solution of GA_3 50ppm took minimum days to first seed germination (14.27) and 50 percent seeds germination (22.48) while maximum days taken by the untreated seed. In case of germination percent at 30 days the maximum (62.55) was found with $GA_3$100ppm. The promising effect of GA_3 as pre-sowing treatments to the seeds replaced the dormancy mechanism of the seeds resulting in early germination (Khan, 1981). Gibberellic acid acts on the embryo and causes synthesis of hydrolyzing enzymes particularly amylase and protease and this hydrolyzed food is utilized for growth of embryo and thereby enhanced the germination (Paleg, 1965). Similar result have been reported by Meena *et al.,* (2003) who revealed that papaya seeds treated with GA_3 100ppm at pre sowing taken minimum days for germination and found maximum percentage of germination. This was due to the fact that GA_3 plays a key role in the initiation of germination. The findings of Venkatrao and Reddy (2005) are close to the conformity of the findings.

The interactions of growing media and GA_3 also enhance the germination rate and soil+ sand+ azotobacter with GA_3 (1:1+ 5g/kg + GA_3 50ppm) and took minimum days for first seed germination (9.33), 50% first seeds germination (19.66), and maximum germination percent (76.68) at 30 days was found in soil+ sand+ azotobacter with GA_3 (1:1+ 5g/kg + GA_3 100ppm). It may be because of faster germination which was facilitate by the media in combinations of GA_3 application, where the combination of soil, sand, azotobacter and GA_3 provides adequate nutrients and enhance both the physical properties of the water holding capacity of the soil on the other hand GA_3 helps in promoting the germination percentage due to its participation in the activities of hydrolyzing enzyme and alpha-amylase at initial stage of germination and thus, facilited the germination process (Singh *et al.,* 1979). Enhancement of seed germination by growth regulators might be due to increase of transcription and/or translation during protein synthesis.

The mobilization of protein and lipid storage bodies upon specific enzymes, which hydrolyze stored molecules and catalyze result into the production of energy and substrates and provide the structural components essential for growth and emergence of the embryo.

This result is in agreement with the findings of Feza Ahmad (2010).The findings are also in agreement with the findings of Sinish *et al.,* (2005) and Syamal *et al., (*2012) who reported the earlier germination with better germination percentage by the application of organic manure (biofertilizer). El-zaher (2008) also found similar result.

Height of plants (cm), Girth of stem (mm), No. of branches / plant, Number of leaves at 30, 60, 90 and 120 DAS (Table 2, 3, 4, 5)

The data showed that almost all the growing media and GA_3 treatments showed significant effect on height of plant, girth of stem (mm), number of branches / plant, number of leaves.

Table.1 Effect of growing media and gibberellic acid on days taken to first seed germination, 50 per sent seed germination and overall germination percentage

Treatments	Seed Germination		
Growing media	Days taken to First seed germination	Days taken to 50% seed germination	Germination per sent at 30 DAS
M_1 (Soil)	18.44	27.00	46.97
M_2 (Soil+ Sand; 1:1)	17.00	25.33	50.52
M_3 (Soil+ Sand+ VC;1:1:1)	16.44	24.89	59.43
M_4 (Soil+ VC; 1:1)	16.22	24.11	60.55
M_5 (Soil+ Sand+ AZO; 1:1+5g AZO)	11.33	20.55	74.80
M_6 (Soil+ Sand+ VC+ AZO;1:1:1+5g AZO)	15.88	25.55	67.06
SEm±	0.75	0.63	0.67
CD at 5 % level	2.17	1.83	1.94
Gibberellic acid			
G_0 (GA_3 0_{ppm})	17.44	26.11	54.97
G_1 (GA_3 50_{ppm})	14.27	22.88	62.14
G_2 (GA_3 100_{ppm})	15.94	24.44	62.55
SEm±	0.53	0.45	0.47
CD at 5% level	1.53	1.30	1.37
Treatments combination			
M_1G_0	20.33	29.33	43.52
M_1G_1	17.33	25.33	46.98
M_1G_2	17.66	26.33	50.43
M_2G_0	17.66	25.66	40.64
M_2G_1	13.66	23.33	53.98
M_2G_2	19.66	27.00	56.96
M_3G_0	16.00	24.33	54.09
M_3G_1	16.66	25.00	63.70
M_3G_2	16.66	25.33	60.51
M_4G_0	19.66	28.66	50.62
M_4G_1	14.33	21.33	63.03
M_4G_2	14.66	22.33	67.00
M_5G_0	11.33	20.66	73.74
M_5G_1	9.33	19.66	73.99
M_5G_2	13.33	21.33	76.68
M_6G_0	19.66	28.00	67.25
M_6G_1	14.27	22.66	70.19
M_6G_2	15.94	24.33	63.74
SEm±	1.30	1.10	1.16
CD at 5% level	3.77	3.18	3.36

Table.2 Effect of growing media and gibberellic acid on seedling height at 30, 60 and 90 DAS of Acid lime seedlings

Treatments	Seedling height (cm)		
Growing media	30 DAS	60 DAS	90 DAS
M_1 (Soil)	3.98	4.65	5.43
M_2 (Soil+ Sand; 1:1)	4.79	5.92	6.45
M_3 (Soil+ Sand+ VC;1:1:1)	4.77	5.65	6.49
M_4 (Soil+ VC; 1:1)	5.30	6.77	6.99
M_5 (Soil+ Sand+ AZO; 1:1+5g AZO)	5.67	6.94	7.81
M_6 (Soil+ Sand+ VC+ AZO;1:1:1+5g AZO)	4.85	5.32	6.66
SEm±	0.012	0.018	0.020
CD at 5 % level	0.036	0.053	0.059
Gibberellic acid			
G_0 (GA_3 0_{ppm})	4.57	5.57	6.37
G_1 (GA_3 50_{ppm})	5.22	6.17	6.91
G_2 (GA_3 100_{ppm})	4.89	5.87	6.63
SEm±	0.009	0.013	0.008
CD at 5% level	0.025	0.037	0.024
Treatments combination			
M_1G_0	3.64	4.30	5.15
M_1G_1	4.00	4.70	5.60
M_1G_2	4.30	4.96	5.55
M_2G_0	5.16	4.60	7.03
M_2G_1	5.23	5.90	6.32
M_2G_2	4.00	5.20	6.00
M_3G_0	4.00	5.23	6.12
M_3G_1	5.50	6.16	6.66
M_3G_2	5.26	5.56	6.70
M_4G_0	5.33	6.62	6.77
M_4G_1	5.00	6.94	7.22
M_4G_2	5.26	6.75	6.98
M_5G_0	5.33	6.50	7.66
M_5G_1	5.96	7.22	8.36
M_5G_2	5.73	7.12	7.43
M_6G_0	3.66	4.16	5.49
M_6G_1	5.63	6.12	7.33
M_6G_2	5.26	5.68	7.16
SEm±	0.021	0.032	0.020
CD at 5% level	0.062	0.091	0.059

Table.3 Effect of growing media and gibberellic acid on stem girth at 30, 60 and 90 DAS of Acid lime seedlings

Treatments	Stem girth (mm)		
Growing media	30 DAS	60 DAS	90 DAS
M_1 (Soil)	0.760	1.40	2.04
M_2 (Soil+ Sand; 1:1)	1.147	1.44	2.12
M_3 (Soil+ Sand+ VC;1:1:1)	1.130	1.48	2.09
M_4 (Soil+ VC; 1:1)	1.197	1.49	2.18
M_5 (Soil+ Sand+ AZO; 1:1+5g AZO)	1.270	1.73	2.57
M_6 (Soil+ Sand+ VC+ AZO;1:1:1+5g AZO)	1.137	1.48	2.23
SEm±	0.014	0.033	0.040
CD at 5 % level	0.041	0.096	0.114
Gibberellic acid			
G_0 (GA_3 0_{ppm})	1.04	1.39	2.12
G_1 (GA_3 50_{ppm})	1.15	1.64	2.30
G_2 (GA_3 100_{ppm})	1.12	1.47	2.19
SEm±	0.010	0.023	0.028
CD at 5% level	0.029	0.068	0.081
Treatments combination			
M_1G_0	0.73	1.12	1.71
M_1G_1	0.79	1.65	2.25
M_1G_2	0.76	1.45	2.17
M_2G_0	1.09	1.32	1.90
M_2G_1	1.21	1.53	2.25
M_2G_2	1.14	1.48	2.23
M_3G_0	1.15	1.71	2.25
M_3G_1	1.13	1.52	2.08
M_3G_2	1.11	1.22	1.96
M_4G_0	1.02	1.29	2.21
M_4G_1	1.17	1.68	2.17
M_4G_2	1.40	1.51	2.16
M_5G_0	1.20	1.62	2.55
M_5G_1	1.50	1.85	2.74
M_5G_2	1.11	1.73	2.42
M_6G_0	1.07	1.33	2.11
M_6G_1	1.11	1.66	2.34
M_6G_2	1.12	1.45	2.24
SEm±	0.024	0.057	0.069
CD at 5% level	0.70	0.166	0.198

Table.4 Effect of growing media and gibberellic acid on number of leaves per plant at 30, 60 and 90 DAS of Acid lime seedlings

Treatments	Number of leaves per seedling		
Growing media	30 DAS	60 DAS	90 DAS
M_1 (Soil)	3.23	4.88	5.98
M_2 (Soil+ Sand; 1:1)	4.10	6.55	7.73
M_3 (Soil+ Sand+ VC;1:1:1)	3.76	5.65	6.77
M_4 (Soil+ VC; 1:1)	4.22	6.66	7.99
M_5 (Soil+ Sand+ AZO; 1:1+5g AZO)	6.65	9.10	11.00
M_6 (Soil+ Sand+ VC+ AZO;1:1:1+5g AZO)	3.44	6.51	8.33
SEm±	0.004	0.004	0.045
CD at 5 %	0.011	0.011	0.129
Gibberellic acid			
G_0 (GA_3 0_{ppm})	3.29	5.40	6.88
G_1 (GA_3 50_{ppm})	4.91	7.42	8.73
G_2 (GA_3 100_{ppm})	4.34	6.86	8.28
SEm±	0.003	0.003	0.032
CD at 5%	0.008	0.008	0.091
Treatments combination			
M_1G_0	2.33	3.66	4.66
M_1G_1	3.72	6.00	6.66
M_1G_2	3.66	5.00	6.62
M_2G_0	3.00	5.33	7.00
M_2G_1	5.66	7.22	8.42
M_2G_2	3.66	7.12	7.77
M_3G_0	2.39	3.76	5.33
M_3G_1	4.45	6.66	7.33
M_3G_2	4.44	6.55	7.66
M_4G_0	3.66	6.00	7.33
M_4G_1	4.00	7.33	8.66
M_4G_2	5.00	6.66	8.00
M_5G_0	5.72	8.66	10.00
M_5G_1	7.33	9.66	11.66
M_5G_2	6.00	9.00	11.34
M_6G_0	2.66	5.00	7.00
M_6G_1	4.34	7.70	9.66
M_6G_2	3.33	6.83	8.33
SEm±	0.006	0.003	0.078
CD at 5%	0.019	0.008	0.224

Table.5 Effect of growing media and gibberellic acid on number of branches per plant at 60, 90 and 120 DAS Acid lime seedling

Treatments	Number of branches		
Growing media	60 DAS	90 DAS	120 DAS
M_1 (Soil)	1.33	1.59	2.66
M_2 (Soil+ Sand; 1:1)	1.53	2.18	3.10
M_3 (Soil+ Sand+ VC;1:1:1)	1.40	1.76	2.81
M_4 (Soil+ VC; 1:1)	1.37	1.88	2.95
M_5 (Soil+ Sand+ AZO; 1:1+5g AZO)	1.75	2.81	3.80
M_6 (Soil+ Sand+ VC+ AZO;1:1:1+5g AZO)	1.55	1.67	3.03
SEm±	0.004	0.046	0.011
CD at 5 %	0.013	0.136	0.031
Gibberellic acid			
G_0 (GA_3 0_{ppm})	1.33	1.83	2.89
G_1 (GA_3 50_{ppm})	1.63	2.18	3.24
G_2 (GA_3 100_{ppm})	1.50	1.93	3.04
SEm±	0.003	0.033	0.008
CD at 5%	0.009	0.095	0.022
Treatments combination			
M_1G_0	1.31	1.33	2.46
M_1G_1	1.36	1.68	2.68
M_1G_2	1.33	1.78	2.84
M_2G_0	1.33	2.23	2.97
M_2G_1	1.66	2.33	3.36
M_2G_2	1.62	2.00	2.98
M_3G_0	1.00	1.62	2.66
M_3G_1	1.88	2.00	3.00
M_3G_2	1.33	1.66	2.77
M_4G_0	1.33	1.66	2.66
M_4G_1	1.40	2.33	3.33
M_4G_2	1.33	1.66	2.88
M_5G_0	1.70	2.66	3.66
M_5G_1	1.82	3.00	3.98
M_5G_2	1.75	2.78	3.76
M_6G_0	1.32	1.52	2.96
M_6G_1	1.66	1.76	3.13
M_6G_2	1.67	1.73	3.00
SEm±	0.008	0.080	0.018
CD at 5%	0.022	0.232	0.053

Table.6 Effect of growing media and gibberellic acid on survival percentage of seedlings

Treatments	Results
Growing media	45.73
M_1 (Soil)	50.73
M_2 (Soil+ Sand; 1:1)	55.55
M_3 (Soil+ Sand+ VC;1:1:1)	59.10
M_4 (Soil+ VC; 1:1)	72.21
M_5 (Soil+ Sand+ AZO; 1:1+5g AZO)	61.10
M_6 (Soil+ Sand+ VC+ AZO;1:1:1+5g AZO)	
SEm±	0.004
CD at 5 %	0.012
Gibberellic acid	
G_0 (GA_3 0_{ppm})	53.31
G_1 (GA_3 50_{ppm})	59.81
G_2 (GA_3 100_{ppm})	58.88
SEm±	0.003
CD at 5%	0.009
Treatments combination	
M_1G_0	43.22
M_1G_1	50.66
M_1G_2	43.33
M_2G_0	46.66
M_2G_1	50.91
M_2G_2	53.33
M_3G_0	46.66
M_3G_1	56.66
M_3G_2	63.66
M_4G_0	53.33
M_4G_1	60.66
M_4G_2	63.66
M_5G_0	66.66
M_5G_1	76.66
M_5G_2	73.33
M_6G_0	63.33
M_6G_1	63.33
M_6G_2	56.66
SEm±	000.7
CD at 5%	0.021

The maximum plant height (5.67, 6.94, 7.81 cm), stem girth (1.27, 1.73, 2.57 mm), no. of branches /plant (1.75, 2.81, 3.80), number of leaves (6.65, 9.10, 11.00) were noted under M5 (soil + sand + azotobacter; 1:1+5g/ kg AZO). The minimum plant height (3.98, 4.65, 5.43 cm), stem girth (0.76, 1.40, 2.04 mm), no. of branches / plant (1.33, 1.59, 2.66), no.

of leaves (3.23, 4.88, 5.98) were recorded under M1 (soil). The maximum plant height (5.22, 6.17, 6.91 cm), stem girth (1.15, 1.64, 2.30 mm), no. of branches / plant (1.63, 2.18, 3.24), number of leaves (4.91, 7.42, 8.73) were noted with G1 (GA3 50ppm) and the minimum plant height (4.57, 5.57, 6.37 cm), stem girth (1.04, 1.39, 2.12 mm), no. of branches / plant (1.33, 1.83, 2.89), no. of leaves of (3.29, 5.40, 6.88) were recorded in G1 (control). The maximum plant height (5.96, 7.22, 8.36 cm), stem girth (1.50, 1.85, 2.74 mm), no. of branches / plant (1.82, 3.00, 3.98), no. of leaves (7.33, 9.66, 11.66) were noted under M5G1 (soil + sand + azotobacter; 1:1+5g/ kg AZO and 50ppm GA_3), whereas, the minimum height (3.64, 4.30, 5.15 cm), stem girth (0.73, 1.12, 1.71 mm), no. of branches / plant (1.31, 1.33, 2.46), no. of leaves (2.33, 3.66, 4.66) were recorded under in M1G0 (soil and control). Increase in seedling height, girth, leaves and branches in acid lime seedling significantly may be because of combine effect of medium mixture on water holding capacity, porosity, soil aeration and supply of substantial amount of nutrients required for better growth of seedling (seedling height, stem girth and number of leaves and branches). The increasing seedling height, stem girth, number of leaves and branches due to application soil + sand + Azotobacter (1: 1+ 5g/kg) could be promoting root and shoot growth over control. The increase of height of seedling, stem girth, number of leave and branches with the 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). (Yadav *et al.,* 2012) reported that medium combination of soil + sand + vermicompost + vermiculite + cocopeat (1:1:1:1:1) with Azotobacter had significantly increased the height and stem diameter in acid lime. Increase in number of leaves might be mainly due to corresponding increase in plant height (Govind and Chandra, 1993). The beneficial effect of GA_3 was probably due to quicker multiplication of cells and cell elongation after the germination. The possible reason of significant increase in seedling height, stem girth, number of leaves and branches might be due invigoration of physiological process of plant and stimulatory effect of GA3 to found new cells at faster rate. These results are in conformity with the findings of Venkatrao *et al.,* (2005) Wagh *et al.,* (1998) and Dalal *et al.,* (2002),they have reported GA_3 gave highest percentage of germination and enhance the plant growth or morphological parameter of seedling (plant height, girth and number of leaves) in fruit crops like Mango, Aonla, Rangpur lime etc. Combined application of biofertilizer and GA_3 would have better availability uptake of N, P, K and other plant nutrients which in terms lead to the production of more efficient plant and enhance the overall plant growth while GA_3 increase the plant height by increase in size of meristematic region and it is also significantly enhance the girth, number of leaves. El-zaher *et al.,* (2008) also reported the same results.

Survival percent of seedling (Table 6)

The survival of seedling was significantly influenced by the various growing media and different concentrations of GA_3.

The significantly maximum survival percent (72.21) of seedling were noted in the growing media, in soil+ sand+ azotobacter (1: 1 +5g/kg) at 120 days after sowing of seed, It may be due to holding ample of aeration and optimum soil moisture status for better growth of the seedling, particularly for good development of a root system, ultimately the

overall survival of the seedling. These results are in close agreement with Shamet *et al.*, (1994).Treatment GA_3 50ppm proved superior over other concentrations for influencing the survival percentage (59.81) while untreated seeds gave minimum number of survival percentage. The application of GA_3 50ppm might have boosted the early germination and growth of seedling by cell multiplication and cell elongation subjected to the better plant growth resulting higher rate of seedling survival. This might be due to rapid and early germination which resulted in giving more periods for vegetative growth for better establishment of plants (Sharma *et al.*, 2012).

Combined application of growing medium and GA_3 was found to have significant on survival percentage (76.66) of seedling. The combination of growing media and GA_3 significantly promoted the germination and growth by multiplication and elongation of plant cell. On the other hand, the improved soil condition, soil health, availability of the nutrients, might be supported to appropriate cation exchange capacity and water holding capacity. Thus permitting adequate moisture exchange of gases uptake of nutrients facilitate better and healthy growth resulting maximum survival of seedling under the treatments.

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