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Effect of seed treatment and storage period on physiological parameters of acid lime (*Citrus aurantifolia* Swingle L.)

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Abstract
An experiment was conducted at Instructional farm, Department of Horticulture, College of Agriculture, Ummedganj, Kota (Agriculture University, Kota). The experiment was laid out in a Factorial Completely Randomized Design with 32 treatment combinations and these treatments were replicated thrice. The treatments comprised of 8 pre-sowing seed treatments (T) *viz.*, T_0-Control (Storage without treatment), T_1- Lime juice treatment, T_2- KNO_3 at 1%, T_3- KNO_3 at 2%, T_4- KNO_3 at 3%, T_5- Thiourea at 1%, T_6- Thiourea at 1.5% and T_7- Thiourea at 2% and four Storage periods (S) *viz.*,S_0- sowing 0 days after seed extraction, S_1- sowing 7 days after seed extraction, S_2- sowing 14 days after seed extraction and S_3- sowing 21 days after seed extraction. Irrespective of the chemical treatments Sowing of seed immediately after extraction (fresh seed) observed maximum nitrogen (1.70%) content, Phosphorus (0.38%) content, Potassium (0.96%) content, chlorophyll a (1.34 mg/g), chlorophyll b (0.38 mg/g), total chlorophyll (1.73 mg/g) and maximum relative water content (87.14%) in leaf as well. However seed treatment T_4 KNO_3 @ 3% recorded maximum phosphorus (0.39%), potassium (1.02%), chlorophyll a (1.40%), chlorophyll b (0.38%), total chlorophyll (1.78%) and relative water content (86.50%) in leaves at 60 DAS. Maximum nitrogen content (1.79%) showed that the treatment T_7 thiourea @ 2%. Treatment combination S_0T_4 recorded maximum phosphorus content (0.42%), maximum Potassium content (1.05%) and chlorophyll b (0.43 mg/g) content at 60 DAS in leaves of acid lime.

Keywords: Physiological, parameters, lime, *Citrus aurantifolia* Swingle L.

Introduction
Acid lime (*Citrus aurantifolia* Swingle L.) is one of the major fruit crop grown in India. It belongs to Rutaceae family with chromosome number 2n=18. India is the largest producer of acid lime in the world. The area of acid lime in India is about 305.0 thousand hectares and the production 3482.0 thousand MT (NHB, 2019). It is also known as Pati lime, Kagzi lime, Mexican lime etc. Acid lime contains 6.3-6.6% citric acid. It is an appetizer, stomachic, anti scorbutic, anti helmintic and it checks biliousness besides a good source of nutrients, vitamins and other antioxidant compounds (Chadha, 2002). It is most important multi-fold nutritional and medicinal value fruit. It is used in making candy, chocolate, ice-cream, pastries etc In kagzi lime germination percentage is low and it varies between 27-58 per cent (Anandam and Singh, 1949 and Cheema *et al*. 1954) [9] and Kagzi lime takes about 3 weeks to germinate (Naik, 1949 and Cheema *et al*. 1954) [19, 9]. The growth of acid lime seedling is very slow in nursery as well as in the field (Shant and Rao, 1973).
The use of growth regulators in overcoming the inhibitory action of certain chemical substances that delay the germination has been reported by abohassan *et al*. (1979) in kagzi lime and chaudhari and Chakrawar (1981) in Rangpur lime. Nitrate ions stimulate germination of dormant seeds (Alboresi *et al.* 2005) [3]. To keeping this in view, an experiment entitled "Effect of Seed Treatment and Storage Period on Germination and Seedling Growth of Acid lime (*Citrus aurantifolia* Swingle L.)''

Material and Methods
The experiment was conducted at Department of Horticulture, College of Agriculture, Ummedganj, Kota. Geographically Ummedganj is located in Kota district which lies in south eastern region of Rajasthan situated between $25^0$11'0" North latitude and $75^0$50'0" East longitude. The fully ripened, healthy and uniformly sized acid lime fruits from the lot were selected and used for seed extraction. The extracted seeds were washed in running water. The washed seeds were dipped in water and the dead floating seeds were removed as non - viable

and dead seeds being generally light in weight float on water. Other seeds, which settled at the bottom of the bucket, were considered as viable seeds and were used for sowing. Using pro-tray was laid out in Factorial Complete Randomize Design using 8 seed treatments with 4 storage period comprising 32 treatment combinations. The potting mixture was prepared by thoroughly mixing two parts of soil, one part of fine sand and one part of vermin-compost (soil: sand: vermin-compost:: 2: 1: 1). The pro tray were taken and filled properly with potting mixture. The well filled pro tray (9" × 7" sized) was placed in flat beds at proper spacing. Then the pre-treated seeds were sown manually in these pro trays. Data on nitrogen, phosphorus, potassium, chlorophyll a, chlorophyll b, total chlorophyll and relative water content in leaves of acid lime at 60 DAS.

Result and Discussion

Effect of seed treatments

Seed treatments show the significant effect on physiological parameters. The seed treatment T_4 KNO_3 @ 3% observed maximum phosphorus (0.39%), potassium (1.02%), chlorophyll a (1.40%), chlorophyll b (0.38%), total chlorophyll (1.78%) and relative water content (86.50%) in leaves at 60 DAS. Maximum nitrogen content (1.79%) showed that the treatment T_7 thiourea @ 2%. The most probable reason for superiority of KNO_3 over other treatments could be due to increased K+ ions (Srimathi 2000 and Rajamanickam *et al.* 2002) and also more nitrogen and potassium ions accumulation in seeds (Hegazi *et al.*2011 and Banik *et al.*2015) [14, 5] leading to acceleration in synthesis of amino acids which thereby helped in the promotion of growth in tissues (Waman *et al.* 2017) [24]. Probably, the other reason for increased NPK content may be due to enhanced uptake of nutrients by the roots owning to better growth and development as indicated by increase in the number of secondary roots, root length, dry and fresh weight in treated seeds.

The results corroborated with the findings of Choudhary and Chakrawar (1982), Hegazi *et al.* (2011) [14], Gurung *et al.* (2014), Banik*et al.* (2015) [5], Kalyani *et al.* (2015), Khatana *et al.* (2015), Waman *et al.* (2017) [24], Hota *et al.* (2018) [15] and Patil *et al.* (2018) [21].

Thiourea is a nitrogen and sulphur containing compound which may have acted as a nutrient supplement (Pandey *et al.* 2013 and Perveen *et al.* 2015) [20]. This was reflected by the increased nitrogen content in leaves. Also the seeds treated with thiourea reported to have improved the sugar metabolism and enhance bio-synthesis of crude proteins, soluble sugars, total free amino acids and soluble phenolics in many plants (Amin *et al.* 2013). Due to having biologically important functional groups exogenous application of thiourea promoted the growth (Jocelyn, 1972) [16] including enhancing the nitrogen content in leaves and the total uptake of nutrients by the crops (Saini, 1991) [22]. This simulative effect of thiourea is in consonance with the findings of Centibaz *et al.* (2006) [7] in prunus and Wahid *et al.* (2017) [24].

Effect of storage period

Storage period showed the significant effect on physiological parameters. Sowing of seed immediately after extraction (0 days after seed extraction) observed maximum nitrogen (1.70%) content, Phosphorus (0.38%) content, Potassium (0.96%) content, chlorophyll a (1.34 mg/g), chlorophyll b (0.38 mg/g), total chlorophyll (1.73 mg/g) and maximum relative water content (87.14%). This might be due to the high level of carbohydrate and protein reserve in the seed which responsible for early initiation and germination percentage of seed (Abbas *et al.* 2003) [1]. This might be due to freshly extracted seeds have more vigour, high moisture and more nutrient reserve resulted healthy and vigorous seedling. These results are in accordance with Deepika *et al.* (2014) [11] in karonda, Khopkar *et al.* (2014) [18] in pummel and Srimathi *et al.* (2003) in jamun.

Interaction effect of storage period and seed treatment

Interaction effect of seed treatment and storage period showed the significant effect on physiological parameters. Treatment combination S_0T_4 (0 days after seed extraction + KNO_3 at 3% for 24 hours) recorded phosphorus content (0.42%), maximum Potassium content (1.05%) and chlorophyll b content (0.43 mg/g) at 60 DAS in leaves of acid lime. This might be due to the high level of carbohydrate and protein reserve in the seed which responsible for early initiation and germination percentage of seed (Abbas *et al.* 2003) [1] and significant increase in the seed germination parameters over control with application of KNO_3 at all concentrations can be explained by the fact that KNO_3 stimulates germination by decreasing the endogenous levels of abscisic acid in embryos (Wang *et al.* 1998) causing vacuolation and cell wall weakening of the aleurone layer (Bethke *et al.* 2007) [6] and involvement in the pentose phosphate pathway due to increased oxidation of NADPH to NADP and enzymes that have been encoded to provide nutrients for germination (Finkelstein *et al.*2008) [12].

Table 1: Effect of seed treatment and storage period on per cent Nitrogen, Phosphorus, Potassium content, chlorophyll a, chlorophyll b, total chlorophyll (mg/g) and relative water content (%) in leaves at 60 DAS in acid lime (*Citrus aurantifolia*).

Treatments	Nitrogen content (%)	Phosphorus content (%)	Potassium content (%)	Chlorophyll a (mg/g)	Chlorophyll b (mg/g)	Total chlorophyll (mg/g)	Relative water content (%)
S (Storage days)							
S0	1.70	0.38	0.96	1.34	0.38	1.73	87.14
S1	1.67	0.36	0.94	1.32	0.37	1.69	86.57
S2	1.64	0.35	0.93	1.30	0.34	1.64	84.82
S3	1.61	0.33	0.91	1.25	0.32	1.57	84.46
S.Em±	0.004	0.002	0.002	0.003	0.002	0.004	0.201
CD at 5%	0.011	0.006	0.007	0.010	0.007	0.012	0.569
T (Seed treatment)							
T0	1.38	0.33	0.80	1.11	0.32	1.43	85.07
T1	1.39	0.34	0.82	1.12	0.34	1.46	85.25
T2	1.74	0.35	1.00	1.33	0.35	1.68	86.12
T3	1.75	0.38	1.01	1.37	0.36	1.74	86.26
T4	1.76	0.39	1.02	1.40	0.38	1.78	86.50

T5	1.73	0.35	0.93	1.34	0.35	1.69	85.65
T6	1.74	0.34	0.94	1.36	0.36	1.72	85.56
T7	1.79	0.36	0.96	1.38	0.36	1.74	85.57
S.Em±	0.005	0.003	0.003	0.005	0.003	0.006	0.285
CD at 5%	0.015	0.008	0.010	0.014	0.009	0.017	0.804

Table 2: Interaction effect of seed treatment and storage period on per cent Nitrogen, Phosphorus, Potassium content, chlorophyll a, chlorophyll b, total chlorophyll (mg/g) and relative water content (%) in leaves at 60 DAS in acid lime (*Citrus aurantifolia*).

Treatments	Phosphorus content (%)	Potassium content (%)	Chlorophyll b (mg/g)
S_0T_0	0.35	0.81	0.35
S_0T_1	0.36	0.83	0.36
S_0T_2	0.38	1.03	0.38
S_0T_3	0.40	1.04	0.39
S_0T_4	0.42	1.05	0.43
S_0T_5	0.35	0.95	0.37
S_0T_6	0.36	0.97	0.39
S_0T_7	0.37	0.99	0.41
S_1T_0	0.34	0.81	0.34
S_1T_1	0.35	0.82	0.35
S_1T_2	0.36	1.01	0.36
S_1T_3	0.38	1.01	0.38
S_1T_4	0.41	1.03	0.40
S_1T_5	0.33	0.93	0.35
S_1T_6	0.34	0.95	0.37
S_1T_7	0.36	0.96	0.38
S_2T_0	0.32	0.81	0.31
S_2T_1	0.33	0.82	0.33
S_2T_2	0.35	0.98	0.34
S_2T_3	0.37	1.01	0.35
S_2T_4	0.38	1.02	0.36
S_2T_5	0.36	0.92	0.34
S_2T_6	0.34	0.93	0.35
S_2T_7	0.35	0.95	0.35
S_3T_0	0.31	0.78	0.28
S_3T_1	0.32	0.79	0.31
S_3T_2	0.33	0.97	0.32
S_3T_3	0.35	0.99	0.33
S_3T_4	0.36	0.96	0.34
S_3T_5	0.34	0.91	0.33
S_3T_6	0.33	0.92	0.32
S_3T_7	0.34	0.93	0.32
S.Em±	0.006	0.007	0.007
CD at 5%	0.016	0.019	0.019

Conclusion

Best physiological parameters of acid lime seedling *viz*. nitrogen content, phosphorus, potassium content, chlorophyll a, b and total chlorophyll and relative water content were found when seed sown immediately after extraction with seed treatment KNO_3 @ 3%.

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