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Effect of PGRs and chemicals on seed germination and physiological parameters of rough lemon (*Citrus jambhiri* L.) under hydroponic condition

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Abstract
This study aimed to investigate the possibility of enhanced germination attributes upon rough lemon's seeds treated with plant growth regulators and chemicals. The experiment was laid out in Completely Randomized Design (CRD) with thirteen treatments replicated thrice. Before the sowing, the seeds were soaked with different concentrations of GA_3, NAA, KNO_3 and thiourea for 12 hours, while the distilled water were taken as control. The study revealed that the earliest germination (10.33 days), highest percentage of germination (88.83%), Survival percentage (90.47%) and total chlorophyll content in leaves (3.50 mg/g) were recorded with soaking seeds in GA_3 at 150 ppm concentration which were superior to other treatments. However, all treatments have not shown significant effect on accumulation of proline in the seedling leaves.

Keywords: PGRs, pre-soaking, germination, rough lemon

Introduction

Rough lemon (*Citrus jambhiri* L.) is apparently a hybrid due to its high degree of polyembryony as compared to other lemon species, and considered to be originated from North Eastern Region of India. Amongst the citrus rootstocks, rough lemon has been widely used for the scion cultivars in India. This rootstock is preferred due to high resistant to citrus tristeza virus (CTV), citrus exocortis as well as for its tolerance against drought and salt. This rootstock makes the plant resistant to numerous viruses and produces high yield with large size fruits in grafted scion cultivars (Altaf *et al.*, 2008) [1]. However, it is highly susceptible to foot rot (Naqvi 2004; Castle 2010) [18, 7]. This rootstock has good adaptability to sandy well drained soil and has tolerance to alkaline and moderate tolerance to salinity. The good yield performance has been shown by rough lemon rootstock in virgin soils and should not be used in replant soils.

Generally, seed germination is the common procedure for large scale production of rough lemon. Due to recalcitrant nature, the seeds have to be sown immediately after extraction. Seed germination is one of the critical stages in the nursery production. Some problems encountered in the process of producing rough lemon rootstock seedlings include slow seed germination as well as the long period between the first and last seeds to germinate. The slow seed germination may be due to the certain inhibitors in the seed coat, deficiency of some endogenous growth promoters or excess of endogenous growth inhibitors. Therefore, the entire process of seed germination and seedling growth will be time consuming, labors intensive and increases cost of production. A large number of plant growth regulators and chemicals in proper concentration may regulate growth behavior in many citrus crops, and could lead to increase seed germination and enhancement of seedling growth. The germination behaviours, seedling height, number of leaves and number of roots are affected by various pre-soaking treatments with growth regulators and chemicals like GA_3, NAA, KNO_3 and thiourea in different crop species (Rajamanickam *et al.*, 2004) [21]. Application of gibberellin increases plasticity of the cell wall and reduces water potential in the cell through the hydrolysis of starch to sugar, hence allowing entry of water into cell which causes elongation (Arteca, 1996) [3]. Plant hormones have most important functions in controlling and coordinating cell division, growth and differentiation (Hooley, 1994) [12].

Also, potassium nitrate is widely used chemical as a seed treatment as it promotes seed germination in various crops. Pre-soaking seeds improves seed germination in various citrus rootstocks (Mehanna and Mohammed, 1989) [17].

Naturally, all citrus rootstock seeds germinate irregularly causing poor seedling uniformity (Aubert and Vullin, 1998) [4]. Therefore, seed treatment is most important for better plant materials, vigorous seedling growth, uniformity and shortening germination period, and to prevent irregularity in seed germination. The role of various plant hormones in plant has been intensively studied. Different plant activities can be affected by plant hormones including seed dormancy and germination (Graeber *et al*., 2012) [10]. Based on these facts, the present investigation has been undertaken to increase the germination potential and survivability of the seedling with different plant growth regulators and chemicals.

Materials and Methods

The present investigation was carried out under hydroponic condition at Fruit Science Laboratory, Department of Fruit Science, College of Horticulture and Forestry, CAU, Pasighat, Arunachal Pradesh during the year 2019-20. The experiment was laid out with Completely Randomized Design (CRD) having thirteen treatments *viz.* T1 - GA_3 50 ppm, T2 - GA_3 100 ppm, T3 - GA_3 150 ppm, T4 - NAA 50 ppm, T5 - NAA 100 ppm, T6 - NAA 150 ppm, T7 - KNO_3 0.5%, T8 - KNO_3 1.0%, T9 - KNO_3 1.5%, T10 - Thiourea 0.5%, T11 - Thiourea 1.0%, T12 - Thiourea 1.5% and T13 - control (Distilled water). The treatments replicated trice using 30 seeds in each treatment of a replication. The required quantities of GA_3, NAA, KNO_3 and thiourea were weighed using electronic balance and dissolved separately in 10 ml ethyl alcohol (99%) in different beakers. The distilled water was added to make up the volume equal to one liter to obtain desired concentrations of solutions. The seeds extracted from the healthy, ripe and uniform size of rough lemon fruits were soaked for 12 hours and sown during last week of November, 2019 under hydroponic condition. The observation, days taken to first germination was recorded when the plumule emergenced at three days interval. The germination percentage was recorded at 45 days after sowing. It was calculated by dividing total number of seeds sown with the number of seeds germinated and multiplied by 100. Survival percentage after transplanting to polybags was calculated by the following formula.

$$\text{Survival (\%)} = \frac{\text{Number of survived seedlings}}{\text{Number of transplanted seedlings}} \times 100$$

Estimation of total chlorophyll content was done as per the method of Arnon (1949) [2]. The plant materials for the estimation of proline were taken during the last week of February, 2020 for each treatment and estimated as per the method prescribed by Bates *et al.,* (1973) [5].

Results and discussion

Days taken for first germination

The data of present research work (Table 1) revealed that the plant growth regulators and chemicals showed significant effect on seed germination characters. The earliest germination (10.33 days) was recorded in the seeds treated with GA_3 150 ppm while the late germination (18.33 days) was observed under control. The promoting of germination may be due to the antagonistic effect of GA_3 against influence of inhibitors (Brain and Hemming, 1958 [6] and Wareing *et al*., 1968 [25]) and endogenous gibberellin increased by soaking (Mathur *et al*., 1971) [16]. These results are in accordance with the results obtained by Shinde *et al.* (2008) [23] in rangpur lime, Tandon *et al.* (2019) [24] in tamarind, Pratibha *et al*. (2015) [20] in papaya.

Percentage of germination

According to the Table (1), the data displayed that the maximum percentage of germination (88.83%) was recorded with GA_3 at 150 ppm concentration at 45 days after sowing. However, the minimum percentage of germination (43.3%) was obtained under control. The accelerated and enhanced germination might have been due to the activation of GA_3 in the synthesis of hydrolytic enzymes as alpha-amylase and other hydrolases into endosperm, where amylase causes the conversion of starch into essential sugar utilizing in growth process of growing embryo (Copeland and Mc- Donald, 1995) [8]. Furthermore, GA_3 is involved in breaking the photo dormancy, thermo-dormancy, dormancy imposed by incomplete embryo development, mechanical barriers and presence of germination inhibitors (Diaz and Martin, 1971) [9]. The results are in agreement with the findings of Khopkar *et al.* (2017) [14] in pummelo and Kalyani *et al.* (2014) [13] in guava.

Table 1: Effect of PGRs and chemicals on germination parameters of rough lemon

Tr. No.	Treatments	Days taken to first germination	Percentage of germination at 45 days	Survival percentage
T_1	GA_3 (50 ppm)	15	48.87	79.05
T_2	GA_3 (100 ppm)	12.67	73.3	86.62
T_3	GA_3 (150 ppm)	10.33	88.83	90.47
T_4	NAA (50 ppm)	16.33	49.97	74.73
T_5	NAA (100 ppm)	17.67	44.43	77.25
T_6	NAA (150 ppm)	17.33	45.5	76.34
T_7	KNO_3 (0.5%)	15.33	64.4	83.9
T_8	KNO_3 (1.0%)	13.67	79.97	86.26
T_9	KNO_3 (1.5%)	11.67	85.53	88.49
T_{10}	Thiourea (0.5%)	15.33	54.43	75.18
T_{11}	Thiourea (1.0%)	13.33	56.63	82.32
T_{12}	Thiourea (1.5%)	16.67	54.4	87.65
T_{13}	Control	18.33	43.3	73.82
S.Em ±		1.27	3.03	2.49
C.D 5% level		4.05	9.72	7.77

Survival percentage

Data regarding survival percentage after transplanting to polybags was observed at 90 days after sowing. From Table (1), it is clear that the response of pre-sowing treatments on survival percentage were significant over control. The maximum survival percentage (90.47%) was recorded in treatment GA_3 150 ppm, whereas the minimum survival percentage (73.82%) was observed in T13 (control). The higher survival percentage of translocated seedlings under GA_3 treatment might be due to the early germination which helps in successful acclimatization, fast development of root and shoot, which make the seedling withstand the transplanting shock and root diseases, and higher germination percentage obtained by GA_3 150 ppm. These results are in harmony with those obtained by Sharaf *et al*. (2016) [22] in cleopatra mandarin and rangpur lime, Patel *et al*. (2017) [19] in mango.

Chlorophyll content

Data presented in Table (2) revealed that the maximum chlorophyll (a) (2.42 mg/g) was observed by the treatment GA_3 150 ppm at 90 days after sowing, while the minimum chlorophyll (a) (0.82 mg/g) was recorded with treatment control. The maximum chlorophyll (b) was observed with the treatment GA_3 100 ppm. However, the minimum chlorophyll (b) (0.63 mg/g) was obtained with treatment control.

Table 2: Effect of PGRs and chemicals on physiological parameters of rough lemon

Tr. No.	Treatments	Chlorophyll (a)	Chlorophyll (b)	Total Chlorophyll (mg/g)	Proline (µ mole/g)
T_1	GA_3 (50 ppm)	1.11	0.64	1.75	7.5
T_2	GA_3 (100 ppm)	1.63	1.22	2.85	6.65
T_3	GA_3 (150 ppm)	2.42	1.08	3.5	8.58
T_4	NAA (50 ppm)	1.18	0.79	1.97	7.53
T_5	NAA (100 ppm)	1.28	0.66	1.94	6.58
T_6	NAA (150 ppm)	0.94	0.64	1.57	6.39
T_7	KNO_3 (0.5%)	0.83	0.8	1.63	8.46
T_8	KNO_3 (1.0%)	1.62	1.14	2.77	8.42
T_9	KNO_3 (1.5%)	2.02	1.2	3.22	8.63
T_{10}	Thiourea (0.5%)	1.4	1.19	2.58	8.51
T_{11}	Thiourea (1.0%)	1.25	0.81	2.06	7.13
T_{12}	Thiourea (1.5%)	1.45	0.74	2.19	6.75
T_{13}	Control	0.82	0.63	1.45	6.3
S.Em ±		0.29	0.12	0.28	0.65
C.D 5% level		0.89	0.47	0.93	N/S

The maximum total chlorophyll content (3.50 mg/g) was obtained with the treatment GA_3 150 ppm, whereas the minimum total chlorophyll content (1.45 mg/g) was recorded under control (seeds soaked with distilled water) at 90 days after sowing. The enhancement in total chlorophyll content of leaf might be due to the stimulatory effect of plant growth regulators may have affected in formation of leaves at faster rates with more chlorophyll content in leaves. The present results are in line with those previously found by Hoda *et al.* (2010) [11] in sour orange, and Manthri and Bharad (2017) [15] in guava variety L-49.

Estimation of proline

The seeds pre-soaking in different plant growth regulators and chemicals have not shown significant effect on the accumulation of proline in the seedling leaves at 90 days after sowing (Table 2).

Conclusion

It can be concluded that, treatment T3 (GA_3 150 ppm) was found superior over rest of the treatments under study, which significantly recorded the desirable values of seed germination, survival percentage, total chlorophyll content in leaves. However, all treatments have not shown significant effect on accumulation of proline in the seedling leaves.

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