

Effect of Gibberellic Acid on Germination and Growth Responses of Pummelo Seedlings under Eastern Himalayan Foothills

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ABSTRACT

The research was conducted at Uttar Banga Krishi Viswavidyalaya, Cooch Behar district of West Bengal, under eastern Himalayan foothills during the year 2019–2020. This trial was aimed to evaluate the effect of seed treatment of pummelo with varying concentrations of gibberellic acid (GA₃) on germination and successive growth behaviour of seedlings. Seven treatments were employed viz., Control, 50 ppm GA₃, 100 ppm GA₃, 150 ppm GA₃, 200 ppm GA₃, 250 ppm GA₃ and 300 ppm GA₃ and each treatment was replicated three times. All treatments significantly performed better as compared to control. According to the results obtained, treatment with 300 ppm GA₃ was found to be superior among all the treatments with highest germination percentage (98.33 %), germination value (5.30), shortest minimum days to germination (18.83), mean germination time (27.95), maximum number of leaves (13.67), number of roots (29.00), shoot length (26.33 cm), root length (21.40 cm), seedling length (47.73 cm), seedling vigour index (4693.90), fresh root weight (1.80 g), fresh shoot weight (1.33 g), fresh leaf weight (3.67 g), dry root weight (0.77 g), dry shoot weight (0.52 g), dry leaf weight (0.96 g), root:shoot ratio (0.52) and total biomass (2.25 g plant⁻¹). Therefore, pre-sowing treatment of pummelo seeds with 300 ppm GA₃ may be recommended to the growers to enhance germination and growth of seedlings leading to the production of vigorous plants which can be utilized in crop improvement programme as well as rootstock studies.

1. Introduction

Pummelo [*Citrus grandis* (L.) Osbeck] belonging to the family Rutaceae is regarded as the citrus species bearing largest fruit. It is indigenous to Malayan and East Indian Archipelago and is now cultivated in many tropical and semi-tropical countries. This fruit is also commonly known as *Batabi*, *Rabab*, *Papanas*, *Chakotra* or *Shaddock* (Uzun and Yesiloglu 2012; Chattopadhyay 2007). Typically, the fruit is around 15–25 cm in diameter, usually weighing 1–2 kg, pale green to yellow when ripe with sweet white or pink flesh, thick albedo and distinctly winged leaf petioles.

Pummelo has numerous health benefits owing to the presence of different bio-active substances such as ascorbic acid, vitamin B complex, phenol, pectin, carotenoids, coumarins, tannin, lycopene, dietary fibre, naringin and other flavonoids in various parts of the fruit (Gupta *et al.*, 2021; Tocmo *et al.*, 2020; Nishad *et al.*, 2018).

Naringin, hesperidin, neohesperidin and naringenin extract of pummelo possess antiglycation properties for preventing diabetic complications (Caengprasath *et al.*, 2013). The peel of white variety of pummelo is a potential source of natural antioxidants (Toh *et al.*, 2013). Ethanolic extract of pummelo seeds and pulp is reported to have antimicrobial effect, which makes it a natural preservative ingredient for food and cosmetic (Sahlan *et al.*, 2018).

Since pummelo is monoembryonic in nature, the resulting seedling is not true to type to the mother plant. So, it is commonly propagated vegetatively by air layering or budding (Sharaf *et al.*, 2016). However, sexual propagation through seed is highly essential in performing a successful crop improvement work and study of rootstocks. Pummelo seeds are usually large, plump, pale yellowish white, flattened and angular. Since citrus seeds are recalcitrant in nature, they are best to be sown immediately after extraction

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from fully ripe fruits to avoid from moisture loss. The germination and growth rate of citrus seeds are very slow which hinders the process of hybridization (Khopkar *et al.*, 2017).

Gibberellic acid is a phytohormone which is essential for various plant development processes, including seed germination (Cornea-Cipcigan *et al.*, 2020; Ma *et al.*, 2018; Urbanova and Leubner-Metzger 2018), leaf expansion, stem elongation (Oh *et al.*, 2015; Oh and Kim 2014), root proliferation, pollination, flower induction and fruit development (Alshakhaly and Qrunfleh 2018; Vishal and Kumar 2018; Amin *et al.*, 2017; Dong *et al.*, 2017). It is well known for its role in promoting enzymes gene expression that helps in the movement of reserved food materials stored in the endosperm (starch, protein and lipids) thereby enhancing the biological yield of the plant (Mayur *et al.*, 2022). It is also widely applied to break dormancy of seed and facilitate germination by activating embryo growth, mobilization of photosynthates and weakening of the endosperm layer (Pallaoro *et al.*, 2016; Baskin and Baskin 2014).

The effect of GA₃ has been studied in different fruit crops including guava (Hosseini *et al.*, 2020; Brijwal and Kumar 2013), papaya (Anjanawe *et al.*, 2013), apple (Grzesiket *et al.*, 2017), loquat (Al-Hawezy 2013), jackfruit (Maiti *et al.*, 2003) etc. Several researchers have reported that pre sowing treatment of citrus seeds with gibberellic acid (GA₃) promotes better germination and uniform growth of seedlings (Dilipet *et al.*, 2017; Khopkar *et al.*, 2017; Sharaf *et al.*, 2016;) but very few papers were found where effect of GA₃ on pummelo seed germination was studied. Therefore, the present trial was conducted with an attempt to shorten the duration taken for seed germination as well as enhance the growth of pummelo seedlings in response to different concentrations of GA₃ treatments.

2. Materials and Methods

The experiment was performed during the year 2019-2020 under net house condition of Department of Pomology and Post-Harvest Technology at Uttar Banga Krishi Viswavidyalaya, Cooch Behar district, West Bengal, India. Geographically the district lies in the foothills of the eastern Himalayas and is located at 28°58'86" N latitude, 81°66'73" E longitude at an elevation of 42 m above mean sea level. The experiment was laid out in Complete Randomized Design (CRD) with seven treatments each replicated thrice. The seven treatments employed were: T₁ (Control), T₂ (50 ppm GA₃), T₃ (100 ppm GA₃), T₄ (150 ppm GA₃), T₅ (200 ppm GA₃), T₆ (250 ppm GA₃) and T₇ (300 ppm GA₃).

The seeds were extracted from fully matured and ripe fruits collected from the college campus and washed properly with tap water followed by soaking them in different concentrations of GA₃ for overnight. On the next day, the

treated seeds were sown in polybags filled with homogeneous mixture of soil:FYM (1:1) and irrigated lightly thereafter. In total, 100 seeds were sown in each replication. The seeds were irrigated whenever necessary and observed periodically for any sign of seed germination and data was recorded. Four months after germination, ten representative seedlings were selected from each replication of a treatment to measure seedling growth parameters. A total of 18 parameters were evaluated under this experiment. Germination percentage and seedling growth parameters like seedling length (cm), fresh weight (g), dry weight (g) and total biomass (g plant⁻¹) were recorded according to ISTA, 1999. The parameters recorded are listed below:

1. Germination Percentage (%)

$$= \frac{\text{Total number of seeds germinated}}{\text{Total number of seeds sown}} \times 100$$

2. Germination value (GV) = It is a composite value that includes both total germination and germination speed. It was calculated using the formula given by Djavanshir and Pourbeik (1976).

$$GV = (\sum DGS/N) GP/10$$

where, GP= germination percentage at the end of the test,

DGS= daily mean germination speed obtained by dividing the cumulative germination percentage by the number of days since sowing,

$\sum DGS$ = the total germination obtained by adding every DGS value obtained from the daily counts,

N= the total number of daily counts starting from the date of first germination.

3. Minimum days to germination: Number of days taken for the first seed to germinate was noted.

4. Mean germination time (MGT): The total time period taken to accomplish germination was recorded as MGT following Bonner (1983) and Phartyal *et al.* (2001) as given below:

$$MGT = \frac{\sum (\text{Daily germination} \times \text{Days})}{\text{Number of seeds sown}}$$

Un-germinated seeds at the end of the test were given values of n+1, where n= number of days in the test.

5. Number of leaves: Total number of leaves per plant was noted.

6. Number of secondary roots: Total number of secondary roots per plant was recorded.

7. Length of primary root (cm): Length of root was measured from the tip to the base of the tap root by using a scale.

8. Shoot length (cm): Length of the shoot was measured from the tip to the base of the seedling by using a scale.

9. Seedling length (cm) = Shoot length (cm) + Length of primary root (cm)

10. Seedling Vigour Index (SVI) = Germination (%) x Seedling length (cm) (Abdul-Baki and Anderson 1973)
11. Fresh root weight (g): Fresh root weight per plant was determined using a precision weighing balance after washing and drying the roots properly with filter paper.
12. Fresh shoot weight (g): Fresh weight of the shoot portion was measured using a precision weighing balance.
13. Fresh leaf weight (g): The total fresh leaf weight per plant was measured using a precision weighing balance.
14. Dry root weight (g): Roots were detached from the stem, washed and kept in hot air oven at 60 °C to 65 °C until a constant value was obtained and then weighed using a precision weighing balance.
15. Dry shoot weight (g): Shoot portion was detached and dry weight was noted as mentioned above.
16. Dry leaf weight (g): Leaves were detached and dry weight was noted as mentioned above.
17. Root:Shoot ratio = $\frac{\text{Dry weight of root}}{\text{Dry weight of top portion of plant}}$ (Racey *et al.*, 1983)
18. Total biomass (g plant⁻¹) = Dry root weight (g) + Dry shoot weight (g) + Dry leaf weight (g)

Observations recorded during the experiment were subjected to the statistical analysis of variance for Complete Randomized Design (CRD). Significance and non-significance of the variance due to different treatments were governed by determining the respective 'F' values based on the method given by Gomez and Gomez (2010).

3. Results and Discussion

3.1. Seed germination

The results shown in Table 1 revealed that GA₃ treatments significantly improved all the germination parameters. Highest germination percentage of 98.33 % was found in treatment 300 ppm GA₃ and lowest was found in control (93.50 %). Initiation of seed germination was earliest (18.83 days) in 300 ppm GA₃ treated seeds while it started at 28.00 days in control. Mean germination time (MGT) was minimum in 300 ppm GA₃ (27.95) as compared to control (37.99). Germination value (GV) was also found to be highest in 300 ppm GA₃ (5.30) whereas it was lowest in control (3.78). The accelerated and improved germination in this treatment may be due to the role of exogenous GA₃ in enhancing various aspects of germination.

During germination, GA₃ can promote embryo growth and facilitate radicle protrusion through the seed coat (Sun 2008) by overcoming the inhibitory effects of the seed coat and abscisic acid-related embryo dormancy on seed germination (Groot and Karssen 1987; Debeaujon and Koornneef 2000). The advance germination of seeds may also be due to the role of GA₃ in facilitating enzyme activities thereby enhancing the production of food reserves for growth and emergence of the embryo (Mayur *et al.*, 2022). GA₃ also

provides better water absorption and cell growth by stimulating transmission of calcium from cell wall to the cytoplasm (Al-Hawezy 2013). Improvement in germination of seeds with the application of GA₃ has also been reported by Hosseini *et al.* (2020) in guava, Dilip *et al.* (2017) in Rangpur lime, Joshi *et al.* (2015) and Kalalbandi *et al.* (2003) in acid lime, Khopkar *et al.* (2014) in pummelo, Al-Hawezy (2013) in loquat, Anburani and Shakila (2010) in papaya and Maiti *et al.* (2003) in jackfruit.

3.2. Seedling growth

Data in Table 1 and Table 2 shows that numbers of leaves, number of roots, shoot length, root length and seedling length were significantly increased by different treatments. Highest number of leaves was observed in 300 ppm GA₃ treatment (13.67) while the lowest was recorded in control (8.00). Highest number of leaves in this treatment may be because of the effect of GA₃ in promoting cell division and cell elongation which leads to the increase development of young leaves (Salisbury and Ross, 1988). Pararell results were also reported by Dilip *et al.* (2017) in Rangpur lime and Joshi *et al.* (2015) in acid lime.

Maximum number of roots (29.00) germination speed and length of primary root (21.40 cm) was also found in seedlings treated with 300 ppm GA₃ and lowest was recorded in control (16.33 and 15.07 cm). It might be due to increased photosynthetic activity and enhanced movement of food materials through phloem to the root zone thereby increasing the number and length of roots (Shukla *et al.*, 1997). These data are in concordance with the reports of Khopkar *et al.* (2017) in pummelo and Ramteke *et al.* (2015) in papaya.

Seedlings treated with 300 ppm GA₃ recorded maximum shoot length (26.33 cm) and minimum value was found in control (17.50 cm). Seedling length was also observed to be maximum in those treated with 300 ppm GA₃ (47.73 cm) and minimum was found in control (32.57 cm). This may be due to enhanced cell elongation (Shanmugavelu 1970) and cell division (Stowe and Yamaki 1957) which will consequently extend the intermodal length. This observation is in harmony with the results of Anjanawe *et al.* (2013) in papaya, Dilip *et al.* (2017) in Rangpur lime and Sharaf *et al.* (2016) in Cleopatra mandarin and Rangpur lime

Seedling Vigour Index (SVI), which determines the quality of seed during germination and seedling development was found to be significantly varied among different treatments (Table 2). Highest value of SVI was recorded in 300 ppm GA₃ (4693.90) while lowest value was recorded in control (3063.00). The vigorous growth of seedlings may be the result of increased photosynthetic rate and translocation of the photosynthetic products to different parts of the plant due to GA₃ treatments. This finding is

supported by Al-Hawezy (2013) in loquat and Anburani and Shakila (2010) in papaya.

It is apparent from Table 2 and Table 3 that fresh weight and dry weight of seedlings were significantly influenced by different treatments. Maximum fresh root weight (1.80 g), fresh shoot weight (1.33 g) and fresh leaf weight (3.67 g) was recorded in 300 ppm GA₃ treatment and lowest was recorded in control. Dry weight of seedling is an essential factor in study of seeds as it is directly correlated with seedling vigour. The seeds with higher seedling dry weight along with better germination are regarded as favourable for longer storage (Sharma *et al.*, 2016). Maximum dry root weight (0.77 g), dry shoot weight (0.52 g) and dry leaf weight (0.96 g) was recorded in 300 ppm GA₃ treatment while lowest value was obtained in control. Apparently, total biomass content was recorded highest in 300 ppm GA₃ treatment (2.25 g/plant) and lowest content was recorded in control (0.80 g/plant).

The increase in fresh weight, dry weight and total biomass of the seedlings may be attributed to the influence of GA₃ in promoting transportation of water and nutrients to various parts of the plant leading to enhanced rate of photosynthesis and resulted in greater accumulation of food reserves in the plant cell and increased vegetative growth (Mayur *et al.*, 2022). These results are in conformity with the studies of Khatana *et al.* (2015) in Kagzi lime and Dilip *et al.* (2017) in Rangpur lime. Effect of different treatments on root:shoot ratio was resulted to be highly significant (Table 3) and maximum value was obtained in 300 ppm GA₃ treatment (0.52) while lowest was recorded in control (0.28). This finding is in close conformity with Meshram *et al.* (2015) in acid lime and Patel *et al.* (2018) in tamarind.

4. Conclusion

Pre-sowing treatment of pummelo seeds with 300 ppm GA₃ for overnight can be recommended to achieve higher seed germination as well as enhanced successive development of seedlings.

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Treat-ments	Germination percentage (%)			Germination value (GV)			Minimum days to germination			Mean Germination time (MGT)			Number of leaves			Number of secondary roots		
	2019	2020	Pool	2019	2020	Pool	2019	2020	Pool	2019	2020	Pool	2019	2020	Pool	2019	2020	Pool
T ₁	94.33 (9.76)	92.67 (9.68)	93.50 (9.72)	3.87	3.69	3.78	28.67	27.33	28.00	37.79	38.19	37.99	9.00	7.00	8.00	18.67	14.00	16.33
T ₂	94.67 (9.78)	93.00 (9.70)	93.83 (9.74)	3.97	3.79	3.88	25.67	24.67	25.17	36.07	36.65	36.36	9.67	9.67	9.67	22.33	17.00	19.67
T ₃	95.33 (9.82)	94.00 (9.75)	94.67 (9.78)	4.20	3.91	4.06	23.67	23.33	23.50	33.96	35.72	34.84	12.67	10.67	11.67	19.33	17.33	18.33
T ₄	95.67 (9.83)	96.00 (9.85)	95.83 (9.84)	4.35	4.25	4.30	23.00	22.67	22.83	33.44	34.17	33.80	13.00	11.00	12.00	24.33	19.00	21.67
T ₅	97.00 (9.90)	97.00 (9.90)	97.00 (9.90)	4.52	4.49	4.51	22.67	21.33	22.00	32.75	33.06	32.91	13.67	11.00	12.33	25.33	23.33	24.33
T ₆	97.67 (9.93)	97.67 (9.33)	97.67 (9.93)	4.88	4.98	4.93	20.67	19.33	20.00	29.99	29.76	29.88	14.00	12.67	13.33	27.00	25.67	26.33
T ₇	98.00 (9.95)	98.67 (9.98)	98.33 (9.97)	5.21	5.39	5.30	19.33	18.33	18.83	28.57	27.33	27.95	14.33	13.00	13.67	30.67	27.33	29.00
SEm ±	0.08	0.05	0.05	0.18	0.09	0.12	0.49	0.40	0.33	0.49	0.38	0.33	0.55	1.73	0.92	2.51	2.94	2.48
CD (5%)	NS	0.14	0.15	0.54	0.29	0.36	1.48	1.21	0.99	1.48	1.16	1.00	1.66	NS	2.75	7.60	8.91	7.52

* T₁ (Control), T₂ (50 ppm GA₃), T₃ (100 ppm GA₃), T₄ (150 ppm GA₃), T₅ (200 ppm GA₃), T₆ (250 ppm GA₃) and T₇ (300 ppm GA₃)

*Figures in the parenthesis are the square root transformed values

Treat-ments	Length of primary root (cm)			Shoot length (cm)			Seedling length (cm)			Seedling Vigour Index (SVI)			Fresh root weight (g)			Fresh shoot weight (g)		
	2019	2020	Pool	2019	2020	Pool	2019	2020	Pool	2019	2020	Pool	2019	2020	Pool	2019	2020	Pool
T ₁	14.47	15.67	15.07	19.47	15.53	17.50	33.93	31.20	32.57	3233.57	2892.43	3063.00	0.80	0.56	0.68	0.67	0.43	0.55
T ₂	14.57	15.90	15.23	20.77	18.77	19.77	35.33	34.67	35.00	3369.23	3258.23	3313.73	0.98	0.61	0.79	1.29	0.85	1.07
T ₃	15.13	17.13	16.13	24.23	20.23	22.23	39.37	37.37	38.37	3744.23	3477.37	3610.80	1.05	0.76	0.90	1.27	0.79	1.03

T₄	15.17	17.10	16.13	25.70	21.70	23.70	40.87	38.80	39.83	3896.47	3765.47	3830.97	1.53	1.04	1.28	1.28	0.98	1.13
T₅	16.20	18.73	17.47	27.37	22.70	25.03	43.57	41.43	42.50	4253.00	4050.00	4151.50	1.58	1.13	1.36	1.36	1.23	1.29
T₆	16.27	20.27	18.27	28.57	23.57	26.07	44.83	43.83	44.33	4349.37	4224.57	4286.97	1.81	1.18	1.50	1.34	1.22	1.28
T₇	19.87	22.93	21.40	29.00	23.67	26.33	48.87	46.60	47.73	4789.33	4598.47	4693.90	2.34	1.26	1.80	1.42	1.25	1.33
SEm ±	1.07	1.09	0.95	1.41	1.74	1.50	1.63	1.54	1.42	200.29	170.96	164.95	0.30	0.15	0.20	0.15	0.18	0.12
CD (5%)	3.25	3.29	2.90	4.28	5.27	4.56	4.95	4.69	4.32	607.51	518.55	500.33	0.91	0.46	0.62	0.45	0.54	0.36

*T₁ (Control), T₂ (50 ppm GA₃), T₃ (100 ppmGA₃), T₄ (150 ppmGA₃), T₅ (200 ppm GA₃), T₆ (250 ppm GA₃) and T₇ (300 ppmGA₃)

Treat-ments	Fresh leaf weight (g)			Dry root weight (g)			Dry shoot weight (g)			Dry leaf weight (g)			Root:Shoot ratio			Total biomass (g/plant)		
	2019	2020	Pool	2019	2020	Pool	2019	2020	Pool	2019	2020	Pool	2019	2020	Pool	2019	2020	Pool
T₁	2.26	1.12	1.69	0.19	0.15	0.17	0.20	0.15	0.18	0.50	0.34	0.42	0.27	0.31	0.28	0.90	0.64	0.77
T₂	2.96	1.83	2.39	0.35	0.22	0.28	0.41	0.23	0.32	0.74	0.57	0.66	0.30	0.28	0.29	1.50	1.02	1.26
T₃	3.18	2.05	2.62	0.45	0.27	0.36	0.39	0.24	0.32	0.78	0.61	0.70	0.39	0.32	0.36	1.62	1.13	1.37
T₄	3.22	1.86	2.54	0.57	0.31	0.44	0.53	0.39	0.46	0.82	0.65	0.74	0.43	0.31	0.37	1.93	1.35	1.64
T₅	3.28	2.14	2.71	0.64	0.35	0.49	0.52	0.33	0.42	0.98	0.81	0.89	0.43	0.31	0.38	2.13	1.49	1.81
T₆	3.77	2.64	3.20	0.81	0.41	0.61	0.49	0.32	0.40	0.98	0.72	0.85	0.56	0.41	0.50	2.27	1.45	1.86
T₇	4.23	3.10	3.67	1.03	0.51	0.77	0.59	0.44	0.52	1.05	0.88	0.96	0.63	0.39	0.52	2.67	1.84	2.25
SEm ±	0.35	0.28	0.25	0.09	0.06	0.07	0.05	0.06	0.05	0.08	0.08	0.08	0.07	0.07	0.05	0.16	0.14	0.14
CD (5%)	1.06	0.84	0.77	0.27	0.20	0.20	0.16	0.17	0.15	0.25	0.25	0.23	0.21	NS	0.14	0.48	0.42	0.44

* T₁ (Control), T₂ (50 ppm GA₃), T₃ (100 ppmGA₃), T₄ (150 ppmGA₃), T₅ (200 ppm GA₃), T₆ (250 ppm GA₃) and T₇ (300 ppmGA₃)

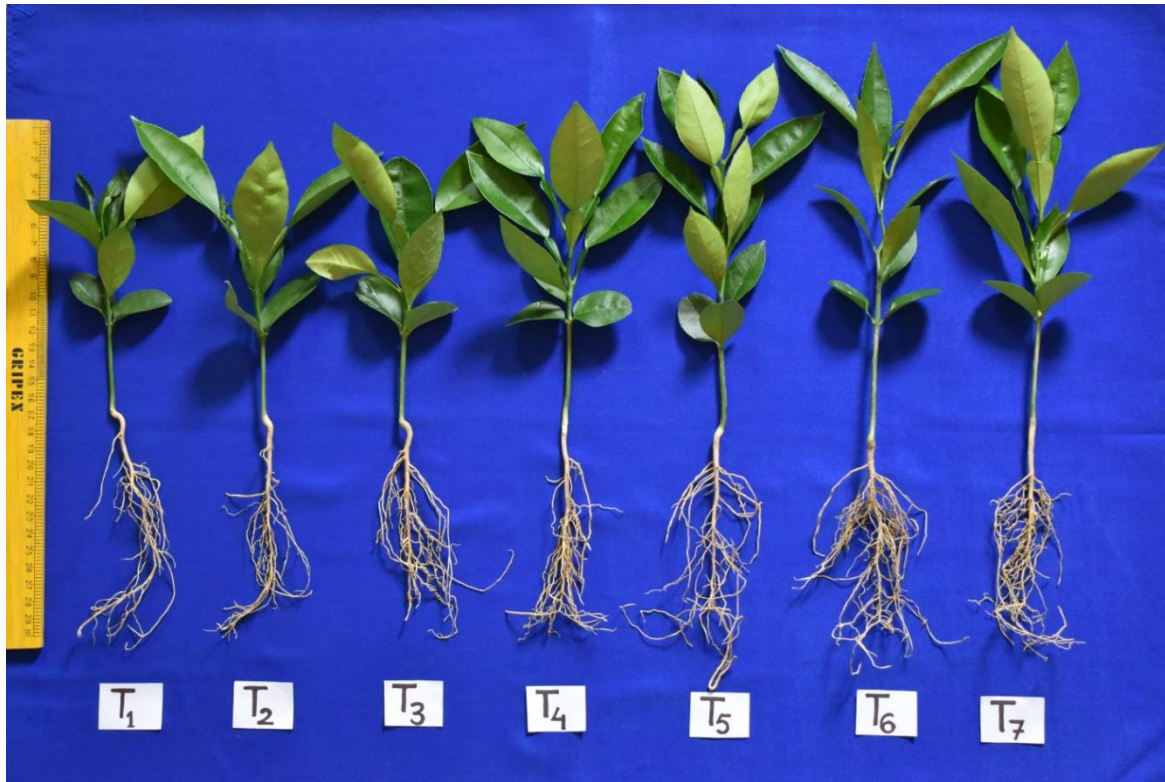


Figure 1. Comparison of seedling growth under different treatments