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Effect of soaking of seeds in bioregulators on growth of kokum seedling

RM Pawar, GM Golvankar, HR Agare and SB Thorat

Abstract

The present investigation entitled with effect of soaking of seeds in bioregulators on growth of kokum seedling was conducted at fruit crop nursery, Department of Horticulture, College of Agriculture, Dapoli, Dist. Ratnagiri (M.S.) during year 2013. The experiment was conducted in RBD with ten pre-sowing seed treatments and replicate in thrice. The results regarding pre-soaking of seed in bioregulators on growth parameters in kokum showed the significantly varied result at 180 DAS. Treatment T₅ had recorded better result regarding height of seedling (18.94 cm) and girth of seedling (0.37) and number of leaves (17.93). Leaf area (55.29) was significantly highest at T₅ which was at par with T₄ (53.85) and T₆ (46.65).

Keywords: *Garcinia indica*, kokum, bioregulators, pre-soaking, growth etc.

Introduction

Kokum (*Garcinia indica* Choisy) is a member of family Guttiferae, mainly found along West Coast of Konkan, Goa, Karnataka and North Malabar. It is also known as *Brindon* to the Portuguese in Goa, *Bhirand* or *Amsul* in Konkani and Marathi, *Murgal* in Kannada and *Punampuli* in Malayalam. French botanists Laurence Garcin collected and studied the tree of this genus during his stay in India and name this tree as *Garcinia*. It occurs from the sea level plains upto an elevation of about 800 m along the westward slopes of the Western Ghats (Muhammed *et al.* 1994) [9].

As per a base line survey (Anon., 2010) [2] conducted by Dr. B. S. Konkan Krishi Vidyapeeth, Dapoli, Maharashtra around 1000 ha area is occupied by kokum in Konkan region with production of 4500 MT fruits. According to survey conducted earlier by Chief Conservator of Forest in 2010 out of the total 46600 Kokum trees in the state of Maharashtra; 43000 trees existed in Ratnagiri and Sindhudurg Districts. It was also reported that in South Konkan 1674 MT of Kokum fruits were used for dried Kokum rind, 757 MT for preparation of Kokum syrup and 40 MT for Kokum butter. In Sindhudurg district, estimated area under Kokum is about 108 hectares scattered along riverbanks, streams, valleys, roadsides and backyard wastelands. Trees are also observed in coconut and arecanut gardens. In India, kokum trees are endemic to Konkan belt and the estimated production is 10,200 tons, of which 9000 tons are processed (Sawant, 2005) [14].

There is great scope for cultivation of kokum in the Konkan region, since the soil and climatic condition are ideal for its growth; however, its cultivation has not extended because of its dioecious nature. The sex of plant is known only after completion of juvenile period; fifty per cent plants being male are unproductive.

Besides low seed viability, poor seed germination and slow seedling growth etc. are the major problems in seedling raising and producing and planting material of elite kokum. Hence, it is essential to standardize proper germination media and their proportions, use of plant growth regulators to improve kokum seed further germination and growth at nursery stage. Therefore, the importance of crop and information on seed treatment with bioregulators and effect on growth was lacking in Konkan region of Maharashtra. Hence present investigation were undertaken with to study the effect of soaking of seeds in bioregulators on growth of kokum seedling.

Materials and methods

The field experiment was carried out at Department of Horticulture, College of Agriculture, Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli.

Experimental details

1. Crop: Kokum
2. Design: Randomized Block Design
3. Replications: Three
4. No. of treatments: Ten
5. No. of plants per treatment: Fifty

Collection of seed material

The seed material of kokum required for the present studies was collected from the bearing trees of kokum planted in orchard of Department of Horticulture. The seed material was collected during the fruiting season. The seeds were extracted from pulp with hand and were washed thoroughly with water to remove all adhering pulp and used for carrying out different experiments.

Treatments Details**Pre sowing seeds treatments**

- T₁:** Control (seeds without any treatment)
T₂: Soaking of seeds in 10 ppm BA solution for 24 hrs.
T₃: Soaking of seeds in 20 ppm BA solution for 24 hrs.
T₄: Soaking of seeds in 25 ppm GA solution for 24 hrs.
T₅: Soaking of seeds in 50 ppm GA solution for 24 hrs.
T₆: Soaking of seeds in 10 ppm IBA solution for 24 hrs.
T₇: Soaking of seeds in 20 ppm IBA solution for 24 hrs.
T₈: Soaking of seeds in 5 ppm BA + 15 ppm GA solution for 24 hrs.
T₉: Soaking of seeds in 10 ppm BA + 30 ppm GA solution for 24 hrs.
T₁₀: Soaking of seeds in 5 ppm BA + 15 ppm GA + 5 ppm IBA solution for 24 hrs.

Preparation of Growth Regulator

The stock solution of 100 ppm GA₃ was prepared by dissolving 100 mg of pure GA₃ in 10 ml alcohol and making volume to one liter with distilled water. The working solution of desired strength was prepared by taking appropriate quantities of stock solution and then diluting the same with distilled water to get required concentration of GA₃ solution *i.e.* 25 ppm and 50 ppm.

The stock solution of 100 ppm BA was prepared by dissolving 100 mg of pure BA in 10 ml alcohol and making volume to one liter with distilled water. The working solution of desired strength was prepared by taking appropriate quantities of stock solution and then diluting the same with distilled water to get required concentration of BA solution *i.e.* 10 ppm and 20 ppm.

The working solution of treatment T₈, T₉ and T₁₀ was prepared by taking required quantity of stock solution of GA, BA and IBA respectively and then diluted with distilled water to get required concentration of 5 ppm BA+ 15 ppm GA, 10 ppm BA+ 30 ppm GA, 5 ppm BA+ 15 ppm GA+ 5 ppm IBA solutions.

Soaking of seeds

The required number of seeds was soaked in 1000 ml solution of BA, GA, IBA solution at the concentration of given ppm solution separately for 24 hrs.

Preparation of potting mixture, filling of polythene bags and sowing of treated seeds

Potting mixture was prepared by using three parts of soil and one part of well decomposed FYM. Then the mixture was filled in polythene bags of size (8cm x 20cm). The treated seeds were sown in polythene bags and placed in rows. The

proper care was taken, while sowing that the seeds will not be placed too deep. The depth of sowing was maintained up to 2 cm. The care was also taken to cover these seeds with thin layer of potting mixture.

Intercultural operation

The intercultural operation like irrigation, weeding was done as per the requirement. The irrigation was given with the help of watering can and proper care was taken to maintain the optimum soil moisture in polythene bags. Weeding was done to remove weeds germinating in polythene bags in order to avoid competition of weeds with seedling for nutrient and moisture. The following observations were recorded.

Observations recorded**Growth observations**

The observations on growth parameters in respect of height of seedling, girth at collar region, number of leaves were recorded at monthly interval from sowing to six months.

1. Height of seedling (cm)

The height of five seedlings from each treatment combination and each replication was measured from the ground level up to the growing tip and after computing the mean, it was recorded as height of seedling in centimeter at the monthly interval from sowing upto six months.

2. Girth of seedling at collar region (cm)

Girth of five selected seedling from each replication of treatment combination at collar region was measured with the help of vernier caliper, from the base and after computing the mean, it was recorded as the average girth of seedlings in centimeter at the monthly interval from sowing upto six months.

3. Number of leaves

Total numbers of leaves per plant were counted from five selected plants from each replication of treatment combination and after computing the mean, it was recorded as a leaves per plant at the monthly interval from sowing upto six months.

4. Leaf area (cm²)

Three leaves from five tagged plants from each replication of treatment combinations were selected randomly. The leaf area of each leaf was estimated with leaf area meter at the age of six month and recorded in square centimeters. The averages of these three representative leaf samples were multiplied with number of leaves to estimate the leaf area per plant.

Statistical method

The data in the present investigation was statistically analyzed by the method suggested by Panse and Sukhatme (1995) ^[12].

Results and Discussion**Effect of bioregulators seed treatment on growth observation in kokum****1. Height of seedlings (cm)**

The plant height is one of the important characters, in growth and development of seedling.

The data regarding height as affected by different preparing plant bioregulators seed treatment are presented in Table 1.

At 30 DAS the highest height of seedlings was recorded in the T₅ (5.05) was significantly superior over all other treatments.

It was lowest in the treatment T₁ (3.33) and was at par with T₁₀ (3.79) and T₄ (3.81).

Similarly at 60 DAS the highest height of seedlings was recorded in the T₅ (6.78) which was significantly superior over all other treatments. It was lowest in the treatment T₁ (5.12).

At 90 DAS the highest height of seedlings was recorded in the T₅ (8.85) which was significantly superior over all other treatments. It was lowest in the treatment T₁ (7.01).

At 120 DAS the highest height of seedlings was recorded in the T₅ (11.53) which was significantly superior over other treatments. It was lowest in the treatment T₁ (8.46) it at par with T₆ (9.43), T₂ (9.43), T₄ (9.41), T₉ (9.26) and T₁₀ (8.93).

The perusal of data presented in Table 1 revealed that effect of various seed treatments on height of seedlings in kokum was significant and in descending order it was as follows.

30 DAS	T ₅ > T ₇ > T ₃ > T ₂ > T ₆ > T ₈ > T ₉ > T ₄ > T ₁₀ > T ₁
60 DAS	T ₅ > T ₃ > T ₇ > T ₈ > T ₂ > T ₆ > T ₄ > T ₉ > T ₁₀ > T ₁
90 DAS	T ₅ > T ₃ > T ₇ > T ₈ > T ₆ > T ₂ > T ₄ > T ₁₀ > T ₉ > T ₁
120 DAS	T ₅ > T ₇ > T ₃ > T ₈ > T ₆ > T ₂ > T ₄ > T ₉ > T ₁₀ > T ₁
150 DAS	T ₅ > T ₃ > T ₈ > T ₇ > T ₂ > T ₄ > T ₉ > T ₆ > T ₁₀ > T ₁
180 DAS	T ₅ > T ₃ > T ₈ > T ₇ > T ₄ > T ₉ > T ₂ > T ₆ > T ₁₀ > T ₁

At 150 DAS the highest height of seedlings was recorded in the T₅ (15.79) which was significantly superior over other treatments. It was lowest in the treatment T₁ (10.43) it was at par with T₁₀ (11.34), T₆ (11.85) and T₉ (11.85).

At 180 DAS the highest height of seedlings was recorded in the T₅ (18.94) which was significantly superior over other treatments. It was lowest in the treatment T₁ (12.60) it was at par with T₁₀ (14.19).

The higher shoot length with GA presoaking treatment of seeds might be attributed to the cell multiplication and elongation in the cambium tissue of the intermodal region (Donoho and Walker 1957) [4].

The result indicated that all the treatment improved height of seedlings than control. The treatment T₅ had significantly highest upto 180 DAS. The similar increase has been reported by Gharge *et al.*, (2011) [6] and Kudmulwar (2006) [8] in custard apple, Kalalbandi *et al.* (2003) [7] in kagzi lime.

2. Girth of plant at collar region (cm)

Girth at collar region is an important factor for giving the support to seedling at initial stage, which is a vital character in health of the seedling.

The data regarding girth of plant as affected by different plant growth regulators are presented in Table 2.

At 30 DAS the highest girth of seedlings was recorded in the T₃ (0.26). It was lowest in the treatment T₁ (0.11).

At 60 DAS the highest girth of seedlings was recorded in the T₄ (0.16). It was lowest in the treatment T₁ (0.12).

At 90 DAS the highest girth of seedlings was recorded in the T₃ (0.19) which was at par with T₅ (0.19) and was significantly superior over all other treatments. It was lowest in the treatment T₁ (0.14).

At 120 DAS the highest girth of seedlings was recorded in the T₅ (0.21) which was at par with T₃ (0.21) and T₉ (0.21) and was significantly superior over all other treatments. It was lowest in the treatment T₁ (0.15).

At 150 DAS the highest girth of seedlings was recorded in the T₃ (0.30) which was at par with T₅ (0.28) and T₉ (0.28) and was significantly superior over rest of the treatments. It was lowest in the treatment T₁ (0.19).

Similarly at 180 DAS the highest girth of seedlings was recorded in the T₅ (0.37) which was at par with T₃ (0.36), T₉ (0.35) and T₄ (0.34) and was significantly superior over other treatments. It was lowest in the treatment T₁ (0.24).

The perusal of data presented in Table 2 revealed that effect of various seed treatments on girth of seedlings at collar region in kokum was significant upto 180 DAS and in descending order was as follows,

30 DAS	T ₃ > T ₂ > T ₄ > T ₇ > T ₅ > T ₈ > T ₁₀ > T ₉ > T ₆ > T ₁
60 DAS	T ₄ > T ₃ > T ₅ > T ₂ > T ₈ > T ₇ > T ₁₀ > T ₉ > T ₆ > T ₁
90 DAS	T ₃ > T ₅ > T ₉ > T ₂ > T ₄ > T ₈ > T ₁₀ > T ₇ > T ₆ > T ₁
120 DAS	T ₅ > T ₃ > T ₉ > T ₄ > T ₈ > T ₂ > T ₁₀ > T ₇ > T ₆ > T ₁
150 DAS	T ₃ > T ₅ > T ₉ > T ₄ > T ₈ > T ₂ > T ₆ > T ₇ > T ₁₀ > T ₁
180 DAS	T ₅ > T ₃ > T ₉ > T ₄ > T ₈ > T ₂ > T ₆ > T ₁₀ > T ₇ > T ₁

Thus, all the treatments showed better results than control. Among different treatment GA₃ 50 ppm shows the better result regarding collar region girth of kokum seedling.

The data revealed that all the treatment improved girth of kokum seedling upto 180 DAS. The treatment T₅ was the best upto 180 DAS, which was followed by T₃. The similar finding were observed by Gawade (2008) [5] and Ratan and Reddy (2004) [13] in custard apple, Pal and Dhaka (2010) [10] in sweet orange.

3. Number of Leaves

From the physiological point of view, leaf is the most important photosynthetic site of the plant. It is the source from which the plant derives energy for its metabolic activities. The primary function of leaves is the carbon assimilation. Thus, leaf forms photosynthetic apparatus of plants.

The data regarding number of leaves per seedlings as affected by different plant bioregulators are presented in Table 3.

Numbers of leaves were increased through the course of study and was significantly varied among the treatment.

At 30 DAS the highest leaves of seedlings was recorded in the T₉ (1.87) which was at par with T₅ (1.80), T₇ (1.80), T₈ (1.80), T₁₀ (1.73) and T₆ (1.73) and was significantly superior over rest of the other treatments. It was lowest in the treatment T₄ (1.33) it was at par with T₃ (1.47), T₂ (1.47) and T₁ (1.47).

Similarly at 60 DAS the highest leaves of seedlings was recorded in the T₅ (4.00) which was at par with T₇ (3.80), T₁₀ (3.80), T₈ (3.67) and T₆ (3.47) and was significantly superior over rest of the treatments and it was lowest in the treatment T₃ (2.87) it was at par with T₂ (3.00), T₁ (3.07) T₄ (3.20) and T₉ (3.27).

At 90 DAS the highest leaves of seedlings was recorded in the T₅ (7.33) which was at par with T₄ (6.93), T₈ (6.87), T₆ (6.73) and T₇ (6.60). It was lowest in the treatment T₁ (5.67) it was at par with T₁₀ (5.93), T₃ (6.00) and T₉ (6.33).

Similarly at 120 DAS the highest leaves of seedlings was recorded in the T₅ (11.87) which was significantly superior over all other treatments. It was lowest in the treatment T₁ (9.20) and was at par with T₃ (9.47), T₂ (9.67) and T₆ (10.07).

At 150 DAS the highest leaves of seedlings was recorded in the T₅ (15.13) which was significantly superior over all other treatments. It was lowest in the treatment T₁ (11.73) it was at par with T₇ (12.33), T₆ (12.33), T₁₀ (12.47), T₂ (12.47) and T₃ (12.53).

At 180 DAS the highest leaves of seedlings was recorded in the T₅ (17.93) which was significantly superior over all other treatments. It was lowest in the treatment T₁ (13.73).

The perusal of data presented in Table 3 revealed that effect of various seed treatments on leaves of seedlings in kokum was significant.

30 DAS	T ₉ > T ₅ > T ₇ > T ₈ > T ₁₀ > T ₆ > T ₁ > T ₂ > T ₃ > T ₄
60 DAS	T ₅ > T ₇ > T ₁₀ > T ₈ > T ₆ > T ₉ > T ₄ > T ₁ > T ₂ > T ₃
90 DAS	T ₅ > T ₄ > T ₈ > T ₆ > T ₇ > T ₂ > T ₉ > T ₃ > T ₁₀ > T ₁
120 DAS	T ₅ > T ₉ > T ₇ > T ₄ > T ₁₀ > T ₈ > T ₆ > T ₂ > T ₃ > T ₁
150 DAS	T ₅ > T ₄ > T ₉ > T ₈ > T ₃ > T ₂ > T ₁₀ > T ₆ > T ₇ > T ₁
180 DAS	T ₅ > T ₄ > T ₇ > T ₁₀ > T ₆ > T ₃ > T ₈ > T ₂ > T ₉ > T ₁

The increased in number of leaves with GA treatment might be due to the promotion of physiological processes and stimulatory action of GA to form new leaves at faster rate and promotion of linear growth and accelerated translocation of food material in the tissue, which create an ideal condition to develop new leaf primordium (Chhonkar and Singh 1964)^[3]. Result indicated that highest number of leaves was in T₅, while lowest in control. The effect of presoaking of seed in bioregulators on seed germination and growth of seedling revealed that presoaking of seeds in GA at 400 ppm resulted higher number of leaves per seedling (Pampanna and Sulikeri 2001)^[11]. These results are in line with those of Aagawade

(1986)^[1], Kudmulwar (2006)^[8], Gawade (2008)^[5] in custard apple and Pal and Dhaka (2010)^[10] in sweet orange.

4. Leaf area (cm²)

Leaf area is an important parameter in determining the size of the photosynthetic site. Leaf area provides scope for absorption of solar radiation and there by leads to synthesis of photosynthates.

The data regarding leaf area of kokum seedling as affected significantly by different plant growth regulators are presented in Table 4.

The highest leaf area of kokum seedlings was recorded in T₅ (989.58), which was at par with T₄ (885.62) and T₆ (702.10) and was significantly superior over rest of the treatments. It was lowest in the treatment T₁ (542.63).

The perusal of data presented in Table 4 revealed that effect of various seed treatments on leaf area of seedlings in kokum was significant. It was in the descending order as follows,

T₅ > T₄ > T₆ > T₂ > T₉ > T₃ > T₈ > T₇ > T₁₀ > T₁

The result indicated that bioregulators improved leaf area of kokum leaves over untreated control at 180 DAS. The effect was significant. Similar result was obtained by Gawade (2008)^[5] and Kudmulwar (2006)^[8] in custard apple.

Table 1: Effect of pre-sowing bioregulators seed treatment on height in kokum seedlings (cm)

Treatments		30-DAS	60-DAS	90-DAS	120-DAS	150-DAS	180-DAS
T ₁	Control	3.33	5.12	7.01	8.46	10.43	12.60
T ₂	10 ppm BA	4.15	6.03	7.97	9.43	12.33	15.35
T ₃	20 ppm BA	4.22	6.39	8.39	9.75	12.25	16.60
T ₄	25 ppm GA	3.81	5.87	7.97	9.41	12.02	15.71
T ₅	50 ppm GA	5.05	6.78	8.85	11.53	15.79	18.94
T ₆	10 ppm IBA	4.09	5.99	8.00	9.43	11.85	14.93
T ₇	20 ppm IBA	4.28	6.20	8.09	10.19	12.65	16.13
T ₈	5 ppm BA + 15 ppm GA	4.03	6.12	8.08	9.71	12.87	16.25
T ₉	10 ppm BA + 30 ppm GA	4.00	5.82	7.67	9.26	11.85	15.37
T ₁₀	5 ppm BA+15 ppm GA+ 5 ppm IBA	3.79	5.73	7.82	8.93	11.34	14.19
	Range	3.33-5.05	5.12-6.78	7.01-8.85	8.46-11.53	10.43-15.79	12.60-18.94
	Mean	4.07	6.00	7.99	9.61	12.44	15.61
	Result	SIG	SIG	SIG	SIG	SIG	SIG
	S.Em±	0.16	0.11	0.12	0.33	0.51	0.63
	CD at 5%	0.49	0.33	0.35	0.97	1.52	1.88

DAS – Days After Sowing

Table 2: Effect of pre-sowing bioregulators seed treatment on girth at collar region in kokum seedlings

Treatments		30-DAS	60-DAS	90-DAS	120-DAS	150-DAS	180-DAS
T ₁	Control	0.11	0.12	0.14	0.15	0.19	0.24
T ₂	10 ppm BA	0.13	0.15	0.17	0.19	0.24	0.31
T ₃	20 ppm BA	0.26	0.15	0.19	0.21	0.30	0.36
T ₄	25 ppm GA	0.12	0.16	0.17	0.19	0.25	0.34
T ₅	50 ppm GA	0.12	0.15	0.19	0.21	0.28	0.37
T ₆	10 ppm IBA	0.11	0.13	0.16	0.18	0.23	0.30
T ₇	20 ppm IBA	0.12	0.14	0.16	0.18	0.23	0.29
T ₈	5 ppm BA + 15 ppm GA	0.12	0.15	0.16	0.19	0.24	0.31
T ₉	10 ppm BA + 30 ppm GA	0.11	0.13	0.17	0.21	0.28	0.35
T ₁₀	5 ppm BA+ 15 ppm GA + 5 ppm IBA	0.12	0.14	0.16	0.19	0.23	0.30
	Range	0.11-0.26	0.12-0.16	0.14-0.19	0.15-0.21	0.19-0.30	0.24-0.37
	Mean	0.13	0.14	0.17	0.19	0.25	0.32
	Result	NS	NS	SIG	SIG	SIG	SIG
	S.Em±	0.05	0.01	0.00	0.00	0.01	0.01
	CD at 5%	--	--	0.01	0.01	0.03	0.04

DAS – Days After Sowing

Table 3: Effect of pre-sowing bioregulators seed treatment on number of leaves in kokum seedlings

Treatments		30-DAS	60-DAS	90-DAS	120-DAS	150-DAS	180-DAS
T ₁	Control	1.47	3.07	5.67	9.20	11.73	13.73
T ₂	10 ppm BA	1.47	3.00	6.47	9.67	12.47	14.93
T ₃	20 ppm BA	1.47	2.87	6.00	9.47	12.53	15.00
T ₄	25 ppm GA	1.33	3.20	6.93	10.27	13.60	16.53
T ₅	50 ppm GA	1.80	4.00	7.33	11.87	15.13	17.93
T ₆	10 ppm IBA	1.73	3.47	6.73	10.07	12.33	15.07
T ₇	20 ppm IBA	1.80	3.80	6.60	10.33	12.33	15.13
T ₈	5 ppm BA + 15 ppm GA	1.80	3.67	6.87	10.20	12.60	15.00
T ₉	10 ppm BA + 30 ppm GA	1.87	3.27	6.33	10.67	12.87	14.67
T ₁₀	5 ppm BA+ 15 ppm GA+ 5 ppm IBA	1.80	3.80	5.93	10.27	12.47	15.13
	Range	1.33-1.87	2.87-4.00	5.67-7.33	9.20-11.87	11.73-15.13	13.73-17.93
	Mean	1.65	3.41	6.49	10.20	12.81	15.31
	Result	SIG	SIG	SIG	SIG	SIG	SIG
	S.Em±	0.12	0.23	0.25	0.31	0.27	0.31
	CD at 5%	0.35	0.68	0.75	0.93	0.80	0.91

DAS – Days After Sowing

Table 4: Effect of pre-sowing bioregulators seed treatment on leaf area in kokum seedlings

Treatments		180-DAS
T ₁	Control	542.63
T ₂	10 ppm BA	683.87
T ₃	20 ppm BA	671.86
T ₄	25 ppm GA	885.62
T ₅	50 ppm GA	989.58
T ₆	10 ppm IBA	702.10
T ₇	20 ppm IBA	636.97
T ₈	5 ppm BA + 15 ppm GA	651.12
T ₉	10 ppm BA + 30 ppm GA	686.35
T ₁₀	5 ppm BA+ 15 ppm GA+ 5 ppm IBA	632.89
	Range	542.63-989.58
	Mean	708.30
	Result	SIG
	S.Em±	46.17
	CD at 5%	137.18

DAS – Days After Sowing

Conclusion

From the present investigation, it can be concluded that the plant growth regulators play important role in various stages of plant. The treatment T₅ i.e. soaking of seeds in 50 ppm GA solution for 24 hours had recorded better result regarding height of seedling (18.94 cm) and girth of seedling (0.37) and number of leaves (17.93). Leaf area (55.29) was significantly highest at T₅ which was at par with T₄ (53.85) and T₆ (46.65).

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