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STUDIES ON EFFECT OF GROWTH REGULATORS AND BIOFERTILIZERS ON SEED GERMINATION AND SEEDLING GROWTH OF TAMARIND (*TAMARINDUS INDICA* L.)

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Abstract

The seeds of tamarind were subjected to pre-sowing treatments with GA_3 (200 and 300 ppm), NAA (200 and 300 ppm), KNO_3 (1 and 2 per cent), Biofertilizers like Phosphate Solubilising Bacteria (PSB), Azospirillum, Azatobactor, Vesicular Arbuscular Mycorrhiza and mixture of biofertilizers [Biomix (PSB + *Azospirillum* + *Trichoderma viridae* + *Pseudomonas fluorescens*)] / 50g/kg of seeds, scarifications like Mechanical Scarification. Acid scarification also seeds were soaked in cow urine 5% and distilled water as control for sixteen hours. Germination and seedling growth parameters at definite intervals were recorded to find out the effect of these pre-treatments on germination of tamarind. Increase in germination percentage (97.78%), rate of germination (2.12) and decrease in number of days taken (5.33 days) for initiation of germination was noticed in seeds subjected to mechanical scarification and GA_3 200 ppm treatment. The maximum plant height (40.57cm), seedling girth (1.99 cm), number of leaves (30.47), fresh and dry weight of shoots (23.99 g and 8.07g, respectively), fresh and dry weight of roots (7.90 g and 4.80g, respectively), vigour index-I (8039.24) and vigour index-II (1260.61) was recorded in GA_3 200 ppm at 150 days after sowing. The minimum values were recorded in control.

Key words : GA_3, NAA, KNO_3, vigour index, Biofertilizers and scarification.

Introduction

Tamarind (*Tamarindus indica* L.) is a member of dicotyledonous family *Fabaceae* and belongs to the sub family *Caesalpinoideae*. It is a diploid species with chromosome number 2n = 24 (Purseglove, 1987). The name of tamarind is derived from an Arabic word "Tamarind- E- Hind" meaning "Date of India" popularly known as "Indian Date". Tamarind is a short trunked, multistemmed, large, evergreen or semi-evergreen tree growing up to 30 m with a trunk of about 8 m circumference and a crown diameter of up to 12m. Tamarind trees starts bearing the fruits at the age of 13 to 14 years and continue to produce fruits even after 60 years and some up to 200 years. Tamarind half the pod weight is contributed by pulp. Pulp contains both sugars (30-40%) and organic acids (8-18%), predominantly tartaric acid. The pulp is also a rich source of vitamins, minerals and calcium.

The pulp is widely used as a spice for souring curries, chutneys and certain beverages. Tamarind is native of the Dry Savanna of Tropical Africa and probably some parts of South India. It is cultivated throughout the tropics and subtropics of the world and has become naturalized at many places. India is the main producer and consumer of tamarind in the world. It is estimated that India produces 3 million tons of fruits and exports the tamarind products worth of Rs. 50 cores per annum (Kotech and Kadam, 2002).

Tamarind is traditionally propagated from seed; tamarind produces relatively large seeds that average about 11-12.5 mm in diameter. They are flattish, shiny brown to blackish, with a hard impermeable seed coat. Germination of tamarind seed is epigeal. On an average, tamarind seeds begin to germinate about 13 days after sowing, but may take a month to complete (Joker, 2000).

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The main disadvantage of seed propagation is freshly harvested seeds of tamarind exhibit poor germination percentage even if exposed to favorable conditions of germination owing to seed dormancy. It may be due to morphological factor such as hard, thick testa or due to incorrect storage or handling (secondary dormancy). Such seeds may require special treatments like stratification, scarification, soaking in water, growth regulators, biofertilizers etc., for overcoming dormancy. Pre-sowing seed treatment with chemicals like GA_3, KNO_3, NAA and thiourea (Rajamanickam *et al*., 2002) and Bio-fertilizers, *viz*; *Azotobacter*, *Azospirillum*, Phosphate Solubilizing Bacteria and Vesicular Arbuscular Mycorrhiza fungi (VAM) improve the seed germination and seedling growth through producing several growth regulators substances like Indole Acetic Acid (IAA), Gibberlic Acid (GA) and vitamins besides fixation of atmospheric nitrogen (Fallick *et al*., 1989 and Ruan *et al*., 1973).

Materials and Methods

The present investigation was carried out at Regional Horticultural Research and Extension Centre, University of Horticultural Sciences, Gandhi Krishi Vignyan Kendra (West), Bangalore-560065 (Karnataka), India during 2012-2013. The experimental field is located at an altitude of 930 meters above mean sea level 12° 58 North latitude and 77° 35 longitude.

The experimental design selected was Completely Randomized Design. Fifteen different treatments were imposed including control. Fifteen seeds were used for each treatment, which was replicated thrice. The treatments as follows $\mathbf{T_1}$- Distilled water (control), $\mathbf{T_2}$ - GA_3 at 200, $\mathbf{T_3}$- GA_3 at 300 ppm $\mathbf{T_4}$ - NAA at 200, $\mathbf{T_5}$- NAA at 300 ppm, $\mathbf{T_6}$- KNO_3 at 1 per cent, $\mathbf{T_8}$- KNO_3 at 2 per cent, bio-fertilizers like $\mathbf{T_9}$- Phosphate Solubilising Bacteria (PSB), $\mathbf{T_{10}}$- Azospirillum, Azatobactor, $\mathbf{T_{11}}$- Vesicular Arbuscular Mycorrhiza and mixture of biofertilizers $\mathbf{T_{12}}$- [Biomix (PSB + *Azospirillum* + *Trichoderma viridae* + *Pseudomonas fluorescens*)]/ 50g/kg of seeds were treated by soaking for 16 hours in prepared solutions, scarification treatments imposed to seeds, which were $\mathbf{T_{13}}$- Mechanical Scarification, $\mathbf{T_{14}}$- Acid scarification and also seeds were soaked in cow $\mathbf{T_{15}}$- urine 5%. After imposing treatments, the seeds were shade dried for 10 minutes and were sown in polythene bag containing media 2:1:1 ratio (sand, soil, FYM) at 0.5 cm depth in 2 cm apart and were kept in the shade house. The polythene bags were watered daily till final data were recorded. Observations were recorded daily on germination parameters and monthly for vegetative parameters like plant height, number of leaves, stem girth, fresh weight, dry weight by keep the seedlings in hot air oven at the temperature of 60^0C till constant weight was attained and seedling vigour for up to 150 days after sowing. The data collected from the five labelled seedlings in each treatment were averaged and completely randomised design (CRD) was employed to find out the significance among different treatments with the help of 'F' test (Sunderaraju *et al*., 1972). The rate of germination and seedling vigour was calculated based on the following formulas (Bewley and Black, 1982).

1. $C = \Sigma G_n / \Sigma G_n.D_n \times 100$

Where,

G_n – Number of seeds germinated on a day n

D_n – Days from initial sowing

2. Vigour index – I (cm) = Mean seedling length × per cent germination

   Vigour index – II (g) = Dry weight of seedling × per cent germination.

Results and Discussion

Seed germination characters

Pre-sowing treatments influenced germination characters of seeds, resulting in their improved germination (table 1).

The seeds subjected to mechanical scarification were recorded earliest germination (5.33 days). This was on par with seeds treated with KNO_3 2 per cent (5.67 days), GA_3 300 ppm for 16 hour (6 days) and KNO_3 1 per cent (6 days). The late (10 days) germination was noticed in control. The seeds treated with GA_3 200 ppm for 16 hours, VAM 50g kg^{-1} of seeds and mechanical scarification recorded the highest germination percentage (97.78). The lowest germination recorded in control (80.00%). The seeds subjected to mechanical scarification recorded significantly faster rate of germination (2.12). Whereas, the slow rate of germination was noticed in control (0.94). The seed germination in tamarind is erratic due to the possession of various degrees of physical dormancy (Heit, 1967) caused due to hard seed coat, which is impermeable to water and oxygen (Bewley and Black, 1982). The mechanical scarification treatment removed the seed coat there by increased the permeability of air and water through seed which favors the earliest germination.

The treatment with GA_3 200 ppm for 16 hours of soaking proved to be good treatment. The exogenous application of Gibberelic acid antagonizes the ill effect of inhibitors (Brain and Hemming, 1958; Wareing *et al*., 1968) and the higher seed germination percentage in GA_3

Table 1 : Effect of pre-sowing seed treatments on germination attributes in tamarind.

Treatments	Initiation of seed germination (days)	Germination percentage	Rate of germination
T_1	10.67	80.00	0.94
T_2	6.33	97.78	1.70
T_3	6.00	93.33	1.58
T_4	8.33	86.66	1.11
T_5	8.67	88.88	1.18
T_6	6.00	91.11	1.54
T_7	5.67	95.55	1.69
T_8	9.33	86.66	1.02
T_9	8.00	91.11	1.25
T_{10}	9.67	88.89	1.04
T_{11}	6.33	97.78	1.58
T_{12}	7.67	95.55	1.41
T_{13}	5.33	97.78	2.12
T_{14}	6.33	91.11	1.61
T_{15}	10.33	86.66	0.93
F test	*	*	*
SE.m±	0.55	3.49	0.10
CD @ 5 %	1.61	10.08	0.29
CV	12.63	6.62	13.01

*significant.

was due to instigative action of GA_3 for germination of seeds. GA_3 induces the denovo synthesis of proteolytic enzymes like α-amylase and ribonuclease. Amylases in turn hydrolase starch in the endosperm, providing the essential sugars for the initiation of growth processes (Copeland and Mc Donald, 1995). GA_3 treatment is also known to overrule the photo dormancy, thermo-dormancy, dormancy imposed by incomplete embryo development, mechanical barriers and presence of germination inhibitors (Diaz and Martin, 1971).

Vegetative characters

Pre-sowing treatments influenced vegetative characters of seedling, resulting in their improved growth and development of seedling (table 2).

At the end of experiment at 150 days after sowing the treatment with GA_3 200 ppm has recorded the maximum seedling height (40.57 cm), which was on par with KNO_3 2 per cent (36.67 cm) treatments whereas, minimum was in control (26.70 cm) also GA_3 200 ppm recorded maximum seedling collar girth (1.99 cm) and this was on par with KNO_3 2 percent (1.89 cm), whereas minimum seedling collar girth (1.49cm) was noticed in control and maximum number of leaves was noticed in treatment with GA_3 200 ppm and this was on par with KNO_3 2 per cent, while minimum was recorded in control.

Basically, plant height is a genetically controlled character. But, several studies have indicated that plant height can be increased by application of synthetic plant growth regulators. However, in the present investigation a significant difference in plant height was noticed among the treatments by the application of different plant growth regulators and biofertilizers used. Among these, GA_3 recorded maximum plant height at all the stages. It was due to GA_3 effect on elongation of internodes, as GA_3 is known to enhance cell elongation (Krishnamoorthy and Sandooja, 1981). The application of growth promotive substances increased the plant height and such effect was due to increased photosynthetic activity, enhancement in the mobilization of photosynthates and change in the membrane permeability (Shukla *et al.*, 1997). The regulation of growth by gibberellins and KNO_3 relates almost extensively to its stem elongation properties. Influence of gibberllic acid and potassium nitrate on stem elongation is by two ways. (1) They have direct effect on stem elongation by including cell wall loosening, by increasing cell wall extensibility, stimulating the wall synthesis, reducing the rigidity of cell wall and by increasing cell division leading to more growth. (2) The direct effect of these chemicals on stem elongation is by increasing the synthesis of IAA (Leopold and Kriedeman, 1983). The increase in seedling height and girth by application of gibberllic acid and potassium nitrate was also reported by earlier workers such as Venkata Rao and Reddy (2005) in mango. The production of more number of leaves in KNO_3 and GA_3 treatments may be due to the vigorous growth induced by GA_3 and KNO_3, more number of branches which facilitates better harvest of sunshine by the plants to produce more number of leaves. Results obtained on this aspect are in agreement with Venkata Rao (2002) in mango.

The fresh weight of shoots significantly differed due to invigouration of seeds. With regard to the seed treatment of different growth regulators and biofertilizers, which influenced the fresh weight of seedlings, the highest fresh (23.99 g) and dry weight of shoots (8.07g) was recorded in the treatment of GA_3 200 ppm. Whereas, minimum fresh weight (11.33 g) and dry weight (3.12g) was noticed in control (table 3). The biomass produced by the plant is the net gain of interplay between various anabolic and catabolic processes in plants. Plant growth regulators, particularly GA_3 exhibited profound influence on dry matter accumulation in different plant parts, which could be due to its effect in stimulating cell division, cell elongation, auxin metabolism, cell wall plasticity and

Table 2 : Effect of pre-sowing seed treatments on seedling growth parameters of tamarind.

Treatments	Seedling height (cm)			Seedling collar girth (cm)			Number of leaves per plant		
	60DAS	90DAS	120 DAS	150DAS	60DAS	90DAS	120 DAS	150DAS	60DAS	90DAS	120 DAS	150DAS
T_1	12.80	16.80	22.73	26.70	0.76	0.94	1.26	1.49	12.80	17.90	23.11	30.47
T_2	20.73	27.70	33.50	40.57	0.99	1.32	1.71	1.99	20.73	28.53	37.34	49.40
T_3	16.13	21.58	26.61	33.17	0.90	1.06	1.49	1.76	16.13	22.20	29.64	39.93
T_4	13.33	19.90	24.77	30.50	0.89	1.07	1.50	1.72	13.33	20.27	27.73	36.53
T_5	14.33	20.20	25.65	30.90	0.88	1.11	1.47	1.60	14.33	20.10	26.71	34.90
T_6	16.93	20.98	26.77	31.80	0.91	1.13	1.47	1.79	16.93	24.07	32.13	40.60
T_7	18.84	25.23	31.30	36.67	0.92	1.20	1.60	1.89	18.30	25.60	34.64	43.90
T_8	14.73	18.15	23.60	28.63	0.90	1.11	1.51	1.65	14.73	19.27	26.27	36.00
T_9	17.27	21.65	26.60	32.20	0.90	1.05	1.47	1.60	17.27	24.20	31.47	41.20
T_{10}	16.33	22.03	28.13	33.20	0.88	1.12	1.49	1.60	16.33	22.07	28.07	38.73
T_{11}	17.80	22.57	28.00	33.37	0.89	1.12	1.50	1.75	17.80	24.33	30.67	41.20
T_{12}	18.15	22.65	29.57	34.80	0.92	1.20	1.55	1.82	18.15	25.00	33.40	41.67
T_{13}	18.80	23.03	29.57	35.23	0.93	1.18	1.51	1.81	18.80	24.67	31.50	42.20
T_{14}	16.00	20.37	25.37	30.37	0.85	1.13	1.49	1.72	16.00	22.33	28.40	37.60
T_{15}	14.67	19.79	24.86	28.93	0.87	1.10	1.39	1.63	14.67	19.60	26.53	33.20
F test	*	*	*	*	*	*	*	*	*	*	*	*
SE.m±	0.98	0.87	1.11	1.45	0.02	0.04	0.05	0.06	0.86	1.08	1.29	1.93
CD @ 5 %	2.83	2.52	3.21	4.19	0.07	0.12	0.17	0.17	2.51	3.14	3.74	5.57
CV	10.33	7.03	7.10	7.75	5.02	6.61	6.86	6.11	9.17	8.31	7.53	8.53

*Significant, DAS- Days after sowing.

permeability of cell membrane leading to enhanced growth. Increase in the dry weight of different plant parts due to biofertilizers could be due to improved soil fertility, thereby rendering more availability of nutrients required for plant growth and development. The results are in accordance with Ratan and Reddy (2004) in annona.

Root characters

Maximum root length (41.67 cm) was noticed in treatment GA_3 200 ppm concentration. The minimum of 28.00 cm was recorded in the control treatment (table 3). Exogenous application of GA_3 induced the activity of gluconeogenic enzymes during early stages of seed germination and this could be the reason for improved germination and vigour characteristics that is reflected in terms of increase in root length.

The significant highest fresh weight of roots (7.90 g) and dry weight of roots (4.80g) was recorded in the treatment GA_3 200 ppm concentration. Whereas, minimum fresh weight (4.77 g) and dry weight (2.67g) was noticed in control (table 3). The seeds treated with GA_3 will accelerates the translocation and assimilation of auxins, reasons for better root growth and vegetative characters are due to the overall assimilation and redistribution of materials with in plants enhance the growth attributes (Pandiyan *et al*., 2011).

Increase in fresh weight of roots is due to the influence of GA_3 on different plant parts, which could be due to its effect in stimulating cell division, cell elongation, auxin metabolism, cell wall plasticity and permeability of cell membrane leading to enhanced growth. Increase in the dry weight of different plant parts due to improved mobilization of nutrients due to the application of GA_3, which promotes plant growth and development.

Seedling vigour

The seedling vigor significantly (table 3) differed due to invigouration of seeds.

Table 3 : Effect of pre-sowing seed treatments on seedling biomass, rootlength, vigour and days taken to attain graftable size in tamarind.

Treatments	Fresh weight shoot (g)	Fresh weight root (g)	Dry weight shoot (g)	Dry weight root (g)	Root length (cm)	Vigour index 1	Vigour index 2	Number of days taken to attain graftable size
T_1	11.33	4.77	3.12	2.67	28.00	4376.00	463.20	201.40
T_2	23.99	7.90	8.07	4.80	41.67	8039.24	1260.61	156.46
T_3	16.87	6.27	6.05	3.53	37.00	6538.21	894.10	179.30
T_4	15.85	5.77	5.33	2.60	31.00	5328.90	690.35	183.40
T_5	15.57	4.60	5.23	3.00	35.67	5930.75	731.51	194.60
T_6	16.10	5.77	5.45	2.60	33.33	5939.27	733.41	180.50
T_7	21.93	6.80	6.70	3.60	37.00	7023.83	984.20	167.00
T_8	16.23	6.33	5.80	3.20	33.33	5367.73	779.97	193.20
T_9	17.83	6.50	5.90	3.70	35.67	6181.03	870.18	197.40
T_{10}	16.40	6.95	5.98	3.47	32.33	5849.41	839.98	196.60
T_{11}	17.47	6.80	5.40	3.53	35.67	6748.37	873.15	179.20
T_{12}	19.70	7.00	6.33	3.96	40.33	7172.94	983.24	174.30
T_{13}	19.98	6.98	6.00	3.75	37.00	7061.03	953.32	177.37
T_{14}	16.10	5.53	5.13	3.10	31.33	5611.93	749.84	183.60
T_{15}	14.95	5.12	4.30	3.45	32.00	5264.84	671.64	192.20
F test	*	*	*	*	*	*	*	*
SE.m±	0.74	0.29	0.32	0.25	1.59	280.37	50.70	7.34
CD @ 5%	2.15	0.86	0.93	0.74	4.59	809.78	146.44	21.21
CV	7.46	8.32	9.96	13.16	7.93	7.88	10.55	6.92

* Significant, DAS- Days after sowing.

All the treatments promoted significantly higher vigour of seedlings, when compared to control. Particularly, treatment with GA_3 200 ppm recorded significantly highest vigour index-I (8039.24) and vigour index-II (1260.61). The least vigour index- I (4376.00) and vigour index-II (463.20) was noticed in control. The highest seedling vigour in GA_3 was attributed to enlarged embryos, higher rate of metabolic activity and respiration, better utilization and mobilization of metabolites to growth points and higher activity of enzymes. Enzymatic and hormonal mechanism stimulate metabolic process such as sugar mobilization, protein hydrolysis, oxidation etc. (Earlplus and Lambeth, 1974), which leads to increase in root length, shoot length and seedling dry weight, in turn increase in seedling vigour.

Number of days taken for attaining graftable size

The minimum number of days (156.46) taken to reach graftable size was recorded in treatments with GA_3 200 ppm, which was on par with KNO_3 2% (167 days), Biomix (174.30) and Scarification (177.37) whereas, maximum number of days (201.40) taken to reach graftable size (2.04 cm) was noticed in control (table 3).

The minimum number of days taken to reach graftable size may be due to the growth induced by the gibberllic acid. The promotion of growth either in terms of increase in plant height or the stem girth and leaf number has been thought to be by increasing plasticity of the cell wall followed by hydrolysis of starch to sugars, which lowers the water potential of cell, resulting in the entry of water into the cell causing elongation. These osmotic driven responses under the influence of gibberellins might have attributed for increase in photosynthetic activity, accelerated translocation and efficiency of utilizing photosynthetic products, thus resulting in increased cell elongation and rapid cell division in the growing portion (Sargent, 1965).

Conclusion

From the present investigation, it was concluded that increase in germination percentage, rate of germination and decrease in number of days taken to initiation of germination, was noticed in seeds subjected to mechanical scarification and also due to application of GA_3 200 ppm. The maximum plant height, seedling girth, number of leaves, seedling biomass and vigour index-I (cm) and vigour index-II (g) was recorded in GA_3 200 ppm. The minimum germination percentage, speed of germination, seedling vigour, plant height, stem girth, number of leaves and seedling biomass were recorded in control treatment.

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