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Studies on Effect of Plant Growth Regulators on Seed Germination and Seedling Growth of Marking Nut (*Semecarpus anacardium* L)

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ABSTRACT

An experiment was conducted to study the effect of plant growth regulators on seed germination and seedling growth of marking nut (*Semecarpus anacardium* L) at Department of Horticulture, College of Agriculture, Latur (Maharashtra). The treatments comprised of GA_3, NAA, Ethrel and Kinetin at three different concentrations (150 to 400 ppm). Seeds pre-soaked with 400 ppm GA_3 for 24 hours significantly influenced the germination and growth attributes. Germination parameters like minimum days taken for initiation of germination (24.06), germination vigour index (0.54) and germination percentage (72.50) were recorded maximum in seeds pre-soaked with 400 ppm GA_3 for 24 hours. The growth and biomass characters i.e. plant height (18.72cm) and leaf area (22.28 cm^2) fresh and dry weight of shoot (9.60 and 2.86 g) root (31.34 and 6.96 g) with significantly higher shoot: root ratios (0.31 and 0.41 on fresh and dry weight basis) respectively, recorded in the same treatment. The seedlings characters like fresh weight of seedling (36.48 g), dry weight of seedling (15.20 g) and per cent survival (91.67) of seedlings found maximum in treatment consist of GA_3 at 400 ppm, compared to non treated control. Thus, present study envisage that, pre-soaking of seeds with plant growth regulators can lead to better germination and further growth of seedlings in marking nut.

Key words: *Semecarpus anacardium*, Marking nut, PGR's germination, Seedling growth

Marking nut (*Semecarpus anacardium* L) is one of the important dry land fruit crop belonging to family anacardiaceous and akin to Mango, Cashew, Pistachio and Charoli which are of same members of the family. It is widely grown and distributed in Sub-Himalayan tract, extending from Beas river to the eastern states like Assam, Khasi hills, Central India and West India includes Gujarat, Konkan, South Maharashtra and deciduous forest of South India are the chief marking nut growing areas of India. It is also known as Bhallatak in India and it's called "marking nut" by Europeans, because it was used by washermen to mark cloth and clothing before washing, as it imparted a water insoluble mark to the cloth. The fruits mature from January to March and botanically oblong drupe with seed of shining black colour when ripe, seated on an orange red to yellow coloured receptacle formed of the disk called as fleshy hypocarp (Chopra *et al.* 1956). The fleshy orange and cup shaped hypocarp is eaten when fruit is quite ripe. It is either eaten by roasting the fresh hypocarp or consumed as dry fruit. Various parts of these plants are commonly used in the Ayurvedic system of medicine for the treatment of various ailments, mainly alimentary tract and certain dermatological problems. Reports have shown noticeable impact on illnesses related to heart, blood pressure, respiration, cancer and neurological disorders. The seed is a rich source of vitamin-A about 185.50 mg, thiamine (B_1) 0.39 mg, riboflavin (B_2) 0.15 mg per 100g of edible hypocarp (Bhalerao 1990). The vesicant juice extracted from pericarp of marking nut is known as Bhilawan Shell Liquid (BSL) in trade is a rich source of phenol. Non-vascicating semi solids or solid resins from BSL are utilised as a base for the manufacture of varnishes, lacquers, enamels, paints, moulding composition, water proofing and insulating (electrical) materials.

Marking nut is propagated by seeds and the seed germination is not uniform, making sexual propagation difficult. The initial growth of seedling is very slow and it takes 180-240 days to attain the stage of planting. The reason for less germination is hard seed coat. Therefore treatment of seeds with growth regulators like gibberellic acid, Naphthalene acetic acid, Kinetin, Ethrel is very important to improve germination. As GA_3 helps in breaking seed dormancy which results in early and enhanced germination due to diffusion of endogenous auxin and

gibberellins like substances (Pawshe *et al.* 1997). Ethrel is also known to improve seed germination. Kinetin significantly improves seed germination in many species having hard seed coats (Joseph *et al*. 2007). NAA increases the germination percentage in many fruit species (Kalita *et al.* 1995). The germination percentage as well as growth rate of seedling is very poor and very scanty information is available about the use of different growth regulators for improving germination in marking nut. Considering the above mentioned facts, the present investigation was carried to find out suitable growth regulator to improve the seed germination and subsequent seedling growth of marking nut (*Semecarpus anacardium* L) by pre-soaking of seeds.

MATERIALS AND METHODS

The present study was carried out under the 50% shade house nursery condition at Department of Horticulture, College of Agriculture, Latur during the year 2015-16. Geographically the experiment site situated at an altitude of 633.85 MSL, 18° 24 North latitude and 76° 36 longitude on the balaghat plates. The experimental area falls under the semi arid tropics. The average mean annual precipitation (worked on the basis of last 33 years) of the area is 840 mm and mostly concentrated during the monsoon months from June to October. The minimum and maximum temperatures inside the shade house recorded during the period of experimentation were 14.0°C and 32.9°C respectively. The relative humidity ranged from 47.0 to 84.0%. The experiment was laid out in a complete randomized block design with 13 treatments and replicated thrice with 20 seeds used for each treatment. The details of treatments imposed in the present investigation are T_1 (GA_3 200 ppm), T_2 (GA_3 300 ppm), T_3 (GA_3 400 ppm), T_4 (NAA 200 ppm), T_5 (NAA 250 ppm), T_6 (NAA 300 ppm), T_7 (Kinetin 150 ppm), T_8 (Kinetin 200 ppm), T_9 (Kinetin 250 ppm), T_{10} (Ethrel 200ppm), T_{11} (Ethrel 300 ppm), T_{12} (Ethrel 400 ppm), T_{13} (Non-treated control i.e. Soaked in tap water).

Seeds were collected from the ripen fruits of healthy, heavy bearing and good yielding marking nut trees from Kandha, Nanded district. The stock solution of 1000 ppm GA_3 was prepared by dissolving 1 g commercial grade of GA_3 in 50 ml alcohol and makeup the volume to one litre with distilled water. The similar way of stock solutions preparation is followed for remaining PGR's. Then, the working solutions of desired concentrations (ppm) as per treatments were prepared from stock solution and then diluted the same with distilled water. Healthy, uniform age, size and weight of marking nut seeds were subjected to each treatment and soaked for 24 hrs in the solutions of different growth regulators as per the treatments. Potting mixture was prepared by mixing of equal quantities of sand, soil and well rotten FYM and filled in polythene bags of size 15cm x 20cm. Then, seeds treated with various treatments were sown with stalk end facing upward in polythene bags with sufficient pores to have the proper drainage. Then 20 bags of each treatment in each replication were arranged in rows and kept in 50 per cent green colour shade net house and intercultural operations like watering, weeding, plant protection measures were carried out as per the need during the course of investigation.

The days required for initiation of germination was calculated by computing the difference between the date of sowing and the date of first plumule emergence and expressed in terms of days. The germination of the seeds were counted daily, seeds with protruding radical and plumule were scored as germinated over the time period till the potential germination. Then, per cent germination was also recorded. Germination vigour index was computed using the formula:

$$GVI = \frac{X_1}{d_1} + \frac{X_2}{D_2} + \frac{X_3}{d_3} + \frac{\dots}{\dots} + \frac{X_n}{d_n}$$

Where $X_1,X_2,X_3.....X_n$ were the number of seeds germinated and $d_1,d_2,d_3....d_n$ are the days taken for germination, respectively (Kumar *et al.* 2008). The seedling growth parameters like plant height, stem girth, leaf area were recorded at 30 days interval from date of sowing to 180 days after sowing. The seedling biomass parameters like fresh weight of seedling, dry weight of seedling, number of primary roots, number of secondary roots, length of tap root, fresh weight of roots and shoot and dry weight of roots and shoot were recorded at 180 days after sowing. The data obtained in present investigation were statistically analyzed by the method suggested by Panse and Sukhatme (1989).

Table 1 Effect of plant growth regulator treatments on germination of marking nut

Treatments	Days taken for complete germination	Germination (%)	Germination vigour index
T_1: GA_3 @ 200 ppm	24.83	68.25	0.50
T_2: GA_3 @ 300 ppm	24.25	70.00	0.53
T_3: GA_3 @ 400 ppm	24.06	72.50	0.54
T_4: NAA @ 200 ppm	26.77	59.50	0.48
T_5: NAA @ 250 ppm	26.19	63.00	0.52
T_6: NAA @ 300 ppm	26.19	63.00	0.52
T_7: Kinetin @ 150 ppm	28.04	51.00	0.39
T_8: Kinetin @ 200 ppm	27.21	54.25	0.43
T_9: Kinetin @ 250 ppm	26.88	56.25	0.45
T_{10}: Ethrel @ 200 ppm	25.94	63.75	0.47
T_{11}: Ethrel @ 300 ppm	25.13	64.25	0.48
T_{12}: Ethrel @ 400 ppm	25.06	65.50	0.50
T_{13}: Control	28.43	45.00	0.32
Mean	26.11	61.17	0.47
S.E±	1.38	3.01	0.03
C.D at 5%	2.84	6.18	0.07

RESULTS AND DISCUSSION

Germination parameters

The data presented in (Table 1) revealed that among the pre-soaking treatments, GA_3 400 ppm has recorded the minimum number of days (24.06) required for germination and this was followed by GA_3 300 ppm (24.25). The highest value of germination vigour index (0.54) and germination percentage (72.50) were also recorded in GA_3 400ppm followed by GA_3 300 ppm (0.53, 70.00) respectively. This

enhancement of seed germination by GA_3 might be due to increase of transcription and or translation during protein and lipid synthesis. Thus, mobilization and hydrolysis of these protein and lipid storage reserves upon specific enzymes includes protease and lipase thus utilized for growth of embryo and thereby might have enhanced the germination (Buckeridge *et al*. 2000).

Seedling growth parameters

The data presented in (Table 2) pertaining to plant height revealed that the plant height was gradually increased from 30 days after sowing up to 150 days and followed consistence height at 180 DAS. It was consistently higher in T_3 (seeds pre-soaked with GA_3 @ 400 ppm) from 30, 60, 90, 120, 150 and 180 days after sowing (11.20, 13.25, 14.50 17.10, 18.65 and 18.69 cm) followed by T_2 (11.02, 13.07, 14.32 16.92, 18.42 and 18.44 cm). Whereas, the lowest plant height was recorded in untreated control (5.35, 7.40, 8.65, 11.25, 12.75 and 12.79 cm) respectively at all the stages of seedling growth. The regulation of growth by gibberellins relates almost extensively to its stem elongation properties. Influence of gibberllic acid on stem elongation is by two ways either by 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 (Hartmann *et al.* 2002, Harris *et al*. 2004) or direct effect of these chemicals on stem elongation is by increasing the synthesis of IAA (Leopold and Kriedeman 1983).

Table 2 Effect of plant growth regulator treatments on plant height (cm) of marking nut

Treatments	30 DAS	60 DAS	90 DAS	120 DAS	150 DAS	180 DAS
T_1: GA_3 @ 200 ppm	10.85	12.90	14.15	16.75	18.25	18.34
T_2: GA_3 @ 300 ppm	11.02	13.07	14.32	16.92	18.42	18.52
T_3: GA_3 @ 400 ppm	11.20	13.25	14.50	17.10	18.60	18.72
T_4: NAA @ 200 ppm	6.20	8.25	9.50	12.10	13.60	13.68
T_5: NAA @ 250 ppm	6.95	9.00	10.25	12.85	14.35	14.44
T_6: NAA @ 300 ppm	7.50	9.55	10.80	13.40	14.90	15.02
T_7: Kinetin @ 150 ppm	9.62	11.67	12.92	15.52	17.02	17.13
T_8: Kinetin @ 200 ppm	10.00	12.05	13.30	15.90	17.40	17.56
T_9: Kinetin @ 250 ppm	10.25	12.30	13.55	16.15	17.65	17.76
T_{10}: Ethrel @ 200 ppm	8.15	10.20	11.45	14.05	15.55	15.64
T_{11}: Ethrel @ 300 ppm	8.75	10.80	12.05	14.65	16.15	16.23
T_{12}: Ethrel @ 400 ppm	9.36	11.41	12.66	15.26	16.76	16.82
T_{13}: Control	5.35	7.40	8.65	11.25	12.75	12.81
Mean	8.86	10.90	12.16	14.76	16.26	17.72
S.E±	0.60	0.72	1.00	0.65	0.68	0.54
C.D at 5%	1.53	1.88	2.50	1.58	1.65	1.35

Table 3 Effect of plant growth regulator treatments on leaf area (cm^2) of marking nut

Treatments	30 DAS	60 DAS	90 DAS	120 DAS	150 DAS	180 DAS
T_1: GA_3 @ 200 ppm	9.70	11.85	14.35	17.15	19.75	22.00
T_2: GA_3 @ 300 ppm	9.85	12.01	14.56	17.31	19.93	22.16
T_3: GA_3 @ 400 ppm	9.98	12.13	14.63	17.43	20.03	22.28
T_4: NAA @ 200 ppm	6.80	9.00	11.30	14.02	16.47	18.62
T_5: NAA @ 250 ppm	7.25	9.45	11.75	14.47	16.92	19.07
T_6: NAA @ 300 ppm	7.65	9.85	12.15	14.87	17.32	19.47
T_7: Kinetin @ 150 ppm	8.75	10.90	13.40	16.20	18.80	21.05
T_8: Kinetin @ 200 ppm	9.05	11.20	13.70	16.50	19.10	21.35
T_9: Kinetin @ 250 ppm	9.30	11.45	13.95	16.75	19.35	21.60
T_{10}: Ethrel @ 200 ppm	7.95	10.15	12.45	15.17	17.62	19.77
T_{11}: Ethrel @ 300 ppm	8.20	10.40	12.70	15.42	17.87	20.02
T_{12}: Ethrel @ 400 ppm	8.55	10.70	13.20	16.00	18.60	20.85
T_{13}: Control	6.45	8.65	10.95	13.67	16.12	18.27
Mean	8.42	10.60	13.01	15.77	18.30	20.50
S.E±	0.54	0.65	0.68	0.62	0.64	0.60
C.D at 5%	1.45	1.95	2.06	0.18	0.18	0.17

Leaf area exhibited significant difference due to pre-sowing seeds treatments with different plant growth regulators (Table 3). The maximum leaf area was recorded in seeds pre-soaked with GA_3 @ 400 ppm (T_3) from 30, 60, 90, 120, 150 and 180 days after sowing (9.98, 12.13, 14.63, 17.43, 20.03 and 22.28 cm^2) followed by T_2 (9.85, 12.01, 14.56, 17.31, 19.93 and 22.16 cm^2). Whereas, minimum leaf area was recorded in untreated control (6.45, 8.65, 10.95,

13.67, 16.12 and 18.27 cm^2) respectively. This might be due to more number of leaves produced in the same treatment and also could be attributed to the faster cell division, cell elongation and cell multiplication processes results in producing maximum leaf area in seedlings of this treatment. Further, GA_3 activity at apical meristem results in production of more nucleoproteins responsible for initiation and expansion of leaves.

Seedling biomass parameters

The influence of different plant growth regulators as pre-sowing treatments significantly increased the fresh weight of the seedling and is presented (Fig 1). The maximum fresh weight of seedling (36.48g) was recorded in GA_3 400 ppm treatment (T_3) followed by GA_3 @ 300 ppm (35.20 g). Minimum fresh weight of seedlings (19.46g) was recorded in untreated control. The dry weight of seedling was significantly influenced by different plant growth regulators as pre-sowing treatments (Fig 1). The maximum dry weight of seedling (15.20 g) was recorded in GA_3 @ 400 ppm treatment (T_3) followed by GA_3 @ 300 ppm (14.45 g). While, minimum fresh weight of seedlings (5.56 g) was recorded in untreated control. The influence of different pre-soaking treatments on survival percentage of marking nut seedlings was found to be significant (Fig 2). The highest survival percentage (91.67) was recorded in GA_3 @ 400 ppm treatment (T_3) followed by GA_3 @ 300 ppm (88.23). The lowest seedlings survival percentage (58.33) was recorded in untreated control.

This is due to correspond increase in maximum leaf area per plant as recorded in the same treatment results in production of more quality carbohydrates that might have increased the total biomass of seedling leads to production of more fresh weight, dry weight in the seedlings. 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 permeability of cell membrane leading to enhanced growth.

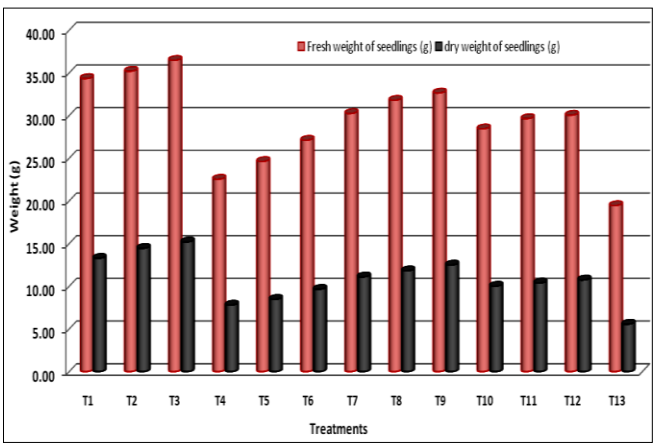

Fig 1 Effect of plant growth regulators on fresh weight and dry weight of marking nut (*Semecarpus anacardium* L.) seedlings

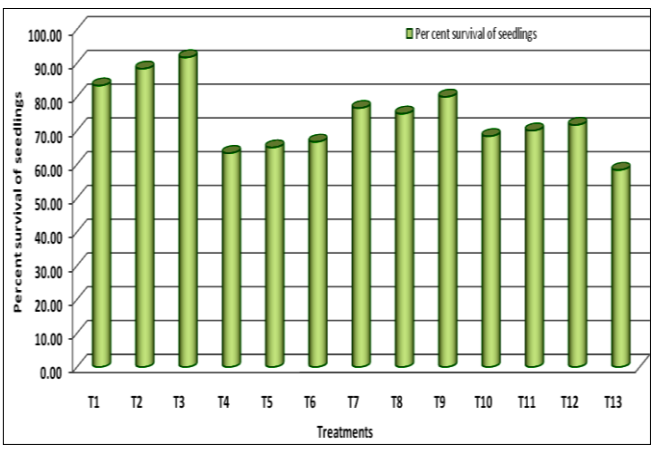

Fig 2 Effect of plant growth regulators on percent survival of marking nut (*Semecarpus anacardium* L.) seedlings

Table 4 Effect of plant growth regulator treatments on root characters of marking nut at 180 days after sowing

Treatments	Number of primary roots/ plant	Number of secondary roots/ plant	Length of tap root (cm)
T_1: GA_3 @ 200 ppm	28.25	49.50	22.30
T_2: GA_3 @ 300 ppm	29.10	53.30	23.20
T_3: GA_3 @ 400 ppm	30.50	55.60	24.05
T_4: NAA @ 200 ppm	18.45	21.62	13.65
T_5: NAA @ 250 ppm	19.80	24.30	14.50
T_6: NAA @ 300 ppm	20.65	26.85	15.35
T_7: Kinetin @ 150 ppm	23.62	40.65	17.25
T_8: Kinetin @ 200 ppm	24.15	43.25	18.62
T_9: Kinetin @ 250 ppm	26.54	45.85	20.38
T_{10}: Ethrel @ 200 ppm	21.35	30.75	15.90
T_{11}: Ethrel @ 300 ppm	22.30	34.62	16.35
T_{12}: Ethrel @ 400 ppm	23.16	38.28	16.80
T_{13}: Control	15.20	18.00	11.85
Mean	23.31	37.04	17.71
S.E±	1.10	1.45	0.65
C.D at 5%	3.35	4.30	1.95

Root biomass characters

The perusal data on number of primary and secondary roots varied significantly due to pre-sowing seed treatments with different plant growth regulators in marking nut (Table 4). The maximum number of primary and secondary roots (30.50 and 55.60) recorded in seeds pre- soaked with 400 ppm GA_3 followed 300 ppm GA_3 (29.10 and 53.30) respectively. The minimum numbers of primary and secondary roots (15.20 and 18.00) were recorded in untreated control (T_{13}). This might be due to GA_3 role cell division, cell elongation and cell multiplication results in more leaf area which inturn increased the synthesis of carbohydrates and efficient translocation of produced energy towards the underground growing part *i.e*, root which might have resulted in production of more number of primary and secondary roots which are essential absorption and translocation of water and nutrients for the developing seedlings.

Significant differences were observed for length of tap root among all the treatments due to pre-sowing seed treatments with different plant growth regulators (Table 4). The maximum length of tap root (24.05cm) was recorded in seeds pre-soaked with 400 ppm GA_3 (T_3) followed by T_2 (23.20 cm). The minimum was length of tap root registered in untreated control (11.85 cm). 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 role of GA_3 in root initiation and development is well known and as the required concentration of GA_3 @ 400 ppm might have increased the cell elongation, cell division and cell multiplication in the cambium tissues results in increased length of tap root (Harris *et al.* 2004).

The significant variation in fresh weight of shoot and roots were noticed due to different pre-sowing growth regulator treatments (Fig 3). The maximum fresh weight of shoot and roots (9.60 and 31.34 g) were recorded in seeds pre-soaked with treatment T_3 (GA_3 400 ppm). Production of maximum leaf area leads to higher photosynthesis and 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 ultimately resulted in production of more fresh weight of shoot and root in seedlings.

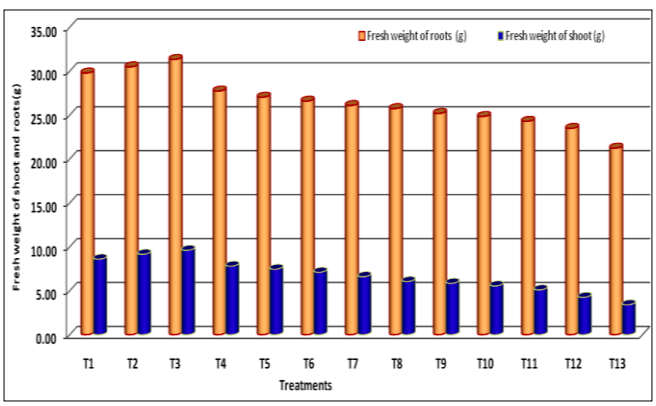

Fig 3 Effect of plant growth regulator treatments on fresh weight of shoot and roots of Marking nut seedlings

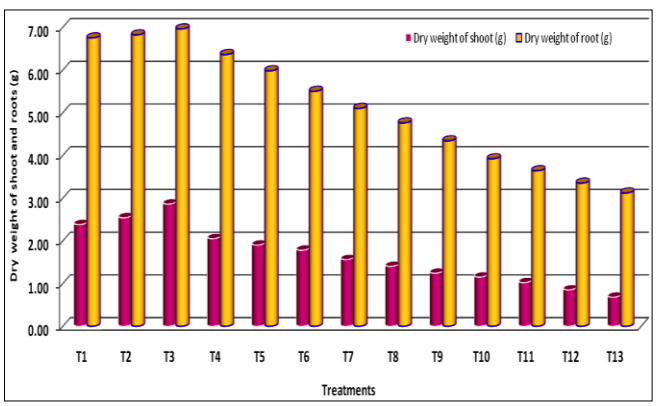

Fig 4 Effect of plant growth regulators on dry weight of shoot and roots of Marking nut (*Semecarpus anacardium* L.) seedlings

The significant variation in dry weight of shoot and roots were noticed due to different pre-sowing growth regulator treatments (Fig 4). The maximum dry weight of shoot and roots (2.86 and 6.96 g) were recorded in seeds pre soaked with treatment T_3 (GA_3 ppm). It is obvious that, the plant produced higher fresh weight will ultimately give high dry weight. The maximum dry weight of shoot in the seedlings of GA_3 400 ppm might be due to the production of maximum fresh weight of shoot in this treatment that ultimately resulted in production of maximum dry weight of shoot. The maximum dry weight of root in GA_3 500 ppm may be due to the fact that as this treatment has produced the maximum fresh weight of root that ultimately might have resulted in production of maximum dry weight of root.

Significant difference was noticed among the treatments for shoot to root ratio (both fresh and dry weight basis) due to pre-sowing seed treatments with different plant growth regulators (Fig 5). The treatment T_3 has recorded the maximum shoot to root ratio (0.31 and 0.41) on fresh weight and dry weight basis respectively. The production of significantly maximum fresh and dry weight of shoot and moderate level of fresh and dry weight of root leading to highest shoot: root ratio in this treatment. This could be attributed due to the availability of harmones at required level for the root growth and resulting in production of maximum number of primary and secondary roots needed for the absorption of water and nutrients from the soil.

The enhanced impact of cytokinin on germination, growth and biomass of seedlings was earlier studied by several workers Nabil and Imam (2007) in Phalsa, Singh *et al*. (2004) in Ber and Murugesh *et al*. (2000) in Aonla. In the present study, apart of from gibberellins, the cytokinin also results at par in most of the observations. Cytokinins is among the most important hormones in regulating cell division and has the capacity to initiate division in quiescent or nondividing cells, in addition to stimulating cell division,

cytokinins also influence shoot and root differentiation, the growth of lateral buds, leaf expansion, chloroplast development and leaf senescence. The application of Kinetin @ 250 ppm results in maximum per cent germination, fresh and dry weight of shoot and root and per cent survival in the present investigation. This might be due to direct role of cytokinin on delay of onset senescence, stimulating protein synthesis and maintain protein levels, prevent chlorophyll breakdown and outmost function of nutrient mobilization and retention by stimulating metabolism (Hopkins and Huner 2004). In spite of cytokinins dose not appear essential for seed germination but during germination, cytokinins appear to offset the effect of inhibitors, notably ABA. Furthermore, it has been described as playing a permissive role in germination in allowing gibberellins to function (Hartmann *et al.* 2002).

Results showed that the pre-soaking treatment with GA_3 400 ppm for 24 hours to marking nut seeds has improved germination percentage and reduced the number of days taken for initiation of germination. The maximum values for most of the growth attributes were recorded with the same treatment. Hence, it will be advisable to treat the seeds of marking nut with GA_3 400 ppm for a period of 24 hours before sowing for obtaining maximum germination and better growth of the seedling.

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