Original Research Article

Effect of Different Growth Hormones in the Micropropagation of Banana cv. GRAND NAINE

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ABSTRACT

Banana (*Musa sapientum*) is one of the most important fruit crop cultivated in India. It is widely grown in more than 150 countries, producing on an average of 105 million tons of fruit per annum. Grand naine Banana variety is the most commonly cultivated banana with high yielding plantain. This variety mainly grown in the region of Uttar Pradesh state. Present study mainly focuses on the micro propagation method which is described for banana cv. Grand naine. In the present study, healthy sword suckers of three months old banana plantlets would be used as explants. The explants sterilized thoroughly and cultured on MS (Murashige and Skoog) media supplemented with the different concentration and combination of auxin and cytokinin like BAP, IAA and NAA which were used for the optimization of best plant growth regulator combination for multiple shoot generation and shoot growth. Among all the tested cytokinin 5 mg/l BAP along with 1mg/l IAA in medium concentration performed well and produced healthy shoots further at higher concentration of all tested cytokinins showed declining effect within the growth parameters. For the rooting purpose, MS media was supplemented with different levels of IBA, IAA and NAA. Results indicated that rooting was highly promoted in the medium supplemented with 0.5 mg/L IAA + 1.5 mg/L IBA.

Keywords: Food security, *in-vitro* micropropagation, *Musa*, Plant Growth Hormones, rooting, shoots proliferation

1. INTRODUCTION

Banana is the oldest and healthy fruit which have originated from Malaysia through a process of hybridization (Novak, 1992). India is the largest producer of banana, which is contributing to almost 27% of world's banana production. The area and production of banana in India (2019-2020) is approximately 866 thousand ha and 32 million metric tons and it will increase to 34.53 metric tons (FAO STAT, 2022). In India production of banana has surpassed the mango production. Tamil Nadu is the leading producer of banana in India, followed by Maharashtra. Banana is one of the most consumed and cheapest fruit worldwide and it is the fifth largest agricultural commodity in the world trade after cereals, sugar, coffee and cocoa. (Esan *et al*. 2022, Tripathi *et al*. 2020). India, Ecuador, Brazil and China alone produced half of the total bananas of the world. The main advantage of this fruit is its availability throughout the year. It is most

popularly grown in large quantities in different states such as, Tamil nadu, Maharashtra, Gujrat, Andhra Pradesh, Karnataka, Madhya Pradesh, Bihar, Uttar Pradesh, West Bengal and Assam. Banana is a tropical crop of socio-economic interest, as it is a much needed fruit crop in developing countries that producing biomass of sugar, starch and cellulose which can further used for paper, textiles and fuel. In addition to its socio-economic value, along with this banana is the first non-Poaceae belongs to grass family monocotyledon for which a high continuity whole genome sequence is available. The 472Mb sequenced assembly was generated from DH-Pahang, it is a doubled-haploid genotype, obtained from the Musa acuminata subspecies (Gaetan *et al*. 2013). In India, there are around 20 varieties of Banana which are widely used for farming system viz, Grand naine, Monthan, Dwarf Cavendish, Robusta, Poovan, Nendran, Red Banana, Nyali, Safed Velchi, Basarai, Ardhapuri, Rasthali, Karpurvalli and karthali etc., Grand naine is characterized among the most commonly cultivated variety in uttar pradesh. Fruits of Grand naine Banana variety are found delicious to eat and yellow in colour with better self-life, quality and productivity than other cultivars. Banana is the major staple food which is categorized as one of the most important sources of energy and starchy food for the people of tropical humid regions. (Rajoriya, 2011) Furthermore, bananas are highly rich in nutrients, starch, sugar, carbohydrates, vitamins A and C, potassium, calcium, sodium, magnesium and digestible food fibers, which are present in the fruits in sizeable levels (Kaliaperumal *et al*. 2018).

Banana plants reproduce asexually by shooting suckers. Suckers can continue to grow from a single mat year after the year, making the banana a perennial crop. Uses of conventional breeding methods for their improvement programmes are difficult. Banana growth production is limited by costly high quality planting materials. The alternative traditional propagation methods are unable to cope with the demand of new planting material. (Juliet Akello *et al*. 2009, Shongwe *et al*. 2008). The material used for the process of conventional propagation includes such as corms, large and small suckers (Cronauer and krikorian, 1984), (Arias, 1992). Suckers produced by the farmers are the good transmitters of insect, pests and diseases (Rahman *et al*. 2004) and also have low multiplication rate. This has gain interest for the use of *in- vitro* tissue culture technique. Plant tissue culture techniques proves to be an efficient and reliable method. Micropropagation is one such a significant technique through which can produce true to type propagation of selected plant species through aseptic shoot tip/meristem culture artificial media called MS media (Murashige and Skoog, 1962). Micropropagation is a great approach for obtaining the disease-free and high-quality planting material. Micropropagation provides genetically uniform plants in large number and an alternative technique for rapid multiplication (Wong, 1986).

2. METHODOLOGY

Source of plant material

The plant material sword suckers of banana cultivar, Grand naine used in the present research methodology were collected from the Horticultural Research Center (HRC), College of Horticulture, S.V.P.U.A & T. Meerut. Suckers of about 30-60 cm along with the rhizome were

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collected which were healthy and vigorously growing.

Preparation of explants

In this study, fresh banana sucker were used as explants. The suckers were carefully washed under running tap water to remove all the mud and other debris present over the outer surface. Roots and outer extra leaf sheath were removed carefully by using a stainless-steel knife in such a way that shoot tip was not destroyed. (Hasan *et al*. 2020). Further rhizomes were cut it off and trimmed into the appropriate size according to their length x breadth x height enclosing the shoot apex.

Pre-treatment of explants

The trimmed banana explants were pre-treated with 1.0% w/v solution of commercial Bavistin for an hour and washed in running fresh tap water before surface sterilization. The explants were then kept in an antioxidant solution of citric acid and ascorbic acid each (100 mg/l) for one hour.

Surface sterilization of explants

The selected grand naine banana suckers were thoroughly washed under the running tap water. Shoot tips were prepared for the experiment by trimming off the roots and outer leaf sheaths from the suckers. The selected explants were kept immersed in tap water for almost about 5 min, further they were pre-treated with 5 ml Tween 20 for about 10 min and a combination of 5 ml Dettol and 45 ml savlon for 30 min with constant swirling and subsequently draining off the disinfectants by washing them using tap water for about 2-3 times to remove the traces of the disinfectants used and then the explants were again washed under running tap water for 60 min. Standardization of surface sterilization was carried out by treating the banana explants with various combinations of different chemical sterilant at different concentrations viz. Bavistin TM, Mercuric chloride, Sodium hypochlorite and Ethanol to optimize minimum contamination, explant survival and culture establishment. The explants were washed three times with autoclaved de-ionized water after each treatment. (Jyoti *et al*. 2022). The explants were prepared by carefully removing upper extended outer leaf sheaths and excised without disturbing upper meristematic shoot bud using scalpel blade in 1.5-2.0 cm.

Experimental treatment and design

In the current study, the culture medium used for banana culture was modified, Murashige and Skoog basal medium for shoot initiation as well as shoot multiplication containing 30 g/l sucrose and gelled by using 8 g/l of agar. The pH of the medium was adjusted to 5.7 using NaOH or HCl (0.1 or 1N) before autoclaving. PGRs were added to the medium before sterilization process. The medium was autoclaved at 1.2 KPa and 121ºC for 20 min, and then cooled at room temperature before use for which BAP (MS + 2mg/L ,3mg/L, 4mg/L, 5mg/L, 6mg/L and 7mg/L) alone and in combination with IAA and NAA at different concentration (BAP+IAA) and (BAP+IAA+NAA) were tested.

Culture Conditions

All the culture jam-bottles were incubated in growth chamber at the temperature of 25 ± 2^0 C with relative humidity of 55 ± 5% under 16 hr illumination of cool white fluorescent tubes with a light intensity of 4000 lux and some callus culture in dark. For regeneration, the callus was placed under a 16-hr photoperiod (4000 lux) provided by cool fluorescent tube lights. After one week, the

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cultures were inspected for contamination. If contaminated was there, the jars were discarded. Only clean and healthy cultures were maintained for further growth and induction of multiplication. The clean explants, after removing the darkened tissue were longitudinally dissected into two halves and transferred to fresh respective medium in order to induce multiplication. This experiment was designed in completely randomized design (CRD) with three replications of each treatment. Observed data were subjected to statistical analysis using SPSS software.

Optimization of best media combination for multiple shoot formation

To establish cultures for the initial shoot initiation different concentration of hormones, BAP, MS + 2mg/L ,3mg/L, 4mg/L, 5mg/L, 6mg/L and 7mg/L) alone and in combination with IAA and NAA were supplemented with MS media mentioned below. The observation of data includes percentage response, number of shoots, shoot length, number of leaves of explant were recorded

***In-Vitro* Rooting of Shoots**

In root development stage, healthy and well grown shoots with expanded leaves were excised and transferred singly to fresh half strength basal rooting MS medium fortified with different concentrations and combinations of Indole-3-acetic acid (IAA) (0.5, 1.0, 1.5 mg/l), Indole butyric acid (IBA) (0.5, 1.0, 1.5 mg/L) and naphthalene acetic acid (NAA) (0.5, 1.0, 1.5 mg/l). Root growth and development was assessed 6 weeks after shoots were transferred to rooting medium for investigating their effects on *in-vitro* root initiation and proliferation. The details of the rooting media composition used were mentioned below.

3. RESULTS AND DISCUSSION

Effect of plant growth regulators on shoot initiation and proliferation

The data presented in Table 1 showed the percentage response of explant, average number of shoots per explants, average shoot length and average number of leaves per explant. Variations were observed among different treatments of cytokinin and auxin on percent response of explants (%) in Banana grand naine variety cultured in MS media in the laboratory condition the results of which have been presented in the Table 1. The highest percent response of explants of G9 was observed in treatment of BAP 5.0 mg/L+ 1mg/L IAA. The second highest was observed in treatment of 5.0 mg/L BAP and the lowest percent response of explants of G9 was observed in control.

Variability was recorded in shoot growth in Grand naine variety of banana among the different media formulations. Highest number of shoots per explant was observed in BSM 11 media (Fig 2). BAP in combination with IAA (BAP 5mg/L+ IAA 1mg/L). (10.0±0.57). Second highest was (8.6 ± 0.66) when supplemented with (BAP 6mg/l) whereas, BSM17 media i.e., BAP in combination with NAA (BAP 8mg/L + NAA 1mg/L) showed lowest number of shoots. Media formulations BSM6, 6mg/L BAP alone showed highest shoot length i.e., (9.5 ± 0.21), whereas lowest shoot length was observed in BSM 17 medium. Similarly highest number of leaves were

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seen in BSM 11 medium (11.6±1.45) and lowest number of leaves were seen when BAP was used in combination with NAA. i.e., BSM17 (2.0±0.14) medium. The 5.0 mg/l of BAP also showed good performance over the control.

In the present research work our findings were found similar with the findings of Chidananda (S *et al*. 2016). Whereas several other (Shahnawaz Ahmed *et al*. 2014), (Asmare Dagnew *et al*. 2012), (Allah Jurio *et al*. 2021) and (Alango Kelta *et al*. 2018) also reported best growth of banana explants when cultured in the medium when supplemented with the growth hormone BAP and IAA. Khanam *et al*. (1996), Rabbani *et al*. (1996), Gebeyehu, (2015) also found best results when the explants are supplemented with high concentration of BAP.

Table 1 Effect of different culture media combination used for *in-vitro* multiplication in banana genotype Grand naine after 4 weeks of culture inoculation

Basal Medium Treatment		Percent response of explant	Average no. of shoots/ explants	Average shoot length(cm)	Average no. of leaves/ explant
BSM 1	**Basal Medium**	35.0± 2.00^{i}	2.3 ± 0.33^{h}	2.0± 0.57^{i}	3.0± 0.57^{i}
BSM 2	**MS Medium + BAP (2.0 mg/l)**	56.0± 2.02^{efg}	4.3 ± 0.33^{ef}	4.6± 0.33^{gh}	7.0± 1.15^{cde}
BSM 3	**MS Medium + BAP (3.0 mg/l)**	53.0± 2.18^{efg}	3.6± 0.66^{ef}	2.6± 0.56^{gh}	3.6± 0.88^{i}
BSM 4	**MS Medium + BAP (4.0 mg/l)**	72.8± 1.33^{ab}	6.3± 0.88^{bc}	7.3± 0.66^{bcd}	8.3± 0.66^{c}
BSM 5	**MS Medium + BAP (5.0 mg/l)**	80.3± 3.28^{b}	8.3± 0.88^{ab}	8.0± 0.88^{c}	8.0± 0.57^{cd}
BSM 6	**MS Medium + BAP (6.0 mg/l)**	70.0± 1.52^{bc}	8.6 ± 0.66^{ab}	9.5± 0.21^{a}	9.0± 0.57^{b}
BSM 7	**MS Medium + BAP (7.0mg/l)**	46.3± 1.45^{efg}	4.0± 0.33^{ef}	2.3 ± 0.33^{h}	3.6± 0.33^{i}
BSM 8	**MS Medium + BAP (2.0 mg/l) + IAA (1.0 mg/l)**	57.5± 2.91^{ef}	3.6 ± 0.33^{f}	5.0± 1.00^{ef}	6.3± 0.88^{d}
BSM 9	**MS Medium + BAP (3.0 mg/l) + IAA (1.0 mg/l)**	55.6 ± 3.6^{efg}	3.3 ± 0.88^{ab}	2.3± 0.25^{i}	4.3± 0.88^{ef}
BSM 10	**MS Medium + BAP (4.0 mg/l) + IAA (1.0 mg/l)**	75.3 ± 1.33^{ab}	8.0 ± 0.57^{ab}	7.6± 0.18^{d}	8.0± 0.56^{cd}
BSM 11	**MS Medium + BAP (5.0 mg/l) + IAA (1.0 mg/l)**	82.6 ± 1.76^{a}	10.0± 0.57^{a}	8.5± 0.21^{b}	11.6± 1.45^{a}
BSM 12	**MS Medium + BAP (6.0 mg/l) + IAA (1.0mg/l)**	70.5 ± 1.52^{bc}	5.3± 1.33^{bc}	7.3± 1.11^{d}	8.6± 1.20^{c}
BSM 13	**MS Medium + BAP (7.0 mg/l) + IAA (1.0mg/l)**	45 ± 2.04^{fg}	3.6 ± 0.33^{f}	2.3± 0.44^{ab}	2.3± 0.88^{i}
BSM 14	**MS Medium + BAP (2.0 mg/l) + NAA (1.0 mg/l)**	65 ± 1.16^{bcd}	6.3± 0.33^{bc}	5.6± 0.88^{ef}	4.0± 0.57^{gh}
BSM 15	**MS Medium + BAP (4.0 mg/l) + NAA (1.0 mg/l)**	60.5± 2.82^{bcd}	5.3± 1.45^{def}	5.0± 1.00^{ef}	4.3± 0.66^{gh}
BSM 16	**MS Medium + BAP (6.0 mg/l) + NAA (1.0 mg/l)**	60.0± 1.05^{bcd}	4.0± 0.57^{ef}	4.6± 1.2^{gh}	6.6± 0.66^{d}

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BSM 17 MS Medium + BAP (8.0 mg/l) + NAA (1.0 mg/l)	42.6± 3.52^{fg}	3.0± 0.57^{cd}	2.0 ± 0.57^{i}	2.0± 0.14^{i}
BSM 18 MS Medium + BAP (2.0mg/l) + IAA(1.0mg/l)+ NAA (1.0mg/l)	55± 2.1^{efg}	4.3± 0.33^{ef}	4.3± 0.33^{gh}	6.0± 0.67^{d}
BSM 19 MS Medium + BAP (4.0 mg/l) + IAA(1.0 mg/l)+NAA (1.0mg/l)	54± 2.42^{efg}	4.0± 0.57^{ef}	4.0± 0.57^{gh}	6.3± 0.54^{d}
BSM 20 MS Medium + BAP (6.0mg/l) + IAA (1.0 mg/l) + NAA (1.0mg/l)	50±1.15^{efg}	3.0±0.57^{efg}	3.3±0.33^{gh}	5.0±0.57^{ef}
BSM 21 MS Medium + BAP (8.0mg/l) + IAA (1.0 mg/l) + NAA (1.0mg/l)	40±2.14^{g}	3.0± 0.57^{g}	2.3±0.33^{i}	4.0±0.52^{gh}

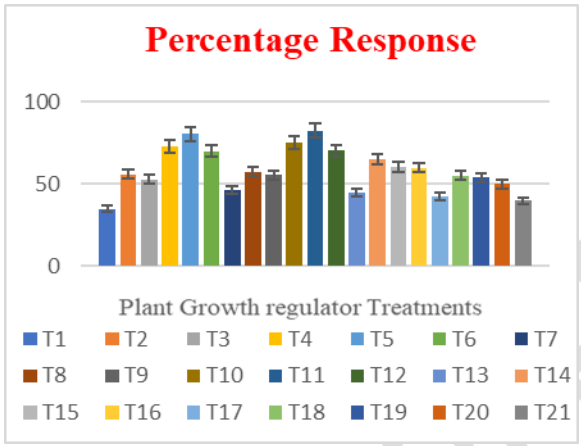

Fig 1

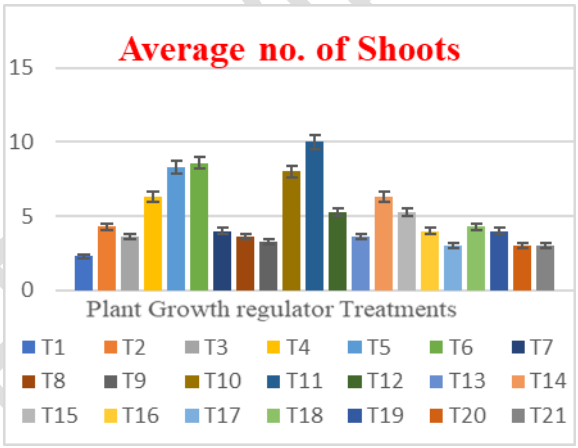

Fig 2

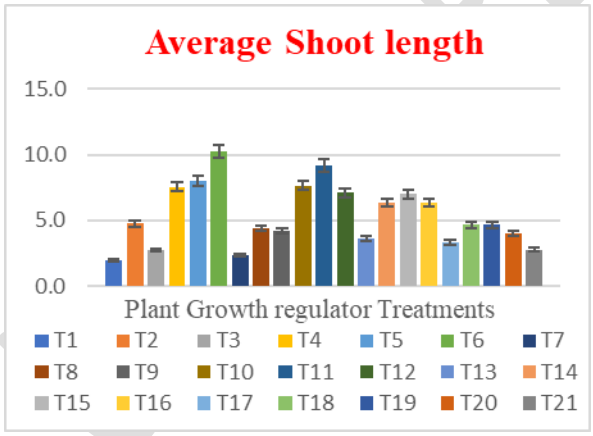

Fig 3

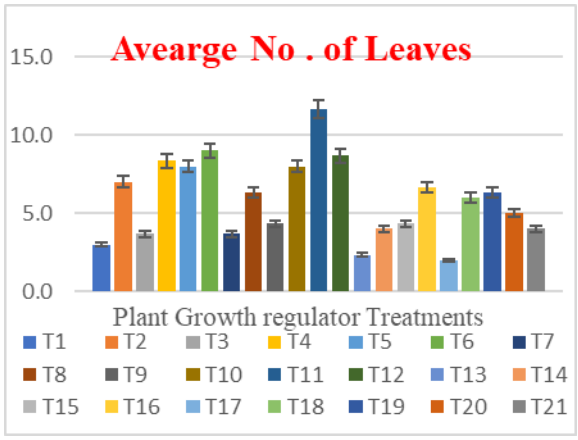

Fig 4

Fig 5 *In-vitro* high frequency shoots multiplication

Fig 6 *In-vitro* high frequency root development

Table 2. Showed the results which were recorded on the basis of number of roots, maximum root length. The highest root number in Grand naine banana were achieved in the medium supplemented with (Basal ½-MS+ 1.5 mg/L IBA) at which the average number of roots per explant was recoded (7.8±1.66) followed by (Basal ½-MS+ 0.5 mg/L IAA + 1.5 mg/L IBA) i.e., (7.2±0.57) and the maximum average root length was recorded (7.5± 0.20) when rooting media supplemented with (Basal ½-MS+ 0.5 mg/L IAA + 1.5 mg/L IBA) and second highest was achieved (7.2±0.12) when supplemented with (Basal ½-MS+ 0.5 mg/L IAA + 0.5 mg/L IBA).

The well developed and healthy rooted *in-vitro* cultured banana shoots were transplanted in the polybags and placed in shaded area. After 30 days, further acclimatized in the pot and transferred into the green-house. Our results somehow are similar with the findings of (Gubbuk and Pekmezci ,2004) and (Molla M *et al.* 2006) as they examined the maximum root length when the shoots are further cultured on rooting media supplemented with IBA and IAA.

Table 2 Effect of Different plant growth regulators on *in-vitro* rooting of Grand naine Banana

Half Basal Medium Treatment	Average no. of roots/explants	Average Root length (cm)	Root growth
Basal ½-MS+ 0.5 mg/L IAA	7.0±0.66 a	6.5±0.20 ^{b}	+++
Basal ½-MS+ 1.0 mg/L IAA	6.8± 1.52 ^{ab}	4.5±0.10 ^{cde}	++
Basal ½-MS+ 1.5 mg/L IAA	6.4± 0.33 ^{ab}	4.2±0.15 ^{ef}	+
Basal ½-MS+ 0.5 mg/L IBA	5.6±0.66 ^{abc}	5.6±0.20 ^{c}	++
Basal ½-MS+ 1.0 mg/L IBA	6.0±0.33 ^{abc}	5.5±0.10 ^{cd}	++
Basal ½-MS+ 1.5 mg/L IBA	7.8 ± 1.66 ^{a}	4.6±0.27 ^{e}	+
Basal ½-MS+ 0.5 mg/L NAA	1.8±0.11 ^{e}	1.2±0.11 ^{gh}	+
Basal ½-MS+ 1.0 mg/L NAA	5.4± 0.57 ^{abcd}	4.6±0.14 ^{ef}	+

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Basal ½-MS+ 1.5 mg/L NAA	5.0±0.12 ^{abc}	4.3±0.11 ^{g}	+
Basal ½-MS+ 0.5 mg/L IAA + 0.5 mg/L IBA	6.1±1.00 ^{abc}	7.2±0.12 ^{a}	+++
Basal ½-MS+ 0.5 mg/L IAA + 1.5 mg/L IBA	7.2±0.57 ^{a}	7.5±0.20 ^{a}	++
Basal ½-MS + 1.0 mg/L IAA + 1.5 mg/L IBA	5.2±0.14 ^{abcd}	3.6±0.11 ^{h}	+
Basal ½-MS+ 0.5 mg/L IBA + 0.5 mg/L NAA	2.6±0.22 ^{ef}	1.5±0.10 ^{i}	+
Basal ½-MS+ 0.5 mg/L IBA + 1.5 mg/L NAA	4.3±0.33 ^{bcd}	4.1±0.33 ^{g}	+
Basal ½-MS+ 1.0 mg/L IBA + 1.5 mg/L NAA	3.4±0.16 ^{cde}	3.6±0.20 ^{h}	+
Basal ½-MS+ 0.5 mg/L IAA + 0.5 mg/L NAA	4.2± 1.52 ^{bcd}	5.2±0.27 ^{c}	++
Basal ½-MS+ 0.5 mg/L IAA + 1.5 mg/L NAA	2.2± 0.57 ef	5.5±0.21 ^{c}	+++
Basal ½-MS+ 1.0 mg/L IAA+ 1.5 mg/L NAA	3.2± 0.33 ^{cd}	5.6±0.14 ^{c}	++

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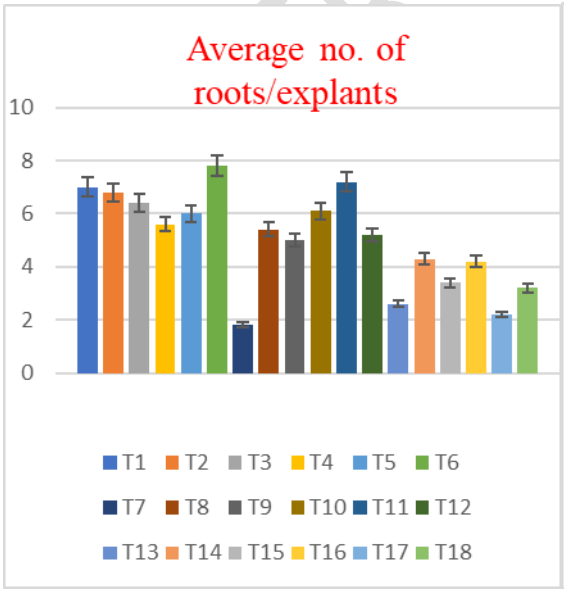

Fig 7

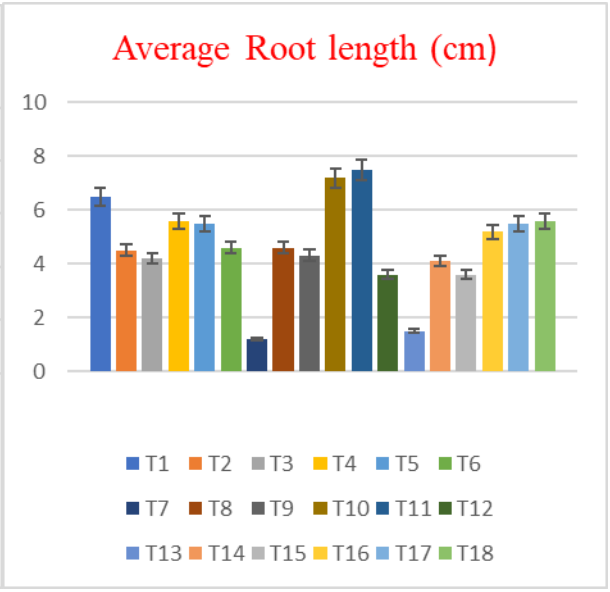

Fig 8

4. CONCLUSION

Bananas are the most essential and widely grown fruit crop in tropical areas. *In-vitro* micropropagation is most appropriate technique by which we can produce multiple plants by using single explant. Micropropagation is a novel technique to produce disease free and true to type plants. On the basis of results obtained in this investigation, following conclusion was obtained. The Grand naine Banana cultivar exhibited variation in shoot initiation, shoot multiplication and rooting when tested with different combinations of growth regulators. On the basis of above research, it was observed that BAP alone not effective for shoot initiation and multiplication. Whereas, combination of 5mg/l BAP and 1mg/l IAA was found most effective for shoot initiation as well as shoot multiplication. For rooting Basal ½-MS+ 0.5 mg/L IAA + 1.5 mg/L IBA showed best response in *in-vitro* cultivation in banana cultivar. Thus, this research study revealed important information related to produce disease free plantlets by *in-vitro* micropropagation. Which can be highly useful to the farmers, entrepreneurs as well as to the commercial growers to develop mass production of disease-free quality banana plants by following this tissue culture protocol.

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