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In vitro micropropagation of ten different Banana genotypes by using single *in vitro* culture media

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ABSTRACT

A study was carried out to investigate the response to ten different banana genotype *viz*., Gaint, Panchadum, cv. Rose, Rajapuri, Lalkel, Cheankadai, Raja Balae, Mas, Udhayam and Chinali on Murashige and Skoog (MS) basal medium supplemented with BA (3.0 mg/l) + adenine sulphate (2.0mg/l) for shoot multiplication and *in vitro* shoot rooting ((½ MS + 1.0 mg/l IBA) using shoot-tip culture. Lalkel and Cheankadai banana genotype showed significantly highest shoot multiplication on MS medium supplemented with BA (3.0 mg/l) + adenine sulphate (2.0mg/l) as compare to other genotypes. Better rooting was obtained when the shoots were cultured on ½ MS with 1.0 mg/l IBA for all 10 banana genotypes. Lalkel and Cheankadai banana genotype showed significantly showed highest number of root and root length as compare to other genotype. All the ten banana genotypes had shown better *in vitro* growth using shoot tip culture.

KEYWORDS

Banana, Genotype, BA, Murashige, Skoog

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Bananas (*Musa* spp.) are one of the most important fruit crops of tropical and subtropical areas of the world and also important source of food millions of people and commercial grown throughout the year (Lescot, 2020; Nayar, 2010). With an estimated global production of 140 million metric tons, bananas and plantains (*Musa* spp.) are the world's leading fruit crop (Evans *et al*., 2020). Presently, Asia is the largest producer of bananas followed by Africa, America, the Pacific islands, Oceania and Europe (FAO, 2022).

It can be used either table purpose as well as culinary fruit and along with banana fruit, other parts of banana plant are also used for various purpose including cattle feed. As banana production is limited by threat from pests and diseases and cultivation is typically done by vegetatively propagated which hinder the growth and yield of crop. Most commercial bananas are propagated vegetatively due to the high degree of sterility and polyploidy of the edible varieties (Stover and Simmonds, 1987).

In order to augment conventional propagation and to avoid constraints imposed by some pathogens, *in vitro approach* has been considered (Tripathi, 2003). Several researchers have reported the regeneration of banana genotypes via *in vitro* micropropagation (Cronauer and Krikorian, 1984; Kagera *et al*., 2004; Madhulatha *et al*., 2004). With the success of large scale multiplication of banana genotypes using shoot tip culture, there are reports available on shoot tip culture plants could show variation under *in vitro* conditions due to genotypes, explant type, age of the mother plant, specialized culture media composition required for each different genotypes and other factors. Therefore, this study was initiated to optimize rapid and reproducible *in vitro* micropropagation protocol for ten different genotypes of banana using common basal media supplemented with plant hormone. For this purpose, the ten different banana genotypes were subject to common MS basal media supplemented with BA in combination with adenine sulphate for shoot multiplication and further shoot were exposed to IBA for better root growth.

Materials and Methods

Plant Materials

Ten widely cultivated banana genotypes, namely Gaint, Panchadum, cv. Rose, Rajapuri, Lalkel, Cheankadai, Raja Balae, Mas, Udhayam and Chinali were used as experimental materials in this study. The explants were obtained from healthy looking, field grown sword suckers of these varieties from Fruit Research Station, NAU, Gandevi, Gujarat, India. The pseudostems at the lower parts of the suckers containing meristems were used as explants. The shoot tips (meristem and a few leaf primordia) were the starting materials. This study was conducted at the Plant Tissue Culture Laboratory, Department of Plant Physiology, NMCA, NAU, Navsari, Gujarat, India.

Explant Preparation and Surface Sterilization

Explants were excised from young suckers (0.75 to 1.0 m) of the ten banana genotypes namely Gaint, Panchadum, cv. Rose, Rajapuri, Lalkel, Cheankadai, Raja Balae, Mas, Udhayam and Chinali. Explants were washed thoroughly in running tap water for 25 min with detergent solution to remove adherent soils.

To study the effect of sterilization treatment on ten different banana genotypes, explants were treated with Bavistin (2.5mg/l) + Chloromphenicol (500mg/l) + 1.0 % $HgCl_2$ for 10 min for measuring the contamination (%), mortality (%) and culture establishment (%). After rinsing three times with sterile distilled water, the explants were excised into final size (about 5 mm) under aseptic conditions.

Experimental Treatment and Design

In this study, was used MS medium (Murashige and Skoog, 1961) as a basal media for shoot establishment and MS medium containing (BA (3.0 mg/l) + adenine sulphate (2.0mg/l) for shoot multiplication for ten banana genotypes, namely Gaint, Panchadum, cv. Rose, Rajapuri, Lalkel, Cheankadai , Raja Balae, Mas, Udhayam and Chinali. All the cultures of ten banana genotypes were maintained at 22^{o}C with a light/ dark cycle of 16/8 hr. White fluorescent light with an intensity of 120 µmol $m^{-2}s^{-1}$ was used for illumination. Multiple shoots of ten banana genotypes were transferred on half MS + 1.0 mg/l IBA for root induction.

Data Collection and Analysis

The data such as contamination (%), mortality (%), culture establishment (%), shoot number per explant, root number per plantlets and root length were recorded after specific interval of time. The experiment was carried out with three replications. The collected data also was analyzed by using statistical significance two ways analysis of variance (ANOVA).

Results and Discussion

Response of Ten Banana Genotypes on Surface Sterilization Treatment

In present investigation, the shoot tip of ten different banana genotypes were surface sterilized and treatment bavistine 2.5 mg/l and chloromphenicol 500 mg/l (45 min.) + 1.0% HgCl2 (10 min.) registered significantly maximum cultured establishment along with minimum contamination (%) and maximum survival (%) in Lalkel (T5), Cheankadai (T6) and Rajapuri (T4) banana genotypes as compare to other banana genotypes (fig 1 and 2).

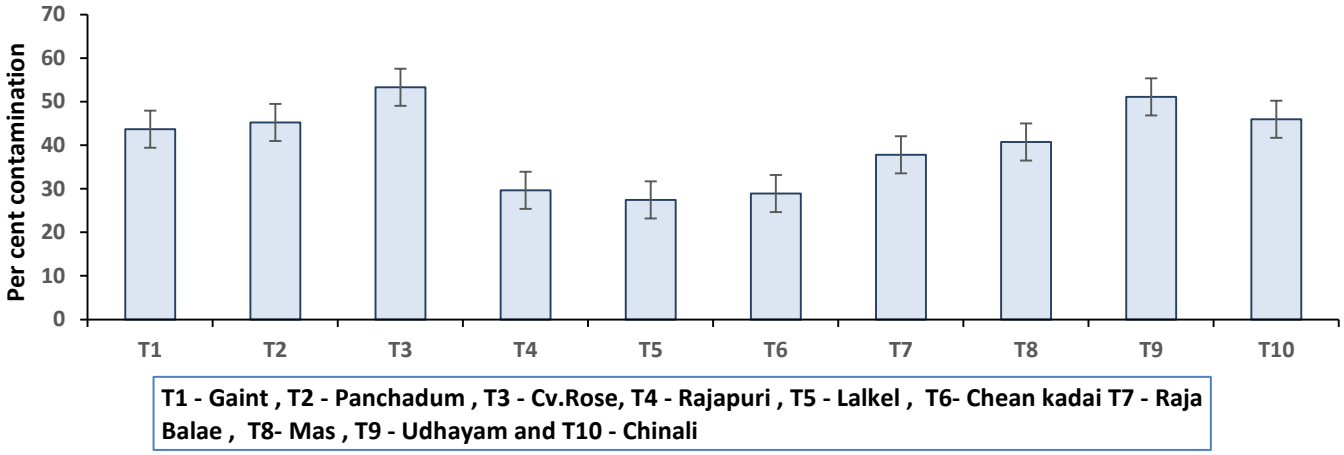

Fig 1. Effect of surface sterilization treatment on per cent contamination of explants different banana genotypes

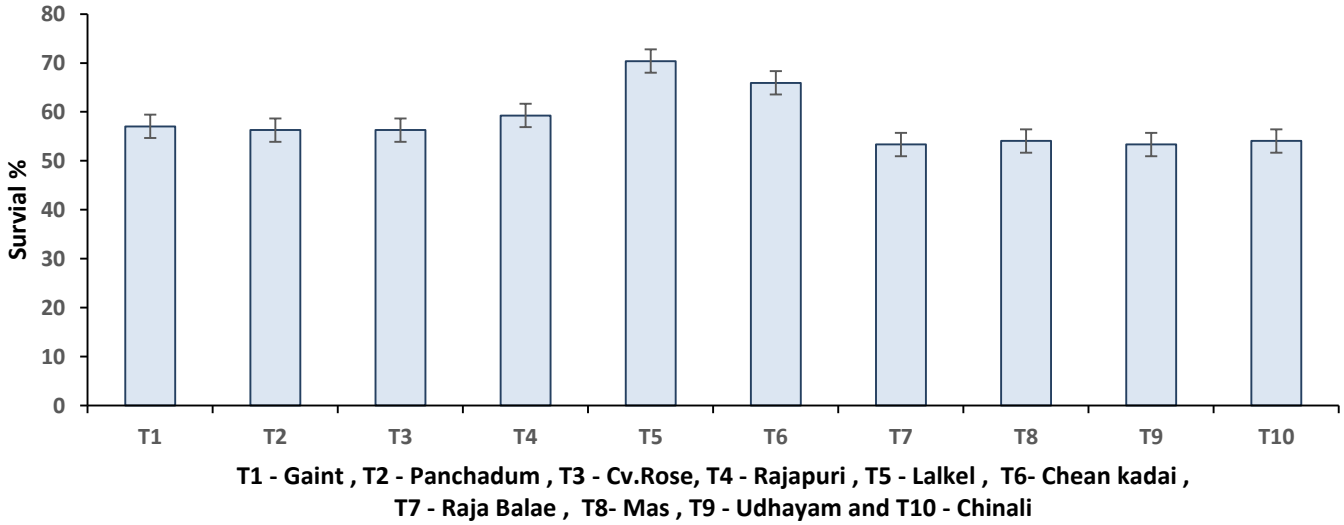

Fig 2. Effect of surface sterilization treatment on survival per cent of explants different banana genotypes

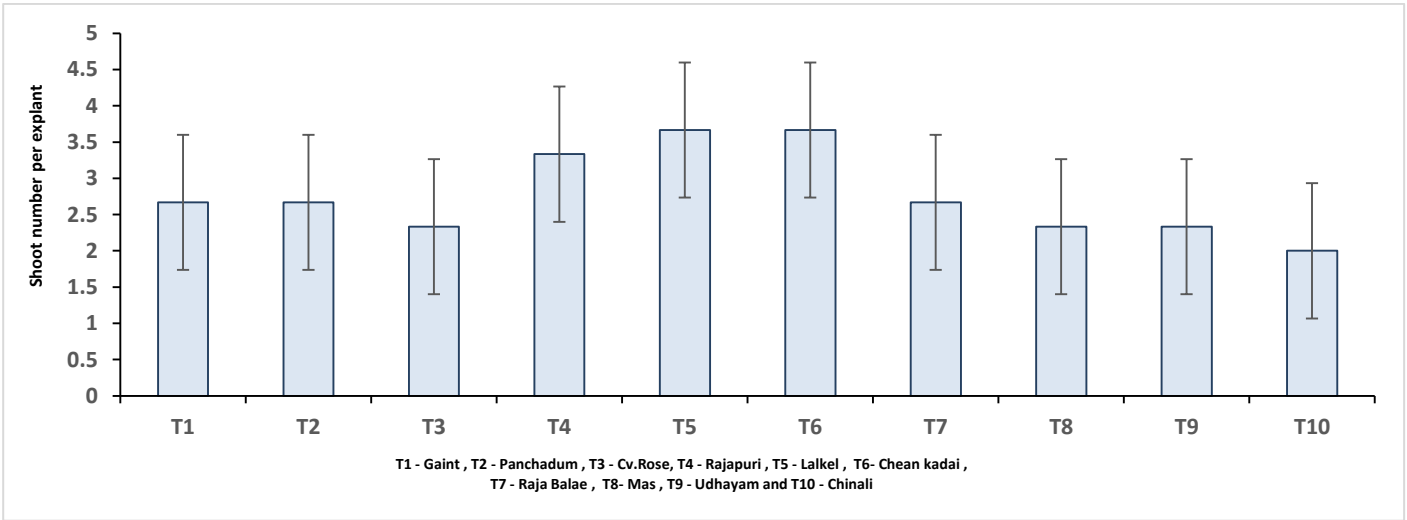

Fig 3. Effect of shoot multiplication media [MS + BA (3.0 mg/l) + adenine sulphate (2.0mg/l)] on shoot number per explant of different banana genotypes

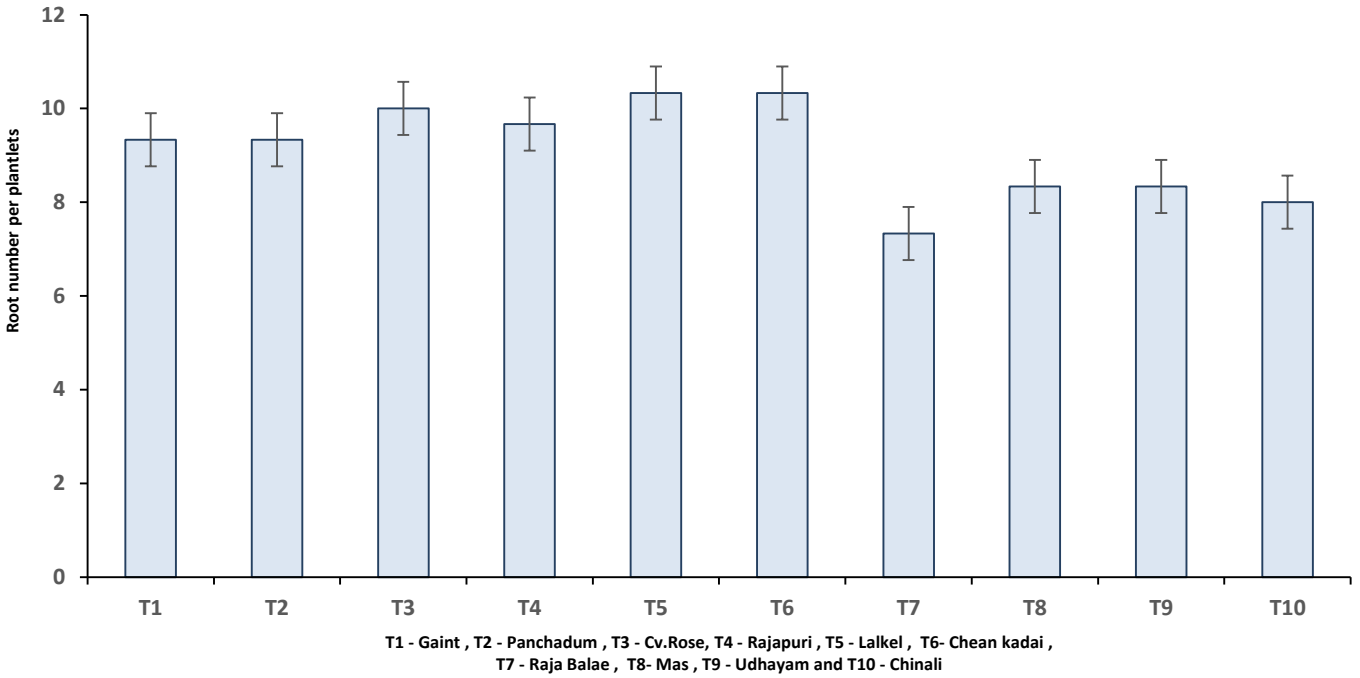

Fig 4. Effect of rooting media [half MS + 1.0 mg/l IBA] on root number per plantlets of different banana genotypes

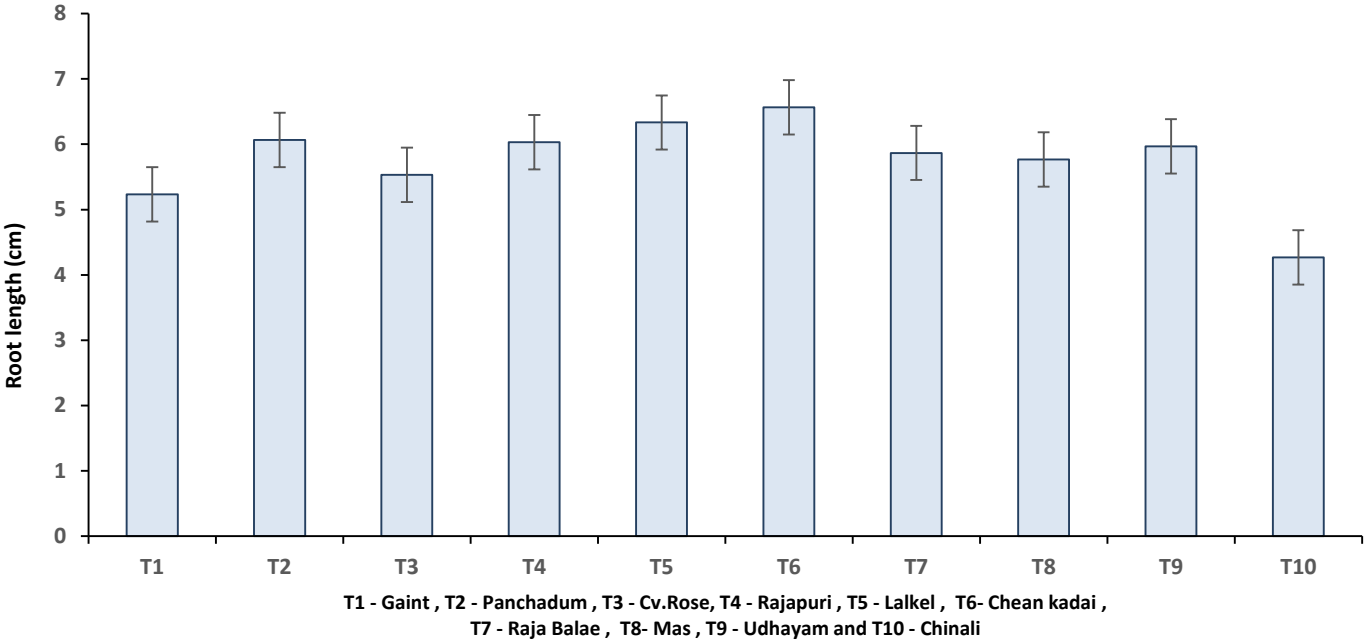

Fig 5. Effect of rooting media [half MS + 1.0 mg/l IBA] on root length (cm) of plantlets of different banana genotypes

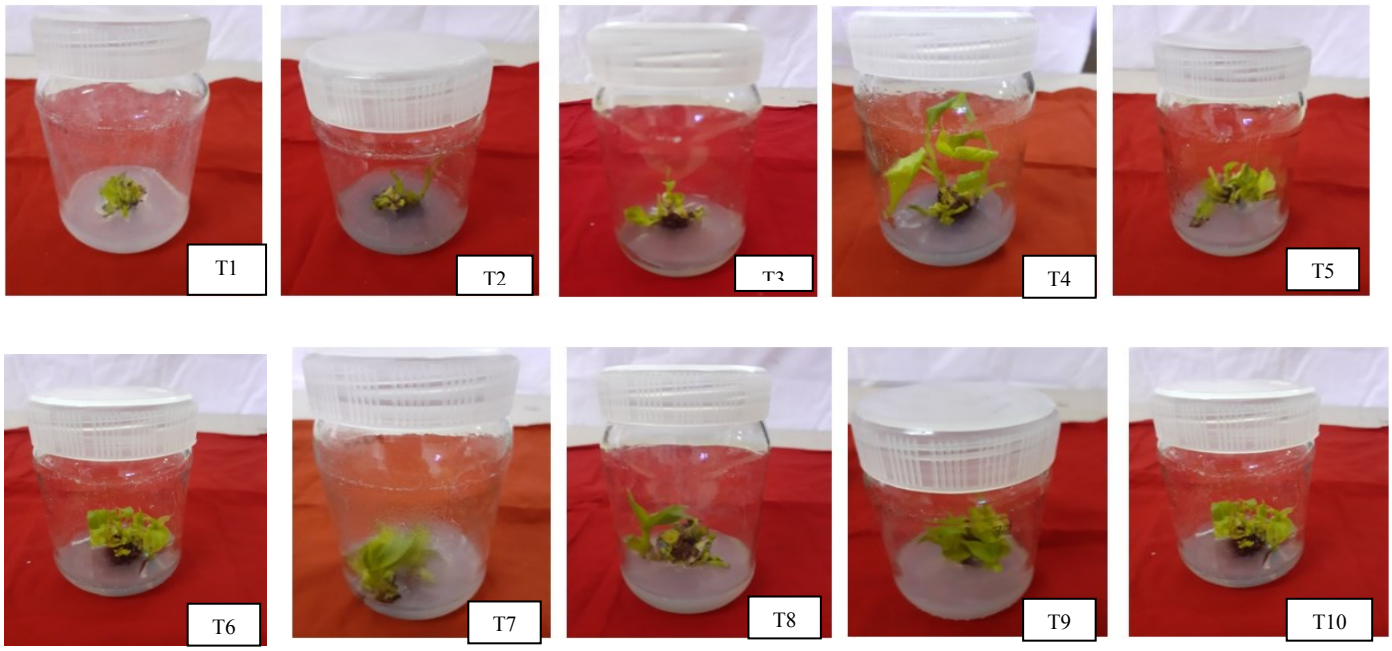

Plate 1. Effect of shoot multiplication media [MS + BA (3.0 mg/l) + adenine sulphate (2.0mg/l)] on shoot number per explant of different banana genotypes

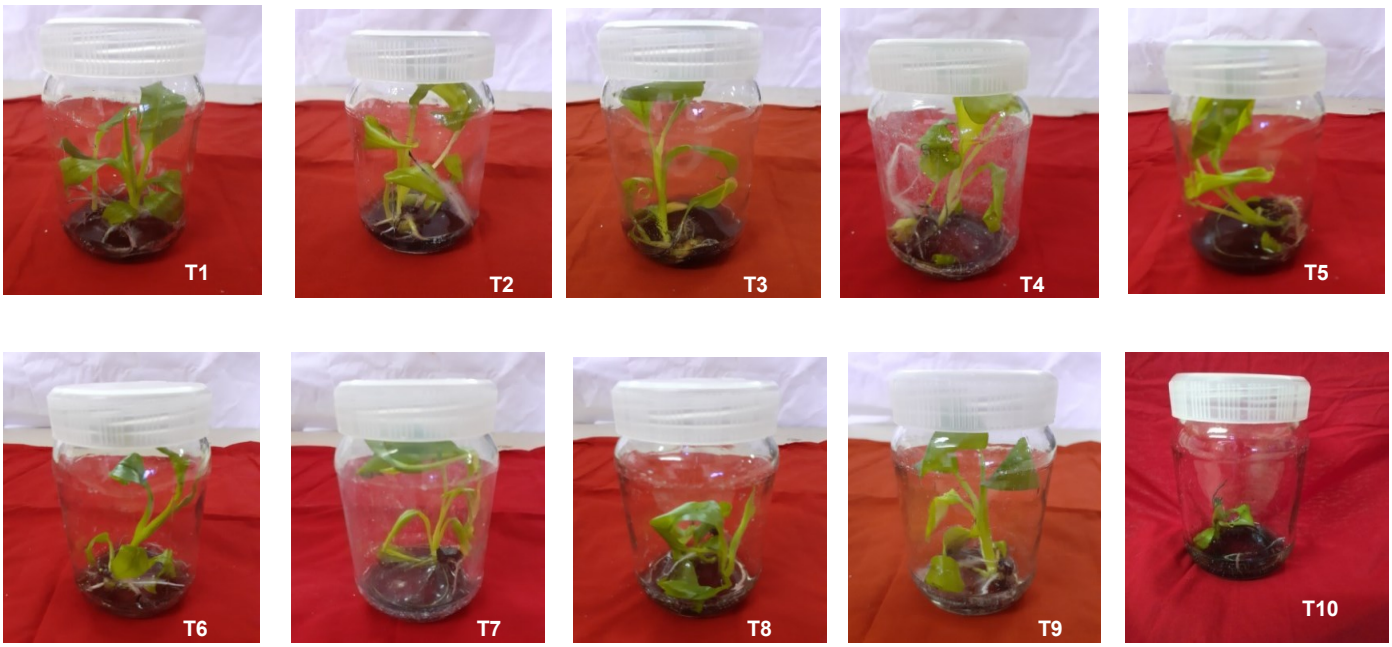

Plate 2. Effect of rooting media [half MS + 1.0 mg/l IBA] on root growth per plantlets of different banana genotypes

The successfully established shoot tip were further culture on MS medium supplemented with (BA (3.0 mg/l) + adenine sulphate (2.0mg/l) for shoot multiplication and observed that all ten banana genotypes responded differently for production of multiple shoots in the same treatments BA (3.0 mg/l) + adenine sulphate (2.0mg/l). Lalkel (T5) and Cheankadai (T6) banana genotypes recorded significantly maximum number of shoot followed by Rajapuri (T4) banana genotype (fig 3, plate 1).

In this study, the difference in multiplication rate of shoot tip was found and this might be due to differences in genetic background of various genotypes of banana. Previous study also reported that the same kind of differences in rate of multiplication of different *Musa* spp., genotypes (Abdullah *et al*., 1997; Vuylsteke, 1998).

Regenerated shoots were cultured in half strength of MS medium supplemented with (½ MS + 1.0 mg/l IBA) and recorded significantly highest number of roots per shoot in Lalkel (T5) and Cheankadai (T6) followed by Rajapuri (T4) as compare to other banana genotypes with same rooting media (fig. 4). The vigorous rooting of *in vitro* grown plantlets observed on half MS medium fortified with 1.0 mg/l IBA (plate 2). In case of root length, significantly maximum root length (cm) was observed in Cheankadai (T6), Lalkel (T5) and Rajapuri (T4) as compare to other banana genotypes (fig 5). The present results are in line with the findings of (Gubbuk and Pekmezci, 2004 and Molla *et al.,* 2006). The source of variation in number of root and root length may difference in cultivars genotype.

Conclusion

In the present study *in vitro* micropropagation of ten different banana genotype on common culture media using shoot tip culture was successfully demonstrated. As in many of the previous reports on banana micropropagation that used more than one type of media for initiation, multiplication and rooting (Cronauer and Krikorian, 1986; Jarret, 1986; Kagera *et al*., 2004). Among the ten banana genotypes tested, all genotypes responded to shoot multiplication and root initiation media and Lalkel and Cheankadai found to be more responsive as the rate of shoot multiplication, root length and root number was recorded highest among the banana genotypes. Banana plantlets production through *In vitro* plant tissue culture techniques seems to be very effective for rapid and large scale multiplication of banana cultivars.

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