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Effect of sterilants on explant of Red Banana (*Musa acuminata*) cv. Lal Velchi *in-vitro* plantlets regeneration

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Abstract
The present study on 'Effect of sterilants on explants of Red Banana (*Musa acuminata*) cv. Lal Velchi *in-vitro* plantlets regeneration' through shoot tip culture of suckers from mother plant aimed to standardize surface sterilization for establishment. As the red banana species highly prone to microbial contamination, resulting to death of explants. Therefore present investigation was performed on surface sterilization of shoot tip before inoculation. The treatments of different combination of sterilants on explant of cv. Lal velchi was done and contamination percentage was recorded during an experiment.

Keywords: Sucker, explants, sterilants, microbial, contamination

Introduction
The fruit crop 'Banana' belongs to the genus Musa of the family Musaceae. It considered to have originated in the hot tropical regions of south-east Asia. Banana is the second most important fruit crop in India next to Mango and fourth most important fruit in world. It is more preferable because of affordable, it's year round availability irrespective to season, taste, nutritive and medicinal values. It has also good export potential and all year demand in Gulf countries. In India banana ranks first in production and third in area among fruit crops. It accounts for 13% of the total area and 33% of the production of fruits. Production is highest in Maharashtra (3924.1 thousand tones) followed by Tamil Nadu (3543.8 thousand tons). In India, Maharashtra has the highest productivity of 65.70 metric tons /ha against national average of 30.5 tons/ha. The other major banana producing states are Karnataka, Gujarat, Andhra Pradesh and Assam. (National Horticulture Board, 2001-02). Conventional propagation of Banana is through suckers and production of suckers per plant is very less. About 70% farmers in India are using suckers while the remaining 30% of the farmers are using tissue culture developed banana plants (NHB). However, the suckers obtained is more tend to be associated with soil borne disease and viral infections like Bunchy top, resulting losses in commercial production. The plots grown by using suckers as planting material seems non-uniform growth. Banana is a prominent crop in India that benefits significantly from tissue culture technology. India has a substantial capacity to produce tissue culture raised planting material, with an installed production capacity of 650 million plantlets annually. However, the current supply stands at 455 million plantlets per year. India's annual requirement for banana planting material is estimated at 3436 million suckers or plantlets. Given this demand, there is considerable potential for expanding tissue culture production to serve better the needs of farmers. By bridging the gap between demand and supply, tissue culture can play a vital role in enhancing banana cultivation across the country. For contamination free cultures and mass multiplication of the explants with further rooting, protocol standardization is required. Now a days popular variety like G9 having huge commercialization and market place due to efficient protocol has already standardized by researchers, hence become more popular that about 5 million tissue cultured plantlets of G9 were planted which is 17% of total cultivated banana area in one year. Unlike with G9 and other varieties of banana, the technique of *in-vitro* regeneration in Red Banana is less popular due to the improper standardization of infection free protocol which is required for profitable

multiplications and production of desired plantings. To avoid microbial contamination, use of systematic disinfectants with antioxidants helps to surface sterilize the explant. Sodium hypochlorite is commonly used disinfectant for surface sterilization of banana explants (Muhammad *et al*., 2004) similarly, Trivedi *et al*., (2018) stated that Bavistin and $HgCl_2$ combination is effective for broad spectrum anti-microbial sterilization to shoot-tip explants. This will be helpful for promoting the Red banana cultivation under micropropagation techniques. During present investigation, the use of different combination of sterilants on explant of cv. Lal velchi was done and contamination percentage were recorded to check the efficiency for contamination free growth of explant.

Materials and Methods

The suckers of Red banana were collected from healthy mother block nursery. Plant with best agronomic character been considered for selection of mother plant. Two-three months old sword suckers were excised from healthy mother plant. Broad corms with narrow, sword like leaves were key characteristics of good suckers. Plant should be disease and pest free with good girth of its base.

The suckers were obtained from first season healthy mother plant, later the suckers were washed under pressured fresh water such that the soil and dirt get washed away. The cleaned suckers (explant) were trimmed using stainless steel knife by 4 to 6 cm and diameter at leaf base by 2 to 4 cm were used for studying the effect of sterilants.

Pretreatment of explants

Suckers (explant) were thoroughly washed under pressured running tap water. Cleaned shoot tips were collected in tray and kept in 1% bavistin solution for 15 min before going under laboratory for surface sterilization, as the bavistin is systemic fungicide action which helps to suppress the fungal growth within the tissues.

Surface sterilization of explants

Obtaining sterilized explants is tough because during the sterilization process biological activity of living material should be maintained and only the fungal and bacterial contaminant needs to be eliminated. Various surface sterilization agents were used for sterilization of explants. Cleaned and trimmed shoot tip were collected in tray and kept in 1% bavistin for 15 min, later one upper layer of treated shoot tips is removed and again shaked on rotatory shaker with 1% bavistin. After this explants were pre-treated with chemicals like Tween 20, citric and ascorbic acid. Further operations were carried out under aseptic condition of Laminar air flow by treating with 10% NaOCl, 0.1% $HgCl_2$. Later one upper layer is removed after finishing one or two treatment, followed by with or without distilled water washing as per given in Table 1.

Observations recorded

Each combination of treatment was replicated 3 times for getting unbiased results. Observation of cultures were done periodically and % of explant showed initiation without any contamination were recorded.

Completely randomized design was used for conducting the experiment and data collected was analysed for mean.

Incubation of Culture

All the cultures were incubated at a temperature of 25±2 °C under fluorescent light in a 16:8 hour's photoperiod and light intensity of 2500 lux.

Results and Discussion

The experiment entitled "Effect of sterilants on explants of Red Banana (*Musa acuminata*) cv. Lal Velchi *in-vitro* plantlets regeneration" was carried out in a Tissue Culture Unit, Biotechnology Laboratory, Botany Division, College of Agriculture, Pune during the year 2023-2025. The development of an efficient sterilization protocol for Red Banana using shoot tip as explant was carried out. The shoot tip explant was taken from the healthy plants of Red Banana from mother block nursery. The preparation of explant, sterilization treatment and inoculation was done according to methodology provided in the chapter of materials and methodology.

Effect of Sterilization

Sterilization of shoot tip explant carried out by using six different treatments, treatment S1 showed highest contamination percentage of 60 % and S5 showed the lowest contamination percentage of 20% as shown in table 1. The process carried out in S5 is by trimming the explant into desired size then the trimmed explants were cleaned thoroughly under pressured running tap water. Cleaned and trimmed shoot tip were collected in tray and kept in 1% bavistin for 15 min for primary disinfection. Later one upper layer of treated shoot tips were removed and again shaked in 1% bavistin for 25 min for deep disinfection. Another upper layer of treated shoot tips is removed and treated with Tween20 for 20 min. The autoclaved distilled water was used to rinse the explant two time for removing the chemical traces of fungicide. The cleaned explants were treated with citric acid and ascorbic acid (both 0.01%) for 10 min for checking the oxidation process of treated explant. Later the explants brought into the UV sterilized laminar chamber and treated with 5% NaOCl for 5 min and then finally treated with 0.1 % $HgCl_2$ for 15 min. The traces of $HgCl_2$ is removed by washing it with double distilled water thrice and finally sterilized explant ready for inoculation on the MS medium.

Table 1: Effect of treatments of explants with various sterilants.

Treatment Number	Treatment details	% of explant showed initiation without any contamination (a)	Contamination percentage(b), where, b= 100-a
S1	1% Bavistin (5 min) + 1% Bavistin (5 min) + Tween 20 (5 min) + 0.1% $HgCl_2$ (2 min)	40	60
S2	1% Bavistin (10 min) + 1% Bavistin (10 min) + Tween 20 (5 min) + 0.1% $HgCl_2$ (2 min)	50	50
S3	1% Bavistin (10 min) + 1% Bavistin (10 min) + Tween 20 (10 min) + 0.1% $HgCl_2$ (5 min))	60	40
S4	1% Bavistin (10 min) + 1% Bavistin (15 min) + Tween 20 (15 min) + citric acid and ascorbic acid (5 min) + 5% NaOCl (5 min) + 0.1% $HgCl_2$ (5 min)	60	40
S5	1% Bavistin (15 min) + 1% Bavistin (25 min) + Tween 20 (20 min) + citric acid and ascorbic acid (10 min) + 5% NaOCl (5 min) + 0.1% $HgCl_2$ (15 min)	80	20
S6	1% Bavistin (15 min) + 1% Bavistin (30 min) + Tween 20 (25 min) + citric acid and ascorbic acid (10 min) + 5% NaOCl (10 min) + 0.1% $HgCl_2$ (15 min)	70	30

Plate 1: Sterilization explants

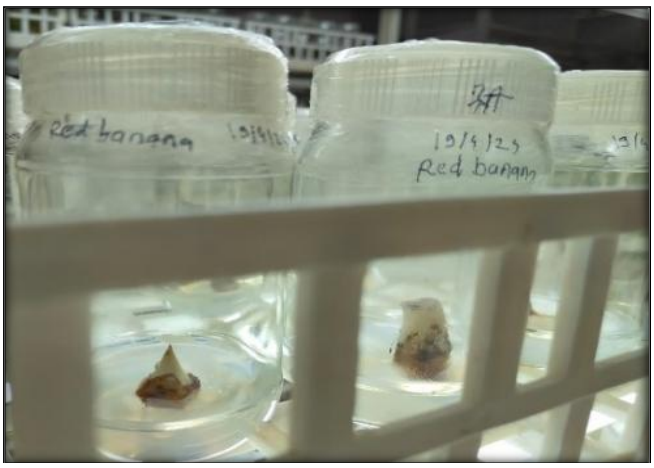

Plate 2: Inoculation of sterilized explants

Plate 3: Establishment of sterilized explants

Plate 4: Contaminated explant

Plates 1 to 3: Treatment S5 on explants
Plate 4: Effect of treatment S1 on explant

Conclusion

Among the all treatments, S5 found to be most effective. The explants remained free from contamination when subjected to a sequential surface sterilization protocol. Initially, explants were treated with 1% Bavistin for 15 minutes, followed by shaking of trimmed explants in 1% Bavistin solution for 25 minutes. This was succeeded by immersion in a solution containing Tween-20 for 20 minutes, supplemented with citric acid and ascorbic acid for 10 minutes to minimize oxidative browning. Subsequently, explants were treated with 5% sodium hypochlorite (NaOCl) for 5 minutes and finally exposed to 0.1% mercuric chloride ($HgCl_2$) for 15 minutes. This sterilization sequence of disinfectants and sterilants proved to be highly effective in achieving contamination-free explants, ensuring better survival and establishment during *in-vitro* regeneration studies.

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