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EFFECTS OF STERILANTS USED IN MICROPROPAGATION OF *Musa paradisiaca*

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Abstract

For removal of micro contamination from the explants many sterilizing chemicals have been used. The disinfectants have many advantages as well as disadvantages. The effects of three commonly used sterilants i.e. mercuric chloride, sodium hypochlorite and calcium hypochlorite on explants treated at different concentration and exposure time are studied in the present experiment. Result showed that the bacterial contamination was reduced to 10 % in mercuric chloride (S8), 20 % in sodium hypochlorite (S16) and 15 % in calcium hypochlorite (S22) where as the fungal contamination was reduced to 5 % in all the cases. But the percentage of explants survived decreases with increase in concentration and exposure time due to the toxic effects of the sterilant.

Keywords: *Musa paradisiaca*, mercury chloride, sodium hypochlorite, calcium hypochlorite, BAP- 6-Benzyl amino purin

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Introduction

Plant tissue culture techniques are essential to many types of academic inquiry, as well as to many applied aspects of plant science. In the past, plant tissue culture techniques have been used in academic investigations of totipotency and the roles of hormones in cytodifferentiation and organogenesis. Losses due to contamination in *in vitro* condition average between 3 and 15% at every subculture in the majority of commercial and scientific plant tissue culture laboratories (Boxus & Terzi, 1987, 1988), the majority of which is caused by fungal, yeast and bacterial contaminants (Leifert et al., 1994).

The rate of contamination in banana tissue culture is higher during initial culture as the explants (Suckers) used for micropropagation of banana is collected directly from the soil. To remove the contaminant on the explants surface a powerful sterilizing agent mercuric chloride is used. Mercury is a xenobiotic metal that is a highly deleterious environmental pollutant. The biotransformation of mercury chloride ($HgCl_2$) into methylmercury chloride (CH_3HgCl) in aquatic environments is well-known and humans are exposed by consumption of contaminated fish, shellfish and algae.

As an alternate sterilizing agent sodium hypochlorite (NaOCl) is the most commonly used disinfectant for surface sterilization of banana explants (Muhammad et at., 2004). Calcium hypochlorite [$Ca(ClO)_2$] has also been used as surface sterilizing agent. In the present study the main objective is to find out better alternate sterilizing agent in place of mercuric chloride for in vitro propagation of banana. Each observation was taken at every 2 days interval till 10th day of initial cultures.

MATERIAL AND METHODS:-

Plant materials:

Banana and plantain varieties that belong to *Musa paradisiaca* are most widely cultivated in Odisha. Mother plants were healthy, true to type and free from diseases and pests, especially virus diseases.

Suckers were collected from the banana mother block of Regional Plant Resource Centre, Bhubaneswar in February 2015 and washed thoroughly under running tap water for 10-15 min. The suckers were then chopped off about 5-6 cm in length and 3-4 cm in diameter.

Explants sterilization:

Suckers after processing were washed in Labolene before treatment with mercuric chloride, Sodium hypochlorite and calcium hypochlorite. Before using the mercuric chloride solution and calcium hypochlorite (bleaching powder) were autoclaved at 15 lbs p.s.i pressure and 121°C for 20 minutes.

Then the explants were washed 2 – 3 times with autoclaved double distilled water followed with 70 % alcohol for 1 minute

Finally, explants were washed with autoclaved double distilled water for 2 – 3 times

Culture medium:

The medium used here for banana tissue culture was Murashige & Skoog Medium (MS) with 4 mg L^{-1} BAP. All the media were autoclaved at 15 lbs p.s.i pressure and 121°C for 20 minutes. The autoclaved molten media were then dispensed into sterilized culture vessel inside a laminar air flow cabinet.

Table 1- Concentration and exposure time of sterilants.

Sterilizing treatments (S.T)	Disinfec-tants	Conc. (gm/100ml)	Exposure time (Min.)
S1	$HgCl_2$	0.2	15
S2	$HgCl_2$	0.2	30
S3	$HgCl_2$	0.3	15
S4	$HgCl_2$	0.3	30
S5	$HgCl_2$	0.4	15
S6	$HgCl_2$	0.4	30
S7	$HgCl_2$	0.5	15
S8	$HgCl_2$	0.5	30
S9	NaOCl	1.0	15
S10	NaOCl	1.0	30
S11	NaOCl	1.2	15
S12	NaOCl	1.2	30
S13	NaOCl	1.4	15
S14	NaOCl	1.4	30
S15	NaOCl	1.6	15
S16	NaOCl	1.6	30
S17	$Ca(ClO)_2$	10	15
S18	$Ca(ClO)_2$	10	30
S19	$Ca(ClO)_2$	20	15
S20	$Ca(ClO)_2$	20	30
S21	$Ca(ClO)_2$	30	15
S22	$Ca(ClO)_2$	30	30
S23	$Ca(ClO)_2$	40	15
S24	$Ca(ClO)_2$	40	30

Inoculation:

The working area of the laminar airflow cabinet was first wiped with cotton moistened with ethanol and then irradiated with ultraviolet light for 30 minutes before inoculation. The explants were surface sterilized and cut aseptically by a sterile surgical scalpel and inoculated in the culture vessel containing induction medium. Healthy explants and contamination percentage at every 2 days interval till 10th day of initial cultures were recorded and the contaminated cultures were autoclaved at 121^0 C and 15 lbs p.s.i pressure for 30 minutes and discarded immediately.

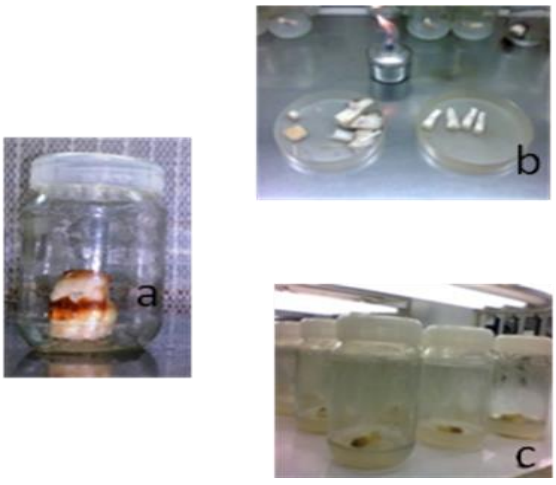

Figure 1 - a. Sterilized suckers, b. cutting the sucker into four explants and c. inoculated explants.

Culture condition:

The culture vessels containing the explants on solid media were kept in culture rack. The culture was maintained at 22°C to 25°C, 16 hr photo period of 35-50µEm-2s-1 intensity provided by cool white fluorescent tubes.

RESULTS

The present study was conducted to standardize the best sterilization protocol using three different sterilizing agents for in vitro propagation of Musa cv. Gaja bantal.

1. Effect of sterilants on explants survival:

Table 2- Effect of different sterilizing treatments on explants survival.

(S.T)	Explants survived (%)				
	2nd day	4th day	6th day	8th day	10th day
S1	100	100	100	100	95
S2	100	100	95	90	90
S3	100	100	100	95	95
S4	100	95	95	90	90
S5	100	100	100	95	90
S6	100	95	90	90	85
S7	100	100	95	95	90
S8	100	95	85	85	75
S9	100	100	100	100	100
S10	100	100	100	100	95
S11	100	100	95	95	95
S12	100	100	100	95	90
S13	100	100	100	95	95
S14	100	95	95	90	90
S15	100	100	100	95	90
S16	100	95	90	85	80
S17	100	100	100	100	100
S18	100	100	100	95	95
S19	100	100	100	95	90
S20	100	100	95	90	90
S21	100	100	95	95	90
S22	100	100	90	85	85
S23	100	90	80	65	55
S24	90	75	55	30	20

From the above result it was seen that the percentage of explants survived decreases gradually with increase in concentration as well as the exposal time. The explants treated with calcium hypochlorite at 40 % (S23 and S24) proved to be highly toxic but mercuric chloride (S8) is comparatively more harmful than sodium hypochlorite (S16) and calcium hypochlorite (S22) at high concentration and exposal time.

2. Effect of sterilants on contamination:

2a. Bacterial contamination in explants:

The percentage of contaminated explants decreases gradually with increase in concentration as well as the exposal time. The explants treated with calcium hypochlorite at 40 % (S23 and S24) were highly effective against bacterial contamination but due to its toxic effect these treatments cannot be used. Mercuric chloride (S8) is comparatively more useful disinfectant than calcium hypochlorite (S22) and sodium hypochlorite (S16).

Table 3- Effect of different sterilizing treatments on bacterial contamination.

(S.T)	Bacterial contamination (%)				
	2nd day	4th day	6th day	8th day	10th day
S1	35	50	80	100	100
S2	20	40	65	85	90
S3	20	35	60	75	75
S4	10	25	30	35	45
S5	5	10	25	40	50
S6	0	5	15	20	25
S7	0	10	20	30	35
S8	0	0	5	10	10
S9	40	55	70	90	100
S10	35	55	70	85	90
S11	25	45	70	90	100
S12	25	50	65	75	80
S13	15	25	40	55	65
S14	5	10	20	40	50
S15	0	0	10	25	35
S16	0	0	5	15	20
S17	45	60	85	100	100
S18	35	55	75	90	90
S19	25	40	55	70	75
S20	10	20	30	40	50
S21	5	15	25	25	35
S22	0	5	5	10	15
S23	0	0	0	5	10
S24	0	0	0	0	5

2b. Fungal contamination in explants:

The fungal contamination is less and mainly caused due to handling errors. The percentage of contaminated explants decreases gradually with increase in concentration as well as the exposal time. From the above observation it was found out that mercuric chloride (S8), sodium hypochlorite (S16) and calcium hypochlorite (S22) have similar effect on fungal contamination. The explant treated with calcium hypochlorite at 40 % (S24) was highly effective against fungal contamination but this treatment cannot be used.

Table 4- Effect of different sterilizing treatments on fungal contamination.

(S.T)	Fungal contamination (%)				
	2nd day	4th day	6th day	8th day	10th day
S1	0	5	10	30	40
S2	0	5	15	25	30
S3	0	5	15	30	30
S4	0	5	10	20	25
S5	0	0	5	10	15
S6	0	5	5	10	10
S7	0	0	0	5	10
S8	0	0	0	0	5
S9	0	5	10	35	45
S10	0	5	10	20	30
S11	0	0	10	15	25
S12	0	5	5	10	15
S13	0	5	5	10	10
S14	0	0	0	5	10
S15	0	0	5	5	5
S16	0	0	0	0	5
S17	0	5	15	25	40
S18	0	5	10	20	35
S19	0	5	10	25	30
S20	0	0	5	10	15
S21	0	5	10	10	20
S22	0	0	0	0	5
S23	0	0	0	5	10
S24	0	0	0	0	0

3. Effect of sterilants on explants growth:

Table 5- Effect of different sterilizing treatments on growth of explants.

(S.T)	Explants biomass (gm)				
	2nd day	4th day	6th day	8th day	10th day
S1	1.5	1.8	2.1	2.3	2.5
S2	1.5	1.7	2.0	2.2	2.4
S3	1.5	1.7	2.0	2.2	2.4
S4	1.5	1.6	1.9	2.1	2.2
S5	1.5	1.7	1.9	2.0	2.2
S6	1.5	1.6	1.7	1.9	2.1
S7	1.5	1.7	1.9	2.0	2.2
S8	1.5	1.6	1.7	1.9	2.0
S9	1.5	1.8	2.1	2.3	2.6
S10	1.5	1.7	1.9	2.1	2.4
S11	1.5	1.8	2.0	2.2	2.5
S12	1.5	1.7	2.0	2.2	2.4
S13	1.5	1.6	1.9	2.1	2.4
S14	1.5	1.7	2.0	2.2	2.3
S15	1.5	1.7	1.9	2.1	2.3
S16	1.5	1.7	1.9	2.0	2.2
S17	1.5	1.8	2.0	2.2	2.5
S18	1.5	1.7	2.0	2.2	2.4
S19	1.5	1.6	1.9	2.1	2.4
S20	1.5	1.7	2.0	2.2	2.3
S21	1.5	1.7	1.9	2.0	2.2
S22	1.5	1.7	1.9	2.0	2.2

Biomass of explant treated with mercuric chloride (S8) is comparative less than the explant treated with sodium hypochlorite (S16) and calcium hypochlorite (S22) after 10 days of inoculation. This showed that due to its high toxic nature, mercuric chloride at higher concentration affects the growth of explants.

DISCUSSION

For high rate of success in tissue culture maintenance of aseptic condition and proper sterilization of explants is necessary.

Onuoha *et al.* achieved the contamination free Plantain culture (100%) in the explants treated with $HgCL_2$ for 6 min.

Calcium hypochlorite had also been used as a mild sterilant. **Nozeran *et al.*,** sterilized potato sprouts by dipping them in alcohol and a few drops of Teepol and then placed them in Calcium hypochlorite solution for 15-25 minutes. **Goodwin *et al.*,** disinfected the sprouts with Sodium hypochlorite in which available chlorine was sterilized single node cuttings of eight different cultivars in 1% aqueous sodium hypochlorite.

CONCLUSION

Sodium hypochlorite is most widely used disinfectant in place of mercuric chloride and calcium hypochlorite is used as alternate sterilizing agent in place of sodium hypochlorite. Using calcium hypochlorite for surface sterilization of explants in *in vitro* culture of *Musa paradisiaca* make the process safe and cost effective.

Amongst the three sterilants i.e. $Ca(ClO)_2$, NaOCl and $HgCl_2$, NaOCl was found better for controlling the infection but $Ca(ClO)_2$ was proved to be more effective for *in vitro* propagation of *Musa paradisiaca* although calcium hypochlorite (S23 and S24) treatments have shown high toxic effect. Explants treated with calcium hypochlorite (S22) showed minimum bacterial and fungal contamination and high survival rate.

Mercuric chloride (S8) and sodium hypochlorite (S16) have also been proved to be an effective surface disinfectant but due to the toxic effects of mercuric chloride it should be used in little quantity.

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References

Boxus, P. H., and J. M. Terzi, 1987. Big losses due to bacterial contamination can be avoided in mass propagation schemes. *Acta Horticulturae*, 212: 91-93.

Boxus, P. H., and J. M. Terzi, 1988. Control of accidental contaminations during mass propagation ISHS. *Acta Horticulturae*, 225: 198-190.

Goodwin, P. B., Y. C. Kim and T. Adisarwanto, 1980. Propagation of potato by shoot tip culture. *Potato Res.*, 23:9-18

Leifert, C., E. C. Morrris and M. W. Waites, 1994. Ecology of microbial saprophytes and pathogens in tissue culture and field grown plants: reasons for contamination problems in vitro. *Plant sciences*, 13(2): 139-183.

Muhammad Aish, Hussain Iqbal, Naqvi S. M. Saqlan & Rashid Hamid., 2004. Banana Plantlet Production through Tissue Culture. *Pak. J. Bot.*, 36(3): 617-620.

Murashige T., and F. Skoog, 1962, A revised medium for rapid growth and bioassays with tobacco tissue culture. *Physiol Plant*., 15: 473 – 497.

Nozeran, R. B., andilho, L. Rossignol and S. Glenan, 1977. Nouvelles possibilities etde multiplication rapie de clones sains de pomme de erre (*Solanum tuberosum* L.), C.R. *Acad Sci*., 285;1: 37-40.

Onuoha Innocent Chimereze, Eze Chinonye Jemimah & Unamba Chibuikem I. N., 2011. In Vitro Prevention of Browning in Plantain Culture. *Journal of Biological Sciences*, 11 (1): 13-17.