INCORPORATION OF PLANT PRESERVATIVE MIXTURE (PPMTM) TO IMPROVE SURFACE STERILIZATION OF TINOSPORA CRISPA IN VITRO

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ABSTRACT

Tinospora crispa (L) Hook. F. & Thomson is locally known as Patawali and belongs to Menispermaceae family. It is mostly found in Asia and Africa and well known for its traditional medicinal properties. It is used as traditional medicine against jaundice, rheumatism, fever, malaria, diabetes, hypertension and can reduce body temperature. Micropropagation techniques can be an alternative method for mass propagation of this species particularly when using samples from selected clones. Young and green nodal segments are more recommendable to use in this study as they are more juvenile and easier to be micropropagated than older part of the plants. The purpose of this paper is to provide information regarding the incorporation of biocide PPMTM for surface sterilization of young nodal segments of Tinospora crispa to improve the percentage of clean explants obtained.

Keywords: *Tinospora crispa, Patawali, PPMTM, micropropagation*

INTRODUCTION

It is generally known that to obtain clean cultures from explants that were taken from field (mature plants) are quite challenging. It is due to the high microbial contamination of explants from mature plants taken from field. Surface sterilization with ethyl alcohol and sodium hypochloride usually are not sufficient to obtain higher percentage of clean cultures. Incorporation of anti microbial agent during surface sterilization and into the medium is important to get sufficient number of clean explants. PPM^{TM} (Plant Preservative Mixture) is a broad spectrum, heat stable preservative/biocide which was reported can be used to effectively prevent or reduces microbial contamination in plant tissue culture. PPM^{TM} kills bacteria and fungi cells, prevent germination of spores and in higher concentrations can eliminate explants of endogenous contamination.

OBJECTIVE

The objective of the study is to evaluate the effectiveness of using PPM^{TM} during surface sterilization and incorporation into the nutrient medium to obtain higher percentage of clean Patawali explants taken from the field plot.

METHODOLOGY

Nodal segments were taken from *T. crispa* plants planted in FRIM's Research Station in Maran, Pahang (Figure 1). Two selected clones were used; clone 159 and A037. These young green nodal segments, which were approximately 30-40 cm long from shoots were harvested and cut into 4 cm long with at least one axillary bud.The leaves were removed and the explants were put into Schott bottle. Prior to surface sterilization, samples were soaked in Teepol and fungicide Thiram, and were rinsed with sterile distilled water three times (SDW). Plant material manipulations from this step onwards were done under laminar flow hood in contamination-free conditions. Explants were soaked in 70% (v/v) ethanol added with three drops of Tween 20 for 3 minutes followed with immersion in 50% (v/v) Clorox (2.63% sodium hypochlorite) for 20 minutes and were finally rinsed three times in SDW. After further trimming into 2 cm long explants, nodal segments were soaked in 0.1% citric acid to prevent browning. Then the explants were soaked and shake in 4% (v/v) PPM^{TM} for 3 hours, followed with 3 times rinsing with SDW. Then explants were immersed again in 20% (v/v) Clorox (0.53% sodium hypochlorite) for 10 minutes then rinsed thoroughly with SDW.

Murashige and Skoog medium (MS, 1962), each supplemented with 30 g/L sucrose and 0.5 mg/L of 6-Benzylaminopurine (BAP) and with or without 0.2% (v/v) of PPM^{TM} were prepared. After pH adjustment at 5.7 with HCl and NaOH, and addition of 2.75 g/L gelrite agar plus Bacto agar, the media were heated at about 80°C then poured into 25x150 mm test tubes (Schott Duran), each receiving 10 ml of medium and covered with polypropylene cap prior to autoclaving at 121°C and 103 kPa for 15 minutes. The cultures were grown at 23 ± 2° C under 16-h photoperiod with light intensity of 22.22 µmol $m^{-2}sec^{-1}$ supplied by LED lights. Just after disinfection, the explants were inoculated (one explants per culture tube) onto the media for a month. The total number of explants used was 30 per treatment. Observation was done after a month in culture.

Figure 1: Field Plot of Tinospora crispa (Patawali) in FRIM's Maran Research Station, Pahang.

Figure 2: Nodal segment explants were taken from young stems of T. crispa (a) and were cut into smaller segments prior to surface sterilization (b).

(a)

(b)

RESULTS AND DISCUSSION

From the results obtained for two clones, it showed that both clones achieved higher clean cultures when explants were soaked in 4% (v/v) PPM^{TM} for 3 hours and cultured onto MS medium incorporated with 0.2% PPM^{TM} compared to medium without PPM^{TM}. For clone A037, 35% clean explants was obtained compared to 25.5% without PPM^{TM} in the medium (Figure 2). And for clone 159, 43.8 % clean explants was obtained compared to 18.8% without PPM^{TM} in the medium after a month in culture (Figure 2). Previous report on surface sterilization of *Tinospora cordifolia* used 0.1 % Mercuric chloride to obtain clean cultures (Mridula *et al*., 2017, Sivakumar *et al*., 2014). Figure 3a and 3b showed shoots initiated from explants in MS medium incorporated with 0.5 mg/L BAP and PPM after a month and two months in culture.

Figure 2: Percentage of clean and responded explants (by producing shoots) obtained after 1 month in culture in the MS medium with or without 0.2% PPM.

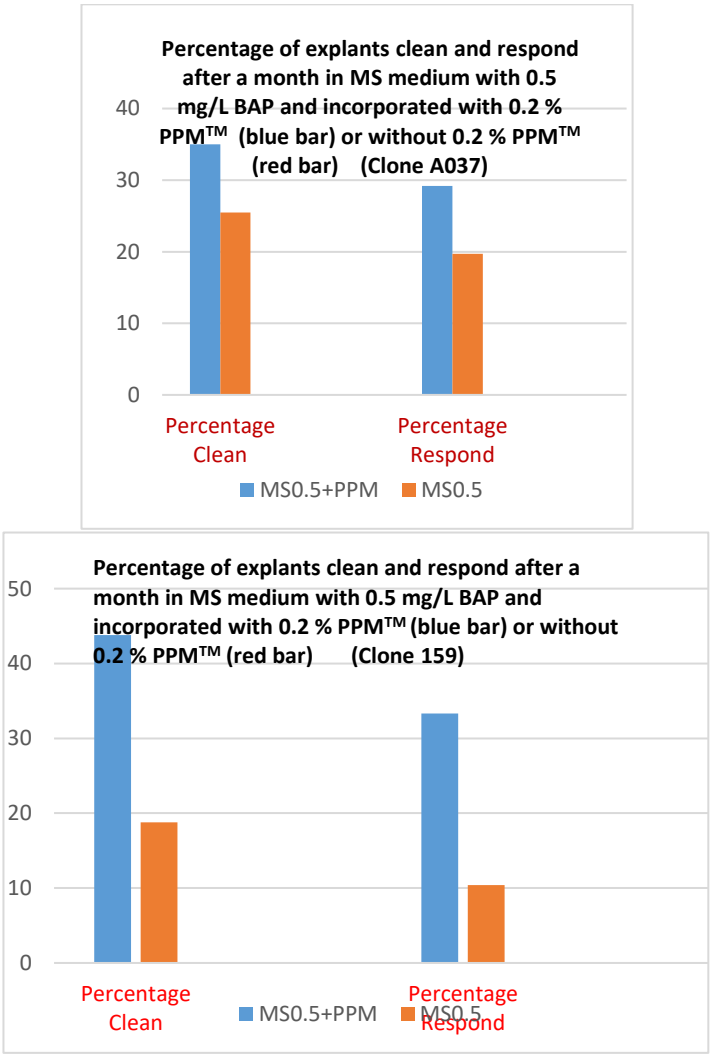

Figure 3: Shoots initiated from clean explants after a month (a) and two month (b) in culture.

(a)

(b)

CONCLUSION

Incorporation of PPM^{TM} during surface sterilization and into the nutrient medium did give a higher percentage of clean Patawali explants compared to the explants in medium without PPM^{TM}. Further work is ongoing to obtain the best medium for shoot multiplication and rooting of Patawali.

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