ISSN: 0976-3376

Available Online at http://www.journalajst.com

ASIAN JOURNAL OF SCIENCE AND TECHNOLOGY

*Asian Journal of Science and Technology*
*Vol. 09, Issue, 10, pp.8833-8836, October, 2018*

## RESEARCH ARTICLE

# RAPID MICROPROPAGATION OF MD2 PINEAPPLE (*ANANAS COMOSUS* L.) USING THE TEMPORARY IMMERSION SYSTEM (TIS) AND LIQUID-SHAKE CULTURE (LSC)

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## ARTICLE INFO

***Article History:***
Received 15th July, 2018
Received in revised form
20th August, 2018
Accepted 24th September, 2018
Published online 30th October, 2018

***Key words:***
Pineapple MD2,
BAP, micropropagation,
Temporary Immersion System (TIS),
liquid-shake culture (LSC),
Solid agar medium.

## ABSTRACT

Efficient and rapid vegetative micro propagation of MD2 pineapple was achieved using two liquid culture medium systems, viz. a culture vessel system (Temporary Immersion System, TIS) and liquid-shake culture (LSC). Multiplication of plantlets using these systems was better than on solid agar medium. Compared with the solid agar medium, the number of plantlets obtained by the liquid media systems was significantly higher, attaining an increase of up to four-fold. These in vitro systems are easy to manage and they facilitate uniform production of high quality MD2 pineapple plantlets. They are also amenable to up-scaling for commercial production.

**Citation:** ***Zuraida Ab. Rahman, Hartinee Abbas and Ayu Nazreena Othman*, 2018.** "Rapid micropropagation of MD2 pineapple (Ananas comosus L.) using the Temporary Immersion System (TIS) and liquid-shake culture (LSC)", *Asian Journal of Science and Technology*, 09, (10), 8833-8836.



## INTRODUCTION

The pineapple (*Ananas comosus*), a tropical species, is the most economically significant plant in the family Bromeliaceae (Morton, 1987; Coppens *et al.*, 2013). Pineapples can be cultivated from cuttings from the top of the fruit. The plants begin flowering after five to ten months and set fruit in the following six months (Birgit Bradtke, 2010). The pineapple is a major tropical fruit in terms of world production (FAOSTAT, 2010), with the MD2 variety being among the highly commercialized cultivars especially popular in the European Union market (Anonymous, 2006). The pineapple can be propagated vegetatively by lateral shoots, basal suckers or crowns. While such methods are easy, the multiplication rate is low, ranging from about 11 to 17 plants over a period of 5 months (Lieu *et al*., 2004). Moreover, these conventional techniques are cumbersome and time consuming (Mhatre, 2007). In pineapple breeding, clonal selection is tedious and requires several generations of backcrossing to develop a stable cultivar with desired traits. With the planting area for pineapple planting expanding in Malaysia, there is increasing demand for MD2 planting materials, the shortage of which has become a major problem facing Malaysia's pineapple industry (Hamid *et al.*, 2013). To address this problem, *in vitro* micropropagation is being explored with the objective of making MD2 planting material available on a scale that meets the industry's demand. Micropropagation of MD2 pineapple has been reported through direct *in vitro* micropropagation (Danso *et al.*, 2008) and indirect regeneration (Hamid *et al*., 2013). As such, the development of a stable micropropagation procedure based on shoot multiplication appears promising as a future commercial production system. Here, we report a simple, economical and rapid protocol for mass propagation of MD2 pineapple *via* the Temporary Immersion System (TIS) and liquid-shake culture (LSC).

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## MATERIALS AND METHODS

**Plant materials:** One hundred and fifty pineapple suckers of the MD2 variety were purchased from Koperasi Serbaguna Anak-Anak Selangor in Banting, Selangor. From these, pieces of approximately 25 - 30 cm were used as explants for *in vitro* culture. After removal of the outer leaves, the explants were trimmed to 5 – 8 cm, washed thoroughly in running tap water and detergent, and then immersed in fungicide (5% w/v

Benlate) for one hour before rinsing with sterile distilled water. The cleansed explants were soaked in in 50% (v/v) Clorox™ (5.25% sodium hypochlorite) solution for 15 minutes in a laminar flow chamber, followed by soaking in 20% (v/v) Clorox™ for 10 mins and then rinsed thrice with sterilized distilled water. The sterilized explants were then cut to 1-2 cm pieces.

**Micropropagation of shoots and acclimatization:** Sterilized explants were cultured on solid Murashige and Skoog (MS) medium (Murashige and Skoog, 1962) that was supplemented with 30 mg/L sucrose, 5 mg/L benzyl aminopurine (BAP) and 0.3% agar, after autoclaving at 121° C for 15 minutes. The cultures were maintained in the medium for three months before sub-culturing on to fresh medium at monthly intervals. After a further three months, the microshoots obtained were transferred on to three types of fresh media: (a) solid medium (with 0.3% gelrite agar) as control, (b) liquid medium in 250 ml shaking flasks (liquid-shake culture, LSC) oscillating at 100 rpm, or (c) the Temporary Immersion System, TIS, apparatus (Plant Form, Sweden). All cultures were maintained in the growth room at 25°C under cool-white fluorescent lighting (12 hours light/darkness). The explants were sub-cultured twice at four-week intervals and the number of plantlets that were produced was recorded. Actively growing plantlets were removed from culture flasks or the TIS apparatus and washed with tap water before transferring to soil in polybags. The plants were maintained in the green house for hardening before they were transplanted in the field in a completely randomized design, with 50 plants for each treatment. Statistical analyses were undertaken using SPSS.

## RESULTS AND DISCUSSION

Micropropagation cultures using solid medium as well as the two liquid medium systems, namely TIS and LSC, were tested for the effect of BAP (1 mg/L and 3 mg/L) on shoot production. When explants from the suckers of MD2 pineapple were cultured on solid MS medium, supplementation with 1 or 3 mg/L BAP produced similar amounts of plantlets, numbering no more than 94 plantlets per treatment (Figure 1).

Compared with the solid culture medium (Figure 2a-c), the results were more promising with the two liquid systems, LSC (Figure 2d-g) and TIS (Figure 2h-k), generating significantly more plantlets. Nevertheless, there was again no difference between the two BAP concentrations in both liquid-based systems (Figure 1). In addition, there was also no statistically significant difference in the number of plantlets initiated *via* LSC or TIS, even though the latter produced more plantlets (531 and 421 plantlets with 3 and 1 mg/L BAP, respectively). Transfer of 5 – 8 cm tall MD2 pineapple plantlets without roots to the soil resulted in almost 99% survival under greenhouse conditions (Figure 2l). In this study, explants grown on BAP-containing media had varying success in the production of plantlets, depending on the system used. As mentioned, TIS and LSC performed better in producing multiple shoots as compared with solid medium. Akin-Idowu *et al*. (2014) who studied *in vitro* micropropagation of pineapple (*Ananas comosus* L. var. Smooth Cayenne) found that supplementation with 1.5 mg/L BAP produced the most shoots as compared with other concentrations of BAP. In our pervious study on Maspine pineapple, we observed that 1.0 mg/L BAP in the culture medium produced a higher number of shoots than 5 mg/L BAP after four weeks of culture (Zuraida *et al*., 2011). Furthermore, Hamad and Taha (2008) reported that shoot initiation was highest with BAP at 1.5 mg/L in four cycles of culture. The positive effect of BAP and NAA in promoting shoot proliferation was also reported by Hashimah *et al*. (2015), who noted that MS medium supplemented with a combination of 2.0 mg/L NAA and 1.5 mg/L BAP was optimal for shoot formation of *Echinocereus cinerascens*. Significant differences were observed between the liquid-based media systems and the solid medium system that served as control. The liquid systems obtained up to a four-fold increase in the number of plantlets. Similarly, according to Marta *et al*. (2013), more frequent immersions promoted a 7.5-fold increase in the fresh weight of cork oak. This might be due to high humidity maintained inside the culture vessel that affected the culture positively (Etienne and Berthouly, 2002). The cultures in TIS were also more uniform due to complete immersion of the cultured tissues which might have promoted better nutrient uptake from the medium (Marta *et al*., 2013).

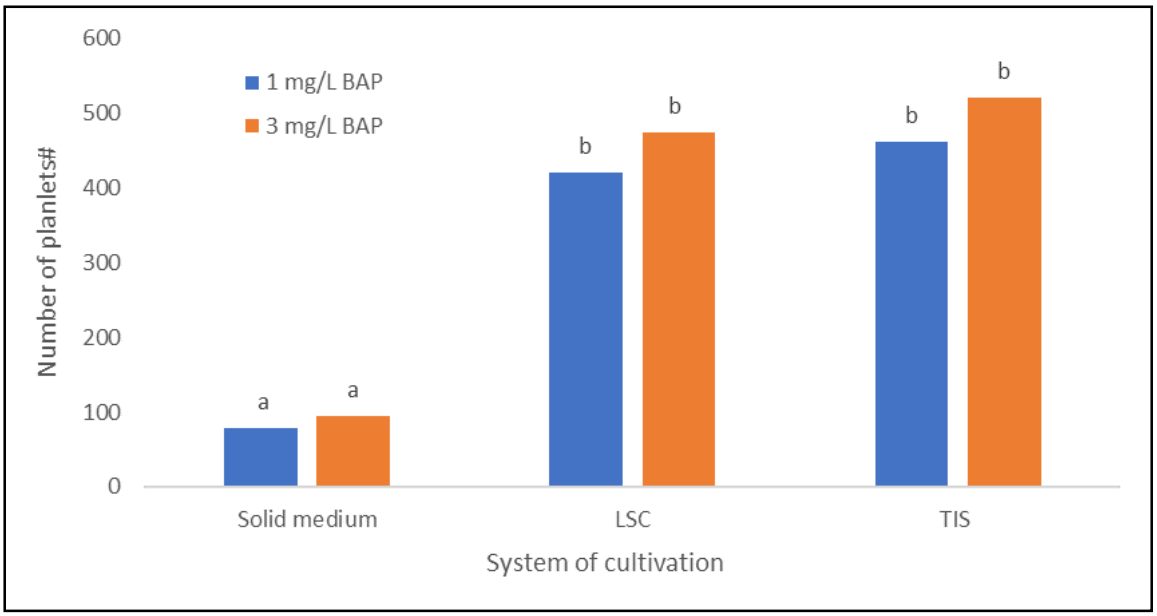


**Figure 1. Plantlets produced using different systems of culture. Each culture medium contained 1 or 3 mg/L BAP. Values marked with the same letter are not significantly different at the $P \leq 0.05$, (Duncan's Multiple Range Test)**

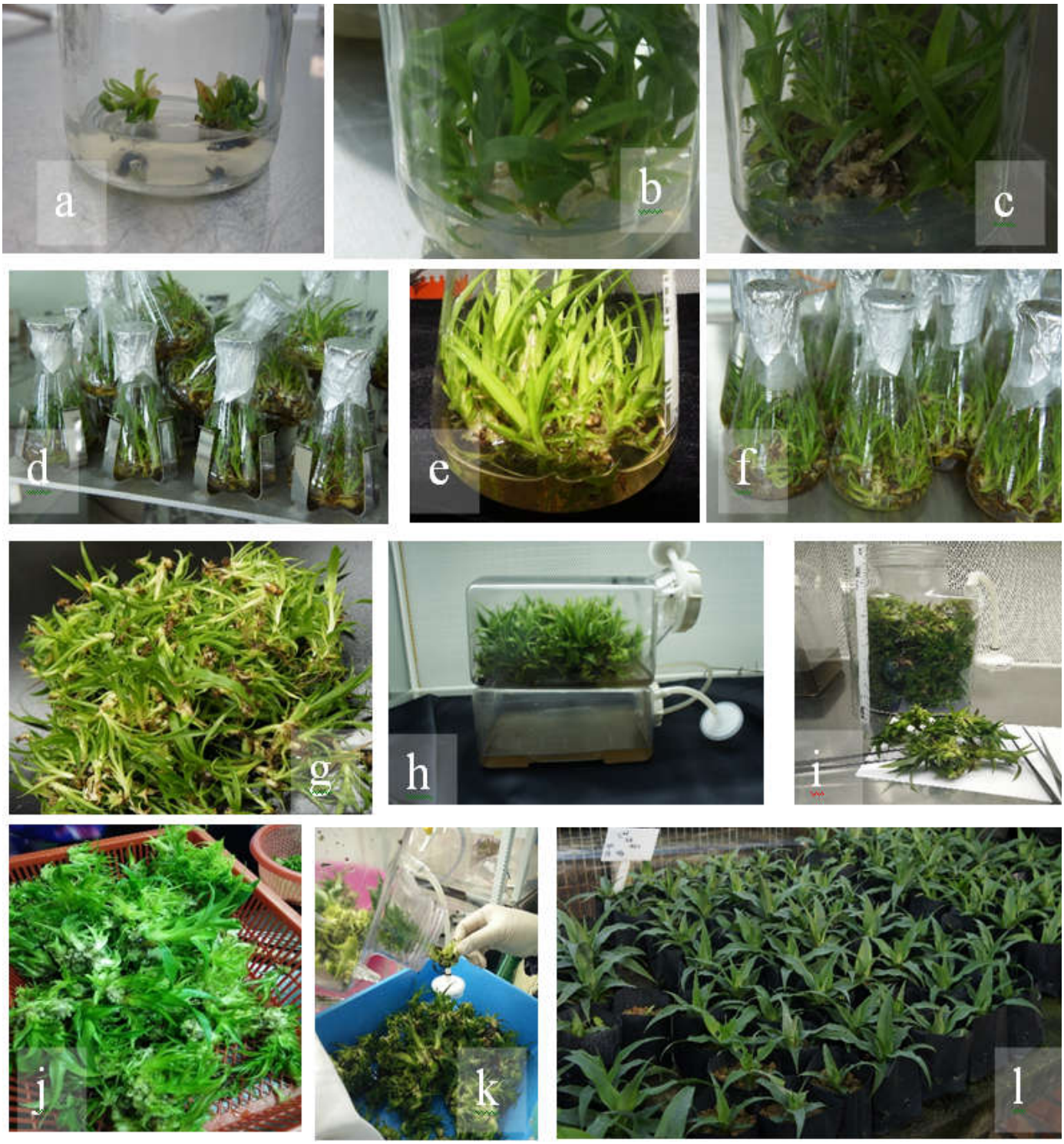


**Figure 2. Shoot proliferation of MD2 pineapple using different culture media. a) Cultures after 2-3 months of initial culture on solid medium. b-c) Plantlets cultured on solid medium after 4-5 months. d-g) Micropropagation of plantlets by liquid-shake culture (LSC). h-k) Micropropagation of plantlets by the Temporary Immersion System (TIS). l) Acclimatization of plants in the greenhouse**

In the same way, the continuous shaking in LSC could have facilitated increased uptake of the nutrients in the medium (Akin-Idowu *et al.*, 2014). For example, liquid culture explants of *Eucalyptus citriodora* displayed improved growth rate compared with those on solid or semi-liquid cultures (Gupta *et al*., 1981). Generally, liquid culture systems tend to enhance growth and propagation as compared with solid medium cultures. Ziv (2005) attributes high growth rates and also optimal plant quality to TIS. Since no reports are available for MD2 pineapple cultured in TIS and LSC, our results from this study bridge this knowledge gap.

## Conclusion

Both TIS and LSC liquid medium systems can be applied in the tissue culture of MD2 pineapple for rapid and efficient clonal propagation. This work alludes to considerable potential for upscaling of liquid cultures for bioreactor production of MD2 pineapple.

**Acknowledgement:** The authors would like to thank the Malaysian Agricultural Research and Development Institute (MARDI) for providing financial support under the RMK11 Research Grant.

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