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Abstract

The aim of this experiment was to study the optimal age of Stemona curtisii plantlets for acclimatization. The in vitro shoots of S. curtisii were cultured on Murashige and Skoog solid medium supplemented with 1 mg L-1 naphthalene acetic acid to induce roots. Then, the plantlets from in vitro culture of 4, 8 and 12 week-old were transferred into the soil and their survival rate during the acclimatization process in the greenhouse was investigated. It was found that the 8 week-old plantlets had the highest survival rate of 80%. However, to enhance the survival rate of these plantlets, a soilless culture technique as a possible approach for the acclimatization was considered. Eight week-old plantlets were transferred into either, soil, a hydroponic system (nutrient film technique), coconut fiber or sand. The plantlets which were cultivated in coconut fiber or hydroponic culture showed 100% survival rate with the highest average number of new roots per plant. However, the mean root length of the plantlets grown in hydroponic system was significantly higher than that grown in coconut fiber.

Keywords

curtisii, stemona, acclimatization, influence, culture, f, soilless, different, age, plantlet, hook, CMMB

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Influence of Plantlet Age and Different Soilless Culture on Acclimatization of *Stemona curtisii* Hook.f.

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Abstract

The aim of this experiment was to study the optimal age of *Stemona curtisii* plantlets for acclimatization. The *in vitro* shoots of *S. curtisii* were cultured on Murashige and Skoog solid medium supplemented with 1 mg/L naphthalene acetic acid to induce roots. Then, the plantlets from *in vitro* culture of 4, 8 and 12 week-old were transferred into the soil and their survival rate during the acclimatization process in the greenhouse was investigated. It was found that the 8 week–old plantlets had the highest survival rate of 80%. However, to enhance the survival rate of these plantlets, a soilless culture technique as a possible approach for the acclimatization was considered. Eight week–old plantlets were transferred into either, soil, a hydroponic system (nutrient film technique), coconut fiber or sand. The plantlets which were cultivated in coconut fiber or hydroponic culture showed 100% survival rate with the highest average number of new roots per plant. However, the mean root length of the plantlets grown in hydroponic system was significantly higher than that grown in coconut fiber.

Key words: acclimatization, growing media, hydroponic culture, plantlet age, soilless culture, *Stemona curtisii* Hook.f., substrate culture

Introduction

Acclimatization is an important process for the adaptation of micropropagated plants to the new environmental conditions, *i.e.* greenhouse or fields. Normally, *in vitro* plantlets are cultured under controlled conditions as enclosed environments, limited gas exchanges, high relative humidity, low light intensity and up-taking of carbon sources from sugars in the culture medium (Preece and Sutter, 1991; Sciutti and Morini, 1993; Pospísilová *et al*., 1999). Those conditions could be due to the abnormal characteristics of *in vitro* plantlets especially in the plant leaves. Hazarika (2006) indicated that unusual stomatal structure, malfunction of stomata, less development of cuticle or epicuticular wax on the surface of *in vitro* leaves are the influential factors contributing to excessive water loss resulting in the high mortality of plantlets or difficult acclimatization. Many researchers tried to solve the problems of acclimatization. Whish *et al.* (1992) found that a reduction in relative humidity during *in vitro* culture increased plant survival rate after transferring to soil. Lamhamedi *et al.* (2003) indicated that the decrease in relative humidity induced the epicuticular wax formation of plantlets. However, there are many factors which could affect the plantlets under greenhouse e.g. planting bed or substrate, plantlet age and shading level (Rodrigues *et al.*, 2005; Hassanpanah and Khodadadi, 2009). Padilla *et al.* (2003) reported that the survival rate of *Prunus domestica* plantlets was affected by the shoot height rather than the number and length of roots. While, Thomas (1998) showed that the 3 week-old *Vitis vinifera* plantlets was more advantageous with enhanced vigor than the 4 and 5 week-old plantlets.

S. curtisii is an insecticidal herbaceous plant in the Family Stemonaceae. The root extract of this plant was shown to have larvicidal activity against *Anopheles minimus* (Mungkornasawakul *et al.*, 2004). The utilization of *S. curtisii* extract as biopesticide was reported by Sastraruji (2006), who found that this pesticide was effective against some pests e.g. *Phyllotreta chontanica*, *Plutella xylostella*, *Lipaphis erysimi*, *Trichoplusia ni* and *Spodoptera littoralis* in agricultural field trials. The commercial demand of *S. curtisii* roots is increasing, but the natural propagation is rather slow and difficult. Tissue culture technique is an alternative for rapid propagation of *S. curtisii*. However, there are some limiting factors such as the survival rate of *S. curtisii* plantlets is less than 50% when transfer to the greenhouse. Thus, the aim of this study was to investigate the optimal age of *S. curtisii* plantlets for acclimatization. Moreover, the use of soilless cultures, such as hydroponics and substrate cultures with sand and coconut fiber were considered with the view of improving the survival rate of the plantlets and to study the growth of these plantlets in soilless culture.

Materials and Methods

Plant materials and *in vitro* propagation of *Stemona* plantlets

This research project was conducted for 11 months from October, 2010 to August, 2011. The intact plants of *S. curtisii* were collected from Trang Province, Thailand. A voucher specimen (No. 17581) was identified and deposited at the herbarium of the Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai, Thailand. Shoot tip and axillary bud explants were excised, washed with running tap water and then surface sterilized with 15% clorox solution for 15 minutes followed by washing 3 times with sterile distilled water. After sterilization, the explants were cultured on solid MS media (Murashige and Skoog, 1962) supplemented with 3% (w/v) sucrose, 0.2% (w/v) gelrite™, 2

mg/L benzyladenine purine and adjusted to pH 5.8±0.02 before autoclaving. The cultures were maintained at a temperature of 25±2ºC with a photoperiod of 16 hr per day for 4 weeks to produce multiple shoots. Each single shoot was then transferred onto solid MS medium supplemented with 1 mg/L naphthalene acetic acid to induce roots.

The optimal age of *S. curtisii* plantlets for acclimatization

In order to increase the survival rate of *S. curtisii* plantlets, the effect of plantlets age was examined. *S. curtisii* plantlets from *in vitro* cultures of 4, 8 and 12 weeks-old (Figure 1) were washed with running tap water to remove agar on the roots and soaked with 0.2% solution of fungicide (Carbendazim) for 30 minutes. Twenty plantlets in each age group were transplanted individually into plastic grow bag (4 inch width x 8 inch length) containing soil. The plantlets in each experiment were grown in the greenhouse at a temperature range 25 – 30 ºC and 11 - 13 hour photoperiod. The plantlets were irrigated two times per day in morning and evening. The survival rate of the plantlets from each age group was recorded every 2 weeks for 8 weeks. Plantlets survival is defined as the plantlets which are still alive.

Soilless cultivation of *Stemona* plantlets

S. curtisii plantlets with optimal age from the previous experiment were used as the plant material to determine the most effective way to improve the survival rate and to study the growth of *S. curtisii* plantlets in soilless culture. The plantlets were washed with running tap water to remove agar on the roots and soaked with 0.2% Carbendazim solution for 30 minutes. These plantlets were shifted individually to plastic grow bag (4 inch width x 8 inch length) containing different substrates: soil, coconut fiber and sand (Figure 2). While in hydroponic culture (nutrient film technique), the plantlets were transplanted individually to

hydroponics tray having a mixture of perlite and vermiculite in the ratio of 1:1 (v/v). After that, twenty plantlets in each culture type were maintained in the greenhouse for 8 weeks and watered two times per day (the same as in the previous experiment). For the survival rate, mean root length and average number of new roots per plant in each soilless culture were recorded at the end of experiment.

Statistical analysis

All the experiments were repeated three replicates. The values are expressed as the Mean ± SD. The data were analyzed by using one-way analysis of variance (ANOVA) followed by Turkey test. All statistical tests were considered significant at $P \leq 0.05$.

Results and Discussion

Influence of plantlet age on survival rate of *S. curtisii* plantlets during acclimatization

The effect of plantlet age is presented in Figure 3. It was found that the percentage of survival rate of plantlets at each age decreased continuously after they were transferred to the soil. At 6 to 8 weeks, the stability of survival rate was noticed in 4 and 8 week-old plantlets which the highest percentage of survival rate (80%) was observed in the 8 week-old plantlets. The percentage of survival rate of 12 week-old plantlets slightly decreased and became stable after week 10 with the survival rate lower than 50% (data not shown) indicating that 12 week-old plantlets were not suitable for the acclimatization. The mortality of the plantlet may be due to the number of *in vitro* leaves per plant. In 12 week-old plantlets, the number of *in vitro* leaves (*ca.* 7 – 10 leaves) is more than that found in the 4 and 8 week-old plantlets (*ca.* 2 – 5 leaves) resulting in a more rapid water loss from these plantlets.

In addition, the characteristics of the leaves and roots of the plantlets were observed continuously for 8 weeks after transferred to the soil. It was found that the formation of new leaves and roots appeared in the survived plantlets but the leaves formed *in vitro* showed wilting or necrosis of the leaf blade. Moreover, the length of *in vitro* roots obviously increased in the survived plantlets and numerous rootlets were produced in both new roots and *in vitro* roots (Figure 4, soil). The abnormal characteristics of the *in vitro* leaves resulted from the tissue culture conditions have been reported in many plant species such as less developed cuticle in *Liquidambar styraciflua* (Wetzstein and Sommer, 1982), malfunction of stomata in sweetgum (Lee *et al.*, 1988) and a few numbers of epidermal hairs in *Rubus idaeus* (Donnelly and Vidaver, 1984). Wardle *et al.* (1983) showed that high relative humidity (RH) during *in vitro* process inhibited the production of surface wax in *Brassica oleracea* plantlets, which agreed with the report by Grantz (1990) and Gilly *et al.* (1997). As a result, *in vitro* leaves are more sensitive to water loss than *ex vitro* leaves (Lavanya *et al.*, 2009) resulting in the wilting or necrosis of the leaf blade. Furthermore, the effects of low light intensity, sugars level in culture medium and restricted gas exchanges (especially CO_2) under *in vitro* conditions may be the causes of abnormality of *in vitro* leaves and roots (Kozai *et al.,* 1991; Zobayed *et al.*, 2000; Serret and Trillas, 2000), which is an important reason for the mortality of plantlets or difficulty in acclimatization.

Effects of different soilless culture on survival rate and growth of *S. curtisii* plantlets

Eight week-old plantlets, the plantlets of optimum ages from the previous experiments, were used as plant materials. The plantlets with eight roots and the root length *ca.* 1.5-2.0 cm were transferred into the soil, hydroponics (NFT), coconut fiber and sand, and then maintained in the greenhouse. The effects of each culture system on the survival rate of the plantlets after 8 weeks are shown in Table 1. It was found that the survival rate of

plantlets in all soilless cultures was higher than that in the soil culture. The maximum survival rate of 100% was observed in hydroponic and coconut fiber cultures followed by those cultured in sand and soil, respectively. Nhut *et al.* (2004) also showed that taro (*Colocasia esculenta*) plantlets cultivated in hydroponic system had higher survival rate (100%) than those cultivated in soil (85%). While, Kurtar *et al.* (2010) reported that the lowest survival rate (12.5% – 16.7%) of winter squash (*Cucurbita maxima*) and pumpkin (*C. moschata*) plantlets was found when cultivated in sand and soil. The effect of growing media on the survival rate of *Tamarindus indica* seedling was studied by Kung'u *et al.* (2008). It was found that farm soil substrate gave the minimum survival rate of 43.5%. It might be due to the higher level of water holding capacity that gave rise to water logging. Water logging is known to hamper gaseous exchange which inhibits growth and ultimately leads to seedling mortality.

Table 1 also presents the growth of survived plantlets in different soilless cultures. The highest average number of new roots per plant was obtained from the plantlets cultivated in coconut fiber, hydroponic and sand. While, the plantlets grown in soil had the lowest number of new roots per plant (6.4 roots). The roots in coconut fiber culture were short with the mean length of 5.9 cm (Figure 4), whereas those cultured in sand, soil and hydroponic were significantly longer with an average length of 8.0, 7.8 and 7.4 cm, respectively. Moreover, new rootlets were found in plantlets cultivated in sand, soil and hydroponic but was not found in those cultured in coconut fiber. This result indicated that the survival rate and growth of plantlets were affected by the different soilless cultures or growing media, which was in accordance with the report by Albaho *et al.* (2009) and Kurtar *et al.* (2010). In *Heliconia bihai*, the high survival rate of plantlets was found in sand and PlantMax substrates but low in vermiculite (Rodrigues *et al.*, 2005). Nhut *et al.* (2004) reported that the survival rate, plant height and leaves number of *Colocasia esculenta* plantlets cultivated in hydroponic

were higher than those in soil culture. Moreover, microtuber formation was only found in the plantlets grown in hydroponics for 15 or 30 days.

Conclusion

The present study revealed that hydroponics was suitable for acclimatization of *S. curtisii* plantlets which gave 100% survival rate, high root length and the number of new roots per plant. Hydroponic cultures will be applied for further study on the production of secondary metabolites especially *Stemona* alkaloids in *S. curtisii*.

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Fig. 1 *S. curtisii* plantlets at different ages

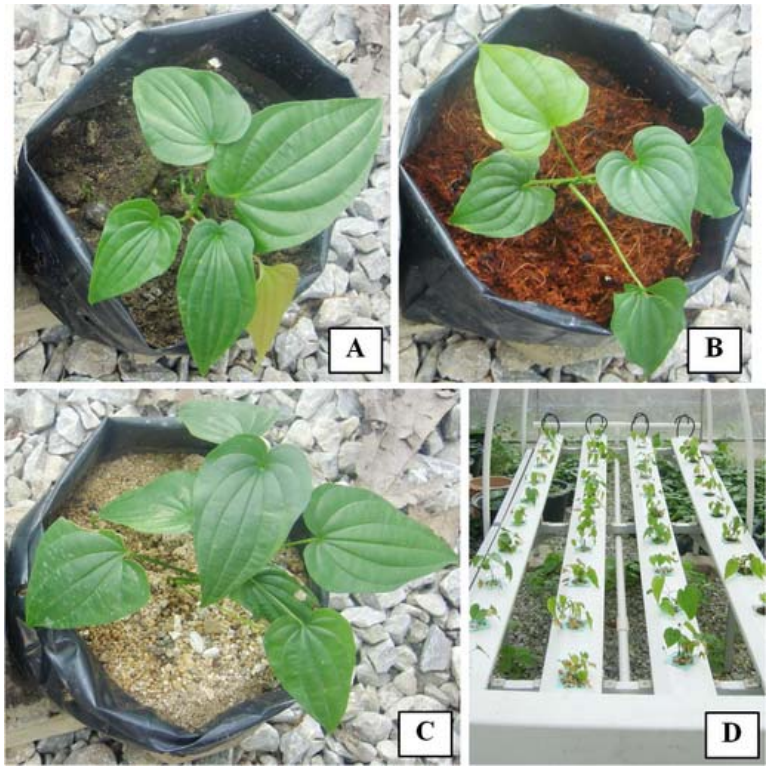

Fig. 2 *Stemona* plantlets cultivated in soil (A), coconut fiber (B), sand (C) and hydroponic (D) culture

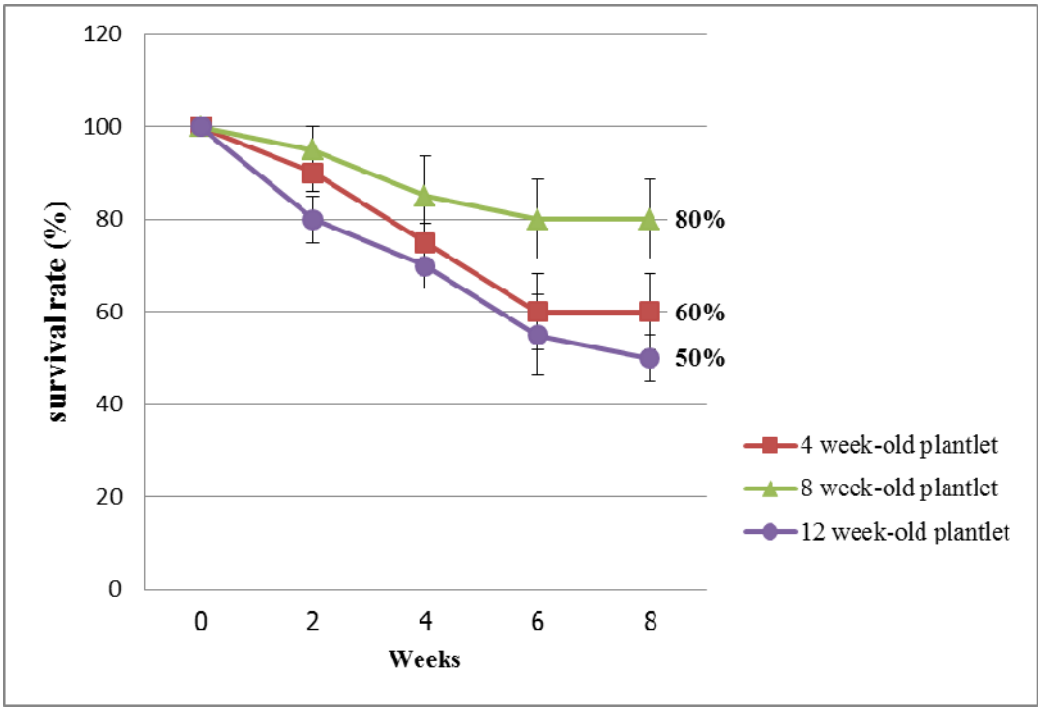

Fig. 3 The percentages of survival rate of 4, 8 and 12 week-old plantlets after transferred to the soil, Values are Means ± SD of three replicates

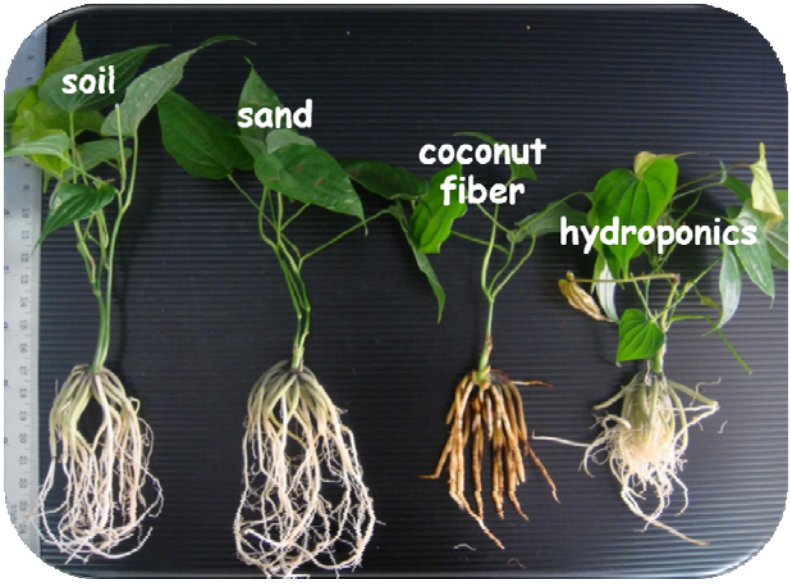

Fig. 4 Root characteristics of *S. curtisii* plantlets in different soilless cultures

Table 1 Effects of different soilless cultures on the survival rate and growth of *S. curtisii* plantlets in greenhouse after 8 weeks

Types of culture	Survival rate (%)	Number of new roots per plant*	Root length (cm)*
Soil	80	6.4 ± 0.3 [b]	7.8 ± 0.3 [a]
Sand	90	8.0 ± 0.4 [a]	8.0 ± 0.4 [a]
Coconut fiber	100	8.5 ± 0.3 [a]	5.9 ± 0.4 [b]
Hydroponic	100	8.2 ± 0.3 [a]	7.4 ± 0.4 [a]

The experiments were repeated three replicates. Each replicate had 20 plantlets per culture type.

*Values showing the Mean ± SD in a column followed by similar letters do not differ significantly at $p < 0.05$.