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In vitro Shoot Cultures of *Tupistra albiflora* K. Larsen

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Abstract

To evaluate an efficient protocol for the micropropagation of *Tupistra albiflora* K. Larsen, the effects of N6-benzylaminopurine (BA) and naphthalene acetic acid (NAA) concentrations on multiple shoot and root induction were examined. *In vitro* shoots were used as the explant materials which were cultured on Murashige and Skoog (MS) agar medium supplemented with 0, 1, 2, 3 and 4 mg/L BA for 4 weeks to induce multiple shoots. It was found that the MS medium containing 3 mg/L BA induced 100 % shoot formation with the highest number of 3.2 shoots per explant (2.4-fold significantly higher than the control). For root induction, *in vitro* shoots were cultured on MS agar medium supplemented with 0, 1, 2, 3 and 4 mg/L NAA for 8 weeks. The results showed that the MS medium containing 1 mg/L NAA induced 100 % root formation with the highest number of 6.6 roots per explant (1.8-fold significantly higher than the control).

Keywords: *Tupistra albiflora*, micropropagation, *in vitro* shoot culture, BA, NAA

Introduction

Tupistra albiflora K. Larsen (syn. *T. muricata*) is a monocotyledonous plant which belongs to the family of Asparagaceae [1,2]. It is a native plant which is widely distributed in the northern region of Thailand, e.g. Doisaket district in Chiang Mai and Wiang Pa Pao district in Chiang Rai. The botanical characteristics of *T. albiflora* were presented in **Figure 1**. The flowers and the young fruits of this plant contain several nutritional benefits and thus, can be eaten. Khuntasa and co-workers [3] reported that the protein from *T. albiflora* flowers varied from 26.0 - 29.6 % per 100 g due to different flower colors. Moreover, vitamin B1, B2 and B6 were also found in the flowers. Additionally, this research indicated that the roots of *T. albiflora* can preserve soil moisture which can prevent landslides. Such benefits of *T. albiflora*, the demand of its flowers and the whole plant has increased rapidly. However, the natural propagation by seed has been found to be rather slow and difficult because of the very thick and hard seed coat. Therefore, the natural seed-propagation of *T. albiflora* was examined. Palee [4] reported that the seed germination of *T. albiflora* was 75 % when the seeds were transplanted into sand for 24 weeks. However, the germination rate increased when the seeds were soaked in warm-water overnight before the transplanting process, which resulted to the germination rate of 84 % within 8 weeks.

Plant tissue culture is one of the alternative ways for rapid propagation of *T. albiflora*. This technique has been successfully used in various plant species, e.g. *Stemona* plants [5,6] *Boscia senegalensis* [7] *Bacopa monaneiri* [8] *Aegle marmelos* [9] and *Hypericum perforatum* [10]. In *T. albiflora*, the *in vitro* mature seed culture and embryo culture were reported by Palee[4]. This research demonstrated that the germination rate of the embryo culture was 100 % within 8 weeks, which was higher than that found in the *in vitro* mature seed culture (40 %). Also, the effect of BA on *in vitro* embryo culture of *T. albiflora* was studied and it was found that the MS agar medium containing 0.5 mg/L BA induced 100 % of shoot and root formation with the highest number of 4.0 shoots per explant and 2.0 roots per explant. The number of roots per plant is, however, still low.

Thus, the purpose of this research was to investigate the effects of BA concentrations on multiple shoot induction and NAA concentrations on root induction from *in vitro* shoot cultures of *Tupistra albiflora* K. Larsen. It was also hope that the findings from the present endeavor could lead to detecting an efficient protocol for the micropropagation of *T. albiflora*.

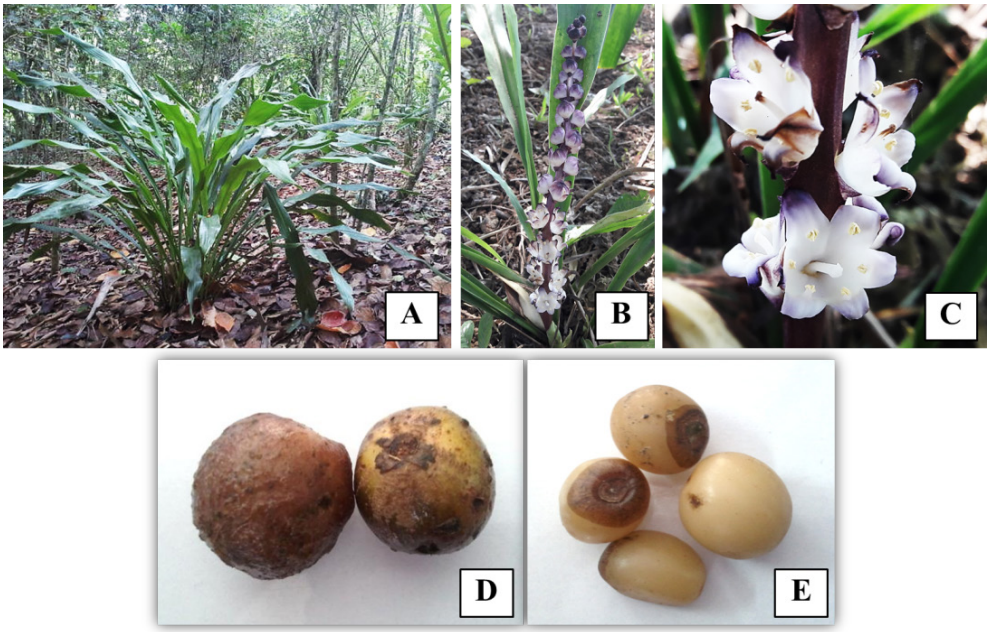

Figure 1 The botanical characteristics of *T. albiflora* K. Larsen (A) habit (B) inflorescence (C) flowers (C) fruits and (D) seeds

Materials and methods

Plant materials

The intact plants of *T. albiflora* (**Figure 2A**) were collected in Wiang Pa Pao district, Chiang Rai Province in Thailand. *In vitro* culture of axillary buds was used to produce shoot materials following the method described by Palee *et al*. [5]. Axillary buds (**Figure 2B**) were excised, washed with running tap water to remove any remaining particles and soil and then soaked in 0.2 % solution of fungicide (Carbendazim) for 20 min. The bud explants were then surface sterilized with 15 % Clorox solution for 10 min followed by washing 3 times with sterile distilled water. Lastly, the axillary buds were cultured on MS [11] agar media supplemented with 3 % (w/v) sucrose, and 0.2 % (w/v) Gellan Gum and adjusted to a pH of 5.8±0.02 before being autoclaved. The cultures were maintained at a temperature of 25±2 °C with a photoperiod of 16 h. Regenerating shoots were subcultured to the fresh medium (MS agar medium without growth regulators) every 4 weeks, for 8 weeks (2 subcultures) to produce shoot materials (**Figure 2C**) for the experimentation of multiple shoot and root induction.

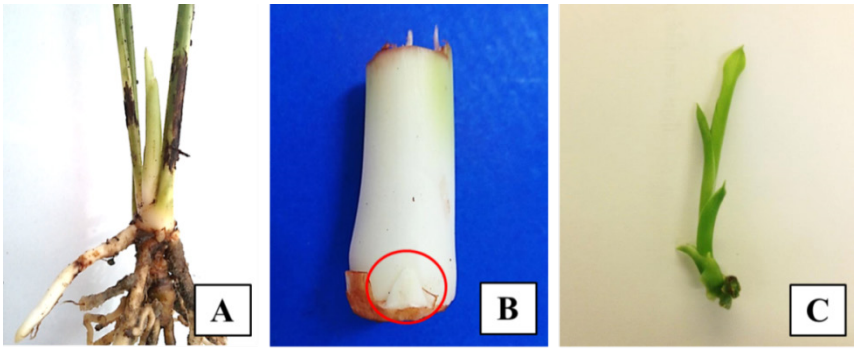

Figure 2 (A) Intact plant (B) Axillary bud and (C) *In vitro* shoot of *T. albiflora*

Multiple shoot induction

The effects of the different concentrations of BA on multiple shoot induction were examined following the method described by Palee *et al*. [5]. The *in vitro* shoots of *T. albiflora* (**Figure 2B**) were used as explant materials, which were cultured on MS agar medium supplemented with 0, 1, 2, 3 and 4 mg/L BA. All cultures were maintained at a room temperature of 25±2 °C with a photoperiod of 16 h for 4 weeks. The percentage of new shoot induction, callus induction, and average number of shoots per explant were recorded.

Root induction

The influence of NAA on root induction was also studied following the method described by Palee *et al.* [5]. The *in vitro* shoots of *T. albiflora* were cultured on MS agar medium supplemented with 0, 1, 2, 3 and 4 mg/L NAA. All treatments were maintained under the same condition of shoot induction for 8 weeks. The percentage of root induction, callus induction, and average number of roots per shoot were recorded.

Statistical analysis

The experiment was laid out in completely randomized design (CRD). All the experiments were conducted in three replicates with 12 plantlets per treatment. The values were expressed as the Mean±SD. The data were analyzed using one-way analysis of variance (ANOVA) followed by Tukey test. All statistical tests were considered significant at $P \leq 0.05$.

Results and discussion

Influence of BA on multiple shoot induction

The results of *in vitro* shoot culture of *T. albiflora* on MS agar medium supplemented with 0, 1, 2, 3 and 4 mg/L BA are shown in **Table 1**. It was found that the stability of shoot induction percentage was noticed in all medium after 4 weeks of culturing. BA had a positive effect on the enhancement of shoot formation. The shoot induction percentage increased when the various concentration of BA were added into the medium. The MS medium containing 2 - 4 mg/L BA induced 100 % shoot formation, which was 2-fold higher than the control (50 %). While, the percentage of shoot induction in the medium containing 1 mg/L BA was at 85 %. Also, the number of shoots per explant was recorded. The results showed that the maximum number of shoots per explant was found in the medium supplemented with 3 mg/L BA (**Figure 3**), which was 3.2 shoots per explant (2.4-fold significantly higher than the control), followed by those treated with 4 mg/L BA (2.6 shoots per explant, 1.6-fold significantly higher than the control). In addition, the treatment of BA also induced the callus formation. The best percentage of callus induction (33 %) was detected in the MS medium containing 3 - 4 mg/L BA, which resulted in the compact callus formation (**Figure 3**).

Thus, the study revealed that the best concentration of BA for the multiple shoot induction of *T. albiflora* was 3 mg/L, which gave 100 % shoot induction with the highest number of 3.2 shoots per explant and induced 33 % callus formation. Similar results of 3 mg/L BA on multiple shoot induction were found in the micropropagation of *Etlingera elatior* [12], *Stemona* sp. [13], *Calotropis procera* and *Calotropis gigantean* [14], *Boscia senegalensis* [7] and *Musa* sp. cv. Namwa Mali-Ong [15]. Furthermore, BA was also reported to have influence on *Etlingera elatior* [16], *Musa sapientum* [17], *Bacopa monaneiri* [8], *Amygdalus communis* [18] and *Lilium longiflorum* [19]. For the embryo culture of *T. albiflora*, Palee [4] reported that the highest number of shoots per explant was found in the MS agar medium containing 0.5 mg/L BA. On the other hand, BA in concentration of 1 - 4 mg/L did not have positive effect on the multiple shoot induction of the embryo culture of *T. albiflora*. The difference in the responses of BA concentration may be due to the explant type. The influence of explant types and plant growth regulators on plant tissue culture was examined in many plants, e.g., *Pterocarpus santalinus* [20] and *Jatropha curcas* [21]. In *Agastache foeniculum*, Moharami *et al*. [22] indicated that the shoot regeneration frequency was affected by the explant type and BAP concentration. The highest shoot regeneration frequency was recorded for nodal explants, followed by cotyledons, shoot tips and hypocotyls.

Table 1 Effects of different concentrations of BA on multiple shoot induction of *T. albiflora* after 4 weeks of culture

BA concentration (mg/L)	Shoot induction (%)	Number of shoots per explant (Mean ± SD)*	Callus induction (%)
0	50%	1.4 ± 0.5 ^{d}	0%
1	85%	1.9 ± 0.3 ^{cd}	15%
2	100%	2.2 ± 0.4 ^{bc}	27%
3	100%	3.2 ± 0.6 ^{a}	33%
4	100%	2.6 ± 0.5 ^{b}	33%

*Values expressing the Mean ± SD followed by the same letter in a column indicates a non-significant difference at $P < 0.05$ (ANOVA; Tukey test).

Figure 3 Multiple shoot formation in MS agar medium containing 3 mg/L BA for 4 weeks

Influence of NAA on root induction

When the shoot explants were cultured on MS agar medium containing 0, 1, 2, 3 and 4 mg/L NAA for 8 weeks, it was found that the control medium (MS medium without NAA) and each concentration of NAA in MS media produced 100 % root formation (**Table 2**). The enhancement of the root number per explant was found in the MS medium supplemented with 1 - 3 mg/L NAA. The MS agar medium containing 1 mg/L NAA gave the maximum number of roots per explant (**Figure 4A**), which was 6.6 roots per explant (1.8-fold significantly higher than the control), followed by those treated with 2 mg/L NAA (5.3 roots per explant, 1.5-fold significantly higher than the control). On the other hand, the number of roots per explant in the medium containing 4 mg/L NAA was 2.8 roots, which was less than those found in the control medium (3.6 roots). The percentage of callus formation is also shown in **Table 2**. The result revealed that NAA in concentration of 1 - 4 mg/L induced 30 - 64 % callus formation, and caused the increase in size of compact callus than those found in the media containing BA. The highest percentage of callus formation was found in the medium supplemented with 3 and 4 mg/L NAA (**Figure 4B**), which was 64 and 62 %, respectively.

Table 2 Effects of NAA on root formation of *T. albiflora* after 8 weeks of culture

BA concentration (mg/L)	Root induction (%)	Number of roots per explant (Mean ± SD)*	Callus induction (%)
0	100%	3.6 ± 0.5 ^{c}	0%
1	100%	6.6 ± 0.7 ^{a}	30%
2	100%	5.3 ± 0.5 ^{b}	30%
3	100%	4.0 ± 0.4 ^{c}	64%
4	100%	2.8 ± 0.4 ^{d}	62%

*Values expressing the Mean ± SD followed by the same letter in a column indicates a non-significant difference at $P < 0.05$ (ANOVA; Tukey test).

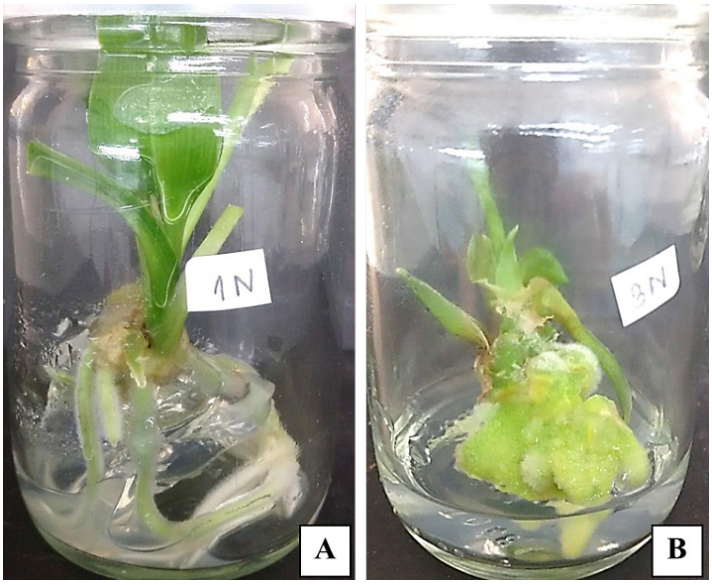

Figure 4 (A) Root formation in MS medium containing 1 mg/L NAA for 8 weeks and (B) Callus formation in MS medium containing 3 mg/L NAA for 8 weeks

Therefore, the current study demonstrated that the optimal concentration of NAA for the root formation of *T. albiflora* was found to be 1 mg/L, which induced root formation at a rate of 100 % with the highest number of 6.6 roots per explant. This result is also in line with the previous studies of Palee *et al*. [5] in *Stemona curtisii*, Chidburee *et al*. [23] in *Stemona tuberosa*, Mukhtar *et al*. [24] in *Citrus reticulata* and Priyakumari and Sheela [25] in *Gladiolus grandiflorus*. Moreover, NAA was also reported to have a positive effect on root induction in tissue culture of *Bacopa monneiri* [8], *Etlingera elatior* [12], *Musa* sp. cv. Namwa Mali-Ong [15] and *Curcuma parviflora* [26]. Ali *et al.* [27] revealed that auxin is essential on root induction of the olive micro-cuttings, which the root formation was not observed in the control medium without auxin. Hausman [28] reported that the high concentration of auxin (higher than optimal concentration) led to a decrease in the number of roots per explant. Baker and Wetzstein [29] indicated that higher concentration of auxin can induce higher level of degradative metabolites in tissues, which may block the regeneration process.

Conclusions

This research has presented an efficient protocol for the proliferation of *T. albiflora*. The adding of plant growth regulator BA and NAA into the culture medium had a positive effect on the enhancement of shoot and root formation from *in vitro* shoot culture of *T. albiflora*. The optimum concentration of BA for the multiple shoot induction was found to be 3 mg/L, which gave 100 % shoot induction with the highest number of 3.2 shoots per explant within 4 weeks. Likewise, 1 mg/L NAA is the best concentration of NAA for the root formation, which gave 100 % root induction with the highest number of 6.6 roots per explant within 8 weeks. Thus, these results revealed that this methodology can produce numerous plantlets of *T. albiflora* within 12 weeks with the number of roots per plantlet higher than the previous experiment (2.0 roots per explant). Future research can examine the acclimatization of *in vitro T. albiflora* plantlets.

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