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ABSTRACT

Objective: To determine the effects of different strength of Murashige and Skoog (MS) media (full, $1/2$ and $1/4$) in solid and liquid media on *in vitro* growth of *Typhonium flagelliforme* (*T. flagelliforme*), whereby an optimum media composition can be provided for mass propagation of *T. flagelliforme*.

Methods: Rhizome bud of *T. flagelliforme* was obtained from the axenic *in vitro* established *T. flagelliforme* plantlets in Plant Tissue Culture Laboratory, Universiti Teknologi MARA, Shah Alam. Rhizome bud was used as explant and cultured onto shoot proliferation medium under different strength of MS media (full, $1/2$, $1/4$) in solid and liquid culture media.

Results: After 6 weeks of culture, the number of shoot, number of leaf, number of root, height of shoot, fresh weight, dry weight and chlorophyll content of *T. flagelliforme* were analyzed. A comparison was made between liquid and solid culture media. The results revealed that the liquid culture media were more effective for all the growth parameters (shoot height, shoot number, leaf number, root number, fresh weight, dry weight, chlorophyll a and chlorophyll b content) compared to solid culture media. Apart from that, this study revealed the positive relationship between strength of MS media and type of culture media (solid and liquid media) to the growth of *T. flagelliforme*. Growth of *T. flagelliforme* was improved when MS strength was increased in liquid media. In contrast, growth of *T. flagelliforme* was improved when MS strength was decreased in solid media.

Conclusions: Through this study, an optimum media composition for mass propagation of *T. flagelliforme* had been established by observing effects of MS media strength and type of culture media (solid and liquid media) on the growth of *T. flagelliforme*.

1. Introduction

Typhonium flagelliforme L. (*T. flagelliforme*), commonly known as 'rodent tuber' has been recognized as a valuable medicinal herb that belongs to the family Araceae (Arum). This plant can be found in disturbed wastelands including soft, damp and shady habitats. The leaves of *T. flagelliforme* are extremely variable, elliptical to ovate, cordate or hastate with 30 cm long petiole. This plant height can increase to 30 cm with white tubers [1].

The use of *T. flagelliforme* as medicinal herbs had long been practiced by the Filipinos and Chinese tribe. This plant had been taken orally by the Chinese for the treatment of injury and the Filipinos used the flowers as anticoagulant [2]. Traditionally, the whole plant of *T. flagelliforme* has been taken as juice with honey to sweeten and increase patient acceptability for detoxification, anti-inflammation, antiviral and anticancer bioactivities [3]. Apart from that, it can be consumed raw, where the leaves are wrapped in longan flesh.

Scientific study had documented the medicinal value of *T. flagelliforme* for anticancer [4], antibacterial [5], anti-inflammatory and antioxidant properties [5]. Since this species is well recognized as important medicinal plants, it is sought after for further research and uses. This plant requires specific condition to grow and become dormant during dry season. Micropropagation using plant tissue culture technique allows its continuous plant materials to supply under control condition.

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Micropropagation through plant tissue culture refers to rapid multiplication of plants *in vitro*. In plant tissue culture, media composition is the determining factor that influences plant growth. Micropropagation of *T. flagelliforme* had been done in the previous studies by manipulating the plant growth regulators [2,6,7]. However, other factors such as strength of Murashige and Skoog (MS) media and culture system (solid or liquid) have not been studied. Previous research showed that MS media strength could affect the number of shoots obtained in *Pogostemon cablin* [8].

Study by Fadel *et al.* [9] proved that half-strength MS could result in maximum number of shoots and roots of *Mentha spicata*. Solid and liquid culture media would eventually affect the nutrient uptake by plant as well as plant growth. Suthar *et al.* [10] revealed that liquid media produce the highest shoot length, rooting percentage, leaf number, fresh weight (FW), dry weight (DW), and chlorophyll content for *Boswellia serrata* Roxb. Thus, this study was aimed to determine the effects of different strength of MS (full, $\frac{1}{2}$ and $\frac{1}{4}$) in liquid and solid media on growth of *T. flagelliforme*.

2. Materials and methods

2.1. Surface sterilization and establishment of initial culture

The mother plant of *T. flagelliforme* species was collected from Pagoh, Johor in December 2015 and maintained in greenhouse. The species was identified by taxonomist with voucher specimen number 40336 and was deposited in Universiti Kebangsaan Malaysia.

Rhizome bud of *T. flagelliforme* was used as explant. For surface sterilization, rhizome buds were washed thoroughly under running tap water for 30 min to remove all the soil debris. The explant was then treated with 70% alcohol for 1 min. Under sterile condition, the explants were immersed in 20% of Clorox with 2 drops of Tween 20 for 15 min. Then, the explants were rinsed with sterile distilled water 6–8 times to remove all traces of Clorox.

The culture medium consisted of 4.4 g/L MS media (Duchefa Biochemie, Netherland) supplemented with 30 g/L of sucrose (Duchefa Biochemie, Netherland) and 7 g/L gelrite powder (Duchefa Biochemie, Netherland). Plant growth regulator was added to the media as suggested by Sai *et al.* [6] [0.3 mg/L 6-benzylaminopurine (BAP) + 0.5 mg/L indole-3-butyric acid (IBA)]. The pH was adjusted to 5.7–5.8 and the media was autoclaved for 15 min at 121 °C and 1.05 kg/cm². The autoclaved media was poured into sterile pill bottle with 20 mL of media each. After surface sterilization, the explants were cultured onto the media prepared. The cultures were then incubated in culture room under 24 h light at (25 ± 1) °C in postgraduate plant tissue laboratory of Universiti Teknologi MARA (UiTM) Shah Alam.

2.2. Different strength of MS media in solid and liquid media

Rhizome bud of *T. flagelliforme* was obtained from *in vitro* established *T. flagelliforme* plantlets in postgraduate plant tissue laboratory of UiTM Shah Alam. Plantlets in healthy condition were selected and rhizome buds were used as explants. The explants were cultured onto shoot proliferation medium under different culture conditions.

To study the effect of different strength of MS media, the explants were cultured onto three different strength of MS

medium (full, half and quarter) [full strength MS medium (4.4 mg/L MS), half strength MS medium (2.2 mg/L MS) and quarter strength MS medium (1.1 mg/L MS)].

To study the effect of solid and liquid media, the explants were cultured onto MS media with and without addition of 7 g/L gelrite. For solid media, the explants were cultured onto MS media, containing 30 g/L sucrose, 0.3 mg/L BAP + 0.5 mg/L IBA and 7 g/L gelrite. For liquid media, the explants were cultured onto MS media, containing 30 mg/L sucrose and 0.3 mg/L BAP + 0.5 mg/L IBA only. Each treatment consisted of 15 replications.

2.3. Data collection and statistical analysis

Average shoot number, shoot length, leaves number and roots number of plants produced were observed and recorded every week until the 6th week of culture. Chlorophyll content, FW and DW were recorded on the 6th week of culture. The mean ± SE were analyzed statistically using SPSS 18.0 (2009). By using Two-way ANOVA, the variances of mean would be determined with the significance value $P \leq 0.05$.

3. Results

Figures 1 and 2 show growth patterns of *T. flagelliforme* *in vitro* in regard to number of shoots and height of shoots taken after 6 weeks of culture. The height and the number of shoots were positively correlated with each other where the highest mean value was recorded in full-strength MS liquid medium (shoots number: 4.400 ± 0.986 , shoots height: 24.467 ± 5.273). The lowest shoots number and shoots height were recorded in control treatment (full-strength MS solid media) (shoots number: 2.133 ± 0.743 , shoots height: 3.233 ± 1.083). In solid media, increased MS strength resulted in a decrease of height and number of shoots. However, in liquid media, the number and height of shoots were decreased when MS strength was reduced. Overall, the highest number and height of shoots were recorded in liquid media (shoots number: 3.600 ± 1.750 , shoots height: 17.756 ± 10.390).

The leaves and roots of *T. flagelliforme* were formed after 2 weeks of incubation. In Figures 3 and 4, liquid media recorded the highest number of leaves and roots (leaves number: 14.911 ± 9.172 , roots number: 36.556 ± 24.805) compared to solid media (leaves number: 7.178 ± 5.597 , roots number: 11.533 ± 10.332). The highest leaves number was recorded in full-strength MS liquid media (18.733 ± 4.008) and the lowest was in

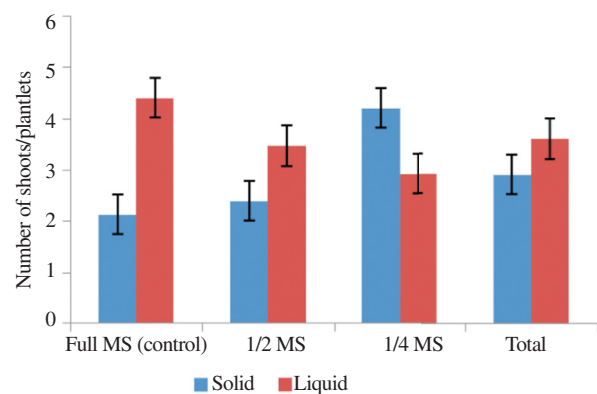


Figure 1. Number of shoots of *T. flagelliforme* affected by different strength of MS in solid and liquid media.

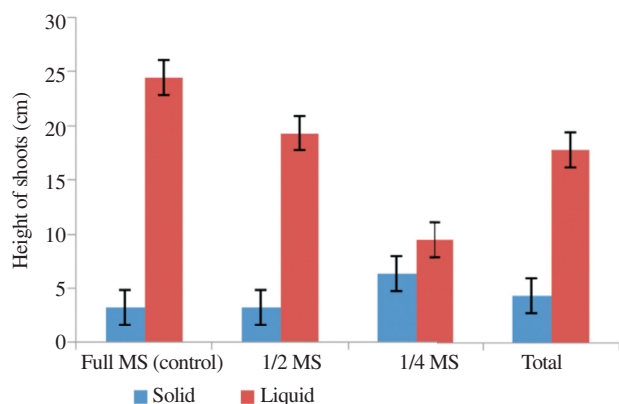


Figure 2. Height of shoots of *T. flagelliforme* affected by different strength of MS in solid and liquid media.

Data were presented in the form of mean \pm SE. Significance is $P \leq 0.05$ using Tukey's simultaneous test.

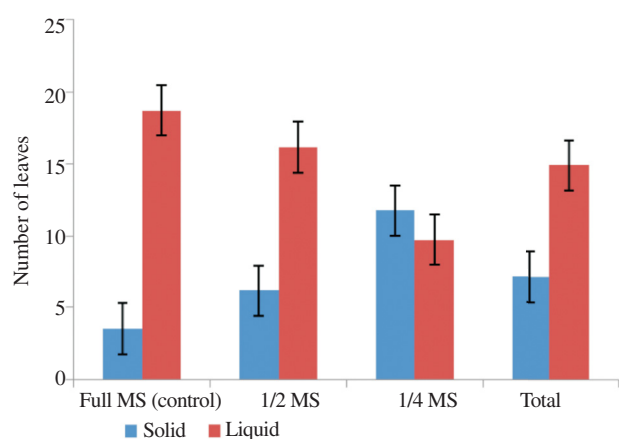


Figure 3. Number of leaves of *T. flagelliforme* affected by different strength of MS in solid and liquid media.

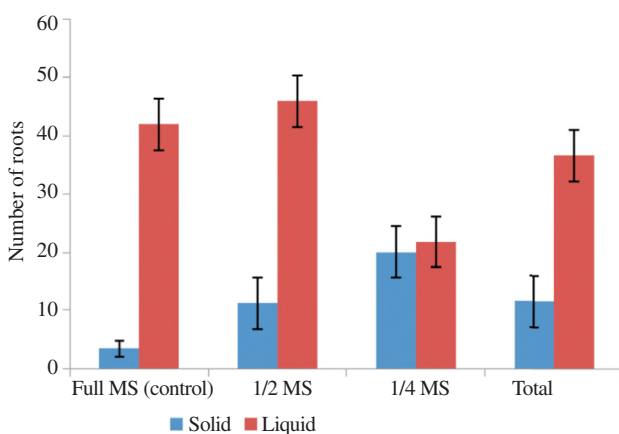


Figure 4. Number of roots of *T. flagelliforme* affected by different strength of MS in solid and liquid media.

Data were presented in the form of mean \pm SE. Significance is $P \leq 0.05$ using Tukey's simultaneous test.

full-strength MS solid media (3.533 ± 1.807). The results obtained for number of leaves and roots were corresponding with the height and number of shoots. When MS strength decreased in solid media, the number of leaves and roots increased. Whereas in liquid media, when MS strength decreased, leaves and roots number also decreased.

From Figures 5 and 6, different strength of MS media also influenced FW and DW of *T. flagelliforme*. In liquid media, full-strength MS media recorded the highest FW (5.774 ± 0.841) and DW (0.610 ± 0.064) followed by half (FW: 5.716 ± 0.970 , DW: 0.564 ± 0.035) and quarter-strength MS (FW: 2.103 ± 0.482 , DW: 0.426 ± 0.083). However, in solid media, the highest FW and DW were recorded in quarter-strength MS media followed by half and full-strength MS. Besides that, solid and liquid media significantly affected FW and DW of *T. flagelliforme*. Liquid media resulted in the highest FW (4.531 ± 1.917) and DW (0.533 ± 0.100) of *T. flagelliforme*. In contrast, the lowest FW (0.699 ± 0.491) and DW (0.124 ± 0.066) of *T. flagelliforme* were recorded in solid media.

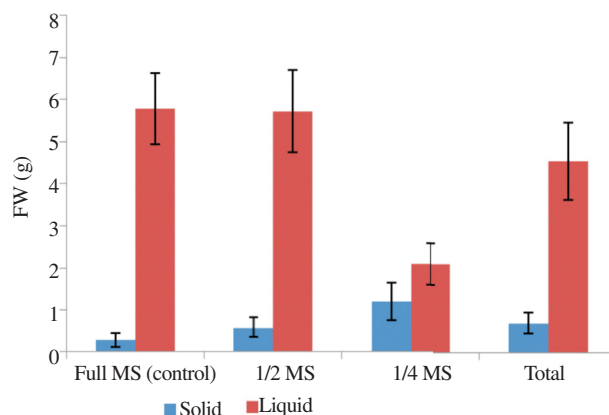


Figure 5. FW of *T. flagelliforme* affected by different strength of MS in solid and liquid media.

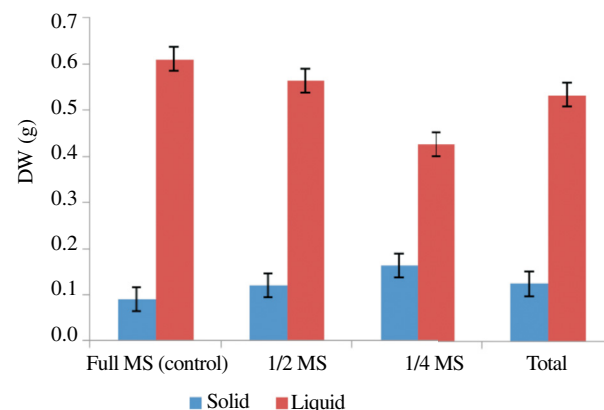


Figure 6. DW of *T. flagelliforme* affected by different strength of MS in solid and liquid media.

Data were presented in the form of mean \pm SE. Significance is $P \leq 0.05$ using Tukey's simultaneous test.

MS strength and culture system (solid and liquid media) also influenced chlorophyll content of *T. flagelliforme*. In Figures 7–9, total chlorophyll, chlorophyll a and chlorophyll b were positively correlated with each other. The highest chlorophyll a, b and total chlorophyll were recorded in quarter-strength MS liquid media (total chlorophyll: 14.663 ± 5.763 , chlorophyll a: 2.115 ± 0.008 , chlorophyll b: 9.215 ± 0.016). Meanwhile, half-strength MS in solid and liquid media resulted in the lowest chlorophyll a (0.614 ± 0.001), chlorophyll b (1.826 ± 0.006) and total chlorophyll (2.446 ± 0.006). Generally, liquid media clearly enhanced chlorophyll content in *T. flagelliforme* (6.803 ± 6.573), compared to solid media (3.303 ± 0.476).

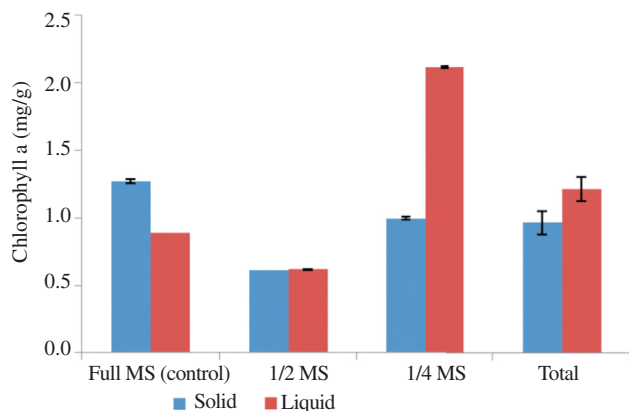


Figure 7. Chlorophyll a concentration in *T. flagelliforme* affected by different strength of MS in solid and liquid media.

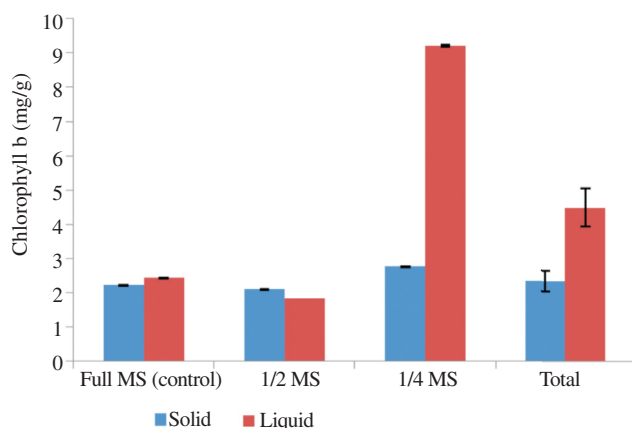


Figure 8. Chlorophyll b concentration in *T. flagelliforme* affected by different strength of MS in solid and liquid media.

Data were presented in the form of mean \pm SE. Significance is $P \leq 0.05$ using Tukey's simultaneous test.

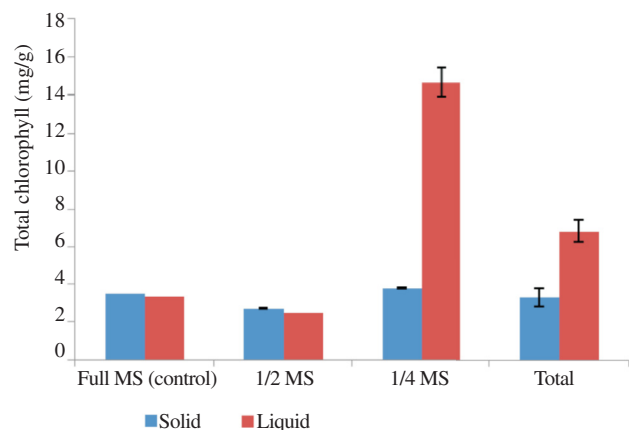


Figure 9. Total chlorophyll concentration in *T. flagelliforme* affected by different strength of MS in solid and liquid media.

Data were presented in the form of mean \pm SE. Significance is $P \leq 0.05$ using Tukey's simultaneous test.

Figure 10 shows *T. flagelliforme* growth after 6 weeks of culture in different MS strength in solid and liquid media. From Figure 10, growth response of the plantlets improved in the order of D, E, F, C, B and A. Plantlets in A, B and C were cultured in liquid media whereas D, E, and F were cultured in solid media. Growth response of plantlets in liquid media was bigger and healthier than plantlets in solid media.

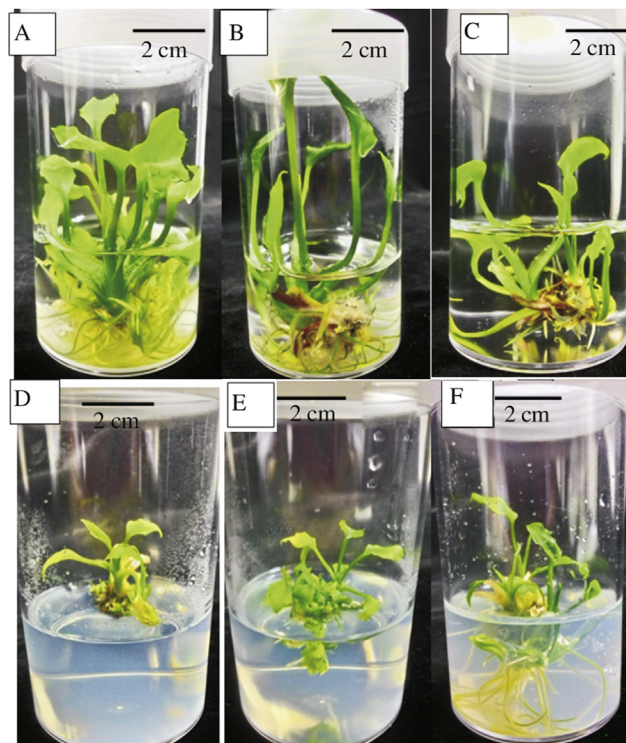


Figure 10. The plantlets after 6 weeks of culture in different strength of MS in solid and liquid media.

A: Full-strength MS in liquid media; B: Half-strength MS in liquid media; C: Quarter-strength MS in liquid media; D: Full-strength MS in solid media; E: Half-strength MS in solid media; F: Quarter-strength MS in solid media.

4. Discussion

From this study, a comparison was made in terms of plant growth response, between solid and liquid culture media. The effects of different MS strength and the interaction between MS strength and liquid and solid culture medium were also discussed.

4.1. The effect of solid and liquid culture media on growth of *T. flagelliforme*

Generally, all explants of *T. flagelliforme* survived in the different type of media. After 6 weeks of culture, significant difference in shoot number (Figure 1), shoot height (Figure 2), leaf number (Figure 3), root number (Figure 4), FW (Figure 5), DW (Figure 6), chlorophyll a (Figure 7) and chlorophyll b content (Figure 8) of *T. flagelliforme* were observed between liquid and solid culture media. Two-way ANOVA showed that the type of culture medium strongly affected height of shoots, number of roots, number of leaves, DW, FW, chlorophyll a and chlorophyll b content of *T. flagelliforme* with P value less than 0.0001. The number of shoot in solid and liquid media was significantly different with P value less than 0.05 ($P = 0.03$). The effect of solid and liquid culture media is supported by Hussien *et al.* [11] which suggested that the physical state of the culture medium strongly influenced growth of micropropagated plant.

From the result, liquid culture media recorded the greatest plant growth response in terms of all growth parameters (shoot height, shoot number, leaf number, root number, FW, DW, chlorophyll a, chlorophyll b and total chlorophyll). These findings are in agreement with Mbiyu *et al.* [12], which reported that liquid culture media produced better shoot growth, more leaves, more roots and dry matter of micropropagated potato. Suthar

et al. [10] also revealed that liquid culture media produced the highest rooting percentage, shoot length, leaf number, FW, DW, and chlorophyll content for *Boswellia serrata* Roxb.

Leaf chlorophyll content provides information about the physiological status in plant. Figures 7–9 show that liquid culture media gave the highest chlorophyll a, chlorophyll b and total chlorophyll concentration compared to solid culture media. Higher chlorophyll content in liquid media was also recorded in *Chlorophytum borivilianum*, *Terminalia bellerica* and *Feronia limonia* [13]. Higher chlorophyll content specifies higher photosynthetic potential and nutrient in plants. This study clearly indicated that the growth of *T. flagelliforme* was enhanced by the liquid culture media which allows adequate contact of the plant tissue with the media. The close contact between plant tissue and liquid culture media could stimulate and facilitate the uptake of nutrients and phytohormones, which then leads to a better plant growth and chlorophyll formation in *T. flagelliforme*.

4.2. The effects of MS strength on the growth of *T. flagelliforme*

There was statistically significant difference in shoot height ($P = 0.002$), FW ($P < 0.0001$), chlorophyll a ($P < 0.0001$) and chlorophyll b ($P < 0.0001$) content of *T. flagelliforme* in full-, half- and quarter-strength MS. It has been observed that, maximum shoot number (Figure 1), shoot height (Figure 2), leaf number (Figure 3), root number (Figure 4), FW (Figure 5) and DW (Figure 6) were recorded in full-strength MS media which were then followed by half- and quarter-strength MS media. Thus, increasing the strength of MS resulting in better shoot number, shoot height, leaf number, root number, FW and DW of *T. flagelliforme*. Generally, the obtained result indicated that full-strength MS media improved most parameters under investigation. The results are in coordination with findings from Mustafa et al. [14] on *Ficus*.

In contrast, total chlorophyll, chlorophyll a and chlorophyll b concentration were found highest in quarter-strength MS (Figures 7–9). Thus, reducing the strength of MS media could increase total chlorophyll, chlorophyll a and chlorophyll b concentration. Since MS media contains all the macronutrients, micronutrients and vitamin, changing the strength or concentration of MS media may alter the nutrients component. Therefore, the effect of different medium strength could also affect the plant organogenesis *in vitro*. The amount of nutrients in culture media for successful culture may vary for different plant species and genotype. A study by Mukherjee et al. [15] proved that shoot proliferation of grape rootstock was increased when cultured on MS with half of nitrate medium. Other than that, Fakhru et al. [16] stated that macronutrients manipulation in MS media could affect the growth of *Stevia rebaudiana*.

4.3. Interaction between MS strength and type of culture medium (solid and liquid media)

MS strength and culture media were negatively correlated with each other in terms of shoot number, shoot height, leaf number, root number, FW and DW. It was found that in liquid culture media, maximum number of shoots was recorded in full-strength MS and the lowest number of shoots was recorded in quarter-strength MS media, as shown in Figure 1. However, the

opposite results were sighted in solid media where quarter-strength MS media had the maximum shoot number and the lowest was recorded in full-strength MS media. Similar results were recorded for shoot height, leaf number, root number, FW and DW (Figures 2–6). In liquid culture media, increased MS strength resulted in better *T. flagelliforme* growth whereas in solid culture media, a reduction in MS strength resulted in better *T. flagelliforme* growth. Generally, full-strength MS liquid media gives better growth performance of *T. flagelliforme* compared to other type of media.

Conversely, Figures 8 and 9 show that there are positive correlation between MS strength and solid and liquid culture media in terms of total chlorophyll and chlorophyll b content in leaves of *T. flagelliforme*. Maximum total chlorophyll, and chlorophyll b were recorded in quarter-strength MS liquid and solid media followed by full- and half-strength. As for chlorophyll a, in Figure 7, the greatest chlorophyll a concentration was shown in quarter-strength MS in liquid media. The result is similar with Abou Dahab et al. [17] which stated that quarter-strength of MS medium can increase chlorophyll a content in the leaves of *Ruscus hypoglossum* L. However, in solid media, full-strength MS gives the highest chlorophyll a concentration. Overall, the highest chlorophyll a concentration was recorded in quarter-strength MS liquid media. Hence, it can be concluded that the growth response of *T. Flagelliforme* was affected by the amount of MS salts and the presence of agar in the media.

T. flagelliforme growth was better in MS liquid media compared to solid media. Apart from that, increasing the strength of MS media resulted in better shoot number, shoot height, leaf number, root number, FW and DW of *T. flagelliforme*. However, reducing the strength of MS could increase total chlorophyll, chlorophyll a and chlorophyll b content. Generally, full-strength MS liquid medium offers maximum growth of *T. flagelliforme* in terms of shoot number, shoot height, leaves number, root number, FW and DW.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

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