Growth response of shallot (*Allium ascalonicum* L.) seedlings cultured on MS solid and liquid medium supplemented with BAP, Thiamine and Adenine Sulphate

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**Abstract.** True Shallot Seeds (TSS) is the best choice of cultivation over bulb materials because it offers disease-free plants, produces larger size bulbs, and reduces cost production. *In vitro* propagation is an alternative method to overcome the TSS development problems. This research aimed to evaluate the growth response of shallot (*Allium ascalonicum*) cv Tuktuk seedlings, cultured on MS solid and liquid media with agitation supplemented with BAP, Thiamine and Adenine Sulphate. The solid MS medium was used to germinate TSS, followed by growth seedlings at control and treatment media. The experiments were carried out in a Completely Randomized Design with 4 replicates. Data were collected 8 weeks after planting. *In vitro* germination results showed that 5.96% of seeds started to germinate at the first week and reached 19.82% after four weeks of planting. After 8 weeks in the culture of TSS seedlings, shoots had better growth on solid medium compared to in liquid medium. The addition of BAP, Thiamine and Adenine did not significantly influence the growth of seedlings culture. Bulbs-like bodies were formed in all media. A higher concentration of plant growth regulators might be needed to enhance *in vitro* propagation of TSS seedlings.

1. **Introduction**

Shallot (*Allium ascalonicum* L.) is an annual plant that belongs to the Alliaceae family with a high economic value and prospective market in many countries, including Indonesia. Shallot is not only useful commonly as a condiment, but it also has an antioxidants property, improves heart health, prevents cancer and diabetes, anti-inflammatory, antimicrobial, and reduces obesity allergies [1].

The biggest export of vegetables from Indonesia is shallot with a net weight of 6.48 thousand tons and export value of 8.81 million, in other words, this plant contributes the highest ‘devisa’ [2]. In Indonesia, the total value of vegetable export in 2017 decreased by 28.88% compared to 2016. Therefore, an increase in shallot productivity is very much needed and have non fluctuated prices needs to be maintained. Shallots production and price are very fluctuations because shallot is the horticultural product that easily damaged or rotten commodity. The shallot price is categorized as inelastic, meaning that shallots are the main condiment that is difficult to replace. The demand of shallot commodity is not too affected by price changes [3], especially in Indonesia at offseason condition [4].

Research on micropropagation of shallot had been carried out using cultivars of Huruta and Minjar [5], Lembah Palu [6-8], Tiron [9], Sumenep [10], Bima Curut [11] as well as Biru Lancor [12]. Mostly
they used bulbs as explants to induce embryo somatic callus. Cells derived from competent source tissue were cultured to form callus [13], and it needs more time and treatment to regenerate callus into micro shoots. The organogenesis process has a lower efficiency than somatic embryogenesis. However, it could produce a “true to type” plant [14]. The use of explant in the form of botanical seeds or known as True Shallot Seed (TSS) has several advantages compared to bulbs [15,16]. Using TSS in micropropagation is easier than bulb because it carries fewer soilborne pathogens carried by bulbs and could reduce culture contamination. Moreover, the cost for TSS distribution and the space of storage are cheaper and easier than using bulbs [17]. Tuktuk is one of the true shallot seed cultivars produced commercially so that the identity is clear and as a source of explants for many seeds also can be found easily.

Cytokinins such as Benzyl Amino Purine (BAP) is common to add to the culture medium. It has an important role in the development and morphogenesis of explants. BAP induces cell division and differentiation of shoots, adventitious bud formation, and reduced apical dominance and root formation. However, BAP can not stimulate plant growth completely. BAP has an unexpected effect directly or indirectly in organogenic variations in Physalis peruviana [18]. Different BAP concentrations affect the number and size of shoots and root formation in banana cultivars [19]. BAP is also the most responsive cytokinin for shoot induction; however, proliferated shoots showed a rosette appearance, grow slowly, stunted with leaf abscission and shoot tip necrosis in Syzygium cumini [20]. In addition, Adenine Sulphate may reduce this problem. To accelerate the productivity of transplants, a combination of BAP with other types of growth regulators is required [21].

Thiamine and Adenine Sulphate are organic compounds known as vitamins B4 and B1 [22]. Thiamine is an important co-factor in carbohydrate metabolism, and it is directly related to the biosynthesis of several amino acids. Adenine Sulphate acts as a precursor of cytokinin synthesis or increases the biosynthesis of cytokinins [23]. Related research on the combination of BAP with Adenine Sulphate on in vitro culture medium has been carried out on Syzygium cumini [20], Centella asiatica [24] and Phaseolus vulgaris [25]. A combination of BAP and Thiamine has been carried out on Abaca banana plants [26]. Medium taro containing BAP combined with Thiamine and Adenine increases the multiplication rate of shoots almost twice [27].

Compared with a solid medium, using a liquid medium in plant tissue culture reduces production costs and be more compatible for automation. In liquid culture, the culture environment conditions are more uniform, the medium can be refilled easily without changing the culture tubs, sterilizing the medium can be done using a microfilter, cleaning the culture tubs after one culture period becomes easier, the use of a larger culture container can be applied so that the frequency of subcultures can be reduced [28,29]. Another advantage is that it can reduce explant oxidative stress, facilitate acclimatization easier because cleaning explants manually could damage the roots so that the remaining can increase microbial growth [29]. However, in vitro culture using a liquid medium system is faced with several problems such as hyperhydricity of the tissue, rapid spread of contamination, plantlet asphyxiation and geotropic changes [30]. In shallot culture, percentage of hiperhidricity or vitrous symptoms was relatively high in culture treatment in a combination of 2,4-D with cytokinins BAP, 2iP and TDZ [10] and shown up at culture treatment with NAA and BAP [12]. The addition of Thiamine and Adenine Sulphate to the culture medium was expected could reduce hyperhydricity and the explants could grow better.

Manipulation of in vitro condition on liquid culture medium could be enhanced the growth and development of red ginger (Zingiber officinale Rocs.) explant, even red ginger propagation in the liquid medium could be done without plant growth regulators addition [31]. Shoot multiplication rate in liquid culture medium on Chlorophytum borivilianum, Celastrus paniculatus, Terminalia bellerica and Boswellia serrata were increased 4.75; 7;4.5 and 2 times fold respectively compared with in solid culture medium. Furthermore, after 42 days of culture, there was no adverse effect such as hyperhydricity was seen and plantlets could be acclimatized easily [30]. This research aimed to evaluate the growth response of shallot (Allium ascalonicum L.) cv Tuktuk seedlings, cultured on MS solid and liquid media with agitation supplemented with BAP, Thiamine and Adenine Sulphate.
2. Methods

2.1. In vitro germination of True Shallot (A. ascalonicum L.) Seeds
The plant material used in this research was the A. ascalonicum cv Tuktuk produced by a seed company. Before sterilization, TSS seeds were wrapped in cotton material and tied with a rubber band, then they were soaked in liquid detergent while shaking for 5 min. Seeds were then left in running water for 15 min. The seeds were soaked in 3% fungicide Dithane solution, shaken for 30 min. Inside the laminar air flow cabinet, the seeds were rinsed with sterile water 3 times. Furthermore, TSS seeds were soaked in 70% ethanol for 5 min, rinsed again with sterile water. At the final stage of sterilization, the seeds were soaked in 30% commercial bleaching solutions (containing 5.25% sodium hypochlorite of active compound) for 15 min, then rinsed again with sterile water 3 times. Before being planted the seeds were air-dried in laminar airflow. Seeds were planted on MS medium containing 30 g/l sugar without plant growth regulators. Culture medium was sterilized by autoclave at 1 atm, 120°C for 15 min. Each culture bottle contained 10 TSS seeds, the number of bottles was 57. The culture was incubated in the culture room at 25-27°C for 4 weeks. Aseptic germination rate was observed every week until 4 weeks in culture.

2.2. Shallot (A. ascalonicum L.) Seedlings culture for growth establishment
Four-weeks old of the aseptic culture of A. ascalonicum L. seedlings from the germination process were isolated from their roots and leaves. About 1-2 cm long of explants from the basal leaves were cultured on 5 types of medium, namely MS medium [32] without the addition of plant growth regulators (as control); MS with 0.5 mg/l BAP; MS with BAP at the same concentration with the addition of 1 mg/l Thiamine; 2 mg/l Adenine Sulphate; and both with Thiamine and Adenine Sulphate also given at the same concentration. This experiment used solid and liquid medium. Solid medium containing Gelzan agar 4 g/l, the liquid medium without addition of Gelzan. The liquid medium culture was placed on the shaker with 100 rpm. On solid medium, one bottle contained 2 explants with 4 replicates. In liquid medium, each Erlenmeyer contained 1 explant. The experiment has 4 replicates. The culture was incubated in the culture room at 25-27°C for 8 weeks in the incubation room with continuous light condition. Growth observation included number and length of leaves, number and length of roots, bulb-like body diameter at the bottom, middle and top parts, also fresh and dry weights of plantlets. Photographs were taken 8 weeks after culture.

2.3. Statistical design and analysis
Experiments were carried out by a Completely Randomized Design. Data were analyzed by software DSAASTAT, followed by Duncan Multiple’s Range Test (DMRT) at a 95% level of significance.

3. Results and Discussion

3.1. In vitro germination of True Shallot (A. ascalonicum L.) Seeds
Aseptic germination percentage of True Shallot Seed, from 1 to 4 weeks after planting is presented in figures 1 and 2. Seeds started to germinate 3 days after planting, but data presented in Figure 1 was recorded from the 1st to 4th weeks in culture. Germination percentage increased sharply from the first week to the second week then slightly increase after that. At 4 weeks after planting, the percentage of germination of Tuktuk cultivar was 19.82%. Low germination rate may be influenced by the genetic characteristics of the variety, seed longevity and the process of in vitro culture. In vitro germination aimed to obtain an aseptic source of explants for further experiments on shoot propagation and bulblet induction. Suitable growth regulators are needed to propagate shoots through organogenesis, embryogenesis, or bulblet formation.

Figure 1 shows that in vitro germination rate was low. In non-aseptic condition, True Shallot Seeds of Tuktuk cultivar had an 82.25% germination rate [33]. In vitro% germination process was started with explant surface sterilization by soaking the seeds in various sterilant solutions in sequence, such as
detergent, fungicide, ethanol followed by sodium hypochlorite. In this sterilization process contamination percentage was still high, i.e. 24.5%, and soaking seeds in a series of chemicals may reduce seeds germination. In a bottle of culture free from contamination 0-10 seeds could germinate, so the conditions of the seeds were still viable. Low in vitro germination rate was also reported on Amaranthaceae seeds [34].

![Figure 1](image1.png)

**Figure 1.** Aseptic germination percentage of True Shallot (A. ascalonicum L.) Seeds after 0-4 weeks of culture

![Figure 2](image2.png)

**Figure 2.** *In vitro* seed germination of True Shallot (A. Ascalonicum L.) Seeds A. Seeds on day-0; B. One week; C. Two weeks, D. Three weeks, and E. Four weeks of culture on MS solid medium without plant growth regulators

3.2. *Seedlings growth of True Shallot (A. ascalonicum L.) Seeds cultured in solid medium*

The addition of 0.5 mg/l BAP with or without 1 mg/l Thiamine and or both with 2 mg/l Adenine Sulphate in solid MS medium, significantly influenced the growth of shallot shoots, but not for the number of roots (table 1). The lowest leaf length was found on MS medium containing only 0.5 mg/l BAP and significantly different with MS control medium. The addition of organic compounds such as 1 mg/l Thiamine and 2 mg/l Adenine Sulphate into MS medium containing 0.5 mg/l BAP was not significantly different from the control medium. MS medium containing 0.5 mg/l BAP, 1 mg/l Thiamine and 2 mg/l Adenine Sulphate had little effect on higher leaves length than in MS medium. The highest number of leaves was found in the MS control medium, but it was significantly different from the MS medium containing only 0.5 mg/l BAP. On MS control medium, the number of green leaves was also had a similar response with the total number of leaves, that it was significantly
different with MS containing 0.5 mg/l BAP. MS containing 0.5 mg/l BAP combined with 2 mg/l Adenine sulphate or Thiamine, reduced root length compared with MS control medium. In this experiment, MS medium supplemented with 0.5mg/l BAP, 1mg/l Thiamine and 2 mg/l Adenine Sulphate for seedlings culture from true shallot seed, up to 8 weeks of culture still unable to produce adventitious shoots. Further research in the form of optimization of BAP and Adenin Sulphate still needs to be done for seedlings explant.

Table 1. Seedling culture growth of Shallot (A. Ascalonicum L.) after 8 weeks cultured in MS solid medium supplemented with BAP, Thiamine and Adenine Sulphate

| Medium                        | Leaf length (cm) | Total leaves number | Green leaves number | Root length (cm) | Roots number |
|-------------------------------|------------------|---------------------|--------------------|------------------|-------------|
| MS0                           | 17.00±3.29a      | 4.25±0.21a          | 1.75±0.21a         | 4.00±1.03a       | 9.25±2.40   |
| MS+0.5 mg/l BAP               | 9.37±3.11b       | 2.00±0.00b          | 1.00±0.00b         | 3.25±0.67a      | 5.25±0.96   |
| MS+0.5 mg/l BAP+1 mg/l Thiamine | 16.37±2.43a     | 4.00±0.61a          | 1.00±0.35ab        | 2.00±0.61ab      | 6.75±0.96   |
| MS+0.5 mg/l BAP+2 mg/l Adenine Sulphate | 19.12±5.94a | 3.25±0.73ab        | 1.50±0.25a         | 1.75±0.57a       | 5.25±1.78   |
| MS+0.5 mg/l BAP +1 mg/l Thiamine+2 mg/l Adenine Sulphate | 19.25±0.73a | 3.50±0.25ab        | 1.25±0.21a         | 1.75±0.21b       | 7.00±0.35   |

Note: Value followed by the same letter on the same column is not significantly different according to DMRT (P=0.05).

Shallot shoots formed bulbs-like bodies formation on the leaf base. The results showed that the addition of 0.5 mg/l BAP with or without 1 mg/l Thiamin and 2 mg/l Adenine Sulphate or organic compound on solid MS medium did not significantly affect bulb-like bodies diameter on the upper, middle and bottom parts, fresh weight and dry weight of plantlet. Solid MS control medium gave larger bulbs-like bodies diameter at top, middle and bottom parts. Furthermore, explants grown in MS control medium had the highest fresh and dry weights (table 2).

Table 2. The diameter of bulbs-like bodies, fresh weight and dry weight of planlet from seedling culture of Shallot (A. ascalonicum L.) after 8 weeks cultured in MS solid medium supplemented with BAP, Thiamine and Adenine Sulphate

| Medium                        | Diameters of bulbs-like bodies (mm) | Total fresh weight (g) | Total dry weight(g) |
|-------------------------------|--------------------------------------|------------------------|---------------------|
|                              | Upper part                           | Middle part            | Bottom part         |                     |                     |
| MS0                           | 3.10±0.56a                          | 3.36±0.58a             | 2.32±0.27a          | 0.38±0.06a         | 0.03±0.006a         |
| MS+0.5 mg/l BAP               | 1.70±0.14b                          | 2.12±0.17ab            | 1.26±0.18bc         | 0.11±0.01b         | 0.01±0.002b         |
| MS+0.5 mg/l BAP+1 mg/l Thiamine | 1.97±0.33ab                        | 2.18±0.25ab            | 1.48±0.14bc         | 0.12±0.03b         | 0.01±0.002b         |
| MS+0.5 mg/l BAP+2 mg/l Adenine Sulphate | 1.61±0.38b  | 1.74±0.38b           | 1.01±0.14c          | 0.09±0.03b         | 0.01±0.003b         |
| MS+0.5 mg/l BAP +1 mg/l Thiamine+2 mg/l Adenine Sulphate | 1.87±0.17ab | 2.13±0.13ab          | 1.78±0.05ab         | 0.14±0.02b         | 0.02±0.006ab         |

Note: Value followed by the same letter on the same column is not significantly different according to DMRT (P=0.05).

The addition of plant growth regulator did not significantly influence the length of the leaf, total number of leaves, number of green leaves, length and number of roots, diameters of bulbs-like bodies as well total fresh and dry weights, even tend to reduce the growth of seedlings culture of TSS. The
use of different BAP concentrations, Thiamine and Adenine Sulphate may be modified to find the best growth of shallots. Different types of plant growth regulators may also be tested to find out the best medium for growth. It seems that the concentration of cytokinin BAP at 0.5 mg/l and two kinds of organic compounds i.e 1 mg/l Thiamine and 2 mg/l Adenine Sulphate in this study may be too low, hence, there was not sufficient yet to produce the adventive shoot. A high concentration of cytokinin 2,iP, combined with coconut water was the best plant growth regulator for supporting shallot var Sumenep growth [35]. Precisely addition of 8.0 mg/l 2,iP, combined with 20% coconut water resulted in 6.3 leaves at 8 weeks after culture [35]. On MS medium containing B5 Vitamin combined with NAA 1 mg/l and BAP 1 mg/l for supporting the growth of basal plate tuber explants of Biru Lancor cultivar of shallot, gave 5.67 leaves per explant [12]. In this case, bulbs grown in a higher concentration of cytokinin combined with organic cytokinin or auxin gave more leaves. However, a low number of leaves indicated that several buds are still dormant, and they will develop into adventitious shoots further when the outer leaves dry out, and new leaves inside the bulbs will appear. Experiments using bulb meristem tissue from Katumi cultivar of shallot showed that the combination of 2 mg/l Kinetin with 0.01 mg/l NAA gave slightly better shoot growth with an average increase of just 0.78 shoots [37].

Adenine sulphate has been widely used in tissue culture medium with different concentrations to enhance the growth of some plant species. The addition of 2 mg/l Adenine Sulphate also increased the growth culture of other monocot plants such as taro (Colocasia esculenta L.) [27] and at 40 mg/l was best for banana multiplication [38] as well in shallots [27], and 40 mg/l Adenine Sulphate was best for multiplication of Stevia rebaudiana [39]. Adenine is a component of the enzyme Adenine Phosphoribosyl Transferase which is the main enzyme that plays an important role in catalysis conversion of cytokinins from a nitrogenous base (a nucleic base) to a nucleotide. This finding was proven in an experiment using Arabidopsis as a model plant [39]. In tissue culture medium, thiamine is found in Murashige & Skoog medium at 0.1 mg/l. Thiamine is a co-factor in carboxylase reactions and amino acid biosynthesis [40]. An increase in Thiamine concentration has been widely used in tissue culture medium such as addition at 1 mg/l on taro multiplication [27], at 0.4 mg/l on Allium neapolitanum micropropagation in combination with 5 mg/l BAP and 0.1 mg/l NAA [41]. In this research, using a single plant growth regulator with a low concentration of cytokinin-BAP was intended to lead shoot formation. It was also expected that the formation of shoots would be a more vigorous increase in numbers supplemented with two kinds of organic compounds to make it easier for their further growth and multiplication.

3.3. Growth of shallot seedlings (A. ascalonicum L.) culture in liquid medium

Growth of in vitro TSS seedlings culture in liquid medium 8 weeks after planting is shown in tables 3 and 4. The addition of 0.5 mg/l BAP with or without 1 mg/l Thiamine and 2 mg/l Adenine Sulphate or both organic compound, on the Liquid MS medium (without the addition of gelling agents) and agitation, only significantly affected on the total number of leaves. Length and number of green leaves, length and number of roots were not significantly different with the control treatment (table 3). The diameter of bulb-like bodies at the top, middle and bottom parts, and the fresh and dry weight of plantlet 8 weeks after culture were not significantly different with control medium (table 4).

Fresh and dry weights of shallot plantlets showed that the liquid medium was higher than that planted in solid medium (Tables 2 and 4). This shows that the use of liquid culture medium at in vitro seedling culture of TSS Tuktuk cultivar is more effective in supporting plantlet growth than using the solid medium for biomass production purposes. The use of liquid culture on Zingiber officinale Rosc. also supporting higher shoots multiplication [42] as well as in potato shoots culture with nodal cutting explant [43]. Increasing aeration during incubations by periodical immersion of explants in the culture medium may enhance the growth of explants.
Table 3. Shallot Seedling (A. ascalonicum L.) growth after 8 weeks cultured in MS liquid medium supplemented with BAP, Thiamine and Adenine Sulphate

| Medium                          | Leaf length (cm) | Total leaves number | Green leaves number | Roots length (cm) | Roots Number |
|--------------------------------|------------------|---------------------|---------------------|-------------------|--------------|
| MS0                            | 14.75±2.76       | 3.00±0.35           | 0.75±0.21           | 17.25±4.08        | 6.75±0.81    |
| MS+0.5 mg/l BAP                 | 8.62±1.21        | 1.25±0.21           | 1.25±0.21           | 12.87±4.62        | 3.75±1.34    |
| MS+0.5 mg/l BAP+1 mg/l Thiamine | 10.77±4.30       | 1.50±0.43           | 1.00±0.35           | 10.00±2.15        | 4.25±0.54    |
| MS+0.5 mg/l BAP+2 mg/l Adenine Sulphate | 7.25±1.98        | 1.25±0.21           | 1.00±0.35           | 15.75±5.41        | 4.50±0.82    |
| MS+0.5 mg/l BAP +1 mg/l Thiamine+2 mg/l Adenine Sulphate | 6.37±1.72        | 1.75±0.21           | 1.50±0.25           | 17.50±6.25        | 4.50±1.03    |

Note: Value followed by the same letter on the same column is not significantly different according to DMRT (P=0.05).

Table 4. Diameter of bulbs-like bodies, total fresh weight and dry weight of plantlet (A. ascalonicum L.) after 8 weeks cultured in MS liquid medium supplemented with BAP, Thiamine and Adenine Sulphate

| Medium                          | Diameters of bulbs-like bodies(mm) | Total fresh weight (g) | Total dry weight(g) |
|--------------------------------|------------------------------------|------------------------|---------------------|
|                                | Upper part | Middle part | Bottom part |                     |                     |
| MS0                            | 2.64±0.48 | 3.64±0.54   | 2.74±0.34   | 0.71±0.35           | 0.09±0.04           |
| MS+0.5 mg/l BAP                 | 2.99±0.30 | 4.47±0.68   | 2.47±0.26   | 0.34±0.17           | 0.04±0.02           |
| MS+0.5 mg/l BAP+1 mg/l Thiamine | 2.94±0.62 | 3.77±0.56   | 2.10±0.10   | 0.52±0.26           | 0.06±0.03           |
| MS+0.5 mg/l BAP+2 mg/l Adenine Sulphate | 1.73±0.15 | 3.16±0.79   | 2.61±0.93   | 0.34±0.18           | 0.04±0.02           |
| MS+0.5 mg/l BAP +1 mg/l Thiamine+2 mg/l Adenine Sulphate | 2.45±0.47 | 4.03±0.72   | 1.81±0.13   | 1.04±0.52           | 0.11±0.05           |

3.4. Performance seedlings culture of shallot (A. ascalonicum L.) in solid and liquid media

Performance of *in vitro* seedlings culture of shallot grown on solid and liquid MS medium containing 0.5 mg/l BAP, 1 mg/l Thiamine and 2 mg/l Adenine Sulphate is presented in figure 3. Shoots grown on solid medium appeared to grow upward with the roots directed grew downward into the medium. Unlikely, in liquid medium shoots grew circular. That is caused by the absence of a solidifying agent that functions as shoots supporting growth.

After 8 weeks of culture, the total number of leaves in the solid medium was higher than that in the liquid culture medium. However, the leaves in solid medium mostly easily senescence, dried up with brown color and unfresh. In a solid medium, the addition of 0.5 mg/l BAP combined with 2 mg/l Adenin Sulphate and the addition of 0.5 mg/l BAP combined with 1 mg/l Thiamine and 2 mg/l Adenine Sulphate produced more intense green colour of leaves and more vigorous shoots compared to other treatments. Similarly, on liquid culture explants grown on MS medium containing 0.5 mg/l BAP combined with 1 mg/l Thiamine and 2 mg/l Adenine Sulphate also showed a more intense green colour of leaves and vigorous explants (figure 3.E. in solid and liquid culture).
Figure 3. Performance of shallot (*A. ascalonicum* L.) seedling after 8 weeks cultured in solid and liquid MS medium. A. Without plant growths regulators; B. MS+0.5 mg/l BAP; C. MS+0.5 mg/l BAP+2 mg/l Adenine Sulphate; D. MS+0.5 mg/l BAP+2 mg/l Adenine Sulphate; and E. MS+0.5 mg/l BAP +1 mg/l Thiamine+ 2 mg/l Adenine Sulphate.

Different growth responses between leaves and roots of plantlets were found in solid and liquid medium (figure 4, tables 1 and 3). In liquid culture medium, root grew longer than using the solid medium at 8 weeks after planting. Contrary to a solid medium, leaves were more prolonged than that in the liquid medium. The root length formed in liquid medium was 4.4 times longer than roots that have grown in a solid culture medium. However, the number of roots in a solid medium was 1.37 times higher than that of the number of roots in a liquid medium. Length and number of leaves observed in the solid medium culture were higher than that in the liquid culture medium (figure 4, tables 1 and 3). The leaf length in solid medium was 1.30 times longer than that in the liquid medium. On the contrary, many leaves in the solid medium were 1.42 times that in the liquid medium. In potato propagation [43], the liquid medium gave more roots per plantlet than in the solid medium, it might be caused that the explant could have direct contact with the medium as a result, that roots are easier to absorb nutrient from the culture medium.
**Figure 4.** Morphology of shallot (A. ascalonicum L.) seedling cultured after 8 weeks in solid and liquid MS medium. A. without plant growth regulators; B. MS+0.5 mg/l BAP; C. MS+0.5 mg/l BAP+2 mg/l Adenine Sulphate; D. MS+0.5 mg/l BAP+2 mg/l Adenine Sulphate; and E. MS+0.5 mg/l BAP+1 mg/l Thiamine+ 2 mg/l Adenine Sulphate.

The bulbs-like bodies formation in solid and liquid cultures containing 0.5 mg/l BAP, 1 mg/l Thiamine, and 2 mg/l Adenine Sulphate after 8 weeks planting is shown in Figure 5. In MS solid without any addition of BAP, Thiamine, or Adenine Sulphate (control), explants formed larger bulbs-like bodies diameter at top, middle and bottom parts. However, after 8 weeks of culture, the old leaf tips turned dry and brown, the younger leaves grew fresh and green. On the contrary, all leaves were fresh in the liquid medium, with reddish-green because the plantlets were submerged in the medium solution. Roots formed in solid medium culture were shorter but with a higher number than that in the liquid medium. More studies are required to increase bulbs-like bodies formation both in the solid and the liquid medium. This can be done by altering the medium composition optimized by the addition of several types of plant growth regulators, as well as with modification of the *in vitro* environment.

**Figure 5.** Morphology of bulbs-like bodies from shallot (A. ascalonicum L.) seedlings after 8 weeks cultured in solid and liquid MS medium. A. without plant growths regulators; B. MS+0.5 mg/l BAP; C. MS+0.5 mg/l BAP+2 mg/l Adenine Sulphate; D. MS+0.5 mg/l BAP+2 mg/l Adenine Sulphate; and E. MS+0.5 mg/l BAP+1 mg/l Thiamine+ 2 mg/l Adenine Sulphate.
4. Conclusion

In vitro germination of true shallot (Allium ascalonicum L.) seeds were low (19.8%) 4 weeks after culture. The addition of 0.5 mg/l BAP, 1mg /l Thiamine and 2 mg/l Adenine Sulphate on solid MS medium of isolated seedlings cultured of True Shallot Seed had little effect on leaf length. In liquid medium, all observed growth parameters were not significantly different from control. In liquid medium, shoot culture had longer roots and more intense green colour of leaves, resulting in higher fresh and dry weights than solid medium culture. However, in a solid medium, shoots had a higher number of roots and leaves and longer leaves.

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