Comunicata Scientiae
Horticultural Journal

ORIGINAL ARTICLE
published: 30 December 2025
https://doi.org/10.14295/CS.v16.4352

# Optimizing in vitro shoot propagation of Indonesian true seeds of shallot (TSS) using MS and DKW media with BAP

Deritha Ellfy Rantau[1]*, Dyah Retno Wulandari[1], Awang Maharijaya[2], Erwin Al-Hafiizh[1], Andri Fadillah Martin[1]

[1]National Research and Innovation Agency, Bogor, Indonesia
[2]Institut Pertanian Bogor, Bogor, Indonesia
*Corresponding author, e-mail: deri001@brin.go.id

## Abstract

Using shallot botanical seeds or true seed of shallot (TSS) as planting material can produce plants free from bulb-borne disease and be more technically and economically efficient. However, its application in the field still has limitations. The objective of this study was to evaluate the *in vitro* propagation media containing a combination of MS and DKW media with the addition of BAP on the growth parameters and multiplication frequency as well as plantlets condition of three cultivars of TSS to initiation support the supply of quality seeds in the field. The seeds were germinated *in vitro* using an MS medium, and then aseptic TSS seedlings were arranged using a completely randomized design with two factors and twelve replications. The first factor was media composition. MS (Murashige and Skoog) and DKW (Driver Kuniyaki Walnut) media were used for the *in vitro* shoot propagation media in combination with BAP (Benzyl Amino Purine) at the level of concentrations: 0, 1, 2 and 4 $mgL^{-1}$. The second factor was TSS cultivars: Bima Brebes, Trisula and Tuktuk. The result showed that Bima Brebes and Trisula cultivars significantly differed in multiplication frequency from the Tuktuk cultivar. DKW medium with 4 $mgL^{-1}$ BAP produced the highest multiplication frequency but not significantly different with 4 $mgL^{-1}$ BAP in MS and 2 $mgL^{-1}$ BAP in DKW media. Adding 1-2 $mgL^{-1}$ BAP in the DKW medium could induce new shoots on the Bima Brebes cultivar with better performance, though with lower multiplication frequency.

**Keywords:** *Allium cepa var aggregatum*, botanical seed, correlation analysis

## Introduction

Shallot (*Allium cepa* L. var *aggregatum*) is a vegetable product with potential health benefits. It is highly sought after in Indonesia as a culinary ingredient. Using shallot bulbs to grow new plants has drawbacks: the quality deteriorates over time, they can carry pests and diseases, storing and distributing them is difficult. Furthermore, there is competition between using bulbs for planting and eating. True sallot (TSS) or botanical seeds offer advantages. It needs less volume of TSS to plant in the same area (2-3 $kgha^{-1}$ vs 1-1.2 $tonha^{-1}$ for bulbs), making storage, including distribution, easier and cheaper (Prakoso & Alpandari, 2021). In addition, TSS lasts longer, remaining viable for 1-2 years compared to about four months for bulbs (Hakim et al., 2022). Tissue culture is expected to provide an early selection of high-yielding shallots and ensure a supply of quality plants from TSS. Mendes et al., (2017) stated that plant varieties, type and tissue explant, type and medium composition, plant growth regulator addition, and culture condition determine the progress of *in vitro* culture.

Organogenesis is a micropropagation technique for massive plant propagation from small tissue (Pasternak & Steinmacher, 2024). A study by Ossai et al. (2023) denoted that organogenesis produced 24% more nodes and significantly more tubers in the genotypes of white and water yams compared to somatic embryogenesis. Masekesa et al., (2021) study revealed that sweet potato plantlets generated through somatic embryogenesis were characterized by weakness and a lack of viability, leading to their death within four weeks after germination. Despite visible shoot apical meristems, these plantlets failed to progress beyond the two-leaf stage of development. Dharmayanti et al. (2018) used TSS on Tuktuk and Trisula cultivars, successfully initiating callus and embryogenesis, but plantlet regeneration still needs


Received: 11 June 2025
Accepted: 17 June 2025

to be carried out further.

The growth medium contains substantial nutrients for plant tissue development, including macronutrients, micronutrients, vitamins, amino acids, nitrogen sources, organic supplements, carbon sources, solidifying agents, and plant growth regulators (Sidik et al., 2024). Cytokinin is a plant growth regulator that helps cell division and shoots growth, with benzyl amino purine (BAP) being the most common use (Devano & Setiawan, 2020). Prior experiments using BAP on TSS seedlings of Tuktuk cultivar in MS enrichment with 0,5 mgL$^{-1}$ BAP, 1 mgL$^{-1}$ Thiamine and 2 mgL$^{-1}$ Adenine sulphate in solid and liquid culture media did not produce new shoots (Rantau et al., 2021). Similarly, works on the TSS Lokananta cultivar using full and half strength MS medium supplemented with 1-4 mgL$^{-1}$ BAP (Handayani et al., 2021), no further information on tiller production. This study aimed to evaluate different combinations of MS and DKW media with varying levels of BAP on the growth and multiplication as well as plantlets condition of three TSS cultivars (Bima Brebes, Trisula, and Tuktuk) grown from TSS.

**Materials and Methods**

*Preparation of In Vitro TSS aseptic seedlings*

Three TSS cultivars were used: Bima Brebes, Trisula, and Tuktuk. TSS was wrapped in gauze, tied with rubber, and then passed through a series of sterilization processes with the following stages: 5% liquid soap 10', running water 15', 5% hydrogen peroxide solution 10', and 30% commercial bleaching solution contains 5,25% Sodium hypochlorite for 15'. After each step, the seeds were rinsed 3 times in sterile water before being planted in MS medium with vitamins from TM Cassonslabs for 4 weeks. The contamination percentage, aseptic seedlings and ungerminated seeds were observed, but this paper does not show the data.

The culture media were adjusted to a pH of 5.8, solidified with 3 gL$^{-1}$ gellan gum (produced by Caissonlabs) and then sterilized by autoclaving at 1 atm for 20'. Furthermore, the seeds and explants were cultured in a glass bottle 12 cm in height and diameter of 6 cm; then, they were incubated in a culture room at 20-22$^{0}$C in continuous light.

*In Vitro Shoot Propagation of TSS Seedlings with BAP in MS and DKW Media*

An experiment was conducted to determine the effect of BAP treatment at concentrations of 0,1,2 and 4 mgL$^{-1}$ with MS and DKW media on *in-vitro* shoot propagation. The experiment was arranged using a two-factor, completely randomized design. The first factor was the composition of the *in vitro* culture media for shoot propagation. The media used was MS and DKW with their vitamins produced by Cassonlabs, combined with 4 levels of BAP, namely 0, 1, 2 and 4 mgL$^{-1}$. The composition of the treatment media were follows; MS+0 mgL$^{-1}$ BAP (MB0); MS+1 mgL$^{-1}$ BAP (MB1); MS+2 mgL$^{-1}$ BAP (MB2); MS+4 mgL$^{-1}$ BAP (MB4); DKW+0 mgL$^{-1}$ BAP (DB0); DKW+1 mgL$^{-1}$ BAP (DB1); DKW+2 mgL$^{-1}$ BAP (DB2) and DKW+4 mgL$^{-1}$ BAP (DB4) with 30 gL$^{-1}$ Sucrose. The second factor was three cultivars of aseptic seedlings derived from TSS: Bima Brebes, Trisula and Tuktuk. Each treatment consisted of four bottles as replicates filled with three explants.

Culture observation and documentation were carried out 8 weeks after culture (WOC) by removing the explant from the culture bottle, then counting the number of roots, including green leaves and total leaves, measuring the length of roots and leaves, and taking the weight of plantlets. The number of new shoots or tillers was presented descriptively, while the percentage of multiplication and vitrification frequency was calculated in percentage with three repetitions from twelve explants in the same treatment.

*Data analysis*

Analysis of variance (ANOVA) was carried out to determine the differences between two factors for *in vitro* propagation experiment. The sqrt formula first transforms the percentage value before analysis to normalize the data. The significantly different treatments were then further tested using Duncan's Multiple Range Test (DMRT) at a 95% confidence level to identify specific significant differences among multiple means. DSAASTAT ver 1.021 (open-source software) was used to analyse ANOVA and DMRT. For correlation analysis between growth parameters, the formulation on MS Excel was validated by Pearson's Correlation Table.

**Results and Discussion**

The experimental results on shoot propagation of three cultivar shallot seedlings treated in a combination of MS and DKW media added with 0-4 mgL$^{-1}$ BAP at 8 WOC showed that the cultivar factor influenced the growth of leaf as well as root length, number of roots, and the multiplication frequency. In contrast, the media composition affects all observed growth parameters, including leaf length, total number of leaves, green leaves, root length, number of roots, plantlet fresh weight, multiplication, and vitrification frequency. Conversely, the cultivars factor did not affect the total number of leaves, number of green leaves, plantlet fresh weight, and vitrification frequency. The interaction between cultivar and media treatments for initiation of *in-vitro* shoot propagation was found in the leaf length, number of green leaves, and root length.

The response of three TSS cultivars plantlets to the treatment of MS and DKW media composition with the addition of 0-4 mgL$^{-1}$ BAP is illustrated in **Figure 1**. The *In vitro* culture of Trisula and Tuktuk cultivars had leaf and root length characteristics that were not significantly different. In contrast, the Bima Brebes cultivar had a shorter leaf length with longer root characteristics than the Trisula and Tuktuk cultivars. The Tuktuk cultivar had the longest root and most significant number of roots with the lowest multiplication frequency compared to Bima Brebes and Trisula.

In this experiment, Bima Brebes and Trisula cultivars had multiplication frequency percentages of 15.3 ± 6,23% and 13,9 ± 4,68% respectively. *In vitro*, culture of the three cultivars of TSS had a vitrification frequency that was not significantly different from each other, which was in the range 34,4 ± 3,28% to 37,2 ± 9,08 %. Bima Brebes is a local cultivar from Brebes - Central Java. Trisula and Bima Brebes have registered as breeder seed by number 4580/Kpts/SR.120/11/2011 and 594/Kpts/TP.240/8/1984 respectively (PSIH-BSIP, 2025). while Trisula is a hybridization between B2558 and B4127 developed by the Ex–Indonesian Vegetable Crop Research Institute (Ex-Balitsa), this time known as Vegetable Plant Assembly and Testing Center (BRMP-TS)– Ministry of Agriculture in 2011, and The Tuktuk cultivar is a 5607 (F) and 5607 (M) recombinant seed produced by PT East-West that was released as an open polination variety with registration number 361/Kpts/SR.120/5/2006 (Adin et al., 2023).

From the description of each cultivar, it is known that the shallot cultivar for Bima Brebes has a broader range of leaves, namely 15-50 leaves, than the Trisula with 28-39 leaves and Tuktuk having 7-14 leaves. The number of leaves formed is related to the amount of tillers. The Tuktuk cultivar only produces a range of 1-2 tillers, while the Trisula and Bima Brebes can produce tillers ranging from 5-8 and 7-12, respectively. In this study, the Bima Brebes cultivar also showed a more varied number of tillers than the Trisula and Tuktuk.

The other experiment using three cultivars and the same planting materials as this study but carried out on a field scale by a planting distance treatment of 10x10 cm on dry land (approximately altitude 275m above sea level) showed that the Bima Brebes cultivar had higher in plant height growth, number of leaves, number of tillers and bulbs production compared to Tuktuk and Trisula (Maintang et al., 2019). However, in the experiment planting the same three TSS cultivars in the wet highland (altitude 1340 m above sea level) using 0-2000 kgha$^{-1}$ of organic fish fertilizer mostly showed that the Trisula and Tuktuk cultivars significantly produced better growth in the plant height, number of leaves, number of tillers and bulbs production compared to Bima Brebes cultivar (Karo et al., 2022). Shallot plants are adaptable because they can produce bulbs in lowlands and highlands. Still, not all superior plant genotypes can provide satisfactory results in various growing environments (Zulfahmi & Tiara, 2024). The plant's potential to achieve maximum productivity from plant genotype is influenced by environmental factors, the ecosystem, and the ability of used genotypes to adapt to their agroecosystem.

In general, leaf length, root length, and the number of roots in MS and DKW media without adding 1-4 mgL-1 BAP tend to be longer and have more roots than those supplemented with BAP. However, DKW

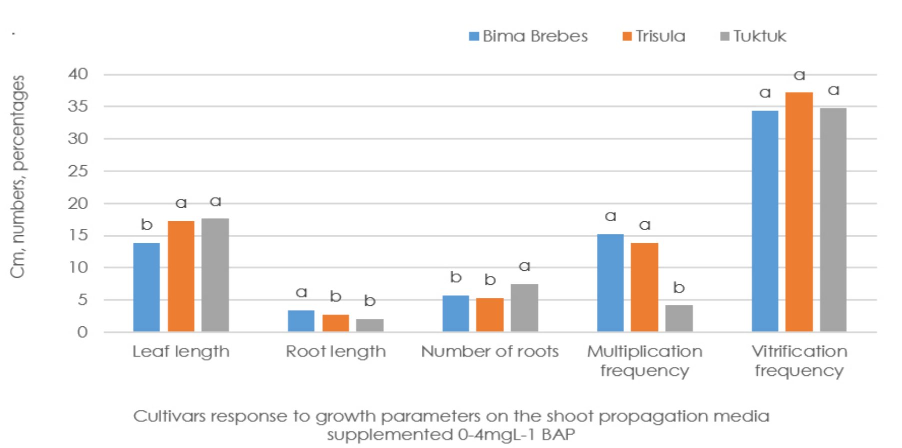


**Figure 1.** Leaf length (cm), root length (cm), number of roots, multiplication and vitrification frequency (%) of plantlets from three TSS cultivars treated with shoot propagation media supplemented 0-4 mgL$^{-1}$ BAP, 8 WOC. (Note: The same letters at the same growth parameter of charts are not significantly different)

media without adding BAP produced the longest leaves and the most significant number of roots and produced the highest fresh weight of shallot plantlets than the other media in this experiment. The highest plantlets' fresh weight in this *in vitro* culture was 0,60±0,10 g, and the most extended leaf length reached 22,13±1,88 cm in DKW media without adding BAP. Adding 1-4 mgL-1 BAP to MS and DKW media reduced the plantlet's fresh weight significantly after 8 WAC. The highest leaf lengths were not significantly different with MS media without adding BAP and 1 mg L$^{-1}$ BAP in MS media. DKW media produced a higher total number of leaves and green leaves than MS media. For root growth, the longest root parameters were found in the MS media without the addition of BAP, i.e. 7,53±0,80 cm, while the highest number of roots was found in the DKW medium without BAP addition was 15,69 ±1,43 roots. The roots length and number of roots in the 1-4 mgL$^{-1}$ BAP treatment were not significantly different between MS and DKW media (**Figure 2**).

Umoh et al. (2020) reveal that roots also respond to chemical acclivity. They proliferate in soil regions with high concentrations of specific ions, such as nitrate or phosphate (a salt of potassium or sodium-containing phosphorus). As a phosphorous and potassium source, DKW media has a $KH_2PO4$ concentration 1.5 times higher than MS media. Based on morphological characteristics, shallots are categorized as monocotyledons with a fibrous root system. The main root of the fibrous monocot root stops growing or dies early in development, but many adventitious roots and crown roots grow from the base of the hypocotyl and the lower stem nodes (Zhang et al., 2023). Apart from that, DKW media with the addition of 2-4 mgL$^{-1}$ BAP also produced the highest total number of leaves, and the supplementation of 1-4 mgL$^{-1}$ BAP led to a higher number of green leaves than the other media (**Figure 2**). DKW media also contains twice the higher macroelement MgSO4 than MS media. Magnesium (Mg) is necessary for some enzymes involved in phosphate transfer, and it is an integral part of the ribosome and the primary constituent of chlorophyll. At the same time, sulfur (S) plays a key role in cellular energy and the synthesis of amino acids and lipids (Shrestha et al., 2020; Zewide & Melash, 2021).

The highest multiplication frequency was found in the combination of DKW medium with the addition of 4 mgL$^{-1}$ BAP, namely 22,21% ± 7,86 and significantly different from MS medium with the addition of 1 mgL$^{-1}$ BAP as well as MS and DKW medium without the addition of BAP (**Figure 3A**). The addition of 1-4 mgL$^{-1}$ BAP in DKW medium could be used to initiate new shoots. Despite that, using an MS medium requires a higher concentration of BAP to produce tillers. MS and DKW media, without adding BAP up to 8 WOC, could not produce tillers or new shoots for the three TSS cultivars used. Thus, using a DKW medium with an additional concentration of 1 mgL-1 BAP produced a better multiplication frequency than an MS medium with the addition of 1 mgL$^{-1}$ BAP(**Figure 3B**).

The Bima Brebes cultivar produced new shoots on DKW medium with the addition of the three BAP concentration levels tested, while the Trisula and Tuktuk cultivars did not. The Bima Brebes cultivar was more responsive to the DKW medium in initiating *in-vitro* shoot propagation to produce several tillers than Trisula and Tuktuk. In this experiment, the Bima Brebes cultivar had a relatively similar distribution in the number of tillers in the treatment of DKW medium with the addition of 1-4 mgL$^{-1}$

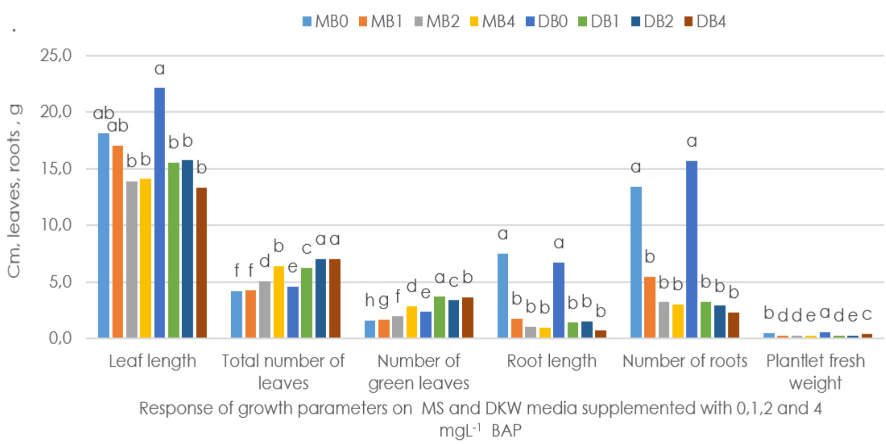


**Figure 2.** Leaf length (cm), the total number of leaves (leaves), number of green leaves (leaves), root length (cm), number of roots (roots), and plantlets fresh weight (gr), 8 WOC on MS and DKW media with 0-4 mg L$^{-1}$ BAP. MB0=MS without BAP; MB1= MS with 1mg L$^{-1}$; MB2 = MS with 2mg L$^{-1}$ BAP; MB4= MS with 4 mg L$^{-1}$ BAP; DB0 = DKW without BAP, DB1= DKW with BAP 1 mg L$^{-1}$; DB2= DKW with 2 mg L$^{-1}$ BAP; and DB4= DKW with 4mg L$^{-1}$ BAP. (Note: The same letters at the same parameter of charts are not significantly different).

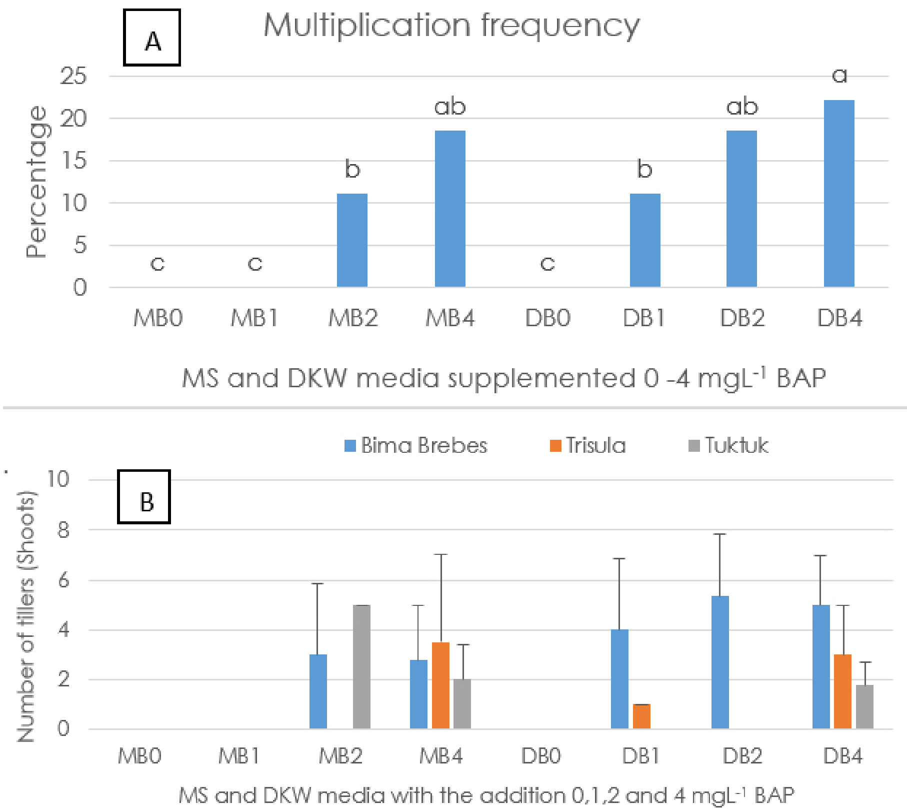


**Figure 3.** Multiplication frequency of *in-vitro* shoot propagation media **(A)** and variation in the number of the tiller produced by three cultivars of TSS seedlings, namely BB=Bima Brebes TS=Trisula TT=Tuktuk **(B)** on MS and DKW medium with the addition of 0-4 mgL$^{-1}$ BAP, 8 WOC. MB0=MS without BAP; MB1= MS with 1mgL$^{-1}$; MB2 = MS with 2mgL$^{-1}$ BAP; MB4= MS with 4 mgL$^{-1}$ BAP; DB0 = DKW without BAP, DB1= DKW with BAP 1 mgL$^{-1}$; DB2= DKW with 2 mgL$^{-1}$ BAP; and DB4= DKW with 4mgL$^{-1}$ BAP. (Note: 1. The same letters in Figure 3A are not significantly different; 2. The averages and standard errors were calculated only from the multiplied plantlets (3B).

BAP and MS medium with 2-4 mgL$^{-1}$ BAP.

Many of the tillers produced by the Bima Brebes cultivar have more than one frequency so that the standard deviation can be calculated. Thought at the beginning of *in vitro* germination, the Bima Brebes TSS cultivar had a considerably higher percentage of ungerminated seeds than the Trisula and Tuktuk TSS cultivar i.e. 33% (data do not show).

The Trisula cultivar had a higher range number of tillers than the Bima Brebes and Tuktuk cultivars when treated with MS media with 4 mgL$^{-1}$ BAP, but only appeared just once frequency and only one new shoot formed on DKW medium with 1 mgL$^{-1}$ BAP. As for the Tuktuk cultivar, apart from being unable to produce new shoots on DKW medium with BAP concentration below 4mg L-1, it also had the lowest range of new shoots. However, when treated with MS medium with 2 mgL$^{-1}$ BAP, the Tuktuk cultivar could produce as many as five new shoots even with one occurrence. The Tuktuk cultivar requires higher BAP concentrations to produce several tillers, whereas, at the early stages of culture initiation, the Tuktuk cultivar produced the highest significantly aseptic seedlings, namely 55.6% (data do not show).

Commonly, the three cultivars of TSS could be propagated on media containing BAP at a concentration of 4 mgL-1 with the range number of tillers 1-7 new shoots in MS and DKW media. In the *in-vitro* shoot initiation using shallot's bulb as an explant, using half strength of MS medium with the addition of 1 mgL-1 BAP can produce just an average of 1,36 shoots per explant (Marlin et al., 2021).

BAP is a synthetic cytokinin that can represent cytokinin activity on plant growth through external application (Liu et al., 2020) and is categorized as an aromatic cytokinin that is widely used in micropropagation due to its low cost and high biological activity as well as its resistance to degradation (Bryksova et al., 2020). The aromaticity arises from the cyclic arrangement of conjugated electrons in the compound structure. This cyclic arrangement increases stability and unique chemical properties, making these aromatic compounds very useful and valuable in chemical reactions (Yasuda, 2023).

Cytokinin biosynthesis varies depending on the kind of cell and tissue. Wu et al. (2021) further indicated that cytokinin concentration and the spatial signal

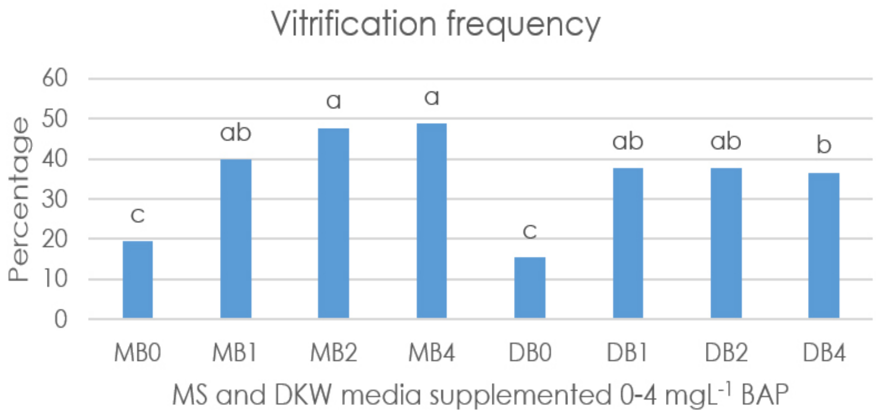


**Figure 4.** Vitrification frequency of the *in-vitro* shoot propagation media on MS and DKW medium with the addition of 0-4 $mgL^{-1}$ BAP, 8 WOC. MB0=MS without BAP; MB1= MS with 1$mgL^{-1}$; MB2 = MS with 2$mgL^{-1}$ BAP; MB4= MS with 4 $mgL^{-1}$ BAP; DB0 = DKW without BAP, DB1= DKW with BAP 1 $mgL^{-1}$; DB2= DKW with 2 $mgL^{-1}$ BAP; and DB4= DKW with 4$mgL^{-1}$ BAP. (Note: The same letters at bars are not significantly different).

transduction of cytokinin determine their effects and activities in distinct cells in different zones. Specific genes modify cytokinin sensitivity by controlling the cytokinin signalling pathway, which controls the shoot apical meristem size (SAM). Cytokinin preserves the SAM's pluripotency or capacity to expand, which supplies stem cells to generate leaf primordia in the early leaf formation stage. The SAM's size and activity, along with the size of the organ that emerges from it, are significantly impacted by the status of cytokinin. SAM's with a higher cytokinin status have more cells and take longer to differentiate, while a lower cytokinin status generates smaller due to fewer cells and earlier differentiation (Werner et al., 2021).

One of the most significant issues with plant tissue culture is vitrification. The mechanisms of vitrification are also known as hyperhydricity or glassiness. The characteristic of vitrified plantlets is that their stems and leaves appear translucent and watery. Typically, vitrified plantlets have thick, curly, elongated dark leaves that cause abnormal development of leaf, stem and root physiology (Bayhan & Yücesan, 2024).

A lack of chlorophyll, accumulation of large starch granules in plastids, cell hydration by the presence of excess fluid in the intercellular spaces, a decrease in cell adhesion and development of large intercellular spaces in the mesophyll, hypo lignification, a reduction in the formation of the epicuticular layer on the leaf surface, alteration in enzyme activity and modifications in protein synthesis as a result of disruption of regular metabolic processes are further signs of hyperhydricity (Polivanova & Bedarev, 2022).

Culture media affected the vitrification frequency in shoot propagation experiment. MS and DKW media supplemented with 1-4 $mgL^{-1}$ BAP had a significantly higher vitrification frequency than MS and DKW media without the addition of BAP. The highest vitrification frequency was found in the MS medium with 2-4 $mgL^{-1}$ BAP. The percentage of vitrification or hyperhydricity in shoot propagation experiments without the addition of BAP ranged from 17,78±7,78 – 18,33 ± 7,46 %, while with the addition of 1-4 $mgL^{-1}$ BAP, the percentage of vitrification tended to be higher, namely ranging from 35,19 ± 8,24 - 50,00 ± 10,45 % in both MS and DKW media than without the addition of BAP (**Figure 4**).

Although most of the macronutrients in the DKW medium, including N, P, K, C, H, O, S, Ca, Mg and vitamins (Thiamin-HCl as well as Nicotinic acid) are higher than in the MS medium (Mirah et al., 2021), their effect was not significantly different of vitrification frequency in this study. According to Polivanova & Bedarev (2022), The main factors influencing the level of hyperhydricity in the plant of *in vitro* culture, besides minerals and hormonal composition of the medium, is the cultivation condition, particularly the aeration of cultivation vessels.

The Bima Brebes cultivar on DKW medium with 1-2 $mgL^{-1}$ BAP induced new shoots with more optimal growth in the number and better appearance of new shoots compared to the addition of 4 $mgL^{-1}$ BAP. The growth of the Bima Brebes cultivar on a DKW medium with 4 mgL-1 BAP appeared to be dwarf and smaller, with less developed leaf shapes (**Figure 5A**). The three TSS cultivars had varied responses to using MS and DKW media with 4mg $L^{-1}$ BAP in plantlet performance. The appearance of the Bima Brebes and Trisula cultivars is almost the same on DKW medium with 4 $mgL^{-1}$ BAP, which appears smaller with more stunted growth than MS media with 4 $mgL^{-1}$ BAP, and this is different from the Tuktuk cultivar which appears more vigour on the same

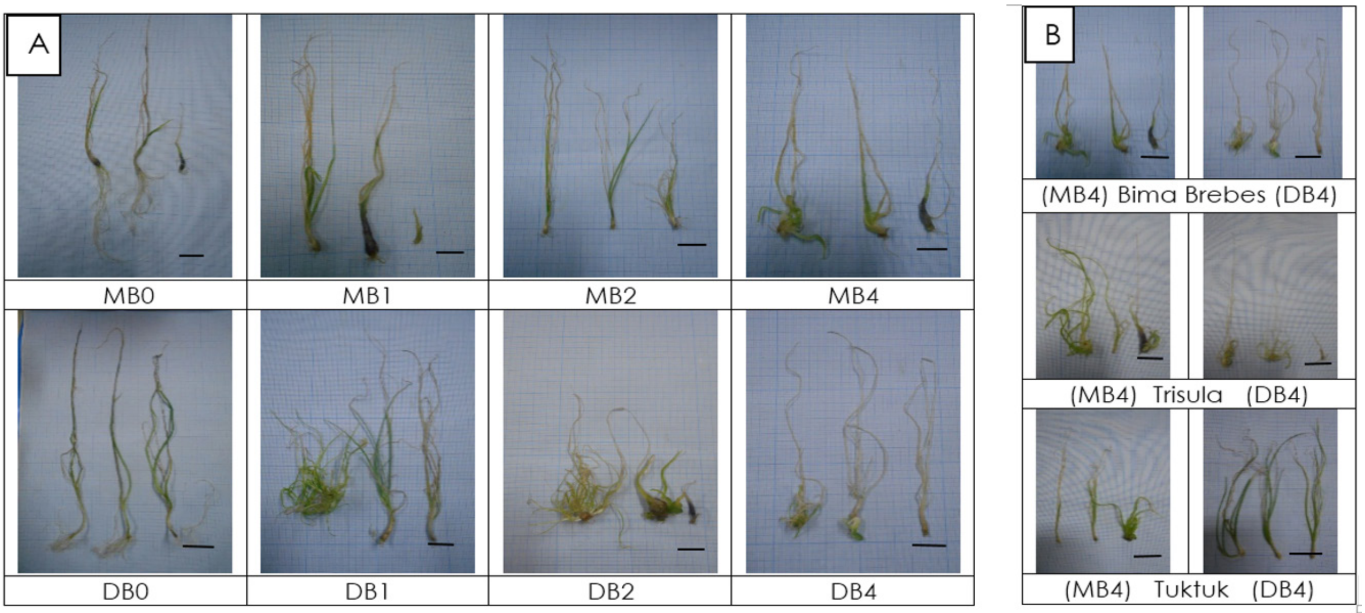


**Figure 5.** The appearance of shoot propagation of *in vitro* TSS seedlings on the combination of MS and DKW medium with the addition of 0 - 4 mg $L^{-1}$ BAP in the Bima Brebes cultivar (5A) and the growth of the tillers from three cultivars of TSS seedlings on MS and DKW media with the addition 4mg $L^{-1}$ BAP (5B), 8 WOC. Note: MB0=MS without BAP; MB1= MS with 1mg $L^{-1}$; MB2 = MS with 2mg $L^{-1}$ BAP; MB4= MS with 4 mg $L^{-1}$ BAP; DB0 = DKW without BAP, DB1= DKW with BAP 1 mg $L^{-1}$; DB2= DKW with 2 mg $L^{-1}$ BAP; BAP; DB4= DKW with 4mg $L^{-1}$ BAP, and = 1 cm.

media with fewer number of new shoot (**Figure 5B**). High multiplication rates using plant growth regulators can steer to more variation of plantlets, and the use of BAP as a plant growth regulator has provided various benefits in shoot cultures in promoting axillary growth, adventitious buds and foliar development. However, abnormalities and off-type plants might occur due to high cytokinin levels, which can lead to somaclonal variation (Razani et al., 2020).

Correlation analysis results between growth parameters of *in-vitro* shoot propagation experiments using MS and DKW media with the addition of 0-4 mg$L^{-1}$ BAP at 8 WOC are illustrated in **Table 1**. The plantlet's fresh weight in this experiment significantly correlated with the root length, number of roots, and leaf length.

The total number of leaves and the number of green leaves had a weak negative correlation ($r \leq 0.399$) with the plantlet fresh weight. Measuring the whole explant's fresh weight is thought to indicate the vegetative growth of the explants and the leaves and roots. In this shoot propagation experiment, the roots part had a stronger correlation with plantlet fresh weight than the leaves part due to BAP use.

The multiplication frequency had a significant correlation with the total number of leaves and a weak correlation with the number of green leaves. Otherwise, the length of leaves, the length of roots and the number of roots had a significantly negative correlation with the multiplication frequency. Even the plantlet fresh weight had a weak negative correlation in this growth parameter. Multiplication in this term describes the process of shoot propagation in plant tissue culture, which aims to increase the number of shoots produced by the explants. In this experiment, vitrification frequency had a significantly negative correlation with the leaf length, the root length, the number of roots, and a weak negative correlation with plantlet fresh weight. However, the vitrification frequency still had a weak correlation with the total number of green leaves and multiplication frequency.

Yang et al. (2023) explained that In seed plants, postembryonic shoot apical meristems develop in leaf axils and are termed axillary meristems, which become buds. As secondary growth begins, axillary meristem has the potential to form branches. Axillary bud can also stay dormant for some time or even permanently. The developmental potential of axillary meristems to form branches is the same as that of embryonic shoot apical meristems. Therefore, the multiplication frequency had a weak correlation with the number of green leaves and a negative correlation with the plantlet fresh weight.

A classically physiological response associated with cytokinins is the regulation of source-sink and nutrient allocation in plants. Sources are photosynthetically active leaves that export photoassimilate to heterotrophic sink tissues, whereas sinks are defined as any organ

**Table 1.** Correlation between TSS aseptic seedling explants growth parameters on *in vitro* shoot propagation media using MS and DKW media with the addition 0-4mgL-1 BAP, 8 WOC.

| | Leaf length (cm) | Total number of leaves (sheet) | Number of green leaves (sheet) | Root length (cm) | Number of roots (piece) | Plantlet fresh weight (gr) | Multiplication frequency (%) | Vitrification frequency (%) |
|---|---|---|---|---|---|---|---|---|
| Leaf length (cm) | 1 | | | | | | | |
| Total number of leaves (sheet) | -0.44 | 1 | | | | | | |
| Number of green leaves (sheet) | -0.33 | 0.89** | 1 | | | | | |
| Root length (cm) | 0.58* | -0.42 | -0.27 | 1 | | | | |
| Number of roots (piece) | 0.58* | -0.48 | -0.32 | 0.88** | 1 | | | |
| Plantlet fresh weight (gr) | 0.56* | -0.22 | -0.06 | 0.74** | 0.74** | 1 | | |
| Multiplication frequency (%) | -0.54* | 0.46* | 0.30 | -0.59* | -0.64** | -0.35 | 1 | |
| Vitrification frequency (%) | -0.47* | 0.16 | 0.08 | -0.49* | -0.52* | -0.21 | 0.15 | 1 |

Note: * significance at $P<0.05$ , ** significance at $P<0.01$

dependent on the import of sugars and amino acids to support growth and development. Sinks include young leaves, reproductive organs, and roots (McIntyre et al., 2021). For a plant to grow and develop properly, the source-sink relationship is essential for vegetative axillary buds, whose activity will determine the branching pattern and, eventually, the architecture of the plant. The axillary bud is an actual sink organ, unable to produce the organic molecules (photoasimilates) needed to promote its growth and development. However, the formation of axillary buds in each leaf axil is genetically controlled but not influenced by endogenous and environmental factors (Doidy et al., 2024).

**Conclusions**

The TSS of Bima Brebes and Trisula cultivars had a significantly different multiplication frequency than the Tuktuk cultivar. DKW media supplemented with 4 mgL-1 BAP had the highest multiplication frequencies but was not significantly different from 4 mgL-1 BAP in MS and 2 mgL-1 BAP in DKW media. The three cultivars had varying responses in shoot appearance to a combination of MS and DKW media with the addition of 1-4 mgL-1 BAP. However, a 1-2 mgL-1 BAP concentration in the Bima Brebes cultivar induced new shoots with a better appearance, even though the multiplication frequency was low. Multiplication frequency negatively correlated with leaf length, root length and number of roots.

**Acknowledgements**

The authors are grateful to The Head of National Research and Innovation Agency (BRIN) and the Deputy for Life Sciences who has initiated the Research-Based Learning Program Scholarship.

**References**

Adin, A., Rohim, F., Haerudin, Fatkhu, R., Harpenas, A. 2023. A Review : TSS (True Shallot Seed) Development in Indonesia and Its Health Benefit. Conference Management System - SEAVEG 2021:208–223.

Bayhan, N., Yücesan, B. 2024. The Impact of Sucrose and 6-Benzylaminopurine on Shoot Propagation and Vitrification in Aronia melanocarpa (black chokeberry). Plant Cell, Tissue and Organ Culture 156: 1–12.

Bryksova, M., Dabravolski, S., Kučerova, Z., Kokás, F., Špundova, M., Plíhalova, L., Takáč, T., Grúz, J., Hudeček, M., Hloušková, V., Koprna, R., Novák, O., Strnad, M., Plíha, O., Doležal, K. 2020. Aromatic Cytokinin Arabinosides Promote PAMP-like Responses and Positively Regulate Leaf Longevity □. ACS Chem. Biol, 15: 1419–1963.

Devano, A., Setiawan, I. 2020. The Effect of Benzyl Amino Purine ( BAP ) Concentration on the Growth Amount of the Explant of Dendrobium spectabile Orchid by. International Journal of Multi Discipline Science 3:33–38.

Dharmayanti, K., Sulistyaningsih, E., Wulandari, R.A. 2018. Callus Induction on True Shallot Seed Explant Using a Combination of BA and 2,4-D. Ilmu Pertanian (Agricultural Science), 2: 137.

Doidy, J., Wang, Y., Gouaille, L., Goma-louamba, I., Jiang, Z., Pourtau, N., Gourrierec, L., Sakr, S. 2024. Sugar Transport and Signaling in Shoot Branching. International Journal of Molecular Sciences 25: 1–17.

Hakim, T., Luta, D. A., Sitepu, D. S. 2022. Teknologi True Shallots Seed dan Pemanfaatan Limbah Pertanian pada Pertumbuhan Produksi Bawang Merah (Allium ascalonicum L.). Seminar Nasional UNIBA - Surakarta, 250–264.

Handayani, E., Basri, Z., Maemunah. 2021. The Growth of Seed Derived Onion on Various Strenght of MS Medium and BAP (Benzyl Amino Purine) Concentrations. Mitra Sains 16–25.

Karo, K., AE, M., Barus, S., Hutabarat, R., Tarigan, R. 2022. Increased Yield of Three True Shallot Seed Varieties by Application of Fish Organic Fertilizer in Tropical Wet Highland (in Indonesian). Jurnal Kultivasi 21: 97–104.

Liu, Y., Zhang, M., Meng, Z., Wang, B., Chen, M. 2020. Research Progress on the Roles of Cytokinin in Plant Response to Stress. International Journal of Molecular Sciences 21: 1–18.

Maintang, Rauf, A. W., Ilyas, A., Syamsuri, R. 2019. The Influence of Varieties and Spacing on Shallot Cultivation from Seeds (True Shallot Seeds/TSS) in Bantaeng District (in Indonesian). Jurnal Pengkajian Dan Pengembangan Teknologi Pertanian 22: 97–106.

Marlin, M., Handajani, M., Yulian, Y., Rustikawati, R., Herawati, R. 2021. Induction of Plantlet Regeneration on Shallot (*Allium cepa* var. *aggregatum*). Proceedings of the International Seminar on Promoting Local Resources for Sustainable Agriculture and Development 13: 239–244.

Masekesa, R. T., Gasura, E., Kujeke, G. T., Ngadze, E., Chidzwondo, F. 2021. Induction of Somatic Embryogenesis and Organogenesis in Zimbabwean Sweet Potato ( cv Brondal ). Advances in Agriculture, Article ID 9976800, 8p.

Mcintyre, K.E., Bush, D.R., Argueso, C.T. 2021. Cytokinin Regulation of Source-Sink Relationships in Plant-Pathogen Interactions. Frontiers in Plant Science 12, 1–13.

Mendes, L.D.S., Eugenia, M., Carvalho, A., Macedo, W.R., Roberto, P., Camargo, D. 2017. In vitro development of sugarcane seedlings using ethephon or gibberellin. Comunicata Scientiae 8: 389–395.

Mirah, T., Undang, Sunarya, Y., Ermayanti, T.M. 2021. The Effect of Cytokinin and Type of Media on Growth of Internode Explants of Stevia (*Stevia rebaudiana* Bert.). Media Pertanian 6: 1–11.

Ossai, C.O., Balogun, M.O., Maroya, N.G. 2023. Organogenesis Versus Somatic Embryogenesis Pathway Efficiencies in In Vitro Propagation of White and Water yams. In Vitro Cellular & Development Biology - Plant 27: 1–11.

Pasternak, T.P., Steinmacher, D. 2024. Plant Growth Regulation in Cell and Tissue Culture In Vitro. Plants, 13: 1–24.

Polivanova, O.B., Bedarev, V.A. 2022. Hyperhydricity in Plant Tissue Culture. Plants 11: 1–12.

Prakoso, T., Alpandari, H. 2021. Potential of Planting Technique Material TSS (True Shallot Seed) on Shallot (*Allium ascalonicum* L.). Agrisintech; Journal of Agribusiness and Agrotechnology 2: 59–66.

PSIH-BSIP. 2025. Pusat Standardisasi Instrumen Hortikultura - Badan Standardisasi Instrumen Pertanian. Benih Sumber Hortikultura: Tanaman Sayuran. online: https://benih-horti.puslithorti.net/frontend/varietas/detail_varietas/2013002. <Accessed on 15 March 2025>

Rantau, D.E., Wulandari, D.R., Maharijaya, A. 2021. Growth Response of Shallot (*Allium ascalonicum* L.) Seedlings Cultured on MS Solid and Liquid Medium Supplemented with BAP, Thiamine and Adenine Sulphate. IOP Conference Series: Earth and Environmental Science 762: 012035.

Razani, M., Kayat, F., Redwan, R. M., Susanto, D. 2020. Detection of Abnormal Banana Plantlets Produced by High BAP Concentration and Number of Subcultures using Representational Difference Analysis. International Journal Agriculture & Biology 23: 541–548.

Shrestha, J., Kandel, M., Subedi, S., Shah, K.K. 2020. Role of Nutrients in Rice (*Oryza sativa* L.): A Review. Agrica 9: 53–62.

Sidik, N.J., Agha, H.M., Alkamil, A.A., Mohammed, M., Alsayadi, S., Mohammed, A.A. 2024. A Mini Review of Plant Tissue Culture : The Role of Media Optimization , Growth Regulators in Modern Agriculture, Callus Induction and the Applications. AUIQ Complementary Biological System 1: 96–109.

Umoh, O.T., Uyoh, V.E., Effiong, E.B. 2020. Review on the Root , Stem and Leaf Initiations in Plants. Asian Plant Research Journal 5: 1–18.

Werner, S., Bartrina, I., Strnad, M., Werner, T. 2021. The Cytokinin Status of the Epidermis Regulates Aspects of Vegetative and Reproductive Development in *Arabidopsis thaliana*. Frontier in Plant Science 12: 1–16.

Wu, W., Du, K., Kang, X., Wei, H. 2021. The Diverse Roles of Cytokinins in Regulating Leaf Development. Horticulture Research 8: 118.

Yang, T., Jiao, Y., Wang, Y. 2023. Stem Cell Basis of Shoot Branching Special Issue – Mini Review. Plant Cell Physol 64: 291–296.

Yasuda, Y. 2023. Organic Chemistry Aromatic Compounds : Understanding the Fragrant World of Organic Chemistry. Journal of Medicinal and Organic Chemistry 6: 43–45.

Zewide, I., & Melash, W. 2021. Review on Macronutrient in Agronomy Crops. Journal of Nutrition and Food Processing 4: 1–7.

Zhang, Y., Ma, Y., Zhao, D., Tang, Z., Zhang, T., & Zhang, K. (2023). Genetic Regulation of Lateral Root Development. Plant Signaling & Behavior 18: 1–17.

Zulfahmi, R., & Tiara, D. 2024. Yield Evaluation Of Seven Shallot Genotypes. Jurnal Agrotek Lestari 10:129–136.

**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.