Optimalization and regeneration of in vitro seedling of Shallot variety Lembah Palu in providing good quality seedling

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Abstract. This research aimed to determine suitable bulb size and proper concentration of benzylamino purine (BAP) to produce vigor plantlets. The research was arranged as factorial completely randomized design with two factors. The first factor was bulb diameter with three levels (0.8, 1.0 and 1.2 ± 0.05 cm). The second factor wasBAP concentration with three levels (2, 4 and 6 mg/L). All treatments were replicated 4 times, thus there were 36 experimental units. Each unit consisted of 3 explants, so there were 108 samples. The results showed that largest bulb of 1.2 cm ± 0.05 in diameter and addition of BAP 4 mg/L to Murashige Skoog basal medium were excellent for producing vigour planlets. There were interactions between the two treatments on number of leaves and root paremeters.

1. Introduction

Shallot var. lembah palu (*Allium wakegi* Araki) is a horticulture commodity as the ingredient for Palu's fried onion. However, its productivity is still low due to the less availability of qualified seeds and limited seed production technology. Statistic data showed that in 2011, the productivity reached 7.84 tons per ha declining to 4.12 tons per ha in 2012 and 3.37 tons per ha in 2013 [1]. One of the causes of the decline was the less availability of qualified seeds and seed production technologies [2].

Conventionally, farmers use bulbs as seeds, while the bulbs have a period of dormancy, requiring storage time prior to germinate [3]. Thus, it is urgent to develop a protocol to provide a rapid multiplication of the seeds, through in vitro culture. Through this method, uniform-free of pests and diseasesand true to type planlets can be produced, in a relatively shorter time, regardless climatic factors [4,5].

Generally, embryogenesis studies on onions were directed to produce plantlets through several experiments [6,7]. While the production of planlet through organogenesis has several advantages, among others, require shorter time and the resulted planlets are more vigour as they grow from larger explants. According to Taji *et al* [8], small size of explants is very risky to damage and failure at the stage of initiation. However, in some studies, it is not directly proportional to the results obtained [9. 10]. Constraints faced in the production of plantlets through organogenesis using large explants include high level of contamination and inappropriate use of growth regulators.

Benzylamino purine (BAP) is a synthetic growth regulator of cytokinin group frequently used for in vitro shoots induction of various species. The concentration used in Allium genus such as garlic [11,12,13] and onion was various. In micro propagation of *Allium cepa,* Kamstaityte and Stanys [14] reported that number of micro shoots formed was genotype dependent and number of shoots per explant

increased 1.0 to 2.1 as BAP concentrations increased from 0.9 to 4.4 μM, while higher concentrations significantly reduced the shoots generated. Using explant of somatic embryo cultured in MS basal medium [15] enriched with 0.1 mg/L naphthalene acetic acid, [9]. Recommended the addition of 5.0 mg/L BAP to generate micro shoots. Different study, using somatic embryos. recommended the addition of 1.5 mg/L BAP for plantlet regeneration. Therefore, it is crucial to conduct research on producing planlets of lembah palu shallot in various bulb diameter and benzylamino purine concentrations.

2. Materials and Methods

2.1. Explant Source

Bulbs of the shallot (var. Lembah Palu) were used as explant with criterions: healthy, shiny of outer shells, solid, pest-and-disease-free and dry. The bulbs were grouped into three sizes based on their diameters namely: 0.8, 1.0 and 1.2 ± 0.05 cm, noted as D1, D2 and D3 respectively. Each experimental unit consisted of three explants and replicated 4 times. The samples were then sterilized according to [2], modified by chilling in 9 ^{o}C in 100 mg/L vitamin C for 24 hours before planting.

The basal media used was Murashige-Skoog with 30% sucrose, enriched with 0.1 mg/L NAA and BAP in three different concentrations (2, 4 and 6 mg/L), solidified with 8.0 g/L agar. The pH of each media was adjusted to 5.7 to 5.8 using 0.5 N NaOH or 0.5 N HCl, prior to autoclaving at 121^{o}C, 17.5 psi for 15 minutes.

2.2. Experimental Design

The experimental research was arranged as factorial completely randomized design with two factors. The first factor was bulb diameter consisting of three levels: 0.8, 1.0 and 1.2 cm ± 0.05. The second factor was BAP concentration consisting of three levels: 2, 4 and 6 mg/L. All treatment combinations were replicated 4 times, with 3 explants per experimental unit, thus there were 108 total samples. Observed parameters included time when shoots appeared, number of shoots, roots and leaves formed and leaf length 20 days after planting. Effect of the treatments on the observed parameters was analyzed using analysis of variance (ANOVA) and the mean differences between the treatments were tested using Honest Significant Differences [8] [10] at the 5% level calculated statistically using Microsoft Excel 2010.

2.3. Culture Conditions

All cultures were incubated in a sterile room, placed on a shelf under fluorescent lamps (TL 40 Watt, 2000 lux) with continuous light 24 hours. Room temperature is maintained at 20 ˚C *with air conditioner.*

3. Results and discussion

There were interactions between the treatments in number of leaves and roots formed. Recapitulation of the analysis is shown in table 1.

Table 1. Recapitulation of Analysis of Variance on The Observed Parameters.

Sources of Variancy	Number of Shoots	Number of Roots	Number of leaves	LeavesLength (cm)
Bulb diameter (D)	**	**	**	**
BAP Concentration (C)	**	*	**	**
DxC Interaction	ns	**	**	ns

Notes: ** Significant at 1% level*Significant at 5% level ^{ns}Non significant

The average number of shoots, roots and leaves obtained from the various BAP concentrations in the three different bulb diameters were shown in figures 1, 2, 3 and 4.

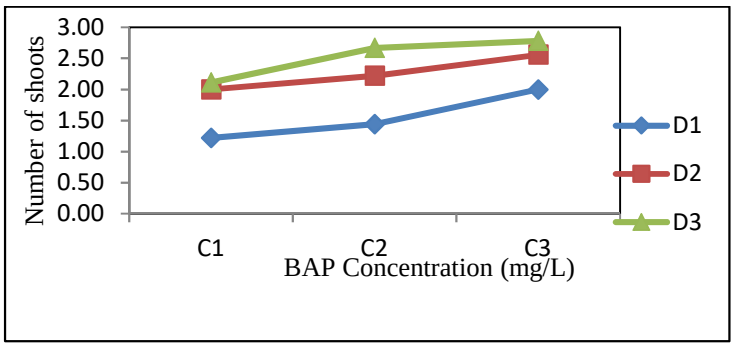

Figure 1. Number of Shoots at Various BAP Concentrations and Bulb Diameters.

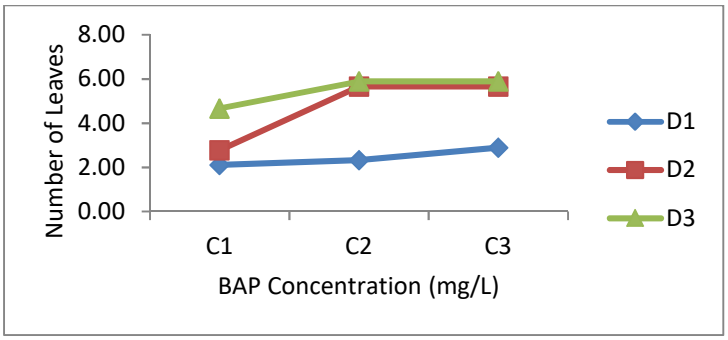

Figure 2. Number of Leaves at Various BAP Concentrations and Bulb Diameters

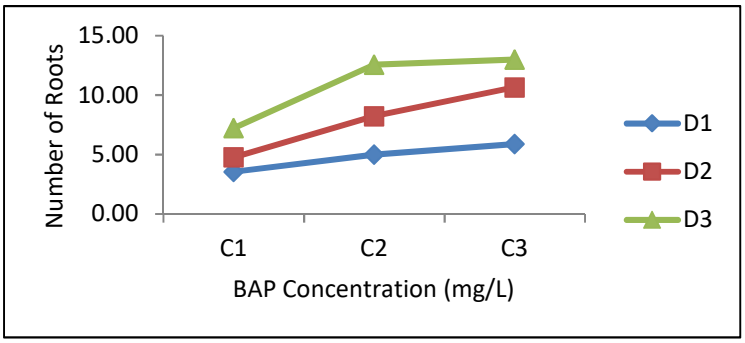

Figure 3. Number of Roots at Various BAP Concentrations and Bulb Diameters.

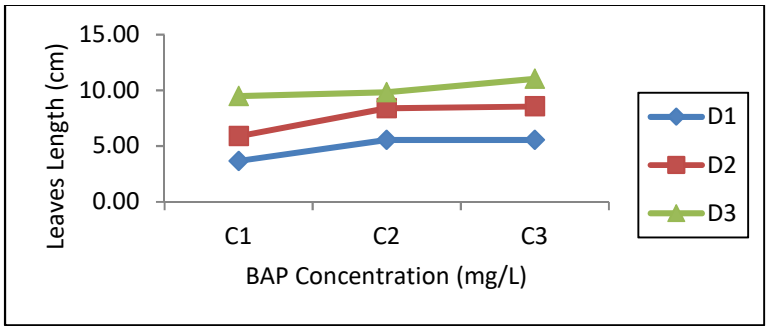

Figure 4. Leaf Length at Various BAP Concentrations and Bulb Diameters.

The largest bulb size (1.2 cm ± 0.05 in diameter) gave the most vigorous planlets. Growth responses seemed to be faster between 2 - 4 mg/L BAP and tended to slow down between 4-6 mg/L (Figures 1-3), especially in the largest bulbs. This is in line with [11] statement that plant growth regulators in plants work at low concentrations, and too high concentration will inhibit the growth. Although 6 mg/L gave the highest value on some parameters, the number was not significantly different from those in 4 mg/L. This indicates that the concentration of 4 mg/L BAP is better to be applied for the growth of the bulbs. Compared to 5.0 mg/L BAP used to regenerate micro shoots [9], using this procedure, it could be obtained more vigor plantlets using lower BAP concentration (4 mg/L).

Visual observations showed that the quality of plantlets produced at 4 mg/L BAP with large diameter of bulbs was better with relatively larger leaves, longer lasting green color, fresher than planlets at 6 mg/L turning into senesence phase earlierly (Figure 6). In some species, multiplication responses to the addition of BAP were better than other cytokinines [1],[3].

In propagation of Sumenep onion using apical meristems, [13] recommended the addition of 2 mg/L BAP, with the highest number of leaves and roots obtained were 4 and <2 per explant, respectively. The use of large explants is meant to provide more energy to grow. As expected, that the explants contain much reserves of nutrients and endogenous plant growth regulators, although the potential for contamination is also greater and can affect survival rate as in other species [6]. The large explants in this experiment, better planlets obtained with maximum number of shoots (2,52), root (10,93), leaf (5,48) and leaf length (10,12 cm) (figure5).

Figure 5. Plant Growth in Various BAP Concentration of the Largest Bulbs (1.2 ± 0.05 cm) 20 HST. A. 2 mg/L B. 4 mg/L, and C. 6 mg/L

To accelerate the bulbs growth, one third of the bulb tips were removed, as the procedure gave better growth compared to removing a half and two-thirds of the tip s*ex vitro* [18] (figure 6). Addition of BAP did not affect the time of shoots appeared, which in average occurred very quickly (1 day after planting). This indicates that the process of germination in the shallot is highly determined by internal growth potencies of the explants rather than media composition. [11] explained that seed germination is initiated by water imbibition process into the outer seed coat by matrix pressure due to hydraulic properties of water. Furthermore, [19] stated that the essential environmental conditions for germination are water, oxygen and temperature during imbibition process and even in the next stage. [14] reported that germination progresses faster at lower media strength, which was thought to have higher matrix pressures.

This *Allium wakegi* Araki species does not produce flower [16], so it cannot produce true seeds that is more sterile to be used as explants, as in other *Allium* species [19] and [20].

Figure 6. Early Growth of Shallots from Different Bulb Sizes.
A. Initial bulbs growth of different sizes. B. Bulbs of various diameter

Therefore, improved sterilization techniques should be performed to increase the percentage of sterile cultures. Soaking explants at low temperature (chilling) to a solution of 100 mg/L of vitamin C for 24 hours, gave a good result with a decrease in the percentage of contamination (preliminary test, data not shown). [21] reported, chilling tulip bulbs at temperatures 5 ˚C a few weeks could reduce contamination. [22] recommended cold store for 3-8 weeks and an increase in cytokinin level for cultures with no multiplication due to lack of cytokinin, dormancy and need for low temperature. [23] reported, soaking explants *Platanus occidentalis* in vitamin C 10 mg/L reduced levels of contamination and browning effectively.

4. Conclusion

It can be concluded that large diameter bulb of lembah palu shallot and the addition of BAP 4 mg /L into basal Murashige Skoog media are recomended to produce vigor planlets. There were interactions between the two treatments on number of leaves and roots formed at 20 days after planting.

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