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The effect of tryptone and tomato juice addition on the growth performance of strawberry (*Fragaria ananassa*) explant under *in vitro* condition

Abstract. Strawberries are a fruit commodity that has high economic value. The obstacle in strawberry production is the lack of high-quality seed availability with disease-free properties. Tissue culture offers a promising solution to increase both the quantity and quality of strawberry seeds. This study aims to assess the effect of different concentrations of tomato juice and tryptone on the growth of strawberry explants under *in vitro* growing condition. The research was conducted at the Plant Breeding Laboratory, Faculty of Agriculture, Universitas Jenderal Soedirman, from December 2022 to May 2023. The study employed a Completely Randomized Design (CRD) with two factors. The first factor was tomato juice (Z), consisting of Z0 = 0 mL/L, Z1 = 50 mL/L, Z2 = 100 mL/L. The second factor was tryptone (T), consisting of T0 = 0 g/L, T1 = 1 g/L, T2 = 2 g/L, T3 = 3 g/L. The addition of 50 mL/L tomato juice solely (T0Z1) resulted in the highest plant height, number of leaves, number of branches, and leaf width. Meanwhile, the addition of2 g/L tryptone solely showed the highest germination rate.

Keywords: Disease-free explant · Tomato juice · Tryptone

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Introduction

Strawberries are a sub-tropical fruit commodity with significant potential for development in Indonesia, particularly in highland areas. Strawberries are rich in phytochemicals, especially phenolic compounds, which offer health benefits (Nofrianinda et al., 2018). The Indonesian Central Statistics Agency reported that strawberry production in Indonesia in 2021 was 9,860 tonnes, marking a decrease from 2017 when it was capable of producing up to 12,225 tonnes. The decrease in strawberry production is partially related to the availability of an adequate supply of disease-free seeds, because the propagation via stolons is the potential transmission of diseases from the parent plant to the daughter plant, such as *Plomobacter* (Dittmer et al., 2021).

Utilizing tissue culture for strawberry propagation can serve as a solution to increase strawberry seed production and produce disease-free seedlings (Mohapatra & Batra, 2017). Tissue culture is a technique that involves isolating plant components like cells, tissues, or organs and cultivating them in a sterile environment to enable these components to reproduce and regenerate into complete plants (Loyola-Vargas & Ochoa-Alejo, 2018). Furthermore, the propagation through tissue culture offer the benefits of requiring minimal storage space, enabling the acceleration of plant growth cycles, and uniform seedling growth by controlled environment (Espinosa-Leal et al., 2018).

The composition of the culture medium utilized depends on the specific plant being propagated. This medium typically includes agar, mineral salts, vitamins, and growth regulators such as plant hormones (Semiarti, 2018). The medium commonly used in strawberry plant culture is Murashige and Skoog (MS) medium, characterized by its high content of inorganic salts. Increasing the regeneration of explants requires the addition of growth regulators to the growth medium. Tomato juice accelerates seed growth because it contains various organic compounds such as carbohydrates, amino acids, and growth hormones (Yunita et al., 2023). The growth regulator present in tomato juice plays a role in chlorophyll formation in plants. The cytokinin content is involved in cell division within meristem tissue (Dewi et al., 2021). Furthermore, adding tomato extract to the medium can accelerate germination, increase chlorophyll biosynthesis, and suppress embryo death (Muharyati et al., 2016).

The application of amino acids to plants can enhance growth by increasing plant dry weight, chlorophyll content, nitrate reductase activity, and glutamine synthetase enzyme activity (Jalali et al., 2020). Tryptone, as a source of amino acids, has been reported to promote the growth of *Cymbidium* orchids (*Cymbidium kanran* Makino). Tryptone provides a diverse array of nutrients, including nitrogen, carbon, sulfur, and various micronutrients, which can serve as a nutrient source for plants instead of other types of amino acids (Zhu et al., 2019) Additionally, the nitrogen content in tryptone, which is as high as 13.3%, is reported can increase the number of nodes in *Eustoma grandiflorum* flowers (Ohta et al., 2004). This research aimed to evaluate the influence of various concentrations of tomato juice and tryptone on the growth of strawberry explant under *in vitro* condition.

Materials and Methods

This research was conducted at the Plant Breeding and Biotechnology Laboratory, Faculty of Agriculture, Jenderal Soedirman University, from December 2022 to May 2023. The materials used in this research included Mencir strawberry seeds, Murashige and Skoog medium, distilled water, tomatoes, tryptone, sucrose, agarose, KOH solution, HCl solution, and 70% alcohol. Additionally, the following equipment were used: Laminar Air Flow Cabinet, tape, plastic wrap, autoclave, analytical balance, pH meter, magnetic stirrer, culture bottle, alcohol bottle, beaker, measuring flask, gas stove, pan, stirrer, micropipette, spatula, tweezers, blade, scalpel, masking, Petri dishes, matches, and Bunsen burner.

Sterilization using 30% Clorox (with 5.25% NaOCl content) soaking for 10 minutes. Then rinsed with sterile distilled water which was repeated 3 times.

This study used a Completely Randomized Design (CRD) with two factors, which were the treatments involving tomato juice and tryptone. The first factor, i.e., tomato juice, consisted of three levels: Z0 (control or no tomato juice), Z1 (50 mL/L), and Z2 (100 mL/L), while the second

factor, i.e., tryptone, consisted of four levels: T0 (control or no tryptone), T1 (1 g/L), T2 (2 g/L), T3 (3 g/L).

The determined tryptone concentration was followed previous study by Silva (2014), which suggests 2 g/L of tryptone for MS enrichment to enhance the growth and development of *Protocorm Like Bodies* (PLBs) in *Cymbidium*. While the concentration of tomato juice used refers Dwiyani et al. (2015), i.e., tomato juice concentration of 100 mL/L for stimulating explant growth.

Tomato juice was prepared in a 1:1 ratio (1000 g of tomatoes mixed with 1000 ml of distilled water) and subsequently blended until it achieved a smooth consistency. The resulting tomato pulp was filtered into an Erlenmeyer flask to obtain a stock solution of tomato juice with a concentration of 100%. This tomato juice stock was then utilized to create treatments of 50 mL/L and 100 mL/L.

The variables observed were germination percentage, germination rate, plant height, number of leaves, number of branches, leaf width, and root length. The experimental design used was a CRD with four replications with a total of 96 experimental units. The data obtained was tested using analysis of variance or ANOVA. Variations between treatment means were analyzed using the Duncan Multiple Range Test (DMRT) with an error rate of 5%.

Results and Discussion

Germination Percentage. Germination percentage is a parameter used to measure the success rate or the percentage of seeds that successfully germinate in this research. The analysis of variance showed that each treatment of tomato juice and tryptone affected the percentage of strawberry germination (Table 1).

The analysis showed that the highest germination percentage occurred in the treatment without tomato juice (0 mL/L), with a result of 0.52%. Applying 50 mL/L and 100 mL/L of tomato juice significantly reduced the germination percentage. The reduction in germination percentage is associated with the inhibitory properties of tomato juice, which contains organic acids known to have a growth-retarding effect (Bidhari et al., 2018). In line with research conducted by Yunita et al., (2023), the utilization of 12 mL/L tomato extract led to a reduced number of shoots compared to the absence of tomato extract application. Excessive application of tomato fruit extract can lead to an imbalance of nutrients in the growth medium. While tomato fruit extract may contain beneficial nutrients for plant growth, such as vitamins and minerals, an overabundance of certain components may disrupt nutrient uptake and utilization by the plants, thereby hindering their growth (Mastuti et al., 2017). Additionally, its acidity and the presence of caffeic acid and ferulic acid are linked to the suppression of plant growth (Bubna et al., 2011).

The use of 1 g/L tryptone led to a decreased germination percentage, although it did not differ significantly from the application without tryptone. However, increasing tryptone concentrations to 2 and 3 g/L further decreased the germination percentage. This aligns with research findings of Kovács et al. (2012) that high concentrations of amino acids in the growth medium can induce osmotic stress on plants. So that, excessive amino acids may limit the plant's access to essential resources needed for growth.

Table 1. The effect of various concentrations of tomato juice and tryptone on the germination percentage of strawberry explants

Treatment	Germination percentage	
Tomato juice		
0 mL/L	0.524	a
50 mL/L	0.333	b
100 mL/L	0.290	b
Tryptone		
0 g/L	0.528	a
1 g/L	0.421	a
2 g/L	0.360	ab
3 g/L	0.222	b

Note: Numbers followed by different letters in the same factor of treatment show significant differences based on Duncan's Multiple Range Test (DMRT) at an error level of 5%

Germination Rate. The analysis of variance showed that tomato juice affected the percentage of strawberry germination (Table 2).

The highest germination rate was found in the tryptone treatment with a concentration of 2 g/L, without tomato juice. Haghighi et al., (2022) explained that as an amino acid, tryptone plays a role in stimulating plant growth and development. Al-Juthery et al., (2019) also added that Amino acids are important in the synthesis of hormones and secondary metabolites. In addition, amino acids play a role in the

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formation of coenzymes and play a role in plant metabolism. Amino acids contribute to increasing the ability to absorb nutrients from the growing medium to increase vegetative growth (Baqir et al., 2019).

The application of tomato juice without tryptone was not able to increase the germination rate in strawberry seeds. However, when tomato juice was combined with tryptone, it resulted in a decrease in the germination rate across all treatment combinations. Tomato juice and amino acids can serve as substrates for microbial growth in the growth medium. This result related with the research of Saepudin et al. (2020), applying organic materials to the media can elevate microbial contamination. Microbial activity may alter the chemical composition of the medium, produce metabolites that affect plant growth, or compete with plants for resources. Changes in microbial communities or metabolite production can influence plant growth negatively.

Table 2. The effect of various concentrations of tomato juice and tryptone on the germination rate of strawberry explants

Tomato juice (mL/L)	Tryptone			
	0 g/L	1 g/L	2 g/L	3 g/L
0	0.57 A b	0.61 A b	1.05 A a	0.97 A a
50	0.70 A a	0.33 B bc	0.63 B ab	0.12 C c
100	0.70 A a	0.65 A a	0.37 C b	0.58 B ab

Note: Numbers followed by different capital letters in the same column or lowercase letters at the same line show significant differences based on Duncan's Multiple Range Test (DMRT) at an error level of 5%.

Plant Height. Enhancing plant height is a physiological process involving cell division and elongation. This process is a form of the influence of plants on the surrounding environment. The results of the analysis of variance showed that there was a significant effect on the treatment of tomato juice and tryptone on strawberry plants. The effect of tomato juice and tryptone concentrations on strawberry plant height is presented in Table 3.

Medium enriched with 50 mL/L tomato juice and without tryptone gave the best results for plant height, namely 6.78 cm, but it is not significantly different from without the application of tomato juice. This is related to the cytokinin content in tomato juice which actively plays a role in cell division and bud formation (Setiari et al., 2016). In addition, tomatoes contain phosphorus, calcium, iron, tyramine, vitamins A, C and K, which can increase plant growth and development (Semiarti et al., 2010).

Table 3. The effect of various concentrations of tomato juice and tryptone on the strawberry plantlet height

Tomato juice (mL/L)	Tryptone			
	0 g/L	1 g/L	2 g/L	3 g/L
0	4.60 B a	1.62 A b	4.76 A a	2.70 A b
50	6.78 A a	0.41 A b	0.41 B b	0.11 B b
100	0.65 C a	0.70 A a	0.78 B a	0.72 B a

Note: Numbers followed by different capital letters in the same column or lowercase letters at the same line show significant differences based on Duncan's Multiple Range Test (DMRT) at an error level of 5%.

Number of leaves. The results of the analysis of variance showed that there was a significant effect on the treatment of tomato juice and tryptone on strawberry plants. The effect of tomato juice and tryptone concentrations on number of strawberry leaves is presented in Table 4.

Table 4. The effect of various concentrations of tomato juice and tryptone on the number of leaves of strawberry plantlet

Tomato juice (mL/L)	Tryptone			
	0 g/L	1 g/L	2 g/L	3 g/L
0	3.37 A a	0.37 A b	3.50 A a	1.62 A ab
50	3.62 A a	0.12 A b	0.12 B b	0.00 A b
100	0.12 B a	0.75 A a	0.25 B a	0.75 A a

Note: Numbers followed by different capital letters in the same column or lowercase letters at the same line show significant differences based on Duncan's Multiple Range Test (DMRT) at an error level of 5%.

Table 4 showed that the of tomato juice and tryptone concentrations was able to produce the highest number of leaves. However, the number of leaves of plantlets grown on MS 0 + 50 mL/L tomato juice and MS 0 + 2 g/L tryptone media was similar compared to plantlets grown on MS 0 media. Plantlets with the higher average number of leaves were found in MS 0 medium, medium enriched with 2 g/L tryptone, and medium enriched with 50 mL/L tomato juice respectively 3.37; 3.50; and 3.62 leaves. The increase in the number of leaves is expected to enhance plantlet growth due to the higher energy generated through photosynthesis.

The medium enriched with 50 mL/L of tomato juice, but no real difference from controls, resulted the highest number of leaves because tomato juice serves as a source of

vitamins, fats, proteins, and growth regulators, including cytokinin. The cytokinin hormone is involved in leaf development and can influence the process of cell division in meristem tissue. This is in accordance with the research from Mokoginta et al. (2021) that cytokinin application in *in vitro* culture of *Dendrobium* sp. orchids can induce shoots and increase the rate of plant multiplication, where cytokinin play a role in plant cell division activity.

Medium enriched with 2 g/L tryptone increases the number of leaves because tryptone, as a source of amino acids play critical roles in various cellular processes, including cell division, elongation, and differentiation (Takatsuka & Umeda, 2014). During leaf formation, nitrogen is incorporated into proteins that are involved in structural components of cells, enzymes, and regulatory proteins (Shah et al., 2017) necessary for leaf growth and development.

Number of branches. Branches are shoot organs that extend laterally and function to help supply carbohydrates to the plantlets. The analysis of variance showed an influence of tomato juice and tryptone on the number of strawberry explant branches (Table 5).

Table 5. The effect of various concentrations of tomato juice and tryptone on the number of branches of strawberry plantlets

Tomato juice	Tryptone			
(mL/L)	0 g/L	1 g/L	2 g/L	3 g/L
0	3.37 A a	0.37 A b	3.50 A a	1.62 A ab
50	3.62 A a	0.12 A b	0.12 B b	0.00 A b
100	0.12 B a	0.75 A a	0.25 B a	0.75 A a

Note: Numbers followed by different capital letters in the same column or lowercase letters at the same line show significant differences based on Duncan's Multiple Range Test (DMRT) at an error level of 5%.

Table 5 showed that the combination of tomato juice and tryptone concentrations was able to produce the highest number of branches, with an average number of leaves of 3.625 found in MS 0 medium, medium enriched with 2 g/L tryptone, and medium enriched with 50 mL/L tomato juice respectively 3.37; 3.50; and 3.62 leaves.

Medium enriched with 50 mL/L tomato juice showed that branches reached 3.62, but not significantly different from tryptone 2 g/L. The increase in the number of branches is related to the cytokinin content in tomato juice. Aiman et al. (2022) also said that the cytokinin ZPT contained in tomato juice accelerates the cell division process, producing more branches. This follows the opinion of Wróblewska (2013) that growth regulators, especially cytokinins, can stimulate branching (axillary buds) growth by triggering apical buds' dormancy to produce branches.

Medium enriched with 2 g/L tryptone increased the number of leaves. This is related to amino acids, which can function to improve nutrient availability and uptake, which can increase photosynthetic activity and photosynthate accumulation (Wróblewska, 2013). Amino acids are the building blocks of proteins, including enzymes involved in photosynthesis such as rubisco. Adequate amino acid availability supports the synthesis of these enzymes, optimizing photosynthetic efficiency (Rydzy et al., 2021).

Figure 1. illustrates that applying 50 mL/L of tomato juice without tryptone results in a broader leaf area, whereas applying 2g/L of tryptone without tomato juice leads to a higher number of branches. This suggests that the tomato extract content, particularly the phytohormone cytokinin, may stimulate the development of wider leaves, while the application of tryptone as a source of amino acids, the precursors of proteins, can enhance branching. Proteins play a crucial role in various biological functions, including cell structure and plant metabolism.

Figure 1. a. Plantlet growth on MS0 medium; b. Plantlet growth on MS0 + 50 mL/L tomato juice c. Plantlet growth on MS 0 + 2 g/L tyrptone

Leaf width. The analysis of variance showed an influence of tomato juice and tryptone on the leaf width of strawberry explants (Table 6).

Table 6. The effect of various concentrations of tomato juice and tryptone on the leaf width (cm) of strawberry plantlets

Tomato juice	Tryptone			
(mL/L)	0 g/L	1 g/L	2 g/L	3 g/L
0	1.77 B a	0.24 A b	1.78 A a	0.54 A b
50	2.55 A a	0.00 A b	0.05 B b	0.00 A b
100	0.05 C a	0.24 A a	0.00 B a	0.35 A a

Note: Numbers followed by different capital letters in the same column or lowercase letters at the same line show significant differences based on Duncan's Multiple Range Test (DMRT) at an error level of 5%.

Table 6 shows the broadest leaf width in the medium treatment enriched with 50 mL/L tomato juice, namely 2.55 mm. Bidhari et al., (2018) reported that the application of tomato extract also increased the length of banana cv Ambon leaves under *in vitro* condition. Malinda et al., (2022) also stated that the auxin content in tomatoes can help the process of organogenesis, somatic embryogenesis, and shoot growth in micropropagation.

Large leaf area increases the rate of plant photosynthesis. Photosynthate will support plant tissue cells' work, thereby accelerating growth and development, for example, leaves, stems, and roots (Hemon et al., 2022). The accumulated photosynthate forms new organs and is proven by the plant height and leaf area being higher than others.

Conclusion

Applying 50 mL/L tomato juice increased plantlet height, number of leaves, branches, and leaf width but reduced the germination percentage. In addition, applying 2 g/L tryptone increased the germination rate and number of leaves and branches of strawberry plantlet under *in vitro* condition.

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