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Effect of plant growth regulators and drought duration on physiology, growth and yield of soybeans (*Glycine max* (L.) Merrill) at the pod growth stage

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ARTICLE INFO	ABSTRACT
Research Paper Received: December 06, 2023 Revised: February 19, 2024 Accepted: February 29, 2024 Keywords Brassinolide Drought stress Gibberellic acid Salicylic acid Soybeans *Corresponding author Phan Hai Van Email: phvan@hcmuaf.edu.vn	During the pod growth stage, soybeans (<i>Glycine max</i> (L.) Merrill) are very susceptible to drought. The research was carried out to investigate the extent of damages by different durations of drought on soybean plants during pod growth, and the capabilities of different plant growth regulators (PGRs) to mitigate these damages. The two-factor experiment was conducted in a completely randomized design, including 12 treatments with 3 replications. Factor A was the drought durations: 0 days (D0), 4 days without irrigation (D4), 8 days without irrigation (D8); and factor R was the PGR treatments: water (W), salicylic acid (SA), brassinolide (BR), and gibberellic acid GA₃ (GA). Results showed that no differences in plant growth and development due to drought durations or PGRs were observed. However, longer drought durations severely reduced the yield by about 83.8% compared with control. Among the three PGRs used, brassinolide was most effective in mitigating drought stress effects on soybean plants, improving the total number of firm pods (35.0 pods), firm seeds (70.8 seeds), the weight of 100 seeds (16.6 g), and productivity (195.9 g). However, spraying brassinolide did not affect the protein, proline, and pigment contents.

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1. Introduction

Soybeans (*Glycine max* (L.) Merrill) are a major food source with high nutritional and economic value, and an essential short-term industrial crop, the second most important source of cooking oil globally, and accounting for more than two third of global vegetable protein

meal (OECD-FAO, 2020). In addition, soybeans are also a major source of food for livestock and raw materials for the processing industry (Ngo et al., 1999), as well as being important for soil improvement.

However, soybeans are also very susceptible to drought (Dede et al., 2022). Moderate to

severe drought could cause loss in yield of soybean from 10.4% to 21.8%, 1.56 to 3.25 times higher than that of maize (Wang et al., 2020). Especially, if drought occurs during the pod growth stage, which is an important stage that determines seeds formation and growth, it can result in major loss in yield and seed quality, which could be up to 72 - 82% (Wei et al., 2018).

The use of plant growth regulators (PGRs) to induce drought tolerance in soybeans is receiving attention because many recently discovered PGRs show the capabilities to induce stress tolerance and adaptation responses (Dede et al., 2022). However, the effectiveness of different PGRs may vary depending on the duration of drought, but studies on this topic are still very limited. Therefore, the study “Effect of plant growth regulators and duration of drought on physiology, growth and yield of soybeans (*Glycine max* (L.) Merrill) at the pod growth stage” was conducted.

2. Materials and Methods

2.1. Time and location

The experiment was conducted at the Experimental Farm of the Faculty of Agronomy, Nong Lam University Ho Chi Minh City, Vietnam from May 2022 to November 2022.

2.2. Materials

The soybean variety HLDN 910 was selected from the cross combination of HL 203 × OMDN 1.

Black plastic pots (28 × 23 × 23 cm in size, top diameter × bottom diameter × height)

Salicylic acid (SA): Merck, Germany

Brassinolide (BR): Merck, Germany

Gibberellic acid (GA₃): Merck, Germany

Fertilizer formula: 40 - 60 - 60 (N - P₂O₅ - K₂O)

Soil was taken from Xuan Thoi Thuong commune, Hoc Mon, Ho Chi Minh City, and analyzed at the Research Institute for Biotechnology and Environment, Nong Lam University, Ho Chi Minh City (Table 1).

Table 1. Physical and chemical properties of soil used in the experiment

Texture (%)				pH	Organic matter (%)	Total N (%)	Total P ₂ O ₅ (%)	Total K ₂ O (%)
Clay	Silt	Fine sand	Coarse sand					
11.47	18.68	31.89	37.96	8.04	2.62	0.10	0.24	0.23

2.3. Experimental design

The two-factor experiment was arranged in a completely randomized design (CRD), with 3 replications, each with 12 pots, 36 pots in total (3 plants/pot). The factors included type of PGR (R factor) with four types: W (water), SA (salicylic acid, 69 ppm), BR (brassinolide, 0.1 ppm), GA (GA₃, 75 ppm); and duration of drought stress

(D factor) with 3 levels: D0 (no drought), D4 (drought in 4 days), D8 (drought in 8 days).

2.4. Cultivation and treatment application methods

Seeds were sown in pots containing 10 kg soil/pot. Pots were watered until leaking every day. The fertilizer formula (kg/ha) of 40 - 60 - 60

(N - P_2O_5 - K_2O ; Nguyen et al., 2018) was used for all treatments, equivalent to 0.84 g urea, 3.6 g superphosphate, and 0.96 g potassium chloride per pot. All the phosphate fertilizer was mixed with the soil before potting. Half the amount of nitrogen and potassium was applied by top-dressing when the plants had 1 - 2 true leaves, the rest was applied when the plants reached 4 - 5 true leaves.

When 50% of plants had pods longer than 0.5 cm (49 days after sowing (DAS)), the PGRs were applied as foliar spray (100 mL per experimental plot). The next day, drought treatments were started by withholding irrigation for the durations according to each treatment. After the drought periods, plants were watered normally until harvest time.

2.5. Measured traits

Five plants of five different pots in each plot were selected randomly for the measurement of growth, yield components, and physiological traits. The plant height and number of leaves were measured on 54 DAS, and the root length, fresh and dry biomass was recorded on 60 DAS. The weight of 100 seeds (g) was calculated as the average of 3 samples, each with 100 seeds. The actual yield was calculated as the total weight of all the seeds in the whole experimental plot. Chlorophyll and carotenoid were extracted from 1 g of mature leaves with ethanol 95% for 24 h, then the absorbance at 470 nm, 664.2 nm and 664.8 nm were measured, and the chlorophyll a, b and total carotenoid contents were calculated

according to Lichtenthaler (1987). Proline content of the leaves was measured according to Paquin & Lechasseur (1979). Perform a colour reaction of 0.5 mL of the extract with 1 mL of 1% ninhydrin mixture, 60% acetic acid, 20% ethanol, boil in a water bath at 95°C for 20 min, then cool. The absorbance at 520 nm was measured, and the proline content was calculated based on a standard curve. Leaf protein content was measured with the Bradford method (Bradford, 1976). Leaf ethanol extract was reacted with Bradford reagent (Coomassie Brilliant Blue 0.01%, ethanol 4.7%, and phosphoric acid 8.5%). The absorbance at 595 nm was measured, and the protein content was calculated based on a standard curve.

2.6. Data analysis

Data were analyzed using R (version 4.2.3). After analysis of variance (ANOVA) gave significant results, the treatments were ranked using the least significant difference (LSD) test at a 5% level of probability.

3. Results and Discussion

Neither drought durations nor type of PGRs affected growth parameters such as plant height and number of leaves (Table 2). This is probably because by the time of PGR application and drought (49 and 50 DAS, respectively), the plants had already completed the vegetative growth cycle. Therefore, no significant effects of drought or PGRs were observed in these measurements.

Table 2. Effects of plant growth regulators (PGRs) and drought durations on height, number of leaves and root length of soybean plants

Parameters	PGRs (R) ¹	Drought durations (D)			Average R
		D0	D4	D8	
Plant height (cm)	W	89.8	79.0	91.4	86.7
	SA	80.2	84.3	76.2	80.2
	BR	75.7	83.2	84.8	81.2
	GA	86.4	83.0	84.1	84.5
	Average D	83.0	82.4	84.1	
CV (%) = 11.3; F _D = 0.1 ^{ns} ; F _R = 0.8 ^{ns} ; F _{DR} = 0.9 ^{ns}					
Number of leaves (leaves/plant)	W	8.8	6.3	6.6	7.2
	SA	9.6	8.2	7.7	8.5
	BR	8.8	9.7	7.8	8.8
	GA	7.3	7.6	5.8	6.9
	Average D	8.6	8.0	7.0	
CV (%) = 25.5; F _D = 2.0 ^{ns} ; F _R = 1.9 ^{ns} ; F _{DR} = 0.4 ^{ns}					
Root length (cm)	W	20.1	31.8	30.1	27.3 ^a
	SA	31.2	32.3	32.6	32.0 ^{bc}
	BR	40.7	36.0	40.7	39.1 ^a
	GA	29.7	37.5	33.3	33.5 ^a
	Average D	30.4	34.4	34.2	
CV (%) = 12.8; F _D = 3.5 ^{ns} ; F _R = 11.8 ^{**} ; F _{DR} = 2.4 ^{ns}					

Within a group of means, values followed by the same letter are not significantly different at 5% level.

^{ns}: non significant; ^{**}: significant at 1% level.

¹W: water; SA: salicylic acid; BR: brassinolide; GA: gibberellic acid.

Figure 1 shows the comparison of root lengths of soybean among the treatments. Only types of PGRs had effects on root length of soybean, with brassinolide giving the highest root length (39.1 cm), significantly higher than the root length of soybean

plants water and salicylic acid treatment, but were not significantly different from root length of soybean plants GA₃ treatment. Elongation of roots is beneficial for plants under drought (Comas et al., 2013) as the roots can access water in more areas of the soil.

**Figure 1.** Root length in different treatments of drought durations and plant growth regulators.

Plant biomass is an indicator of the impact of drought on crops. The results in Table 3 show that both factors and their interaction had significant effects on both fresh and dry biomass of the plants. Prolonged drought up to 8 days significantly reduced fresh biomass of soybeans

compared to non-drought condition. Research has also shown that the main effect of drought stress on plant morphology is a reduction in plant size while a reduction in photosynthetic rate is also an important factor leading to a reduction in plant size and biomass production.

Table 3. Effects of plant growth regulators (PGRs) and drought durations on biomass of soybean plants

Biomass	PGRs (R) ¹	Drought durations (D)			Average R
		D0	D4	D8	
Fresh (g/ plant)	W	15.5 ^{cde}	13.1 ^{de}	5.9 ^e	11.5 ^C
	SA	31.7 ^{a-d}	15.5 ^{cde}	25.6 ^{b-e}	24.3 ^B
	BR	45.0 ^{ab}	40.3 ^{ab}	32.8 ^{abc}	39.4 ^A
	GA	26.2 ^{a-e}	47.2 ^a	11.1 ^{de}	28.1 ^B
	Average D	29.6 ^A	29.0 ^A	18.8 ^B	
CV (%) = 25.8 ; F _D = 9.8 ^{**} ; F _R = 26.7 ^{**} ; F _{DR} = 6.9 ^{**}					
Dry (g/plant)	W	2.5 ^d	4.2 ^{cd}	2.8 ^d	3.2 ^C
	SA	10.3 ^{abc}	3.7 ^d	3.4 ^d	5.8 ^{BC}
	BR	14.0 ^a	14.8 ^a	15.2 ^a	14.6 ^A
	GA	6.3 ^{bcd}	12.5 ^{ab}	3.9 ^{cd}	7.6 ^B
	Average D	8.3 ^{AB}	8.8 ^A	6.3 ^B	
CV (%) = 26.3; F _D = 4.7 [*] ; F _R = 50.9 ^{**} ; F _{DR} = 6.9 ^{**}					

Within a group of means, values followed by the same letter are not significantly different at 5% level

. *: significant at 5% level; **: significant at 1% level.

¹W: water; SA: salicylic acid; BR: brassinolide; GA: gibberellic acid.

Among the PGRs, brassinolide treatment gave the highest results for both fresh and dry biomass. Regarding the interaction between two factors, only brassinolide application showed consistent improvement of soybean plant biomass in all drought durations, while GA₃ application only improved plant biomass in case of 4 days without irrigation, and salicylic acid did not show any effects. This highlights the difference in the effectiveness of these PGRs under different drought durations. According to Zhang et al. (2008), brassinolide participates in many developmental processes including flowering, leaf senescence, chloroplast development, and root growth and development. Brassinolide also

regulates biotic and abiotic stress responses, helps plants limit water loss and increases the biomass accumulation ability of soybeans.

Chlorophyll and carotenoid contents are parameters that are strongly impacted by drought stress. Chlorophyll contents typically decrease under drought, which could be a result of pigment photo-oxidation and chlorophyll degradation as a symptom of oxidative stress (Anjum et al., 2011). Data in Table 4 show that under a short duration of drought, chlorophyll b and carotenoid contents remained unchanged statistically compared to non-drought condition, while chlorophyll a content increased to 26.6 mg/g. This increase could be due to

overcompensation during the recovering period (Dong et al., 2019), showing that soybean plants could recover from short-term drought in terms of leaf physiology. However, under more prolonged drought of 8 days, both chlorophyll a and b contents decreased significantly (23.4 mg/g

and 13.0 mg/g, respectively), while carotenoid content increased, indicating that the plants were under severe stress beyond recovery, and substantial amount of chlorophyll was lost due to oxidative damage while carotenoid accumulated to combat the oxidative stress.

Table 4. Effects of plant growth regulators (PGRs) and drought durations on chlorophyll a, chlorophyll b and carotenoid content of soybean plants

Parameters	PGRs (R) ¹	Drought durations (D)			Average R
		D0	D4	D8	
Chlorophyll a content (mg/g)	W	25.6 ^{abc}	27.8 ^a	24.1 ^{cd}	25.8 ^A
	SA	24.2 ^{cd}	26.2 ^{abc}	23.2 ^d	24.6 ^B
	BR	24.5 ^{bcd}	26.1 ^{abc}	27.5 ^a	26.0 ^A
	GA	27.3 ^a	26.5 ^{ab}	18.9 ^e	24.2 ^B
	Average D	25.4 ^B	26.6 ^A	23.4 ^C	
CV (%) = 2.7; F _D = 64.2 ^{**} ; F _R = 14.4 ^{**} ; F _{DR} = 38.3 ^{**}					
Chlorophyll b content (mg/g)	W	12.7 ^{de}	24.2 ^{ab}	11.5 ^{de}	16.1 ^{AB}
	SA	11.6 ^{de}	16.5 ^{cd}	11.4 ^{de}	13.2 ^B
	BR	11.8 ^{de}	13.3 ^{de}	20.9 ^{bc}	15.3 ^{AB}
	GA	29.1 ^a	15.1 ^{cde}	8.3 ^e	17.5 ^A
	Average D	16.3 ^A	17.3 ^A	13.0 ^B	
CV (%) = 14.7; F _D = 11.3 ^{**} ; F _R = 5.6 ^{**} ; F _{DR} = 32.8 ^{**}					
Carotenoid content (mg/g)	W	5.7 ^{ab}	4.4 ^b	5.8 ^{ab}	5.3 ^A
	SA	5.5 ^{ab}	5.2 ^{ab}	6.9 ^a	5.9 ^A
	BR	6.1 ^{ab}	5.6 ^{ab}	5.2 ^{ab}	5.7 ^A
	GA	2.2 ^c	5.9 ^{ab}	5.0 ^{ab}	4.3 ^B
	Average D	4.9 ^B	5.3 ^{AB}	5.7 ^A	
CV (%) = 11.9; F _D = 4.9 [*] ; F _R = 10.2 ^{**} ; F _{DR} = 11.1 ^{**}					

Within a group of means, values followed by the same letter are not significantly different at 5% level.

*: significant at 5% level; **: significant at 1% level.

¹W: water; SA: salicylic acid; BR: brassinolide; GA: gibberellic acid.

Among the PGRs, only brassinolide was capable of improving leaf chlorophyll a and b contents (27.5 mg/g and 20.9 mg/g, respectively) compared to treatment with water (24.1 mg/g and 11.5 mg/g, respectively) under 8 days with no irrigation. This once again shows the

potential of brassinolide in mitigating the effects of prolonged drought in soybeans. However, carotenoid content did not show any changes statistically among treatments of different PGRs after 8 days without irrigation.

The improvement effects of brassinolide on chlorophyll contents of soybean leaves could help soybean plants maintain their photosynthetic ability, especially during the crucial period of seed setting and filling, which could determine the weight of seeds and yield of the crop. According to Nagasuga (2019), there is a strong association between photosynthesis and yield during the seed filling stage. The study of Rahdari et al. (2012) showed that drought can damage the photosynthetic apparatus including thylakoid membranes, photosynthetic pigments and enzymes, leading to a decrease in chlorophyll content in leaves. These damages depend on the duration and severity of the drought and result in reduced photosynthesis.

Soybean plants that were not watered for 4 and 8 days had a significantly reduced total number of pods and total number of firm seeds compared to normal irrigation, and the reduction was more severe under longer drought (Table 5). Specifically, the total number of firm pods on plants under 4 and 8 days of drought decreased by 17.8% (21.7 pods/plant) and 33.3% (17.6 pods/plant), respectively, compared to non-drought condition (26.4 pods/plant), and the total number of firm seeds decreased by 17.1% (42.7 seeds/plant) and 30.1% (36.0 seeds/plant), respectively, compared to non-drought condition (51.5 seeds/plant). Among the PGRs used, brassinolide had the most positive effects on the total number of firm pods and seeds of soybeans under drought condition, reaching 28.8 (pods/plant) and 56.9 (seeds/plant), respectively.

Table 5. Effects of plant growth regulators (PGRs) and drought durations on pod and seed parameters of soybean plants

Parameters	PGRs (R) ¹	Drought durations (D)			Average R
		D0	D4	D8	
Number of firm pods (pods/plant)	W	32.0 ^{ab}	18.9 ^{de}	10.6 ^{fg}	20.5 ^B
	SA	27.8 ^{bc}	18.6 ^{de}	15.4 ^{ef}	20.6 ^B
	BR	23.0 ^{cd}	28.3 ^{bc}	35.0 ^a	28.8 ^A
	GA	22.8 ^{cd}	21.0 ^{ed}	9.4 ^g	17.8 ^C
	Average D	26.4 ^A	21.7 ^B	17.6 ^C	
CV (%) = 8.3; F _D = 70.0 ^{**} ; F _R = 61.3 ^{**} ; F _{DR} = 51.0 ^{**}					
Number of firm seeds (seeds/plant)	W	64.0 ^{ab}	35.5 ^{cd}	23.3 ^{ef}	40.9 ^B
	SA	55.5 ^b	36.4 ^{cd}	30.7 ^{de}	40.9 ^B
	BR	43.8 ^c	56.2 ^b	70.8 ^a	56.9 ^A
	GA	42.7 ^c	42.7 ^c	19.1 ^f	34.8 ^C
	Average D	51.5 ^A	42.7 ^B	36.0 ^C	
CV (%) = 8.3; F _D = 55.9 ^{**} ; F _R = 62.1 ^{**} ; F _{DR} = 56.2 ^{**}					
Weight of 100 seeds (g)	W	15.6 ^{ab}	11.1 ^{def}	6.8 ^g	11.1 ^C
	SA	17.5 ^a	14.5 ^{abc}	9.4 ^{efg}	13.8 ^B
	BR	17.4 ^a	13.9 ^{bcd}	16.6 ^{ab}	15.9 ^A
	GA	14.0 ^{bcd}	12.0 ^{cde}	8.4 ^{fg}	11.5 ^C
	Average D	16.1 ^A	12.9 ^B	10.3 ^C	
CV (%) = 7.6; F _D = 102.6 ^{**} ; F _R = 45.2 ^{**} ; F _{DR} = 13.6 ^{**}					

Within a group of means, values followed by the same letter are not significantly different at 5% level. **, significant at 1% level.

¹W: water; SA: salicylic acid; BR: brassinolide; GA: gibberellic acid.



Figure 2. Seeds and pods of soybean plants in different treatments of drought durations and plant growth regulators.

When considering the interaction between the two factors, it can be observed that brassinolide actually reduced the number of firm pods and seeds of soybeans under non-drought condition (23.0 pods/plant and 43.8 seeds/plant, respectively) compared to treatment with water (32.0 pods/plant and 64.0 seeds/plant, respectively). However, under drought condition of both durations (4 and 8 days), brassinolide significantly improved the number of firm pods (28.3 and 32.0 pods/plant, respectively) and seeds (56.2 and 70.8 seeds/plant, respectively) compared to all other treatments. Interestingly, GA_3 failed to provide any improvement in these two parameters in any drought or non-drought conditions, despite improving plant biomass in some of these treatments. These results highlight the role of brassinolide as a stress PGRs, and the role of GA_3 as a growth inducing substance rather than a stress regulator.

Similarly, decrease in seed weight was observed to be more severe the longer the drought lasted (Table 5 and Figure 2), dropping from 16.1 g/100 seeds with normal irrigation to 12.9 g/100 seeds under 4 days without irrigation (20% reduction), and 10.3 g/100 seeds under 8 days without irrigation (36% reduction). When considering the interaction of the factors, GA_3 applications showed no statistical improvement compared to

water treatments under both drought durations. Interestingly, salicylic acid treatment proved to be able to improve seed weight (14.5 g/100 seeds) under short drought duration, despite previously showing no improvement in other parameters. This once again implies the different mechanisms by which salicylic acid, brassinolide and GA_3 induce stress response in plants, that focus on different organs and physiological processes of the plants. However, when the drought lasted to 8 days, only brassinolide treatment showed to be able to rescue the seed weight consistently, while salicylic acid and GA_3 failed to have any significant improvement compared to water treatment.

The combination results of the yield components led to superior performance of plants in treatments with brassinolide in terms of actual yield (Table 6). Both under short-term (4 days) and long-term drought (8 days), treatments with brassinolide had the highest actual yield compared to all other treatments at the same drought durations. At 8 days of drought, treatment with brassinolide showed no reduction in yield (195.9 g) compared to non-drought treatment. However, at 4 days of drought, treatment with brassinolide showed some reduction in yield (150.6 g) compared to non-drought treatment, suggesting that this PGR

may be slow acting, and may be more effective for prolonged drought. Besides brassinolide, salicylic acid also showed improvement effects, but to a lesser extent, on actual yield of soybeans

under drought condition, mostly through improving seed weight as mentioned above. On the other hand, GA₃ also exhibited no mitigation effects on the damage to yield of drought stress.

Table 6. Effects of plant growth regulators (PGRs) and drought durations on actual yield of soybeans

Parameters	PGRs (R) ¹	Drought durations (D)			PGRs (R)
		D0	D4	D8	
Actual yield (g)	W	193.0 ^a	65.8 ^{de}	31.2 ^f	96.6 ^B
	SA	161.1 ^b	83.6 ^{cd}	71.1 ^{de}	105.3 ^B
	BR	201.5 ^a	150.6 ^b	195.9 ^a	182.7 ^A
	GA	106.2 ^c	58.5 ^e	47.3 ^{ef}	70.7 ^C
	Average D	165.4 ^A	89.6 ^B	86.3 ^B	
CV (%) = 6.9; F _D = 393.2 ^{**} ; F _R = 342.6 ^{**} ; F _{DR} = 55.5 ^{**}					

Within a group of means, values followed by the same letter are not significantly different at 5% level.

^{**}: significant at 1% level.

¹W: water; SA: salicylic acid; BR: brassinolide; GA: gibberellic acid.

In order to study the mechanism of the drought mitigation effects of these PGRs in soybeans, we measured the proline and protein contents in the leaves (Table 7). Proline is known as a stress-induced amino acid, acting as an osmolyte to regulate osmotic pressure,

maintain protein and cell membrane stability, as well as helping protect the cells from oxidative stress (Wang et al., 2022). Proline biosynthesis and degradation also play an important role in the energy balance between chloroplasts and mitochondria (Lisar et al., 2010).

Table 7. Effects of plant growth regulators (PGRs) and drought durations on proline content and protein content of soybean leaves

Parameters	PGRs (R) ¹	Drought durations (D)			PGR (R)
		D0	D4	D8	
Proline content (mg/g)	W	9.7 ^{efg}	27.6 ^b	22.3 ^c	19.9 ^B
	SA	10.4 ^{efg}	9.2 ^{fg}	11.1 ^{ef}	10.2 ^D
	BR	8.0 ^g	16.3 ^d	12.2 ^e	12.1 ^C
	GA	8.9 ^{fg}	25.9 ^b	31.6 ^a	22.1 ^A
	Average D	9.2 ^B	19.8 ^A	19.3 ^A	
CV (%) = 4.8; F _D = 699.7 ^{**} ; F _R = 499.1 ^{**} ; F _{DR} = 167.6 ^{**}					
Protein content (mg/g)	W	91.1 ^{ef}	115.4 ^b	107.3 ^c	104.6 ^A
	SA	85.9 ^{fg}	83.4 ^g	121.6 ^a	96.9 ^B
	BR	84.3 ^g	97.2 ^d	93.6 ^{de}	91.7 ^C
	GA	86.7 ^{fg}	105.6 ^c	97.0 ^d	96.4 ^B
	Average D	165.4 ^A	89.6 ^B	86.3 ^B	
CV (%) = 1.8; F _D = 307.3 ^{**} ; F _R = 75.8 ^{**} ; F _{DR} = 117.9 ^{**}					

Within a group of means, values followed by the same letter are not significantly different at 5% level.

^{**}: significant at 1% level.

¹W: water; SA: salicylic acid; BR: brassinolide; GA: gibberellic acid.

The results in Table 7 show that both salicylic acid and brassinolide, the two PGRs that exhibited drought mitigation effects in soybeans, reduced the content of proline in the leaves at both 4 days and 8 days without irrigation compared to the treatments with only water. Similar results were observed for protein content, except for salicylic acid treatment at 8 days without irrigation, which was higher than water treatment at the same drought duration. These results suggested that both salicylic acid and brassinolide induce drought tolerance in soybeans through mechanisms unrelated to proline accumulation. The stress mitigation effects of salicylic acid and brassinolide might in fact reduce the stress level experienced by leaves, and consequently the leaves did not have to accumulate large amount of proline in order to cope with the stress. According to Krishna (2003), brassinolide are a group of natural plant steroid compounds that help increase crop yields by altering metabolism and protecting plants from environmental stresses. In recent years, genetic and biochemical studies have established the essential role of brassinolide in their ability to increase plant resistance to various stresses, such as low temperature, drought, salt and pathogen attack.

4. Conclusions

Drought at the stage of pod growth and seed filling in soybeans did not affect the growth and development of the vegetative parts of the plants, but greatly affected yield. Brassinolide were the most effective PGR in mitigating the effects of drought at pod growth stage on soybeans, maintaining the yield and seed quality comparable to non-drought condition. However, the proline contents in brassinolide treatments were lower than those of water treatments, suggesting that brassinolide may act through a mechanism unrelated to proline accumulation.

Conflict of interest

The authors have no conflicts of interest to declare.

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Fertilizer practices and soil properties in durian orchards during the fruit-bearing stage: A case in Cai Lay district, Tien Giang province

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ARTICLE INFO	ABSTRACT
Research Paper Received: February 01, 2024 Revised: February 29, 2024 Accepted: March 13, 2024 Keywords Durian orchard Fertilizer practices Fruit-bearing stage Soil properties *Corresponding author Thai Nguyen Diem Huong Email: tndhuong@hcmuaf.edu.vn	This research aimed to assess the fertilizer practices during the fruit-bearing stage of durian cultivation. A total of 60 durian farmers in Cai Lay district were interviewed using structured questionnaires. Subsequently, 20 soil samples (0 - 50 cm) were collected from durian orchards post-harvest to analyze basic physical-chemical properties. Findings revealed substantial variation in fertilizer utilization among durian orchards in the Cai Lay district, Tien Giang province, with 68.18% employing imported organic fertilizers. The application of inorganic fertilizers displayed an imbalanced pattern, notably characterized by excessive phosphorus supplementation. Primary use of inorganic fertilizers was in the form of multi-macronutrient (NPK) formulations, strategically applied during critical developmental stages, including new shoot emergence, flowering, and fruit development. The practice of incorporating lime, applied as limestone directly to the soil surface post-harvest, combined with extensive chemical fertilizer use, led to be acidic to very acidic soil. The soil analysis indicated that the soil in the durian orchards prominently exhibited a heavy mechanical composition classified as clay, clay loam and loam. Despite farmers' attention to applying organic fertilizer on durian, the content of organic matter in the soil remained low (1.4 - 3.8%). The soil in the study area demonstrated varying levels of total and available nutrients, with total nitrogen ranging from 0.15% to 0.25%, total potassium (K₂O) from 1.7% to 1.9%, and exchangeable K from 0.4 to 0.8 meq/100 g. Additionally, exchangeable calcium ranged from 5.5 to 9.5 meq/100 g, and exchangeable magnesium from 1.7 to 3.7 meq/100 g. The phosphorus content was notably high, with total phosphorus ranging from 0.13% to 0.23% and available phosphorus from 39.4 to 81.6 mg/100g. Cation exchange capacity varied from low to medium, spanning from 11.6 to 19.8 meq/100 g.

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1. Introduction

Durian (*Durio zibethinus* Murr.), a key fruit crop earmarked for strategic plan until 2030 according to the Ministry of Agriculture and Rural Development (MARD, 2022), holds particular significance in the Mekong River Delta. Tien Giang province, hosting the largest durian cultivation area in the region, notably in Cai Lay district, has demonstrated its pivotal role, encompassing 9,125 ha in 2019 and standing as the oldest durian cultivation area in Tien Giang (MARD, 2021). In optimizing durian productivity, meticulous attention to fertilization practices, alongside cultivar selection and pest management, play a critically important role. Despite the presence of fertilizer programs designed for durian cultivation, significant disparities persist owing to variables such as soil characteristics, sites, cultivar diversity, and the age of the trees. This variety is noticeable in recommendations from distinct sources, including SOFRI (2003), MARD (2021) in Vietnam, Somsri (2008) and Salakpetch (2000) in Thailand. As a result, farmers striving for increased durian fruit productivity often engage in excessive fertilizer application. This practice not only jeopardizes nutrient absorption by plants but also leads to soil pollution and heightened greenhouse gas emissions (Serpil, 2012; Chali, 2023). Therefore, the objectives of the research were to investigate fertilizer application patterns in Cai Lay district, Tien Giang province and to assess the nutritional status of durian soil through both soil physical and chemical analysis.

2. Materials and Methods

2.1. Surveying the fertilizer practices on durian orchards in Cai Lay district, Tien Giang province

Sites: Tam Binh, Ngu Hiep, and Long Tien communes which have large areas of bearing durian orchards in the district (over 1,400 ha) were chosen as the survey sites.

Orchards: The orchards selected randomly for inclusion in this study comprise durian cultivation sites currently in the bearing stage, possessing a minimum area of 2,000 m². Furthermore, these orchards are requisite to possess a minimum of seven years of experiential expertise in the cultivation of durian.

Survey method: The survey employed direct interviews with farmers utilizing pre-prepared questionnaire forms. The survey encompassed inquiries into general farmer information, specifics regarding fertilizer dosage, type, and the timing of application.

Number of participating farmers: The study involved interviews with a total of 60 farmers, distributed across different communes, including 30 farmers from Tam Binh, 20 from Ngu Hiep, and 10 from Long Tien. Each farmer completed an individual survey form.

2.2. Assessing properties of durian growing soil in Cai Lay, Tien Giang province

Selected sites for soil sampling:

In accordance with survey findings, a randomized selection was undertaken to identify 20 durian orchards aged between 17 and 21 years. The selected orchards exhibited a uniform utilization of fertilizers at a standard level within the local context. Soil samples were collected from the orchards owned by the surveyed farmers. Specifically, orchards in the post-harvest stage, yet to undergo fertilization for the forthcoming crop, were chosen as the sampling units.

Soil sampling procedure:

For each durian orchard, five samples corresponding to five distinct trees, were randomly selected within the orchard using a zig-zag pattern. Each tree contributed subsamples from four positions situated in four different directions, extracted from the soil layer from 0 to 50 cm. During the sampling process, the topsoil layer (0 - 2 cm) was carefully removed to eliminate any residual plant material. A sterilized auger, treated with alcohol as per established protocols, was utilized to extract soil samples

at a depth of 0 to 50 cm, specifically at $\frac{3}{4}$ of the canopy diameter (Masri, 1991). Subsequently, the collected subsamples from each tree were mixed to form a composite 1-kg sample. Immediate transportation to the laboratory ensued following collection, where samples were stored in a dry environment, placed within labeled zip bags, clearly denoting the site and specific sampling location, in accordance with established procedures (Forster, 1995). General information about 20 orchards collecting soil samples is shown in Table 1.

Table 1. General information of soil sampling orchards

Number	Commune	Location	Age of durian (year)	Number	Commune	Location	Age of durian (year)
1	Tam Binh	10°19'11,8"N, 106°9'13,2"E	18	11	Ngu Hiep	10°18'15,8"N, 106°9'38,4"E	17
2	Tam Binh	10°19'17,2"N, 106°9'10,4"E	20	12	Ngu Hiep	10°18'4,5"N, 106°9'1,4"E	17
3	Tam Binh	10°19'11,5"N, 106°9'7,9"E	18	13	Ngu Hiep	10°18'9,2"N, 106°8'36,8"E	21
4	Tam Binh	10°19'12,8"N, 106°9'4,7"E	20	14	Ngu Hiep	10°18'2,8"N, 106°6'27,2"E	20
5	Tam Binh	10°19'20,1"N, 106°9'14,7"E	21	15	Long Tien	10°20'45,6"N, 106°9'39,5"E	18
6	Tam Binh	10°19'28"N, 106°9'4,3"E	21	16	Long Tien	10°21'22,7"N, 106°9'4,5"E	17
7	Tam Binh	10°19'36,2"N, 106°9'12,3"E	19	17	Long Tien	10°19'59,2"N, 106°9'43,3"E	19
8	Ngu Hiep	10°17'30,9"N, 106°8'50,5"E	21	18	Long Tien	10°20'47,6"N, 106°9'0,8"E	20
9	Ngu Hiep	10°17'39,2"N, 106°9'9,4"E	19	19	Long Tien	10°21'7,1"N, 106°9'26,5"E	20
10	Ngu Hiep	10°17'36,9"N, 106°7'6,9"E	18	20	Long Tien	10°20'30,1"N, 106°9'12,5"E	19

Soil properties measurement.

The soil analysis, incorporating both physical and chemical assessments, was conducted in adherence to established procedures. The following standard methods were employed:

Soil texture was determined using a hydrometer (Lavkulich, 1981; Gee & Bauder, 1986). Soil pH was measured using a pH meter. Organic matter content was assessed using the Walkley-Black method (FAO & GLOSOLAN, 2019). Total nitrogen was quantified using the Kjeldahl method (FAO & GLOSOLAN, 2021). Total phosphorus was analyzed using the Olsen & Sommer method (1982), while available phosphorus content was determined using the Bray & Kurtz P-1 method (1945). Total potassium was measured using a Flame photometer. Exchangeable potassium, calcium, and magnesium were quantified using Atomic Absorption Spectrophotometry (FAO & GLOSOLAN, 2022). Cation exchange capacity (CEC) was assessed using the ammonium acetate method at pH = 7 (FAO & GLOSOLAN, 2022).

Data analysis

The collected data were analyzed by Microsoft Excel software. Standard deviation was employed as a statistical measure to categorize and group certain survey information, ensuring a robust and systematic approach to data examination.

3. Results and Discussion

3.1. Information about the durian orchards

In Cai Lay district, a major durian-growing region in Tien Giang province, farmers tend to have relatively small durian orchards (less than 6,000 m²). Because of their limited area, meticulous agricultural practices, including fertilization, fruit thinning, and pesticide

application, are consistently employed by farmers to ensure the optimal care of their durian trees. An examination of the age distribution of durian orchards in the region reveals a noteworthy distribution, with approximately 50% falling within the 16 to 20 years age bracket. Additionally, a substantial portion (26.67%) is observed in the 20 to 22 years age category, while the remaining orchards are younger, aged below 16 years (Table 2). The economic significance of durian in Cai Lay is evident, particularly during the fruit-bearing stage when market prices are favorable, underscoring the role of durian as a primary income source for local farmers. The Ri6 cultivar emerges as the predominant choice among durian cultivators in Cai Lay, with 20% exclusively cultivating this variety. Moreover, a significant proportion (66.67%) engage in intercropping, combining the Ri6 with the Monthong cultivar, while 11.67% concurrently intercrop the Ri6 with both Monthong and Chuong Bo cultivars. The appeal of Ri6 durian in the market is attributed to its distinctive features, including yellow flesh and a flat seed.

Significant variations in durian planting spacing were notable among orchards, ranging from 9 x 9 m to 5 x 5 m, corresponding to tree densities of 120 - 400 trees per ha. The 7 x 7 m planting spacing was most prevalent, accounting for 33.33% of cases, followed by 6 x 6 m and 8 x 8 m, utilized by 21.67% and 20% of farmers, respectively. The "Others" category, comprising alternative planting spacings, predominantly featured the 6.5 x 6.5 m configuration. Notably, the durian planting spacing observed in the sites appeared to be narrower and characterized by high tree density comparing with durian cultivation in Southeast and Central Highland provinces.

Table 2. General information about durian orchards

Parameters	Level	Orchard (%)
Area (m ²)	< 6,000	46.67
	6,000 - < 9,000	30.00
	≥ 9,000	23.33
	Mean: 6,233.33; SD: ± 2,757.83; Min: 2,000; Max: 15,000	
Tree age (year)	< 16	23.33
	16 - < 20	50.00
	≥ 20	26.67
	Mean: 15.88; SD: ± 4.23; Min: 7; Max: 22	
Cultivar	Ri 6	20.00
	Ri 6 + Monthong	66.67
	Monthong + Chuong bo	1.67
	Ri 6 + Monthong + Chuong bo	11.67
Tree spacing (m x m)	6 x 6	21.67
	7 x 7	33.33
	8 x 8	20.00
	Others	25.00

3.2. Lime application practices in durian orchards in Cai Lay district, Tien Giang province

Within the agroecosystem of the region, the conscientious implementation of lime application practices in durian cultivation is a remarkable aspect of agricultural management. Survey outcomes revealed a pervasive adherence to lime application among durian farmers, with a 100% prevalence rate. This annual practice is consistently executed during the post-harvest stage and primarily entails the widespread distribution of lime onto the soil. Lime dosage ranged from 1.5 to 4.5 kg/tree per year, typically

falling within the range of 2 to 3 kg/tree per year. It was predominantly administered in the form of limestone. Especially, a subset of durian farmers (21.7%) opted for the application of quicklime (CaO) at a lower dosage of approximately 2 kg per tree annually and the number of farmers in Long Tien commune who used this type was higher than in other communes (Table 3). However, a subsequent field assessment conducted one year post-lime application unveiled a significant residual presence of undissolved lime, particularly notable in cases where limestone was employed. This observation underscores the inefficacy of limestone-based applications.

Table 3. Lime application practices in durian orchards across various communes in Cai Lay district, Tien Giang province

Lime dosage (kg/tree per year)	Proportion of farmers (%) used			
	Tam Binh	Ngu Hiep	Long Tien	Mean
≤ 2	26.7	10.0	30.0	21.7
$> 2 - 3$	50.0	50.0	60.0	51.7
> 3	23.3	40.0	10.0	26.6

3.3. Fertilizer utilization for durian in Cai Lay district, Tien Giang province

3.3.1. Organic fertilizer

In alignment with contemporary development emphasizing green and sustainable agricultural practices, the application of fertilizers has garnered substantial attention, particularly within the context of high-value crops such as durian. Additionally, it is essential to ensure that the concentration of soil organic matter remains above the threshold level in order to enhance soil quality (Dadi & Damtev, 2021). Survey results underscored the popular integration of organic fertilizer among durian farmers in the region, with a full adoption rate of 100%. The selection of organic fertilizers employed exhibited remarkable diversity, comprising 22 distinct types. A predominant proportion (68.18%) was sourced from international markets, while the remaining 31.82% originated from local entities with longstanding presence in the domestic market. Noteworthy variations surfaced among durian farmers concerning the selection and application of organic fertilizers. The array of

organic fertilizers utilized exhibited considerable heterogeneity. The Table 4 shows at Tam Binh commune, there was 33.3% of farmers applied Organic Booster fertilizer to durian orchards. Meanwhile, the farmers at Ngu Hiep commune were interested in using organic fertilizer on durian, the common organic fertilizer in Cai Lay district was applied by 20 - 30% of farmers with Black Bounce fertilizer was the most common (30.0% of farmers used). At Long Tien commune, 60.0% of farmers used imported chicken manure from Japan and 50% of farmers employed Super Humic fertilizer from Hop Tri company on their durian orchards.

The majority of farmers employed one to three types annually. Moreover, the frequency of organic fertilizer application displayed considerable variance, averaging between 3 to 7 times per year. Each fertilizer type was specifically applied at different stages; cow manure, for example, was mainly used after harvest, while other organic fertilizers were strategically applied during the new shoot stage or at the beginning of the rainy season.

Table 4. Types of organic fertilizer used in durian across various communes in Cai Lay district, Tien Giang province

Types of organic fertilizer	Composition	Origin	Proportion of farmers (%) used			
			Tam Binh	Ngu Hiep	Long Tien	Mean
Con Ca	22% OM (organic matter), 5% N, 2% P ₂ O ₅ , 2% K ₂ O, 2% humic acid	ADC fertilizer factory	6.7	20.0	30.0	15.0
Cow manure		Domestic	16.7	10.0	10.0	13.3
Bat guano		Domestic	20.0	25.0	20.0	21.7
Bounce Back	≥ 40% OM, 2% N, 2% P ₂ O ₅ , 2% K ₂ O	Australia	10.0	30.0	0.0	15.0
Hop Tri Super Humic	70% Humic acid	Hop Tri Investment Corporation	16.7	20.0	50.0	23.3
Fertiplus 65 OM	65% OM, 3.5% N, 2% K ₂ O, C/N: 12	Netherland (Imported and distributed by Vinh Thanh Trading Company Limited)	13.3	20.0	10.0	15.00
Chicken manure	≥ 60% OM	Japan (Imported by many companies)	16.7	20.0	60.0	23.3
Organic Booster	65% OM, 2% N, 2% P ₂ O ₅ , 2.9% K ₂ O	Australia (Distributed by TOBA Company Limited)	33.3	25.0	10.0	26.7

3.3.2. Inorganic fertilizer

The incorporation of inorganic fertilizers holds pivotal significance in the cultivation of crops, with particular relevance to durian cultivation. Inorganic fertilizers play a crucial role in delivering timely and efficient nutrient supply to plants (Omidire et al., 2015). Yet, it is crucial to recognize that improper use of inorganic fertilizers may undermine their effectiveness in promoting plant growth. The survey results underline the widespread reliance on inorganic fertilizers among durian farmers in the research region. Farmers reported utilizing approximately 3 to 5 distinct types of inorganic fertilizers within a single fruit crop, corresponding to a one-year

cycle. Despite the diversity in fertilizer types, the frequency of fertilization events was notably high, ranging from 9 to 16 times per year, with certain farmers engaging in as many as 22 times (at an interval of once every 15 days). This rigorous fertilization routine exemplifies significant dedication and enthusiasm demonstrated by durian farmers in Cai Lay district, Tien Giang province, towards the cultivation and nurturing of durian trees. This heightened interest may be attributed, in part, to the elevated market price of durian fruit in recent years, prompting farmers to make considerable investments in both financial resources and labor efforts to ensure the optimal growth and development of durian trees within their orchards.

Table 5. Types of inorganic fertilizer used in durian across various communes in Cai Lay district, Tien Giang province

Types of fertilizer	Composition	Proportion of farmers (%) used			
		Tam Binh	Ngu Hiep	Long Tien	Mean
DAP	18% N, 46% P ₂ O ₅	83.3	45.0	40.0	63.3
NPK 12 - 12 - 17	12% N, 12% P ₂ O ₅ , 17% K ₂ O	36.7	5.0	20.0	23.2
NPK 15 - 15 - 15	15% N, 15% P ₂ O ₅ , 15% K ₂ O	36.7	50.0	30.0	40.0
NPK 16 - 16 - 16	16% N, 16% P ₂ O ₅ , 16% K ₂ O	16.7	40.0	50.0	30.0
NPK 20 - 10 - 10	20% N, 10% P ₂ O ₅ , 10% K ₂ O	30.0	15.0	30.0	25.0
Superphosphate	16% P ₂ O ₅ , 10% S, 15% Ca	23.3	40.0	60.0	35.0
YaraMila Complex	12% N, 11% P ₂ O ₅ , 18% K ₂ O, 2.65% MgO, 19.9% SO ₃ , 0.02% Zn, 0.015% B	43.3	35.0	40.0	40.0
YaraLiva Nitrabor	15.4% N, 26% CaO, 0.3% B	40.0	25.0	30.0	33.3

A comprehensive exploration of inorganic fertilizer practices among durian cultivators in Cai Lay district, Tien Giang province, revealed there were 20 inorganic fertilizers used for durian. Diammonium Phosphate (DAP) emerged as the predominant choice, constituting 63.3% of the overall usage, with a significant concentration observed in Tam Binh commune, where 83.3% of durian farmers utilized this fertilizer (Table 5). DAP was initially utilized for flowering treatment although some farmers extended its application to fulfill nutritional needs throughout the flowering and fruit growth phases. The survey also highlighted a substantial reliance on superphosphate, employed regularly by 60.0% of farmers at Long Tien commune to aid in the differentiation of durian flower buds. Consequently, inadvertent soil acidification resulting from this practice was noted, adversely affecting soil pH. The NPK fertilizers, owing to their convenience, were prominently favored in the region with YaraMila Complex (utilized by 40% of farmers) and NPK 15 - 15 - 15 being the most employed types.

The Table 6 shows the farmers at three communes of Cai Lay district applied nitrogen, phosphorus and magnesium to durian at the same level but there was a difference in dosage of potassium and calcium used. Specifically, the dose of potassium was applied to durian at the lowest at Tam Binh commune, while calcium was employed at the highest level at Ngu Hiep commune when comparing the three communes. Farmers engaged in comprehensive fertilization, incorporating nitrogen, phosphorus, potassium, calcium and magnesium. However, a notable disparity was observed in nutrient proportions when compared to established research by Ng & Thamboo (1967) and Jamil (1966). This may be attributed to the elevated amount of phosphorus applied to durian during the reproductive stage in Cai Lay, Tien Giang province. These inorganic fertilizers were predominantly applied during the shoot, flower, and fruit growth stages (Table 6).

Table 6. Status of fertilizer application in durian across various communes in Cai Lay district, Tien Giang province

Element	Dosage (kg/tree per year) *				Time of fertilization
	Tam Binh	Ngu Hiep	Long Tien	Mean	
N	1.15 ± 0.21	1.27 ± 0.38	1.24 ± 0.39	1.21 ± 0.31	Shoot, flower, fruit growth
P ₂₀ 5	1.41 ± 0.1	1.41 ± 0.49	1.55 ± 0.48	1.44 ± 0.50	Shoot, flower induction, flower and fruit growth
K ₂ O	0.67 ± 0.27	0.87 ± 0.28	0.80 ± 0.29	0.76 ± 0.29	Shoot, flower, fruit growth
CaO	0.78 ± 0.35	0.62 ± 0.39	0.85 ± 0.29	0.73 ± 0.36	Flower, fruit growth
MgO	0.54 ± 0.21	0.58 ± 0.20	0.54 ± 0.47	0.55 ± 0.25	Flower, fruit growth

* Account for famers using inorganic fertilizers; Mean ± SD.

3.4. Properties of durian growing soil in Cai Lay, Tien Giang province

Table 7. Physical and chemical properties of durian growing soil across various communes in Cai Lay district, Tien Giang province

Parameter	Tam Binh	Ngu Hiep	Long Tien	Mean
Texture				
- Sand (%)	29.5 ± 7.3	36.2 ± 5.6	28.3 ± 7.5	31.5 ± 7.4
- Silt (%)	23.8 ± 4.9	38.1 ± 4.7	30.0 ± 5.6	30.7 ± 7.8
- Clay (%)	46.7 ± 6.4	25.7 ± 3.7	41.7 ± 12.6	37.8 ± 12.1
pH _{H₂O} (1:5)	5.0 ± 0.4	5.1 ± 0.4	5.0 ± 0.3	5.1 ± 0.4
Organic matter (%)	2.4 ± 1.0	3.0 ± 1.4	2.2 ± 1.1	2.6 ± 1.2
N (%)	0.15 ± 0.07	0.23 ± 0.03	0.21 ± 0.02	0.20 ± 0.05
C/N	12.3 ± 9.8	7.5 ± 3.0	6.1 ± 3.1	8.8 ± 6.6
P (%)	0.15 ± 0.03	0.20 ± 0.06	0.21 ± 0.05	0.18 ± 0.05
K ₂ O (%)	1.9 ± 0.1	1.8 ± 0.1	1.8 ± 0.1	1.8 ± 0.1
P (mg/100 g)	50.1 ± 16.5	68.5 ± 21.9	63.2 ± 23.2	60.5 ± 21.1
K ⁺ (meq/100 g)	0.5 ± 0.1	0.7 ± 0.2	0.6 ± 0.2	0.6 ± 0.2
Ca ²⁺ (meq/100 g)	7.7 ± 1.5	7.3 ± 2.5	7.5 ± 2.3	7.5 ± 2.0
Mg ²⁺ (meq/100 g)	3.0 ± 1.1	1.9 ± 0.4	3.3 ± 1.0	2.7 ± 1.0
CEC (meq/100 g)	16.8 ± 5.1	14.3 ± 2.6	16.0 ± 4.4	15.7 ± 4.1

Mean ± SD; CEC: cation exchange capacity.

The examination of physical and chemical properties in durian growing soil of the research region, plays a pivotal role in understanding the factors influencing crop growth, yield, and quality. These properties are intricately linked to both inherent soil characteristics and anthropogenic interventions, including fertilization, liming, and watering practices. Table 7 shows the distribution of three soil textures across the investigated communes in Cai Lay district, based on USDA classifications (1960): clay (accounting for 45%), clay loam (30%) and loam (25%). Clay texture is predominant in Tam Binh commune, while clay loam texture is commonly found in Long Tien commune, and loam texture is observed in Ngu Hiep commune. The heavy

mechanical composition soil characteristics confer compactness, water and nutrient retention capabilities, albeit with compromised hydraulic conductivity and slow drainage (Phogat et al., 2015; Abdulkadir, 2016; Al-saeedi, 2022).

The active acidity of durian soil, expressed through pH values (pH_{H₂O}), exhibited a spectrum from very acidic to slightly acidic (Slavich & Petterson, 1993). Although lime and fertilizer usage among durian farmers varied among the communes, pH values did not differ. This is partly due to the inappropriate use of fertilizers. For instance, the proportion of farmers in Long Tien commune using quicklime is high, but the use of superphosphate or extensive use of inorganic fertilizers raises concerns about

potential generation upon the release of acid ions into the soil (Jote, 2023). Besides, a large amount of undissolved limestone remaining on the soil surface also partly affects its pH-improving effect in durian soil.

Soil organic content exhibited significant variation, ranging from low to high levels (organic matter from 0.89% to 5.71%) (Rayment & Lions, 2011). Despite 100% of farmers applying organic fertilizer (Table 3), the organic matter content remained predominantly low, potentially due to high rates of organic mineralization in the soil, as indicated by total N content predominantly at good and high levels (Swangjang, 2015). The average C/N ratio of 8.8 suggests a heightened decomposition rate of organic matter, possibly attributed to microbial activity converting organic materials into nitrogen-containing compounds (Mot et al., 2021). Especially, the average C/N ratio in soil samples collected in Long Tien commune was the lowest among the three communes, possibly due to applying a large amount of chicken manure to the soil (Rayment & Lions, 2011).

The analysis divulged that total and available phosphorus, total potassium in the soil recorded at high levels, while exchangeable potassium was at medium to high. Specifically, the exchangeable potassium content in the soil in Ngu Hiep commune was higher than in the other two communes, consistent with the results of the farmer survey; durian farmers in Ngu Hiep commune applied potassium at a higher dose (Table 6). In particular, the use of phosphorus to stimulate durian flower production and the application of NPK throughout durian crop led to a substantial increase in both total and available phosphorus content in the soil. The results showed that the content of available potassium,

calcium and magnesium was at medium level. Consequently, cation exchange capacity (CEC) in soil was at medium level, about 15.7 meq/100 g (Rayment & Lions, 2011).

4. Conclusions

Despite the evident interest among durian farmers in Cai Lay toward the application of organic fertilizers, the prevalence of durian orchards characterized by high organic matter content remains disproportionately low. The existing scenario is further compounded by the consequences of unbalanced fertilization practices, using a large amount of phosphorus to induce durian to flower. The analytical results indicated prominently a heavy mechanical composition of durian growing soil classified as clay and clay loam. The soil analysis also revealed a low pH, a medium level of CEC, with the content of total and available nutrients exhibiting variations from medium to high levels. In particular, the content of total and available phosphorus were at high level in durian soil at the post-harvest stage. Given these findings, targeted interventions are essential to enhance soil conditions by applying lime and organic fertilizer. The primary focus should encompass pH improvement and a meticulous adjustment of the fertilizer formula tailored specifically for durian cultivation.

This comprehensive understanding provides a foundation for implementing strategic soil management practices, ensuring a more favorable environment for durian cultivation. By addressing the identified challenges, durian farmers in Cai Lay, Tien Giang province can enhance both the quality and yield of their crops, contributing to the overall sustainability and success of durian cultivation in the region.

Conflict of interest

The authors have no conflicts of interest to declare.

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Effects of salinity concentrations at different stages on growth, yield and grain quality of ST24 rice variety in pot-planted condition

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ARTICLE INFO	ABSTRACT
Research Paper Received: August 30, 2023 Revised: December 05, 2023 Accepted: December 09, 2023 Keywords Rice quality Salinity concentration ST24 rice variety *Corresponding author Pham Thi Thuy Duong Email: pttduong@hcmuaf.edu.vn	Saltwater intrusion has posed a complex and detrimental impact on rice cultivation in the Mekong Delta. The ST24 is the rice variety with prominent quality, which is being admired by both domestic and international markets. The assessment of salt tolerance at various growth stages aids farmers in minimizing losses during cultivation. This study was carried out to evaluate the effects of salinity concentrations and stages on growth, yield and rice quality of ST24 rice variety in the Summer-Autumn crop in 2023. The rice plants were grown in pots and placed in a greenhouse throughout the experiment. A two-factor experiment was arranged in a complete randomized design (CRD) with three replications. The first factor included three salinity concentrations: 2‰, 4‰, and 6‰, while the second factor comprised three growth stages: the seedling stage, tillering stage, and heading stage. The results indicated that the concentrations and stages of salinization significantly affected the growth, yield and grain quality of ST24 rice variety. The growth parameters tended to decrease as the salinity concentrations increased. The actual yield reached 220.1, 113.5, 46.2 g/plot at salinity concentrations of 2‰, 4‰ and 6‰, respectively. The yield of rice plants was least affected by salinity at the seedling stage (252.8 g/plot) and were most severely affected by salinity at the heading stage (46.8 g/plot). Increasing salinity from 2‰ to 6‰ did not affect gelatinization (at level 2.0), but reduced the size of grains.

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1. Introduction

Rice is a major cereal crop of Vietnam which plays an important role in ensuring food security and export. The Mekong Delta is the largest rice production area of Vietnam, with the total area of rice fields reaching nearly 2 million ha, accounting for 46.9% of the country's rice land

area (Nguyen et al., 2015). However, occasional saltwater intrusion into the river and canal systems in the Mekong Delta has become a major issue, getting more complicated and frequent in recent years due to climate change (Pham et al., 2021), which seriously threatens rice production of the region.

Salinity has negative effects to the quality and potential yield of crops including rice (Muchate, et al., 2016; Kopittke et al., 2019). Many studies indicated that salinity in irrigation water causes inhibition of plant growth and development, thereby negatively affecting yield through impeding the absorption of water and nutrients by plants (Shawquat et al., 2015; Hashem et al., 2018; Rahman et al., 2021), inducing oxidative stress (Hasanuzzaman et al., 2021), inhibiting cellular metabolic activity, and damaging organelles (Benito et al., 2014; Ning et al., 2018; Mohsin et al., 2020). However, plant susceptibility to salinity can change significantly by growth stage (Rad et al., 2012; Zhang et al., 2017). At the same time, salinity concentration can also change drastically throughout the season.

Therefore, the assessment of the salinity tolerance of rice makes an important contribution to helping farmers apply appropriate techniques for cultivation under salinity conditions, in order to ensure economic efficiency. The capacity of plants to flourish, advance, and yield within diverse salinity conditions mirrors the salt tolerance of each crop (Munns & Tester, 2008). The ST24 is a Vietnamese elite rice variety with exceptional rice quality, favored by domestic and foreign consumers; however, salt susceptibility of this rice variety has not been studied in detail. For that reason, this study was carried out to evaluate the effects of salinity concentrations at different stages on growth, yield and grain quality of ST24 rice variety in pot-planted conditions.

2. Materials and Methods

2.1. Time and place of the study

The study was carried out from March to July 2023, at the experimental farm of the Department of Physiology and Biochemistry,

Faculty of Agronomy, Nong Lam University Ho Chi Minh City, Vietnam.

2.2. Experimental materials

- The ST24 rice variety has a growth period of 105 to 110 days, an average height between 110 and 115 cm, low falling rate, potential yield reaching 8 to 9 tons/ha.

- Topsoil was taken from the soil layer with a depth of 0 to 30 cm in the rice growing area in Vung Liem district, Vinh Long province.

- HD Organic Vina organic fertilizer (25% organic matter) was produced and distributed by Hai Duong Co., Ltd, sea salt (81.6% NaCl), KOH 95% (India).

- Plastic tanks have dimensions ($216 \times 110 \times 35$ cm, length $\times$ width $\times$ height), plastic pots ($17 \times 13 \times 25$ cm, mouth diameter $\times$ bottom diameter $\times$ height); equipment including EC Hanna meter (Italy), Opti-Sciences handheld chlorophyll meter (USA), straight ruler, Mitutoyo electronic caliper (Japan), Ohaus electronic scale (USA), SPAD-502Plus (Japan).

2.3. Experimental layout

A two-factor experiment was arranged in a complete randomized design (CRD) with three replications. The first factor included three salinity concentrations (factor N): 2‰, 4‰ and 6‰, and the second factor included three salinity stages (factor T): seedling stage, tillering stage and heading stage.

The experiment consisted of 9 treatments, with 3 replicates corresponding to 27 plots; Each plot has 25 pots and each pot has 2 plants; The total number of pots in the experiment was 675 pots and 1,350 plants.

2.4. Experimental method

Soil used in the experiment was taken from the topsoil layer (0 to 30 cm in depth) in the rice growing area. Soil was placed in plastic pots with a depth of 20 cm (the top of the pot was 5 cm higher than the soil surface). Organic fertilizer was applied to the soil with the amount of 50 g/pot at 15 days before sowing. Seeds were soaked in warm water (50°C) for 24 h and incubated in damp towels for another 48 h to crack the coats, then sown directly into pots with the quantity 4 seeds/pot. The pots were then put in plastic tubs. Each tub was divided into 3 separated watertight cells, lined with plastic to be used as 3 different experimental plots. Water was supplied into each cell and maintained at the soil surface. After sprouting, the seedlings were pruned to 2 plants/pot. The experiment was placed in a greenhouse to ensure all treatments were not affected by rainwater.

For the salinity treatments, sea salt was used to prepare saline waters at different concentrations depending on treatments. The saline waters were used in place of groundwater to fill the cells at different periods depending on treatments: from 15 to 30 DAS (days after sowing) for the seedling stage; from 30 to 50 DAS for the tillering stage; from 50 to 65 DAS for the heading stage. The salinity concentration was checked every three days by measuring electrical conductivity (EC) of the water with an EC meter and adjusting with groundwater or saline water to maintain EC at 3.2, 6.4 or 9.6 dS/m, corresponding to salinity of 2‰, 4‰ or 6‰. After the salinity treatment periods, the saline water was replaced by well water. To harvest when about 85 to 90% of the seeds were ripe.

2.5. Parameters

Growth parameters included length of root (cm), height of plant (cm), number of tillers (tillers/plant), number of leaves (leaves/plant), chlorophyll content index of leaf (SPAD index), survival rate (%), stem fresh and dry weight (g/plant), root fresh and dry weight (g/plant).

Yield parameters included number of panicles (panicles/plant), number of grains (grains/panicle), weight of 1,000 grains (g), rate of firm grains (%), actual yield (g/plot).

Rice grain quality parameters included length (cm) and width (cm) of grains, whiteness of grains and gelatinization of grains by SES scale (IRRI, 1996).

2.6. Data analysis

Data were averaged by Microsoft Excel 2010 software; ANOVA analysis and the Duncan's multiple range test at the significance level $\alpha = 0.05$ by SAS 9.4 software.

3. Results and Discussion

According to Munns & Tester (2008), growth was a measure used to assess the salt tolerance of crops in salinity conditions. The growth parameters of ST24 rice plants at harvest, including length of root, height of plant, number of leaves, number of tillers, leaf chlorophyll content index and survival rate, were presented in Table 1.

Table 1. Effects of salinity concentrations at different stages on growth parameters of ST24 rice variety at harvest

Parameters	Salinity concentrations (‰) (N)	Salinity stages (T)			Average (N)
		Seedling	Tillering	Heading	
Length of root (cm)	2	28.5	17.2	23.7	23.2 ^A
	4	24.7	12.5	18.8	18.7 ^B
	6	17.9	6.9	12.5	12.4 ^C
	Average (T)	23.7 ^A	12.2 ^C	18.4 ^B	
	CV (%) = 10.9; $F_N = 67.5^{**}$; $F_T = 76.7^{**}$; $F_{NT} = 0.1^{ns}$				
Height of plant (cm)	2	79.4	83.7	90.8	84.6 ^A
	4	72.0	79.0	90.5	80.5 ^A
	6	71.7	66.3	86.5	74.8 ^B
	Average (T)	74.4 ^B	76.3 ^B	89.2 ^A	
	CV (%) = 6.7; $F_N = 7.6^{**}$; $F_T = 20.7^{**}$; $F_{NT} = 1.7^*$				
Number of tillers (tillers/ plant)	2	9.5	7.7	10.4	9.2 ^A
	4	8.1	6.1	9.8	8.0 ^B
	6	6.3	4.8	10.1	7.0 ^C
	Average (T)	7.9 ^B	6.2 ^C	10.1 ^A	
	CV (%) = 10.8; $F_N = 13.9^{**}$; $F_T = 31.5^{**}$; $F_{NT} = 2.7^{ns}$				
Number of leaves (leaves/ plant)	2	47.4	52.3	72.3	57.3 ^A
	4	40.8	56.7	68.9	55.5 ^A
	6	33.7	41.4	71.9	49.0 ^B
	Average (T)	40.6 ^C	50.1 ^B	68.3 ^A	
	CV (%) = 11.3; $F_N = 4.6^*$; $F_T = 58.4^{**}$; $F_{NT} = 2.3^{ns}$				
Chlorophyll content index of leaf (SPAD index)	2	37.0	32.3	30.2	33.1 ^A
	4	30.7	32.1	28.3	30.4 ^B
	6	29.8	30.9	27.5	29.4 ^B
	Average (T)	32.5 ^A	31.8 ^A	28.6 ^B	
	CV (%) = 7.6; $F_N = 6.1^*$; $F_T = 6.7^{**}$; $F_{NT} = 1.7^{ns}$				
Rate (%) of survival plants	2	78.0	73.3	68.0	73.1 ^A
	4	71.3	54.0	46.0	57.1 ^B
	6	56.7	44.7	34.7	45.4 ^C
	Average (T)	68.7 ^A	57.3 ^B	49.6 ^C	
	CV (%) = 12.0; $F_N = 35.3^{**}$; $F_T = 16.8^{**}$; $F_{NT} = 1.1^{ns}$				

*In the same group, numbers with the same character are statistically insignificant difference; ns: non-significant; significant difference at $P \leq 0.05$ (°) or $P \leq 0.01$ (**).*

The results of Table 1 illustrated that the growth of ST24 rice variety was significantly affected by the salinity concentrations in irrigation water and the stage of salinity treatment. The higher the salinity in irrigation water was, the lower the growth of rice plants was. The root length of rice plants decreased correspondingly when increasing the salt concentration, the shortest reached 12.4 cm. Length of root (12.4 cm), height of plant (74.8 cm), number of leaves (49.0 leaves/plant), number of tillers (7.2 tillers/plant), and survival rate (45.4%) was lowest when the salinity was 6‰. Khan et al. (1997) concluded that salinity affects growth, photosynthesis and mineral ion accumulation of different rice varieties, thereby reducing the growth parameters of rice. The growth parameters decreased more as salt stress was executed later in the growth cycle. The

leaf SPAD index (28.6 CCI) and the survival rate (49.6%) were lowest when the plants were subjected to salinity at the heading stage.

The interaction between the concentration and the stage of salinity also affected growth parameters of ST24 rice variety (Figure 1). At a salt concentration of 6‰ applied at the seedling stage, the rice plants had the lowest number of leaves (33.7 leaves/plant). Because at this time, the vegetative growth of the plant was not complete, saltwater irrigation interfered with the absorption of water and nutrients, thus inhibiting the elongation as well as new leaf forming. In addition, the survival rate decreased sharply when the plants were irrigated with 6‰ saline water at the heading stage. The leaf chlorophyll content index was not statistically different under the influence of the interaction between the concentrations and the stages of salinity, fluctuating from 27.5 to 37.0.

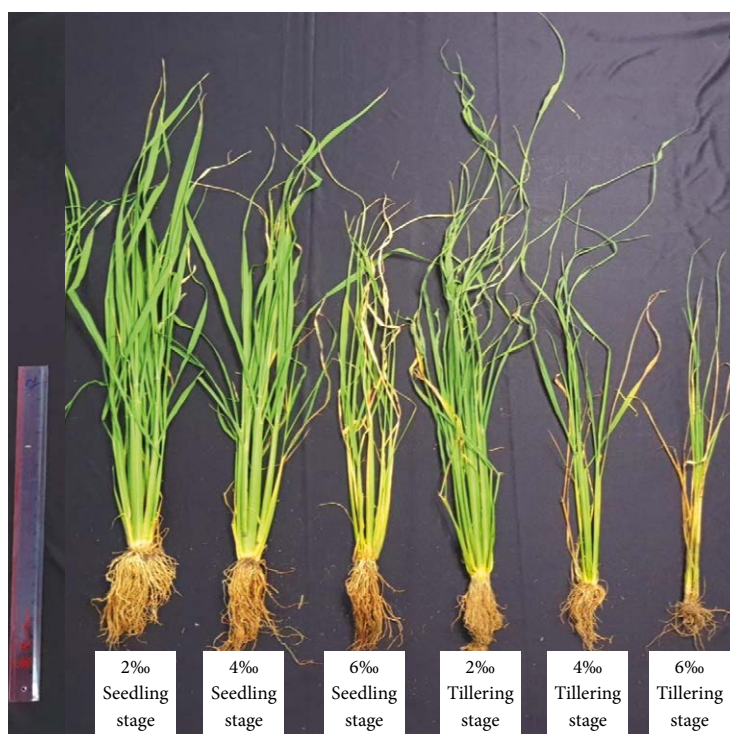


Figure 1. Morphology of ST24 rice variety was watered at different salinity concentrations at seedling and tillering stages at 50 days after sowing.

Salinity also affected the stem and root biomass of rice plants. The differences in stem and root biomass of ST24 at harvest time because of

the influence of different salinity concentrations and stress periods were presented in Table 2.

Table 2. Effects of salinity concentrations at different stages on biomass of stem and root of ST24 rice variety at harvest

Parameters	Salinity concentrations (‰) (N)	Salinity stages (T)			Average (N)
		Seedling	Tillering	Heading	
Fresh biomass of stem (g/ plant)	2	76.1	61.2	59.7	65.7 ^A
	4	60.5	48.8	44.0	51.1 ^B
	6	50.3	33.4	35.1	39.6 ^C
	Average (T)	62.3 ^A	47.8 ^B	46.2 ^B	
CV (%) = 9.9; $F_N = 57.3^{**}$; $F_T = 26.3^{**}$; $F_{NT} = 0.3^*$					
Dry biomass of stem (g/ plant)	2	17.5	14.9	14.2	15.5 ^A
	4	13.9	11.2	10.6	11.9 ^B
	6	12.0	7.8	8.5	9.4 ^C
	Average (T)	14.5 ^A	11.3 ^B	11.1 ^B	
CV (%) = 9.8; $F_N = 59.0^{**}$; $F_T = 22.1^{**}$; $F_{NT} = 0.4^{ns}$					
Fresh biomass of root (g/ plant)	2	38.1	18.9	23.2	26.7 ^A
	4	29.6	11.3	18.7	19.9 ^B
	6	19.6	4.9	13.0	12.5 ^C
	Average (T)	29.1 ^A	11.7 ^C	18.3 ^B	
CV (%) = 12.5; $F_N = 75.1^{**}$; $F_T = 114.8^{**}$; $F_{NT} = 4.7^{ns}$					
Dry biomass of root (g/ plant)	2	10.4	5.1	6.1	7.2 ^A
	4	8.0	3.1	4.9	5.3 ^B
	6	5.0	1.3	3.6	3.3 ^C
	Average (T)	7.8 ^A	3.1 ^C	4.9 ^B	
CV (%) = 14.4; $F_N = 60.0^{**}$; $F_T = 85.7^{**}$; $F_{NT} = 2.9^{ns}$					

In the same group, numbers with the same character are statistically insignificant difference; significant difference at $P \leq 0.05$ () or $P \leq 0.01$ (**).*

The results of Table 2 indicated that fresh and dry weights of stems and roots showed statistically significant differences among treatments. In the same period of saline treatments, the higher the salinity concentration in the irrigation water was, the lower the fresh and dry biomass of

stems and roots were. At the time of harvest, the average fresh and dry weights of stems were the lowest, at 39.6 and 9.4 g/plant, respectively, when irrigated with 6‰ saline water. This was consistent with the influence of salinity on plant growth parameters. When watering with high

salinity concentrations, root length, plant height, number of leaves and number of tillers were reduced considerably, thus reducing stem and root biomasses. The results by Ologundudu et al. (2014) also demonstrated that the dry weights of leaves and roots of rice decreased with increasing salinity concentration in water. The fresh and dry weight of roots was also the lowest at 6‰ salinity, at 12.5 and 3.3 g/plant, respectively.

Significant differences among stem and root biomasses of the treatments were observed. Fresh and dry biomass of stems of ST24 rice variety were the lowest (33.4 and 7.8 g/plant, respectively) when irrigated with 6‰ saline water at tillering stage. Besides, the rice plants which were irrigated with 6‰ saline water at tillering stage also the lowest in terms of fresh and dry biomass of roots (4.9 and 1.3 g/plant, respectively). At the tillering stage, the plants needed to absorb water and nutrients for metabolism to form new plant growth parts. However, increasing salinity in irrigation water inhibited the activity of enzymes and damaged plant metabolism due to excessive accumulation of Na^+ ions (Benito et al., 2014; Hashem et al., 2018; Isayenkov & Maathuis, 2019), thereby causing declines in growth of stems and roots.

Yield is an important factor of primary concern in crop and rice production. Another effect of salinity on plants is reducing photosynthetic activity, affecting carbohydrate synthesis and yield. The yield components of ST24 rice variety under different salinity concentrations applied at different growth stages were presented in Table 3.

The results of Table 3 showed that the yield components and actual yield of ST24 rice variety showed remarkable changes under different salinity concentrations at different stages of growth. When applied at the same stage,

the higher the salt concentration in irrigation water was, the lower the yield components of rice were. Rice plants saw the lowest values in terms of number of panicles (4.1 panicles/plant), number of grains (58.3 grains/plant), weight of 1,000 grains (12.9 g), rate of firm grain (51.4%) and actual yield (46.2 g/plot) at salinity of 6‰. These results agreed with the studies of Singh et al. (2008), Hasamuzzaman et al. (2009), and Khatun & Flowers (1995) which concluded that salinity significantly reduced the rate of firm grains and weight of 1,000 grains of rice. Kronzucker & Britto (2011) proposed that the sodium content in saltwater inhibits carbohydrate metabolism during growth and panicle formation, which is the main cause of yield reduction.

The yield components of rice were also significantly different when irrigated with saline water at different stages. For the same salt concentration, plants experiencing salinity at the seedling stage were least affected, while salinity at the heading stage affected the plants the most in terms of yield components. When irrigated with saline water at the heading stage, the average actual yield was 46.8 g/plot.

Regarding the interaction of salinity concentrations and stress periods, plants irrigated with 6‰ saline water at the heading stage had the lowest yield components and actual yield. Conversely, the yield of ST24 rice variety when irrigated with 2‰ saline water at the seedling stage had the highest actual yield among stress treatments (383.5 g/plot) while the lowest actual yield (8.1 g/plot) was found in treatment of 6‰ salinity applied at the heading stage. The results of Grattan et al. (2002) also showed that salinity at or above 3.0 dS/m considerably affects rice yield.

Table 3. Effects of salinity concentrations at different stages on yield components and yield of ST24 rice variety

Parameters	Salinity concentrations (‰) (N)	Salinity stages (T)			Average (N)
		Seedling	Tillering	Heading	
Number of panicles (panicles/plant)	2	7.2	5.8	7.7	6.9 ^A
	4	6.2	4.2	5.2	5.2 ^B
	6	4.6	3.0	4.7	4.1 ^C
	Average (T)	6.0 ^A	4.2 ^B	5.9 ^A	
CV (%) = 12.1; F _N = 42.0 ^{**} ; F _T = 17.9 ^{**} ; F _{NT} = 1.1 ^{ns}					
Number of grain (grain/panicle)	2	106.0 ^a	81.1 ^{bc}	68.0 ^c	85.0 ^A
	4	97.9 ^{ab}	65.1 ^{cd}	58.4 ^d	73.8 ^B
	6	81.8 ^{bc}	40.7 ^f	53.2 ^e	58.3 ^C
	Average (T)	95.2 ^A	62.3 ^B	59.9 ^B	
CV (%) = 6.5; F _N = 12.9 ^{**} ; F _T = 108.5 ^{**} ; F _{NT} = 15.1 ^{**}					
Weight of 1,000 grains (g)	2	21.4 ^a	18.4 ^b	14.1 ^c	18.0 ^A
	4	20.5 ^a	14.5 ^c	10.4 ^e	15.1 ^B
	6	18.5 ^b	12.2 ^d	8.0 ^f	12.9 ^C
	Average (T)	20.1 ^A	15.0 ^B	10.8 ^C	
CV (%) = 5.5; F _N = 87.0 ^{**} ; F _T = 293.8 ^{**} ; F _{NT} = 4.9 [*]					
Rate (%) of firm grain	2	75.5 ^a	71.8 ^a	61.9 ^b	69.7 ^A
	4	75.3 ^a	60.7 ^b	45.7 ^c	60.6 ^B
	6	71.4 ^a	47.8 ^c	34.9 ^d	51.4 ^C
	Average (T)	74.0 ^A	60.1 ^B	47.5 ^C	
CV (%) = 4.5; F _N = 103.0 ^{**} ; F _T = 216.5 ^{**} ; F _{NT} = 4.9 ^{**}					
Actual yield (g/plot)	2	383.5 ^a	167.3 ^c	109.5 ^d	220.1 ^A
	4	268.0 ^b	49.7 ^e	22.9 ^{ef}	113.5 ^B
	6	107.0 ^d	23.6 ^{ef}	8.1 ^f	46.2 ^C
	Average (T)	252.8 ^A	80.2 ^B	46.8 ^C	
CV (%) = 21.3; F _N = 95.5 ^{**} ; F _T = 151.9 ^{**} ; F _{NT} = 10.5 ^{**}					

In the same group, numbers with the same character are statistically insignificant difference; ns: non-significant; significant difference at $P \leq 0.05$ () or $P \leq 0.01$ (**).*

Besides yield, rice quality is a matter of great concern to producers and consumers. The ST24 rice variety is favored by the market due to its exceptional quality with glutinous texture. Therefore, the parameters of rice quality of ST24 rice variety under different salinity concentrations at different growth stages were evaluated and presented in Table 4.

Table 4. Effects of salinity concentrations and stages to quality of ST24 rice variety

Parameters	Salinity concentrations (‰) (N)	Salinity stages (T)			Average (N)
		Seedling	Tillering	Heading	
Length of grain (mm)	2	8.12 ^a	8.08 ^{ab}	8.06 ^b	8.09 ^A
	4	8.12 ^a	8.08 ^{ab}	7.88 ^c	8.03 ^B
	6	8.10 ^{ab}	8.04 ^b	7.68 ^d	7.94 ^C
	Average (T)	8.11 ^A	8.07 ^B	7.87 ^C	
	CV (%) = 0.41; $F_N = 46.58^{**}$; $F_T = 133.94^{**}$; $F_{NT} = 27.45^{**}$				
Width of grain (mm)	2	1.80 ^a	1.80 ^a	1.77 ^{bc}	1.79 ^A
	4	1.81 ^a	1.77 ^{bc}	1.75 ^d	1.78 ^{AB}
	6	1.78 ^{ab}	1.76 ^{cd}	1.74 ^d	1.76 ^B
	Average (T)	1.80 ^A	1.78 ^B	1.75 ^C	
	CV (%) = 0.60; $F_N = 4.12^*$; $F_T = 41.43^{**}$; $F_{NT} = 2.68^*$				
Whiteness grain level	2	3.0	3.0	3.0	3.0
	4	3.0	3.0	5.0	3.7
	6	5.0	5.0	7.0	5.7
	Average (T)	3.5	3.5	4.5	
Gelatinization grain level	2	2.0	2.0	2.0	2.0
	4	2.0	2.0	2.0	2.0
	6	2.0	2.0	2.0	2.0
	Average (T)	2.0	2.0	2.0	

In the same group, numbers with the same character are statistically insignificant difference; significant difference at $P \leq 0.05$ () or $P \leq 0.01$ (**).*

The results of Table 4 demonstrated that the size of rice grains was significantly different among treatments, while the gelatinization of rice grains were not affected by salinity. Length and width of rice grains tended to decrease at higher salinity concentrations. Rice grains had the lowest length (7.94 mm), width (1.76 mm) when the plants were watered the salinity concentration of 6‰. When supplied with salt water at the heading stage, rice grains also had the lowest length (7.87 mm), width (1.75 mm). Whiteness score fluctuated between 3.0 and 5.7 when rice plants were watered at different salinity concentrations. The gelatinization of rice grains was high (at level 2.0). Gelatinization was the process of breaking the starch chain in the rice grain (Liu et al., 2009), which determines the time and energy required to cook the rice (Hoang et al., 2021). Therefore, the ST24 rice variety needs a long time to cook.

4. Conclusions

The growth and yield parameters of ST24 rice variety in the pot-planted condition were significantly affected by the different salinity concentrations and application stages. Root length, plant height, number of leaves, number of tillers, chlorophyll content index of leaf, and survival rate decreased corresponding increasing salt concentration in irrigating water. The actual yield reached 220.1, 113.5, 46.2 g/plot at salinity concentrations of 2‰, 4‰ and 6‰, respectively. Under saline conditions, rice plants had the highest (252.8 g/plot) and the lowest actual yield (46.8 g/plot) when experiencing salinity at the seedling stage and heading stage, respectively. The gelatinization of rice remained high and was not affected by the salt concentrations and salinity application stages; The rice grain size decreased with the higher salinity concentrations and the later stages of salinity treatment.

Conflict of interest

The authors whose names are listed above certify that they have no any conflict of interest declaration.

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A field study on the evaluation of application of MB-1® live attenuated vaccine in broilers

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ABSTRACT

The research aimed to evaluate the application of MB-1 vaccine at the hatchery to prevent Gumboro disease in commercial broilers. One hundred serum samples were randomly collected from commercial white broilers to examine infectious bursal disease virus (IBDV) and Newcastle disease virus (NDV) antibody titers. The surveyed chickens exhibited an average maternally derived antibody (MDA) level of $3,258 \pm 1,551$ (titers). At 36 and 42 days of age, IBDV antibody titers exceeded the protection level, with very high uniformity: $4,217 \pm 911$ and $4,413 \pm 1,153$ (titers), respectively. In addition, the humoral immune response to the ND vaccine is good with antibody titers of $3,180 \pm 2,103$ at 42 days of age. IBDV of MB-1 vaccine was detected in bursa from 15 days of age and persisted until the last sample was taken at 36 days of age. The bursa lesion scores (BLS) were at normal ranges, gradually increasing from 1 to 2.8 between 18 and 36 days of age. The bursa index (BI) was highest when the chicken was at 15 days old (0.23%) and decreased to the lowest (0.06%) when the chicken was at 36 days old. In summary, the use of MB-1 vaccine in the hatchery has shown early localization of the MB-1 vaccine virus in the bursa, provided a strong immune response to IBDV without affecting the humoral immune response to the ND killed vaccine, and was safe for the bursa when applied to commercial broilers at the hatchery.

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1. Introduction

Infectious bursal disease (IBD) is an acute infectious disease that is highly contagious and causes great economic loss to the poultry industry. Every year, the broiler industry loses

3.9 thousand tons of meat due to the effects of variant IBDV strains (Zachar et al., 2016). The causative agent is the infectious bursal disease virus, a virus belonging to the Birnaviridae family and non-envelope (Dobos et al., 1979; Muller et al., 1979). Initially, IBDV multiplies in

macrophages in the lymph nodes, then the virus attacks the lymph nodes in the bursal, leading to the bursal becoming inflamed, and shrunk, causing immunodeficiency, and therefore making chickens susceptible to secondary infections (Dey et al., 2019).

Vaccination is the best way to control IBDV. Many new vaccine technologies have been researched and brought to the market to increase the effectiveness of IBD prevention including IBD hatchery vaccines (live attenuated, immune complex, and recombinant vaccines (Muller et al., 2012), and on-farm vaccines (live attenuated and inactivated IBD vaccines) (de Wit et al., 2021). Live vaccines have been proven to be a powerful tool in controlling Gumboro disease (Muller et al., 1979; van den Berg et al., 2000; Eterradossi & Saif, 2013). However, the problem encountered when using live vaccines is the interference of MDA (Dey et al., 2019).

In particular, an application of hatchery vaccine is easier to implement, increases standardization, and ensures animal welfare compared to forms of vaccination at the farm (Franzo et al. 2020). According to Phibro Animal Health, MB-1 vaccine contained a naked live Gumboro disease virus with the M.B. strain adapted for in-ovo or subcutaneous injection at the hatchery. The mode of action of MB-1 vaccine is maternal antibodies dependent. According to Wein et al. (2023), the ability of MB-1 virus vaccine to survive within macrophages ensures its survival during chicks with effective MDA protection. After MB-1 vaccination, the vaccine virus was carried by skin resident macrophages and then migrated via the bloodstream to invade the spleen. In addition, MB-1 vaccine virus infects and survives splenic macrophages. This mode of action allows MB-1 vaccine virus to spread, invade, and replicate in Bursa. It provides

a clear explanation of the ability of the MB-1 virus vaccine to survive in maternally immune chicks and replicate properly to allow efficient, early, and continuous immunization against Gumboro disease.

The vaccine virus release and replication in the bursa is individually adjusted to the MDA level and MB-1 vaccine has been proven safe when applied to chicks. The MB-1 vaccine ensures that chicks have been vaccinated at the right time and induced full protection against a vvIBDV for commercial broilers (de Wit et al., 2021). According to Ray et al. (2021), a hatchery vaccine named MB-1 was safety, early onset of immunity, and applied convenience in a hatchery.

Within the past five years, Gumboro field studies have been conducted by Ashash et al. (2019) in Latin, Asia, and Asia. The US, Africa, and Israel have proven to believe that the IBDV M.B. strain from the IBD hatchery vaccine named MB-1 vaccine also gave a good immune response and did not affect productivity indicators. In India, Ray et al. (2021) suggested that the use of live attenuated IBDV vaccine M.B. strain on chicks in the hatchery is effective even when the chickens have high levels of MDA. At the same time, the study also showed that when using the M.B. strain vaccine, chickens at the hatchery had lower bursal lesion scores and a high productivity index compared to using an immune complex vaccine. de Wit et al. (2021) demonstrated that the use of M.B. vaccine strain in commercial broiler hatcheries, it has been successful and has full protection against vvIBDV (very virulent infectious bursal disease virus) strains at 22 and 36 days of age. In Vietnam, the MB-1 application of the hatchery Gumboro vaccine showed that the virus was located early in the bursa, and IBDV antibodies formed and developed quickly and evenly; in particular, it

did not affect the humoral immune response of chickens to Newcastle disease vaccine when applied to Luong Phuong broilers (Nguyen et al., 2022a), Isa Brown layers (Nguyen et al., 2022b) and Ac chicken (Nguyen et al., 2023).

This study aims to evaluate the effectiveness of the immune response of commercial white broilers to the MB-1 vaccine. This is a breed that often has very high levels of MDA (thanks to the work of making inactivated Gumboro vaccine for breeders). Therefore, we proposed improvements and changes in IBD vaccination methods in white commercial broilers to contribute to the development of the overall health of poultry.

Table 1. Vaccine program

Day of age	Vaccine	Brand
1	MB-1/ - 0.1 mL	Phibro
	ND-killed - SC/0.1 mL	MSD
	ND-IB live/Spray	Zoetis
10 - 15	ND-IB-H9-killed	No information
22	ND-IB live/DW	MSD

2.2. Gumboro and Newcastle antibody titers

Chickens were sampled to measure IBDV antibody titers to evaluate development of IBD immune response after MB-1 vaccination. One of the concerns when applying live attenuated IBD vaccines is the risk of immunodeficiency meaning that live virus vaccine affects to humoral immune response to other vaccine antigens, such as ND-killed vaccine. So that was the reason why we measured NDV antibody titers in this study. We randomly selected 20 chickens to take wing vein blood at five-time points at 1, 28, 32, 36 and 42 days after MB-1 vaccination. Blood samples were allowed to clot naturally, then stored at a temperature of 2°C - 8°C on farm and then it was sent to the laboratory to test. A total of 100

2. Materials and Methods

2.1. Experimental design

The study was conducted with a total of 15,000 one-day-old Ross 308 breed chicks vaccinated with the Gumboro MB-1 vaccine and ND-killed vaccine subcutaneously in the same hatchery. Other vaccines on the farm were applied according to the farm's vaccination program (Table 1). All chickens are raised in cold houses in Binh Duong province from August to October in 2023. Farm management followed the instructions of the Emivest breeding company with a good uniform about feeding, drinking water, temperature, humidity, and ventilation.

samples were taken for ELISA testing according to the IDEXX's protocol instructions of IDEXX IBD Ab test and IDEXX ND Ab test.

2.3. Bursal index (BI)

The BI value was calculated to determine the relative increase or decrease in weight of the bursa to the chicken's body weight. In the experiment, 6 chickens were randomly selected at 15, 18, 21, 28 and 36 days of age to weigh, dissect and cut the bursa according to the method described by Cazaban et al. (2015). A total of 30 bursa samples were taken for the survey. The BI ratio was calculated according to the following formula:

$$BI = \text{Bursa weight (g)} \times 100 / \text{body weight (g)}$$
 (Olesen et al., 2018).

2.4. Infectious Bursal Disease identification by RT-PCR and Nucleotide sequence analysis

The bursa samples from the assigned birds were smeared directly into circles of FTA cards on 15, 18, 21, 24 and 36 days of age. Total 6 bursas were smeared each FTA cards per time. They were sent to Clinic for Poultry and Fish Medicine, Department for Farm Animals and Public Health in Veterinarian (Vetmeduni Vienna) for PCR amplification and amplicon sequence analysis of pooled samples. RNA extraction of all samples was performed using QIAamp cadon Pathogen Kit (Qiagen, Hilden, Germany). Initially, all RNA samples were investigated by the RT-PCR for the presence of IBDV RNA, using primers and probes for the VP3 gene in the A segment of the IBDV genome (Escaffre et al., 2010 family Birnaviridae, genus Avibirnavirus; Ashash et al., 2019).

IBDV-positive samples were further subjected in-house RT-PCR to amplify 750 bp region of the VP2 gene. Positive PCR products were excised from the gel and purified with the MinElute Gel Extraction Kit (Qiagen, Valencia, CA). The purified PCR products were submitted for Sanger sequencing using the BigDye Terminator v3.1 Cycle sequencing kit (ThermoFisher Scientific, Waltham, MA) and run on a 3500 Genetic Analyzer (ThermoFisher Scientific). Raw sequence data were analyzed with the Lasergene/EditSeq application, and sequence consensus construction was produced with the

SeqMan application (DNASTAR, 2006). The IBDV strain differentiation analyses utilized the GenBank database and were conducted with the Molecular Evolutionary Genetic Analysis (MEGA) software (Kumar et al., 2004).

2.5. Bursal lesion score (BLS)

The bursa from the assigned birds was removed at 18, 21, 28 and 36 days of age. A total of 24 bursa tissues were fixed in 10% neutral buffered formalin. All samples were sent to the Vet Hospital of Nong Lam University. Based on a level of affected bursa, Muskett et al. (1979) scored in the range of 0 to 5: (0) normal bursa; (1) 1 - 25% of the follicles show lymphoid depletion; (2) 26 - 50% of the follicles show nearly complete lymphoid depletion; (3) 51 - 75% of the follicles show lymphoid depletion; affected follicles show necrosis and severe influx of heterophils is detected; (4) 76 - 100% of the follicles show nearly complete lymphoid depletion, hyperplasia and cyst structure are detected; (5) 100% follicles show nearly complete lymphoid depletion; complete loss of follicular structure, thickened and folded epithelium, and fibrosis of bursal tissue. The average bursal lesion score was calculated by dividing the total of bursal lesion score by the number of bursa samples (Olesen et al., 2018) the histological bursal lesion score (HBLS). A summary of the research parameters is presented in Table 2.

Table 2. Research parameters

Parameters	Day of samples (days of age)	Number of samples/times	Total samples
IBDV/NDV ELISA	1, 28, 32, 36, 42	20	100
Busa Index	15, 18, 21, 28, 36	6	30
RT-PCR	15, 18, 21, 24, 36	6	30
Bursal lesion score	18, 21, 28, 36	6	24

2.6. Statistical analysis

The data were processed and calculated by using Microsoft Excel 2019. The data were presented as averages of the parameters.

3. Results and Discussion

3.1. RT-PCR and sequencing

The RT-PCR and gene sequencing were used to determine the presence of vaccine virus in the chicken's bursa. At the age of 15 and 18 days old, RT-PCR and sequencing results from the bursa showed positive results for IBDV and distinguished the M.B. vaccine virus strain.

In the following sampling times, when the chickens were at 21, 24 and 36 days old, all FTA cards showed positive IBDV results and identified 100% of the virus as belonging to the M.B. strain of vaccine (Table 3). Furthermore, if it was a virus strain different from the vaccine strain, it would have been present a few days later, but the strain identification results only showed the presence of the M.B. vaccine strain, not the presence of the environmental strain. Thus, from 15 days of age, the vaccine virus had located in the bursa and could stimulate B lymphocytes to produce antibodies against IBDV. According to Ashash et al. (2019), from 33% to 100% of the genome of M.B. strain was detected in the bursa of 24-day-old chickens and by 28 days of age, this rate increased from 83% to 100%. Therefore, this study showed that the vaccine virus located in the bursa earlier, when the chickens were 15 days old.

In another experiment on MB-1 vaccine, Ashash et al. (2019) detected 33% - 100% of the MB-1 vaccine virus genome in all bursa samples of chickens vaccinated with MB-1 at the time

of sampling at 24 days of age. In addition, in an experiment comparing the effectiveness of two chicken flocks using MB-1 vaccine with immune complex 228E (Icx), Ray et al. (2021) showed that vaccine virus 228E was detected as early as 17 days of age after Icx injection but disappeared at 21 days of age and recorded that MB-1 virus appeared at 24 days of age and at later times on the animals. Ray et al. (2021) also proved that MB-1 strain's ability to spread and occupy space in the bursa was very good and stronger than 228E, so it can replace 228E when the distance between the house using MB-1 vaccine is too close to the house using 228E vaccine. On the other hand, MB-1 contained free-living viral particles but was not completely neutralized by MDA (Ashash et al., 2019) and its replication in the bursa was not detected before 21 days of age (depends on MDA), which might allow the full differentiation and maturation of bursa-derived lymphocytes (Ray et al., 2021).

Table 3. RT-PCR and sequencing

Days of age	RT-PCR	Sequencing
15	Positive	M.B. Strain
18	Positive	M.B. Strain
21	Positive	M.B. Strain
24	Positive	M.B. Strain
36	Positive	M.B. Strain

3.2. Bursal index and Bursal lesion scores

Bursal index and Bursal lesion scores are used as indicators of body health and immune response. One of the clinically based features to know the localization of vaccine virus is that the bursa size becomes smaller (Etteradossi & Saif, 2013). The cause may be that when the IBD virus replicates in B lymphocytes that are in the differentiation stage and lasts about 7 to 10 days, it may reduce the number of B lymphocytes in

the cortex and medulla causing the reduction in bursa size (Sharma et al., 2000). At 15 and 21 days old, BI was at a high value of 0.17 - 0.20, respectively, then it gradually decreased to 0.13 at 28 days old. The lowest BI (0.06) was found at 36 days old (Table 4). The BI values gradually decreased from 18 to 36 days of age, indicating normal development and maturation of the bursa, consistent with the physiological changes of the bursa as the chicks grew up. The images of bursal lesion score are shown in Figure 1.

Table 4. Bursa index (BI) and bursal lesion scores (BLS)

Days of age	BI	BLS
15	0.17	
18	0.20	1.50
21	0.17	1.00
28	0.13	2.17
36	0.06	2.80



Figure 1. Bursal lesion scores range from 0 to 5 based on the level of affected bursa.

Bursal lesion scores increased from 1.0 when the chicken was 18 days old and increased to 2.8 when the chicken was 36 days old. The cause may be that IBDV replicated in B lymphocytes that were in the differentiation stage and survive for about 7 to 10 days. The virus may have reduced the number of B lymphocytes in the cortex and medulla, causing the bursa to decrease in size (Sharma et al., 2000). According to Ashash et al. (2019), the BLS - after MB-1 vaccination were lower than those reported for other vaccines, suggesting a milder impact on the bursa. According to Mazariegos et al. (1990), a BLS result greater than or equal to 4 in chickens vaccinated with intermediate-virulence IBD vaccine is considered above the standard of vaccination and indicates exposure of the chickens to the virus in the field. The results of this experiment showed that BLS had gradually increased since the time the vaccine virus was located and was at an average level and was all less than 4 points. In an experiment in India, it had been shown that chickens using MB-1 had lower BLS than immune complex vaccines and live vaccines of the M.B strain. normally (Ray et al., 2021). Because the experimental conditions did not extend the sampling time, it was not possible to monitor at later stages to assess recovery via BLS. However, the recovery ability of the bursa through BLS was recorded and reported on Luong Phuong broilers (Nguyen et al., 2022a), Isa Brown layers (Nguyen et al., 2022b).

The effectiveness of the MB-1 vaccine in this study were consistent with previous studies conducted in different regions. Some field studies on MB-1 in Latin America, Africa, and Israel had been shown to be safe and effective in commercial broiler chickens having varying MDA (Ashash et al., 2019). According to Ashash et al. (2019) the mode of action of MB-1, they have found that

MDA mediates the timing of replication, and it is related to the MDA titers of the individual bird.. In Vietnam, the trial of using MB-1 for one-day-old Isa Brown chicks at the hatchery provided the early localization of vaccine virus (21 days old), rapid and uniform development active IBD antibodies; it also did not affect to the immune response of ND vaccination and was safe for the bursa (Nguyen et al., 2022b). In another trial in Luong Phuong broiler, MB-1 vaccination also provided good results for immunity response (Nguyen et al., 2022a). According to Nguyen et al. (2023), Ac chicken had IBD titers at 28 days of age so high with the average titer of 6218. The positive results observed in the immune response and production performance of broilers vaccinated with MB-1 highlight its potential for improving the control and prevention of IBD.

3.3. Humoral immune response

3.3.1. IBD humoral response

Maternal derived antibodies are key to protecting chicks against IBD during the first weeks. The MDA helps B lymphocytes have time to increase in number, mature and can protect chicks for 2 - 3 weeks of age (de Wit et al., 2001). At 1 day old, the average MDA of the surveyed chickens reached $3,258 \pm 1,551$ (titer). According to Kreider et al. (1991), Antibody titers are divided into 3 levels: low level ($< 3,000$), medium level ($3,000 - 5,000$) and high level ($> 5,000$). Thus, the MDA of the surveyed chickens was at an average level and had a good coefficient of variation CV% of 47.61% (Legese et al., 2022). After vaccination with MB-1 vaccine, the time of vaccine virus release to the Bursa will depend on the level of MDA of each chicken. In chicks with low MDA titers the vaccine virus will replicate earlier in comparison to chicks with higher MDA titers, thus onset of immunity

and onset of antibody titer will also be earlier in chicks with low MDA titers (Wein et al., 2023). Therefore, this mode of operation ensures that each chicken is vaccinated at the right time (Ray et al., 2021) and shortens the immune gap when MDA falls below the protective level and active immunity has not yet increased to level of protection (Le Gros et al., 2009). The results of the study showed that the IBD virus was localized from 15 days old and 100% of the M.B. was detected through genetic sequencing at 21 days of age and there was absolutely no existence of field virus in samples taken throughout the sampling process. Therefore, environmental viruses do not have favorable conditions to attack the bursa since the time of MB-1 localization, helping chickens to be partially protected by this positioning mechanism. Furthermore, vaccine virus strain M.B. had the ability to respond quickly and strongly (Quach et al., 2018) so it could shorten the immune gap susceptible to IBDV (Berg et al., 2000).

IBDV antibody titers are an important part of Gumboro's protection mechanism. The average antibody results by age are shown in Table 5 and Figure 2. At 28 days old, the average IBDV antibody titers reached 238 ± 660 (titers), with a very high coefficient of variation of 277.21%. MDA at this time decreased sharply, this showed that the IBDV of MB-1 vaccine had multiplied in the bursa and stimulated active B lymphocytes to create antibodies. The humoral immune response to the MB-1 vaccine was very strong, and elevated IBDV antibody titers was observed at 32, 36 and 42 days of age. At 32 days of age, the average IBDV antibody titers had increased and reached an antibody level of $1,854 \pm 1,144$ (titer), the coefficient of variation CV% was at a fairly

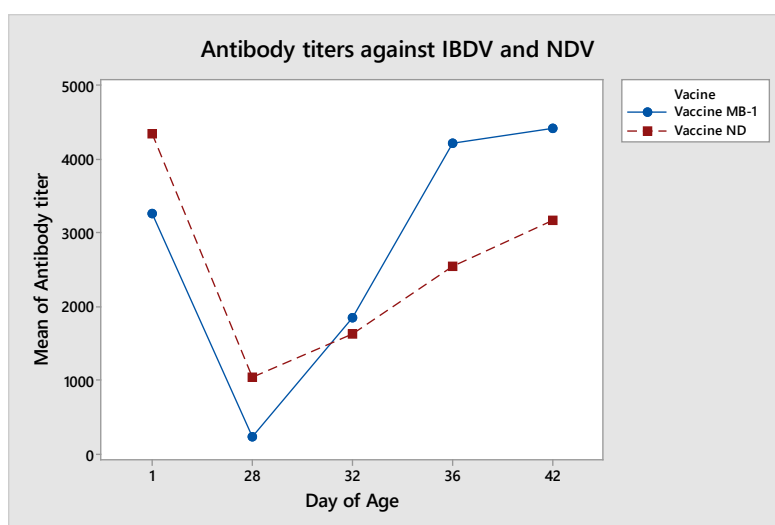
high level of 61.70%. Similarly, at 36 and 42 days old stages, an average IBDV antibody titers had $4,217 \pm 911$ and $4,413 \pm 1,153$ (titer), respectively. According to IDEXX, the average baseline for IBD resistance is recommended at 1,000 (titer). Thus, at 36 and 42 days of age, IBDV antibody titers reached a level above the protection level, with very high IBDV antibody titer uniformity with CV% at 21.6% and 26.13%, respectively. Thanks to the uniformity of the immune response between each individual in the herd, it will help minimize the possibility of spreading IBDV virus into the environment (Quach et al., 2018), further supporting the immune response process when using MB vaccine.

3.3.2. NDV antibodies titers

According to van den Berg et al. (2000) when using vaccines to prevent IBD, one of the concerns is the risk of the virus negatively affecting B lymphocytes, which are responsible for humoral immune response of poultry (ND-killed used frequently for broiler chickens). Therefore, the study conducted a NDV antibody titers when ND-killed vaccination to evaluate the humoral immune response of chickens. Chickens have a good immune response to the NDV antibody titers vaccine that gradually increases at the stage of chickens from 28 to 42 days of age, more uniformly at 42 days of age (Table 5, Figure 2). At 32 days old, the chicken's NDV antibody titers were $1,622 \pm 926$ (titer) with a CV of 57.06% and increased at later days of age. At 36 and 42 days old, NDV antibody titers were $2,550 \pm 2,338$ and $3,180 \pm 2,103$ (titer). Thus, chickens still respond humorally to inactivated ND vaccine when injected on the same day as MB-1 vaccine.

Table 5. Infectious bursal disease virus (IBDV) and Newcastle disease virus (NDV) antibody titers

Date sample	Statistical parameters	IBDV antibody titers	NDV antibody titers
1	$\pm$ SD	3.258 ± 1.551	4.348 ± 1.719
	CV %	47.61	39.54
28	$\pm$ SD	238 ± 660	$1,046 \pm 999$
	CV %	277.21	95.47
32	$\pm$ SD	$1,854 \pm 1.144$	$1,622 \pm 926$
	CV %	61.7	57.06
36	$\pm$ SD	$4,217 \pm 911$	$2,550 \pm 2.338$
	CV %	21.6	91.69
42	$\pm$ SD	$4,413 \pm 1,153$	$3,180 \pm 2,103$
	CV %	26.13	66.14

**Figure 2.** Antibody titers against infectious bursal disease virus (IBDV) and Newcastle disease virus (NDV).

4. Conclusions

The application of the MB-1 vaccine at the hatchery for commercial broilers resulted in early localization of the vaccine virus in the bursa, rapid and uniform development of active IBD antibodies. The MB-1 vaccine did not negatively affect the immune response to Newcastle disease vaccination and was safe for the bursa (a good

BI and BLS) when applied to one-day-old chicks. These findings support the use of the MB-1 vaccine as an effective strategy for improving Gumboro disease.

Conflict of interest

The authors have no conflicts of interest to declare.

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Efficacy of toltrazuril and gleptoferron with dextran on the prevention of coccidiosis and iron deficiency anemia in suckling piglets

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ABSTRACT

The study aimed to evaluate the efficacy of injectable toltrazuril and gleptoferron (combined in one injectable product) in controlling coccidiosis and iron deficiency anemia in piglets. A total of 16 farrowing litters were assigned to 2 treatment groups, 8 litters per group. The piglets in the control group (conventional treatment) were injected with 1 mL of iron dextran (200 mg) and orally administered 1 mL of 5% toltrazuril at 2 days of age (DOA). In the treatment group, the piglets were injected with 1.5 mL of combined product (containing 200 mg of iron and 45 mg of toltrazuril), also at 2 DOA. The mean hemoglobin concentration of 2 DOA piglets in the control group (110.00 ± 17.20 g/L) was similar to that of the treatment group (104.60 ± 15.40 g/L) ($P > 0.05$). However, the mean hemoglobin concentration of the control group (103.30 ± 15.80 g/L) was lower than that of the treatment group (111.60 ± 16.00 g/L) at weaning ($P < 0.05$). Real-time PCR results did not detect oocysts in fecal samples except at 15 DOA. The threshold cycle (Ct) index in the control group at 15 DOA (34.36 - 34.79) was lower than that of the treatment group (35.07 - 36.98). Furthermore, the treatment group had a higher mean body weight at weaning and average daily gain (7.98 kg and 265.20 g/day) than that of the control group (7.40 kg and 225.60 g/day) ($P < 0.001$). In conclusion, combination product provided more benefits for suckling piglets, including controlling coccidiosis and significantly improving weight gain.

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1. Introduction

Toltrazuril is routinely used to control coccidiosis in neonatal piglets on affected farms not only in Vietnam but also worldwide. Its high anticoccidial activity is due to its ability to kill all asexual and sexual stages of coccidia, except the oocyst (Mundt et al., 2003; Joachim & Mundt, 2011). Coccidiosis is a common disease in the swine farming industry globally, caused by a protozoan parasite called *Cystoisospora suis* (formerly *Isospora suis*). This disease is one of the primary causes of diarrhea in suckling piglets, leading to poor productivity and significant economic losses in the pig farming industry (Karembé et al., 2021; Sawhney et al., 2023) and oral formulations have been licensed for many years. Recently, the first parenteral formulation containing TZ in combination with iron (gleptoferron). It is prevalent in all types of farrowing facilities with poor hygiene and management. Toltrazuril is typically administered orally.

Recently, a new injectable combination product (30 mg toltrazuril/mL; 133.4 mg iron/mL as gleptoferron), which can reduce stress and labor costs, has been developed to control coccidiosis and prevent iron deficiency anemia in suckling piglets. A single intramuscular shot (with a recommended dose of 1.5 mL/piglet, equivalent to 45 mg toltrazuril and 200 mg iron) is administered 24 - 96 h after birth (Morales et al., 2018; Hiob et al., 2019; Joachim et al., 2019). Administering toltrazuril by injection ensures accurate and sufficient dosing (avoiding issues where piglets do not swallow or vomit) and combines iron supplementation, reducing stress for suckling piglets and significantly saving labor costs. Although some studies on the pharmacokinetics of combined product

(toltrazuril and gleptoferron) have been conducted by Joachim et al. (2018), experimental data on its efficacy are still limited, particularly in Vietnam. Therefore, the objective of this study was to evaluate the efficacy of combined product in controlling coccidiosis and preventing iron deficiency anemia in suckling piglets during the suckling period.

2. Materials and Methods

2.1. The experimental farm and animals

The experiment was conducted on a farm with a history of coccidiosis, using Pig Improvement Company (PIC) sows crossbred with Duroc boars. Piglets from a total of 16 farrowing litters were randomly selected and divided into two groups, with each group kept separately to prevent the spread of coccidia during the rearing period. Both groups were balanced in terms of sow parity and the average weight of the piglets (Table 1).

The control group: the piglets (2 days old) were injected with 1 mL of iron dextran (200 mg) and orally administered 1 mL of 5% toltrazuril (drop suspension with the pump provided by the supplier).

The treatment group: the piglets (2 days old) were injected with 1.5 mL of the combined product (30 mg toltrazuril/mL; 133.4 mg iron/mL).

Table 1. Body weights of neonatal piglets

Groups	N	Mean body weight ($\pm$ SE)kg	P
Treatment	92	1.54 $\pm$ 0.02	0.565
Control	93	1.52 $\pm$ 0.03	

2.2. Blood sampling và hemoglobin test

A total of 48 piglets (6 piglets - three barrows and three females) in each group were randomly chosen to measure Hb concentration on the farm. Blood was taken from the ear vein of the piglets, two first drops were removed and the test was tested with Hemocue® Hb 201+ system (SE-262 71, Ängelholm, Sweden). Results were displayed in g/L within 15 - 60 sec depending on the hemoglobin concentration. Piglets were classified as follows: Hb levels < 90 g/L were considered to be anemic, Hb levels $\geq$ 90 g/L and $\leq$ 110 g/L were suboptimal and Hb levels > 110 g/L were optimal (Bhattarai & Nielsen, 2015b).

2.3. Fecal sampling and real time PCR

Fecal samples were collected from 5 piglets per litter and then pooled them into one pooled sample (3 - 5 g/sample). Use medical cotton to clean the anus before taking the samples, then use the swab to collect feces inside the rectum with 3 - 5 g/sample. It was put in 15 mL tube and kept cool to deliver to the lab the same day. As 8 litters of each group were examined four times, a total of 32 fecal samples/group were available. The sampling schedule was at 3, 5, 15, and 24 DOA.

DNA extraction

The DNA of *Cystoisospora suis* was extracted from fecal samples following the manufacturer's instruction by using QIAGEN's QIAamp® PowerFecal Pro® DNA Kit.

Real time PCR protocol

The real time PCR reaction mix is based on QIAGEN's QuantiNova Probe RT-PCR kit with the specific oligonucleotides (Samarasinghe et al., 2008). The following oligonucleotides were used: Forward primer: CystoisoITSGF1 (GATCATTCACACGTGGCCCTT);

Reverse primer: CystoisoITSR4 (CCTTTATGCGGCCATTCTGAAGC); and CystoisoITS probe 2 (FAM-GCGTTCGGGGGAAGCTGTG-BHQ1). The total volume was 20 μ L as followed: the reaction mixture consisted of 3.4 μ L distilled water, 1 μ L of each primer (10 μ mol/ μ L), 10 μ L QuantiNova MasterMix (2x), 0.1 μ L ROX, 0.5 μ L probe (10 μ M) and 4 μ L extracted DNA. A thermal cycler was used to perform 95°C for 10 min, 40 cycles (95°C sec, 60°C for 40 sec).

2.4. Produced performance

After farrowing, all neonatal piglets were weighed, those weighing 0.9 kg or more and healthy were selected for raising. At weaning, 6 piglets per litter were randomly weighted at 26 DOA.

2.5. Statistical analysis

Summary statistics were calculated for each variable. This study was performed a two-samples t-Test for body weight and hemoglobin level by using Minitab (17 version).

3. Results and Discussion

3.1. Hemoglobin level

Table 2. Hemoglobin test results

Groups	Age	Optimal		Sub-clinical		Anemia		Total	
		No.	%	No.	%	No.	%	No.	%
Control	2 d old	20	41.67	24	50.00	4	8.33	48	100
	24 d old	15	31.25	24	50.00	9	18.75	48	100
Treatment	2 d old	19	39.58	24	50.00	5	10.42	48	100
	24 d old	29	60.42	15	31.25	4	8.33	48	100

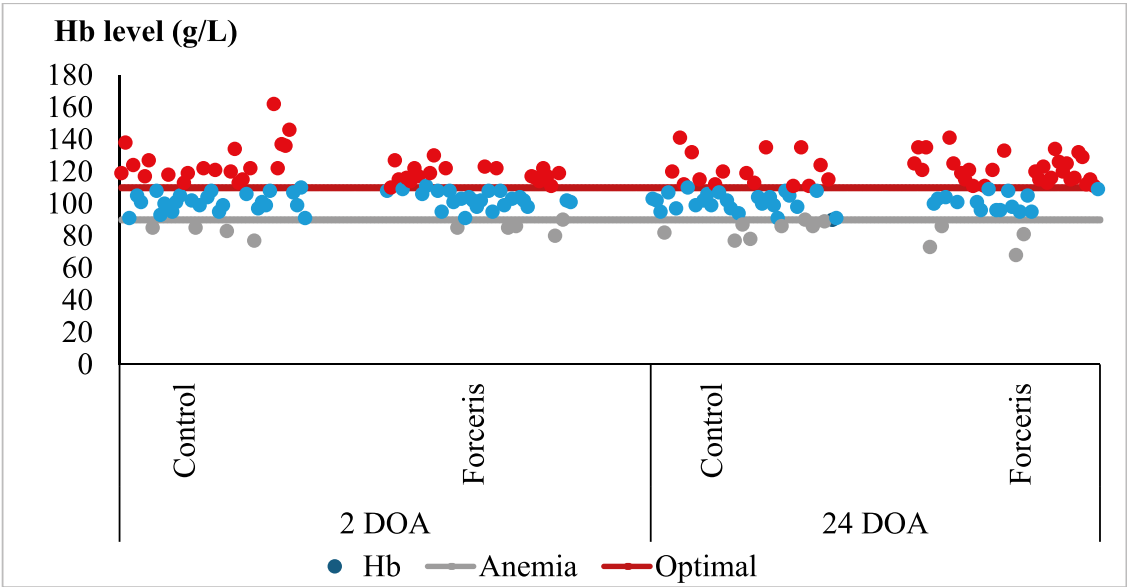


Figure 1. Hemoglobin test results.

Hemoglobin results are shown in Table 2 and Figure 1. The findings indicated that the treatment group showed greater improvement in hemoglobin levels (24 DOA) compared to the control group. At 24 DOA, the hemoglobin (Hb) level of suckling piglets in the treatment group was at optimal levels in 60.42% of cases, compared to 31.25% in the control group. Additionally, the anemia rate in the treatment group (8.33%) was lower than that in the control group (18.75%). Bhattarai & Nielsen (2015a) and Chevalier (2019) confirmed the association

between hematological parameters at weaning and weight gain in piglets. Positive correlations were observed between hemoglobin levels and weight gain, indicating that an increase in hemoglobin corresponds to an improvement in weight gain. The results suggest that combination product performed well in improving Hb levels, consistent with previous reports (Karembe et al., 2021) and oral formulations have been licensed for many years. Recently, the first parenteral formulation containing TZ in combination with iron (gleptoferron).

The study compared the efficacy of gleptoferron and iron dextran in preventing anemia in suckling piglets. Both administered products were safe, but gleptoferron may have greater efficacy in preventing anemia compared to iron dextran. The study highlights the importance of iron supplementation for piglet health and growth. Iron supplementation through parenteral administration is a common practice to prevent iron deficiency anaemia in piglets. Hemoglobin level is the most widely used parameter for detecting anemia and evaluating

the efficacy of iron supplementation. However, Hb values in suckling piglets are more variable, physiological levels and consequently anemia are defined differently. Following the intramuscular administration of 200 mg of iron at 2 DOA, Hb levels in piglets in the treatment group were more optimal, with less anemia observed compared to the control group. It has also been observed that an optimal iron status (Hb > 110 g/L) at weaning can lead to improved growth performance, suggesting a more effective hematinic activity of gleptoferron (Morales et al., 2018; Chevalier, 2019).

3.2. Real time PCR result

Table 3. Real time PCR results

Groups		Results	3 DOA	5 DOA	15 DOA	24 DOA
Control	Positive (%)	Ct = 34.36	-	-	12.50	-
		Ct = 34.79	-	-	12.50	-
	Negative (%)		100	100	75.00	100
Treatment	Positive (%)	Ct = 35.07	-	-	12.50	-
		Ct = 36.98	-	-	12.50	-
	Negative (%)		100	100	75.00	100

Ct: threshold cycle; DOA: days of age.

Diagnosis of coccidiosis is considered a challenging process; it is usually performed by collecting and pooling fecal samples from different piglets of the same litter. The most common diagnostic technique is flotation with oocysts and subsequent counting, often using a McMaster chamber. However, other techniques with higher sensitivity, such as real time PCR, are becoming more popular.

The oocyst detected results in both the treatment and control groups were negative except at 15 DOA. At 15 DOA, the suckling piglets can get a high-risk period for coccidiosis infection with 25% infected proportion (two pooled samples) (Table 3). Interestingly, the

administration of toltrazuril can reduce the infection and harmful effects of coccidia in both experimental piglets. However, the results showed a small difference in the Ct index, with the treatment group having a higher Ct index than the control group (it means that the detection of coccidiosis DNA is lower than the control group) (Table 3). This further confirms that intramuscular administration of toltrazuril using the combined product provides better control of coccidiosis in piglets. This result aligns with the findings of Karembe et al. (2021), which showed a high concentration of toltrazuril in the intestinal tissues of piglets when the combined product was administered at 2 DOA.

Cystoisospora suis is an intracellular parasite that infects intestinal cells, primarily in the jejunum and ileum (Niestrath et al., 2002). The injectable toltrazuril product with an oral toltrazuril product for controlling piglet cystoisosporosis. Both treatments were safe and highly effective in suppressing oocyst excretion, with body weight development significantly improved in the treatment group compared to the control group. In this study, real-time PCR method was used to detect oocyst in fecal samples. The results showed that real time PCR was more sensitivity and specificity to diagnose coccidiosis in swine. The results of this study align with Maes et al. (2007), which reported positive effects of toltrazuril on piglet growth in herds without clinical isosporosis.

3.3. Body weight

Table 4. Body weight at weaning

Groups	N	Mean body weight ($\pm$ SE)kg	P
Treatment	48	7.98 $\pm$ 0.11	< 0.001
Control	48	7.39 $\pm$ 0.10	

The average live weight of neonatal piglets in both groups at the beginning of the experiment was similar (Table 1). At weaning (26 DOA), the mean body weight in the treatment group (7.98 kg) was higher than that of the control group (7.39 kg). The difference in average weaning weight is statistically significant ($P < 0.001$) (Table 4). This result indicates that the combined product can improve weight gain in suckling piglets due to the effectiveness of iron and toltrazuril, as well as the reduction of stress. This leads to healthier and faster weight gain in suckling piglets. This is consistent with the results of Joachim et al. (2018), who compared the effectiveness of the combined product with an oral toltrazuril product. Their study showed that the mean body weight in the treatment group

was higher compared to the control group due to a reduction of coccidia infection and stress. This study also demonstrated that suckling piglets in the treatment group had higher ADG than those in the control group. Growth performance during the suckling period is essential for post-weaning growth, even if the difference is not statistically significant at the start. Improved growth performance may be associated with optimal iron status, enhancing oxygen transport, immune function, vitality, and metabolism (Perri et al., 2016; Chevalier et al., 2021).

4. Conclusions

The optimal Hb levels (60.42%) were higher, and the anemia Hb levels (8.33%) were lower in the treatment group at 24 DOA. The Ct index for coccidia detection using real time PCR was above 35 in the treatment group. Weaning weight gains were significantly higher in the treatment group compared to the control group. The use of a product which combines toltrazuril with gleptoferron, can reduce coccidia infection and increase growth in suckling piglets.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

Author contributions

Nguyen Ba Toan and Vo Phong Vu Anh Tuan conceived and designed this study. Nguyen Ba Toan and Huynh Vo An Khang collected data from the swine farm. Nguyen Ba Toan; Vo Phong Vu Anh Tuan and Nguyen Thi Thuong drafted the manuscript. All authors read and approved the final article.

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Evaluation of the incompatible prevalence by blood crossmatching tests in dogs

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ARTICLE INFO	ABSTRACT
Research Paper Received: November 18, 2024 Revised: January 04, 2025 Accepted: January 08, 2025 Keywords Crossmatching Dogs Ho Chi Minh City Incompatibility *Corresponding author Nguyen Thi Thuong Email: thuong.nguyenthi@hcmuaf.edu.vn	Crossmatching is one of the most important tests before a transfusion in small animal practice, helping to reduce the risk of post-transfusion reaction in dogs with unknown or experienced blood transfusion history. In this study, crossmatching assessments were performed after washing and resuspending the collected blood. Two types of crossmatching methods was conducted: major crossmatching and minor crossmatching. Based on an agglutination reaction, crossmatching allows the detection of naturally occurring alloantibodies or alloantibodies produced as a consequence of sensitization. In this study, there were 10 incompatible cases in total of 26 cases of dogs taken to the veterinary hospital and Greyhounds (incompatible rate 38.46%). The results showed that higher proportions of incompatible cases were recorded in group of dogs from 1 to 5 years old (26.92%), body weight from 5 - 10 kg (19.23%), female (23.08%), foreign breeds (34.62%), commercial feed (34.62%) and in-house (23.08%). The dogs in the same house or neighborhood, there were 38 incompatible cases in the total of 134 cases (28.36%), in which the highest proportion was found in dogs from 1 - 5 years old had (13.43%), female (15.67%), and foreign breeds (15.67%). In the management groups, in-house dogs had a lowerproportion of incompatibility with each other than cage-free dogs, with 2.24% and 26.12% respectively. Although the results indicated that age, body weight, gender, and breed did not affect the pre-transfusion crossmatching results, it is necessary to perform the crossmatching test before a transfusion to reduce the risk of hemolytic transfusion reactions in dogs.

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1. Introduction

Blood transfusion is being applied widely in dogs and cats in veterinary practice in Vietnam. Crossmatching is one of the most important steps before a transfusion in dogs. The main reason of performing crossmatching is to provide safe blood to the right dog at the right time. Crossmatching determines the serological compatibility between the patient and donor blood, based on an agglutination reaction, and allows detection of naturally occurring alloantibodies or alloantibodies produced as a consequence of sensitization (Vidushi et al., 2020). Cross-matching is indicated when the transfusion history is unknown or the dog has been transfused more than 4 days ago (Davidow et al., 2021). Even though crossmatching is performed before the transfusion, it is still possible that the patient can experience post-transfusion reactions. There are two assessments of crossmatching: major cross-match and minor cross-match (Day & Kohn, 2012). Although transfusion is widely applied in veterinary practice, there are almost no studies about factors such as age, body weight, gender, breed, feed and management, which can affect the prevalence of incompatible crossmatching results. Even though crossmatching reduces the risk of hemolytic transfusion reactions, it does not completely eliminate the risk of transfusion reactions in dog (Tocci & Ewing, 2009). Appropriate RBC survival and/or complete elimination of transfusion reactions are not guaranteed, because low titers of anti-RBC antibodies may not cause enough agglutination for detection, even with a compatible crossmatch test (Zaremba et al., 2019).

Factors, such as age, breed, gender, feed and management, have not been studied for any

association with the result of crossmatching in dogs. Greyhound is a good candidate for being a source of blood donor, due to the big size of this dog breed (Gibson & Abrams-Ogg, 2012), however, there are not many researches about using Greyhound's blood for transfusion in dogs in Ho Chi Minh City. Therefore, this study was carried out to evaluate the prevalence of the incompatibility in crossmatching between the blood of Greyhound and dogs that were taken to the vet hospital, and between dogs living in the same house or neighbor. Furthermore, the study was also to evaluate if factors such as age, gender, breed, feed, and management had affected the prevalence of incompatible results.

2. Materials and Methods

2.1. Blood sampling

A total of 26 blood samples were collected from dogs from October 2022 to October 2023 at Nong Lam University Veterinary Hospital and the Veterinary Clinic in Ho Chi Minh City.

2.2. Crossmatching assay procedure

The crossmatching assessments were major cross-match and minor cross-match. The protocol based on the protocol of (Lanevski & Wardrop, 2001). Blood collected from the donors and patients are stored in EDTA (ethylenediaminetetraacetic acid) tubes. The EDTA tubes containing blood are centrifuged with 3.400 rpm. Washing red blood cells (RBCs): resuspend 0.25 mL of RBCs in 2 - 4 mL of saline, then centrifuge in 1 min, and remove the supernatant. Repeat the procedure twice, and finally remove the supernatant. Resuspend 0.1 - 0.2 mL of RBCs in approximately 4.8 mL saline in order to obtain a 2 - 4% RBC solution. In 3 tubes identified "Major", "Minor" and "Control" add

the following Table 1. Incubate tubes in 15 min at 37°C. Reading results: Note serum color and record any hemolysis. Then resuspend the red cell button into the overlying serum layer, noting the presence of agglutinating clumps. Place a drop of resuspended RBCs on a slide, apply coverslip, and read at 100X and 400X microscope. If the crossmatch is compatible, the RBCs should be individually distributed. Hemolysis

(compared to control) or agglutination is seen with an incompatible crossmatch Rouleaux, a physiological plasma-related phenomenon, may sometimes be observed. In order to distinguish this from agglutination, centrifuge the tubes again for 15 sec, remove serum, and add 2 drops of saline; then centrifuge the tubes once more and reexamine the RBC suspensions.

Table 1. Crossmatching composition in the major, minor and control tubes

	“Major” crossmatch	“Minor” crossmatch	Control
Patient	2 drops of serum	1 drop of RBC solution	1 drop of patient RBC solution & 2 drops of patient serum
Donor	1 drop of RBC solution	2 drops of serum	1 drop of donor RBC solution & 2 drops of donor serum

These assessments were performed in Nong Lam Veterinary Hospital’s Laboratory. In the first experiment, the blood of Greyhound dogs from the previous study of Nguyen et al. (2024) was collected for cross-matching with other patients. All dogs were fully vaccinated within the last 12 months and received prophylactic flea treatment regularly. Dogs did not receive any raw meat diet and were completely healthy and relaxed. The dogs were fed with commercial dry feed (France) (900 gr). All dogs were checked physically and all clinical signs, including body temperature, heart and respiratory rate, and appetite were normal. Blood from dogs taken to the veterinary hospital, was also collected in 1 mL EDTA tubes, and then crossmatching was immediately performed with Greyhounds’ blood.

In the second experiment, dog blood living in the same house or neighborhood, was collected in 1 mL EDTA tubes, and then crossmatching was performed immediately between them. The information on age, gender, breed, feed, and management was also collected.

2.3. Statistical analysis

The collected data was analyzed with Microsoft Excel 2016 and test with Minitab 17.

3. Results and Discussion

3.1. Prevalence of incompatibility crossmatching between Greyhounds and other patient dogs

A total of 26 dogs were tested for compatibility with Greyhound dogs from the previous study of Nguyen et al. (2024) to investigate the prevalence of incompatibility crossmatching and evaluate factors that could be relevant to the crossmatching results. In 26 cases in the veterinary clinic, there were 10 dogs whose blood was incompatible with the Greyhounds’ blood (38.46%). The data are summerized in Table 2.

Table 2. The prevalence of incompatibility crossmatching between dogs taken to the veterinary clinic and Greyhounds

	Factor	Total cases	Incompatibility	
			Cases	Prevalence (%)
Age	Below 1 year old	0	0	0.00
	1 - 5 years old	21	7	26.92
	Above 5 years old	5	3	11.54
Body weight	Below 5 kg	11	2	7.69
	5 - 10 kg	8	5	19.23
	Above 10 kg	7	3	11.54
Gender	Male	13	4	15.38
	Female	13	6	23.08
Breed	Domestic	3	1	3.85
	Foreign	23	9	34.62
	Mix	0	0	0.00
Feed	Rice	0	0	0.00
	Commercial feed	21	9	34.62
	Mix	5	1	3.85
Management	In-house	10	6	23.08
	Cage-free	16	4	15.38
	Total	26	10	38.46

In the first experiment, the prevalence of incompatible crossmatching of the group of 1 - 5 years old, which was the highest in three age groups, followed by the group of above 5 years old. In the below 1 year old group, there were no cases of incompatible crossmatching between Greyhounds and dogs taken to the veterinary hospital. The blood of the Greyhounds was tested compatibility with 11 dogs below 5 kg, 8 dogs from 5 - 10 kg, and 7 dogs above 10 kg. The compatible cases were 2, 5 and 3 in dogs of below 5 kg, 5 - 10 kg, and above 10 kg, respectively. In gender groups, the prevalence of male dogs' blood compatible with Greyhounds' blood was 15.38% (4 cases), which was less than the prevalence in the female group (23.08%, 6 cases). In breed groups, the prevalence of positive results in foreign breeds accounted for 34.62% of

total compatible cases, which was much higher than in the domestic group (with 3.85%). There were 9 incompatible cases in a total of 21 dogs fed with commercial feed, while there was only 1 incompatible case in the mixed feed group. In the management group, the cases of in-house dogs whose blood was incompatible with Greyhounds' blood accounted for 23.08%, while cage-free dogs accounted for 15.38%.

Retired Greyhounds are universal blood donors whose blood can be given to most dogs. The main reasons for Greyhounds being ideal blood donors are they have an appropriate size (> 25 kg), a gentle temperament, a high hematocrit, large and easy-to-access jugular veins (Couto, 2005). Previous reports indicated that Greyhounds might have lower levels of some coagulation factors (Lara-García et al., 2008;

Vilar et al., 2008) CBC, platelet count, OSPT, APTT, platelet function with PFA-100. When compared with other breeds, Greyhound's blood had significantly lower concentrations of von Willebrand factor (vWF), coagulation factor X, and fibrinogen. However, they are still suitable for use as plasma donors (Walton et al., 2014). It has been reported that Greyhounds have a larger blood volume (about 110 to 120 mL/kg B.W) than other dog breeds with a similar size (80 to 100 mL/kg B.W) (Lisciandro et al., 2009). The result indicated that the compatible results were not associated with age, body weight, gender, breed, feed and management between dogs taken to the veterinary hospital and the Greyhounds ($P > 0.05$).

3.2. Prevalence of incompatibility crossmatching between dogs in the same house

In many cases requiring the blood transfusion to be performed as soon as possible, a Greyhound as a blood donor or stored blood was usually unavailable. Even though having different hematologic and hemostatic profiles compared to Greyhounds, non-Greyhounds' blood can be used to aid in anemia situations (Chang et al., 2021), and these non-Greyhounds can be other dogs from the same house. Donor dogs should be from 1 to 8 years of age, greater than 23 kg, and provide 450 mL per donation, screening for blood-borne pathogens, up-to-date with vaccinations and dewormed (Wardrop et al., 2016; Kuo & McMichael, 2020). Donation volumes of 15 to 20 mL/kg from dogs (Lanevski & Wardrop, 2001). In the second experiment, the blood of dogs in the same house or neighborhood was compatible with each other. The data were showed in Table 3.

Table 3. The prevalence of incompatibility crossmatching between dogs in the same house or neighbor

Factor		Total cases	Incompatibility	
			Cases	Prevalence (%)
Age	Below 1 year old	65	16	11.94
	1-5 years old	59	18	13.43
	Above 5 years old	10	4	2.99
Body weight	Below 5 kg	58	17	12.69
	5-10 kg	54	16	11.94
	Above 10 kg	22	5	3.73
Gender	Male	75	17	12.69
	Female	59	21	15.67
Breed	Domestic	67	16	11.94
	Foreign	62	21	15.67
	Mix	5	1	0.75
Feed	Rice	89	25	18.66
	Commercial feed	25	7	5.22
	Mix	20	0	0.00
Management	In-house	31	3	2.24
	Cage-free	103	35	26.12
Total		134	38	28.36

In the age groups, the highest proportion of incompatibility was in the group of 1 - 5 years old (13.43%), followed by the below 1-year-old group (11.94%) and above 5 years old (2.99%). The same goes with body weight groups: dogs in the below 5 kg group had a higher percentage of incompatibility (12.69%) than dogs in 5 - 10 kg group (11.94%) and in above 10 kg group (3.73%). The incompatible results of female dogs accounted for 15.67%, which was higher than the male dogs (12.69%). The foreign breed had the highest proportion of positive results (15.67%). The domestic breed's incompatible proportion was 11.94%. There was only 1 incompatible result in the mixed-breed group. Dogs fed with rice had the most cases of incompatible results, with 25 cases (accounting for 18.66%). The commercial feed accounted for 5.22%, with 7 cases. There was no incompatible case in the mixed feed group. In management groups, dogs with cage-free living had more cases than those in-house (35 and 3 dogs, respectively). Even though Greyhounds are great blood donors, they are not always available in many cases. In contrast, dogs from the same house or neighborhood are usually available. Age, body weight, gender and breed did not affect the compatible results ($P > 0.05$).

4. Conclusions

In the studies, age, body weight, gender, and breed were not associated with the prevalence of incompatible crossmatching results between dogs taken to the veterinary hospital and the Greyhounds, or dogs in the same house or neighborhood. Greyhounds are the ideal blood donor due to their size, large jugular veins, and temperament. Dogs in the same house or neighborhood are available blood source in some emergencies. The prevalence of incompatibility crossmatching in dogs in this study was 28.36

- 38.46%. Even though the results indicated that age, body weight, gender, and breed did not affect the pre-transfusion crossmatching results, it is still recommended to perform the crossmatching test before a transfusion in order to reduce the risk of post-transfusion hemolytic reactions. However, more tests should be carefully performed before the transfusion, such as complete blood count, biochemistry profile, infectious diseases scanning and blood typing.

Conflicts of interest

The authors have no conflicts of interest to declare.

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Effect of growth regulators and culture conditions for micropropagation of Vietnamese ginseng (*Panax vietnamensis* Ha et Grushv) on a temporary immersion system

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ABSTRACT

Ngoc Linh ginseng is a valuable medicinal crop recognized for its anti-amnesia, anti-inflammatory, hepatoprotective, and anti-cancer properties. This study was to investigate the variation effect of growth regulators and culture conditions for micropropagation of Ngoc Linh ginseng (*Panax vietnamensis* Ha et Grushv.) on a temporary immersion system. After ten weeks of cultivation, the best callus induction rate in a solid Murashige and Skoog (MS) medium containing 2,4-dichlorophenoxyacetic acid (2,4-D) 1.5 mg/L was 90%. Callus cultured on MS medium (indole-3-butyric acid (IBA) 0.7 mg/L) supplemented with Thidiazuron (TDZ) 0.8 mg/L resulted in the highest embryogenesis rate, reaching 69.29%, with an average of 11.25 embryos per explant. The MS medium supplemented with 6-benzylaminopurine (BAP) 1 mg/L and α -naphthalene acetic acid (NAA) 1 mg/L was shown to be appropriate for shoot growth from Ngoc Linh ginseng embryos. The temporary immersion system showed that with a 6-benzyladenine acid (BA) concentration of 1.5 mg/L for 3 min of immersion, the greatest value for shoot number was 6.00 shoots/explant, and the height was 2.70 cm. Roots developed best at a treatment with a 5 min of immersion and NAA 1 mg/L concentration, root formation frequency, and root length reaching values of (71.25%; 1.61 cm). As a result, the temporary immersion system (TIS) system can be used for high-efficiency *in vitro* propagation of Ngoc Linh ginseng plants.

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1. Introduction

In 1985, Ha & Grushvitsky (1985) discovered and identified Ngoc Linh ginseng as a new medicinal species with the scientific name *Panax vietnamensis* Ha et Grush. Since its discovery, Ngoc Linh ginseng (NLG) has been the focus of comprehensive investigation by domestic and

international institutions, particularly regarding its chemical constituents, therapeutic properties, and breeding techniques. Especially, Duong et al. (2013) have successfully cultivated NLG tissue *in vitro* in multiple investigations. However, cultivating NLG tissue is still complicated. The ability to multiply shoots is poor, and the

culture to produce a plant suitable for growing in the wild is still limited. Many scientists are continuing to develop the optimum methods for growing Ngoc Linh ginseng *in vitro*. Growth and development are determined by plant growth regulators (PGRs), which are responsible for cell division and growth. The PGRs (auxin and cytokinin) regulate the development of different plant organs during *in vitro* growth. Auxins and cytokinins also improve cellular division and induce the formation of adventitious shoots by decreasing apical dominance (Pierik, 1988). Temporary immersion systems (TIS) allow for control of contamination, adequate nutrient and oxygen supply, mixing, relatively infrequent subculturing, ease of medium changes, and limited shear damage. The TIS results in increased biomass with less labor and faster than conventional (semi-solid) techniques. Consequently, they have been adopted by many propagation and breeding programs to save costs and accelerate the production of plantation units of elite clones and/or the release of new cultivars (Watt, 2012).

Recent research by Jekayinoluwa et al. (2019) has demonstrated the use of automated TIS, which is less expensive and provides an ideal environment for *in vitro* plant cell, tissue, and organ cultures with high productivity. The TIS offers a flexible and perspective-oriented cultivation technology that could be adopted for the needs of large-scale production of secondary metabolites by plant *in vitro* shoot cultures. The usefulness of TIS and their positive effects in one or more stages of plant tissue proliferation have been reported for several easily multiplying species, such as *Ananas comosus* (Escalona et al., 1999), *Eucalyptus spp.* (Alister et al., 2005), *Psidium guaiava* (Vicchez & Albany, 2014), *Sorbus domestica* (Valeria & Francesco, 2024),

In TIS, the immersion times vary significantly, according to the research results of Maciel et al. (2016), who showed that long immersion times (1 h every 6 h) were efficient for potatoes, whereas very short immersion times (1 min every 12 h) stimulated somatic embryo production in *Coffea arabica* and *rubber* and increased *grapevine* shoot propagation (Pérez, 2013). Nevertheless, at the moment, to the best of our knowledge, there is a scarcity of studies using the TIS system on Ngoc Linh ginseng. The majority of studies focus on hair root biomass (Ha & Duong, 2017). Nguyen et al. (2020) used the TIS system to survey the effectiveness of embryo multiplication, shoot germination, and small taproot in a $1/2$ SH (Schenk & Hildebrandt) liquid medium. Simultaneously, no research has investigated the effects of the combination of growth regulators and immersion time on Ngoc Linh ginseng shoot propagation and rooting in an MS liquid medium on TIS.

2. Materials and Methods

2.1. Plant materials, chemicals, and reagents

The initial material used for the experiments was a rhizome of a 5-year-old Ngoc Linh ginseng provided by the Medicinal Plant Research Laboratory, Faculty of Biological Sciences, Nong Lam University, Ho Chi Minh City.

Composition of the culture medium used: The basic MS medium (Murashige & Skoog, 1962) was supplemented with 6-benzyladenine acid (BA), α -naphthalene acetic acid (NAA), Thidiazuron (TDZ), 2,4-dichlorophenoxyacetic acid (2,4-D), 6-benzylaminopurine (BAP), agar and sucrose for different culture experiments.

Origin of the temporary immersion system: In this study, the TIS system was designed according

to Escalona et al. (1999) with two glass containers of 1000 mL capacity. One for growing plants and a reservoir for liquid medium. The two containers were connected by silicone and glass tubes. The airflow was sterilized by passage through 0.2 μ m hydrophobic filters (Figure 1). Air pressure

from an air compressor pushed the medium from one container to the other to immerse the plants completely. The airflow was reversed to withdraw the medium from the culture container. Electronic timers controlled the frequency and length of the immersion period.

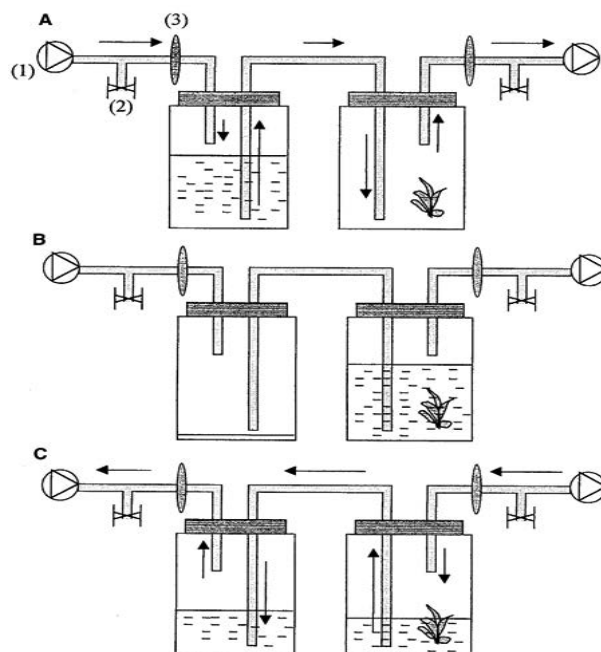


Figure 1. A–C Description of automated temporary immersion system. An (A) solenoid valve is opened, and compressed air forces medium into the plant container, immersing the plants (B).

(C) After a fixed time a second solenoid valve is opened, and air pressure forces medium back into the original container (A). 1 Air compressor, 2 solenoid valve, 3 hydrophobic filters (0.2 μ m) (Escalona et al., 1999).

2.2. Effect of 2,4-D and BAP on callus induction of Ngoc Linh ginseng

The initial material used for the experiments was a rhizome of 5-year-old Ngoc Linh ginseng. The rhizomes were sterilized and transplanted into MS medium using the sterile protocol described by Duong et al. (2010). Thereafter, the explants were used to conduct callus induction tests. The rhizome tissue was cut crosswise into thin, 1 mm-thick slices. Then it was cultivated in MS medium with 7 g/L agar and 3% sucrose. The experiment was set up completely randomized, with two

factors: concentrations of 2,4-D (0; 0.5; 1; 1.5 mg/L) and BAP (0; 0.5; 1.0; 1.5 mg/L). The experiment involved 16 treatments with 3 repetitions per treatment (9 explants per repetition). Explants were cultivated in an all-dark room.

2.3. Effect of TDZ concentrations on embryogenesis induction from callus tissue Ngoc Linh ginseng *in vitro*

Callus was cut into small pieces, transferred into a triangular vase containing MS medium (7 g/L) agar and 3% sucrose, and supplemented

with 0.7 mg/L IBA (indole-3-butyric acid). The experiment was completely randomized with one factor of TDZ at different concentrations (0; 0.2; 0.4; 0.6; 0.8 mg/L). The experiment involved 5 treatments and 3 repetitions (9 explants/repetition). All of the explants were cultured in a standard culture room at $22 \pm 2^\circ\text{C}$, illuminated at an intensity of 2000 lux for 10 h per day.

2.4. Effect of BA and NAA on shoot development of Ngoc Linh ginseng *in vitro*

The embryos from the embryogenesis induction experiment were continually transferred into a triangular vase containing MS medium, agar (7 g/L), and 3% sucrose, supplemented with BA at concentrations (0; 0.5; 1; 1.5 mg/L) and NAA (0; 0.5; 1; 1.5 mg/L). The experiment was arranged with 16 treatments and 3 repetitions (9 explants per repetition). All of the explants were cultured in a standard culture room at $22 \pm 2^\circ\text{C}$, illuminated at an intensity of 2000 lux for 16 h per day.

2.5. Effect of immersion time and growth regulators on shooting of Ngoc Linh ginseng *in vitro* in temporary immersion system

Shoots from the experiment (2.4) were about 0.5 cm in size and transplanted into the TIS system, which contained 150 mL of MS medium supplemented with 3% sucrose, two BA concentrations (0, 0.5, 1.0, and 1.5 mg/L), and a combination of immersion times (1, 3, and 5 min) with a 6-h immersion cycle. The study used 12 treatments and 3 repetitions (9 explants per repetition). Explants were cultivated in a standard culture chamber at $22 \pm 2^\circ\text{C}$, illuminated at 2000 lux for 16 h per day. Data on shoot formation was collected after 10 weeks of culture.

2.6. Effect of immersion time and growth regulators on rooting of Ngoc Linh ginseng *in vitro* in temporary immersion system

Shoots from the experiment (2.4) were about 0.5 cm in size and transplanted into the TIS system, which contained 150 mL of MS medium supplemented with 3% sucrose, two NAA concentrations (0, 0.5, 1.0, and 1.5 mg/L), and a combination of immersion times (1, 3, and 5 min) with a 6-h immersion cycle. The study used 12 treatments and 3 repetitions (9 explants per repetition). Explants were cultivated in a standard culture chamber at $22 \pm 2^\circ\text{C}$, illuminated at 2000 lux for 16 h per day. Data on root formation was collected after 10 weeks of culture.

2.7. Data analysis

All data were processed using Microsoft Excel software. Analyze One Way ANOVA and Two Way ANOVA with Minitab statistical processing software version 16.2.4 using Tukey's test method.

3. Results and Discussion

3.1. Effect of 2,4-D and BAP on callus induction

Studies in subjects of the genus *Panax* show that the callus initiation phase often involves a combination of cytokinins and auxins (Duong et al., 2010). Auxin, especially 2,4-D, plays an essential role in callus formation and proliferation at specific concentrations (Khan, 2019). The combination of BAP and 2,4-D as growth regulators have a positive effect on callus production (Hemmati et al., 2020).

After 6 weeks of culture (Figures 2a & 2c), the majority of the treatments began to exhibit the first signs of induction. Induction of callus formation occurs surrounding or on the surface of the explants. The callus is formed by cutting explants in contact with the medium supplemented with appropriate growth regulators (Yunita et al., 2021).

Table 1. Effect of 2,4-D and BAP on callus induction after 10 weeks of culture

Treatment	BAP (mg/L)	2,4-D (mg/L)	Callus induction frequency (%)
A1	0.0	0.0	-
A2		0.5	50.37 ^{ef}
A3		1.0	83.51 ^{ab}
A4		1.5	90.00 ^a
A5	0.5	0.0	-
A6		0.5	46.06 ^{fg}
A7		1.0	64.76 ^{cde}
A8		1.5	77.02 ^{abc}
A9	1.0	0.0	-
A10		0.5	43.94 ^{fg}
A11		1.0	57.12 ^{def}
A12		1.5	67.65 ^{bcd}
A13	1.5	0.0	-
A14		0.5	32.69 ^g
A15		1.0	54.94 ^{def}
A16		1.5	59.50 ^{def}
CV%			9.15

Mean values in the same column with different letters are statistically significantly different, $P < 0.05$. The percentage data were converted according to the formula $\arcsin\sqrt{(x/100)}$ before statistical processing. (-): The value of zero; 2,4-D: 2,4-dichlorophenoxyacetic acid; BAP: 6-benzylaminopurine.

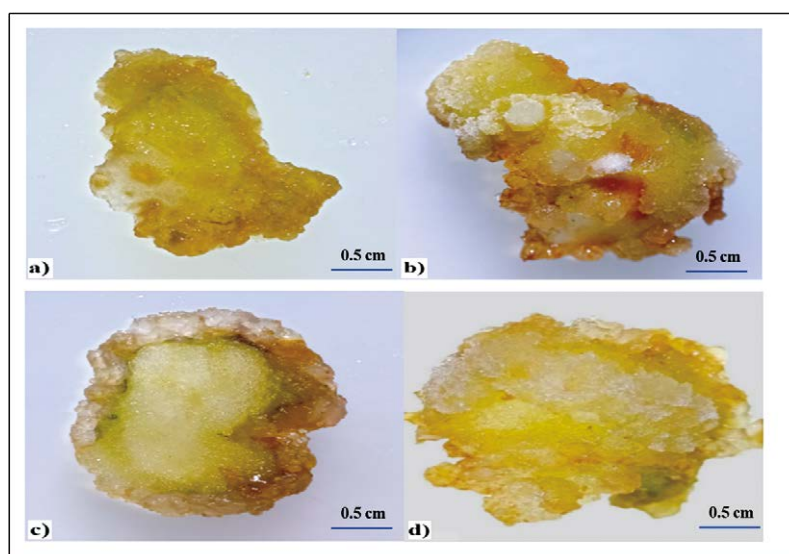


Figure 2. a, b) Callus after 6 and 10 weeks culture in treatment A8 (MS+ BAP 0.5 mg/L + 2,4-D 1.5 mg/L); c, d) Callus after 6 and 10 weeks culture in treatment A4 (MS + 2,4-D 1.5 mg/L). MS: Murashige & skoog; 2,4-D: 2,4-dichlorophenoxyacetic acid; BAP: 6-benzylaminopurine.

The results revealed that 2,4-D and BAP had an impact on the explant's development. After 10 weeks of culture, the whole explant's surface was covered with callus. However, for varying concentrations of plant growth regulators, the rate of callus formation varies with different treatments.

According to the data in Table 1, the frequency of callus formation in the medium supplied with 2,4-D differs significantly. When 2,4-D is added at concentrations ranging from 0.5 to 1.5 mg/L, the frequency of callus production steadily increases as the concentration increases. Based on observation, most calluses are white or yellowish-white. The growth regulators BAP and 2,4-D in the medium both impact the callus color. When auxin concentrations do not match cytokinins or the concentration of 2,4-D combined with BAP in the medium increases, the callus color changes, turning yellow to brown. The callus has a hard texture and an obvious brown surface. According to observations (Figures 2b & 2d), the callus induced with 2,4-D 1.5 mg/L had a yellowish-white and compact texture. More secondary metabolites can

accumulate in a compact callus texture (Indah & Ermavitalini, 2013) and the medium containing 2,4-D 1.5 mg/L had the highest frequency of callus development, at 90% (Table 1). Among all the growth regulators evaluated, 2,4-D gave the highest frequency of callus and somatic embryo formation in *Panax ginseng* (Zhong & Zhong, 1992; Arya et al., 1993) and *Panax Vietnamese ginseng* (Vu et al., 2015).

3.2. Effect of TDZ concentrations on embryogenesis induction from callus tissue Ngoc Linh ginseng *in vitro*

In addition to inducing shoot regeneration, cytokines also initiated somatic embryogenesis. It was reported in *Santalum album L.* that MS medium containing BA alone could induce a high frequency of somatic embryo differentiation (Akhtar & Shahzad, 2019). In recent years, there has been increased interest in the use of TDZ in the somatic embryogenesis regeneration of plants. TDZ, a synthetic phenylurea-type plant growth regulator, was initially thought to induce responses comparable to those generated by natural cytokinins. (Guo et al., 2011; Unal, 2018).

Table 2. Effect of TDZ on embryogenesis induction from callus of Ngoc Linh ginseng *in vitro* after 10 weeks of culture

Treatments	TDZ (mg/L)	IBA (mg/L)	Embryos induction frequency (%)	Average number of embryos (embryo/explant)
B1 (control)	0.0	0.7	33.33 ^c	3.17 ^d ± 0.43
B2	0.2	0.7	43.36 ^{bc}	4.33 ^{cd} ± 0.72
B3	0.4	0.7	54.89 ^{ab}	5.83 ^{bc} ± 0.43
B4	0.6	0.7	54.89 ^{ab}	6.25 ^{bc} ± 0.92
B5	0.8	0.7	69.29 ^a	11.25 ^a ± 0.55
B6	1.0	0.7	60.62 ^{ab}	7.50 ^b ± 0.58

Mean values in the same column with different letters are statistically significantly different, $P < 0.05$. TDZ: thidiazuron; IBA: indole-3-butyric acid.

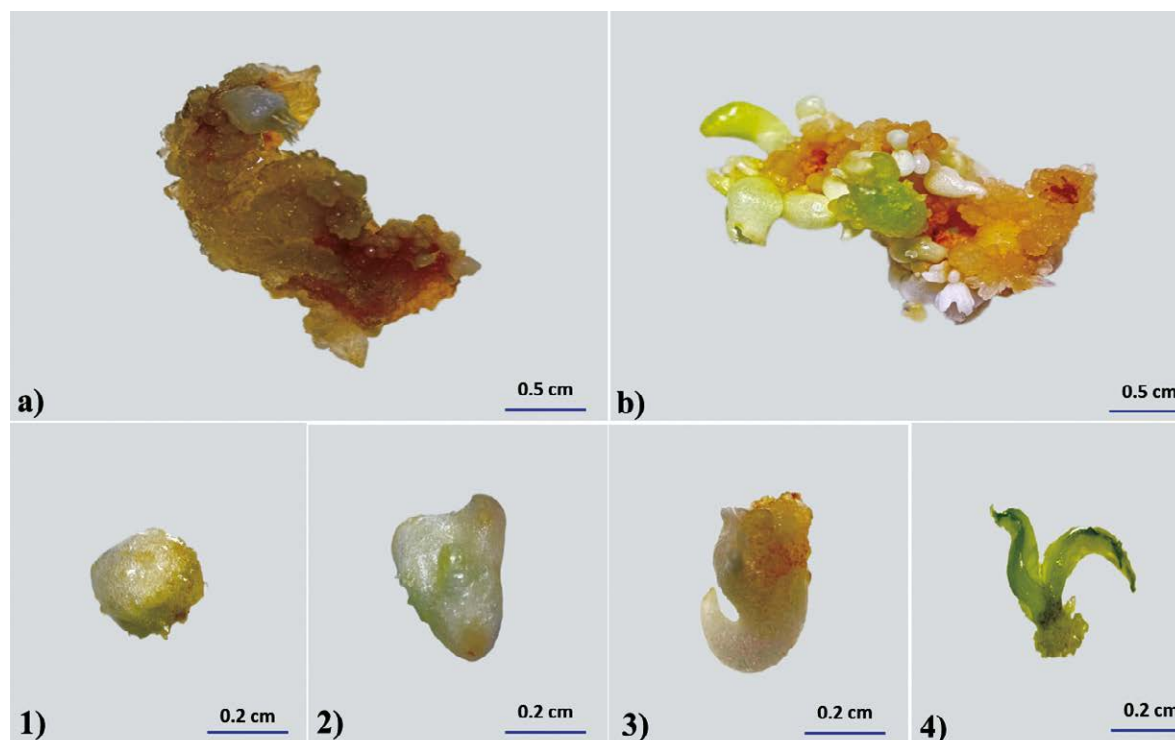


Figure 3. Embryogenesis of Ngoc Linh ginseng after 10 weeks culture. a) embryogenesis explant on medium MS + IBA 0.7 mg/L (control treatment); b) embryogenesis explant on medium MS + IBA 0.7 mg/L + TDZ 0.8 mg/L (1) Global shape, 2) Heart shape, 3) Torpedo-shaped, 4) Cotyledonary). MS: Murashige & skoog; TDZ: thidiazuron; IBA: indole-3-butyric acid.

After 10 weeks of culture, the results in Table 2 show that the embryogenesis frequency and average number of embryos in conditions supplemented with TDZ are significantly different from the control treatment. The embryogenesis frequency and average number of embryos increased with increasing TDZ concentration (0 - 0.8 mg/L), with the highest frequency observed in the B5 treatment. When MS medium was added with TDZ 0.8 mg/L and IBA 0.7 mg/L, the embryogenesis frequency reached 69.29%, with a number average of 11.25 embryos per explant. In B6 treatment (TDZ 1 mg/L and IBA 0.7 mg/L), the embryogenesis frequency and the number of embryos decreased. This shows that the auxin/cytokinin ratio is critical for embryogenesis

induction. At the same time, investigating embryo morphology in treatment B5 showed that the embryo developed through spherical, heart-shaped, torpedo-shaped, and cotyledon-shaped stages (Figure 3). In this study, the use of TDZ in the formation of embryos was achieved at low concentrations. This is in agreement with the study of Giridhar et al. (2004), which reported that low levels of TDZ stimulated direct somatic embryogenesis in coffee.

3.3. Effect of BAP and NAA on shoot development of Ngoc Linh ginseng *in vitro*

Cytokinins are substances that, when combined with auxin, promote the growth of young shoots and the formation of new shoot

apical meristems from the parenchyma. The growth regulator BAP is a cytokinin that helps increase cell growth when auxin is present in the environment. The frequency of shoot

induction varies according to whether the concentration of cytokinin supplied to the medium is appropriate (Bui, 2000).

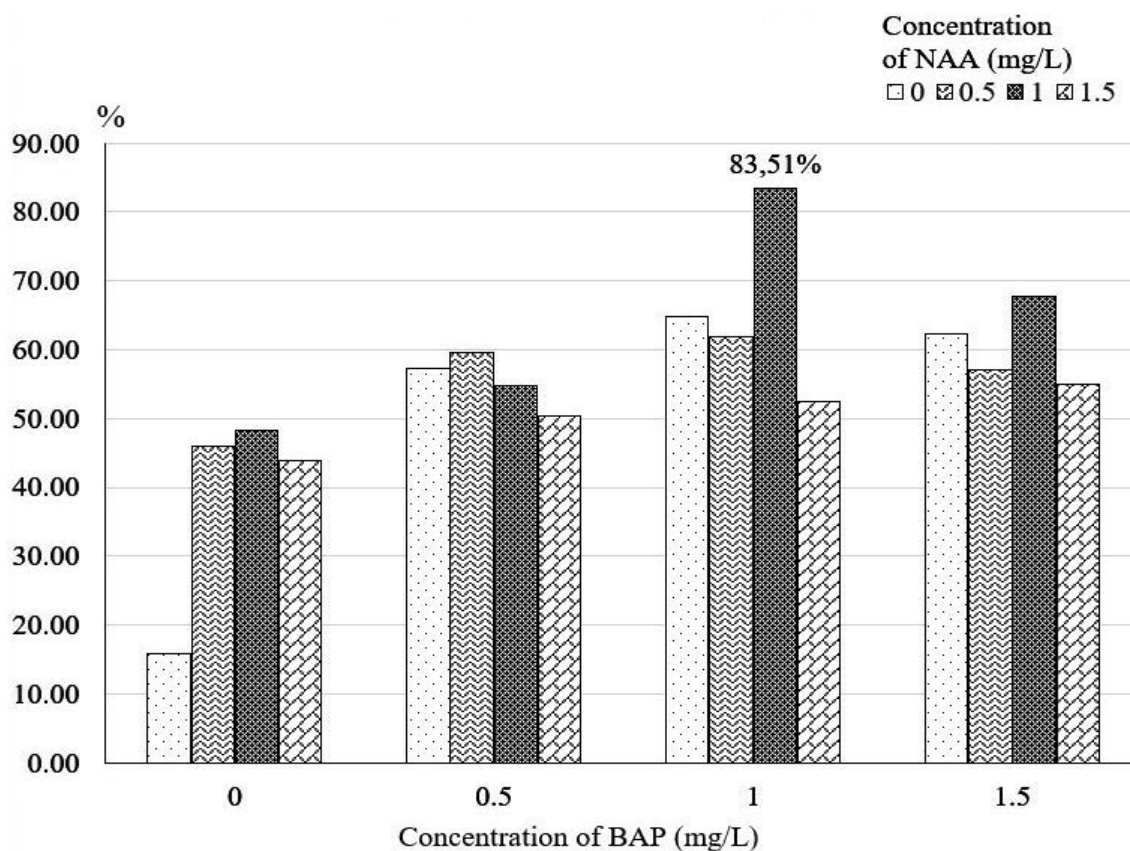


Figure 4. Frequency of shoot development from embryo explants of Ngoc Linh ginseng after 10 weeks of culture. NAA: α -naphthalene acetic acid; BAP: 6-benzylaminopurine.

Table 3. Effect of BAP (6-benzylaminopurine) and NAA (α -naphthalene acetic acid) on shoot development from embryo explants of Ngoc Linh ginseng in vitro after 10 weeks of culture

BAP (mg/L)	NAA (mg/L)	Number of shoots per explant	Shoot height (cm)
0.0	0.0	$0.30^h \pm 0.28$	$0.33^f \pm 0.29$
	0.5	$1.18^g \pm 0.28$	$0.63^f \pm 0.06$
	1.0	$1.44^g \pm 0.40$	$0.53^f \pm 0.06$
	1.5	$1.29^g \pm 0.06$	$0.67^f \pm 0.15$
0.5	0.0	$2.26^f \pm 0.17$	$1.23^e \pm 0.21$
	0.5	$2.59^{ef} \pm 0.07$	$1.47^e \pm 0.25$
	1.0	$3.00^e \pm 0.30$	$1.57^{de} \pm 0.12$
	1.5	$2.55^{ef} \pm 0.23$	$1.50^e \pm 0.10$
1.0	0.0	$4.44^{bcd} \pm 0.28$	$2.47^c \pm 0.15$
	0.5	$4.71^{bc} \pm 0.13$	$3.07^b \pm 0.12$
	1.0	$5.70^a \pm 0.24$	$3.57^a \pm 0.12$
	1.5	$4.85^b \pm 0.17$	$2.27^c \pm 0.25$
1.5	0.0	$4.00^d \pm 0.11$	$2.07^c \pm 0.12$
	0.5	$4.15^{cd} \pm 0.17$	$2.23^c \pm 0.06$
	1.0	$4.26^{bcd} \pm 0.23$	$2.37^c \pm 0.15$
	1.5	$3.85^d \pm 0.17$	$2.03^{cd} \pm 0.15$
CV		6.12	8.38

Mean values in the same column with different letters are statistically significantly different, $P < 0.05$.

Shoot explants cultured on MS basal medium were used as controls; they showed signs of browning and failed to initiate shoots. Growth regulator application improved shoot responsiveness and induction. The frequency of shoot development, as well as the number and height of shoots, vary based on the concentration of plant growth regulators used. After 10 weeks of culture, the results of the experiment showed that increasing the concentrations of BAP and NAA from 0.5 to 1.0 mg/L increased the frequency of shoot development, the average number of shoots, and the average shoot height. This shows that cytokinin, when combined with auxin, increases the growth of young shoots and the production of new shoot apical meristems from the parenchyma. Several shoots were induced with the maximum frequency (83.51%) (Figure 4). The combination of both

growth regulators, BAP and NAA, yielded statistically significant differences in both the average number of shoots and the average shoot height. MS basic medium supplemented with the combinations BAP 1.0 mg/L and NAA 1.0 mg/L demonstrated the highest values, with 5.70 shoots/explant, and the maximum shoot height (3.57 cm) was observed (Table 3). It was most suitable for the shoot growth from the Ngoc Linh ginseng embryos. The result of this experiment is also similar to the findings of Duong et al. (2013), which reported that adding BA and NAA to the culture medium resulted in the highest number of shoots (6.3 shoots).

After the experiment investigating the development of shoots from Ngoc Linh ginseng embryos, a large number of plantlets were created. The roots were removed from the

plantlets, leaving only the shoots. These shoots were used as materials for the next experiment on the TIS system.

3.4. Effect of immersion time and growth regulators on shooting of Ngoc Linh ginseng *in vitro* in temporary immersion system

Cytokinin promotes cell division and cell expansion and growth is stimulated by adding a

cytokinin to a proliferation medium. Cytokinin induces the initiation and capability of axillary meristems which result in shoot formation. In this way, different concentrations of one of the common cytokinin types of plant growth regulator (BA) were applied to effect (Kadota & Niimi, 2003). The results after 10 weeks of culture are shown in Table 4.

Table 4. Effect of immersion time and growth regulators on shooting of Ngoc Linh ginseng *in vitro* on TIS (temporary immersion system) after 10 weeks culture

Immersion Time (min)	BA (mg/L)	Number of shoots (shoot/explant)	Shoot height (cm)
1	0.0	1.96 ^e ± 0.17	0.38 ^g ± 0.04
	0.5	2.93 ^d ± 0.17	1.44 ^d ± 0.05
	1.0	3.26 ^d ± 0.28	1.57 ^c ± 0.04
	1.5	4.04 ^c ± 0.17	2.36 ^b ± 0.13
3	0.0	2.04 ^e ± 0.17	0.49 ^{fg} ± 0.04
	0.5	4.07 ^c ± 0.23	1.50 ^c ± 0.03
	1.0	5.29 ^b ± 0.17	2.68 ^a ± 0.08
	1.5	6.00 ^a ± 0.11	2.70 ^a ± 0.04
5	0.0	-	-
	0.5	2.18 ^e ± 0.23	1.33 ^d ± 0.04
	1.0	1.89 ^e ± 0.11	0.82 ^e ± 0.03
	1.5	1.22 ^f ± 0.22	0.63 ^f ± 0.04
CV%		5.79	3.37

Mean values in the same column with different letters are statistically significantly different, $P < 0.05$. (-): the value of zero. BA: 6-benzyladenine acid.

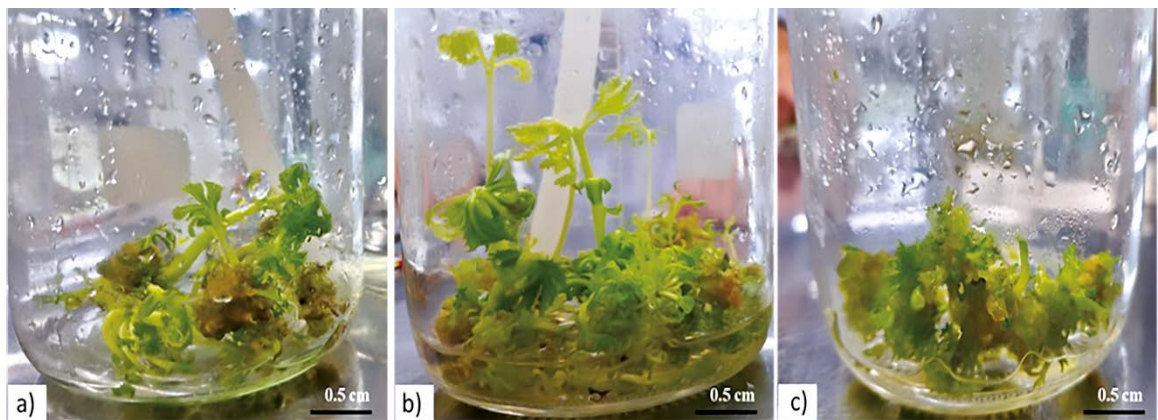


Figure 5. Shoots induction of Ngoc Linh ginseng at a concentration of 6-benzyladenine acid 1.5 mg/L combined with immersion time on temporary immersion system: a) 1 min; b) 3 min; c) 5 min.

The results in Table 4 and Figure 5 show that the number of shoots and shoot height at immersion times of 1 min and 3 min corresponded with additional BA concentrations ranging from 0.0 mg/L to 1.5 mg/L. Specifically, at BA 0 mg/L, there was no statistically significant difference between Ngoc Linh ginseng explants grown for 1 min or 3 min. However, as the BA concentration increased, the number of shoots and shoot height reached their highest at BA 1.5 mg/L within 3 min of immersion, giving 6.00 shoots and 2.70 cm. This observation aligns with the findings of Cung et al. (2007) research on the *Phalaenopsis hybrid*, suggesting that in the TIS system, the well-ventilated environment facilitates optimal growth due to continuous air exchange between the internal and external environments through the pump system and filter membranes. Conversely, at a 5-min immersion duration, the results demonstrated an inverse relationship with the increase in additional BA concentration. Both the number of shoots and shoot height declined compared to the 1-min and 3-min immersion times. Particularly in the absence of BA addition, no shoot formation occurred, with NLG shoots displaying signs of browning and failing to develop. This outcome parallels the findings of Silva et al. (2007), for the result observed the

highest number of shoots and shoot mass at a 3-min immersion duration in a 2-h cycle when investigating pineapple plant propagation on the TIS. The success of the TIS micropropagation protocol depends not only on the number of shoots obtained by explants but also on the morphological quality and vigor present in the seedlings produced (Martre et al., 2001).

3.5. Effect of immersion time and growth regulators on rooting of Ngoc Linh ginseng *in vitro* in temporary immersion system

Roots are an essential component of the plant, absorbing water, mineral salts, and nutrients for growth and development. In contrast to wild plants, *in vitro* plants are unlikely to be able to form roots on their own due to a lack of phytohormones (Nguyen & Nguyen, 2011). Therefore, when constructing the cultivation process, particular emphasis should be placed on nurturing the plant's root system and establishing an optimal environment conducive to root formation and development. The importance of auxin in root development is demonstrated in *in vitro* culture techniques. In the medium containing only auxin, the cultivated tissue only forms roots (Vu et al., 2009). The results after 10 weeks of culture are displayed in the table following:

Table 5. Effect of immersion time and growth regulators on rooting of Ngoc Linh ginseng *in vitro* on temporary immersion system

Immersion time	NAA (mg/L)	Frequency of root induction (%)	Number root per explant	Root length (cm)
1	0.0	-	-	-
	0.5	19.47 ^{ef}	0.11 ^{de} ± 0.00	0.37 ^e ± 0.06
	1.0	30.50 ^{cde}	0.26 ^d ± 0.06	1.29 ^b ± 0.02
	1.5	39.63 ^{bcd}	1.18 ^b ± 0.06	0.73 ^d ± 0.03
3	0.0	-	-	-
	0.5	15.86 ^{ef}	0.11 ^{de} ± 0.11	0.18 ^f ± 0.16
	1.0	50.37 ^{bc}	1.26 ^b ± 0.13	1.20 ^b ± 0.04
	1.5	59.50 ^{ab}	1.93 ^a ± 0.13	0.94 ^c ± 0.02
5	0.0	-	-	-
	0.5	25.24 ^{de}	0.53 ^c ± 0.03	0.42 ^e ± 0.09
	1.0	71.25 ^a	1.85 ^a ± 0.13	1.61 ^a ± 0.09
	1.5	54.74 ^{ab}	1.07 ^b ± 0.06	0.71 ^d ± 0.01
CV%			8.55	6.85

Mean values in the same column with different letters are statistically significantly different, $P < 0.05$. (-): the value of zero. NAA: α -naphthalene acetic acid.



Figure 6. Roots induction of Ngoc Linh ginseng at a concentration of α -naphthalene acetic acid 1 mg/L combined with an immersion time of 5 min/ 6 h on temporary immersion system; a) After 6 weeks of culture, b) After 10 weeks of culture. 1),2),3) Root morphological different types emerged.

Table 5 and Figure 6 show that root initiation of Ngoc Linh ginseng explants began about 4 to 6 weeks after transferring the rooting medium. The highest rooting induction frequency after 10 weeks of culture. The TIS showed minimal callus at the base of roots and a well-developed root system. During immersion times of 1, 3, and 5 min, all NAA concentrations ranging from 0.5 to 1.5 mg/L produced roots. At an NAA concentration of 1 mg/L, both 3-min and 5-min immersion resulted in root number values with no significant difference; however, root length was greatest at the 5-min immersion (1.61 cm) and had the highest root induction frequency of 71.25%. Therefore, the treatment with a 5-min immersion time and NAA 1 mg/L concentration is the best for Ngoc Linh ginseng root induction on the TIS. Also with the TIS system, Marco & Lourdes (2016) evaluated three bioreactor systems for *V. planifolia* micropropagation (multiplication and rooting). After 4 weeks of culture on the TIS system, the number and length of roots increased (4.25 roots and 2.41 cm) with an immersion frequency of 2 min every 4 h.

Finally, by combining appropriate cultural ventilation and occasional contact between shoots and the liquid medium, the microenvironment inside the temporary immersion container was improved, which contributed to improved shoot and root formation of Ngoc Linh ginseng. Plantlets of Ngoc Linh ginseng obtained in this study would probably have positive effects on ex vitro rooting and transplanting in large-scale commercial production. These results agree with those obtained in the micropropagation of other plant species using TIS systems (Escalona et al., 1999; Bernal et al., 2008; Marco & Lourdes, 2016).

4. Conclusions

The results of our study demonstrated the utility of the TIS for micropropagation of Ngoc Linh ginseng with the increased number of shoots and roots. In the current investigation, immersion time was 3 min every 6 h with a concentration of BA of 1.5 mg/L during shoot propagation for the best number of shoots (6.00 shoots/explant) and 2.70 cm in height. Simultaneously, at 5 min of immersion time and a concentration of NAA of 1 mg/L, the results showed the highest frequency of root (71.25%) and root length (1.61 cm).

Conflicts of interest

The authors declare no conflicts of interest.

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Identification and characterization of *Pseudomonas* spp. isolated from durian growing soil in the Central Highlands, Vietnam

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ABSTRACT

Durian cultivation is being developed and expanded in the Central Highlands because of its high economic value. The farmers commonly apply pesticides and chemical fertilizers to control diseases and help durian plants growth. However, an alternate approach, using biological products from microorganisms is a sustainable direction for durian cultivation. In particular, plant growth promoting rhizobacteria (PGPR) is widely used in many countries to support crops and increase productivity. This study aimed to isolate native *Pseudomonas* strains from durian orchards in the Central Highlands and investigate some of their characteristics affecting the growth of durian trees. As a result, four bacterial isolates were identified by morphology, biochemical analysis, and 16S rRNA sequence analysis, including TD-Dak Lak, DN-Lam Dong, T1-Dak Nong, and DT-Gia Lai belonging to *P. fluorescens*, *P. mosselii*, *P. monteilii*, and *P. putida*. The survey of nitrogen fixation, indole-3-acetic acid (IAA) production, siderophore, and phosphorus solubilization abilities of the four strains showed that all of them were capable of N₂ fixation (3.03 to 3.69 mol/mL), IAA production (11.63 to 54.88 µg/mL), solubilizing phosphate (1.43 to 2.26 cm halo ring), and siderophore production. Three strains of *P. putida*, *P. fluorescens*, and *P. monteilii* increased the germination length of durian seeds by 13.6%, 12.2%, and 9.2%, respectively. All four *Pseudomonas* strains increased plant height from 4.25 to 5.66 cm, and the average number of leaves varied from 2.4 to 3.2 leaves, compared to 1.5 leaves in the control at 90 days. The results indicate that four *P. fluorescens* (TD-Dak Lak), *P. putida* (DT-Gia Lai), *P. mosselii* (DN-Lam Dong), and *P. monteilii* (T1-Dak Nong) were beneficial biological agents that can promote the growth and development of durian trees.

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1. Introduction

Plant growth-promoting rhizobacteria (PGPR) directly stimulate the growth of plants by the nitrogen fixation process (Han et al., 2005), the production of plant growth-regulating hormones, and 1-aminocyclopropane-1-carboxylate (ACC) deaminase enzyme production (Correa et al., 2005). Besides, PGPR indirectly inhibits phytopathogenic fungi through the production of chitinases, β -1,3-glucanases, antibiotics, fluorescent pigments, and hydrogen cyanide (HCN) (Pal et al., 2001). *Pseudomonas* spp. is a special species among PGPR genera in terms of root colonization, nitrogen fixation, production of exopolysaccharides, siderophores, HCN, and phytohormones, solubilization of phosphorus, potassium, and zinc, biofilm formation, activity, antioxidant capacity, stress adaptation, and positive interactions with other microbial communities. *Pseudomonas* strains are indeed rhizosphere endophytes promoting good growth of sunflower (*Helianthus annuus*) (Pandey et al., 2013). Ngo et al. (2024) determined the level of nitrogen fertilizer replacement of *Pseudomonas* sp. BT1 and BT2 with high-yielding rice plants grown in pots; replacing 50% N with *Pseudomonas* due to the rice yield in potting increased by 23.9% compared to the control. Chu et al. (2020) found that *Pseudomonas* three strains could stimulate the growth of peanuts under saline conditions both *in vitro* and in greenhouse. *P. fluorescens* was reported to manage several diseases caused by soil-borne pathogens (Wei et al., 1996). *Pseudomonas* spp. is widely studied in disease management strategies due to biological control mechanisms. The most common compounds secreted by *Pseudomonas* include phenazine-1-carboxamide, lipopeptide, 2, 4-diacetylphloroglucinol (DAPG), pyrrolnitrin, pyoluteorin, and phenazine-1-carboxylic acid (PCA) (Dimkić et al., 2022). *Pseudomonas* strains survive well under stressful conditions and control certain fungal plant diseases such

as okra root rot caused by *Fusarium solani* (35), foliage blight disease caused by *Phytophthora nicotianae* (37), root rot caused by *Rhizoctonia solani* (36) and damping-off disease caused by *Pythium* spp. (34).

Root zone management is a method of effectively enhancing soil nutrients to promote plant growth and productivity (Zia et al., 2021). *Pseudomonas* is an environmentally acceptable alternative to chemical fertilizers because of its active growth-promoting activities. They also support plant growth by promoting resistance to biotic and abiotic stress and providing nutritional support to the host plant. However, to achieve this goal, users must first find and research a source of indigenous *Pseudomonas* strains to fully exploit the potential of this bacterial strain on durian trees and in agricultural applications.

2. Materials and Methods

2.1. Isolation of *Pseudomonas* spp from durian orchard soils and roots

Soil and root samples were collected from durian-growing areas in the Central Highlands provinces, including Gia Lai, Dak Lak, Dak Nong, and Lam Dong. Samples were taken from durian gardens with trees older than three years. To collect soil, the top 10 cm of soil was first removed, and then soil was collected from a depth of 10 - 20 cm using a metal spoon. Approximately 2 - 3 tablespoons of soil were collected at each point, with a total of around 500 g per sample taken from multiple points around the roots. In each garden (or farm), five samples were collected: four from diagonal points and one from the center of the garden. Root samples, weighing about 50 - 100 g, were collected simultaneously with the soil samples and placed in plastic bags. A total of seventy soil and root samples were collected from the durian farm.

Table 1. The soil and root samples collected from durian farms

No.	Host	Location	GPS	Samples
1	Durian 3 years; covered grass	ChuPrông, Gia Lai	13°50'38.5"N 107°56'16.0"E	10
2	Durian years; inter-cropping coffee	Đăk Đoa, Gia Lai	13°54'02.7"N 108°02'34.7"E	8
3	Durian 8 years; covered grass	Đạ Huoai, Lâm Đồng	11°22'46.3"N 107°32'51.6"E	10
4	Durian garden of many ages	Bảo Lộc, Lâm Đồng	11°30'36.0"N 107°53'48.1"E	10
5	Durian 6 years; inter-cropping coffee	Cư M'gar, ĐăkLăk	12°46'09.4"N 108°08'15.7"E	10
6	Durian 8 years; covered grass	Buôn Ma Thuột, ĐăkLăk	12°57'35.0"N 108°07'09.6"E	8
7	Durian garden of many ages, covered grass	Gia Nghĩa, Đăk Nông	12°01'14.1"N 107°40'03.9"E	14
Total				70

From the rhizosphere and root samples: Ten grams of rhizosphere soil were added to 90 ml of sterile physiological water and shaken at 120 rpm for 20 min. Then 0.1 mL of dilution at concentrations of 10^{-3} - 10^{-5} was spread onto King's B (Mew & Rosales, 1986).

From the rhizoplane samples: 10 g of root segments were added to 90 mL of sterile physiological water and shaken for 2 h at 120 rpm. Then, 0.1 mL of the dilution was spread onto King's B medium (Mew & Rosales, 1986).

From the endorhizosphere samples: The collected durian roots were washed under running water treated with a 2.5% sodium hypochlorite solution, for 3 min, and rinsed three times with sterile distilled water (Mew & Rosales, 1986). Ten grams of these roots were then added to 90 mL of sterile physiological water and shaken for 2 h at 120 rpm. Afterward, 0.1 mL of each dilution was spread onto King's B

medium and incubated at room temperature ($30 \pm 2^\circ\text{C}$) for 24 - 48 h. Bacterial strains growing on the agar plates were purified and stored in Yeast Dextrose Carbonate (YDC) medium at -80°C with 40% glycerol (Parke et al., 1986).

2.2. Physiological and biochemical characterization of *Pseudomonas* strains

Biochemical tests: According to Cappucino & Sherma (1992), *Pseudomonas* spp. was classified based on catabolic reactions and biochemical, physiological, and nutritional characteristics. Bacterial strains were characterized on King's B plates at 37°C . Biochemical tests were performed on all isolates, including the Gram test, oxidase test, catalase test, mannitol utilization, carbon source utilization, glucose fermentation, motility, nitrate reduction, arginine-dihydrolase, gelatin liquefaction, siderophore production, and IMViC test (Cappucino & Sherman, 1992).

Indole acetic acid (IAA) phytohormone production test: The IAA production test was performed according to Bric et al. (1991). Sterilized LB medium (Sigma, pH 7.5) supplemented with five mM L-tryptophan was poured into 90 mm diameter agar plates. Bacterial strains were inoculated into the central of the agar plates, which were then covered with Whatman paper (82 mm diameter, No. 2). After 48 h of incubation, 5 mL of Salkowski reagent (2% FeCl₃ in 35% perchloric acid) was added. The presence of IAA was indicated by the formation of a characteristic red halo around colonies with a diameter of 2 mm. Each isolate was tested in triplicate.

The bacteria were grown in TSB medium supplemented with 0.1 g/L tryptophan, shaken at 150 rpm and $30 \pm 2^\circ\text{C}$ in the dark for four days. One milliliter of the inoculum was then centrifuged to remove biomass. The supernatant was mixed with an equal volume of improved Salkowski reagent (1:2 ratio of bacteria to reagent) and incubated in the dark for 1 h (Glick, 1995). The presence of IAA was indicated by the development of a pink-red color, and the absorbance was measured at 535 nm. To quantify IAA, a standard curve was created using IAA concentrations ranging from 0 to 50 $\mu\text{g/mL}$ with Salkowski reagent on a spectrophotometer at 530 nm. The calibration curve was generated with the form $y = ax + b$ ($R^2 > 0.95$). Measurements from the samples were compared to this standard curve to determine IAA concentration. Each strain was tested in triplicate.

IAA Quantification: Bacteria were grown in TSB medium supplemented with 0.1 g/L tryptophan, shaken at 150 rpm and $30 \pm 2^\circ\text{C}$ in the dark for four days. Afterward, 1 mL of the inoculum was centrifuged to remove biomass.

The supernatant was mixed with an equal volume of improved Salkowski reagent (1:2 ratio of bacteria to reagent) and incubated in the dark for 1 h (Glick, 1995). The IAA presence was indicated by a pink-red color, and absorbance was measured at 535 nm. Standard IAA isolations, with concentrations ranging from 0 to 50 $\mu\text{g/mL}$, were prepared and measured with Salkowski reagent using a spectrophotometer at 530 nm. A calibration curve ($y = ax + b$, with $R^2 > 0.95$) was generated to standardize the measurements. Each strain was tested in triplicate.

Biological nitrogen fixation: The biological nitrogen fixation capability of *Pseudomonas* spp. was assessed by growing bacterial cultures in peptone broth with Nessler's reagent. The negative control was performed with sterile peptone broth without bacterial inoculation. The positive bacterial strain for biological nitrogen fixation was identified by a color change in the culture medium from brown to yellow after 3 - 7 days of incubation. Subsequently, the optical density of the culture medium was measured at 450 nm with a UV-Vis spectrophotometer. Ammonia concentration was estimated using a standard curve of ammonium sulfate, with concentrations ranging from 0.1 to 1 $\mu\text{mol/mL}$.

Phosphate solubilization: Phosphate solubilization by the isolated *Pseudomonas* spp. was assessed using Pikovskaya agar medium. Bacteria were inoculated in the center of the plates (90 mm in diameter) (Pikovskaya, 1948). Each isolate was tested in triplicate. After nine days of incubation at 30°C , the diameter of the solubilization halo (DSH) was measured using the appropriate formula as follow:

$\text{DSH} = \text{THD} - \text{CD}$ (1), in which, THD is the total halo diameter, CD is the colony diameter.

In addition to the qualitative test, a quantitative phosphate solubilization test in broth using 250 mL flasks containing 100 mL of NBRIP medium (Nautiyal, 1999). One milliliters of a homogeneous bacterial suspension grown for two days was added into the flask. The samples were incubated on a rotary shaker at 130 rpm at $30 \pm 2^\circ\text{C}$ for nine days under the laboratory condition. After incubation, the samples were centrifuged at 12000 rpm for 5 min, and the supernatant was filtered. An aliquot of 5 mL from the supernatant was used to measure the amount of dissolved phosphate. The phosphate concentration in the culture supernatant was estimated using the method described by Olsen & Sommers (1982). Each bacterial isolate tested in triplicate and the control treatment was conducted under the same conditions without bacterial inoculation.

Siderophore production: The ability to produce siderophores was determined using improved CAS (Chrome Azurol S) agar medium (Milagres et al., 1999). Bacteria were cultured on a synthetic siderophore medium. Siderophore production was indicated by a color change around the colonies to lemon yellow or dark yellow. The CAS medium was prepared with the following components: 60.5 mg CAS, 72.9 mg hexadecyltrimethylammonium bromide (HDTMA), 30.24 g piperazine-1,4-bis (2-ethanesulfonic acid) (PIPES), 1 mM $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ in 10 mL 10 mM HCl, and 0.9% (w/v) agar.

Molecular identification and phylogenetic analysis: *Pseudomonas* spp. was identified using molecular analyses based on 16S rRNA sequences. Three to four pure colonies of each

bacterial strain, grown on King's B medium for 24 h were suspended in sterile water. The DNA was extracted using the TopPURE DNA extraction kit (HI - 112, ABT, Vietnam) and checked using gel electrophoresis on a 1% agarose gel. The DNA was stored at -20°C (Yang et al., 2008) and amplified by PCR using the primers 8F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTACGACTT-3') of the 16S rRNA gene, producing a DNA fragment of approximately 1500 bp (Turner, 1999). The PCR reaction was performed in a total volume of 25 μL , including 25 ng of DNA, 2.5 μL of $10 \times$ PCR buffer with 20 mM MgCl_2 , 2.5 μL of dNTPs (2 mM), 0.75 μL of forward primer (10 μM), 1.5 μL of reverse primer (10 μM), and 0.125 μL of FastStart polymerase (5 U/ μL). The PCR conditions included an initial denaturation at 95°C for 3 min, followed by 30 cycles of denaturation at 95°C for 30 sec, annealing at 45°C for 45 sec, and extension at 72°C for 2 min, with a final elongation at 72°C for 7 min. The PCR products were purified. The equipment used included a thermal cycler (Bioer, China), an electrophoresis kit, nuclease-free water, agarose, and a GelDoc It2. The PCR sequences were compared to 16S rDNA sequences in GenBank using the NCBI BLAST program. Bacterial strains were processed with the BioEdit V7.0.9.0 tool. Alignment and clustering were performed using the neighbor-joining method (Tamura, 1992). Phylogenetic trees were constructed using MEGA version 7.0 with the accession number of *Pseudomonas* strains sequence found in Gene Bank in Table 2 (Kumar et al., 2016). Bootstrap analysis with 1000 replicates was conducted to assess the reliability of the phylogenetic tree.

Table 2. Accession number of sixteen sequences in GenBank used for this analysis phylogenetic tree

<i>Pseudomonas</i> species	Accession number
<i>Pseudomonas fluorescens</i> strain CIFRI.BTL.S-10	PP759351.1
<i>Pseudomonas fluorescens</i> strain psf3	MN256389.1
<i>Pseudomonas mosselii</i> strain PtA1	CP024159.1
<i>Pseudomonas mosselii</i> strain BS011	CP023299.1
<i>Pseudomonas mosselii</i> strain PH4	CP104107.1
<i>Pseudomonas plecoglossicida</i> strain MR134	MG674349.1
<i>Pseudomonas putida</i> strain NBRC 12996	AB680362.1
<i>Pseudomonas putida</i> strain GPSD-19 HQ270550.1	
<i>Pseudomonas putida</i> strain HN2010	MT515798.1
<i>Pseudomonas putida</i> strain HN2013	MT515799.1
<i>Pseudomonas monteilii</i> strain EO4	OQ999328.1
<i>Pseudomonas monteilii</i> strain JM13	MN758773.1
<i>Pseudomonas monteilii</i> strain WR9	MN733114.1
<i>Pseudomonas monteilii</i> strain SKPf2	KR422299.1
<i>Pseudomonas aeruginosa</i> PAO1	CP053028.1
<i>Azotobacter vinelandii</i> CA	CP005094.1

2.3. Effects of *Pseudomonas* spp. on durian germination seed ability

The experimental setup included 5 treatments: NT0 (control without bacterial strain), NT1 (*P. monteilii*), NT2 (*P. putida*), NT3 (*P. mosselii*), and NT4 (*P. fluorescens*). Each treatment involved 20 Dona durian seeds and was replicated five times. The seeds were soaked washed, sterilized with 70% alcohol for 30 sec, and then rinsed three times

with sterilized distilled water. Subsequently, the seeds were immersed in bacterial cell suspension (10^7 cfu/mL) for 30 min, dried for 10 min and placed in a plastic box (30-10-5 cm) lined with paper soaked in sterile distilled water. The seeds were then covered with a layer of damp paper and kept at 27°C. The percentage of germinated seeds and the length of the durian sprouts were recorded after 7 days.

$$\text{Germination seeds (\%)} = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds}} \times 100\%$$

2.4. Plant growth assay for testing the effects of the four *Pseudomonas* isolates on durian plant growth

The germination durian seeds were conducted in pots (25 - 20 - 25 cm) filled a substrate consisting of a soil and burned husks mixture (2:1 v/v). The substrate was inoculated with

Pseudomonas strain suspensions (10^8 cfu/mL) at 10 mL per pot, following the method described by Amkraz (2010), and this inoculation was repeated monthly. The pots were placed in a greenhouse maintained at 26°C with $75 \pm 5\%$ relative humidity and were watered every 2 days. Weekly fertilization was carried out using a commercial nutrient solution (NPK 20-20-20)

(Amkraz, 2010). Control plants, derived from untreated seeds, were transplanted into soil substrates without inoculants. All treatments were replicated ten times and arranged in a completely randomized design. After 30, 60, and 90 days, measurements of plant height (aerial region) and the number of leaves were recorded.

2.5. Data analysis

Data were collected, synthesized, and calculated using Microsoft Excel, analyzed with one-way ANOVA, and graded using MINITAB 16.

3. Results and Discussion

3.1. Isolation of *Pseudomonas* spp.

Bacteria strains were purified and examined for colony morphology and pigmentation through gram staining, as well as described on specialized media. Cultural characteristics of isolates were observed, including shape, size, surface, margin, color, odor, and pigmentation, and were recorded according to Bergey's Handbook of Definitive Bacteria (Bergey & Holt, 1994). Cell shape and gram reaction were documented using the standard procedures

outlined by Barthalomew & Mittewar (1950). The results in Table 3 showed that the rhizospheric soil of durians was rich in bacteria with the *Pseudomonas* genus being predominant among the isolates. The bacteria were isolated from three planes: the rhizosphere and roots, the rhizoplane, and the endorhizosphere. Thirteen bacterial strains were isolated from seventy samples collected from various locations on the durian farm: two from the endorhizosphere, six from outside the rhizosphere and roots, and five from the rhizoplane. The *Pseudomonas* spp. group constituted 46.15% of the isolated bacteria, including 7% from the rhizosphere soil, 20% from the rhizoplane, and 0.21% from the endorhizospheric component. Differences in isolation rates were attributed to factors such as crop type, soil type, plant age, sampling season, and cultivation practices. These findings are consistent with those reported by Qessaoui et al. (2019), who identified 19 fluorescent *Pseudomonas* isolates from soil, fruits, and vegetable samples, including 11 isolates from the rhizoplane, five isolates from the endorhizosphere, and three isolates from the rhizospheric soil.

Table 3. Bacterial strains were isolated from position of durian in the Central Highlands, Vietnam

No.	Name of isolates	Origin	Location	Bacterial genus
1	DT-Gia Lai	Rhizoplane	Gia Lai	<i>Pseudomonas</i> spp.
2	ĐTe-Lam Dong 1	Rhizoplane	Lam Dong	<i>Enterobacter</i> sp.
3	Đte-Lam Dong 2	Rhizoplane	Lam Dong	<i>Bacillus</i> sp.
4	TD-DaK Lak	Rhizoplane	Dak Lak	<i>Pseudomonas</i> spp.
5	DN-Lam Dong	Rhizoplane	Lam Dong	<i>Pseudomonas</i> spp.
6	CS-Gia Lai	Rhizosphere and roots	Gia Lai	<i>Pseudomonas</i> spp.
7	GN-Dak Nong 1	Rhizosphere and roots	Dak Nong	<i>Bacillus</i> sp.
8	PL-Gia Lai 1	Rhizosphere and roots	Gia Lai	<i>Enterobacter</i> sp.
9	PL-Gia Lai 2	Rhizosphere and roots	Gia Lai	<i>Pseudomonas</i> spp.
10	PL-Gia Lai 3	Rhizosphere and roots	Gia Lai	<i>Burkholderia</i> sp.
11	T1-Dak Nong	Rhizosphere and roots	Dak Nong	<i>Pseudomonas</i> spp.
12	BMT-Dak Lak	Endorhizosphere	Dak Lak	<i>Bacillus</i> sp.
13	PT-Gia Lai	Endorhizosphere	Gia Lai	<i>Rhizobium</i> sp.

3.2. Morphology and biochemical characterization of *Pseudomonas* strains

The genus *Pseudomonas* consists of Gram-negative, aerobic, non-spore-forming, rod-shaped bacteria. They are a physiologically diverse group, with the potential to form extracellular slime. The diameter of *Pseudomonas* cells is typically less than 1 μm (Lysenko, 1961). In this research, we focused on the biochemical and

molecular characteristics of *Pseudomonas* spp. All six *Pseudomonas* spp. were characterized based on colony morphology on King's B agar medium, cell morphology, and Gram reaction (Table 4). Colonies developed into small to medium-sized, smooth, glistening colonies (Figure 1A). Under the microscope, these isolates appeared as Gram-negative, small, single rods without sporulation. Colonies developed into small to medium-sized, smooth, and glistening colonies (Figure 1A).

Table 4. Growth characteristics of colonies and cells on King's B of six *Pseudomonas* spp.

Isolates	Colonies on King's B agar	Cells
DT-Gia Lai	Colonies are circular, with characteristics that can be flat, raised, convex, or umbonate. They have entire, smooth edges, transparent, and odorless.	Gram-negative rods with polar flagella, motile, and forming extracellular slime irregularly.
T1-Dak Nong PL-Gia Lai 2	Colonies on King' B agar medium are circular and non-pigmented. They are also non-haemolytic on blood agar.	Gram-negative, cells are circular and smooth.
DN-Lam Dong CS-Gia Lai	Colonies are circular and non-pigmented when grown on nutrient agar, measuring 1.8 - 2.0 mm in diameter after 24 h. The bacteria are non-spore-forming rods with polar flagella and are motile.	Gram-negative, with cells that are circular and smooth.
TD-Dak Lak	Colonies are circular, flat, raised, or convex, with entire, smooth edges, transparent appearance, and no odor. They are non-pigmented.	Gram-negative rods that do not form spores. They are motile by polar flagella and may form irregular capsules or extracellular slime.

The isolated bacteria were identified through biochemical characterization according to Bergey's Manual of Systematic Bacteriology (Bergey & Holt, 1994; Boone et al., 2001). Six *Pseudomonas* spp. isolates were tested for various biochemical properties including IMVIC, oxidase, catalase, carbohydrate fermentation,

denitrification, H_2S production, starch hydrolysis, gelatin liquefaction, etc. All six isolates tested positive for fluorescence (Figure 1B), catalase test, oxidase test, gelatine liquefaction, glucose, and citrate utilization. In contrast, they were negative for pyocyanin, starch hydrolysis, ethanol, Voges Prausker's, methyl red, and indole test (Table 5).

Table 5. Biochemical characterization of the six *Pseudomonas* isolates

Test	DT-Gia Lai	DN-Lam Dong CS-Gia Lai	T1-Dak Nong PL-Gia Lai 2	TD-Dak Lak
Gram reaction	-	-	-	-
Fluorescin	+	+	+	+
Pyocyanin	-	-	-	-
Catalase	+	+	+	+
KOH (3%)	+	+	+	+
Gelatine	+	+	+	+
Starch hydrolysis	-	-	-	-
Oxidase	+	+	+	+
Ethanol	-	-	-	-
Glucose	+	+	+	+
Mannitol	-	+	-	-
Maltose	+	+	-	+
H ₂ S	-	+	+	-
Indole	-	-	-	-
MR	-	-	-	-
VP	-	-	-	-
Citrate utilization	+	+	+	+

+: Positive; -: Negative; MR: -Methyl red; VP: - Voges Praskaur's test.

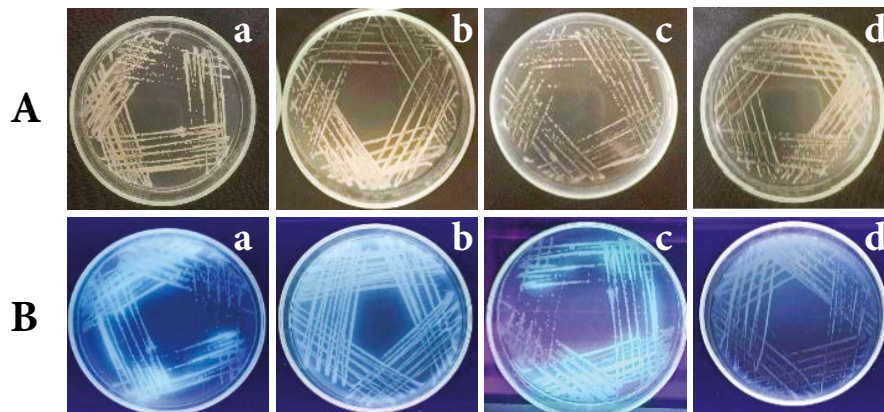


Figure 1. Morphology of 4 *Pseudomonas* spp. on King' s B agar at 24 h, 37 °C (A) and morphology of 4 *Pseudomonas* spp on King' s B agar at 24 h, 37 °C for fluorescens test under UV light (B). a. DT-Gia Lai; b. DN-Lam Dong; c. TD-Dak Lak; d. T1-Dak Nong.

3.3. 16S rRNA gene sequencing and phylogenetic tree

Sequencing of the 16S rRNA gene is a standard method for classifying bacteria into new species or genera. This approach is

particularly useful for identifying particularly useful for identifying new species that have never been successfully cultured (Weisburg et al., 1991). *Pseudomonas* sp. can be isolated from a variety of plant species (Rodríguez et al., 2006). Phylogenetic analysis based on 16S rRNA

gene sequences is a fundamental technique for bacterial identification, as these genes are considered basic to bacterial classification (Zeng et al., 2016). The 16S rRNA sequence of the DT-Gia Lai strain showed 100% similarity with *P. putida* on GenBank. To determine its phylogeny, a phylogenetic tree was constructed and compared with sequences of available *P. putida* strains and closely related species using BLAST sequencing and the software Mega X. The neighbor-joining tree created from the aligned sequences revealed that DT-Gia Lai clustered with *P. putida* HN2013 and *P. putida* HN2010, with a bootstrap value of 65 (Figure 2). The gene sequence analysis of the 16S rRNA region indicated that the DN-Lam Dong strain showed 100% similarity with *P. mosselii*. Figure 2 shows that DN-Lam Dong shares the same branch as *P. mosselii* PtA1, with a bootstrap value of 89. Similarly, the 16S rRNA gene sequence analysis of the T1-Dak Nong strain revealed a 100% sequence similarity with *P. monteilii* on GenBank. T1-Dak Nong aligns with *P. monteilii* SKPf2 and *P. monteilii* EO4 on

the same branch, with a bootstrap value of 85. *P. fluorescens* and *P. putida*, both containing ACC-deaminase have been shown to improve wheat growth even with low doses of nitrogen (Naveed et al., 2008). Similarly, Kavino et al. (2010) found a significant impact of *P. fluorescens* on the leaf nutrient content and yield of bananas. Rakh et al. (2019) reported that, *Pseudomonas* cf. *monteilii* (isolate 29) inhibited *Fusarium oxysporum* the causative agent of *Fusarium* wilt disease in groundnuts, by up to 60.75% in in vitro studies. Additionally, *P. mosselii* BS011 isolated from the rhizosphere soil of rice plants, exhibited strong inhibitory activity against the rice blast fungus *Magnaporthe oryzae*. The gene cluster *c-xtl* was identified as essential for this inhibitory activity, and the crude extract from the BS011 fermentation sample significantly inhibited the development of *M. oryzae* at a concentration of 10 µg/mL (Wu et al., 2018). These findings suggest that PGPR strains could serve as an effective alternative to high-cost fertilizers, supporting sustainable agriculture.

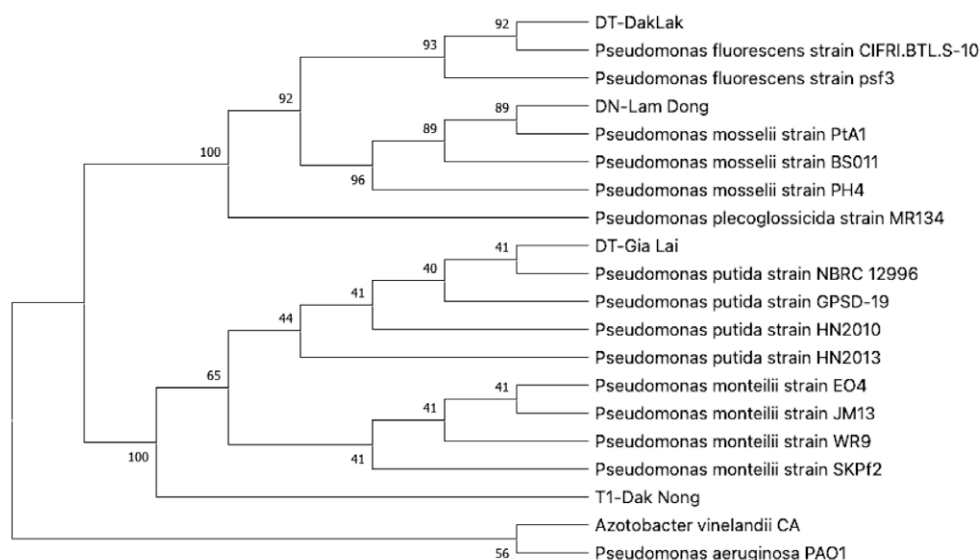


Figure 2. Neighbor-joining phylogenetic analysis of *Pseudomonas* spp. was conducted with similar species from GenBank, based on 16S rRNA gene sequence data. The numbers on each branch represent bootstrap values, which are percentages derived from an analysis of 100 replicates.

The PGPR influence plant growth through several mechanisms, including increasing mineral nutrient solubility, fixing nitrogen, providing nutrients to plants, inhibiting soil-borne pathogens (by producing hydrogen cyanide, siderophores, antibiotics, enzymes such as chitinase, and competing nutrients), enhancing plant tolerance to drought, salinity, and metal toxicity, and producing phytohormones such as indole-3-acetic acid (IAA) (Gupta et al., 2000). Results shown in Table 6 showed that four *Pseudomonas* sp. isolates can produce indole-3-acetic acid, ammonia, solubilized phosphate, and siderophores. The ability to solubilize phosphate

among these four *Pseudomonas* strains was qualitatively assessing, halo ring diameters ranging from 1.43 cm to 2.26 cm. Phosphorus (P) is a macronutrient crucial for plant growth and development (Soetan et al., 2010). However, the content of soluble phosphates in the soil is low due to its strong absorption by soil mineral and particles. Li et al. (2019) reported that *Pseudomonas* species isolated from the soil and rhizomes of various plant are capable of phosphorus solubilization. Additionally, treatment with *Pseudomonas* spp. has been shown to increase biomass yield and phosphorus uptake in *Triticum aestivum* (Ma et al., 2011).

Table 6. Producing indole-3-acetic acid, ammonia, and siderophores and solubilize phosphate of *Pseudomonas* spp.

Isolates	IAA		Nitrogen fixation		Phosphate solubillization		Siderophore
	Q	µg/mL	Q	Mol/mL	Q	HD (cm)	Q
DT-Gia Lai (<i>P. putida</i>)	+	54.88 ^a 1.00	+	3.25 ^b	+	1.43 ^c 0.25	+
DN-Lam Dong (<i>P. mosselii</i>)	+	12.07 ^c 0.93	+	3,14 ^b	+	2.04 ^a 0.40	+
T1-Dak Nong (<i>P. monteilii</i>)	+	19.09 ^b 0.91	+	3,03 ^c 0.55	+	1.72 ^b 0.28	+
TD-Dak Lak (<i>P. fluorescens</i>)	+	11.63 ^c 1.17	+	3,69 ^a	+	2.26 ^a 0.35	+

HD: halo ring diameter, Q: qualitative (+: yes –: no). Values indicate mean values ($\pm$ SD); different letters indicate significant differences within a row or column at $P < 0.01$.

Pseudomonas spp. satisfied the ion needs by consuming siderophores produced by other rhizosphere microorganisms. As soil copper concentrations increase, bacterial strains capable of producing high levels of siderophores experience less inhibition. Bacterial siderophores play a crucial role in host plants by influencing iron homeostasis and immune function (Hesse et al., 2018). Under conditions of iron deficiency, siderophore-producing bacteria assist plants in improving iron absorption. The results showed

that *Pseudomonas* strains produced siderophores on CAS medium (Table 4). The production of the phytohormone IAA (indole-3-acetic acid) by the four *Pseudomonas* strains was notably high, ranging from 11.63 to 54.88 µg/mL. Fluorescent *Pseudomonads*, such as *P. chlororaphis*, *P. putida*, *P. aeruginosa*, *P. monteilii*, *P. plecoglossicida*, *P. fluorescens*, *P. fulva*, and *P. mosselii* are known for their phosphate solubilizing ability (Maheshwari, 2011). Ravindra et al. (2008) reported that the up to 41% of fluorescent *Pseudomonad* bacteria

isolated from banana roots were phosphate-solubilizers. Additionally, some fluorescent *Pseudomonas* species produce various phytohormones including auxin, gibberellin, cytokinin, and abscisic acid, with IAA being particularly important for plant growth (Patten & Glick, 2002). IAA production by *Pseudomonas* spp. enhances the density and length of root hairs, improving the plant's ability to uptake water and nutrients, thereby stimulating plant growth (Kumar et al., 2005). Certain *Pseudomonas* species are also capable of nitrogen fixation, positively impacting plant growth and productivity (Nicole et al., 2003). Andrade et al. (1998) reported that *P. fluorescens* enhanced rhizobacterial symbiosis in peas through the *Nif* gene. The four *Pseudomonas* strains tested were able to fix nitrogen, with ammonia concentrations reaching 3.03-3.69 mol/mL.

The PGPR plant growth, yield, and stress tolerance by improving nutrient uptake from the soil through various mechanisms such as phosphate solubilization, siderophore production, immobilization of proteins, phytohormone production, antifungal activity, and induction of systemic drug resistance. Due to these benefits, PGPR, particularly strains from

the genus *Pseudomonas*, are used as biological alternatives to agrochemical products like fertilizers and pesticides. This approach reduces soil pollution and improves the quality of fruits and vegetables.

3.4. Effects of *Pseudomonas* spp. on germination seed ability and growth of durian plants

Results regarding the effects of *Pseudomonas* strains on germination of durian seeds showed that germination rates were 100% for both the control and the *Pseudomonas*-treated seeds. While germ length in the control and *P. mosselii* strain (DN-LamDong) did not differ significantly, *P. fluorescens* (TD-DakLak), *P. putida* (DT-GiaLai), and *P. monteilii* (T1-DakNong) exhibited significant differences compared to the control. The highest germ lengths were observed in seeds treated with *P. putida*, *P. fluorescens*, and *P. monteilii* measuring 6.98 cm, 6.84 cm, and 6.37 cm, respectively, compared to 5.31 cm in the uninoculated control (Figure 3). *Pseudomonas* strains have been shown to enhance seed germination, seedling vigor, and tomato plant height to 50.66 cm and 48 cm, respectively, compared to 30 cm in untreated plants.

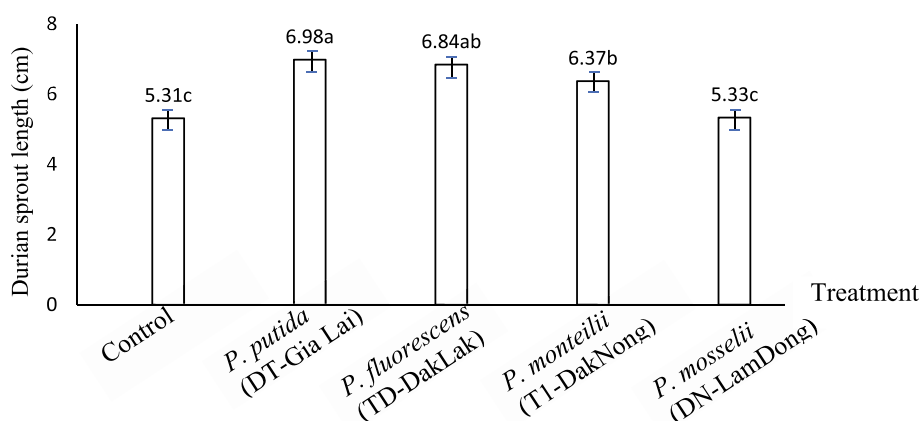


Figure 3. Effect of *Pseudomonas* isolates on durian seedling growth. Bars with the different letters indicate significant differences at $P < 0.01$.

Table 7. Effect of seedling treatment with *Pseudomonas* strains on plant height, leaf number in different days

Treatments	Plant height (cm)						Leaf number (leaves)					
	30 days		60 days		90 days		30 days	60 days	90 days			
DT-Gia Lai (<i>P. putida</i>)	21.64 ^a	0.65	36.92 ^a	0.50	43.16 ^a	0.37	3.0 ^a	0.4	15.1 ^b	0.5	18.3 ^a	0.4
TD-Dak Lak (<i>P. fluorescens</i>)	21.71 ^a	0.51	36.00 ^{ab}	0.98	42.38 ^{ab}	0.50	2.9 ^b	0.3	14.6 ^c	0.7	18.1 ^{ab}	0.3
T1-Dak Nong (<i>P. monteilii</i>)	20.66 ^b	0.54	33.52 ^{bc}	1.30	41.75 ^b	1.05	2.9 ^b	0.4	14.9 ^c	0.6	18.2 ^{ab}	0.4
DN- Lam Dong (<i>P. mosselii</i>)	19.54 ^{bc}	0.56	34.35 ^b	1.18	42.09 ^{ab}	0.56	2.8 ^c	0.5	15.9 ^a	0.8	17.5 ^b	0.5
Control	18.86 ^c	0.44	31.08 ^c	0.62	37.50 ^c	0.67	2.7 ^d		12.0 ^d	0.4	15.1 ^c	0.4

Different letters indicate significant differences within a column at $P < 0.01$.

The recorded data showed a significant increase in plant height of durians plants treated with different *Pseudomonas* strains compared to the untreated control (Table 7). All four *Pseudomonas* strains effectively improved plant height across all treatments compared to the uninoculated control. Specifically, the height of durian trees treated with *P. fluorescens* increased from 4.25 to 5.66 cm over 90 days. Among the strains, *P. putida* (DT-Gia Lai) was found to be most effective in enhancing plant height. Additionally, the number of durians leaves increased by an average of 2.4 to 3.2 leaves per plant in the *Pseudomonas*-treated groups compared to the control after 90 days. These results underscore the effectiveness of *Pseudomonas* strains as PGPR that can significantly enhance plant growth. Supporting this, Sarma & Saikia (2014) reported that *P. fluorescens* and *P. putida* increased rice plant height by 16.2% and 15.6%, respectively, compared to the uninoculated control after 30 days. Similarly, Qessaoui et al. (2019) showed that fluorescent *Pseudomonas* isolates from the rhizosphere significantly increased tomato plant height. Notably, the *Pseudomonas* strain

Q13B increased plant height by 31.5% and collar diameter by 48.57% compared to the control. Additionally, *Pseudomonas* sp. (Q1B) significantly enhanced the number of leaves by 83.33% 20 days after transplantation.

4. Conclusions

Four *Pseudomonas* spp. were isolated from the rhizoplane and rhizosphere durians trees in the Central Highlands, Vietnam. Four bacterial isolates were identified by morphology, biochemical analysis, and 16S rRNA sequence analysis as *P. fluorescens* (TD-Dak Lak), *P. mosselii* (DN-Lam Dong), *P. monteilii* (T1-Dak Nong), and *P. putida* (DT-Gia Lai) siderophore and solubilizing phosphate. They also demonstrated biological nitrogen fixation capacities ranging from 3.03 to 3.69 mol/mL and IAA production levels between 11.63 and 54.88 µg/mL. Inoculation with these *Pseudomonas* strains significantly improved plant height compared to the uninoculated control. Specifically, durian plants inoculated with *P. fluorescens* showed an increase in height from 4.25 cm to 5.66 cm

over 90 days. Among the strains, *P. putida* (DT-Gia Lai) was the most effective in enhancing plant height. Additionally, the number of durian leaves increased by an average of 2.4 to 3.2 leaves per plant in the *Pseudomonas* treated groups compared to the control after 90 days. These findings indicate that *P. fluorescens*, *P. putida*, *P. mosselii*, and *P. monteilii* are beneficial biological agents that promote the growth and development of durian trees. Therefore, these indigenous *Pseudomonas* strains have the potential to be used as biofertilizers or bioproduct to enhance durian cultivation in local agricultural conditions.

Conflict of interest

The authors have no conflicts of interest to declare.

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Plant growth promoting and biocontrol efficacy of *Pseudomonas* spp. for tomato root rot disease caused by *Fusarium solani*

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ABSTRACT

Root rot disease in tomatoes caused by *Fusarium solani* is one of the most serious diseases and causes severe problems in all tomato growing regions. *Pseudomonas* is a genus of bacteria capable of synthesizing valuable active compounds, promoting plant growth, and controlling disease-causing fungi. This study aimed to evaluate the plant growth- promoting potential of selected bacterial strains from the *Pseudomonas* genus and their ability to prevent the occurrence of *Fusarium solani*, the fungus responsible for tomato root rot disease. *In vitro* experiments were conducted to assess the characteristics and effects related to plant growth promoting of the tested *Pseudomonas* strains. The antagonistic capability of the bacteria against the fungi was also evaluated by measuring mycelial growth inhibition. Greenhouse conditions were used to evaluate the effectiveness of *Pseudomonas* as a soil treatment for plant growth promoting and reducing the severity of tomato root rot disease. The results demonstrated a positive influence of *Pseudomonas* spp. on the growth and development of tomato plants, with *Pseudomonas* spp. PN05 exhibited the most significant impact. Moreover, both strains *Pseudomonas* spp. PN02 and PN05 exhibited inhibitory effects on *F. solani* *in vitro* and under greenhouse conditions, limiting root rot disease in tomato plants with a control efficiency ranging from 48.3 to 62.2%.

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1. Introduction

In the context of an increasing population that seriously impacts the environment, ensuring the quality of agricultural products is a significant challenge for both farmers and researchers. The use of biofertilizers and biotic agents, in conjunction with appropriate farming management practices, is a suitable approach that has received significant attention from the community. Among the biological solutions applied in agriculture, the use of plant growth-promoting bacteria (PGPB) has been reported. The PGPB are freely distributed in soil, on root surfaces, or endophytically within roots, and can promote plant growth and inhibit plant diseases. The mechanisms of action of PGPB bacteria include (i) direct mechanisms related to various processes such as phosphorus solubilization, nitrogen fixation, siderophore production, HCN, ammonia, vitamins, and phytohormone production (such as auxin, cytokinin, and gibberellin) and (ii) indirect mechanisms including 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity, antibiotic production, cell wall degradation enzyme production, and induced systemic resistance (ISR) (Jaizme-Vega et al., 2004).

Among PGPBs, *Pseudomonas* spp. have been widely distributed in soil, water, and agricultural ecosystems because of their diversity in nutritional and physiological processes, making them highly adaptable to adverse environmental factors such as temperature, humidity, oxygen content, nutrient availability, and the presence of toxic substances. In addition, their ability to synthesize various valuable nutrients, clean the environment from pollutants, and their extremely simple cultivation conditions, along with their rapid growth rate, make them potential candidates for agricultural applications.

Some species of *Pseudomonas* genus produce 2,4-diacetylphloroglucinol (2,4-DAPG), hydrogen cyanide, pyoluteorin (PLT), phenazine (including *P. chlororaphis*, *P. fluorescens*, and *P. aeruginosa*), and pyocyanin..., secrete cell wall-degrading enzymes such as chitinase, protease inhibiting the development of fungal diseases (Gupta et al., 2005).

Fusarium root rot caused by *Fusarium solani* is a significant problem in tomato production regions (Simões & de Andrade, 2023). *Pseudomonas fluorescence* and *P. aeruginosa* have antagonistic effects on *F. solani*. These *Pseudomonas* strains could inhibit the mycelial growth of *F. solani* in *in vitro* experiments (Kumar et al., 2015; Hofny et al., 2022). In addition, the volatile metabolites produced by *P. aeruginosa* were found to be efficient in reducing the mycelial growth of *F. solani* (Alsudani & Al-Awsi, 2020). Using of *P. fluorescens* as a biocontrol agent decreased disease severity under greenhouse conditions (Cai et al., 2021).

This study aimed to elucidate the characteristics related to the ability of some *Pseudomonas* spp. to promote plant growth and control *Fusarium solani*, the agent causing tomato root rot disease, to provide a basis for the selection and formulation of bio-products for sustainable agriculture.

2. Materials and Methods

2.1. Materials

Six *Pseudomonas* spp. and *Fusarium solani* strains were isolated, identified, and preserved at the Plant Integrated Biology (PIB) Laboratory of Nong Lam University Ho Chi Minh City, Vietnam. Phu Nong F1 (T-11) tomato seeds were used in the present study.

2.2. Determination of the ability of *Pseudomonas* spp. strains to promote plant growth

Pseudomonas spp. were cultured in Tryptone Soya Broth (TSB) for 24 h at 30°C with shaking at 150 rpm. Preparation of the bacterial suspension with an optical density of 0.1 at 600 nm. The experiments were designed in a completely randomized design with 4 replicates. The ability to dissolve phosphate was observed by the appearance of a clear zone of $\text{Ca}_3(\text{PO}_4)_2$ on Pikovskaya's agar medium after 3 days of cultivation at 30°C (Nautiyal, 1999). Indole-3-acetic acid (IAA) production was detected and quantified using the Salkowski reagent colorimetric method at 520 nm (Sasirekha & Shivakumar, 2012). Gibberellic acid (GAs) production was quantified using the extraction method with ethyl acetate, and absorbance was measured at 254 nm (Sasirekha & Shivakumar, 2012). The ability to form biofilms was determined using the method of O'Toole et al. (2000), siderophore production was determined on CAS agar medium according to Srivastava et al. (2018), and nitrogen fixation of the bacterial strains was determined according to Bashan et al. (1993).

2.3. Investigation of the influence of *Pseudomonas* spp. on tomato plant growth

In vitro conditions: Tomato seeds were sterilized and soaked in bacterial suspension (10^9 CFU/mL), and after 3 h, seeds were placed on a plate covered with a layer of sterile filter paper. In each treatment, 30 seeds were sown in Petri dishes containing moistened absorbent cotton and incubated at $25 \pm 1^\circ\text{C}$. Seed germination was monitored after 2 and 3 days, and the germination rate was calculated.

Greenhouse conditions: Healthy tomato seedlings with 3 fully developed leaves were transplanted into pots (25 x 21 cm) containing clean soil, manure, lime, and phosphate fertilizer at a ratio of 4:1:1:1. When the plants had 5 true leaves, 5 mL of bacterial culture (10^7 CFU/mL) was irrigated at the base of each plant, once a week for 4 weeks. Plant height, leaf number, branch number, cluster number, flower number, and fruit quality were monitored.

2.4. Evaluation of the antagonistic ability of bacteria against *Fusarium solani*

The dual culture method was performed according to Ferreira et al. (1991) by measuring the fungal colony radius and calculating the antagonistic efficacy according to the formula of Moayedi et al. (2009) and the rating scale of Soyong (1988). The agar well diffusion method was performed according to Sharma et al. (2017), and the antagonistic activity of bacteria was demonstrated through the formation of inhibition zones after a 48-h incubation time, calculated using the formula of Azman et al. (2017). The experiments were arranged in a completely randomized design, with each bacterial strain as a treatment, repeated 3 times with four petri dishes in each replication.

Evaluation of disease control ability against *Fusarium solani*: seven-day-old tomato seedlings were immersed in a suspension of *F. solani* spores at a density of 10^6 spores/mL and a bacterial suspension at a density of 10^9 CFU/mL (Fs + PN) or in the reverse order (PN + Fs), and then transferred to 1.5 mL tubes. All seedlings were placed at 28°C with a 12-h light cycle. Disease progression was monitored after 7 days. The experiments were arranged in a completely randomized design, with each treatment consisting of 3 seedlings. The experiments were repeated twice.

Healthy tomato seedlings with 3 fully developed leaves were transplanted into pots (25 x 21 cm) containing clean soil, manure, lime, and phosphate fertilizer in a ratio of 4:1:1:1. When the plants had 5 true leaves, their roots were injured, and 5 mL of *F. solani* spore suspension (10^6 spores/mL) was applied. Additionally, 5 mL of bacterial suspension (10^9 CFU/mL) was applied around the plant base, either 24 h before, at the same time, or 24 h after the fungal inoculation, depending on the treatment. The experiments were arranged in a randomized complete block design, with each treatment replicated 3 times, with 4 pots per treatment. The pots were placed in a greenhouse, and watering was provided as per the plant requirements. Disease incidence (%) and disease index (%) after infection at 7, 14, 21, and 28 days were recorded and classified according to Ateka et al. (2001). The efficacy of disease control was calculated using the Abbott (1925) formula.

2.5. Data analysis

The experimental data were analyzed using analysis of variance (ANOVA) to compare means based on Duncan test. The data were transformed to ensure compliance with the normal distribution, if necessary.

3. Results and Discussion

3.1. Characteristics related to the plant growth-promoting ability

All six strains of *Pseudomonas* spp. exhibited the ability to solubilize phosphate, synthesize IAA, form biofilms, and produce siderophores, but did not produce GAs or fix nitrogen. Among them, strain PN04 showed the highest phosphate solubilization, measuring 21.13 mm, while strain PN01 produced the highest concentration of IAA at 23.15 μ g/mL after 120 h in TSB supplemented with tryptophan (Table 1, Figure 1).

Table 1. Characteristics related to the plant growth-promoting ability of *Pseudomonas* strains

Strains	Phosphorus solubilization (mm)	IAA production (mg/mL)	Biofilm formation	Siderophore production	GAs production	Nitrogen fixation
PN01	20.90 ^a	23.15 ^a	+	+	-	-
PN02	14.67 ^d	12.89 ^b	+	+	-	-
PN03	16.97 ^c	7.09 ^c	+	+	-	-
PN04	21.13 ^a	15.00 ^b	+	+	-	-
PN05	19.63 ^b	12.90 ^b	+	+	-	-
PN06	10.97 ^e	3.03 ^c	+	+	-	-
CV (%)	8.87	8.74				
F	5.69 ^{**}	7.92 ^{**}				

In the same column, numbers with the same letters represent statistically insignificant differences; (**) $\alpha = 0.01$. IAA: indole-3-acetic acid; Gas: gibberellic acid.

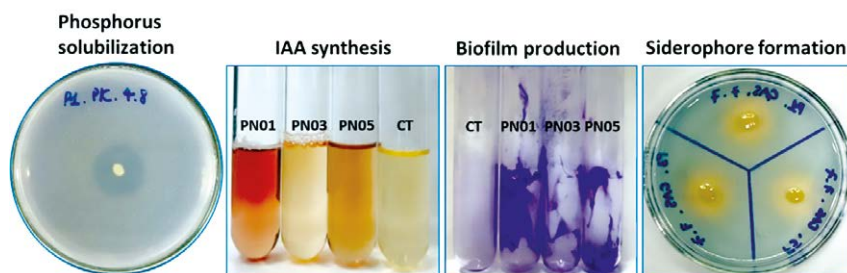


Figure 1. Characteristics related to the plant growth promotion ability of *Pseudomonas* spp.

3.2. Influence of *Pseudomonas* spp. on tomato seed germination *in vitro* condition

After 72 h of cultivation, tomato seeds treated with PN03 strain had the highest germination rate (75.0%), equivalent to the control (71.3%),

whereas the seeds treated with PN04 strain had the lowest germination rate (49.67%). Seeds treated with the remaining four bacterial strains had germination rates ranging from 53.00% to 64.33% ($P < 0.01$) (Figure 2).

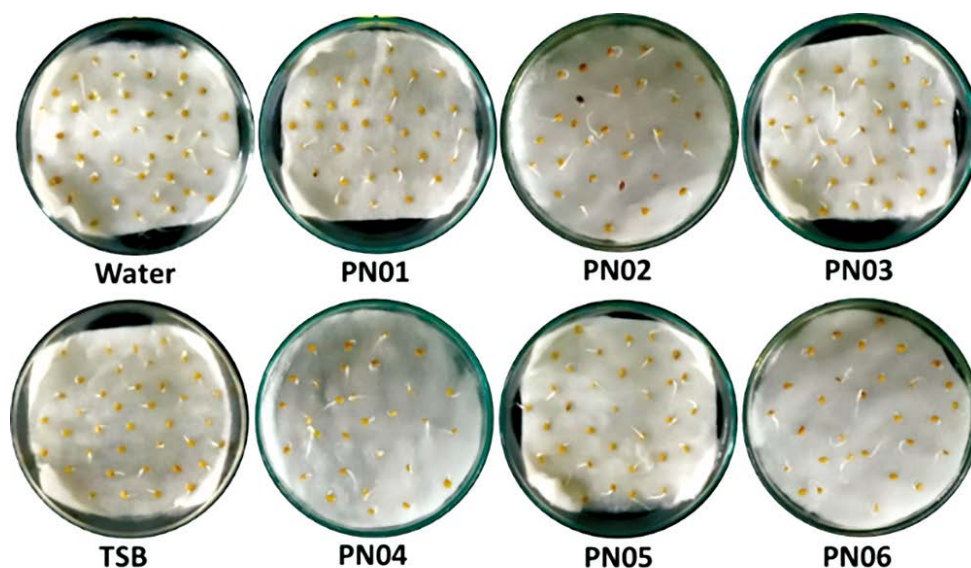


Figure 2. Influence of bacteria on tomato seed germination.

3.3. Growth promotion effect of *Pseudomonas* spp. on tomato plants in greenhouse condition

The results showed that at the 14-day stage, the height of plants treated with strain PN03 reached the highest value of 12.88 cm. Control treatments and strains PN02, PN05, and PN06 had heights ranging from 9.00 cm to 11.75 cm. At the 28-day stage, treatment with strain PN05 had a significant impact on plant growth and development, with a height of 39.88 cm, the highest number of leaves (13.00), branches (2.59), flower clusters (3.50), and flowers (3.25) ($P < 0.05$) (Figure 3, Table 2). After 56 days of inoculation, the highest number of fruits per plant was observed in the treatment with the PN05 strain (8 fruits per plant), whereas the other

bacterial strains did not show any significant difference compared with the control. However, parameters such as fruit length, fruit diameter, and fruit flesh thickness were significantly different compared with the control ($P < 0.05$) (Table 3). The results showed that strain PN05 stands out for its ability to significantly promote both vegetative and reproductive growth in tomato plants, leading to increased plant height, leaf number, branching, flower production, and fruit yield. The other strains also contributed positively to specific growth parameters and fruit quality, highlighting their potential as part of integrated plant growth-promoting strategies. Further research is needed to fully understand the mechanisms behind these effects and to validate the findings in field conditions.

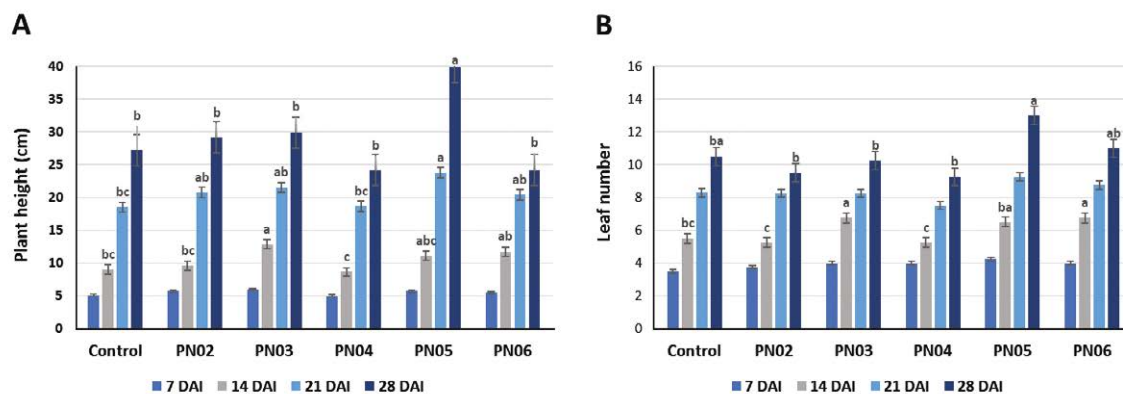


Figure 3. Plant height (A) and leaf number (B) of tomato plants treated with bacteria. DAI: days after inoculation.

Table 2. Influence of bacteria on the growth of tomato plants in greenhouse conditions 28 days after inoculation

Treatments	Branch number	Flower clusters	Flower numbers
Control	0,75 ^b	1,75 ^{ba}	8,00 ^b
PN02	0,25 ^b	1,25 ^c	5,75 ^b
PN03	0,75 ^b	1,75 ^{bc}	7,00 ^b
PN04	0,25 ^b	1,00 ^c	4,50 ^b
PN05	2,59 ^a	3,50 ^a	17,25 ^a
PN06	1,25 ^b	3,00 ^{ba}	10,50 ^{ba}
CV (%)	24.16	18.33	19.99
F	5.74 ^{**}	4.37 [*]	5.67 ^{**}

In the same column, numbers with the same letters represent statistically insignificant differences; (*) $\alpha = 0.05$; (**) $\alpha = 0.01$.

Table 3. Fruit quality at 56 days after inoculation

Treatments	Number	Fresh weight (g)	Length (cm)	Diameter (cm)	Fruit flesh thickness (mm)
Control	4,00 ^b	77,9	3,70 ^b	4,90 ^b	43 ^c
PN02	5,50 ^b	79,8	4,13 ^a	5,37 ^{ba}	50 ^{bac}
PN03	6,25 ^b	80,9	4,20 ^a	5,50 ^a	57 ^{ba}
PN04	4,50 ^b	78,4	4,06 ^a	5,06 ^{ba}	47 ^{bc}
PN05	8,00 ^a	83,1	4,23 ^a	5,63 ^a	60 ^a
PN06	6,50 ^b	80,5	4,10 ^a	5,30 ^{ba}	50 ^{bac}
CV (%)	19.9	14.8	3.01	4.01	11.3
F	5.67 ^{**}	0.86 ^{ns}	7.41 ^{**}	4.93 ^{**}	3.47 [*]

In the same column, numbers with the same letters represent statistically insignificant differences; (ns) no significant difference; (*) $\alpha = 0.05$; (**) $\alpha = 0.01$.

3.4. Antagonistic ability against *Fusarium solani* by *Pseudomonas* strains under *in vitro* conditions

Using the dual culture method, all bacterial strains except PN06 showed varying degrees of antagonism against *F. solani*. Strain PN01 had the lowest resistance rate (48.1%), while strains PN02 and PN05 had the highest resistance rates (60.3% and 58.9%, respectively) ($P \leq 0.05$). In

the agar well diffusion method, all six bacterial strains exhibited resistance against *F. solani*. PN06 had the lowest antagonistic efficiency (32.5%), while PN02 and PN05 had the highest efficiencies (45.8% and 45.3%, respectively) ($P \leq 0.05$) (Figure 4). Strains PN02 and PN05 were further evaluated for their ability to control the disease in tomato plants under *in vitro* and greenhouse conditions.

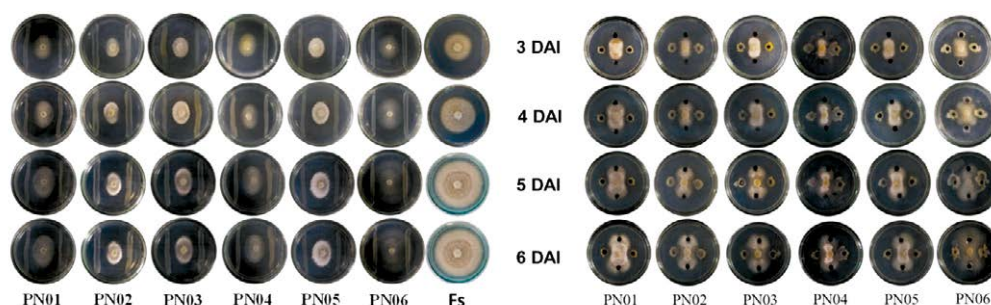


Figure 4. Inhibitory ability against *Fusarium solani* of the six *Pseudomonas* strains. Dual culture method (left) and agar well diffusion method (right). DAI: days after inoculation.

Fs: *Fusarium solani*.

3.5. Antifungal activity of *Pseudomonas* strains against *Fusarium solani* under *in vitro* conditions

After 72 h of inoculating the tomato seedlings with *F. solani*, symptoms of withering appeared, and by 7 DAI, the plants had completely dried up. Treatments with PN02 and PN05 whether applied before fungi (PN02 + Fs, PN05 + Fs), or

after (Fs + PN02, Fs + PN05) showed no symptom of yellowing and exhibited healthy growth (Figure 5). The experimental results indicated that two *Pseudomonas* strains could limit the impact of *F. solani* on young tomato plants *in vitro*. This lays the foundation for further investigation into the disease control abilities of two strains, PN02 and PN05, on mature tomato plants in greenhouse conditions.

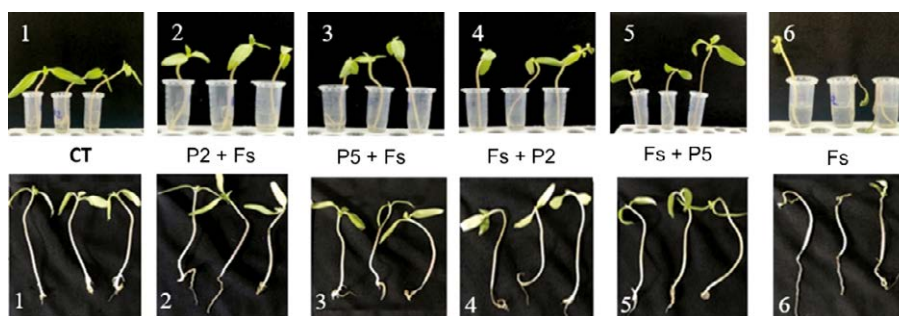


Figure 5. Tomato seedlings treated with *Pseudomonas* bacteria and *Fusarium solani*, 72 h after inoculation (top panel) and 7 days after inoculation (bottom panel). CT: control, Fs: *Fusarium solani*. P2, P5: PN02, PN05 strains.

3.6. Ability to control tomato root rot caused by *Fusarium solani* by *Pseudomonas* spp. strains under greenhouse conditions

At 28 DAI, the disease incidence showed a difference between the two groups. The group with a high disease incidence ranging from 77.8%

to 88.9% was treated with fungal infection before bacterial treatment (*F. solani* + PN02; *F. solani* + PN05; *F. solani* simultaneous with PN05), and the group with a low disease incidence of 55.6% was treated with bacterial inoculation before fungal infection (PN02 + *F. solani*; PN05 + *F. solani*; PN02 simultaneous with *F. solani*) (Table 3).

Table 3. Disease index (%) and control efficacy (%) of tomato root rot caused by *Fusarium solani* of two bacterial strains in greenhouse conditions

Treatments	Height (cm)	Root length (cm)	Disease Incidence (%)	Disease Index (%)			Control efficacy (%)
	28 DAI	28 DAI	28 DAI	14 DAI	21 DAI	28 DAI	28 DAI
Control	41.7 ^{ba}	23.3 ^b	0.00 ^e	0.00	0.00 ^c	0.00 ^d	-
<i>F. solani</i>	36.5 ^c	20.1 ^c	100 ^a	6.66	22.2 ^a	68.9 ^a	-
<i>F. solani</i> + PN02	45.0 ^a	26.7 ^a	88.9 ^b	2.22	11.1 ^b	42.3 ^b	38.8
<i>F. solani</i> + PN05	44.7 ^{ba}	25.8 ^{ba}	88.9 ^b	4.45	20.0 ^{ab}	35.6 ^{bc}	48.5
<i>F. solani</i> - PN02	41.0 ^{bac}	23.3 ^b	55.6 ^d	2.22	15.6 ^{ab}	26.7 ^c	61.2
<i>F. solani</i> - PN05	39.7 ^{bc}	23.1 ^b	77.8 ^c	2.22	17.8 ^{ab}	35.6 ^{bc}	48.3
PN02 + <i>F. solani</i>	40.7 ^{bac}	23.4 ^b	55.6 ^d	4.45	11.1 ^b	40.0 ^{bc}	41.9
PN05 + <i>F. solani</i>	41.2 ^{bac}	23.1 ^b	55.6 ^d	2.22	22.2 ^a	42.2 ^b	38.7
CV (%)	10.59	6.09	31.7	9.46	34.6	35.9	
F	2.88 [*]	7.64 ^{**}	4.53 ^{**}	0.73 ^{ns}	13.3 ^{**}	15.9 ^{**}	

In the same column, numbers with the same letters represent statistically insignificant differences; (ns) no significant difference; (*) $\alpha = 0.05$; (**) $\alpha = 0.01$. DAI: days after inoculation.

At day 28 after inoculation, the disease index increased by 68.9% in the fungal treatment whereas in the bacterial treatments, the disease index

ranged from 26.7 - 42.3% and the disease control efficacy ranged from 38.7% (PN05 + *F. solani*) to 61.2% (*F. solani* - PN02) (Table 3, Figure 6).

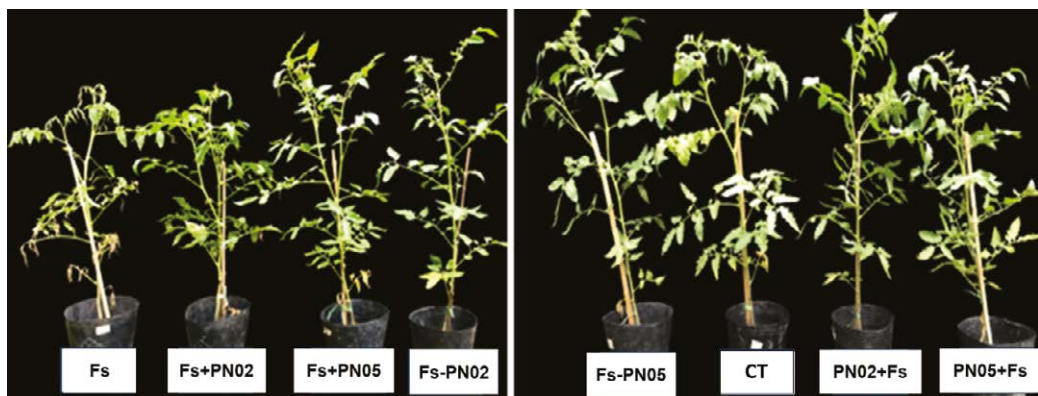


Figure 6. Ability to control root rot disease by *Pseudomonas* sp. strains PN02 and PN05. CT: control, Fs: *Fusarium solani*.

Some PGPR species found worldwide could promote growth in various crops. They can be used as biofertilizers, biopesticides, root bacterial treatments, and bio-stimulants (Ryu et al., 2003). *Pseudomonas* and *Bacillus* are the most extensively studied and widely used PGPRs. The mechanisms by which PGPR promotes plant growth involve the synthesis of certain metabolites (auxin, cytokinin, and gibberellin), the production of siderophores, hydrocyanic acid (CN), antibiotics, and volatile compounds. Competition, inducing systemic resistance, and mineral solubilization (such as phosphorus or potassium) are other factors that *Pseudomonas* uses to enhance crop productivity (de Andrade et al., 2023). This bacterial species also exhibited biological control activity against plant pathogens. In this study, all *Pseudomonas* strains showed the ability to solubilize phosphate, synthesize IAA, form biofilms, and produce siderophores, but did not produce GAs or fix nitrogen. *In vitro* conditions, these strains did not improve tomato seed germination rate after 3 days of incubation. Reports have shown that phytohormones produced by bacteria may reduce plant seed germination (Tabatabaei et al., 2016; Pirog, 2018; Mohammed et al., 2023). The bacterial density and treatment duration for each type of plant seed need to be determined before application. All five tested bacterial strains showed positive effects on plant growth through growth and fruit quality parameters, with *Pseudomonas* sp. strain PN05 having the most pronounced effect.

Pseudomonas spp. can colonize the roots of plants and produce antifungal metabolites represent a real alternative chemical fungicide (Walsh et al., 2001). Studies selecting *Pseudomonas* bacteria using antagonism against plant pathogens have also been conducted. The two bacterial strains PN02 and PN05 showed

effective antagonism against *Fusarium solani*. Strain PN02 was identified as *Pseudomonas aeruginosa* (Tran et al., 2023) whereas PN05 has not been identified. Yasmin et al. (2014) reported an antagonistic *P. aeruginosa* strain that showed effectiveness against *F. oxysporum*, *F. moniliforme*, *F. solani*, and *Rhizoctonia solani* causing diseases in cotton; it inhibited the *in vitro* development of *Colletotrichum gloeosporioides* and *Rhizoctonia solani* (Sasirekha & Srividya, 2016). To date, there have been few reports on the biological control potential of *P. aeruginosa* under field conditions, possibly because of biosafety concerns. *Pseudomonas* strains have been evaluated as biocontrol agents against *Fusarium solani* in multiple studies. Abo-Zaid et al. (2023) found that *P. aeruginosa* F2 and *P. fluorescens* JY3 produced siderophores with high efficiency and showed antagonistic activity against *F. oxysporum* and *R. solani*. Kedia and Sharma isolated *Pseudomonas* strains from tomato rhizosphere soil and found that two strains, *Pseudomonas* VK1 and VK2, showed antagonistic activity against *F. solani* (Kedia et al., 2023). Hu et al. (2021) isolated *P. aeruginosa* strain YY322 from saffron rhizosphere soil and found that it exhibited highly effective antagonistic activity against *F. oxysporum* and *F. solani*. These findings demonstrate the potential of *Pseudomonas* strains as biocontrol agents for plant diseases in Vietnam, offering sustainable alternatives to chemical fertilizers and pesticides.

4. Conclusions

All *Pseudomonas* spp. exhibited no negative effects on tomato growth and development, with *Pseudomonas* sp. strain PN05 having the most pronounced impact. Strains PN02 and PN05 inhibited *F. solani* and reduced root rot disease under *in vitro* and greenhouse conditions.

Further research could assess the persistence of these biocontrol effects over multiple growing seasons and investigate the mechanisms by which *Pseudomonas* inhibits pathogen growth and induces biochemical changes in treated tomato plants.

Conflict of interest

The authors declare that they have no competing interests.

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Effect of urban agriculture on the quality of urban life: A review

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ARTICLE INFO	ABSTRACT
Research Paper Received: November 13, 2023 Revised: May 29, 2024 Accepted: June 10, 2024 Keywords Food security Quality of life Review Urban agriculture Urbanisation *Corresponding author Oyerinde Ismail Adedapo Email: oyerindeismailadedapo@gmail.com	The purpose of this research was to conduct an extensive literature review concerning the correlation between Urban agriculture and the quality of life of individuals residing in urban areas. Employing established systematic review protocols, the study gathered data from a wide array of renowned databases, such as Scopus, Science Direct, Web of Science, and Google Scholar. The criteria for inclusion were determined based on the applicability to the themes of urban agriculture and quality of life, publication within the time frame of 2010 to 2022, and availability in the English language. The synthesis included a comprehensive examination of 20 scholarly papers that were peer-reviewed. The economic impacts suggest that urban agriculture has the capacity to produce earnings, decrease household food costs, and promote local economies. Urban agriculture actively encouraged community engagement, tackled social concerns, and nurtured interpersonal connections. In terms of health, urban agriculture played a role in ensuring food security and improving physical well-being. Urban agriculture’s environmental impacts include its beneficial influence on urban ecosystems, its reduction of carbon emissions as well as its efforts to combat air and noise pollution. Urban agriculture functioned as a cultural medium, facilitating the connection between persons and their ancestry, fostering agricultural expertise, and serving as a cultural hub. Negative externalities include resource exploitation, contamination as well as ecosystem disruption. This study offers an in-depth understanding of the many effects of urban agriculture on the quality of life of urban people. The analysis adds to the existing pool of information, providing insights into the potential of urban agriculture to improve several aspects of humans’ well-being in urban environments.

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1. Introduction

The urgent need to address the global issues of climate change, increasing populations, and resource disputes highlights the importance of investigating sustainable methods to guarantee food and energy security for urban inhabitants (Wiskerke & Viljoen, 2012; Abera & Belayneh, 2017). According to St Clair's (2017), it is predicted that by 2030, more than 60% of the global population would live in urban areas, which will increase the difficulties in sustainable urban planning and administration. These difficulties arise in several forms, such as the reduction of unused land, increased energy use due to commuting, deforestation, water contamination, and the development of peri-urban areas (Frantzeskaki et al., 2017; Skar et al., 2020). Academics and other stakeholders are therefore advocating for cities to become producers of agricultural products themselves (Voleníková & Opršal, 2016; Ibrahim & Salim, 2020).

Urban agriculture is a key endeavour carried out by people living in cities. It has a significant impact on tackling current urban issues such as poverty, social division, lack of food and energy security, and environmental stress (Battersby & Marshak, 2013; Ibrahim & Salim, 2020). In their study, Wadumestrigue et al. (2021) provide a definition of urban agriculture as the production that takes place either inside urban centres or on their periphery, including activities such as agriculture, processing, and distribution. Internationally, urban agriculture has been acknowledged as a socially significant activity that brings considerable advantages to communities and the environment (Mpigi, 2020). According to Akinagbe & Ipinmoye (2022), incorporating agriculture into urban areas improves the

sustainability of cities by using organic matter as fertiliser and constructing green infrastructure. It enhances economic prosperity, fosters physical and mental well-being via leisure pursuits, and alleviates the consequences of climate change (De Zeeuw et al., 2011; Ibrahim & Salim, 2020).

Although the importance of urban agriculture in urban environments is recognised, this study seeks to further review its impact on the general well-being of urban inhabitants. Given the current economic difficulties, which are marked by significant inflation, unpredictable fluctuations in food prices, and meagre wages, it is crucial to understand the intricate consequences of urban agriculture. This review thus aimed to explore the relationship between urban agriculture and quality of life, with the goal of gaining a deeper knowledge of how it contributes to improving the well-being in urban settings.

1.1. The concept of urban agriculture

Agriculture, a long-lasting element of human history, has evolved from ancient civilizations to the contemporary era, establishing a vital connection between humanity and the productive use of land (Ofordu et al., 2022). In the past, there was less clear distinction between urban and rural areas, which resulted in agricultural operations taking place inside metropolitan zones. This created a mutually beneficial interaction between cities and agriculture (Doğan, 2022). The historical setting underscores the inherent link between urban areas and agricultural methods as seen in ancient cities such as Aztec and Maya civilizations (Doğan, 2022). This highlights the widespread occurrence of this practice throughout history. The discussion among scholars about urban agriculture has changed throughout time, characterised by extensive deliberation and

diverse interpretations (Nogeire-McRae et al., 2018). The original definitions, as emphasised by Mbiba (1995), were rudimentary but served a vital purpose in starting conversations about creating an established terminology among academics (Knapp et al., 2016).

Urban agriculture was formally defined in 1996 at the United Nations International Conference on Human Habitats as an economic sector that involves the production, processing, and distribution of food, fuel, and other commodities to fulfil the daily requirements of urban dwellers. This activity encompasses both privately and publicly owned land and water areas throughout urban and suburban zones (Doğan, 2022). Urban agriculture comprises a wide range of activities, including both commercial and non-commercial efforts, as shown by these definitions. The scope extends beyond the manufacturing of food to include non-food products, such as ornamental trees, which display differences in function, form, size, and location (Lin et al., 2015). The functional element of urban agriculture is greatly influenced by global differences, which result in different objectives for industrialised and less developed countries. Advanced countries prioritise the promotion of leisure activities, whereas low-income regions prioritise tackling the urgent problem of food security (Krishnan et al., 2016). Urban agriculture functions as a livelihood strategy in the Global South, with a focus on economic benefits. In industrialised countries, it is more of a recreational activity and a way to pass time (WinklerPrins, 2017).

Urban agriculture is a complex activity with many uses and objectives, as seen from historical, intellectual, and global viewpoints. It encompasses more than just food production and also includes larger economic, socio-

cultural, and livelihood aspects, demonstrating its flexibility to adapt to different socio-economic and geographical conditions. The objective of this study was to further investigate these aspects, examining the intricate relationship between urban agriculture and its effects on residents.

1.2. Quality of life as a concept

The concept of “quality of life” is widely discussed and acknowledged in everyday conversations, including both personal discussions and larger societal or policy debates (Beck, 2012; Bolzan et al., 2023). This idea is complex and may be studied from two different viewpoints: macro and micro. The macro viewpoint focuses on objective and social elements such as education, living circumstances, work prospects, values, well-being, and lifestyle satisfaction (Mihaela & Daniel, 2011). In contrast, the micro view focuses on subjective and personal elements, including individual assessments derived from life experiences, personal beliefs, and goals (Blome & Augustin, 2015). The World Health Organisation (WHO, 1995) defines quality of life as a holistic concept that includes physical health, mental well-being, personal autonomy, social relationships, individual values, and interactions with the environment. This comprehensive comprehension situates quality of life as a notion intricately intertwined with several aspects of human life.

The assessment of personal satisfaction or dissatisfaction in quality of life is shaped by the social or philosophical context (Bolzan et al., 2023). Bolzan et al. (2023) highlights that the assessment of quality of life depends on favourable living conditions, such as having access to healthy food, clean air and water, pleasant experiences in natural surroundings, a harmonious relationship with nature, and a secure society protected from

dangerous substances and radiation (Medvedev & Landhuis, 2018). The concept of quality of life is widely recognised as a complex idea that is crucial in the development of policies, healthcare strategies, and social programmes designed to improve the overall well-being of people (Abolhassani et al., 2019). From a macro view, it is intricately connected to social concerns, whilst a narrower approach emphasises the personal and subjective elements of individual experiences.

The WHO's comprehensive approach coincides with the premise that quality of life goes beyond individual well-being to cover environmental elements and social interactions. This study aims to investigate the complex interaction of these indicators as presented in Table 1, offering a detailed comprehension of how many elements contribute to the total well-being experienced by people in different sociocultural and environmental settings.

Table 1. Dimensions of quality of life

Dimensions	Indicators
Economic dimension	Income levels Employment opportunities Wealth and asset ownership Access to services and resources Standard of living Financial security
Social dimension	Social connections and relationships Social support networks Civic engagement and community participation Social equity and inclusion Safety, security, and social cohesion
Health dimension	Physical health and well-being Mental and emotional well-being Lifestyle factors and health behaviours
Environmental dimension	Access to clean air and water Availability of green spaces and natural areas Environmental safety and security Conservation of natural resources Aesthetics and urban design
Cultural dimension	Cultural diversity and inclusivity Preservation of cultural heritage Access to cultural and artistic events Cultural participation and expression

Source: International Society of Quality-of-Life Studies (ISQOLS, 2023).

2. Methods

The research performed a thorough analysis, using established systematic review techniques, specifically according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) framework (Page et al., 2009), to completely assess scholarly literature on the effects of urban agriculture on the quality of life of urban dwellers. This technique guaranteed a systematic and evidence-based evaluation.

2.1. Data collection

The sources for this study were systematically identified by scanning four well-known bibliographic databases: Scopus, Science Direct, Web of Science, and Google Scholar. The search method used Boolean operators to combine the core term, urban agriculture, and its synonyms with three secondary keywords, namely 'quality of life,' 'well-being,' and 'urban life.'

2.2. Inclusion criteria

Articles were chosen for their inclusion based on four fundamental criteria in order to maintain the highest level of quality. Initially, it is essential that the investigation was carried out in an urban region, as defined by each article. Furthermore, the paper must have primarily emphasised the concept of urban agriculture, as described within the article, while also evaluating the correlation between urban agriculture and quality of life. Furthermore, the essay should have presented primary data or findings derived from an innovative examination of pre-existing data sources. Ultimately, only publications published between the timeframe of 2010 to 2022 were considered. Non-English articles were also excluded.

2.3. Search methodology and coding

A thorough search was conducted in each database using particular search strings and keywords to identify acceptable articles. In addition, a thorough search was conducted in the journal Agriculture and Development to find relevant titles and citations that might help uncover any possibly overlooked research. One team member conducted a comprehensive screening procedure at the title and abstract level, excluding publications that obviously did not fulfil the inclusion requirements. Subsequently, two team members conducted individual assessments of the whole content of the remaining articles, and any discrepancies were handled by a third team member. Two team members autonomously retrieved pertinent data from each article that satisfied the inclusion criteria. Any inconsistencies encountered throughout the extraction procedure were handled by a third member. The data were methodically streamlined using an elementary classification technique, focusing on different aspects of quality of life.

3. Results

The study identified 1,276 titles and abstracts that were presumably relevant for our evaluation. After doing a thorough examination of citation titles, determined that 624 publications are not relevant to our research. The study then reviewed the summaries of the remaining 652 articles and eliminated an additional 493 articles since they were deemed irrelevant. A total of 159 articles were obtained using these approaches for a comprehensive assessment of their full-text content. Out of them, 20 satisfied the criteria for inclusion and were retained for data collection and analytical synthesis (Table 2).

PRISMA Flow Diagram.

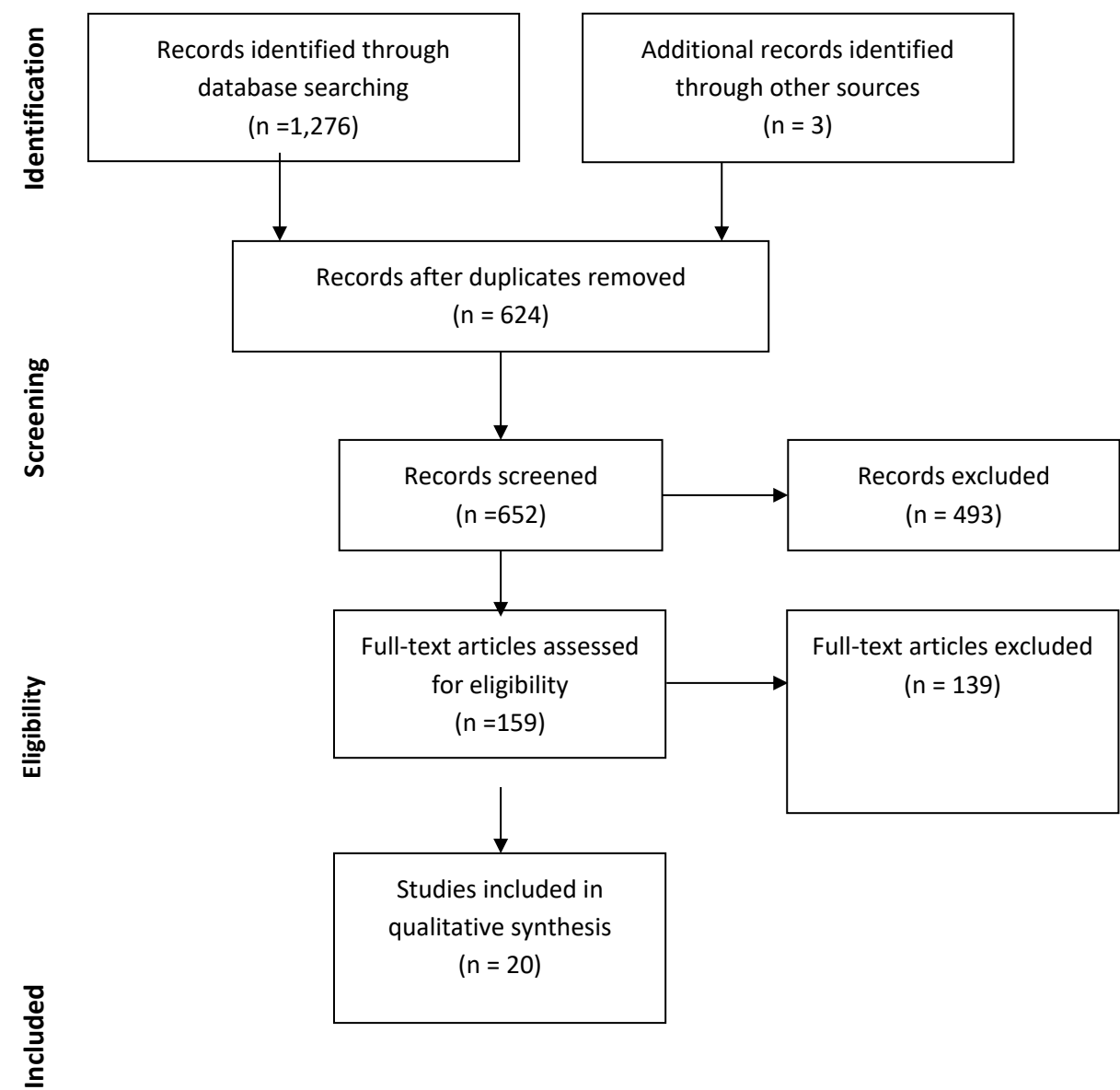


Figure 1. PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) flow diagram.

Table 2. Characteristics of studies

S/N	Author (year)	Location	Objective	Data Collection	Data Analysis
1	Doğan (2022)	Turkey	To define the relationship between urban agriculture and sustainability	Questionnaire and interview	Factor and content analysis
2	Uko (2021)	Nigeria	To assess the significance of urban and peri-urban farming.	Questionnaire administration	Descriptive and Inferential analysis
3	Mpigi (2020)	Nigeria	To assess how poor urban households in engage with urban agricultural production	In-depth Interview	Thematic analysis
4	Mupeta et al. (2020)	Zambia	To determine the impact of urban agriculture on household income	food consumption/ expenditure survey	Propensity score estimates
5	Cabannes & Raposo (2013)	Portugal	To determine what extent can urban and peri-urban agriculture (UPA) contribute to the social inclusion of migrant	Secondary data	Comparative analysis
6	Cadzow & Binns (2016)	Sierra Leone	To evaluate group associations among vegetable farmers in Freetown	Focus groups/ interviews	Content analysis
7	Barbieri et al. (2019)	USA	To assess perceived benefits of agricultural lands offering agritourism	Survey administration	Descriptive and Inferential analysis
8	Chah et al. (2010)	Nigeria	To evaluate the contribution of urban crop agriculture	Questionnaire/interview schedule	Descriptive and Inferential analysis
9	Gaglio et al., (2017)	Italy	To analyze the Land Use/Land Cover (LULC) changes and associated Ecosystem Services (ESs) of a protected wetland area	Spatial data	Transition matrices
10	Okanlade (2017)	Nigeria	To evaluate the impact of urban farming on household	Secondary data	Content analysis
11	Ackerman et al. (2014)	USA	To assess the opportunities and challenges associated with the development of urban agriculture	Survey administration	Inferential analysis
12	Bolang & Osumanu (2019)	Ghana	To assesses the participation of formal sector employees in urban agriculture and their contributions to food supply	Survey administration	Descriptive and Inferential analysis
13	Salau & Attah (2012)	Nigeria	To assess the contribution of UA to the socio-economic development of urban farmers.	Structured interview	Descriptive analysis
14	Tasciotti & Wagner (2015)	Tanzania	To evaluate the effects of practicing agriculture in urban Tanzania on indicators of dietary diversity	Panel Survey	Descriptive analysis
15	Mancebo (2018)	France	To examine how urban design and planning can promote this solution to reconfigure more sustainable and resilient cities	Secondary data	Content analysis
16	Okafor & Mgbenwelu (2023)	Nigeria	To assess the impacts of urban agriculture on the environment	Field survey	Descriptive and Inferential analysis
17	Ackerson & Awuah (2010)	Ghana	To identify health problems related to urban agricultural farming	Structured questionnaires	Descriptive and Inferential analysis
18	Karimi & Ataei (2023)	Iran	To study farmers' behavior in using urban wastewater considering perceived social risk	Survey technique	Inferential analysis
19	Barbillon et al. (2019)	France	To study health risks assessment in urban farms	Field survey	Descriptive and Inferential analysis
20	Aubry & Manouchehri (2019)	Madagascar	To assess the risks of urban agriculture and health	Field survey	Descriptive and Inferential analysis

3.1. Economic effects of urban agriculture

Urban agriculture serves as a stimulus for economic growth, taking several forms. Doğan (2022) demonstrates that the practice has the capacity to strengthen local enterprises by providing options for generating wealth and creating jobs. According to Uko (2021), the act of growing and selling agricultural products provides people and communities with a concrete way to generate funds, therefore empowering them. The economic advantages of urban agriculture are achieved via two main techniques. Initially, the trade of agricultural goods serves as a direct source of income for local economies. Moreover, when households consume food that they have produced on their own plots, it results in cost savings that would otherwise be spent on buying such goods (Mpigi, 2020). The FAO (2012) highlights considerable cost reductions for urban dwellers in the southern area, notably for disadvantaged families that spend a substantial amount of their income, ranging from 60% to 80%, on food expenses.

Urban agriculture, situated inside particular urban areas and adapted to their economic dynamics, plays a crucial role in promoting the local economy by supplying vital food supplies for the city. Mupeta et al. (2020) provide evidence supporting this claim by showing a significant and favourable impact on the financial earnings of households. Urban farm households saw a significant rise in income, ranging from 13.7% to 19.1%. This highlights the potential of urban agriculture to benefit family livelihoods by improving revenue sources.

The revenue generated by the sale of agricultural products, livestock and other goods is allocated to different sectors of the urban economy, such as education, taxes, transportation,

food, apparel, and rents. Urban agriculture, in its many manifestations, serves as a dynamic catalyst for urban economic growth, dependent on the cities' ability to cultivate. The examined research highlights the economic impacts of urban agriculture, emphasising its function as a versatile catalyst for economic development. The studies provided demonstrate the many benefits of urban agriculture, including the generation of revenue, savings, local economic growth, and enhanced family livelihoods.

3.2. Social effects of urban agriculture

Urban agriculture is a potent catalyst for increasing community involvement and promoting interpersonal connections, building a collective feeling of responsibility and obligation within a community. According to Doğan (2022), urban agriculture serves as a cohesive factor, uniting people based on a shared sense of responsibility and promoting a strong feeling of community. The involvement of socioeconomically disadvantaged urban families, including vulnerable populations such as those impacted by HIV/AIDS, those with impairments, widows or female-headed households, unemployed adults, and migrants, is especially vital (Mpigi, 2020). Urban agriculture is considered a viable approach to reducing poverty and fostering social inclusion, particularly for marginalised populations (Cabannes & Raposo, 2013).

Kirby et al. (2021) contends that the major aim of urban agriculture is to promote the inclusion of people into the urban system, guaranteeing sustainable means of living and reducing social problems such as drug misuse and illicit behaviours. The research examined confirms that urban agriculture is vital in

resolving social concerns by offering chances for marginalised populations to actively engage in productive endeavours. Research findings indicate that the adoption of urban agriculture promotes social unity by encouraging joint efforts towards common objectives, such as improving availability of financial services. Urban agricultural members often establish cooperatives and groups as a means of doing this (Cadzow & Binns, 2016). Prominent instances include the formation of the Cassava Grower Association, Poultry Farmers Association, Cocoa Farmers Association, and related alliances. These organisations not only strengthen social connections but also contribute to the financial prosperity of its members.

Urban agriculture is more than just a horticultural profession. It is a collaborative effort that uses public areas to create relationships between people from different backgrounds. Urban agriculture fosters collaboration, encouraging joint efforts to achieve personal and communal goals, so making a substantial contribution to social cohesion and community development. These results are consistent with the viewpoints expressed in the literature, highlighting the significant influence of urban agriculture on the social environment in cities.

Nevertheless, despite its numerous positive effects, urban agriculture also presents certain social risks that need to be addressed. Karimi & Ataei (2023) for example conducted a study on the social risks linked to the utilization of urban wastewater in farm irrigation. Their research shed light on various potential resource exploitation and challenges arising from this practice, emphasizing the importance of adopting proper behavior to address these concerns effectively.

3.3. Health effects of urban agriculture

Urban agriculture has health implications that extend beyond production and are closely connected to food security, nutritional diversity, and general well-being. Urban agriculture is crucial for enhancing food security and promoting dietary diversity among urban dwellers (Tasciotti & Wagner, 2015). Engaging in the practice of growing and using food that is obtained from nearby sources leads to better nutritional status, improved health results, and a reduced probability of diet-related illnesses. This simultaneous effect on both food and nutrition security has a direct impact on the overall welfare of urban households.

Participating in physical activities related to urban agriculture, such as planting and cultivating, fosters a physically demanding lifestyle and enhances overall health and wellness (Okanlade, 2017). Existing research highlights the direct link between active engagement in urban agriculture and enhanced physical well-being, emphasising the significance of practical involvement in promoting a healthy way of life. Urban agriculture has substantial environmental benefits that enhance the total welfare of urban dwellers, in addition to individual health improvements (Papanek et al., 2023). Agricultural operations that generate urban green spaces are essential for generating cooler microclimates in hot urban areas, hence reducing the impact of the urban heat island. Moreover, these areas have a role in the absorption and storage of carbon dioxide, while also offering chances for leisure and recreational pursuits, therefore improving the overall urban living experience.

The practice of growing fresh, locally obtained food not only tackle concerns about the availability of food and the variety of nutrients it

provides, but also promotes physically active and involved lives. Furthermore, the establishment of urban green spaces via agricultural endeavours enhances the overall health of urban areas, hence benefiting the well-being of inhabitants. These results are consistent with academic viewpoints that highlight the crucial role of urban agriculture in influencing health outcomes and improving the overall quality of urban living.

While urban agriculture presents numerous health benefits, it is important to recognize and address the associated health risks. In their study, Ackerson & Awuah (2010) highlighted the negative health effects associated with urban agriculture, particularly concerning water sources and various health complaints reported by respondents. The study identified a range of health issues prevalent among individuals engaged in urban agriculture. Apart from diseases like schistosomiasis and cholera, respondents reported experiencing bacterial infections, nematode infections, malaria, headaches, dermatological problems, visual impairments, cardiac issues, and respiratory problems. Among these health complaints, headaches were the most frequently reported. Barbillon et al. (2019) also noted that the ingestion of soil among urban farmers represents a significant health risk, serving as a source of exposure to contaminants. This is particularly concerning as urban soils may contain various pollutants such as heavy metals, pesticides, and other harmful substances. These findings underscore the urgent need to address the health risks associated with urban agriculture practices.

3.4. Environmental effects of urban agriculture

Urban agriculture has a crucial role in boosting the wellness of ecosystems, improving the capacity of cities to withstand challenges, and supporting

environmental sustainability. Urban agriculture is essential for fostering environmental equity and raising knowledge about health among people of all ages, including younger generations. Domestic food production greatly lowers the distance that food has to travel, therefore mitigating greenhouse emissions and tackling air pollution in metropolitan regions (Ackerman et al., 2014; Gutberlet, 2016). These endeavours foster a more environmentally friendly and ecologically aware urban way of living.

Mancebo (2018) observed that the adoption of urban agriculture initiatives effectively reduces the urban heat island effect by about 4 - 5 degrees Celsius when compared to other agricultural techniques. In addition, the implementation of agroforestry or community gardening along urban highways significantly decreases noise pollution levels. Urban agriculture not only improves environmental quality but also has a favourable influence on the well-being of urban dwellers. Urban agriculture functions as a storage place for trees and fruits, reducing the buildup of dust and offering a sustainable supply of fuelwood to fulfil customer demands (Bolang & Osumanu, 2019). This dual functioning serves the purpose of promoting ecological purity and ensuring the availability of crucial resources, in accordance with the ideals of environmental justice and sustainability.

Urban agriculture may increase the resilience of cities by adding more green space. Urban agriculture not only provides fresh food but also significantly improves the environmental well-being of urban regions. Urban agriculture is a versatile approach for tackling environmental concerns, such as decreasing carbon emissions, managing urban heat islands, and promoting the creation of green areas. These results align with

academic viewpoints that highlight the crucial role of urban agriculture in fostering environmental sustainability and resilience in urban ecosystems. However, many studies underscored the significant negative consequences of urban agriculture on the environment. For instance, Okafor & Mgbenwelu (2023) highlighted how urban agriculture has profoundly influenced the ecology, pointing to various environmental challenges that must be addressed to ensure the long-term viability of the practice. The research findings shed light on the detrimental effects of urban agriculture on the ecosystem, indicating potential risks to biodiversity, soil health, water quality, and air purity. Aubry & Manouchehri (2019) further raised concerns on contamination through the use of chemical fertilizers, pesticides, and herbicides in urban agriculture practices which can contaminate water sources through runoff. These chemicals may leach into nearby water bodies, leading to water pollution and adverse effects on aquatic ecosystems and human health. These negative consequences underscore the importance of adopting sustainable practices to minimize environmental degradation and safeguard the city's ecological balance.

3.5. Cultural effects of urban agriculture

Urban agriculture goes beyond its practical function and serves as a potent means of cultivating links between people and their ancestral heritage and traditions associated with farming and food cultivation. Salau & Attah (2012) highlight the role of urban agriculture in reviving historical agricultural methods and safeguarding cultural heritage by assuring the continuation of traditional knowledge and competence. Cultivating and transmitting agricultural techniques serves as a concrete means of preserving a connection to cultural heritage and ancestral origins.

According to Chah et al. (2010), urban agriculture functions as a means to augment knowledge and comprehension of the food chain. This heightened awareness fosters a deep feeling of respect for the natural world and its ecology. Through active engagement in the growing process, people get a more profound understanding of the many interconnections within the food system. Culture has a substantial impact on how people think, affecting their understanding of society responsibilities, influencing their actions, and offering valuable knowledge for future generations. Urban agriculture serves as a cultural focal point, shaping people's perception and interaction with their surroundings, strengthening their commitment to sustainable practices.

Urban agriculture has the potential to evolve into an enjoyable and educational attraction for visitors, promoting agriculturalism and nurturing innovative pursuits (Barbieri et al., 2019). Gaglio et al. (2016) emphasise the significance of farms and agricultural tourism in promoting agricultural awareness. These areas serve as centres for the sharing of information, education, and the reduction of food waste via community gardening techniques. Urban agriculture not only produces agricultural goods, but also serves as a tangible representation of cultural identity and expertise. Urban agriculture has a significant role in building cultural awareness by maintaining cultural history, promoting understanding, and establishing links with the larger community. These results support academic viewpoints that highlight the diverse cultural effects of urban agriculture and its contribution to supporting sustainable cultural practices in urban areas.

4. Discussion

This research utilises established systematic review methodologies and consolidates data from 20 relevant studies to provide a thorough investigation of the evidence on Urban agriculture and its influence on quality of life. Although data quality difficulties are often encountered, our research, consistent with other studies, revealed some crucial findings.

Urban agriculture has a significant economic effect. Studies highlight the significant capacity of urban agriculture to strengthen local economies, produce revenue, and provide employment prospects. Gripper (2023) emphasises the economic benefits, including the cost reductions that families may get by cultivating their own food. This has an especially large effect on families who are economically challenged, since a substantial percentage of their income is dedicated to spending on food. Furthermore, the research conducted by Mupeta et al. (2020) demonstrates a noteworthy and favourable impact on household income, which has the potential to enhance people's standard of living and provide a valuable contribution to the economic development of urban areas.

Urban agriculture is a potent means of promoting community development and nurturing interpersonal connections. According to Doğan (2022), the communal component is highlighted as he asserts that engaging in the joint activity of planting and producing food fosters a feeling of community. The societal advantages include disadvantaged populations, as emphasised by Mpigi (2020), where urban agriculture functions as a strategy for reducing poverty and fostering social cohesion. The establishment of cooperatives and groups, as shown by Cadzow & Binns (2016), enhances

social cohesiveness, demonstrating that urban agriculture is not just focused on individual farming but rather a collaborative endeavour with shared objectives.

Urban agriculture offers a wide range of health advantages. The presence of fresh, locally obtained food enhances nutritional status and promotes overall health (Papanek et al., 2023). Participating in physical activities associated with urban agriculture, such as planting and cultivating, encourages a lifestyle that involves regular physical exercise (Okanlade, 2017). In addition, the environmental benefits of urban agriculture, such as the creation of green areas, lead to a more sustainable and healthier urban environment (Papanek et al., 2023).

The favourable environmental impacts of urban agriculture are well acknowledged. Gutberlet (2016) states that reducing the lengths of food transportation helps to decrease carbon emissions. Integrating urban agriculture projects may help alleviate the negative impacts of urban heat islands, decrease noise pollution, and serve as a space for growing trees and fruits, resulting in several environmental advantages (Pearson, 2011). Furthermore, urban agriculture is seen as a noteworthy factor in enhancing urban resilience, by fostering sustainability and boosting environmental well-being (FAO, 2013).

Urban agriculture is seen as a way to preserve cultural traditions and promote comprehension of the food chain. Salau & Attah (2012) observe the resurgence of age-old agricultural methods, which play a role in safeguarding cultural heritage. Gaglio et al. (2016) emphasise the importance of urban agriculture in teaching young people and promoting agricultural awareness. They suggest that farms might be transformed into educational attractions for visitors. Urban agriculture is

closely linked to the preservation of culture and the dissemination of knowledge.

Urban agriculture, while offering numerous benefits, also brings about various externalities that extend beyond the immediate participants. These external impacts encompass social, economic, environmental, and policy dimensions, influencing various stakeholders and aspects of urban life.

Socially, urban agriculture can enhance community cohesion and interaction, fostering a sense of belonging and solidarity among residents. However, it may also lead to resource exploitation, tensions and conflicts, particularly if farming activities result in noise, odor, or pest-related issues that affect neighboring communities. Disparities in access to urban farming resources and spaces can further exacerbate existing social inequalities, raising questions about equity and inclusion in urban agriculture initiatives. Economically, urban agriculture can provide employment opportunities, generate income for farmers, and contribute to local food security and affordability. Yet, it may also have unintended consequences, such as driving up property prices and contributing to gentrification in certain neighborhoods. Competition for land and resources, coupled with market dynamics and policy interventions, can influence the economic viability and distributional outcomes of urban agriculture.

From an environmental perspective, urban agriculture holds the potential to enhance biodiversity, improve soil health, and mitigate climate change through carbon sequestration. However, unsustainable farming practices, such as the use of chemical inputs and land degradation, can lead to soil erosion, water pollution, and habitat loss. These environmental

impacts pose risks to human health, aquatic ecosystems, and urban resilience, underscoring the importance of adopting sustainable farming methods and land management practices.

From a health perspective, urban agriculture can offer opportunities for increased physical activity and improved access to fresh produce, contributing to overall well-being. However, there are also health risks associated with urban farming practices, such as exposure to contaminants in soil and water, as well as the use of chemical fertilizers and pesticides. Poor sanitation and hygiene practices in urban farming areas can further exacerbate these risks, potentially leading to the spread of infectious diseases and other health concerns among farmers and nearby residents.

5. Conclusions

This research aims to elucidate the complex interaction between urban agriculture and quality of life in urban environments. The consolidation of results from the relevant research, using established systematic review protocols, has yielded vital insights into this dynamic correlation. Although we recognise the widespread problem of data quality difficulties, our research reveals important discoveries that highlight the potential beneficial impacts of urban agriculture on several elements of quality of life. The studied research consistently highlights economic empowerment, social cohesiveness, better health outcomes, environmental sustainability, and cultural preservation as key recurring themes.

Contributions to theoretical understanding

Our study enhances the theoretical discussion on urban agriculture and quality of

life by providing a detailed viewpoint on the many effects of urban agriculture. The extensive literature analysis sheds light on the economic, social, health, environmental, and cultural aspects of urban agriculture, offering a thorough knowledge of how it affects the well-being of city dwellers.

Limitations

Recognising the constraints of this research is of utmost importance. The presence of data quality issues in the literature under evaluation is a difficulty, and differences in research methodology used in various studies may impact the extent to which the results can be applied to a broader context. Moreover, variations in the surrounding circumstances within metropolitan environments might affect the applicability of findings.

Policy implications

Policymakers should establish clear regulations to ensure urban agriculture practices meet safety and environmental standards. Urban planners need to integrate farming into city planning and designate suitable areas for agriculture to optimize land use and protect ecosystems. Governments should support small-scale urban farmers with training, financial assistance, and marketing support to enhance food security and livelihoods. Encouraging community engagement and partnerships can strengthen social cohesion and ensure projects meet local needs.

Managerial implications

Urban farmers should prioritize sustainable practices and risk management strategies to

protect health and minimize environmental impact. Collaboration with stakeholders can foster innovation and improve productivity. Diversifying marketing channels can expand market opportunities and increase sales. Continuous monitoring and adaptation of farming practices are essential for efficiency and competitiveness.

Scientific implications

Interdisciplinary research is needed to understand urban agriculture's impacts and develop innovative solutions. Data collection and knowledge sharing can inform evidence-based decision-making. Monitoring and evaluation frameworks are necessary to assess interventions' effectiveness. Policy advocacy efforts should promote evidence-based policies that support sustainability and public health.

Conflict of interest

The authors assert that there are no conflicts of interest that might have impacted the design, execution, or interpretation of the study provided in this publication.

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Gelation of Pangasius (*Pangasianodon hypophthalmus*) minced muscle by high pressure processing: The effects on selected qualities

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ARTICLE INFO	ABSTRACT
Research Paper Received: March 27, 2025 Revised: May 05, 2025 Accepted: May 09, 2025 Keywords FTIR Gelation High pressure processing (HPP) Pangasius Scanning electron microscopy (SEM) *Corresponding author Truong Quang Binh Email: tqbinh@hcmuaf.edu.vn	This study evaluated the changes of <i>Pangasius hypophthalmus</i> protein under high-pressure processing (HPP), focusing on key parameters such as color, gel structure, water-holding capacity (WHC), protein solubility, and mechanical properties to determine optimal conditions for improving product quality. Pangasius muscle were minced and subjected to pressures ranging from 100 to 600 MPa, at temperatures between 0 - 30°C, and for 0 - 15 min. The processed samples were then analyzed for color, gel texture, gel strength, WHC, protein solubility, scanning electron microscopy (SEM), fourier transform infrared spectroscopy (FTIR) and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The results showed that treatment at 400 - 450 MPa enhanced the whiteness of Pangasius gels, improved microstructural texture, and increased water-holding capacity (WHC). In contrast, processing at 500 MPa at 30°C for 12.5 min reduced structural stability, led to the formation of porous networks, and decreased protein solubility. The gel's mechanical properties were optimal at 400 - 450 MPa but reduced the hardness at 500 MPa. Overall, the conditions of 400 - 450 MPa, 20 - 30°C, and 10 - 15 min were identified as optimal for enhancing Pangasius gel quality.

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1. Introduction

Pangasius (*Pangasianodon hypophthalmus*) is a species of catfish belonging to the family Pangasiidae, primarily found in the Mekong River basin, including countries such as Laos, Vietnam, Cambodia, and Thailand. Pangasius is widely farmed in Vietnam, particularly in the Mekong Delta region, and in recent years has become the country's leading freshwater fish for export, playing a significant role in the Vietnamese aquaculture industry.

However, the value added products from Pangasius, especially Pangasius gel, still encounter various challenges in maintaining high quality, extending shelf life, and improving sensory attributes. Common issues associated with Pangasius gel products include water loss, reduced gel strength, protein structure alterations, and color changes during storage. These factors not only affect the sensory value but also reduce the market competitiveness of the products. In this context, high pressure processing (HPP) has emerged as a promising solution to enhance the quality of Pangasius gel without compromising its nutritional and sensory properties (Truong et al., 2020). The HPP is a non-thermal food preservation technique that applies high pressure (typically between 100 - 600 MPa) to alter protein structures, thereby improving gel strength, water-holding capacity, and retaining the natural color of the product.

Nevertheless, the determination of the optimal processing conditions-such as pressure level, temperature, and holding time-remains a major challenge. Previous studies have shown that excessive pressurization may lead to undesirable protein denaturation, adversely affecting gel structure and product quality. Therefore, it is essential for researchers to identify suitable HPP

conditions to improve the quality of Pangasius gel.

This study aimed to evaluate the potential of applying HPP to Pangasius gel and to investigate the effects of different HPP conditions on its physicochemical properties. Treatments were conducted at pressure levels ranging from 400 to 500 MPa, temperatures between 20 - 30°C, and holding times of 10 - 15 min, in order to determine the most effective treatment combination for producing high quality Pangasius gel using HPP.

2. Materials and Methods

2.1. Materials

The study was conducted from November 2023 to December 2024 at the laboratories of the Faculty of Fisheries and the Faculty of Food Technology, Nong Lam University (Linh Trung Ward, Thu Duc City, Ho Chi Minh city), as well as the laboratory of the Faculty of Agro-Industry, Chiang Mai University, Thailand.

Pangasius fish (*Pangasianodon hypophthalmus*) weighing between 2 - 3 kg were purchased from local farms in Chiangmai, Thailand and filleted at the laboratory of the Faculty of Agro-Industry, Chiang Mai University, Thailand.

2.2. Experimental Design

Pangasius fillets were cut into small pieces, frozen, and subsequently minced with 2% salt for 2 min. The minced fish was stuffed into collagen sausage casings with a diameter of 24 mm and vacuum-packed. Gelation was carried out using a HPP system (Baotou Kefa High Pressure Technology Co., Ltd., China) at pressure levels ranging from 400 to 500 MPa, with treatment durations of 10 - 15 min at temperatures between 20 & 30°C.

Following HPP, the *Pangasius* gels were heated at 90°C for 30 min then cooling in ice water (4°C). The quality of the final product was assessed immediately after cooling. The treatment conditions were as follows:

NT1: HPP at 400 MPa/20°C in 12.5 min

NT2: HPP at 400 MPa/25°C in 10 min

NT3: HPP at 400 MPa/25°C in 15 min

NT4: HPP at 400 MPa/30°C in 12.5 min

NT5: HPP at 450 MPa/20°C in 10 min

NT6: HPP at 450 MPa/20°C in 15 min

NT7: HPP at 450 MPa/25°C in 12.5 min

NT8: HPP at 450 MPa/30°C in 10 min

NT9: HPP at 450 MPa/30°C in 15 min

NT10: HPP at 500 MPa/20°C in 12.5 min

NT11: HPP at 500 MPa/25°C in 10 min

NT12: HPP at 500 MPa/25°C in 15 min

NT13: HPP at 500 MPa/30°C in 12.5 min

The quality of *Pangasius* gels processed using high-pressure processing (HPP) was compared to that of control products, which were produced using conventional heat treatment (90°C for 30 min).

The following parameters were evaluated: color, mechanical properties (gel strength, hardness and springiness), water holding capacity (WHC), protein solubility, Fourier-transform infrared (FTIR) spectroscopy, sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and scanning electron microscopy (SEM).

2.3. Analytical Methods

2.3.1. Color measurement

The color of *Pangasius* gels were measured by a Konica Minolta chromometer (CR-400, Tokyo, Japan). Color values including L^* (lightness), a^* (red-green), and b^* (yellow-blue) were used to calculate whiteness by the following formulas:

$$\text{Whiteness} = 100 - [(100 - L)^2 + a^2 + b^2]^{1/2}$$

2.3.2. Mechanical Properties

Pangasius gel samples with the size of 2.4 cm × 3 cm were removed from casings and tempered at 25°C for 2 h prior to gel strength analysis. A texture analyzer (TA-XT plus, Stable Micro Systems, UK) with cylindrical plunger (40 mm in diameter) and 200 N load cell was used to investigate gel strength at a speed of 2 mm/min. Gel strength was calculated as breaking force × breaking deformation. Similarly, hardness and springiness of *Pangasius* gel samples (2.4 cm × 3 cm) were measured using the same texture analyzer with cylindrical plunger (40 mm in diameter) and 200 N load cell at a compression rate of 2 mm/min and compressing samples to 50% of fish gels height.

2.3.3. Protein solubility

Protein solubility was measured following the method of Truong et al. (2020) with minor modification. *Pangasius* gel samples (4 g) were homogenized with 40 mL of a 30 g/L NaCl solution for 1 min, followed by centrifugation at 1500 × g for 15 min at 5°C. A 1 mL aliquot of the supernatant was mixed with 10 mL of 30 g/L NaCl solution, and protein concentration was determined spectrophotometrically using the Lowry method at 550 nm with bovine serum albumin as the standard for quantifying protein.

2.3.4. Water-holding capacity

Pangasius gels (about 2 g) was wrapped around by filter papers then centrifuged at $9000 \times g$ at 20°C for 20 min (Universal 320R centrifuge, Hettich, USA). The WHC was calculated as the percentage of water retained relative to the initial water content (Uresti et al., 2005).

2.3.5. Fourier Transform Infrared Spectroscopy (FTIR)

The FTIR was conducted with an FT-IR 4700 spectrometer (JASCO, Tokyo, Japan) equipped with an ATR prism crystal accessory. Pangasius gels (approximately 1 mg) was placed on the ATR crystal surface at room temperature and applying pressure with a flat tip plunger to obtain clear spectral peaks. Infrared spectra were measured within the range of 4000 to 650 cm^{-1} and all measurements were performed in triplicate. The spectral resolution was maintained at 4 cm^{-1} . To improve spectral resolution, background subtraction and second derivative analysis was applied using OriginPro 2024 software (Ojagh et al., 2011).

2.3.6. SDS-Polyacrylamide Gel Electrophoresis (SDS-PAGE)

+ Protein extraction

Total SDS-soluble and sarcoplasmic protein fractions were extracted following the method of Pazos et al. (2015). For the total SDS-soluble proteins, 0.5 g of Pangasius gels was mixed with eight volumes of Tris buffer (10 mM Tris-HCl, pH 7.2) containing 2% SDS as a denaturing agent and 5 mM PMSF as a protease inhibitor and homogenized thoroughly. The homogenate was boiled, then homogenized again using an Ultra-Turrax device, and centrifuged at $40,000 \times g$ for 12 min at 4°C . The collected supernatant was designated as the total SDS-soluble protein fraction.

+ SDS-Polyacrylamide gel electrophoresis

SDS-PAGE was done with 1-D laboratory-prepared 10% (v/v) polyacrylamide gels with an acrylamide:N,N'-ethylene bis-acrylamide ratio of 200:1. The stacking gel (4% polyacrylamide) was loaded with $30\text{ }\mu\text{g}$ of protein for each well, and run at 60V for 30 min, followed by the separating gel at 100V for 90 min in a Mini-PROTEAN 3 cell system (Bio-Rad, Hercules, CA, Singapore) (Laemmli, 1970). The running buffer consisted of 1.44% (w/v) glycine, 0.67% Tris-base, and 0.1% SDS. The gels were stained overnight with Coomassie PhastGel Blue R-350 dye (GE Healthcare, Uppsala, Sweden).

2.3.7. Scanning Electron Microscopy (SEM)

The microstructure of Pangasius gels was observed following the method of Iwasaki et al. (2005) with minor modifications. The centre of gel samples was cut into square blocks (1 mm in thickness $\times 2\text{ cm}^2$ in area) then fixed in 2.5% glutaraldehyde and dehydrated by ethanol with increased concentration (50%, 70%, 90%, and 100%). The dehydrated samples were immersed in 2-methyl-2-propanol to replace ethanol and freeze-dried. Samples were sputter coated in vacuum with platinum-palladium. The microstructure of Pangasius gels was observed by a Hitachi TM4000 plus SEM (Tokyo, Japan) at 10 kV.

2.3.8. Statistical Analysis

All analytical measurements were performed in four replications, and results were expressed as mean $\pm$ standard deviation. Data were analyzed by one-way ANOVA followed by LSD post-hoc tests to assess differences among treatments. Statistical significance was set at $P < 0.05$ (SPSS version 21.0, IBM, USA).

3. Results and Discussion

3.1. Color characteristics of Pangasius gel under different high-pressure processing conditions

Figure 1 shows the color characteristics of Pangasius gel under different treatment conditions. The gels subjected to high-pressure processing (HPP) exhibited whiteness values ranging from 62.72 to 64.12, which were significantly higher than those of the heat treated gel (60.52 ± 0.10) ($P < 0.05$) (data not shown). These results are consistent with the findings of Cando et al. (2015), who observed an increase in lightness in Alaska Pollock gels as pressure levels increased. HPP may induce

greater protein denaturation, contributing to a whiter appearance of the gel. Additionally, high pressure may displace or disrupt the heme and cause globin denaturation, leading to increased whiteness in the gel (de Oliveira et al., 2017). Changes in color are also associated with protein aggregation and cross linking, which result in more compact structures capable of reflecting more light (Uresti et al., 2004). Other studies also have similar results indicated that HPP induced protein aggregation enhances gel whiteness. This observation aligns with previous reports showing that pressures above 200 MPa significantly alter the color of white fish muscle (Buckow & Bull, 2013).



Figure 1. Visual color of Pangasius gel under different high pressure processing conditions.

3.2. Mechanical properties

The results of the mechanical properties of Pangasius gel are presented in Table 1. The hardness of the gel samples ranged from 6.53 N to 10.74 N. High-pressure processing (HPP) significantly increased the hardness of all Pangasius gel treatments compared to

the control group that did not undergo pressure treatment ($P < 0.05$). These results are consistent with previous studies on barramundi (Truong et al., 2017), tilapia (Hwang et al., 2007), and *Nemipterus virgatus* (Ma et al., 2015), which also reported enhanced gel hardness with HPP treatment.

Among the HPP treated samples, the treatments at 400 MPa/20°C in 12.5 min and 450 MPa/20°C in 10 min exhibited the highest hardness values (10.73 N and 10.74 N, respectively), which were significantly higher ($P < 0.05$) than those of treatments at 450 MPa/25°C in 12.5 min, 450 MPa/30°C in 10 min, 450 MPa/30°C in 15 min, 500 MPa/20°C in 12.5 min, 500 MPa/25°C in 10 min, 500 MPa/25°C in 15 min, and 500 MPa/30°C in 12.5 min.

The difference in hardness values among these HPP treatments could be attributed to the interaction between pressure and temperature

conditions. Treatments with moderate pressure and temperature likely promoted optimal protein aggregation and gel network formation, whereas excessively high pressure or temperature may disrupt gel structure, resulting in reduced gel quality and lower hardness values.

In terms of springiness, there were no statistically significant differences ($P \geq 0.05$) between HPP-treated and non-HPP-treated gels. This finding is consistent with the report by Truong et al. (2017), indicating that HPP may have limited influence on the elasticity of Pangasius gels

Table 1. Mechanical properties of Pangasius gel treated under different high pressure processing conditions

Treatment	Hardness (N)	Springiness (mm)	Gel strength (N.mm)
Control	7.21 ^a ± 0.18	0.89 ^a ± 0.01	360.62 ^a ± 18.95
400 MPa/20° in 12.5 min	10.73 ^b ± 1.27	0.86 ^a ± 0.02	514.88 ^{bc} ± 117.71
400 MPa /25° in 10 min	10.62 ^{bc} ± 1.23	0.89 ^a ± 0.08	495.08 ^{bcd} ± 106.14
400 MPa /25° in 15 min	10.29 ^{bcdh} ± 0.62	0.89 ^a ± 0.01	479.37 ^{bcd} ± 55.38
400 MPa /30° in 12.5 min	9.62 ^{cdh} ± 0.33	0.86 ^a ± 0.02	461.32 ^{bcd} ± 5.89
450 MPa /20° in 10 min	10.74 ^b ± 0.18	0.88 ^a ± 0.01	512.99 ^{bc} ± 77.98
450 MPa /20° in 15 min	10.37 ^{bch} ± 0.67	0.87 ^a ± 0.02	504.88 ^{bc} ± 24.19
450 MPa /25° in 12.5 min	9.38 ^{deh} ± 0.24	0.88 ^a ± 0.02	484.11 ^{bcd} ± 53.24
450 MPa /30° in 10 min	8.99 ^{efi} ± 0.36	0.88 ^a ± 0.02	477.47 ^{bcd} ± 35.82
450 MPa /30° in 15 min	8.51 ^{eg} ± 0.36	0.87 ^a ± 0.02	491.62 ^{bcd} ± 77.86
500 MPa /20° in 12.5 min	9.31 ^{ce} ± 1.40	0.86 ^a ± 0.02	494.66 ^{bcd} ± 107.62
500 MPa /25° in 10 min	8.61 ^{efg} ± 0.29	0.87 ^a ± 0.02	427.35 ^{ac} ± 77.76
500 MPa /25° in 15 min	8.24 ^{gi} ± 0.57	0.88 ^a ± 0.03	397.85 ^{ad} ± 19.67
500 MPa /30° in 12.5 min	8.56 ^{efg} ± 0.31	0.88 ^a ± 0.02	359.20 ^a ± 66.27

Different letters (a, b, c, d) indicate significant differences ($P < 0.05$).

The gel strength of Pangasius gels ranged from 359.20 to 514.88 N.mm. High pressure processing (HPP) significantly increased the gel strength of most treatments compared to the control (heat only) samples ($P < 0.05$), except for the treatments at 500 MPa/25°C in 10 min, 500 MPa/25°C in 15 min, and 500 MPa/30°C in

12.5 min, where no significant differences were observed ($P \geq 0.05$). These results suggest that HPP can increase the gel strength of Pangasius muscle under appropriate conditions. However, the enhancement effect of HPP was diminished in treatments with excessive pressurization, temperature, and prolonged holding time.

Among the HPP treatments, gel strength values were closely aligned with hardness trends. Specifically, the treatments at 400 MPa/20°C in 12.5 min and 450 MPa/20°C in 10 min achieved the highest gel strength (514.88 N·mm and 512.99 N·mm, respectively), which were significantly greater ($P < 0.05$) than those observed in 500 MPa/30°C in 12.5 min and 500 MPa/25°C in 15 min. This indicates that higher pressure (500 MPa) combined with elevated temperatures (25 - 30°C) may reduce the gel strength of *Pangasius* gels.

Overall, the application of high pressure improved the mechanical properties of *Pangasius* gels, resulting in higher gel hardness and strength compared to conventional heat treatment. This improvement may be attributed to the differing gelation mechanisms between pressure and heat. During thermal processing, proteins denature and unfold, forming an ordered three-dimensional protein network stabilized by hydrophobic interactions, hydrogen bonds, and disulfide linkages (Colmenero, 2002). In contrast, high-pressure gelation begins with the disruption of hydrophobic interactions in the native protein structure, promoting solubilization and unfolding (Hwang et al., 2007). Disulfide bonds are also formed under pressure, and upon decompression, heat sensitive hydrogen bonds and hydrophobic interactions are established. Consequently, HPP induced gelation is stabilized by three types of intramolecular forces: disulfide bonds, thermally sensitive hydrogen bonds, and hydrophobic interactions (Hwang et al., 2007).

Moreover, in HPP induced gels, myosin filaments are primarily linked via their head regions, as opposed to the tail to tail associations observed in heat-induced gels. This head to head interaction contributes to the specific rheological characteristics of pressure induced gels (Iwasaki et al., 2005).

When pressure exceeds optimal levels (e.g., 500 MPa) and temperatures over 25°C, gel strength declines significantly. This weakening may be attributed to two factors: first, excessive pressure may break down the gel matrix; second, the intense protein denaturation can promote non specific aggregation rather than controlled cross linking, leading to gel instability. These mechanisms likely account for the weakened gel texture observed under extreme conditions (Ma et al., 2015).

3.3. Protein solubility

The protein solubility of *Pangasius* gel is presented in Figure 2, ranging from 7.63 mg to 8.69 mg, with no significant differences observed among the treatments ($P \geq 0.05$). A decrease in protein solubility is indicative of protein denaturation and the precipitation of myofibrillar proteins, particularly myosin, following denaturation (Ramírez et al., 2000). Both high-pressure and thermal treatments induced substantial protein denaturation and precipitation, which contributed to the proper formation of the *Pangasius* gel structure.

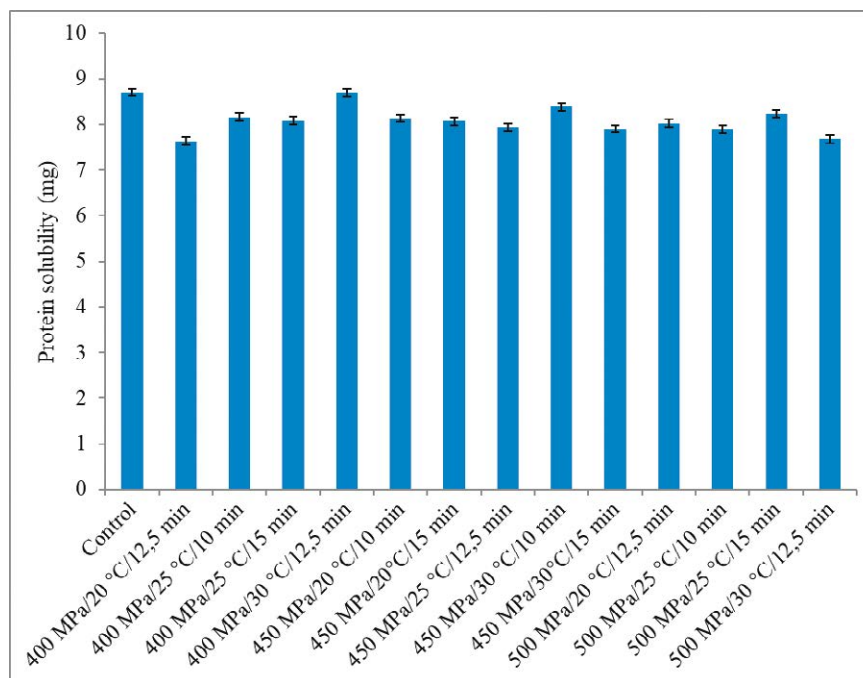


Figure 2. Protein solubility (mg) of Pangasius gel under different high pressure processing conditions. Different letters (a, b, c, d) indicate significant differences ($P < 0.05$).

3.4. Water-Holding Capacity (WHC)

The WHC of Pangasius gel is presented in Figure 3, with the highest value observed at 90.95% for the treatment at 450 MPa/20°C in 10 min, and the lowest at 75.8% in the control (heat-only) group. HPP significantly increased the WHC of all Pangasius gel treatments compared to the control ($P < 0.05$).

Treatments at 400 MPa/20°C in 12.5 min, 400 MPa/25°C in 10 min, 400 MPa/25°C in 15 min, 400 MPa/30°C in 12.5 min, 450 MPa/20°C in 10 min, and 450 MPa/20°C in 15 min showed significantly higher WHC than those at 450 MPa/30°C in 15 min, 500 MPa/20°C in 15 min, 500 MPa/25°C in 10 min, 500 MPa/25°C in 15 min, and 500 MPa/30°C in 12.5 min ($P < 0.05$).

The increase in WHC may be attributed to the ability of high pressure to promote the formation of a stable gel network, which enables the

retention of more immobilized water within the matrix and reduces water loss during cooking. Moreover, HPP enhances protein unfolding and solubility, allowing more free water to interact with protein molecules, thereby improving protein hydration (Tabilo-Munizaga & Barbosa-Cánovas, 2004).

However, when pressure exceeds 450 MPa and temperatures above 25°C, the combined effects of high pressure and elevated temperature appear to reduce WHC. This may be due to the disruption of the three-dimensional gel matrix structure. Excessive pressure can damage the protein network, resulting in a weaker gel matrix that cannot effectively retain water, allowing more free water to be released. Additionally, elevated temperature may denature proteins before the gel network is fully formed, further decreasing the gel's ability to retain moisture.

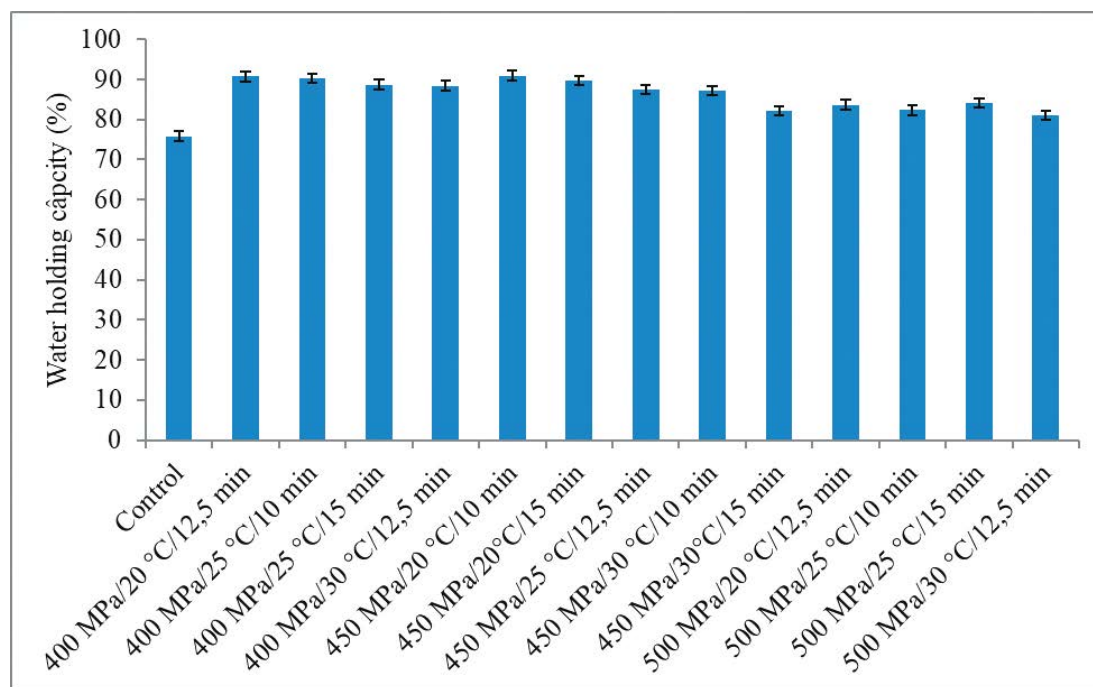


Figure 3. Water holding capacity of Pangasius gel under different high pressure processing conditions. Different letters (a, b, c, d) indicate significant differences ($P < 0.05$).

3.5. Fourier-transform infrared (FTIR) spectroscopic analysis

FTIR spectroscopy was used to obtain the vibrational spectra of each sample, providing information on the secondary structure of proteins after high-pressure treatment under different conditions. Figure 4 shows the spectra of all samples in the wavenumber range of 4000–650 cm^{-1} . In general, all spectra exhibited similar patterns across treatments, with characteristic peaks commonly observed at the following positions: 1038–1061 cm^{-1} , 1131–1146 cm^{-1} , 1207–1218 cm^{-1} , 1397–1399 cm^{-1} , 1452–1455 cm^{-1} , 1538 cm^{-1} , 1626 cm^{-1} , 1703 cm^{-1} , 2809–2836 cm^{-1} , 2901–2907 cm^{-1} , 2930–2936 cm^{-1} , and 3291 cm^{-1} . The peaks between 1038–1061 cm^{-1} are associated with C–O and C–C stretching vibrations, while the peak at 1397–1399 cm^{-1} corresponds to C–H stretching (Ye et al., 2019). The FTIR band at 1452–1455 cm^{-1} is attributed

to the bending vibration of CH_2 or CH_3 groups, originating from the side chains of proteins and/or lipids. Most notably, the amide I peak at 1626 cm^{-1} and the amide II peak at 1538 cm^{-1} are of key importance.

For myofibrillar proteins, the amide I band (1700 – 1600 cm^{-1}) is particularly significant, as it is highly sensitive to hydrogen bonding, dipole–dipole interactions, and the conformation of the polypeptide backbone (Krimm & Bandekar, 1986). Thus, the amide I band is commonly used to assess secondary structural changes in proteins. This band corresponds to the C=O stretching vibration/hydrogen bonding interactions, typically associated with carboxyl groups ($-\text{COO}^-$), while the amide II band reflects N–H bending and C–N stretching. The amide I peak near 1626 cm^{-1} is assigned to β -sheet structures, whereas the amide II peak at approximately 1538 cm^{-1} corresponds to α -helix

structures (Kobayashi et al., 2017). Additionally, the peak at $2930 - 2936 \text{ cm}^{-1}$ is related to C–H stretching, and the broad band at 3291 cm^{-1} , located between 3000 and 3500 cm^{-1} , is referred to as the amide A band, which is commonly used to assess the interaction between protein molecules and water (Ye et al., 2019).

The application of high-pressure processing caused no substantial shifts in the FTIR peak patterns compared to the control group, suggesting that no new secondary protein structures were formed under pressure. However, the variations in peak intensities indicate possible differences in the formation or disruption of specific secondary structures, which need quantitative analysis in future studies

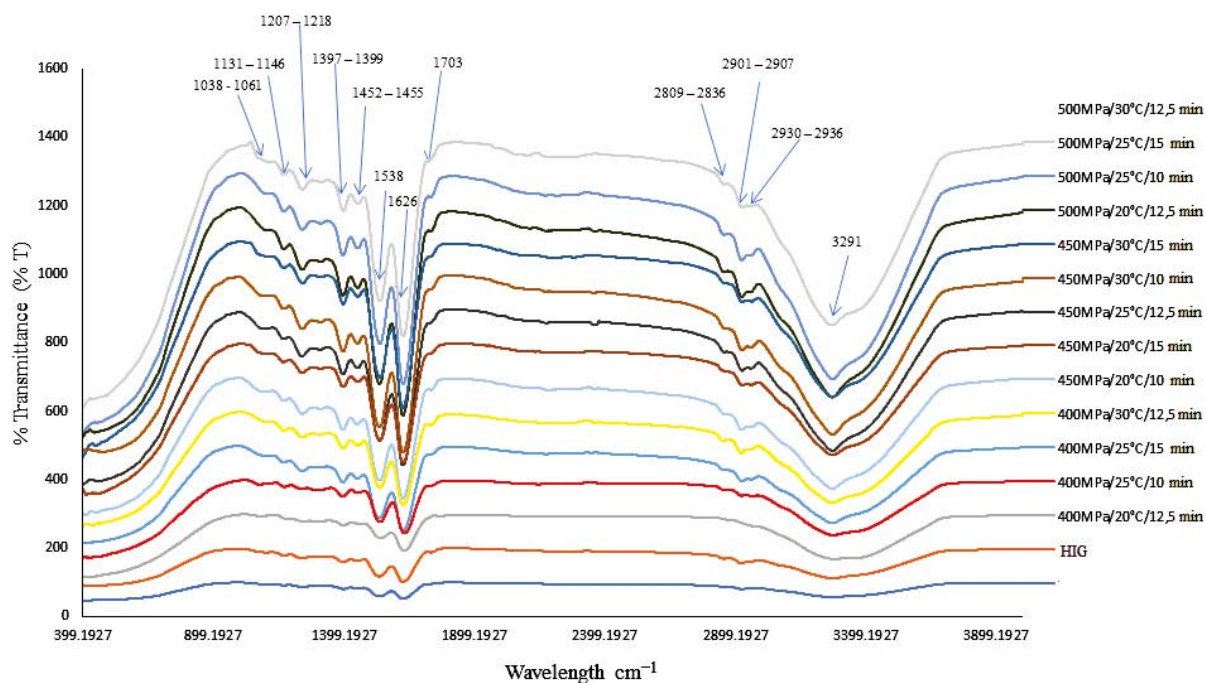


Figure 4. FTIR spectra of Pangasius gel under different high pressure processing conditions at the wavenumber range of $4000 - 650 \text{ cm}^{-1}$.

3.6. SDS-Polyacrylamide Gel Electrophoresis (SDS-PAGE)

The SDS-PAGE results of total SDS-soluble proteins extracted from Pangasius gel are shown in Figure 5. Compared to the heat-treated gel, Pangasius gels subjected to high-pressure processing (HPP) exhibited a similar protein molecular weight profile. Similarly, no major differences were observed in protein composition

among the different HPP treatments. In fish muscle, two key proteins are myosin, a high molecular weight protein ($\sim 200 - 240 \text{ kDa}$), and actin, a lower molecular weight protein ($\sim 45 \text{ kDa}$). Other bands ranging from 21.9 to 94.9 kDa are associated with various myofibrillar proteins such as tropomyosin, troponins, and sarcoplasmic proteins. Myosin is considered the principal protein responsible for gel-forming ability (Uresti et al., 2004).

Several studies have shown that high pressure often affects the intensity of the myosin band in fish gels. In particular, gels subjected to HPP followed by thermal treatment (90°C for 15 min) exhibited a stronger myosin band than those subjected to heat treatment alone, suggesting that less myosin aggregation occurred during heat-induced gelation in HPP-treated samples. Other research has indicated that surimi treated with HPP alone tends to show a gradual decrease in myosin band intensity as pressure increases, due to the formation of non-disulfide bonds under high pressure. Myosin, especially

its head region, is known to be sensitive to high pressure and may aggregate under saline conditions (Uresti et al., 2004).

These observations suggest that the mechanisms of protein unfolding and myosin aggregation differ between HPP and thermal gelation. In the present study, myosin bands in HPP-treated gels appeared at lower molecular weights compared to those in heat-treated gels, indicating that high pressure caused partial denaturation and aggregation of myosin, thereby contributing to a denser gel structure than that formed by thermal treatment alone.

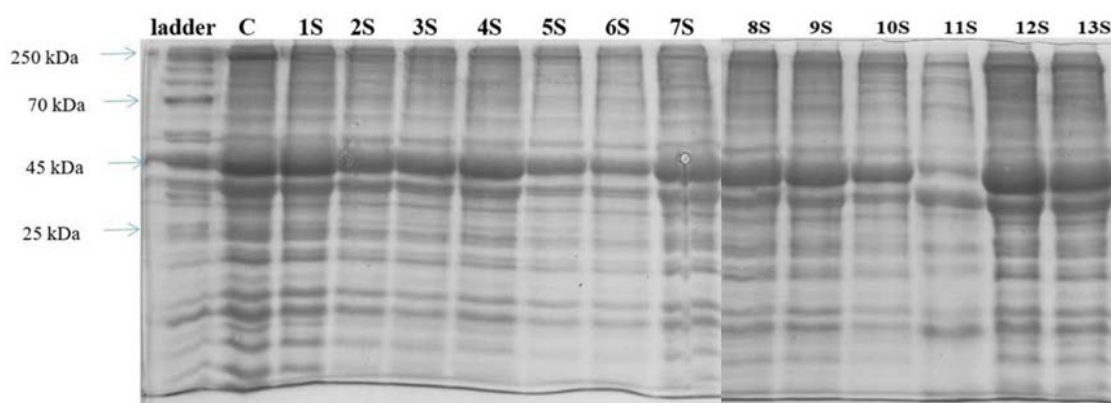


Figure 5. SDS-PAGE profile of total SDS-soluble proteins from *Pangasius* gels induced by different high pressure processing conditions. Control (C); 400 MPa/20°C in 12.5 min (1S); 400 MPa/25°C in 15 min (2S); 400 MPa/25°C in 10 min (3S); 400 MPa/30°C in 12.5 min (4S); 500 MPa/20°C in 12.5 min (5S); 500 MPa/25°C in 10 min (6S); 500 MPa/25°C in 15 min (7S); 450 MPa/20°C in 10 min (8S); 450 MPa/20°C in 15 min (9S); 450 MPa/25°C in 12.5 min (10S); 450 MPa/30°C in 10 min (11S); 450 MPa/30°C in 15 min (12S); 500 MPa/30°C in 12.5 min (13S).

3.7. Scanning electron microscopy (SEM) analysis

As shown in Figure 6, *Pangasius* gels subjected to heat treatment alone exhibited a rough, porous surface with an uneven distribution of the protein network and numerous voids. In contrast, gels treated with high pressure prior to thermal processing showed smoother surfaces and more compact, cohesive structures,

particularly in treatments with moderate HPP conditions such as 400 MPa/20°C in 12.5 min, 400 MPa/25°C in 10 min, and 450 MPa/20°C in 10 min. However, high-pressure treatments at elevated temperature and pressure combinations such as 500 MPa/25°C in 15 min and 500 MPa/30°C in 12.5 min appeared to reduce the effects of HPP, resulting in less uniform, less compact, and rougher gel structures.

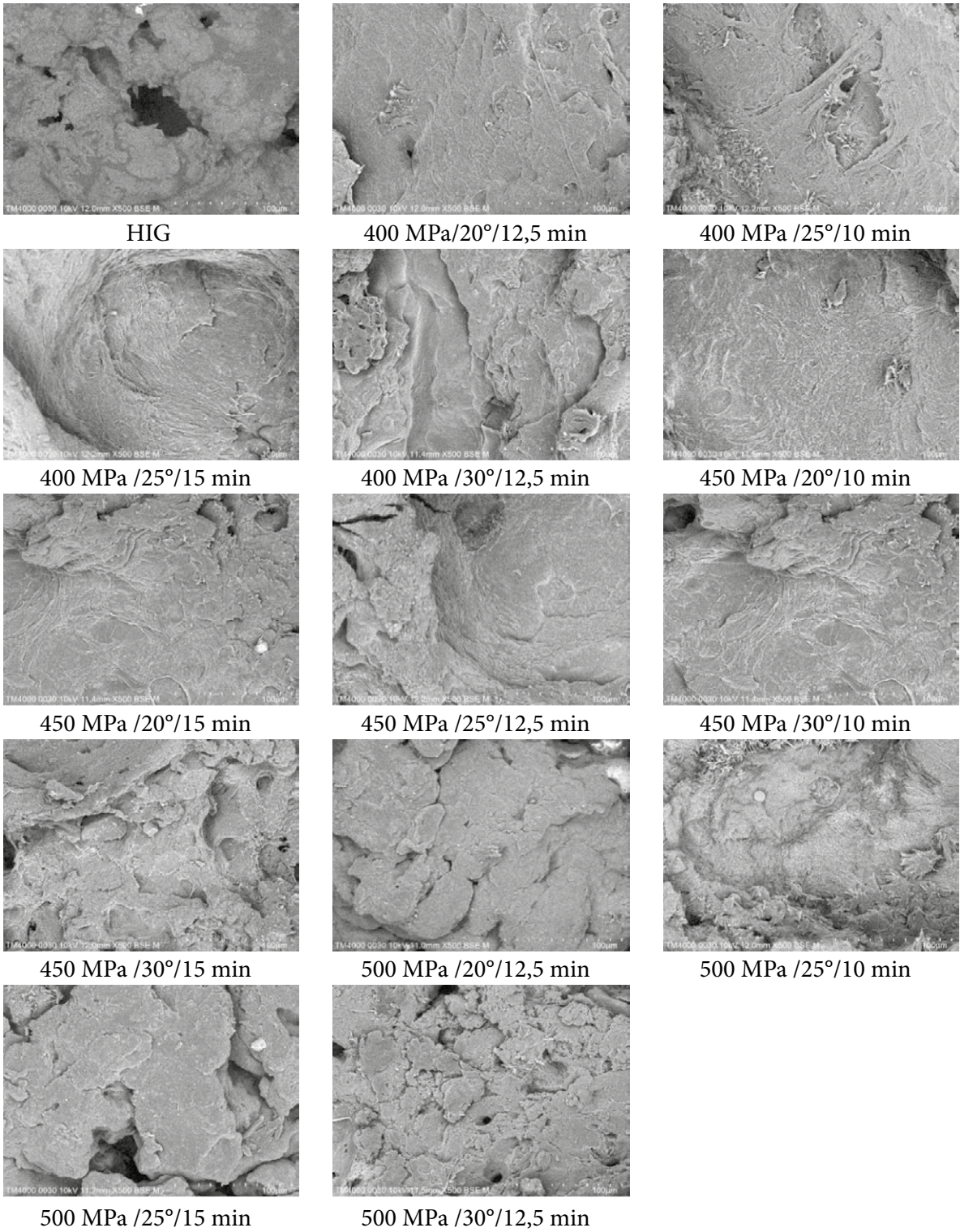


Figure 6. Scanning electron microscopy (SEM) images of Pangasius gels under different high-pressure processing conditions.

The HPP prior to heating promoted the formation of typical pressure induced gel structures, similar to those reported for other fish species such as barramundi gels (Truong et al., 2017), sardine surimi (Perez-Mateos & Montero, 1997), and threadfin bream gels (Ma et al., 2015). The mechanism of pressure-induced gel formation has been explained by greater protein denaturation and/or aggregation under the combined effects of high pressure and subsequent heat, as opposed to heat alone. Under pressure, fibrous proteins such as filamentous actin or globular proteins like sarcoplasmic actin are disrupted, leading to solubilization of myosin (Davis, 1985), actin, and other proteins (Iwasaki et al., 2006) for gel formation.

Protein structure is primarily maintained through ionic bonds, hydrogen bonding, hydrophobic interactions, disulfide bonds, and non-disulfide covalent linkages (Ma et al., 2015). Under high pressure, disruption of hydrophobic and electrostatic interactions, along with formation of hydrogen and disulfide bonds, leads to conformational changes in soluble proteins and promotes protein aggregation (Sikes et al., 2009). Ikeuchi et al. (1992) also proposed that during the heating of pressure treated proteins, more hydrophobic groups is exposed in aqueous solution, reducing solubility and increasing protein aggregation. Furthermore, protein unfolding under pressure may reorganize the protein network, resulting in increased aggregation during subsequent thermal processing (Uresti et al., 2004).

4. Conclusions

The results showed that HPP at 400 - 450 MPa improved the color of Pangasius gel, resulting in a brighter appearance. Gels treated at 400 - 450 MPa exhibited a more uniform, smoother,

and more stable structure compared to control samples. However, at 500 MPa, the gel structure was weakened, with the appearance of voids caused by excessive protein denaturation. The highest water-holding capacity was observed at 400 - 450 MPa; however, when the pressure exceeded this range, WHC decreased due to disruption of the gel network. Regarding mechanical properties, gels treated at 400 - 450 MPa showed the best gel strength, springiness, and hardness. In contrast, gels treated at 500 MPa became fragile and less stable. In conclusion, HPP at 400 - 450 MPa, combined with mild thermal conditions (20 - 30°C for 10 - 15 min), was identified as the optimal range for improving the overall quality of Pangasius gels.

Although this study provides insights into the influence of HPP on Pangasius gel quality, certain limitations remain. Future research should focus on optimizing processing parameters of HPP conditions and investigation of bond formation. In addition, comprehensive evaluations of sensory attributes, nutritional content, and storage stability are necessary to confirm the feasibility and consumer acceptance of Pangasius gels processed using high-pressure techniques

Conflict of interest

The authors declare that they have no competing interests.

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