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Impact of plant growth-promoting bacteria on the growth performance and resistance enzymes of tatsoi mustard in a hydroponic system

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Abstract. *Anastasya NA, Putra AM, Rachmawati SW, Trianti I, Setiawan A, Abadi AL, Aini LQ. 2024. Impact of plant growth-promoting bacteria on the growth performance and resistance enzymes of tatsoi mustard in a hydroponic system. Biodiversitas 25: 3309-3317.* Hydroponic system faces some problems in greenhouse cultivation including various biotic and abiotic stresses. Applying plant growth-promoting bacteria (PGPB) in hydroponic systems can alleviate those problems by serving as nutrient providers, plant growth promoters, and maintaining plant resilience. Therefore, the aim of this study was to evaluate PGPB strains for their potential to enhance plant growth and induce plant defense-related enzymes and compounds in tatsoi mustard plants (*Brassica narinosa* L.H. Bailey) when cultivated in a nutrient film technique (NFT) hydroponic system. The experiment was performed in a randomized block design (RBD) consisting of seven treatments and three replications. The treatments included P1: control with AB mix only; P2: *Pseudomonas versuta* UB36; P3: *Pseudomonas aeruginosa* UB52; P4: *Pseudomonas lundensis* UB53; P5: *Pseudomonas migulae* UB54; P6: *Enterococcus gallinarum* UB55 and; P7: *Lysinibacillus fusiformis* UB64. Based on the results, it was concluded that application of PGPB on tatsoi mustard promoted plant growth and production in hydroponic systems, which was shown in the treatment of *P. ludensis* UB53, *P. migulae* UB54, and *L. fusiformis* UB64. In addition, inoculation of PGPB strains induced resistance enzymes and compounds, namely phenylalanine ammonia-lyase (PAL), polyphenol oxidase (PPO) and peroxidase (PO) enzymes, and phenolic compounds in tatsoi mustard plants. *P. ludensis* UB53, *P. migulae* UB54, *Enterococcus gallinarum* UB55, and *L. fusiformis* UB64 showed higher activity in inducing plant resistance enzymes and compounds. Thus, these results suggested that PGPB improved tatsoi plant resistance against biotic as well as abiotic stresses and has the potential to be developed as an effective strategy for pest and disease management and increasing crop yield of tatsoi mustard grown in hydroponic systems.

Keywords: Hydroponic system, induced systemic resistance, *Lysinibacillus*, PGPB, plant resistance, *Pseudomonas*

INTRODUCTION

Hydroponic systems have become popular in the agricultural sector because they implement soil-less plant cultivation that works efficiently and in a controlled manner (Son et al. 2019). The basic principle in the hydroponic system is to provide nutrients directly to the plant roots through a nutrient solution dissolved in water (Vasdravanidis et al. 2022). Hydroponic systems are widely used due to their ability to produce high-quality food consistently, with lower soil-borne pest incidences, higher yields, and reduced fertilizer compared to conventional agriculture (Nemali 2022). However, despite the promising benefits, hydroponic cultivation faces some problems that are common in greenhouse cultivation, including various biotic and abiotic stresses, such as pest and disease infection as well as unfavorable environmental conditions such as like high temperatures, and high salinity leading to plant deficiency or toxicity, can disrupt plant growth and productivity (Compant et al. 2019; Bruni et al. 2023).

Insect pest and plant disease incidences often cause crop loss and reduced production quality in hydroponic systems. Several water borne plant pathogens, such as *Fusarium*, *Collectotrichum*, *Pythium*, *Phytophthora*, and *Rhizoctonia* are found to be the main problems in hydroponic cultivation (Renault et al. 2018). In addition, thrips and aphids have been reported to be the main insect pests found in crops grown in hydroponic systems (Silva et al. 2023). Various control efforts have been made by farmers, commonly by injecting carbon dioxide and spraying synthetic pesticides containing metalaxil (Fussy and Papenbrock 2022). Exposure to pesticides has been linked to a diverse array of health issues and affects food safety. In addition, the use of pesticides in hydroponic systems can lead to pest and disease resistance (Goddek et al. 2019; Chen et al. 2024).

Environmentally friendly control measures for plant pests and diseases are required in hydroponic systems to reduce crop loss, increase crop productivity, and protect the environment. The control measures that can be safely applied in a hydroponic system include the use of heat treatment, UV radiation, and particularly biocontrol agents (Fussy and Papenbrock 2022; Thomas et al. 2023). Biocontrol agents such as Plant Growth-Promoting Bacteria (PGPB) have been widely used as beneficial microbes for conventional field agriculture. PGPB is also known to support crops in reducing biotic and abiotic stresses through its role as a nutrient provider, plant growth promoter, and inducing plant resistance (Lee and Lee 2015). Some microbial genera included in PGPB are *Azospirillum*, *Pseudomonas*,

Bacillus, *Rhizobium*, *Burkholderia*, and *Enterobacteria* (Souza et al. 2015; Yulistiana et al. 2020).

The indigenous bacteria of the Universitas Brawijaya Education Forest (UB Forest) were examined for their PGPB abilities in previous studies. Out of 78 strains tested, 6 exhibited significant PGPB activity. These strains were identified as *Pseudomonas versuta* UB36, *Pseudomonas aeruginosa* UB52, *Pseudomonas lundensis* UB53, *Pseudomonas migulae* UB54, *Enterococcus gallinarum* UB55, and *Lysinibacillus fusiformis* UB64. The PGPB strains found are also known to produce indol acetic acid (IAA), fix nitrogen, dissolve phosphate, grow well under environmental stress conditions, such as high salinity, low pH, drought, and high temperature, and inhibit the growth of *Xanthomonas campestris* (Aini et al. 2023). Putra et al. (2024) have also tested the inoculation of two PGPB strains on lettuce (*Lactuca sativa* L.) grown in hydroponic systems. The results showed that *P. ludensis* UB53 and *P. migulae* UB54 were able to increase lettuce growth and yields, suppress pest damage, and induce plant resistance enzymes in the form of *Phenylalanine ammonia-lyase* (PAL) and chlorophyll. According to Sajali and Khoiriah (2023), tatsoi mustard is a trendy vegetable with excellent sales prospects in Indonesia. The high demand for fresh vegetables is increasing, especially in urban areas. The aim of this study was to evaluate PGPB strains for their potential to enhance plant growth and induce plant defense related enzymes and compounds in tatsoi mustard plants (*Brassica narinosa* L.H. Bailey) when cultivated in a nutrient film technique (NFT) hydroponic system.

MATERIALS AND METHODS

Plant and PGPB strains

The tatsoi mustard cultivar Take Cai was used in this experiment. The PGPB strains used were *Pseudomonas versuta* UB36, *P. aeruginosa* UB52, *P. lundensis* UB53, *P. migulae* UB54, *Enterococcus gallinarum* UB55, and *Lysinibacillus fusiformis* UB64. The bacterial strains were sourced from the Plant Pathology Laboratory of the Plant Pests and Diseases Department, Faculty of Agriculture, Universitas Brawijaya, Indonesia. These strains were selected based on their significant efficacy as PGPB in a previous study (Aini et al. 2023). The bacterial strains were firstly cultivated on nutrient agar (NA) medium and then propagated on nutrient broth (NB) medium with shaking at 125 rpm for 3 days at 28°C.

Greenhouse NFT hydroponic experiment

In this experiment, tatsoi mustard was grown using the NFT hydroponic system. The experimental design was conducted in a randomized block design (RBD) consisting of seven treatments distributed across three planting periods, serving as replications. The treatments consisted of the inoculation of PGPB strains into hydroponic growing media containing AB mix (nutrient solution as a fertilizer). The treatments were as follows: P1: control without PGPB inoculation; P2: *P. versuta* UB36; P3: *P. aeruginosa* UB52; P4: *P. lundensis* UB53; P5: *P. migulae* UB54; P6: *E. gallinarum* UB55; P7: *L. fusiformis* UB64.

Tatsoi mustard seeds were soaked in a bacterial suspension with a density of 10^9 CFU mL^{-1} for 30 minutes. After soaking, the seeds were sown on rock wool media and kept in a shady place for three days. After the seeds germinated, the rock wool was moved to a place exposed to sunlight to avoid etiolation. At 14 days, seedlings were selected based on their size and health condition. Next, seedlings grown in rockwool media were placed in net pot and transferred to the gully. The gully was irrigated with AB mix fertilizer at an electrical conductivity (EC) of 1200-1400 and mixed with PGPB strain suspension at 10^7 CFU mL^{-1}, except for the control. The growth of tatsoi mustard plants was observed once a week. Plant length, leaf total number, leaf area, pest and disease intensity, fresh and dry root weight, and fresh and dry shoot weight were observed. Biomass and leaf area were measured at 35 days after planting (DAP).

Analysis of damage intensity by pest or disease

The intensity of damage was observed by determining the score of damage caused by pests or diseases occurring naturally on hydroponic tatsoi mustard plants. Damage intensity was calculated based on the damage score value (v) with specific categories. The damage intensity of pest or disease was calculated using the formula of Natawigena (1993):

$$I = \frac{\sum(n \times v)}{Z \times N} \times 100\%$$

Where:

I : damage intensity

n : number of symptomatic plant leaves on the score damage

v : scale value on the score damage

Z : the highest value of the damage score

N : number of leaves observed

The damage score categories were; 0: No infestation; 1: damage <25% of the observed leaf area; 2: damage 26-50% of the observed leaf area; 3: damage 51-75% of the observed leaf area; and 4: damage >76% of the observed leaf area.

Analysis of tatsoi mustard plant defense related compounds

Total phenolic compounds

The total content of phenolic compounds was analyzed on the leaves of tatsoi mustard plants at 35 DAP according to the method of Phuyal et al. (2020). One g of fresh tatsoi mustard leaves was homogenized in 10 mL of 80% ethanol using a mortar and pestle, and then centrifuged for 15 min at 40,000 rpm. To the 50 µL supernatant were added 3000 µL of sterile distilled water, 200 µL of folin Ciocalteu reagent, and 750 µL of 7% Na_2CO_3. The mixture was incubated for 30 minutes and then measured at a wavelength of 765 nm with a gallic acid standard at 1,000 rpm. The total phenolic content was expressed as gallic acid equivalents (GAE) per gram of sample (GAE/g).

Phenylalanine ammonia-lyase (PAL)

Analysis of PAL enzyme in tatsoi mustard leaves was conducted at 35 DAP using the method of Inayati et al. (2020), with slight modifications. Samples of tatsoi mustard leaves were taken as much as 0.5 g and pulverized in a mortar and pestle. Next, 5 mL of 0.1 M borate buffer pH 8.8 was added to prepare the crude enzyme, sonicated for 10 minutes and then centrifuged at 10,000 rpm, 4°C for 10 minutes. The supernatant containing enzyme extract in a volume of 300 mL was mixed with 300 mM sodium borate and 30 mM solution of L-phenylalanine and then adjusted with distilled water up to 3.5 mL. The mixture was then incubated at 30°C for 60 minutes. Absorbance was measured at 290 nm using spectrophotometer and PAL enzyme activity was calculated as millimoles of cinnamic acid produced per minute per gram of fresh weight.

Chlorophyll and carotenoid contents

Chlorophyll and carotenoid contents in tatsoi mustard leaves were analyzed at 35 DAP according to the method of Lichtenthaler and Buschmann (2001). One g samples of tatsoi mustard leaves were homogenized using a mortar and pestle, and then a solution of 90% methanol was added to the sample of as much as 10 mL. Tatsoi mustard leaf extract was centrifuged at 3,000 rpm for 5 minutes. The supernatant liquid was analyzed for chlorophyll a, b, total chlorophyll, and carotenoid content using a spectrophotometer with wavelengths of 665.2 nm, 652.4 nm, and 470 nm. The chlorophyll and carotenoid content were calculated using the following formula by Lichtenthaler (1987):

Chlorophyll a=(16.82 x A665.2) - (9.28 x A652.4)
Chlorophyll b=(36.92 x A652.4) - (16.54 x A665.2)
Total Chlorophyll=Chlorophyll a + Chlorophyll b
Carotenoid (µg/mL)=(1000A470-1.91Ch-a - 95.15Ch-b)/225

Polyphenol oxidase (PPO) and peroxidase (PO)

The analysis of PPO and PO enzyme activities of tatsoi mustard plants was conducted using 35 DAP leaf samples. PPO enzyme analysis was conducted according to the method of Sundravadana et al. (2007), with minor changes. 0.5 g samples of tatsoi mustard leaves were taken and pulverized in a mortar and pestle. The 100 µL of crude enzyme extract from the leaf sample was mixed with 3.0 mL (0.01 M) catechol buffer solution and 0.1 mL phosphate buffer (pH 6.5). The changes in absorbance were measured using a spectrophotometer at a wavelength of 495 nm, and PPO activity was calculated as the absorbance change $min^{-1}g^{-1}$ leaf fresh weight. The PO enzyme activity was determined following the method of Elsharkawy et al. (2022a) with minor modifications. Leaf samples were crushed using 5 mL of sodium phosphate buffer (pH 6.5) and centrifuged at 12000 rpm at 4°C for 10 minutes. The supernatant was then mixed with 2.9 mL of 100 mM sodium phosphate buffer (pH 6.5) that contained 0.25% (v/v) catechol and 100 mM H_2O_2. The change in absorbance per minute interval for a total of 10 minutes was measured using a spectrophotometer at a wavelength of 495 nm.

Data analysis

Quantitative data on plant growth and enzyme assays subjected to ANOVA at a 5% significance level. If significant, Duncan Multiple Range Test (DMRT) was used with a 5% confidence level. Data was analyzed using SPSS v25 software.

RESULTS AND DISCUSSION

Effect of PGPB inoculation on tatsoi mustard growth performance

Plant length

The results showed that treatment with PGPB significantly increased tatsoi mustard plant length at 14 and 28 DAP (Table 1). At 14 DAP, *P. ludensis* UB53 and *P. migulae* UB54 inoculated plants had a higher plant length than the control ($F_{6,14}=5.553$; $p=<0.05$). While at 28 DAP, *P. migulae* UB54 treatment had a higher plant length than the control ($F_{6,14}=1.751$; $p=<0.05$). The results indicated that inoculation of PGPB in a hydroponic system supported tatsoi mustard plant growth. These results were in accordance with the report by Putra et al. (2024), who found that *P. migulae* increased the length of hydroponic lettuce plants by 12.23% compared to the control. PGPB from the genera *Bacillus*, *Pseudomonas*, and *Enterobacter* effectively stimulates plant growth in hydroponic systems (Lee and Lee 2015). The interaction between PGPB and plants in hydroponic systems has various mechanisms for increasing plant growth. Optimizing nutrient absorption and phytohormone production are important mechanisms carried out by PGPB (Sankaranarayanan et al. 2021).

Table 1. Plant length of tatsoi mustard after inoculation with plant growth-promoting bacteria (PGPB)

Treatments	Plant length (cm)±SD at various DAP			
	14	21	28	35
Control	6.33±0.37^{a}	8.66±0.58	10.63±0.48^{ab}	11.15±1.00
Pseudomonas versuta UB36	6.47±0.18^{a}	7.86±0.45	10.32±0.41^{a}	12.16±1.89
Pseudomonas aeruginosa UB52	6.48±0.33^{a}	8.14±0.59	10.83±0.55^{ab}	12.31±1.54
Pseudomonas lundensis UB53	7.35±0.53^{b}	9.25±1.16	11.82±0.79^{bc}	12.90±0.56
Pseudomonas migulae UB54	7.81±0.16^{b}	8.93±0.47	12.45±0.44^{c}	13.21±1.30
Enterococcus gallinarum UB55	6.29±0.66^{a}	9.11±0.72	11.77±0.69^{bc}	13.06±2.27
Lysinibacillus fusiformis UB64	6.58±0.51^{a}	9.11±2.01	11.57±0.86^{bc}	12.28±2.14

Notes: The same letter in a column means no significant difference at the 5% DMRT test. DAP: Day After Planting

Number of leaves

Results showed that treatment with PGPB increased the number of tatsoi mustard leaves at 14, 21, 28, and 35 DAP (Table 2). Tatsoi mustard plants inoculated with *P. ludensis* UB 53, *P. migulae* UB 54, *E. gallinarum* UB 55, and *L. fusiformis* UB 64 had more leaves at 14 DAP ($F_{6,14}=11.553$, $p=<0.05$) and 21 DAP ($F_{6,14}=28.336$, $p=<0.05$) than the control. In addition, *P. migulae* UB54 and *L. fusiformis* UB64 significantly increased the number of leaves on 28 DAP ($F_{6,14}=84.223$; $p=<0.05$) and 35 DAP ($F_{6,14}=36.398$; $p=<0.05$). These results align with the study of Bychkova et al. (2022) who reported that *P. migulae* treated plants can increase vegetative mass by >30%. Nitrogen-fixing activity possessed by beneficial microbes can support leaf formation. Adequate nitrogen needs in plants helps in preparing amino acids in plant growth to increase the number of leaves (Raksun et al. 2019).

Leaf area

Results exhibited that inoculation of PGPB significantly increased tatsoi mustard leaf area (Table 3). DMRT tests showed a significant increase in leaf area of plants treated with *P. ludensis* UB53, *P. migulae* UB54, *E. gallinarum* UB55, and *L. fusiformis* UB64 compared to control ($F_{6,14}=12.663$; $p=<0.05$). The inoculation of PGPB in tatsoi mustard plants is thought to increase nutrient uptake and production of the hormone IAA, which plays a role in the enlargement and elongation of plant cells, hence promoting plants to grow more optimally (Chen et al. 2022). *Pseudomonas* spp. can produce growth compounds and polysaccharides and interact positively with other bacteria when inoculated in plants (Nadeem et al. 2016). Some strains in the genera *Pseudomonas* and *Bacillus* also support fixing nitrogen and dissolving phosphate, which contributes to photosynthesis and plant growth (Stegelmeier et al. 2022).

Effect of PGPB inoculation on pest or disease intensity

Observations results on tatsoi mustard plants showed no disease incidence, but leaf damage was seen due to *Crocidolomia pavonana* (Zeller) (Lepidoptera: Pyralidae) (Figure 1) Symptoms caused by *C. pavonana* were in the form of hole which cause damage on the stems and leaves of plants due to their eating activities. According to Sembiring and Prasetia (2019), *C. pavonana* causes damage to the leaf parts of the plant, and in severe condition, plants fail to form crops.

Table 3. Leaf area of tatsoi mustard after inoculation with PGPB

Treatments	Leaf area (cm)±SD
Control	21.30±0.53 ^{a}
Pseudomonas versuta UB36	22.90±0.81 ^{a}
Pseudomonas aeruginosa UB52	22.96±0.83 ^{a}
Pseudomonas lundensis UB53	28.88±0.96 ^{c}
Pseudomonas migulae UB54	26.90±0.72 ^{bc}
Enterococcus gallinarum UB55	25.32±0.46 ^{b}
Lysinibacillus fusiformis UB64	26.60±0.15 ^{b}

Note: The same letter in a column means no significant difference at the 5% DMRT test

Table 2. Number of tatsoi mustard leaves after inoculation with PGPB

Treatments	Number of leaves ± SD at various DAP			
	14	21	28	35
Control	6.07±0.11 ^{a}	9.95±0.40 ^{a}	19.47±0.32 ^{a}	33.15±0.48 ^{a}
Pseudomonas versuta UB36	6.60±0.20 ^{ab}	9.53±0.33 ^{a}	19.06±0.12 ^{a}	35.43±0.45 ^{ab}
Pseudomonas aeruginosa UB52	6.53±0.30 ^{ab}	9.67±0.18 ^{a}	19.22±0.33 ^{a}	33.07±0.80 ^{ab}
Pseudomonas lundensis UB53	7.97±0.56 ^{c}	10.68±0.18 ^{b}	19.14±0.34 ^{a}	33.17±0.55 ^{ab}
Pseudomonas migulae UB54	7.47±0.15 ^{c}	11.40±0.19 ^{c}	21.94±0.08 ^{c}	37.93±0.49 ^{b}
Enterococcus gallinarum UB55	6.87±0.30 ^{b}	11.40±0.21 ^{c}	21.53±0.10 ^{bc}	34.10±0.51 ^{ab}
Lysinibacillus fusiformis UB64	6.77±0.37 ^{b}	11.27±0.28 ^{c}	21.21±0.19 ^{b}	38.13±0.98 ^{b}

Notes: The same letter in a column means no significant difference at the 5% DMRT test. DAP: Day After Planting

Figure 1. Symptoms of pest damage on tatsoi mustard leaves caused by *C. pavonana*: A. First instar of *C. pavonana;* B. Second instar of *C. pavonana;* C. Instar of *C. pavonana.*

The analysis results showed that treatment of PGPB significantly reduced the intensity of pest damage compared to the control (Table 4). It can also be seen that all PGPB treatments had a lower percentage of pest damage intensity at 21 DAP ($F_{6,14}$=1306.402; p=<0.05), 28 DAP ($F_{6,14}$=1590.975; p=<0.05), and 35 DAP ($F_{6,14}$=132.379; p=<0.05). These results show that inoculation of all PGPB strains can reduce pest damage intensity. The inoculation of rhizobacteria can increase plant resistance through its activity in inducing defense compounds in planta. Saprophytic rhizobacteria have been known to activate plant defense compounds and protect against pest infection (Backer et al. 2018). According to Ruiu (2020), several PGPB species such as *Bacillus*, *Pseudomonas*, *Serratia*, and *Streptomyces* are known to act as bioprotectants against invertebrate pests through various mechanisms, including pathogenesis activity and production of secondary metabolite compounds that function as toxins to pests. Weber et al. (2020) stated that plant-resistance compounds such as phenols are closely related to the process of plant resistance to both abiotic and biotic stress.

Effect of PGPB inoculation on crop production

The application of PGPB has been widely known to affect plant production, which in terms of tatsoi mustard can be measured by the biomass accumulation of fresh and dry weight of crops. The results indicated that inoculation of *P. ludensis* UB53, *P. migulae* UB54, *E. gallinarum* UB55, and *L. fusiformis* UB64 significantly increased the fresh weight of tatsoi mustard leaves compared to the control (Table 5). In addition, PGPB increased the dry weight of crop shoots and roots, but not root fresh weight compared to the control.

These results indicate that PGPB inoculation had different capacities for stimulating the biomass production of tatsoi mustard plants. PGPB has been widely known to associate with roots to produce phytohormones, such as cytokinins, gibberellins, and IAA, which are helpful for plant growth and development. Phytohormones mediated by rhizosphere bacteria promote plant growth and root development (de Andrade et al. 2023). According to Aini et al. (2023), four strains of PGPB, i.e., *P. aeruginosa* UB52, *P. migulae* UB54, *E. gallinarum* UB55, and *L. fusiformis* UB64 could fix nitrogen, dissolved phosphate, and produced IAA. Putra et al. (2024) reported that in NFT hydroponic system inoculation of *P. ludensis* UB53 and *P. migulae* UB54 can increase lettuce production by up to 45.78%. Mei et al. (2023) also found that *Pseudomonas* increased the fresh weight of oakleaf green lettuce up to 55.3% in NFT hydroponic system.

Effect of inoculation of PGPB on compound and enzymes related to plant growth and resistance

Total phenolic compounds, phenylalanine ammonia-lyase (PAL), carotenoid, and chlorophyll contents

Results showed that *P. lundesis* UB53, *P. migulae* UB54, *E. gallinarum* UB55, and *L. fusiformis* UB64 significantly increased phenol content compared to the control (Table 6). The strain *P. migulae* UB54 resulted in the highest phenol value, compared to both control and other treatments.

Table 4. Effect of PGPB inoculation on damage intensity

Treatments	Damage Intensity (%)±SD at various DAP		
	21	28	35
Control	9.03±0.30 ^{e}	25.60±0.45 ^{d}	26.38±0.26 ^{e}
Pseudomonas versuta UB36	4.80±0.05 ^{d}	7.25±0.04 ^{b}	19.96±0.50 ^{b}
Pseudomonas aeruginosa UB52	2.41±0.02 ^{b}	7.58±0.25 ^{b}	18.53±0.50 ^{a}
Pseudomonas lundensis UB53	2.48±0.05 ^{b}	2.84±0.02 ^{a}	23.96±0.25 ^{d}
Pseudomonas migulae UB54	4.36±0.04 ^{c}	2.76±0.04 ^{a}	18.46±0.47 ^{a}
Enterococcus gallinarum UB55	1.35±0.01 ^{a}	7.40±0.02 ^{b}	22.13±0.32 ^{c}
Lysinibacillus fusiformis UB64	4.36±0.03 ^{c}	11.16±0.72 ^{c}	19.70±0.65 ^{b}

Notes: The same letter in a column means no significant difference at the 5% DMRT test. DAP: Day After Planting

Table 5. Effect of PGPB inoculation on crop biomass

Treatments	Crop biomass±SD			
	Plant fresh weight (g)	Plant dry weight (g)	Root fresh weight (g)	Root dry weight (g)
Control	41.71±0.06 ^{a}	3.12±0.02 ^{c}	10.13±1.05 ^{ab}	1.14±0.02 ^{b}
Pseudomonas versuta UB36	37.09±0.42 ^{a}	2.99±0.04 ^{b}	9.56±0.98 ^{a}	1.09±0.03 ^{a}
Pseudomonas aeruginosa UB52	39.08±0.27 ^{a}	3.29±0.03 ^{d}	10.76±2.22 ^{ab}	1.15±0.03 ^{b}
Pseudomonas lundensis UB53	52.31±0.30 ^{bc}	3.50±0.03 ^{e}	13.12±2.12 ^{ab}	1.26±0.02 ^{d}
Pseudomonas migulae UB54	58.07±6.51 ^{d}	3.75±0.05 ^{f}	14.51±3.04 ^{b}	1.28±0.01 ^{d}
Enterococcus gallinarum UB55	56.75±0.70 ^{cd}	2.87±0.02 ^{a}	11.27±2.34 ^{ab}	1.21±0.01 ^{c}
Lysinibacillus fusiformis UB64	50.27±1.43 ^{b}	4.41±0.03 ^{g}	12.64±3.35 ^{ab}	1.30±0.03 ^{d}

Note: The same letter in a column means no significant difference at the 5% DMRT test

These results suggested that the presence of PGPB may be essential in triggering the tatsoi mustard plant to induce phenol accumulation and activate plant defense mechanisms. Cappellari et al. (2017) also showed that inoculation of PGPB from the genera *Pseudomonas* and *Bacillus* increased phenolic compounds' biosynthesis. The phenolic compounds are produced by plants to protect against biotic and abiotic stress (Abideen et al. 2015). The type and concentration of phenol in plants also increase when attacked by pests, where phenol's role in plant defense is related to antibiotic properties (Kousar et al. 2020). Not only biotic factors such as PGPB can affect phenol, but abiotic factors (oxidative stress, nutrient deficiency, hormonal disturbances, temperature stress) can also influence the activity of phenolic compounds (Tariq and Ahmed 2023).

In PAL activity test, inoculation of *P. aeruginosa* UB52, *P. lundensis* UB53, *P. migulae* UB54, *E. gallinarum* UB55, and *L. fusiformis* UB64 had a significant effect on PAL activity compared to control ($F_{6,14}=3.503$, $p=<0.05$) (Table 6). *L. fusiformis* UB64 treatment had the highest PAL activity compared to other treatments. Elsharkawy et al. (2022a) reported that *Pseudomonas* sp. inoculation upregulated PAL gene expression linked to *Rhizoctonia solani* disease incidence. PAL is an enzyme that involved in crucial plant defense mechanisms through phenol formation, jasmonic acid biosynthesis (Elsharkawy et al. 2022a), and entering different biosynthetic pathways leading to lignin synthesis (Solekha et al. 2020). In addition, PAL activity is also related to the synthesis of phenolics that play a role in plant defense. PAL is the most critical enzyme in inducing systemic resistance (ISR) mechanism through phenylpropanoid pathway, where lignin production is also triggered by increasing PAL and activating peroxidase compounds (Singh et al. 2016).

In addition, assays were conducted to determine the impact of PGPB inoculation on the biochemical compounds of tatsoi mustard plants, including carotenoids and chlorophyll. The results indicated that inoculation of PGPB on tatsoi mustard plants had no significant effect on increasing carotenoid ($F_{6,14}=0.954$; $p=<0.05$), chlorophyll a ($F_{6,14}=0.706$; $p=<0.05$), chlorophyll b ($F_{6,14}=0.692$; $p=<0.05$), and total chlorophyll ($F_{6,14}=0.402$; $p=<0.05$) (Table 7). Carotenoids are vital to plant compounds since they have a role in energy transfer to chlorophyll during photosynthesis as photo-protectors, antioxidant compounds, color attractants, and plant hormone precursors (Maoka 2020). The non-significant effect of PGPB inoculation compared to the control on carotenoid and chlorophyll accumulation probably results from the nutrient regime being fulfilled in the hydroponic system. A study by Yama et al. (2020) indicated that inoculating 1000 ppm hydroponic AB mix nutrients could provide functional nitrogen elements for plants to optimize chlorophyll formation. Chlorophyll acts as the main component in carrying out photosynthesis for plant growth. In accordance with Pinto et al. (2014), concentration of chlorophyll and carotenoids in plants is directly related to their mineral content.

Peroxidase (PO) and polyphenol oxidase (PPO) enzymes

PO and PPO enzymes were measured by absorbance changes using a spectrophotometer at a wavelength of 495 nm recorded every 1 minute for 10 minutes. The application of PGPB *P. versuta* UB36 and *P. aeruginosa* UB52 increased the activity of PO enzyme (Figure 2.A). The similar result was shown in Figure 2.B where the activity of PPO enzyme increased after the application of *P. versuta* UB36 and *P. aeruginosa* UB52. It is known that the activity of PO and PPO enzymes contributes to increas plant defense against pests and diseases. When pests impair plants, PO enzymes arise quickly and are involved in wound healing, lignification, and cell wall elongation (Sofo et al. 2015). In stressed conditions, PPO enzymes induce phenol production, which contributes to early defense mechanisms (Elsharkawy and El-Khateeb 2019). PO and PPO enzymes in tatsoi mustard plants inoculated with all PGPB strains were higher and in line with the lower intensity of pest damage that occurs. It is suspected that the higher PO and PPO enzyme activity in tatsoi mustard plants attacked by pests contributes to increasing plant resistance. As in the study by Elsharkawy et al. (2022b), plants infected with pests have a higher level of defense enzymes, and when treated with *P. aeruginosa*, PO enzyme activity increases higher than the control. In addition, similar activity was also shown by PPO enzymes, which were higher after being treated with *Pseudomonas*. The activity of phenolic compounds, PPO, and PO in plants has been known to be influenced by the inoculation of biocontrol agents (Fathi et al. 2018).

Table 6. Effect of PGPB inoculation on phenolic compound and PAL enzyme

Treatments	Phenol (mg/GAE/g)	PAL (mmol cinamic acid/g FW)
Control	1.44±0.08 ^{ab}	0.27±0.01 ^{a}
Pseudomonas versuta UB36	1.40±0.01 ^{a}	0.32±0.05 ^{ab}
Pseudomonas aeruginosa UB52	1.48±0.07 ^{abc}	0.38±0.01 ^{bc}
Pseudomonas lundensis UB53	1.80±0.18 ^{cd}	0.38±0.03 ^{bc}
Pseudomonas migulae UB54	1.88±0.28 ^{d}	0.38±0.10 ^{bc}
Enterococcus gallinarum UB55	1.73±0.28 ^{bcd}	0.37±0.04 ^{bc}
Lysinibacillus fusiformis UB64	1.74±0.01 ^{bcd}	0.43±0.03 ^{c}

Note: The same letter in a column means no significant difference at the 5% DMRT test

Table 7. Effect of PGPB inoculation on chlorophyll and carotenoid contents

Treatments	Carotenoid and chlorophyll contents (µg/mL)±SD			
	Carotenoid	Chlorophyll a	Chlorophyll b	Total chlorophyll
Control	3.30±0.75	5.47±0.37	4.97±0.80	10.44±1.17
Pseudomonas versuta UB36	3.21±0.95	5.63±1.05	5.01±0.64	10.64±1.69
Pseudomonas aeruginosa UB52	2.44±0.56	5.18±0.96	4.82±0.71	10.00±1.62
Pseudomonas lundensis UB53	4.11±0.56	5.99±0.54	5.27±0.74	11.26±1.28
Pseudomonas migulae UB54	3.63±1.03	6.10±0.28	5.42±0.32	11.52±0.29
Enterococcus gallinarum UB55	3.06±0.62	6.02±1.76	5.43±1.06	11.45±2.83
Lysinibacillus fusiformis UB64	3.20±1.46	5.85±0.72	4.79±0.90	10.64±0.58

Note: The same letter in a column means no significant difference at the 5% DMRT test

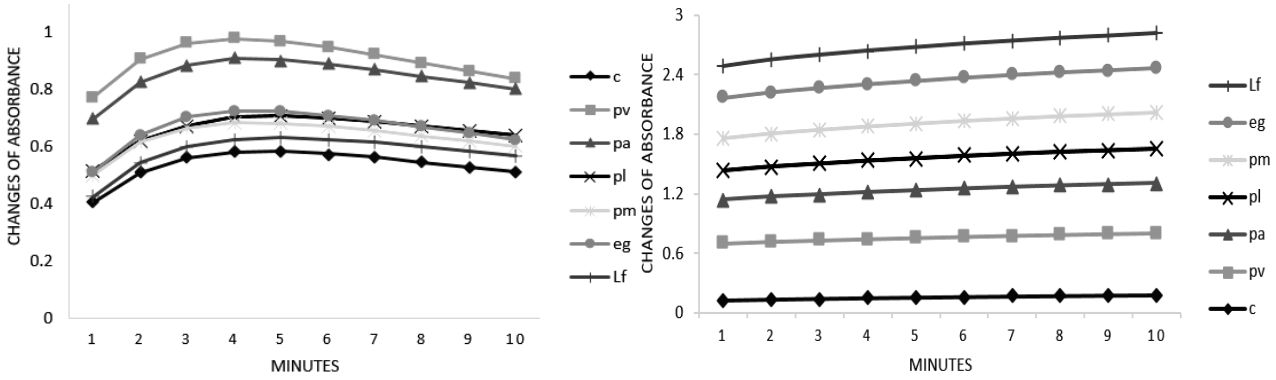

Figure 2. The effect of PGPB inoculation on A. Peroxidase (PO) and; B. Polyphenol oxidase (PPO) enzyme activities in tatsoi mustard grown in NFT hydroponic system. C: control, Ps: *Pseudomonas versuta* UB36, Pa: *Pseudomonas aeruginosa* UB52, Pl: *Pseudomonas lundensis* UB53, Pm: *Pseudomonas migulae* UB54, Es: *Enterococcus gallinarum* UB55, Lf: *Lysinibacillus fusiformis* UB64

Based on the results, it was concluded that tatsoi plants inoculated with PGPB strains promoted growth and biomass production, which was shown in the treatment of *P. ludensis* UB53, *P. migulae* UB54, and *L. fusiformis* UB64. In addition, PGPB in tatsoi plants grown in the NFT hydroponic system can induce plant resistance-related compounds and enzymes, such as phenolic compounds, PAL enzymes, PPO enzymes, and PO enzymes. These results suggested that the inoculation of PGPB improved tatsoi plant resistance against biotic as well as abiotic stresses and has the potential to be developed as an effective strategy for pest and disease management and increasing crop yield of tatsoi mustard grown in hydroponic systems.

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