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# Enhancing phosphate avaibility and maize adaptibility to acidic stress condition, through the inoculation of native acid tolerant rhizosphosphate bacterial isolate

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## Abstract

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The utilization of acidic land for the cultivation of maize is confronted by various limiting factors, including soil acidity and low fertility, mainly due to the fixation of phosphorus by aluminum ions. This research aims to assess the compatibility of selected isolates Phosphate-Solubilizing Rhizobacteria (PSR) from acidic soil, Kentrong Ultisol (KT-1, KT-2, and KT-3), and their capacity to enhance soluble phosphorus and maize plant growth. The experiment employed a randomized complete block factorial design with three replications. The first factor involved PSR isolates at eight levels, comprised individual isolates and their consortium combinations, while the second factor encompassed the acidity of the culture medium with three levels (pH 4.5, 5.5, and 6.5). Results demonstrated that isolates KT-1, KT-2, and KT-3 were compatible each other, and have the ability to form biofilm on plant roots. PSR consortium exhibited synergistic effects in improving root dry weight, PSR population, and soluble P, and there was an interaction between media acidity and bacterial isolates. The interaction of the KT-2 + KT-3 treatment at pH 4.5 significantly influenced and increased soluble P (895%) and PSR population (1693%), while the KT-3 treatment significantly increased root dry weight (98%). The interaction of the KT-2 + KT-3 treatment at pH 4.5 showed no significant difference, but had the potential to increase root length (35%), shoot dry weight (46%), and photosynthate accumulation (52%). The combination of using this inoculant has great potential as a biofertilizer in increasing acid soil fertility and maize growth.

*Keywords:* organic fertilizer; posphate solubilizeng; PGPR; sustainable farming; *Zea mays*

## Introduction

Growing maize in acidic soil environment requires specific strategies. Soil acidity is one of the most limiting factors, affecting the growth and yield of many crops all over the world. Soil acidification influences the solubility of $Al^{3+}$, $Fe^{3+}$, $Mn^{2+}$, $Cu^{2+}$, and $Zn^{2+}$, which can become harmful to plants, when present in excessive amounts. Conversely, it leads to a shortage of crucial plant nutrients like N, P, K, Ca, Mg, and Mo (Wakwoya et al., 2022).

Phosphorous is one of the most essential primary elements for plant growth (Glaser & Lehr, 2019). Phosphorous is essential for plant growth, as it triggers the growth of young plants, accelerate a vigorous start and hastens the maturity. Insufficient supply of P reduced the plant growth. Phosphorous nutrition in plants is linked with some particular growth factors as it strengthens the stalk and stem straw, boosts the formation of the flowers and production of fruits, enhances development of the roots, and also has the consequential role in seed formation. Phosphorus nutrition is also associated with resistance development against diseases in plants (Bargaz et al., 2018; Kaur et al., 2019)challenged by increasing global demand for food, scarcity of arable lands, and resources alongside multiple environment pressures, needs to be managed smartly through sustainable and eco-efficient approaches. Modern agriculture has to be more productive, sustainable, and environmentally friendly. While macronutrients such as nitrogen (N. Phosphorous is present in soil in both organic and inorganic forms. Its content varies in soil from 0.02–0.5% with an average of 0.05% (Ahmed et al., 2019).

The available forms of P for plants ($H_2PO_4^-$ and $HPO_4^{2-}$) are maximized at two main pH conditions: pH 4.5 and 6.5, where the degree of P fixation by calcium (Ca), aluminum (Al), and iron (Fe) is minimized (Penn and Camberato, 2019; Bouray et al., 2021). Soil pH was the main factor affecting phosphorus immobilization in soil (Zhao et al., 2023). Soil P availability is affected by the level of soil acidity, which is optimum at pH range 6.0 to 6.5 (Havlin et al., 2005). According to (Cerozi and Fitzsimmons, 2016), when the soil pH is lower than 5, phosphorus avaibility will decrease. Phosphate Solubilizing Rhizobacteria (PSR) are an alternative, that the P element can be used by plants (Tian et al., 2021)and one of the main limiting elements for biomass production as plant-available P represents only a small fraction of total soil P. Increasing global food demand and modern agricultural consumption of P fertilizers could lead to excessive inputs of inorganic P in intensively managed croplands, consequently rising P losses and ongoing eutrophication of surface waters. Despite phosphate solubilizing microorganisms (PSMs. Furher, many beneficial microbes may be local isolates, which are native to the location and cropping systems and they will be more suitable for adaptation to the local climatic environment (Cho and Tiedje, 2000). Those local PSR isolates can be used to enhanced P avaibility and plant growth in acidic soil.

Application of PSR are believed to provide an eco-friendly and economically sound approach to overcome the P scarcity (Kalayu, 2019)deficiencies can reduce plant growth and development. Though soil possesses total P in the form of organic and inorganic compounds, most of them remain inactive and thus unavailable to plants. Since many farmers cannot afford to use P fertilizers to reduce P deficits, alternative techniques to provide P are needed. Phosphate solubilizing microbes (PSMs. PSR play a dominant role in the plant growth via the synthesis and through a secretion of a plethora of beneficial substances, such as auxins, cytokinins, and gibberellic acid, as well as ethylene, hydrogen cyanide, and siderophores (Wahid et al., 2020; Yu et al., 2022) thus making it immobile in soil. Inoculation of arbuscular mycorrhizal fungi (AMF. Various plant growth-promoting hormones, such as auxins, cytokinins, and gibberellins, can be produced by PSR, and those hormones have important effects on plant root and shoot development, flowering, germination, and xylem differentiation (Puri et al., 2020). Application of such naturally occurring organisms possessing multiple growth-promoting activities holds therefore greater promise for increasing the productivity of many crops (Wang et al., 2022). PSR serve as potent biofertilizers that improve the agricultural yield in harmony with ecological concerns (Rajwar et al., 2018).

## Materials and Method

The bioassay was carried out in the greenhouse of Agriculture Faculty, Universitas Padjadjaran, Jatinangor District, Sumedang Regency, West Java, Indonesia. Factorial randomized block design was used for the experiment, comprised 8 treatments of isolates, 3 treatments of media pH, and 3 replications. The first factor consists of the PSR isolate and its consortia (KT-1, KT-2, and KT-3), while the second factor is the media pH (pH 4.5, 5.5, and 6.5). The PSR isolates used in this study were KT-1, KT-2 and KT-3. These isolates were collected at the Laboratory of Soil Biology, Faculty of Agriculture, Universitas Padjadjaran, which were isolated from agricultural soils and natural forest.

The bioassay of phosphate-solubilizing rhizobacteria was conducted in a modified Murashige & Skoog (MS) liquid medium supplemented with 0.25 $AlPO_4$, and pH variations were controlled for experimentation. Media pH adjustment is done by adding HCl or NaOH. The addition of these materials is done before the media is sterilized in the autoclave. The maize seeds used previously were sterilized and germinated on the straw paper for 72 h. Sprouts that have grown were planted in test tubes filled with 85 mL of MS liquid media and treated with 10 mL of PSR isolates by $10^9$ cfu/mL density. Observations were carried out for 4 weeks, refer to the maize vegetative phase and adjusted to the capacity of the growing medium. PSR population, P-dissolved, biofilm, maize plant growth, and photosynthate accumulation further conducted.

The data were analyzed by means of variance (ANOVA) using SPSS, for treatments had a significant effect, Duncan's multiple distance test was carried out at a significance level of 5% (Gomez and Gomez, 1995).

## Results and Discussion

***Compatibility test***

The compatibility test indicated that the three tested isolates were mutually compatible with each other, showing no antagonistic properties among the isolates. Observations revealed that each inoculum could grow in close proximity, without the presence of inhibitory zones or clear zones (Figure 1).

Each isolate does not exhibit antagonistic behavior towards one another. Antagonistic behavior is characterized by the formation of a clear zone, due to competition for limited nutrient sources, leading each bacterium to produce antibiotics to inhibit the growth of other bacteria (Hibbing et al., 2010). These antibiotics hinder bacterial growth by disrupting cell wall synthesis, protein synthesis, and membrane depolarization, resulting in the formation of a clear zone indicating the absence of bacterial growth (Reygaert, 2018; Martí et al., 2018). This event signifies that isolates displaying antagonistic traits are unsuitable for consortium formation.

Figure 1 illustrates the colony growth of each PSR isolate, which grows in overlapping proximity with one another. This growth signifies compatibility among the isolates. Compatibility occurs when a bacterial consortium is capable of cross-feeding essential metabolites, thereby meeting the nutritional needs among bacterial isolates (Wintermute & Silver, 2010). Additionally, a compatible bacterial consortium can utilize metabolic by-products from other bacterial isolates that may be toxic, but beneficial to other isolates, thereby reducing the accumulation of toxic metabolites produced among bacteria and simultaneously fulfilling the needs of other bacteria (Zuroff and Curtis, 2012). Such interactions represent a form of mutualistic symbiosis among bacteria. In mutualism, each member relies on the other for survival as they exchange essential substances or detoxify each other's waste, allowing each bacterium to continue growing (Roell et al., 2019). Further testing of the compatible consortium will be conducted through bioassay experiments, to assess its impact on plants and other parameters.

***PSR population and P-dissolved***

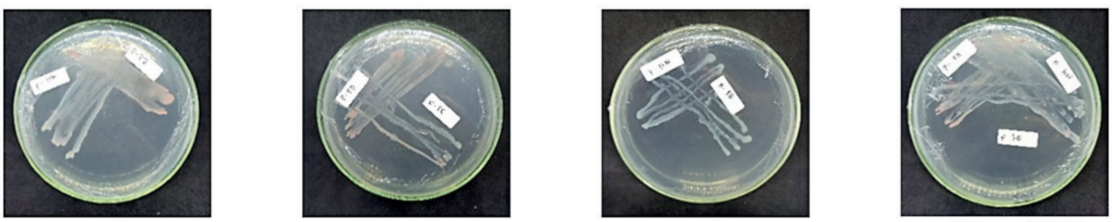
The interaction between PSR inoculation and media pH can significantly increase the population of PSR compared to the control. The KT-2 + KT-3 treatment at pH 4.5 has the ability to enhance the PSR population. This treatment shows the highest PSR population, reaching 19.7 x $10^9$ cfu/mL, significantly better by 1693% compared to the control, demonstrating a significant difference from the control as seen in Table 1.

The results in Table 1 indicate that the inoculation of KT-2 + KT-3 at pH 4.5 has the highest impact on increasing

**Table 1. Interaction between PSR inoculation and media pH on PSR population (cfu/mL)**

| PSR Isolates | PSR population in different pH media (x $10^9$ cfu/mL) | | |
|---|---|---|---|
| | 4.5 | 5.5 | 6.5 |
| Control | 1.1 A a<br>– | 1.9 A a<br>– | 1.1 A a<br>– |
| KT-1 | 4 B b<br>(263%) | 3.3 AB bcd<br>(74%) | 2.5 A bc<br>(123%) |
| KT-2 | 5.6 B c<br>(412%) | 2.2 A ab<br>(16%) | 1.9 A ab<br>(70%) |
| KT-3 | 9.1 C d<br>(727%) | 2.8 B abc<br>(47%) | 1.5 A ab<br>(40%) |
| KT-1 + KT-2 | 12.7 B e<br>(1051%) | 4.3 A d<br>(115%) | 3.4 A c<br>(206%) |
| KT-1 + KT-3 | 15 C f<br>(1263%) | 2.8 B abc<br>(47%) | 1.5 A ab<br>(30%) |
| KT-2 + KT-3 | 19.7 C g<br>(1693%) | 4 B cd<br>(105%) | 2.2 A abc<br>(100%) |
| KT-1 + KT-2 + KT-3 | 8.6 B d<br>(681%) | 2.3 A ab<br>(21%) | 1.4 A abc<br>(30%) |

*Note*: Numbers followed by the same letter are not significantly different based on Duncan's test at a 5% significance level. Lowercase letters are read vertically, and uppercase letters are read horizontally. Percentage values in parentheses indicate the increase in plant height compared to the control



KT-1 and KT-2 | KT-1 and KT-3 | KT-2 and KT-3 | KT-1 and KT-2 and KT-3

**Fig. 1. Each isolate demonstrates compatibility as evidenced by the absence of inhibitory zones at the interface of isolate overlap**

the population of PSR compared to other treatments. This treatment is 218% more effective than the average of single isolate treatments at the same pH, and 738% more effective than the average at other pH levels. This suggests compatibility of the isolates and their suitability for growth in pH 4.5 or acidic media.

Deng and Wang (2016) explained that after exponential growth and depletion of primary nutrients, bacteria in consortium cultures, can continue to grow by utilizing metabolites produced by other bacteria. As a result, consortium cultures achieve higher growth density compared to single cultures that lack other isolates for metabolite exchange, leading to higher population levels. Additionally, consortium treatments create an ideal environment for bacterial growth with an increasing number of bacteria, allowing for inter-bacterial signaling transmission, and enhancing resilience to different pH media (Toyofuku et al., 2016).

The interaction between isolate treatments and media pH shows that the used isolates have the best growth ability at pH 4.5 compared to pH 5.5 and 6.5. PSR population increases with decreasing pH, reflecting the bacteria's adaptability to the surrounding environment. The high population of PSR at pH 4.5 may be due to the isolates' origin from acidic soil habitats. (Suharyono et al., 2012) stated that bacterial adaptation is faster when the growth environment matches the bacterial habitat. This aligns with research of Lund et al. (2020), who is demonstrating the critical role of pH in bacterial growth, as each bacterial species has an optimal pH required for its growth. The high population may also result from minimal competition with other bacteria.

Population density affects the PSR's ability to assist plant growth, including increasing soluble P in the media. The effectiveness of PSR in phosphate solubilization can be observed in Tables 7 and 8, where higher population density correlates with increased soluble P in the culture media. This aligns with Rudrappa et al. (2008) statement, that microbial efficacy depends on population density. Table 8 demonstrates the PSR isolate's ability to solubilize phosphate, which increases with decreasing media pH.The interaction between PSR inoculation and media pH significantly increases soluble P compared to the control. The KT-2 + KT-3 treatment at pH 4.5, shows the highest ability to enhance soluble P, with a concentration of 529.89 ppm, which is 895% better than the control. This significant difference can be observed in Table 2.

The results in Table 2 demonstrate that the inoculation of the KT-2 + KT-3 consortium at pH 4.5 has the highest impact in increasing soluble P compared to other treatments. This treatment with both isolates is 46% more effective than the average of single isolate treatments at the same pH, and 372% more effective than the average of treatments at other

**Table 2. Interaction between PSR noculation and media pH on soluble phosphorus (ppm)**

| PSR Isolates | P-Dissolved in different pH media | | |
|---|---|---|---|
| | 4.5 | 5.5 | 6.5 |
| Control | 53.24 A a<br>– | 82.70 A a<br>– | 35.48 A a<br>– |
| KT-1 | 290.64 A bc<br>(445%) | 303.72 A b<br>(267%) | 189.10 A b<br>(432%) |
| KT-2 | 392.38 B cd<br>(637%) | 197.38 A ab<br>138%) | 136.07 A ab<br>(283%) |
| KT-3 | 404.30 B cd<br>(659%) | 155.03 A a<br>(87%) | 113.09 A ab<br>(218%) |
| KT-1 + KT-2 | 319.27 B bc<br>(499%) | 180.89 A ab<br>(118%) | 198.33 AB b<br>(131%) |
| KT-1 + KT-3 | 238.74 A b<br>(348%) | 132.14 A a<br>(60%) | 131.59 A ab<br>(270%) |
| KT-2 + KT-3 | 529.89 B d<br>(895%) | 126.68 A a<br>(53%) | 97.46 A ab<br>(174%) |
| KT-1 + KT-2 + KT-3 | 304.07 B bc<br>(471%) | 128.95 A a<br>(55%) | 88.53 A ab<br>(149%) |

*Note*: Numbers followed by the same letter are not significantly different based on Duncan's test at a 5% significance level. Lowercase letters are read vertically, and uppercase letters are read horizontally. Percentage values in parentheses indicate the increase in plant height compared to the control

pH levels. Based on this, the PSR treatment at pH 4.5 shows higher effectiveness in phosphate solubilization compared to higher pH values. The concentration of soluble P increases as the media pH decreases. This is attributed to the isolates used in this experiment, which are known to inhabit acidic soils. Fitriatin et al. (2022) reported similar findings, showing that the highest levels of soluble P were obtained at pH 4.5 compared to higher pH values.

The phosphate solubilization by bacteria is related to their ability to produce organic acids, enzymes, or other compounds such as exopolysaccharides (EPS). Under stressful environmental conditions, bacteria produce EPS as a response to the stress. EPS forms complexes with metal ions present in the media (Al3+ > Cu2+ > Zn2+ > Fe3+ > Mg2+ > K+), and this mechanism can be extrapolated as a means of phosphate solubilization by PSR (Ochoa-Loza et al., 2001). Moreover, EPS also assists in maintaining quorum sensing signal molecules, various extracellular enzymes, and other metabolic products, ultimately supporting cell-cell communication and substance degradation (Toyofuku et al., 2016).

***Plant root biofilm***

Inoculation with PSR can form biofilms on plant roots across various pH conditions. Figure 2 illustrates the presence of a white layer on the surface of plant roots inoculated with PSR, whereas in the control treatment, no layer or structure is formed on the surface of plant roots. Biofilms develop in the rhizosphere of plants. In the plant rhizosphere, PSR is known to be more metabolically active, influencing plant growth, yield, and defense mechanisms as part of a mutualistic relationship with plant roots (Vazquez et al., 2000). The high metabolic activity of PSR in the plant rhizosphere may be attributed to the availability of nutrient-rich root exudates, influencing plant growth, yield, and defense mechanisms (Vejan et al., 2016). Root exudates serve as a key factor for PSR, to colonize the surface of plant roots, containing organic acids, amino acids, proteins, sugars, phenolics, and other secondary metabolites required by microorganisms (McNear, 2013). The quantity and quality of root exudates play a crucial role in shaping the density and structure of bacteria in the rhizosphere.

Figure 2 illustrates the formation of a biofilm in the rhizosphere of plants subjected to PSR isolate treatment. Biofilm, as a complex assembly of microorganisms within an extracellular polymer matrix, serves as a protective mechanism against various stress factors, including low pH stress (Flemming et al., 2016). In this experiment, all PSR isolate treatments were capable of forming biofilms with varying thickness in the rhizosphere of plants at different pH levels. Upon reaching optimal microbial density, the biofilm begins to act collectively in a process known as quorum sensing, coordinating the release of compounds, that aid in promoting plant growth through direct and indirect mechanisms. The biofilm supports plant growth through direct mechanisms by releasing plant growth-stimulating compounds, such as auxins and cytokinins. Meanwhile, indirect mechanisms involve the synthesis of antibiotics for pathogen control and induction of systemic resistance (McNear, 2013).

***Plant growth characteristics***

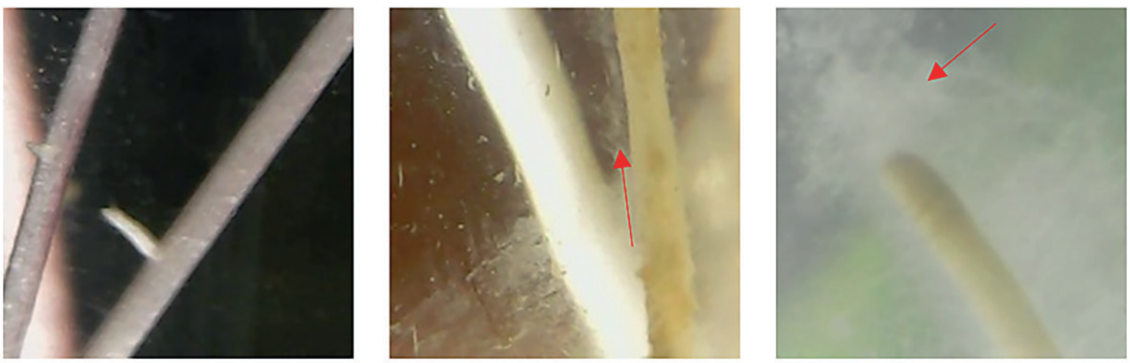
The results indicate that there is an interaction between PSR inoculation treatments and media pH, concerning the parameters of plant height and root length, while the chlorophyll content parameter demonstrates independent effects of both media pH and PSR inoculation. The interaction between PSR inoculation and media pH reveals a significant interaction, although the inoculation treatments did not significantly increase the plant height of maize plants compared to the control. The treatment KT-1 + KT-2 at pH 6.5 exhibited the tallest plant height, reaching 61.0 cm, which was not significantly different from the control Table 3.

The result in Table 3 indicates the tallest plant height in the treatment KT-1 + KT2 at pH 6.5, reaching 61 cm, which was not significantly different from the control. The initial growth phase of maize plants occurs rapidly, and media pH does not affect the rate of shoot growth. Wellmann et al. (2023) reported that newly germinated corn seedlings tend to secrete acidic substances into their environment, which can lower the pH of the media. Corn germinated at pH 7, for example, showed an extreme decrease in media pH to 4, due to the diffusion of hydrogen ions, produced by the pericarp

**Table 3. Interaction between PSR inoculation and media pH on maize plant height (cm)**

| PSR Isolates | Shoot height in different pH media | | |
|---|---|---|---|
| | 4.5 | 5.5 | 6.5 |
| Control | 59.8 A c | 53.7 A c | 60.8 A c |
| KT-1 | 42.1 AB a | 50.5 B bc | 38.8 A a |
| KT-2 | 56.9 B bc | 46.8 A abc | 39 A a |
| KT-3 | 52.3 A bc | 43.4 A ab | 47.9 A ab |
| KT-1 + KT-2 | 49.9 A ab | 52.0 A bc | 61.0 B c |
| KT-1 + KT-3 | 54.9 B bc | 51.4 B bc | 40.2 A a |
| KT-2 + KT-3 | 59.9 B c | 47.5 A abc | 51.5 AB bc |
| KT-1 + KT-2 + KT-3 | 49.0 B ab | 39.2 A a | 45.9 AB ab |

*Note*: Numbers followed by the same letter are not significantly different based on Duncan's test at a 5% significance level. Lowercase letters are read vertically, and uppercase letters are read horizontally. Percentage values in parentheses indicate the increase in plant height compared to the control



(a) (b) (c)

**Fig. 2. PSR biofilm on plant roots (a) control, (b) and (c) PSR inoculation**

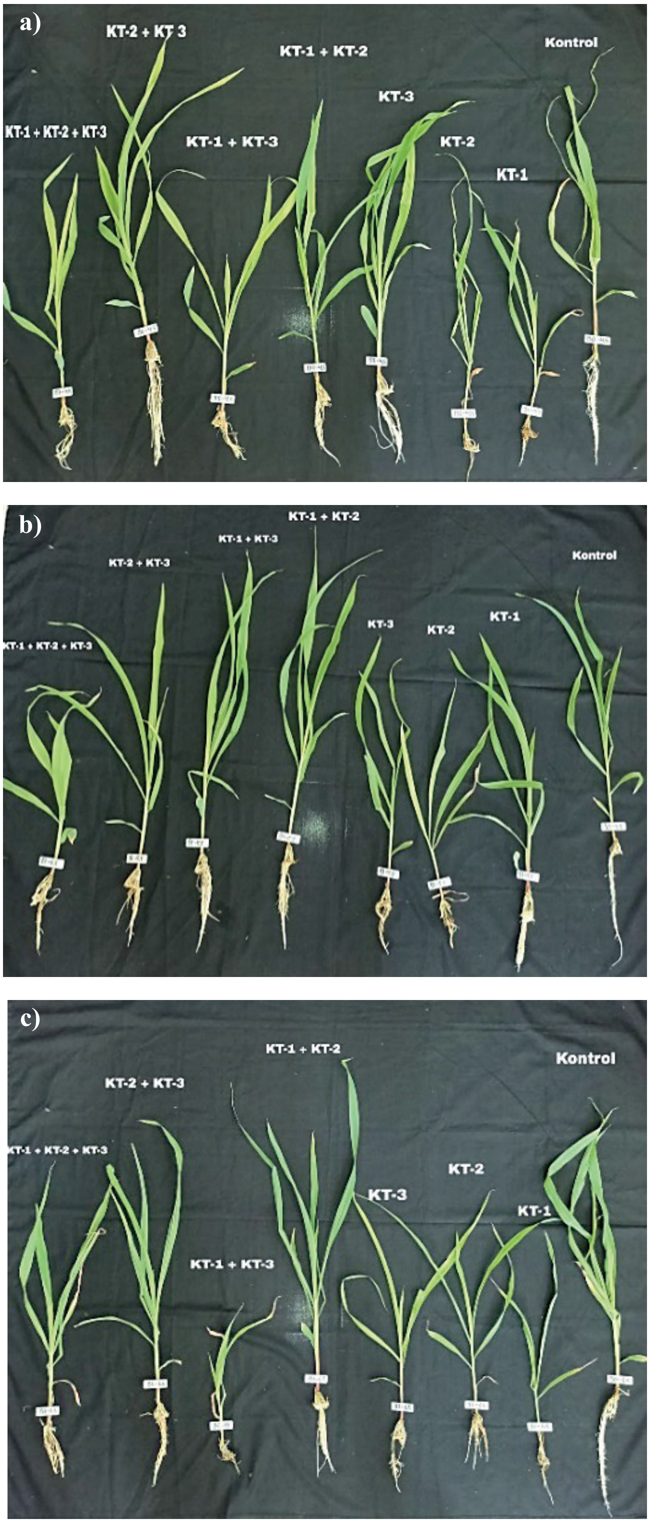



**Fig. 3. Effects of PSR innoculation to maize in different pH media (a) pH 4.5; (b) 5.5; (c) 6.5**

and testa tissues of corn seeds. This explains the ability of maize plants to thrive in acidic media during the early growth phase. Media acidity does not affect shoot height growth in the early growth phase, but media pH does influence chlorophyll content during the early growth phase of maize plants, as apparent from the leaf color differences in Figure 3 and the chlorophyll content results in Table 3.

Figure 3 illustrates the growth outcomes of maize plants over a 28-day period. The figure shows that the plant' height in the control treatment is not significantly different from the consortium inoculation treatment, but significantly differs from the single inoculation treatment. Additionally, root length demonstrates a significant difference between isolate treatments and media pH levels. The control treatment exhibits a proportional increase in root length with an increase in media pH. Media with a pH of 4.5 results in the highest average root length compared to other pH levels. This indicates that the use of isolates can enhance root length at pH 4.5. The interaction between PSR inoculation and media pH reveals a significant interaction, although the inoculation treatments did not significantly increase root length compared to the control. The treatment KT-2 + KT-3 at pH 4.5 has the potential to enhance root length, exhibiting the longest root length at 16.2 cm, a 35% improvement over the control, but it does not show a statistically significant difference from the control, as observed in Table 4.

**Table 4. Interaction between PSR inoculation and media ph on maize plant root length (cm)**

| PSR Isolates | Root length in different pH media | | |
|---|---|---|---|
| | 4.5 | 5.5 | 6.5 |
| Control | 12.0 A abcd<br>– | 14.4 AB b<br>– | 18.0 B d<br>– |
| KT-1 | 9.0 AB ab<br>– | 11.6 B ab<br>– | 6.1 A a<br>– |
| KT-2 | 11.5 A abc<br>– | 11.9 A ab<br>– | 10.7 A bc<br>– |
| KT-3 | 14.3 B cd<br>(19%) | 9.9 A a<br>– | 13.6 B c<br>– |
| KT-1 + KT-2 | 8.4 A a<br>– | 10.3 A ab<br>– | 9.9 A abc<br>– |
| KT-1 + KT-3 | 12.8 B bcd<br>(6%) | 10.2 AB ab<br>– | 6.6 A ab<br>– |
| KT-2 + KT-3 | 16.2 B d<br>(35%) | 8.8 A a<br>– | 8.9 A ab<br>– |
| KT-1 + KT-2 + KT-3 | 10.3 A abc<br>– | 9.7 A a<br>– | 8.7 A ab<br>– |

*Note*: Numbers followed by the same letter are not significantly different based on Duncan's test at a 5% significance level. Lowercase letters are read vertically, and uppercase letters are read horizontally. Percentage values in parentheses indicate the increase in plant height compared to the control

The result in Table 4 indicates that inoculation with KT-2 + KT-3 at pH 4.5 has the highest impact on increasing root length compared to other treatments. This treatment is significantly better by 40% compared to the average single isolate treatment at the same pH, and 83% compared to the average at other pH levels. The treatment demonstrates compatibility between isolates and their ability to enhance the root length of maize plants at pH 4.5.

The observed increase in root length is attributed to the colonization of PSR on plant roots, enhancing the plant roots' resilience to environmental stress. According to Li et al. (2024), root length determines nutrient absorption, because longer roots can easily absorb nutrients within a broader range in the media. The ability of KT-2 + KT-3 to increase root length is also influenced by its high capability to solubilize phosphorus in the media (Table 7). This is crucial as phosphorus plays a vital role in cell division and the development of meristematic tissues, stimulating root growth, especially in seeds and young plants (Liu, 2021).

In addition to root length, another growth parameter in this experiment is chlorophyll content. The interaction effect between PSR inoculation and media pH is not observed in the chlorophyll content parameter. This parameter demonstrates the independent effects of PSR inoculation and media pH, significantly influencing the chlorophyll content in maize plant leaves (Table 5).

The control treatment exhibited the highest chlorophyll content at 15.30 CCI, significantly differing from all other inoculation treatments. Furthermore, independent effects on chlorophyll content were observed for media pH treatments, with pH 6.5 showing the highest chlorophyll content at 12.61 CCI, significantly differing from all other pH treatments. Chlorophyll content in plants indicates the effectiveness of photosynthesis, and environmental stress triggers a significant reduction in leaf chlorophyll content due to its fragile nature (Tan et al., 2012)the serotonin N-acetylating enzyme in plants may differ greatly from the animal AANAT with regard to sequence and structure. This would imply multiple evolutionary origins of enzymes with these catalytic properties. A primary function of melatonin in plants is to serve as the first line of defence against internal and environmental oxidative stressors. The much higher melatonin levels in plants compared with those found in animals are thought to be a compensatory response by plants which lack means of mobility, unlike animals, as a means of coping with harsh environments. Importantly, remarkably high melatonin concentrations have been measured in popular beverages (coffee, tea, wine, and beer. This phenomenon is also evident with acidity in the media.

**Table 5. Independent effects of PSR inoculation and media ph on chlorophyll content in maize plants (CCI)**

| Treatments | Chlorophyll content (CCI) |
|---|---|
| Medium pH | |
| 4.5 | 10.59 a |
| 5.5 | 9.73 a |
| 6.5 | 12.61 b |
| PSR Isolates | |
| Control | 15.30 e |
| KT-1 | 12.58 d |
| KT-2 | 9.72 ab |
| KT-3 | 7.89 a |
| KT-1 + KT-2 | 12.11 cd |
| KT-1 + KT-3 | 9.62 ab |
| KT-2 + KT-3 | 10.49 bcd |
| KT-1 + KT-2 + KT-3 | 10.13 abc |

*Note*: Numbers followed by the same letter do not significantly differ according to Duncan's test at a significance level of 5%

The results indicate that low chlorophyll content at acidic pH is a consequence of environmental stress, while near-neutral media pH results in the highest chlorophyll content. In the PSR inoculation treatments, the control treatment exhibited the highest chlorophyll content. The higher chlorophyll content in the control treatment may be attributed to the young age of the maize plants (28 DAS) and the availability of sufficient nutrients, enabling the plants to adapt and enhance their natural resilience to environmental stress. In contrast, lower chlorophyll content in the inoculation treatments may be due to nutrient competition with bacteria. According to Dworkin & Harwood (2022), bacteria require nutrients for their survival and growth, and apart from root exudates, they utilize nutrients available in their surroundings. Bacteria need nutrients such as water, carbon, nitrogen, vitamins, phosphorus, sulfur, magnesium, calcium, and various mineral salts obtained from their environment. The nitrogen element used by bacteria is also utilized by plants to form chlorophyll (Lefever et al., 2017). This nutrient competition with bacteria may result in higher chlorophyll content in the control treatment compared to the inoculation treatments.

***Plant dry weight***

The analysis of variance results indicates the presence of an interaction between PSR inoculation treatments and media pH on the parameters of shoot dry weight, root dry weight, and photosynthate accumulation. Plant dry weight provides insight into the utilization of chlorophyll and the amount of nutrients, available to plants for growth processes (Fitriatin et al., 2022). In this study, sunlight radiation exposure is assumed to be the same for all treatments, while the quantity of nutrients differs among treatments due to the addition of phosphate nutrients in the form of $AlPO_4$+ and varying media acidity, which can affect phosphate availability. Table 6 shows the shoot dry weight of plants resulting from the influence of PSR inoculation and media pH.

The interaction between PSR inoculation and media pH reveals a significant interaction, although the inoculation treatments did not significantly increase shoot dry weight compared to the control. The treatment KT-2 + KT-3 at pH 4.5 has the potential to increase shoot dry weight. This treatment exhibits the heaviest shoot dry weight at 478 mg, a 46% improvement over the control, but it does not show a statistically significant difference from the control, as observed in Table 6.

**Table 6. Interaction between PSR inoculation and media pH on dry weight of maize plant shoots (mg)**

| PSR Isolates | Shoot dry weight in different pH media | | |
|---|---|---|---|
| | 4.5 | 5.5 | 6.5 |
| Control | 327 AB ab<br>– | 265 A ab<br>– | 497 B c<br>– |
| KT-1 | 226 AB a<br>– | 352 B b<br>(33%) | 95 A a<br>– |
| KT-2 | 351 B ab<br>(7%) | 204 AB ab<br>– | 133 A a<br>– |
| KT-3 | 369 B ab<br>(12%) | 139 A a<br>– | 272 AB ab<br>– |
| KT-1 + KT-2 | 280 A ab<br>– | 306 A ab<br>(15%) | 432 A bc<br>– |
| KT-1 + KT-3 | 388 B ab<br>(19%) | 239 AB ab<br>– | 140 A a<br>– |
| KT-2 + KT-3 | 478 B b<br>(46%) | 211 A ab<br>– | 238 A a<br>– |
| KT-1 + KT-2 + KT-3 | 249 A a<br>– | 139 A a<br>– | 187 A a<br>– |

*Note*: Numbers followed by the same letter are not significantly different based on Duncan‘s test at a 5% significance level. Lowercase letters are read vertically, and uppercase letters are read horizontally. Percentage values in parentheses indicate the increase in plant height compared to the control

The result in Table 6 demonstrates that the combination of isolates KT-2 and KT-3 has the highest impact in increasing shoot dry weight compared to other treatments. This treatment is significantly better by 52% compared to the average single isolates at the same pH, and 112% compared to the average at other pH levels. The treatment shows the compatibility of isolates and their ability to increase the shoot dry weight of maize plants in acidic pH conditions. The KT-2 + KT-3 treatment at pH 4.5, has a higher average soluble phosphorus (P) compared to other treatments. Zhang et al. (2017) reported that an increase in soluble phosphorus in the media can enhance the uptake of micronutrients, and the shoot dry weight of maize plants. Additionally, the reported increase in shoot dry weight not accompanied by an increase in plant height (Table 5). The shoot dry weight values indicate the amount of biomass that plants can absorb during growth, leading to the shoot part of the plant. Therefore, an increase in shoot dry weight suggests the plant is growing well. In addition to the influence of soluble phosphorus, PSR colonization in plant roots also affects plant growth by increasing resistance to environmental stress.

Plant root growth is not only assessed by root length, but also by root dry weight, representing the density of photosynthates formed from nutrient uptake by the plant. Table 7 shows that the interaction between PSR inoculation and media pH has a significant interaction, and the inoculation treatments can significantly increase root dry weight compared to the control. The KT-3 treatment at pH 4.5 has the ability to increase root dry weight. This treatment exhibits the heaviest root dry weight at 218 mg, significantly better by 98% compared to the control, and shows a statistically significant difference from the control, as observed in Table 7.

**Table 7. Interaction between PSR inoculation and media pH on dry weight of maize plant roots (mg)**

| PSR Isolates | Root dry weight in different pH media | | |
|---|---|---|---|
| | 4.5 | 5.5 | 6.5 |
| Control | 110 A a<br>– | 110 A ab<br>– | 179 A b<br>– |
| KT-1 | 110 AB a<br>– | 164 B b<br>(49%) | 63 A a<br>– |
| KT-2 | 196 B b<br>(78%) | 109 A ab<br>– | 78 A a<br>– |
| KT-3 | 218 B b<br>(98%) | 68 A a<br>– | 138 A ab<br>– |
| KT-1 + KT-2 | 165 A ab<br>(50%) | 148 A ab<br>(35%) | 159 A b<br>– |
| KT-1 + KT-3 | 147 A ab<br>(34%) | 95 A ab<br>– | 81 A a<br>– |
| KT-2 + KT-3 | 187 B ab<br>(70%) | 123 AB ab<br>(12%) | 111 A ab<br>– |
| KT-1 + KT-2 + KT-3 | 116 A a<br>(5%) | 114 A ab<br>(3%) | 110 A ab<br>– |

*Note*: Numbers followed by the same letter are not significantly different based on Duncan‘s test at a 5% significance level. Lowercase letters are read vertically, and uppercase letters are read horizontally. Percentage values in parentheses indicate the increase in plant height compared to the control

The results in Table 7 shows that inoculation with KT-3 at pH 4.5 has the highest impact on increasing root dry weight compared to other treatments. This treatment is significantly better by 41% compared to the average consortium treatment at the same pH, and 111% compared to the average at other pH levels. The treatment demonstrates compatibility between isolates and their ability to enhance the root dry weight of maize plants in acidic pH conditions.

Maize plants cannot maintain the accumulation of plant dry weight under environmental stress conditions (Suwa et al., 2010). Photosynthate allocation will be used for the formation of small roots, compared to being used as a biomass contributor to plant roots (Wen et al., 2017). Root growth also plays a role in the growth of plant shoots. Roots take up nutrients from the media and transport them to support plant growth, ultimately leading to an increase in the dry weight of maize plants (Zhang et al., 2023). Additionally, root dry weight is a reflection of photosynthate accumulation during vegetative growth.

Results in Tables 7 and 2 demonstrate that root dry weight is proportional to phosphorus availability and environmental stress in the form of acidic pH. KT-3 at pH 4.5 has a soluble phosphorus content of 404.30 ppm, one of the treatments with the highest values. The treatment without isolates (control) has the lowest dry weight compared to treatments with PSR isolates, which is also related to the significantly lower levels of soluble phosphorus in the inoculation treatments. This is because phosphorus availability affects the growth of plant roots, and phosphorus availability is directly proportional to the increase in the dry weight of plant roots (Gaume et al., 2001). The root dry weight and shoot dry weight, as explained earlier, are accumulated and represent the plant's nutrient uptake synthesized into photosynthates.

The accumulation of plant photosynthates provides an insight into the utilization of sunlight radiation and the amount of nutrients available to plants for growth processes (Qin et al., 2020). Table 6 shows that PSR inoculation and media pH have a significant interaction, although the inoculation treatments did not significantly increase photosynthate accumulation compared to the control. The treatment KT-2 + KT-3 at pH 4.5 has the potential to increase photosynthate accumulation. This treatment exhibits the highest photosynthate accumulation, reaching 665 mg, a 52% improvement over the control, but it does not show a statistically significant difference from the control, as observed in Table 8.

Results in Table 8 indicate that the inoculation of KT-2 + KT-3 at pH 4.5 has the highest impact on increasing photosynthate accumulation compared to other treatments. This treatment is significantly better by 35% compared to the average single isolate treatments at the same pH, and 95% compared to the average at other pH levels. The treatment demonstrates its ability to enhance plant resilience in acidic pH media, making it an ideal environment. The ideal environment can be attributed to bacterial colonization in plant roots, which enhances plant resilience, provides phosphorus nutrients, and produces plant growth-regulating substances (Vejan et al., 2016). An ideal environment encourages plants to produce more assimilates, thereby enhancing overall plant growth (Rahman et al., 2015). According to Rahmah (2015), the increase in biomass is attributed to plants absorbing more water and nutrients, which stimulate organ development, such as roots, enabling the plant to absorb more nutrients and water. Subsequently, photosynthetic activity increases, influencing an increase in plant dry weight.

**Table 8. Interaction between PSR Inoculation and Media pH on photosynthate accumulation in Maize Plants (mg)**

| PSR Isolates | Accumulation of photosynthates in different pH media | | |
|---|---|---|---|
| | 4.5 | 5.5 | 6.5 |
| Control | 436 A ab<br>– | 375 A ab<br>– | 676 B c<br>– |
| KT-1 | 336 AB a<br>– | 516 B b<br>(38%) | 159 A a<br>– |
| KT-2 | 548 B ab<br>(26%) | 313 AB ab<br>– | 212 A a<br>– |
| KT-3 | 587 B ab<br>(35%) | 207 A a<br>– | 410 AB ab<br>– |
| KT-1 + KT-2 | 445 A ab<br>(2%) | 453 A ab<br>(21%) | 591 A bc<br>– |
| KT-1 + KT-3 | 535 B ab<br>(22%) | 334 AB ab<br>– | 221 A a<br>– |
| KT-2 + KT-3 | 665 B b<br>(52%) | 334 A ab<br>– | 349 A a<br>– |
| KT-1 + KT-2 + KT-3 | 365 A ab<br>– | 252 A ab<br>– | 297 A a<br>– |

*Note*: Numbers followed by the same letter are not significantly different based on Duncan's test at a 5% significance level. Lowercase letters are read vertically, and uppercase letters are read horizontally. Percentage values in parentheses indicate the increase in plant height compared to the control

## Conclusion

Phosphate solubilizing rhizobacteria isolates KT-1, KT-2, and KT-3 are exhibited mutual compatibility and demonstrated the capability to form biofilms on plant roots. The PSR consortium displayed synergistic attributes in augmenting parameters such as root dry weight, PSR population, and soluble phosphorus, with observed interactions between media acidity and bacterial isolates. The interaction of the KT-2 + KT-3 treatment at pH 4.5 had a significant impact, resulting in a substantial increase in soluble P (895%) and PSR population (1693%). Additionally, the KT-3 treatment had a significant effect, leading to a notable enhancement in root dry weight (98%). The interaction between the KT-2 + KT-3 treatments demonstrated effects that were not statistically different but exhibited the potential to increase root length (35%), shoot dry weight (46%), and photosynthate accumulation (52%). Conversely, inoculation treatments did not show a significant improvement in shoot height and chlorophyll content in maize plants. The combination of using this inoculant has great potential as a biofertilizer in increasing acid soil fertility and maize growth

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