BioEksakta: Jurnal Ilmiah Biologi Unsoed
Volume 2, Nomor 2 (2020): 261 - 265
E-ISSN: 2714-8564

Inoculation of Rhizobacteria to Red Chili Plant (*Capsicum annum* L.) in Saline Sandy Soil

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History Article :

Submited : 26/08/2019
Accepted : 31/01/2020

Abstract
A red chili plant (*Capsicum* spp.) is one of the horticultural commodities in Indonesia which has good economic value, so it has priority to be developed. Agriculture practices in Java island have partly switched to coastal areas which have characteristics of saline soils. Saline soils are alkaline and nutrient-poor, especially essential nutrients such as P and N. One way to restore fertility in saline soils used beneficial and fertilizing bacteria such as PGPR. Plant growth-promoting rhizobacteria (PGPR) are free-living, soil-borne bacteria, which are capable of enhancing the growth of the plant either directly or indirectly. The objectives of this research were to determain the effect of PGPR isolates to the growth of red chili plants in saline sandy soil and to determine the best PGPR isolate capable to improve the growth of red chili plant in saline sandy soil. The result showed that the inoculation of rhizobacteria significantly affected to plant height and root length of the red chili plant (Capsicum annum). The combination of 3 PGPR isolates (Azospirillum PSA 10, Azotobacter PSA 8, and Marinococcus PSA 1) was the best result in increasing the height and root length of the chili plant (Capsicum annum).
Keywords: *red chili plant (Capsicum annum), PGPR, Saline soil.*

INTRODUCTION

Salinity causes problems for plants in saline soils because of the excessive concentration of dissolved salt in the soil, which causes water difficult to be absorbed by plants, resulting in low turgor pressure. This resulted in a deterioration in plant growth, and changes in anatomical and physiological characteristics (Yulianto *et al*., 2017). The osmotic pressure of saline soil is also one of the natural problems that adversely affected plant growth (Hadijah, 2014). Coastal areas would have the potential for red chili plant cultivation if it managed properly. Certain techniques are needed to improve the growth of plant crops. One way to restore fertility in saline soils used beneficial and fertilizing bacteria such as PGPR (Widawati, 2015). Plant growth-promoting rhizobacteria (PGPR) are free-living, soil-borne bacteria, which are capable of enhancing the growth of the plant either directly or indirectly (Kanchana *et al*., 2014; Pii *et al.*, 2015).

These bacteria are also known to colonize plant roots actively. The roles of PGPR for plants are: 1) as a biofertilizer, PGPR can accelerate the process of plant growth through accelerating nutrient absorption, 2) as bio-stimulants, PGPR can stimulate plant growth through phytohormones production and 3) as a bioprotectant, PGPR protects plants from pathogens (Rai, 2006). PGPR includes several bacteria such as *Rhizobium*, *Azospirillum*, *Azotobacter*. *Azotobacter* sp. PSA 8 and *Azospirillum* sp. PSA 10 was known capable of synthesizing growth-promoting hormone or phytohormo ne such as Indole Acetic Acid (auxin), capable of fixing atmospheric nitrogen and dissolving phosphate. *Marinococcus* sp. PSA 1 was aerobic, have cocci cells, Gram-positive, irregular colony shape, and it can dissolve phosphate. The bacterium was known as saline halotolerant, which was capable of growing in medium containing 5 % NaCl (Kusumawardhani, 2018). The objectives of this research were to determaine the effect of PGPR isolates to the growth of red chili plants in saline sandy soil and to determine the best PGPR isolate is able to improve the growth of red chili plant in saline sandy soil.

MATERIAL AND METHOD

Subculture

Azospirillum sp. PSA 10 was subcultured on Caceres agar medium, *Azotobacter* sp. PSA 8 was subcultured on Ashby mannitol agar medium, and *Marinococcus* sp. PSA 1 was subcultured on Pikovskaya medium. The cultures were incubated for 2 x 24 hours at room temperature (Widawati *et al*., 2015; Hindersah *et al*., 2016).

Measurement of Salinity Soil

The saline sandy soil was sampled from the Sodong beach area, Cilacap. Soil salinity was measured using a calibrated hand refractometer (Martiningsih *et al*., 2015; Putra *et al*., 2018).

Seedling preparation

The saline sandy soils were taken then dried and sifted evenly. The sandy soil was mixed with manure with a ratio of 2: 1 then sterilized in drum sterilizer. Selected red chili seeds (Panex F1 100) with uniform size were planted on the planting

medium slowly and then covered with soil. The seedling that has been grown or already had 3-4 leaves or after 21-24 days were selected for the further experiment (Widayanthi *et al*., 2017).

Inocula Preparation

Amount of 1 loop of the culture of *Azospirillum* sp. PSA 10., *Azotobacter* sp. PSA 8 from the nutrient agar medium was inoculated to nutrient broth medium and incubated in the shaker incubator (150 rpm, room temperature) for 1 x 24 hours at room temperature. Amount of 1 loop of the culture of *Marinococcus* sp. PSA 1 was inoculated in nutrient agar medium with streak continues method then incubated for 1 x 24 hours at room temperature. Amount of 3-4 mL of aquadest was added onto the cultures that already incubated and scraped off using drugalsky, the solutions were placed in a test tube. The amount of 1 mL solution was taken and inoculated in 20 mL nutrient broth medium. The population of bacterial inoculum was determined as many as 10^8 CFU / mL by spectrophotometric method.

Inoculation of red chili plant with PGPR isolates

Selected seedling was planted in a polybag with a diameter of 25 x 20 cm which contained saline sandy soil. The roots of selected red chili seedlings were planted in the polybag in an upright position. The amount of 2 mL of liquid bacterial isolates with a population of 10^8 CFU / mL was sprayed onto the roots in each polybag (Widayanthi *et al*., 2017).

This research was conducted experimentally using a Completely Randomized Design (CRD) with 8 treatments. Each treatment was repeated 3 times. The treatments were comprised of as follows:

1. Control (chili plants without inoculation of PGPR isolates).
2. Red chili plants inoculated with *Azospirillum* sp. PSA 10.
3. Red chili plants inoculated with *Azotobacter* sp. PSA 8.
4. Red chili plants inoculated with *Marinococcus* sp. PSA 1.
5. Red chili plants inoculated with mixture of *Azospirillum* sp. PSA 10 and *Azotobacter* sp. PSA 8.
6. Red chili plants inoculated with mixture of *Azotobacter* sp. PSA 8 and *Marinococcus* sp. PSA 1.
7. Red chili plants inoculated with mixture of *Azospirillum* sp. PSA 10 and *Marinococcus* sp. PSA 1.
8. Red chili plants inoculated with mixture of *Azospirillum* sp. PSA 10, *Azotobacter* sp. PSA8, and *Marinococcus* sp. PSA 1.

Maintenance of Seedling

Maintenance of red chili seedling included watering and weeding. Watering plants were done in the morning and evening or at necessary by spraying water using a hand sprayer. Seedlings that are growing poorly or are not in good condition were replaced with other healthy seedlings. Weeding was done manually to control weeds. Maintenance of the seedling for 35 days in the greenhouse.

Measurement of Plant Growth

Measurement of plant height was done by measuring the height of chili plants from the base of the stem to the highest shoot using a ruler. The root length measurement was done by measuring the length of the root from the root base to the tip of the root using a ruler (Widayanthi *et al*., 2017).

RESULT AND DISCUSSION

The media used for the growth of red chili plants were prepared based on the results of measurements of salinity soil on the Sodong beach Cilacap. The Sodong beach soil had the salinity of 0.5 dS/m. Rachman *et al*. (2018); Sitorus (2012); Thohiron & Prasetyo (2012), stated that the salinity content is classified as very low if it has an Electrical Conductivity value <1 dS/m. Coastal areas usually contained saline soil because there were influenced by tides. Low salinity in coastal areas was due to natural leaching of salt by rainwater, because the sodium contained in saline soil was diluted.

The result of this research showed that the growth of red chili was significantly affected following the application of PGPR isolates especially in increasing the plant height and root length based on Analysis of Variance (ANOVA) at 5 %. This result indicated that PGPR isolates could provide nutrients for plants such as P and N and to produce phytohormone, so they can increase the plant growth. PGPR isolates used were might be able to trigger root development, so that supports absorbing more nutrients and affect plant height. Kusumawardhani (2018) reported that *Azospirillum* sp. PSA 10 and *Azotobacter* sp. *PSA* 8 were capable to fix nitrogen, dissolve phosphate, and produce phytohormones such as IAA, while *Marinococcus* sp. PSA 1 could fix nitrogen and dissolve phosphate. In addition, *Azospirillum* sp. PSA 10, *Azotobacter* sp. PSA 8, and *Marinococcus* sp. PSA 1 was resistant to salinity of NaCl 5 %.

Iswati (2012) found that the addition of PGPR significantly affected the root length of tomato plants. A'yun *et al*. (2013) reported that PGPR (*Azotobacter* sp.) had a significant effect on the chili plant's height. The combination treatment of PGPR isolates (*Azospirillum* sp. PSA 10, *Azotobacter* sp. PSA 8, *Marinoococcus* sp. PSA 1) showed the highest root length and plant height of 9.4 and 14.9 cm(Table 1). This indicated that the PGPR consortium was able to stimulate the chili (*C. annum*) plant height. Anggarwulan *et al*. (2008) also found that the consortium of PGPR isolates was able to stimulate plant growth because bacterial

consortium has an excellent synergistic correlation in the availability of N and P elements. Aiman *et al*. (2017) and Mukhtar *et al*. (2019) reported that *Azospirillum* sp., *Azotobacter* sp., and *Marinococcus* sp., could fix nitrogen, dissolve phosphate, and produce phytohormone thus if the plant inoculated by PGPR consortium produced better plant yield.

Table1. The Average of Red Chili Root Length and Plant Height Inoculated with PGPR isolates

Treatments	Root Length (cm)	Plant Height (cm)
Kontrol	3.6667 b	9.0000 b
PSA 10	6.6000 ab	13.6333 a
PSA 8	4.6667 b	12.8333 a
PSA 1	4.5667 b	13.5667 a
PSA 10 + PSA 8	5.0000 b	11.1667 ab
PSA 10 + PSA 1	5.1667 b	12.6667 a
PSA 8 + PSA 1	3.7333 b	12.3333 ab
PSA 1 + PSA 8 + PSA 10	9.4000 a	14.9333 a

Notes: The numbers accompanied by different letters indicate significant differences at LSD 5 %.

A consortium of PGPR bacteria (*Azospirillum* sp. PSA 10, *Azotobacter* sp. PSA 8, *Marinoococcus* sp. PSA 1) were known to have an ability to fix nitrogen, dissolve phosphate, and produce phytohormones such as IAA (Kusumawardhani, 2018). IAA is an active form of the auxin hormone found in plants and served to enhance cell development, stimulate the formation of new roots and stimulate growth.

PGPR saline-resistant bacteria can help plant growth in extreme environments to obtain the required elements such as N and P. The N element is absorbed by plant roots in the form of ammonia (NH_3), but N element was available in the form of N_2 in the air. The mechanism of N fixing by the PGPR bacteria was by converting N_2 from the atmosphere into ammonia aided by the nitrogenase enzyme (Widawati *et al*., 2015). PGPR hydrolyze organic P into inorganic forms, such as in the form of orthophosphate (H_2PO_4 and HPO_4^{2-}), through the secretion of organic acid, produce phosphatase enzymes such as phosphomonoesterase enzymes and phytase enzymes which responsible for the hydrolysis of organic phosphate into inorganic phosphates. The presence of supply N and P elements as nutrients available in the soil can, therefore, help the plant growth.

Based on Least Significant Difference (LSD) analysis at 5 % of plant height and root length of red chili plant (*C. annum*) showed that the combination treatment of PGPR isolates showed the best result followed by *Azospirillum* sp. PSA 10, *Marinoccocus* sp. PSA 1, and *Azotobacter* sp. PSA 8. Kusumawardhani (2018) reported that *Azospirillum* sp. PSA 10, *Marinoccocus* sp. PSA 1, and *Azotobacter* sp. PSA 8 was capable of fixing nitrogen. *Azospirillum* PSA 10 was able to dissolve phosphate higher than *Azotobacter* sp. PSA8. According to Oedjijono *et al.* (2012), members of the genus *Azospirillum* sp. can fix nitrogen more dominant than other nitrogen-fixing bacteria. Widawati *et al.* (2015) stated that *Azospirillum* sp. bacteria could live and spread widely in the rhizospheric area in several soil ecosystems. Their population was higher in plant rhizosphere than other rhizospheric bacteria.

The ability of *Marinococcus* sp. PSA 1 in dissolving phosphate was higher than *Azotobacter* sp. PSA 8 (Kusumawardhani, 2018). *Marinococcus* sp. is moderate halophilic bacteria means that the bacterium could adapt to a wide range of salt concentrations (Shin *et al*., 2016). *Marinococcus* sp. had a metabolite that functions as osmoprotectant such as ectoine, which able to reverse the plant growth inhibition caused by osmotic stress (Rai, 2000).

CONCLUSION

Based on the results obtained, it can be concluded that PGPR isolates affected the height and root length of Chili plant in saline sandy soil. The combination of PGPR isolates of *Azospirillum* sp. PSA 10, *Azotobacter* sp. PSA 8, and *Marinococcus* sp. PSA 1 showed the highest result in increasing the height, and root length of Chili plants in saline sandy soil.

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