JOURNAL OF DEGRADED AND MINING LANDS MANAGEMENT
ISSN: 2339-076X (p); 2502-2458 (e), Volume 6, Number 3 (April 2019):1771-1777
DOI:10.15243/jdmlm.2019.063.1771

Research Article

Application of bacterial isolates to mitigate the effects of salt stress on red chilli growth and yields

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Received 25 January 2019, Accepted 11 March 2019

Abstract: The objective of this research was to study the effect of bacterial isolates on red pepper at the saline condition. The research conducted in the station of Brawijaya University in Jatikerto village, Malang regency. The study used a randomized block design with bacteria isolated from the soil of saline-prone regions of Lamongan, in coastal East Java Indonesia. The treatments consisted of P0: saline soil without bacteria, P1: saline soil + bacteria SN 13, P2: saline + bacteria SN 22, P3: saline soil + bacteria SN 23, P4: saline soil + bacteria (SN 13 + SN 22), P5: saline soil + bacteria (SN 13 + SN 23), P6: saline soil + bacteria (SN 22 + SN 23), and P7: saline soil + bacterial (SN 13 + SN 22 + SN 23). Red chilli variety used was Gada MK F1. The results showed that the application of bacterial isolates increased leaf area, dry weight of roots and shoot. Uptake of N, P, K and Na also increased with bacterial isolate application. The number of fruit and fruit weight increased by 11.94% and 15.93%, respectively, compared with no bacteria. Proline content increased with the application of bacterial, while the capsaicin content decreased.

Keywords: *Bacillus sp., Bacillus megaterium, capsaicin, proline, Streptomyces sp.*

To cite this article: Aini, N., Yamika, W.S.D., Aini, L.Q. and Wahyuningsih, P. 2019. Application of bacterial isolates to mitigate the effects of salt stress on red chilli growth and yields. J. Degrade. Min. Land Manage. 6(3): 1771-1777, DOI: 10.15243/jdmlm.2019.063.1771.

Introduction

Soil salinity in agriculture related to the high salt dissolved in the rhizosphere. The soil is saline when the electrical conductivity of the saturated groundwater extract is more than 4 dS/m. The total saline area in Indonesia of 9.5 million hectares is the potential for agriculture, but only 4.186 thousand hectares are used for agriculture (Alihamsyah, 2004). Salinity causes ionic stress, osmotic stress, and secondary stress. The most important ion stress is poisoning by Na^+. Excessive Na^+ ion in the root surface can inhibit K^+ uptake by roots. Potassium is an important macronutrient that plays essential roles related to the osmotic adjustment, to maintaining turgor and to regulating the membrane potential, cytoplasmic homeostasis, protein synthesis and enzyme activation under salt stress (Almeida et al., 2017; Hasanuzzaman et al., 2018). Salinity causes reduced growth and production of many crops because salt inhibits plant photosynthesis, protein synthesis and lipid metabolism (Paul and Lade, 2014). Plants accumulate a large number of different types of compatible solutes in response to various pressures. In addition, salt-stressed plants accumulate various molecules found in organic matter such as proline, glucose and glycine betaine as in quaternary compounds in the cytosol for osmoregulation to occur and thereby protecting macromolecules and enzyme activity (Mittler, 2002; Jha and Subramanian, 2013). An environmentally sound effort to mitigate slat stress on plants is the bacterial utilization of PGPR (Plant

Growth Promoting Rhizobacteria). PGPR colonies in plant roots are beneficial for improving plant health and promoting plant growth. Microorganisms around plant roots that live in saline conditions are capable of increasing plant tolerance to saline stress. A preliminary salinity study conducted at Sidomukti Village, Brondong District, Lamongan Regency obtained nine isolates of bacteria tolerant to 5% saline. These bacterial isolates have the potential to increase plant growth under saline conditions. The bacterial isolates proved capable of fixing free nitrogen and phytohormone synthesis such as IAA (Indole Acetic Acid). Cahyaty et al. (2017) found that rhizosphere bacteria identified as *Bacillus megaterium*, isolated from the saline field, proved capable of fixing free nitrogen and synthesizing Indole acetic acid (IAA). The application of *Bacillus megaterium* to cucumber plants on saline conditions can improve growth and yield. The PGPR increased plant growth through the production of phytohormones, increased nutrient uptake, enhanced stress resistance, biocontrol of both major and minor plant pathogens and improved water status (Kumar et al., 2015).

The use of saline tolerant varieties and bacterial applications is expected to improve crop yield on saline conditions. Research on the use of bacteria to increase plant growth in saline conditions has been widely conducted but there is little information on utilizing endemic saline bacteria to increase plant growth under saline conditions. Chilli is an important source for vitamins (A, D3, E, C, K, B2 and B12), protein, fibres, lipid, mineral salts (Ca, P, Fe, K) and Capsaicin (Chigoziri and Ekefan, 2013; Orobiyi et al., 2015). Capsaicin content of chilli gives a spicy flavour to the fruit and are used in sauces and other benefits. According to Yamika et al. (2016), Gada MK is one of red chilli variety that has saline tolerant. Saline tolerant varieties and bacterial applications expected to increase the ability of plants to overcome ionic stress. Bacterial applications in this study are expected to increase the ability of plants to overcome stress on saline conditions. The objective of this study was to elucidate the effect of bacterial isolates on red chilli at the saline condition.

Materials and Methods

Experimental site and plant material

The experiment was conducted at the experimental station of Brawijaya University at Jatikerto village, Kromengan sub-district, Malang Regency in September-January 2017. The experimental station is located at an altitude of ± 303 m above sea level with a daily average temperature of 21-33ºC, and EC growing medium of ±4.5 dS/m. The treatments tested were P0: without bacteria, P1: bacteria SN 13, P2: bacteria SN 22, P3: bacteria SN 23, P4: bacteria (SN 13+ SN 22), P5: bacteria (SN 13+ SN 23), P6: bacteria (SN 22 + SN 23), and P7: bacteria (SN 13 + SN 22 + SN 23). The eight treatments were arranged in a randomized block design with four replicates. The bacteria were isolated from the soil of saline-prone regions of Lamongan coastal in East Java Indonesia. One of them was identified to the species level that was the isolate of SN 22 that has a similarity of 99% with the species of *Bacillus megaterium*. While bacteria isolate of SN 23 was identified to the level of a genus *Bacillus* with a 99% similarity, and isolate SN 13 was from the genus of *Streptomyces*. Bacterial isolates were applied in the root areas with a density of 10^9 CFU/mL and a concentration of 20 mL/plant. The number of plants per treatment was nine.

Soil preparation

Air-dried soil was mixed with cow manure with a ratio of 5: 1. Sterilization of soil using fumigation with formalin 4% (30 L/kg soil). The soil-manure mixture was then covered with polyethylene plastic for 7 days and stirred every 3 days. After 7 days, the plastic cover was opened, and the soil-manure mixture was dried. The planting media used was a non-saline soil that was simulated into a saline soil. Soil salinisation was done by gradually adding NaCl solution to obtain a planting medium EC value of ± 4.5 dS/m (saline medium). To achieve EC ± 4.5 dS/m, NaCl solution was splashed onto the mixed soil medium 4 times with a concentration of 1000 ppm (10 g NaCl dissolved into 1 litre of water). The soil mixture was then placed in a polybag with a weight of 10 kg.

Planting preparation and treatment applications

Red chilli variety grown was Gada MK F1. The chilli variety is known as tolerance to salinity stress. The chilli seeds were soaked in warm water for 1 hour to clean the pesticide. The seeds were then soaked in a suspension containing bacterial isolate according to the treatment with a density of 10^9 CFU/mL for 2 hours. The chilli seeds were sown on a polybag by using mixed media of soil and cow manure with ratio 1:1. Seedlings were planted at 28 DAS (day after sowing) with criteria of seeds having 3-5 leaves, normal growth, and vigorous. Application of bacterial isolate was 5 times, i.e. at the time of soaking of seeds along with transplanting, then 1, 2 and 3 weeks after transplanting. The bacterial suspension sprayed in a root zone of 20 ml per polybag. It was done during the afternoon to avoid high temperatures during the day.

Maintenance of plant and harvest

The plants were irrigated daily with the field capacity conditions. The EC of medium retained about 4 dS/m by adding salt water at ages 4 and 5 WAP (weeks after planting) with a concentration of 5000 ppm. Chilli plants were fertilized 2 times at age 1 WAP as much as 3 g per polybag and 4 WAP of 3 g per polybag using NPK 15-15-15 fertilizer. The lateral shoots were removed two weeks after transplanting. Chilli fruit was harvested with the criteria of 95% -100% red fruit by picking fruit and stem, with harvest interval 3-5 days until all fruits were run out.

Measurement of plant growth and N, P, K, Na uptake

Leaf area per plant, measured using a Leaf Area Meter (Li-3000 type) at 4 and 10 WAP. The dry weight of shoots and roots was weighed at the age of 4 and 10 WAP after drying at 80° C for ± 2 x 24 hours until a constant dry weight was reached. The number of fruits formed was recorded by counting the number of fruits that have been 75% red. Observation of the number of fruits was carried out at intervals of 5 days. Fruit weight per plant was weighed using an analytical scale. Analysis of leaf proline content using a method of Bates (1973). Furthermore, shoots and roots were analyzed for total N accumulation by the Kjeldahl method (Saez-Plaza et al., 2013), total P, and K (dry oxidation method). P concentration was measured using spectrophotometer UV-VIS, and K concentration using flame photometer (Thamrin et al., 2013). Capsaicin levels in fruits were measured according to the procedure of Othman et al. (2011) using High-Performance Liquid Chromatography (HPLC).

Statistical Analysis

The data obtained from the observation were analyzed by using variance analysis (F test) at α = 5%, followed by honestly significant difference (HSD) test at α = 5%.

Results and Discussion

Effects of bacterial isolate on plant growth and yield

The leaf area of red chilli is presented in Table 1. At 4 and 10 WAP, application of bacteria isolates of SN 13, SN 22, and SN 23 bacterial isolate either in single or in combination, affected leaves area. At the age of 4 WAP, leaf area of combination treatment of SN 22 + SN 23, and combination treatment of SN 13 + SN 22 + SN 23 showed higher leaf area with 437.78 cm^2 and 519.62 cm^2/plant, respectively than without application of bacteria (263.51 cm^2). However, among the bacterial isolates did not show significant differences. Similarly, at the age of 10 WAP, the area of red chilli leaves with bacterial isolate application was higher than control. The combination of SN 13 + SN 22 isolates resulted in a higher leaf area of (927.01 cm^2) than other treatments.

Table 1. The leaves area of red chilli due to the effect of endemic saline bacterial at various ages

Treatments	**Leaf area (cm^2/plant)**	
	4 WAP	**10 WAP**
Control	263.51 a	304.86 a
SN 13	366.34 ab	639.70 bc
SN 22	355.70 ab	663.16 bc
SN 23	423.38 ab	682.28 bc
SN 13 + SN 22	389.94 ab	927.01 d
SN 13 + SN 23	358.99 ab	549.40 b
SN 22 + SN 23	437.78 b	636.80 bc
SN 13 + SN 22 + SN 23	437.78 b	793.30 cd
HSD 5%	174.02	161.83

Remarks: Numbers followed by the same letters mean not different based on HSD test at α = 5%; WAP = weeks after planting

Root and shoot dry weight of chilli plant due to the treatment of isolates of endemic saline bacteria can be seen in Tables 2 and 3. Applications of the bacterial isolate could increase the dry weight of chilli roots on saline conditions. At the age of 4 WAP, the weight of roots of red chilli with the treatment of bacterial endemic saline isolate was higher than treatment without saline endemic bacterial isolate. The application of endemic saline bacteria of SN 23 showed the best effect on root dry weight. The treatment of endemic saline bacteria of SN 22, combination of SN 13 + SN 22, SN 22 + SN 23, and SN 13 + SN 22 + SN 23 showed no significant different (Table 2). The endemic saline bacteria had a significant effect on the biomass of the chilli plant. At 4 and 10 WAP, the highest plant biomass was produced by chilli plant inoculated with endemic saline bacteria of SN 13+SN 22 and SN 13+SN 22+SN 23 (Table 3). The increase in leaf area and plant biomass as a result of the application of salt-resistant bacteria isolates was related to the ability of the bacteria to fix free nitrogen and produce IAA. IAA hormones have a function in extending and enlargement of cells to stimulate the vegetative growth of plants of red chilli. Preliminary research results showed that isolates of SN 13, SN 22 and SN 23 were able to synthesize IAA.

Table 2. Dry weight of red chilli roots due to the effect of endemic saline bacterial at various ages

Treatments	**Root dry weight (g/plant)**	
	4 WAP	**10 WAP**
Control	0.60 a	2.25 a
SN 13	0.87 ab	2.83 ab
SN 22	1.09 bc	2.86 ab
SN 23	1.33 c	2.88 ab
SN 13 + SN 22	1.03 bc	3.18 b
SN 13 + SN 23	0.81 ab	3.18 b
SN 22 + SN 23	1.10 bc	3.15 ab
SN 13+SN 22+SN 23	1.20 bc	3.13 ab
HSD 5%	0.42	0.91

Remarks: Numbers followed by the same letters mean not different based on HSD test at $\alpha = 5\%$; WAP = weeks after planting

Table 3. Dry weight of red chilli shoots due to the effect of endemic saline bacteria at various ages

Treatments	**Shoot dry weight (g/plant)**	
	4 WAP	**10 WAP**
SN 13	3.54 bc	19.13 ab
SN 22	3.44 bc	18.56 ab
SN 23	2.74 ab	19.21 ab
SN 13 + SN 22	4.16 c	20.69 b
SN 13 + SN 23	3.41 bc	19.09 ab
SN 22 + SN 23	3.39 bc	19.96 ab
SN 13+SN 22+ SN 23	3.90 c	20.44 b
HSD 5%	1.09	6.42

Remarks: Numbers followed by the same letters mean not different based on HSD test at $\alpha = 5\%$; WAP = weeks after planting

IAA helps in the production of longer roots with an increased quantity of root laterals and root hair, which are involved in nutrients uptake (Datta and Basu, 2000; Jatav et al., 2017). The wider root surface will affect the absorption of water and nutrients from the soil more, allowing more water and nutrient absorption to increase plant growth. This result was in line with Goswami et al. (2014) PGPR of groundnut by *Bacillus licheniformis* strain A2 showed 39% increase in root length, 43% increase in fresh biomass and 44% increase in dry biomass under saline biomass. The plants inoculated with a combination of endemic saline bacteria of SN 13 + SN 22 + SN 23 and SN 13 + SN 23 had the higher number of fruits, i.e. the average of 39.50 and 35.25 fruits/plant, compared to number fruits of chilli with no addition of bacteria (Table 4). In addition, fruit weight of chilli fruit also increased with the application of bacteria. Egamberdiyeva et al. (2017) reported that the application of the plant growth promoting rhizobacteria *Pseudomonas chlororaphis* TSAU13 and *Pseudomonas extremorientalis* TSAU20 strains significantly increased fruit yield of tomato compared to un-inoculated plants under saline condition. The bacteria rhizosphere have the ability to produce growth regulating substances of IAA and to increase P availability, as well to induce N and P assimilation. The results of research Cahyaty et al. (2017) showed that the application of rhizosphere bacteria on saline soil increased the weight of fruit per plant 16.1% compared with no bacterial application.

Table 4. Number and weight of fruit due to the effect of endemic saline bacterial

Treatments	**Number of fruit**	**Weight of fruit (g/plant)**
Control	18.00 a	118.82 a
SN 13	31.25 ab	284.13 b
SN 22	33.50 b	320.10 b
SN 23	32.25 b	261.66 ab
SN 13 + SN 22	35.25 b	273.52 b
SN 13 + SN 23	32.80 b	273.96 b
SN 22 + SN 23	28.50 ab	297.21 b
SN13+SN22+SN 23	39.50 b	301.28 b
HSD 5%	13.75	148.99

Remarks: Numbers followed by the same letters mean not different based on HSD test at $\alpha = 5\%$; WAP = weeks after planting

In this study, the application of rhizosphere bacteria was capable of increasing the production of red chilli on saline conditions. Yao et al. (2010) reported that application of rhizosphere bacteria helps the growth of plants grown in saline soil. The high yield of a plant is affected by plant growth. Based on the result of this study, the application of rhizosphere bacteria was able to increase the area of leaves and biomass of red chilli plants. Yamika et al. (2017) reported that grain yield is the effect of plant growth. Application of gypsum on varieties of Argopuro/IAC, 100 increased dry weight of grain yield under saline condition.

Effects of bacterial isolate on N, P, K, Na uptake

Saline endemic bacteria increased the absorption of P, K, and Na nutrients. The highest P absorption was found at chilli inoculated SN 13+SN 23 bacteria, while the highest K absorption occurred at chilli plant inoculated with SN 22+SN 23 bacteria. The highest Na absorption was found at chilli plant with the treatment of SN 13+ SN 22 bacteria (Table 5). The increasing P, K, Na absorption might be related to the role of the bacteria in producing Indole Acetic Acid (IAA). The role of IAA hormone is increasing root growth and development. While the spread of chilli root affected water and nutrition absorption of the plant. This study was in line with Kumari et al. (2018) who found that both the identified species *P. aeruginosa* BHU B13-398 and *B. subtilis* BHU M were found to enhance most of the growth characteristics associated with PGPR including phosphate solubilization, IAA, ammonia, siderophore, and HCN production. Gechemba et al. (2015) reported that the species of *Pseudomonas* and *Bacillus* have the potential for phosphate solubilization, IAA production, and siderophore activity. Egamberdiyeva (2007) reported that the rhizosphere bacteria strains of *Pseudomonas alcaligenes* PsA15, *Bacillus polymyxa* BcP26, and *Mycobacterium phlei* MbP18 had a much better stimulatory effect on plant growth and nitrogen (N), phosphorus (P) and potassium (K) uptake by maize in saline soil.

Table 5.Total N uptake, P, K, and Na of root due to the effect of endemic saline bacteria

Treatments	Nutrient uptake (mg/plant)			
	N	P	K	Na
Control	5.40	0.59 a	4.10 ab	1.73 a
SN13	7.10	0.74 ab	3.54 a	3.08 b
SN 22	6.44	0.66 ab	4.32 ab	2.40 ab
SN 23	5.81	0.66 ab	3.94 ab	2.30 ab
SN13+SN22	6.05	0.57 a	4.01 ab	2.61 b
SN13+SN23	6.00	0.86 b	3.30 a	2.03 a
SN22+SN23	6.90	0.63 a	5.10 b	2.05 a
SN13+SN22+SN23	6.25	0.75 ab	4.75 ab	2.09 a
HSD 5%	Ns	0.26	1.78	0.89

Remarks: Numbers followed by the same letters mean not different based on HSD test at α = 5%; WAP = weeks after planting. ns = not significant

Effects of bacterial isolate on proline and capsaicin contents

Inoculation of bacterial isolates of SN 13 and SN 23 increased proline content of chilli leaves by 101.25 µg/g and 102.75 25 µg/g, respectively. On the contrary, chilli plant with no addition of bacteria (control) had the lowest proline content (49.75 µg/g) (Figure 1). This result was in line with the study of El-Samad et al. (2010) that protein and amino acids of shoots and roots of salinized maize and broad bean plants were accompanied by a marked increase in the proline content. Proline contents in shoots and roots of both cultivars increased significantly with increased salinity. In addition, Han and Lee (2005) reported that the increase of PGPR plant tolerance to saline stress was due to the accumulation of osmoregulatory compounds such as proline in plant tissue. The results showed that the application of bacterial isolates either single or combination decreased proline content on red chilli compared to no bacterial isolate application. Single bacterial isolate application showed higher proline content than the combination of isolate application. This result was supported by research of Cahyaty et al. (2017) that the bacteria SN 22 application decreased proline content of cucumber compared to without bacterial isolate application. The capsaicin content of red chilli was lower with the application of bacterial isolate than without bacteria isolate application (Figure 2).

The application of a single bacterial isolate also resulted in lower capsaicin content than the combination of bacteria isolates. Capsaicin content on red chilli increased with the high salinity stress condition compared to no salinity stress condition (Arrowsmith et al., 2012). According to Jalil and Ansari (2018), plants and microbiomes are interacted with each other through the different metabolic cross and formed stress tolerance strategies. Microbes produced various metabolites as signals during stress conditions, and plants have a mechanism to recognize certain compounds released by microbes and also activate defence mechanisms in response to stressful conditions.

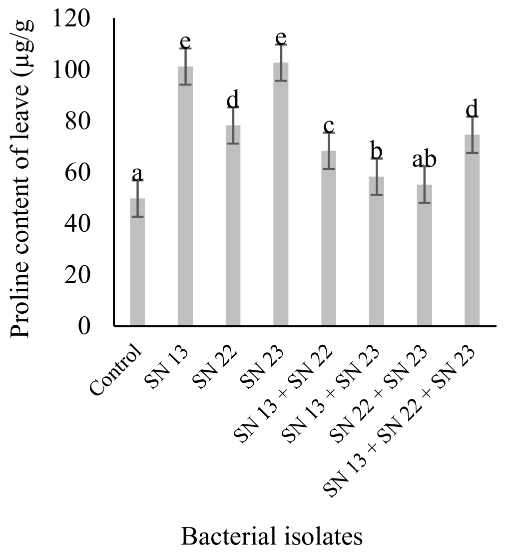

Figure 1. Proline content of chilli due to the application of bacterial isolates at 4 weeks after transplanting

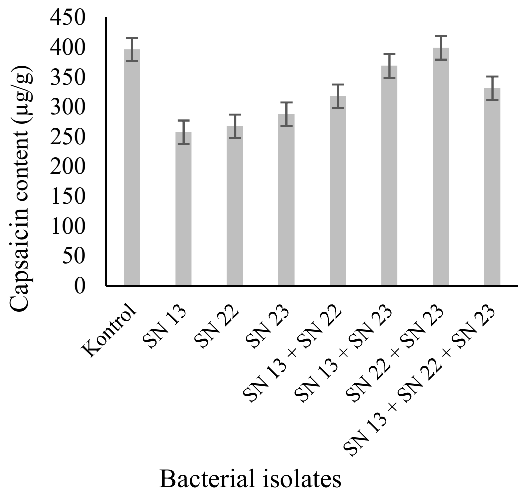

Figure 2. Capsaicin content of red chilli due to the application of bacterial isolates at 10 weeks after transplanting

Conclusion

Application of saline endemic bacteria increased the yield of red chilli and had potential as biofertilizer on the plant under saline condition. For future recommendation, the application saline endemic bacteria could have a positive effect on mitigating the reduction of plants growth and production caused due to saline stress.

Acknowledgements

This study was supported by the research grant of the Ministry of Research, Technology, and Higher Education through the Competitive Research Grant Program for Decentralization Research 2018 (338.139/UN10.C10/PN/2018). The authors thank Elok Sukmarani, a graduate of Faculty of Agriculture, Brawijaya University.

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