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# Application of saline tolerant bacteria and soil ameliorants improved growth, yield and nutrient uptake of tomato in saline land

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## Abstract

Salinity is one of the problems in agricultural sector widespread in almost all countries in the world, which could reduce crops yield. This research was carried out to study the effect of frequency application of consortium saline-tolerant bacteria isolates with different soil ameliorants on growth and yield of tomato grown saline lands. Research was conducted using split plot design with three replications. Soil ameliorants (Cow manure and gypsum) were the main plot while sub-plots were frequency applications of consortium bacterial isolates (without bacterial, once, twice, three and four times). In this study, bacteria consortium was SN13 (*Streptomyces* sp.) + SN22 (*Bacillus* sp.) and SN23 (*Corynebacterium* sp.) which isolated from the soil of saline-prone regions of Lamongan, in coastal East Java Indonesia. Results showed that gypsum and cow manure as soil ameliorant did not give significant effect on yield and nutrient uptake of tomato while application of saline-tolerant bacteria was able to improve yield and nutrient uptake of tomato. However, the four times application of saline-tolerant bacteria significantly improved plant height (23.36 %), leaves area (96,49 %), total chlorophyll content (11.86 %), dry weight of plant (103.59 %), fresh weight of fruit (85.51 %), N uptake (135.22 %), P uptake (132.99 %), K uptake (141.68 %) and declined Na uptake (16.28 %), compared to without bacteria application. In conclusion, four times application of saline-tolerant bacteria can alleviate salinity stress of tomato plants in saline lands

## Keywords: Bacillus sp., Chlorophyll content, Na uptake, Streptomyces sp.

**Abbreviations:** ACC\_ 1-aminocyclopropane-1-carboxylic acid; APX\_ ascorbate peroxidase; ATP\_ adenosine tri-phosphate; CAT\_ catalase; CEC\_ cation exchange capacity; CFU\_ colony forming unit; EC\_ Electrical Conductivity; IAA\_ indole-3-acetic acid; HSD\_ honestly significant different; MDA\_ malondialdehyde; PGPR\_ plant growth promoting rhizobacteria; SAR\_ sodium adsorption ratio; SOD\_ superoxide dismutase; TDI\_ total dissolved ion; TDS\_ total dissolved solids

## Introduction

Saline soils, the soils with high concentration of soluble salt, have the ability to decrease the growth and yield of crops. Salinity can be determined by measuring electrical conductivity (EC) of soil water extract, soluble ion concentrations of soil water, mass of total dissolved solids (TDS), or total dissolved ion (TDI) (Hardie and Doyle, 2012). Soil salinity can be classified based on the value of electrical conductivity (EC). Soil can be regarded as saline when it has electrical conductivity more than 4 dS m<sup>-1</sup> (equivalent to 40 mM NaCl) (Shrivastava and Kumar, 2014). Water soluble salts such as Sodium (Na<sup>+</sup>), Magnesium (Mg<sup>2+</sup>), Calcium (Ca<sup>2+</sup>), Potassium (K<sup>+</sup>), Chloride (Cl<sup>-</sup>), Sulfate (SO<sub>4</sub><sup>2-</sup>), Carbonate (CO<sub>3<sup>2-</sup></sub>) and Bicarbonate (HCO<sup>3-</sup>) accumulated in the soil can potentially cause soil salinity are which rises in the dry season (Sabareshwari and Ramya, 2018; Paul and Lade, 2014). Accumulation of sodium (Na<sup>+</sup>) in the soil causes deterioration of physical properties, such as changes in exchangeable soil solutions ion and soil pH, destabilization of soil structure and increased susceptibility to crusting, run off and erosion. It also causes nutritional imbalances in plant (Qadir and Schubert, 2002). Salinity stress affects morphological, physiological and biochemical conditions of a

plant (Asghari and Ahmadvand, 2018). Salinity has been reported to decrease photosynthetic rate, total chlorophyll content and cell membrane stability in wheat (Bagdi et al., 2011; Yadav et al., 2011). The main effect of salinity stress is water deficit caused by lowered osmotic potential of soil water, limiting the capacity of plant in absorbing water (Maksimovic and Ilin, 2012; Unlukara et al., 2017). Nutrient availability is one of factors affecting plant growth and development. Salinity stress causes nutrient imbalance of a plant. Salinity increases Na accumulation in plant tissue and reduces nutrient essential such as nitrogen and potassium content (Sathee et al., 2013; Talei et al., 2012; Gomes et al., 2011). With the right management, saline soil can be used for agricultural purposes. However, the productivity of saline soil is still low. Therefore, proper technological innovations are needed to increase the productivity of saline soils. Approaches which can be employed to alleviate the effect of salinity stress are plant selection, application of soil ameliorant, and the use of biotechnology such as salinetolerant bacteria. Plants have different tolerance level to salinity stress. Plants can be classified into sensitive, moderately sensitive, moderate tolerant and tolerant, according to their ability to grow in different levels of salinity (Grieve et al., 2014). Saline soils can be reclaimed by adding material (organic or inorganic) called ameliorant which can improve the physical or chemical characteristic of the soil. Ahmed et al. (2015) reported that soil physical-chemical characteristics, such as bulk density, organic matter, hydraulic conductivity, pH, EC, SAR and fodder beet yield were significantly improved by the application of soil ameliorants presented as gypsum and farm manure under saline-sodic conditions. Ameliorant decreases salinity level by replacing Na<sup>+</sup> with another ion such as Ca<sup>2+</sup>. The common ameliorants used in saline soil are gypsum and cow manure. Gypsum has been reported to be able to decrease level of Na<sup>+</sup>, EC, pH and Sodium Adsorption ratio (SAR) (Abdel-Fattah, 2012; Cha-um et al., 2011; Daur and Tatar, 2011). Cow manure is usually applied to increase C-organic of soil. Increasing organic material would decrease the amount of Na in the soil through Na absorption by colloids of organic material. Organic manure is used as a long-term ameliorant for degrading the negative effect of saline soil (Buttar et al., 2017).

The use of biotechnology, such as the application of microorganism, plays an important role as bio stimulant and bio fertilizer. It is an alternative to improve plants tolerance to salinity stress. Some bacteria can increase plant growth under salinity stress through mechanisms of producing phytohormones such as auxin, IAA and ACC deaminase (Egamberdieva et al., 2009; Igbal et al., 2016; Pourbabaee et al., 2016; Yu et al., 2016). Rhizobacteria has also been reported to increase salt-tolerance in plant through regulation of plant antioxidant enzymes such as SOD, CAT, APX, and MDA, which reduce oxidative stress in plant (Yilmaz and Kulaz, 2019). Utilization of indigenous bacteria from areas containing high salinity will be more effective than introducing bacteria from other environments due to their tolerance to saline condition. Some bacteria isolated from rhizosphere of plants grown in saline land can be called saline-tolerant bacteria. Combining the effect of applying saline-tolerance bacteria and soil ameliorant is expected to be more effective for alleviating salinity stress. Furthermore, the effect of applying saline-tolerant bacteria with different frequencies combined with ameliorants has not been reported. Therefore, this research was conducted with a hypothesis that frequency of applying of saline-tolerant bacteria on different ameliorants can affect growth, yield and nutrient uptake of tomato in saline land.

## **Results and discussion**

## Growth and Yield of tomato

Salinity stress decreases growth and yield of plant. The results (Table 1.) showed that gypsum and cow manure as soil ameliorant had the same effect on the growth and yield of tomatoes in saline land, while the application of consortium saline tolerant bacteria significantly increased the growth and yield of tomatoes in saline land. This research revealed that three- and four-times application of consortium saline tolerance bacteria were able to increase plant height up to 23% and both leaves area and dry weight of plant up to 90 %, compared to control treatment. Increasing growth of plant due to the application of bacteria was also reported by Egamberdieva et al. (2017) suggesting bacteria that inoculation of from Pseudomonas extremorientalis can increase plant height of tomato plants up to 27% under saline conditions. Mayak et al. (2004) had

also reported that the application of PGPR bacteria could increase both the fresh and dry weight of tomato plants in saline stress conditions.

The research showed that the application of consortium saline tolerance bacteria was able to improve the yield of tomato (Table 1). Fresh weight of fruit per plant increased by 85.51 % with four times application of saline tolerance bacteria, compared to control treatment. It showed that fresh weight of fruits was increased in line with the application of saline-tolerant bacteria. Egamberdieva et al. (2017) also reported that inoculation of *Pseudomonas extremorientalis* increased the fresh weight of tomato fruit per plot  $(1 \text{ m}^2)$  by 22%, compared to un-inoculated plants.

The total chlorophyll content of tomato leaves was measured on reproductive stage (Figure 1.). It showed 8-13% increase due to the application of consortium salinetolerant bacteria for all frequency. Total chlorophyll content of tomato leaf decreased under salinity stress (Taffouo et al., 2010). There was an increase in total chlorophyll content through the application of saline-tolerant bacteria. Total chlorophyll content was widely influenced by nutrient sufficiency. The plant response to high amounts of nitrogen was increase in the green pigment, which is in line with the total chlorophyll. Similarly, the increasing of chlorophyll content under salinity stress due to application rhizobacteria was also reported by Nadeem et al. (2006) on maize and Metwali et al. (2015) on faba bean.

Four times application of consortium saline tolerance bacteria also improved fruit quality according to the value of total sugar. The total sugar was increased equally with the application frequency of consortium saline tolerance bacteria. It means that the application of bacteria can improve the quality of tomato fruits under salinity stress. Shen et al. (2012) showed that application of PGPR bacteria from Bacillus pumilis species was able to improve the quality of tomato fruits of irrigated salt-water seeds by increasing total dissolved sugars. The results of research by Rodriguez et al. (2018) showed that inoculation of PGPR from Bacillus sp. was able to increase total dissolved solids in tomato fruits compared to plants un-inoculated with bacteria and inoculated with Pseudomonas and Aeromonas sp. The results of the research showed that the total sugar content ranged from 8.8 – 11.5%. This value is still higher compared to the standard total value of tomato sugars at full maturity level, which is about 9.00 % (Teka, 2013).

Bacteria have their own mechanism to adapt or live in stressed environments such as saline soil. They also have a mechanism to help plant grow well under salinity stress by playing their roles as bio-stimulant and bio-fertilizer. Bacteria are able to stimulate plant growth through the production of indole acetic acid (IAA) which has an important role in stimulating root growth. Patten and Glick (2002) reported that inoculated canola seeds using IAAproducing bacteria from Pseudomonas peptide species increased root length compared to un-inoculated plants and inoculated plants with mutant bacteria. Iqbal et al. (2016) reported that auxin-producing bacteria from also Pseudomonas fluorescens and Serratia proteamaculans were able to increase the grain yield of maize up to 31 %. In addition, one of the plant responses to salinity stress is the overproduction of ethylene, which can inhibit plant growth. Bacteria from rhizobacteria species have been known to be able to produce Aminocyclopropane-1-Carboxylate Deaminase (ACC Deaminase) enzyme, which might regulate

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Treatments	Plant Height (cm)	Leaf Area (cm² plant¹)	Dry Weight of Plant	Fresh Weight of Fruits
			(g plant <sup>-⊥</sup> )	(g plant <sup>-1</sup> )
Soil				
Ameliorant				
Gypsum	81.33 ± 7.34	1041.06 ± 101.24	51.33 ± 2.08	519.93 ± 85.43
Cow Manure	77.87 ± 2.00	877.38 ± 77.28	51.51 ± 4.31	440.54 ± 68.86
HSD (5%)	ns	ns	ns	ns
Frequency Applica	tion of Consortium Bacteria			
Control	70.89 ± 2.09 a	635.47 ± 72.58 a	35.41 ± 2.20 a	353.53 ± 43.03 a
Once	75.89 ± 3.13 ab	847.35 ± 128.80 ab	45.68 ± 8.13 a	404.91 ± 39.69 ab
Twice	79.72 ± 5.16 ab	964.26 ± 92.96 ab	45.96 ± 1.72 a	467.02 ± 127.4 ab
3 Times	84.06 ± 8.11 b	1100.40 ± 236.65 b	57.96 ± 7.97 b	519.61 ± 108.3 ab
4 Times	87.45 ± 10.86 b	1248.63 ± 118.78 b	72.09 ± 5.26 b	655.86 ± 36.47 b
HSD (5%)	9.24	452.36	15.75	258.65

 Table 1. Effect of Ameliorant and Frequency Application of Consortium Saline Tolerance Bacteria on Growth and Yield of

 Tomato in Saline Land

Note. Mean ± standard deviation. Means followed by different letter indicated significant difference at 0.05 significance level. ns: not significant



**Fig 1.** Total Chlorophyll Content of Tomato Leaves Affected by Ameliorants (a) and Application of Consortium Saline Tolerant Bacteria (b). Values followed by different letter indicated significant difference at 0.05 significance level.



**Fig 2.** Total Sugar of Tomato Fruits Affected by Ameliorants (a) and Application of Consortium Saline Tolerant Bacteria (b). Values followed by different letter indicated significant difference at 0.05 significance level.



**Fig 3.** Nitogene Uptake of Tomato Affected by Soil Ameliorants (a) and Aplication of Consortium Saline Tolerant Bacteria (b); Phosporus Uptake of Tomato Affected by Soil Ameliorants (c) and Aplication of Consortium Saline Tolerant Bacteria (d); Potassium Uptake of Tomato Affected by Soil Ameliorants (e) and Aplication of Consortium Saline Tolerant Bacteria (f). Natrium Uptake of Tomato Affected by Soil Ameliorants (g) and Aplication of Consortium Saline Tolerant Bacteria (h). Values followed by different letter indicated significant difference at 0.05 significance level.



**Fig 4.** Regression Between Potassium Uptake and Fresh weight of Tomato Fruits (a) and Potassium Uptake and Total Sugar of Tomato Fruits (b).

the production of ACC, a precursor of ethylene (Glick, 2014). This shows that ACC-deaminase producing bacteria are able to improve the tolerance of plant growth under salinity stress. Zafar-ul-Hye et al. (2014) reported that PGPR-containing ACC-deaminase from *Pseudomonas syringae* and *Pseudomonas fluorescens* species may improve yield of maize under drought and salinity stress.

## Nutrient Uptake

Under salinity stress, the rate of nutrient uptake was limited. Generally, the result of this research (Table 2.) showed that gypsum and cow manure had the same effect on N, P, K and Na uptake of shoots. Nutrient uptake increased by the application of consortium saline tolerant bacteria. Three and four applications had a better effect on N, P and K uptake compared to the control treatment. Application of consortium saline-tolerant bacteria was able to alleviate salinity stress related to the value of Na which had a declining trend along with the increasing application frequency of saline-tolerant bacteria (Figure 3.). N, P and K uptake increased 85-135 % while Na uptake was reduced 8-16%, compared to control.

The results of the research indicated that the application of consortium bacteria increased total nitrogen, phosphorous, and potassium uptake in shoots while sodium uptake showed a declining trend although it was not statistically significant. This shows that the application of bacteria can potentially increase nutrient uptake. Zameer et al. (2016) reported that PGPR is a group of bacteria that colonizes roots and helps plants take up nutrients such as N, P and K. Meanwhile, Castilo-Aguilar et al. (2017) showed that even seedlings inoculated with Panebacillus polymyxa were not significantly different in the nitrogen uptake of Capsicum chinense, but higher amounts of P and K uptake were identified compared to un-inoculated seedlings. Consortium application of bacteria is more effective than the application of single bacteria. Sharafzadeh (2012) reported that the application of Pseudomonas, Azotobacter and Azospirillum as a consortium resulted in higher amounts of N, P and K uptake of tomato compared to single application of those bacteria. Na uptake in plants treated with bacteria application is lower than that in plants treated without bacteria. These results indicate that the application of bacteria can alleviate stress of the plant as evidenced by the decrease of Na<sup>+</sup> levels in the plant tissue. According to

Maathuis (2014), Na<sup>+</sup> is an ion that easily accumulates in cells of plants and at certain levels is toxic for plants. Ashraf et al. (2004) reported that rhizobacteria were able to produce exopolysaccharide compounds that can bind Na<sup>+</sup> in plant tissue and alleviate salinity stress of plants.

Yield and quality of fruit are greatly influenced by the adequacy of nutrients, especially K (potassium). Linear regression showed that fresh weight and total sugar of fruits are strongly influenced by potassium uptake (Figure 4). Potassium plays an important role in biochemical processes such as enzymes activation, carbohydrate metabolism, protein synthesis and ATP production which is dominant in regulating photosynthetic rate (Hasanuzzaman et al., 2018). Increasing fruit yield of tomato under salinity due to application of potassium was also reported by Amjad et al. (2014) and Sajyan et al. (2018). Potassium is also known to play a role in biosynthesis and sugar transfer. So, it also determines the quality of tomato fruits, especially the total sugar content. Javaria et al. (2012) reported that increasing K fertilization could increase total sugars and titratable acidity in tomato fruit, by 11.03 % and 121.87 %, respectively.

## Materials and methods

#### Plant materials

Plant material used in this research was tomato seed Variety of Tymoti F1. Seed were sowed into plastic nursery and transplanted to the field after 21 days after sowing.

#### Bacteria isolates preparation

Bacteria used in this study was consortium of three isolates namely SN13+ SN22+ SN 23. They were isolated from rhizosphere of plants grown in the soil of saline-prone regions of coastal area of Lamongan, in coastal East Java Indonesia. These three isolates were developed and tested (selection of salinity tolerance, hypersensitivity test on tobacco leaves, IAA production test and nitrogen fixing test) at Bacteriology Laboratory of Pests and Plant Disease Department, Faculty of Agriculture, University of Brawijaya. The molecular identification from our previous research (Cahyaty et al. 2017) showed that SN 13, SN 22 and SN 23 belong to the species of *Streptomyces* sp., *Bacillus* sp. and *Corynebacterium* sp. Each bacteria isolate was cultured on Nutrient Agar (NA) medium with strike plate methods and then incubated for 24 hours. Bacteria grown from NA, were collected using inoculation needles and then dipped in the 100 ml Nutrient Broth (NB) liquid medium. Bacteria suspensions were shaken for 2 x 24 hours at a speed of 150 rpm and then obtained the Optical Density (OD) using spectrophotometer. After obtaining OD value  $\geq$  1, the bacterial suspensions were diluted to a density of 1 x 10<sup>9</sup> cfu ml<sup>-1</sup>. Preparation of consortium bacteria was done by mixing each bacteria with the same ratio.

## Experimental site and design

Field experiment was conducted at Sidomukti Village District of Brondong Lamongan East Java, Indonesia. The soil had EC 4.12 dS m<sup>-1</sup>, pH 7.1, C-organic 1.39 %, N-total 0.16 %, P 8.61 ppm, K 0.4 me 100 g<sup>-1</sup>, Na 1.68 me 100 g<sup>-1</sup> and CEC 37.8 me 100 g<sup>-1</sup>. Research was arranged using split plot design with three replications. The main plot was soil ameliorants namely A1: Gypsum and A2: Cow manure while subplots were application frequency of consortium saline tolerance bacteria namely FO: Control (without bacterial isolates, F1: Once (soaking seeds), F2: Twice (soaking the seeds and transplanting), F3: Three times (soaking seeds, transplanting and 2 WAT). F4: Four times (soaking seeds, transplanting, 2 and 4 WAT). Application ameliorants of gypsum with doses 5 ton ha<sup>-1</sup> and Cow manure cow manure 20 ton ha<sup>-1</sup>. Gypsum and cow manure were given to the soil 10 days before transplanting according to treatments.

#### Seed treatment and maintenance

Tomato seeds were treated with bacteria by soaking the seeds into warmed water (32 °C) for about fifteen minutes and then soaking into bacteria suspension (1 x 10<sup>9</sup> cfu mL<sup>-1</sup>) with a concentration of 5 mL  $L^{-1}$  for about 2 hours. Seeds untreated with bacteria were only soaked with distilled water. Seeds were sown using a plastic nursery with soil and cow manure as medium. After 3 weeks, the tomato seedlings were ready to be transplanted. Tomato seedlings were transplanted to bedded land covered with plastic mulch with plant spacing of 50 x 50 cm. Standard agronomic practices were followed to allow the plant to grow well. Replanting was done up to 2 weeks after transplanting. Watering was done manually using buckets once a day and every day after the plants were just transplanted up to 1 week after transplanting, then continued once every 2 days up to 14 weeks after transplanting (at harvesting). The applied fertilizers were urea, super phosphate (SP 36), and NPK fertilizer (16-16-16). Urea and SP 36 were applied with 30% doses of 150 kg hectare<sup>-1</sup> of Nitrogen and 120 kg hectare<sup>-1</sup> P<sub>2</sub>O<sub>5</sub> as basal fertilizer. NPK fertilizer was applied in three splits (15, 30, and 45 days after transplanting) with a dose of 500 kg hectare<sup>-1</sup>. Pest and diseases control were done using organic pesticide OrgaNeem extracted from Azadirachta indica at a concentration of 5 mL L<sup>-1</sup>.

## Application of bacteria

Consortium bacteria isolates were applied according to the treatments by drenching the soil around each plant with 20 mL of bacteria suspension (1 x  $10^9$  cfu mL<sup>-1</sup>) with concentration 22.5 mL L<sup>-1</sup>. Bacteria was applied in the afternoon to keep the bacteria isolates from direct solar radiation.

## Growth and Yield Measurement

Plant growth parameters including plant height, leaf area, and dry weight of plants were measured by random selection of four plants at 8 weeks after transplanting for each treatment. Meanwhile, yield parameters including fresh weight of fruit per plant were measured from 15 selected plants of each treatment. Fruits quality (total sugar of fruit) was recorded from 5 selected fruits at the full ripening maturity stage using the spectrophotometry method.

Total chlorophyll content of tomato leaves was measured using chlorophyll meter SPAD-502 at the reproductive stage and converted according to Jiang (2017) by the following equation:

Total chlorophyll (g mg<sup>-1</sup>) = [0.0306 (x) + 0.1443](x = value read by chlorophyll meter)

#### Plant analysis

Dried materials of plants were analyzed for nutrient contents such as total nitrogen, phosphorus, potassium and sodium. Total nitrogen content was determined by Micro Kjeldahl method (Stuart, 1936). Phosphorus content was determined by spectrophotometer method (Cook and Daughton, 1981). Potassium and sodium were determined by flame photometer method (Hald, 1946). Nutrient uptake was determined by multiplying the value of each nutrient and dry weight of shoots.

#### Statistical analysis

The collected data were analyzed using Analysis of Variance, then examined with F test at 5 % error level and further with Honestly Significant Difference (HSD) at 5 % error level. The correlation and regression among parameters were examined using SPSS version 16.

#### Conclusions

Application of consortium saline tolerant bacteria at four times applications (soaking seeds, transplanting, 2 and 4 weeks after transplanting) can improve yield and nutrient uptake of tomato grown in saline land. Application of consortium saline tolerant bacteria are not affected by soil ameliorant type. Gypsum and cow manure as soil ameliorant showed no different effect on yield and nutrient uptake of tomato grown in saline land. On tomato plant, consortium of saline tolerant bacteria was more effective when applied several times. It can be applied as early as seed treatment then repeated once or twice in the vegetative phase.

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