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EFFECTIVENESS OF SALT-TOLERANT RHIZOBIUM AND FORAGE LEGUMES SYMBIOSIS ON SALINE SOIL

E. Fuskhah [1], R.D. Soetrisno [2], S.P.S. Budhi [2], and A. Ma'as [3]

[1] Faculty of Animal Husbandry and Agriculture, Diponegoro University Semarang
[2] Faculty of Animal Husbandry, Gadjah Mada University Yogyakarta
[3] Faculty of Agriculture, Gadjah Mada University Yogyakarta
eny_fuskhah@yahoo.com

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ABSTRAK

[EFEKTIVITAS SIMBIOSIS RHIZOBIUM TAHAN GARAM DAN LEGUMINOSA PAKAN PADA TANAH SALIN] Pertumbuhan legume dan kemampuannya bersimbiosis dengan rizhobium sering terhambat oleh cekaman salinitas tanah. Tujuan dari penelitian ini adalah untuk membandingkan pertumbuhan dan tingkat nodulasi tiga spesies legume pakan yang diinokulasi dengan isolat yang toleran terhadap salinitas. Leucaena, Calopo, and Centro dinokulasi dengan tiga sumber rhizobium (isolat yang berasal dari Calopo, Leucaena, dan kombinasi Calopo dan Leucaena) dalam susunan factorial dengan tanpa isolat sebagai kontrol. Tanaman yang telah diinokulasi ditanaman secara individu dalam pot yang berisi tanah salin dengan konduktivitas elektrik 6.4 mmhos cm^{-1} dan pH 5.8. Pengamatan dilakukan pada 12 minggu setelah tanam terhadap pertumbuhan tanaman dan tingkat nodulasi. Hasil menunjukkan bahwa isolate maupun interaksi tidak berpengaruh nyata pada semua variabel yang diamati. Calopo dan Centro menghasilkan pertumbuhan dan bobot nodul yang lebih tinggi dibanding Leucaena.

Kata kunci: *Calopogonium, Centrosema*, lamtoro, isolat, garam terlarut.

ABSTRACT

Legumes growth and their symbiosis with rhizobium are often hindered by soil salinity stresses. Objectives of this study were to compare the growth and nodulation of three forage legume species on saline soil as inoculated with saline tolerant isolates. Leucaena, Calopo, and Centro were inoculated with three sources of rhizobium (Calopo, Leucaena, and combination of Calopo and Leucaena isolates) in a factorial arrangement with no isolate as the control treatment. The inoculated plants were individually grown on the experimental plot containing saline soil with electrical conductivity 6.4 mmhos cm^{-1} and pH 5.8. Each legumes. Observations were made at 12 week after planting on the plant growth and root nodulation. Neither isolate nor interaction had significant effects on all variables observed. Calopo and Centro produced higher growth performances and fresh weight of efective nodules than leucaena.

Keywords : *Calopogonium, Centrosema, Leucaena,* isolates, soluble salt.

INTRODUCTION

The use of legumes as forage crops has long been known among ruminant growers. In most cases, forage of legumes is regarded to have superior feeding value than grasses (Dewhurst *et al*., 2009) and their beneficial effects on feed intake, animal growth, and production performances are widely documented (Dewhurst, 2013; Steinshamn, 2010; Paulson *et al*., 2008;). Apart from their nutritional values, legumes can be considered as versatile crop due to their wide area of adaptation. With the capability of fixing atmospheric nitrogen, legumes can grow on marginal environments where other forage crops may not viable. Nevertheless, it has been an axiomatic that less favorable environments would hinder the optimal performances of the crops, including the ability to develop a mutual symbiosis with *Rhizobia* for atmospheric nitrogen fixation.

Under arid environment with soil salinity problems, plants may undergo stress associated with high concentration of soluble salts in the soil. Ions of soluble salt most commonly accumulate in saline soil include the anions chloride (Cl^-), sulfate ($SO_4^=$), carbonate (HCO_3^-), and sometimes nitrate (NO_3^-) and the cations sodium (Na^+), calcium (Ca^{++}), magnesium (Mg^{++}), and sometimes potassium (K^+) (Lamond and Whitney, 1992).These of soluble salts through their high osmotic pressures affect plant growth by restricting the uptake of water by the roots, specific-ion toxicity and/or nutritional disorders (Tester and Devenport, 2003; Läuchli and Epstein, 1990). The general symptoms of salinity stress are growth inhibition, accelerated development and senescence and death during prolonged exposure (Jouyban, 2012).

As with most agricultural crops, each legume species differs in the ability to tolerate salt accumulation in the soil (Lamond and Whitney, 1992; Abdel-Wahab and Zahran, 1981). Earlier study also indicated that Sesbania and Leucaena (tree legumes) were more tolerant to high level of salinity than Calopo and Centro (herbaceous legumes) (Fuskhah *et al.,* 2007). Similarly, the salinity tolerance is naturally occurred amongst rhizobia strains. Singleton *et al*. (1982) showed that some rhizobia strains can survive and grow at salt concentrations which are inhibitory to most agricultural legumes. Although symbiosis and nodule functions are commonly more sensitive to salinity stress than the host legumes or the rhizobia (Delgado *et al*., 1994), several symbiotic systems of legumes and rhizobia have the inherent ability to develop a salinity stress tolerance (El-Sheikh and Wood, 1995; Craig *et al.*, 1991; El-Mokadem *et al.*, 1991) and other symbiotic systems can be enhanced the tolerance by inoculating the legumes with salt-tolerant strains of rhizobia (Sharma *et al*., 2013; Shamseldin and Werner, 2005).

Most of the saline soils available in Indonesia are underutilized for agriculture production. Establishment of grassland legume on such areas would represent a good strategy for supplying forage legume and land restorations. Objective of this study was to compare the growth and nodulation of three legume species on saline soil as inoculated with three saline tolerant isolates collected from their natural habitats.

MATERIALS AND METHOD

The pot experiment was conducted in a glasshouse of forage crops Science Laboratory Animal Husbandry Faculty, Diponegoro University, Semarang. A completely randomized design with three replications was employed to allocate three legume species, i.e. leucaena (*Leucaena leucocephala*), calopo (*Calopogonium mucunoides)*, and centro (*Centrosema pubescens*) in factorial arrangement with three sources of rhizobium (isolate from calopo, isolate from leucaena, isolates from calopoand leucaena). No isolate was used as the control treatment. *Leucaena* was identified as legume that very selective to rhizobium,*Calopogonium* was less selective, and *Centrocema* was moderately selective.

The pots were filled with 6 kg saline soil from Tugu Semarang with electrical conductivity (EC) 6.4 mmhos cm^{-1} and pH 5.8. Each isolate was collected from the nodules of the corresponding species in their natural habitats. Prior to rhizobium inoculation, each isolate was rejuvenated in yeast manitol agar (YMA) with congo red and proliferated in yeast extract manitol (YEM). Rhizobium inoculations were made on the scarified legume seeds as the isolates reached an exponential stage. The inoculated seeds were then sown on the soil media and the plants were maintained in the pots till harvest at 12 weeks after planting (WAP).

Observations were made on stem length, plant fresh and dry weight, and fresh weight of effective nodules. Analysis of variance was performed the collected data with F-test at $P<0.05$. The mean separation was made using Duncan's multiple range testat $P<0.05$.

RESULTS AND DISCUSSION

Analysis of variance indicated that both plant growth and rhizobia proliferation at 12 WAP were significantly determined by legume species ($P<0.05$), but not by the source of isolates or their interaction effects. Table 1 shows that both Calopo and Centro produced stem elongation faster than Leucaena. Aswida (2009) reported that Calopo and Centro had the same growth rate. As short-lived perennial herbaceous plants, Calopo and Centro are widely known as aggressive creepers. At 12 WAP their stems are succulent and do not become woody for at least 18 months after planting (FAO, 2013). On the other hand, Leucaena is a woody tree that produces stem elongation comparatively slower than the herbaceous plants. At 12 WAP, Leucaena plant can be considered as seedling and under favorable conditions it usually will be only about 50 cm in height. In this study, the introduced salinity in the growing media might have slowed down the stem elongation. Soil containing soluble salt with EC 6.4 mmhos cm^{-1} is commonly considered as highly saline (Lamond and Whitney, 1992). Francois and Mass (1999) reported that most forage legumes were less tolerant to soil salinity than cereals. Munns *et al.* (2006) noted on cereals that early growth stages are sensitive periods in the plants life, regardless their inherent sensitivity to soil salinity. Reduced leaf area and stunted shoot are the general expression shoot growth under salinity stress (Läuchli and Epstein, 1990).

Similar features were also exhibited by plant fresh and dry weight (Table 2 and Table 3). Calopo and Centro produced higher biomass production than Leucaena. With fast stem elongation and an extensive branching system, Calopo and Centro will result in rapid land coverage, whereas the growth rate of Leucaena usually increases after 3 months. It has been the general natural characteristic of tree legume that grows very slowly in the first stage of the growth cycle.

The absence of isolate effectsand interaction effects on all observed traits implied that the introduced rhizobia by inoculation were not effective in stimulating the plant growth. Furthermore, no significant different between the control plants (no isolate) and the inoculated plants on the weight of effective root nodules(Table 4) suggested that the resulted nodulation did not come from the rhizobia provided during seed inoculation but from the inherent population in the soil media.The inoculated rhizobia might be able to withstand and successfully multiply under salinity stress, their infectibility and nodulating ability may be decreased (Zahran, 1991). The contribution of inherent rhizobia to the plants

Table 1. Average stem length (cm) of three legumes as inoculated with two *Rhizobium* isolates

Source of Rhizobium	Legumes			$Average^{ns}$
	Leucaena	Calopo	Centro	
No isolate	19.42	113.17	104.17	78.92
Calopo isolate	37.25	121.67	106.83	88.58
Leucaena isolate	39.25	113.17	117.33	89.92
Calopo+Leucaena isolates	13.75	119.33	115.00	82.69
Average	7.42^{b}	16.83^{a}	110.83^{a}	

Notes: averages of legume stem length followed by the same letter is not significantly different at the 5 % level by Duncan's test.

Table 2. Average plant fresh weight production of legumes (kg/ha) as inoculated with two *Rhizobium* isolates

Source of Rhizobium	Legumes			$Average^{ns}$
	Leucaena	Calopo	Centro	
No isolate	210.00	998.07	994.67	734.24
Calopo isolate	306.67	1286.53	924.13	839.11
Leucaena isolate	400.00	1199.20	1068.00	889.07
Calopo+Leucaena isolates	106.67	1553.07	1123.60	927.78
Average	255.83^{b}	1259.22^{a}	1027.60^{a}	

Notes: averages of legume stem length followed by the same letter is not significantly different at the 5 % level by Duncan's test.

Table 3. Average plant dry weight production (kg/ha) as inoculated with two *Rhizobium* isolates

Source of Rhizobium	Legumes			Average[ns]
	Leucaena	Calopo	Centro	
No isolate	69.67	296.20	295.53	220.47
Calopo isolate	134.13	420.40	292.27	282.27
Leucaena isolate	127.20	338.13	296.60	253.98
Calopo+Leucaena isolates	46.73	415.67	387.53	283.31
Average	94.43[b]	367.60[a]	317.98[a]	

Notes: averages of legume stem length followed by the same letter is not significantly different at the 5 % level by Duncan's test.

Table 4. Average fresh weight of effective root nodules of legumes (kg/ha) inoculated with two *Rhizobium* isolates

Source of Rhizobium	Legumes			Average[ns]
	Leucaena	Calopo	Centro	
No isolate	26.67	46.67	110.00	61.11
Calopo isolate	60.00	63.33	96.67	73.33
Leucaena isolate	36.67	53.33	113.33	67.78
Calopo+Leucaena isolates	10.00	76.67	150.00	78.89
Average	33.33[c]	60.00[b]	117.50[a]	

Notes: averages of legume stem length followed by the same letter is not significantly different at the 5 % level by Duncan's test.

growth was notably lowest on Leucaena. Root nodulation in Leucaena was occurred about 2 months after planting (Mannetje and Jones, 2000) and its effect on the Leucaena plant was not evident until 1-2 years after planting (Homchan *et al*., 1989).

CONCLUSION

Rhizobia inoculations using the isolates endemic soil saline were not necessarily improve the growth of the host plants under high salinity. At 12 WAP, the different in plant growth performances and rate of nodulation were mainly due to the growth habit of the legume species. With faster biomass production, both Calopo and Centrosema (herbaceous legumes) can provide forage earlier than Leucaena (tree legume). Under saline soil the rhizobium inoculated from isolates had no effect on the plant growth or root nodulation.

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