RESPONSE OF SALT-TOLERANT SOYBEAN (*GLYCINE MAX* (L.) MERR.) GENOTYPES TO PHOSPHORUS FERTILIZER ON SALINE SOILS OF INDONESIA

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Abstract. Due to global climate change the salt-affected agricultural land has enlarged in Indonesia, especially in coastal areas. Chemical soil properties, such as pH, Na and Cl contents, as well as soil nutrients including availability of soil phosphorus, are changed by higher soil salinity. Phosphorus fertilizer management on saline soils must be done properly since improper dosage of P can worsen the severity of the effects of salinity. The study aimed to determine appropriate dose of P fertilization for salt-tolerant soybean genotypes in saline soil. The experiment was conducted on an agricultural field during dry season 2018 in Tuban District, East Java Province, Indonesia. The treatment consisted of two P fertilizer dosage (72 and 108 kg P_2O_5/ha) on two salt-tolerant of soybean genotypes (Anjasmoro variety and K-13 line). The treatment was arranged in randomized complete block design, with fourteen replicates. Soil EC at the study site during the growing season was 9-17 dS/m, while EC of irrigation water was 6-16 dS/m. Results showed that among the two tested salt-tolerant genotype, Anjasmoro variety revealed better performance than K-13 line according to growth parameters, as well as yield and yield attributes. Appropriate P fertilizer dosage for both genotypes was 108 kg P_2O_5/ha.
Keywords: *application, dosage, nutrient, salinity, yield*

Introduction

Vulnerability to sea level rise, drought and flooding are new criteria in land suitability evaluation in the global climate change era (Sukarman et al., 2018). Climate change caused sea level rise and increased soil or groundwater salinity, especially in coastal areas. Indonesia has a large coastal area with about a 100,000 km coastline, which is the second longest in the world following Canada. Based on vulnerability to sea level rise, there is 12.02 million ha lands that is prone to salinization (Karolinoerita and Yusuf, 2020). The problem of salinity stress for plants is not only related to the adverse direct effects of salinity and toxicity of Na or Cl elements, but also to the indirect effect related to high pH and nutrient immobilization, especially phosphorus.

Phosphorous deficiency often occurs in saline soils because of absorption inhibition (Kaya et al., 2001; Hirpara et al., 2005), low availability due to Ca fixation and high soil pH (Chhabra and Thakur, 2000; Mahmood et al., 2013; Penn and Camberato, 2019; Tang et al., 2019). Increasing the availability of P in saline soils through fertilization improves growth and increases yield (Zribi et al., 2018; Belouchrani et al., 2020). The P fertilizer effectiveness and crop yield increase are higher when combined with organic fertilizers (Mahmood et al., 2013; Meena et al., 2018; Ding et al., 2020).

The use of salinity-tolerant genotypes is the key to increase crop productivity. Soybean (*Glycine max* (L.) Merr.) line IAC100/Bur/Malabar-10-KP-21-50 and K-13 were identified tolerant to salinity up to 12.2 dS/m (Purwaningrahayu et al., 2015,

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2016). Soybean line K-13 and Anjasmoro variety were also reported tolerant to salinity in different mechanism. Salt-tolerance of Anjasmoro was due to more K uptake, while in K-13 it was due to inhibition of Na uptake (Taufiq et al., 2016).

Combining salinity-tolerant genotypes with best soil management resulted in better effect as well as yield. Soybean yield of Anjasmoro variety and K-13 line increased by 36% in saline soil with salinity of 10 dS/m amended with 2.5 t/ha manure and 1.5 t/ha gypsum (Taufiq et al., 2016; Purwaningrahayu and Taufiq, 2018). In previous studies on saline soil with soil conductivity 6- > 10 dS/m, soybean K-13 line productivity reached 1.5 t/ha in saline soil with fertilization at 46 kg N/ha and 108 kg P_2O_5/ha (Purwaningrahayu and Taufiq, 2018a). Phosphorous fertilization of saline soil must be done properly. Tang et al. (2019) showed that maize in saline environment absorbed more Na from well P supplied soil compared with low P or P deficient soil. This research aimed to determine the optimum dosage of P fertilization in saline soil for two saline-tolerant of soybean genotypes.

Methodology

Place and time of study

Field trial was conducted in saline land at Gesikharjo Village, Palang Sub District, Tuban District, East Java Province (6°54'19.5196"S; 112°8'17.7947"E; 4 m asl) from July to October 2018 (dry season). The trial site was located at about 500 m from coastline with soil properties presented in *Table 1*. There was no rainfall during the field trial, and average air temperature was 27.9 °C (*Fig. 1*). Soybean planted in paddy field after rice following planting pattern of rice-soybean. In fact, farmer in this land only grow rice once during rainy season and then fallow because of high salinity of soil as well as irrigation water.

Research design

The treatment consisted of two factors. The first factor was two dosages of P fertilizer (72 kg P_2O_5/ha and 108 kg P_2O_5/ha) using SP36 (36% P_2O_5), and the second factor was two soybean genotypes (Anjasmoro variety and K-13 line). Treatment of P fertilizer dosage use was based on the optimum dosage of a previous research. Each treatment combination was applied in a plot of 24 m^2 (4 m wide and 6 m length) that were laid out according to randomized complete block design, with 14 replications.

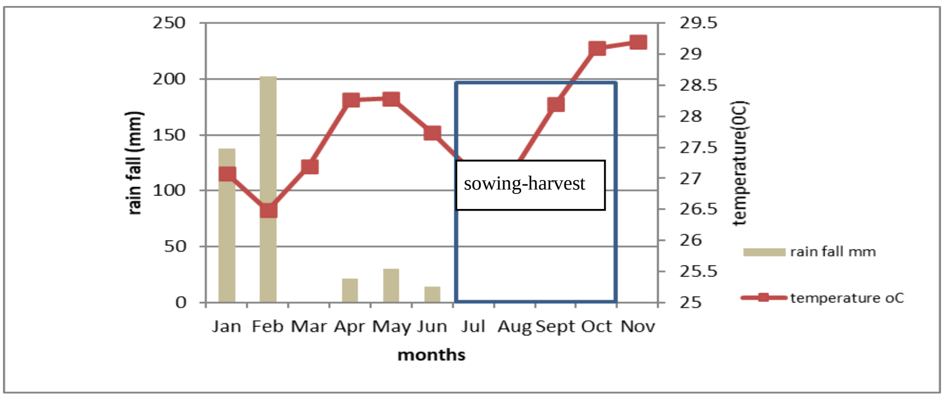

Figure 1. *Rain fall and air temperature in Palang Sub District, Tuban District in 2018*

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Table 1. *Chemical and physical properties of top 0-20 cm composite soil sample at preplanting. Tuban, dry season 2018*

Properties	Methods	Value	Classification[1)]
Soil fraction (%)	Pipet		
• Clay		74	
• Silt		23	
• Sand		3	
• Texture class		Clay	
Bulk density (g/cm^3)		1.1	
Moisture content (%w/w)			
• pF 2.5	Pressure plate	44.5	
• pF 4.2		24.5	
Salinity (dS/m)	Field measurement (Portable EC meter)	14.4	VH
pH-H_2O	1:5[2)]	8.2	Slight alkaline
Total-N (%)	Kjedahl[3)]	0.12	L
C-organic (%)	Walky & Black[4)]	1.70	L
P_2O_5 (mg/kg)	Olsen[5)]	17.73	L
SO_4 (mg/kg)	1 N NH_4OAc pH 4,8[6)]	997	VH
Cu (mg/kg)	DTPA extraction	4.9	M
Fe (mg/kg)	DTPA extraction	1.12	L
Exchangeable cations (cmol$^+$/kg)	1 N NH_4OAc pH 7.07[7)]		
• K		3.05	VH
• Na		3.55	VH
• Mg		4.87	VH
• Ca		24.97	VH
CEC (cmol$^+$/kg)	1 N NH_4OAc pH 7	48.04	H
Na saturation (ESP, %)	(Na/CEC)*100%	7.38	H

[1)]L: low; H: high; VH: very high; M: moderate according to Sulaeman et al. (2005); [2)]Mclean (1982); [3)]Bremner (1960); [4)]Walkley and Black (1934); [5)]Olsen and Sommers (1982); [6)]Tabatabai (1982); [7)]Peech (1945)

Rice straw clearing, as well as soil tillage, were conducted before planting. Prior to planting, soybean seeds treated with insecticide of thiametoxam active ingredient for protection against pest. Seed planting was done manually by dibbling 2-3 seeds into the hole at planting distance of 40 cm interrow and 15 cm interplant, and then covered with manure at a dosage of 5 t/ha. All plots were fertilized with 46 kg N/ha as urea (46% N) and 60 kg K_2O/ha as MOP (60% K_2O). Phosphorous fertilizer at dosage according to the treatment and all dosage of MOP fertilizer broadcast applied once at planting time, and then 3.5 t/ha of rice straw applied as mulch. Urea fertilizer applied at 15 DAP and 30 DAP (days after planting) with 50% of the dosage, respectively. Weeding was done twice (20 and 45 DAP) using herbicide, and chemical pesticide sprayed five times for pest control. Soybean crops were irrigated five times during the growth using water from well around at the trial site.

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Data collection and analysis

Moisture content (gravimetric method) and electrical conductivity (portable EC meter Hanna instruments) were measured at 0-20 cm soil depth starting from 15 DAP to 75 DAP with 15 days interval. Plant height and Chlorophyll content index (CCI) were observed from 15 DAP to 75 DAP with 15 days interval. The CCI was measured in the second and the third top leaves of ten samples using SPAD-502 (Minolta instruments). Proline, K and Na content of leaf were measured at 60 DAP (pod filling stage). Considering yield and yield attributes (number of filled and empty pods, weight of a 100 seeds) yield was measured based on dry seed (12% moisture content) from the plot, while yield attributes from 10 samples of each plot. The population of growing plants was calculated on an experimental plot of 24 m^2 (6 m × 2.4 m) or about 780 plants at plant spacing of 40 cm × 15 cm, 2 plants per hole. Plant population growth was calculated at 15 DAP, then the percentage was determined by the number of plants growing theoretically (780 plants). Similarly, at harvest time, the number of harvested plants was calculated on a 24 m^2 tiled plot, then the percentage was calculated by the number of growing plants.

The collected data were analyzed according to analysis of variance (ANOVA), and the mean comparison analysis using LSD 5%. All statistical analyses were proceeded with a statistical program package.

Results and discussion

Soil properties

In the soil of the study site clay fraction dominated, and so that the percentage of available water was low (20%) even water content at field capacity was quite high. Soil salinity, pH, exchangeable cations, CEC, S, and Na saturation were high, N, P and C-organic content were low. Micronutrient Cu was sufficient, but Fe was low, which might have been due to high soil pH (*Table 1*). Based on pH, salinity, and Na saturation values, soil in the study site was classified as high salinity soil according to Jones (2003). High soil salinity, Na, and pH as well as low P might be a limiting factor for soybean growth. Deficiency of P become more severe because of high soil salinity, pH, Na and Ca content. These soil properties not only inhibit P uptake, but also immobilized P due to Na and Ca fixation (Chhabra, 2002; Qadir et al., 2003; Ghafoor et al., 2004).

Soil salinity vs soil moisture

Soil EC (ECs), soil moisture content (SMC), as well as EC of irrigation water (ECw) changed during crop growth (*Fig. 2*). SMC tend to decrease linearly as soybean crop become older, and the lowest SMC was about physiological maturity stage. In contrast, ECs increased as SMC became lower. ECs was relatively stable and lower at 15 days after planting (DAP) and 30 DAP, then increased drastically at 45 DAP, but it was a little bit lower and relatively constant after 45 DAP (*Fig. 2A*). ECs at study site was about 9 dS/m during vegetative stage and increased to 15-17 dS/m during generative stage. ECw increased from 6 dS/m at planting to 14-16 dS/m at 15-60 DAP (*Fig. 2B*). Irrigation sources were wells located in the research location, because during the period of growth to harvest there was no additional rainwater (*Fig. 1*). Irrigation was carried out eight (8) times during the plant growth period at 0, 18, 23, 27, 29, 32, 44 and 51 DAP (*Fig. 2B*). Means that soybean experiencing higher salinity stress during generative stage than

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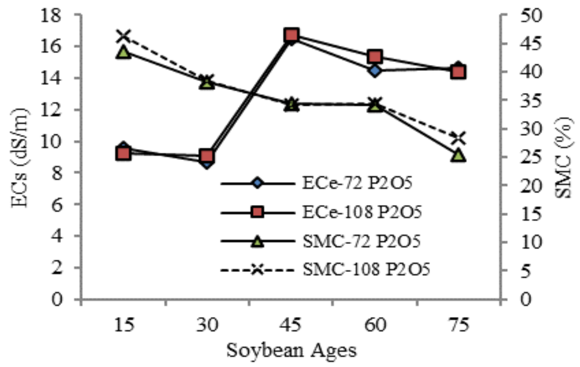
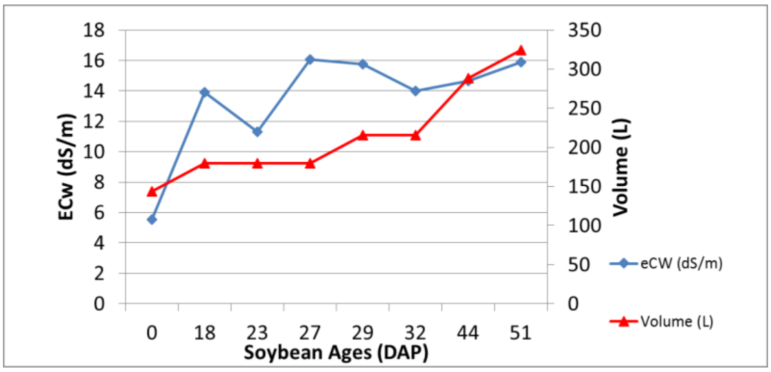
vegetative stage. Soybean was also subjected to double salinity stress, namely soil and irrigation water, and also sudden peak salinity stress that resulted in more adverse effect than constant salinity according to Bustingorri and Lavado (2011).



(A)



(B)

***Figure 2**. Soil EC and moisture content (A) in saline soil and EC and volume of irrigation water (B) during soybean growth. Tuban, dry season 2018*

Application of P fertilizer had no effect on ECs or SMC (*Fig. 2A*). Therefore, increasing ECs during crop growth could be related to reducing SMC and increasing ECs. Soybeans were irrigated five times during their growth, using water from bored wells located in the experimental site. During the dry season bore wells were the only source of irrigation water in this area. Unfortunately, ECw in the dry season was almost twice higher than in the rainy season. EC from water irrigation could have an adverse effect to crop growth according to Aydinşakir et al. (2015), and even plant death (Singh et al., 2004).

Plant growth

Soybean growth as indicated by plant height and shoot biomass at 45 DAP and at harvest was affected significantly by P fertilization. Application of 108 kg P_2O_5/ha increased plant height by 29.6% at 45 DAP and by 16.4% at harvest compared to a dose of 72 kg P_2O_5/ha (*Fig. 3A*), and hence shoot biomass at 45 DAP and at harvest was 30.9% and 38.8% higher than at a dose of 72 kg P_2O_5/ha (*Fig. 3B*). It means that increasing P fertilizer rates improve soybean growth. This result is in accordance with Faozi et al. (2019) from their trial in sandy saline soil in coastal land in Yogyakarta. Phosphorous nutrient has an important role in photosynthetic activity of soybean (Marschner et al., 1996).

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The genotype factor as well as its interaction with P fertilization had no significant effect on plant height and shoot biomass at all observation dates. Anjasmoro variety grows taller than K-13 line and higher shoot biomass than K-13 line at all observation dates, but the difference in shoot biomass was not significant (*Fig. 3C, D*). Anjasmoro and K-13 were tolerant to high salinity stress (Taufiq et al., 2016). Salinity stress reduced root, stem, and shoot biomass, and the reduction of salt-tolerant soybean genotype was lower than susceptible genotype (Hamayun et al., 2019). The result indicates that Ajasmoro variety seems to be more tolerant to salinity stress than K-13 line.

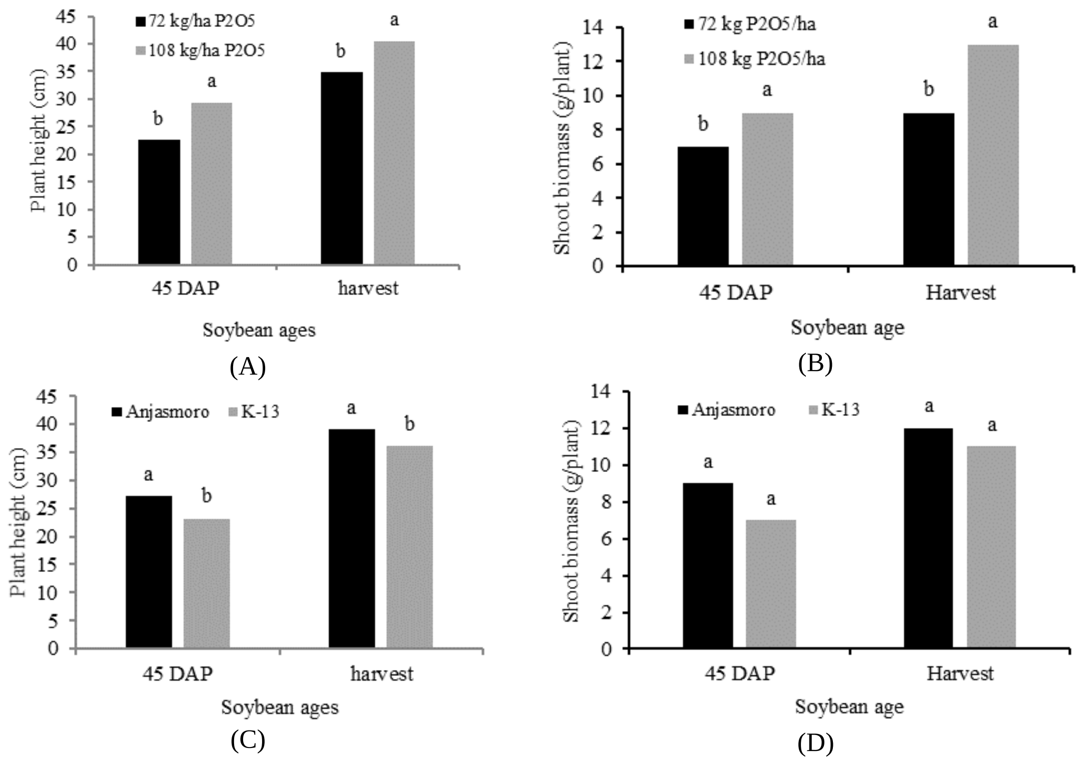

***Figure 3.** Effect of P fertilization on plant height (A), shoot biomass (B), and effect of soybean genotype on plant height (C) and shoot biomass (D) in saline soil. (Same letter above bar in every chart and observation date means no significant difference based on LSD 5% test)*

Chlorophyll content index (CCI)

Soil salinity increases with the age of the crop, and this condition was also followed by a decrease in CCI (*Fig. 5*). Increasing salinity levels indicate increasing salt content, and this condition reduces chlorophyll content (Ghassemi-Golezani et al., 2011), because of the ultra-structure of leaf chloroplast damage the effect becomes severe as crop becomes older (Mitsuya et al., 2003). The CCI at different P fertilization dosage showed different trend, even though it was not significant statistically. The CCI at P fertilizer dosage of 72 kg P_2O_5/ha decreased continuously. However, CCI at P dosage of 108 kg P_2O_5/ha tended to increase and stabiles after 30 DAP, even the CCI was lower than in the early growth (*Fig. 4A*). Leaves analysis at 60 DAP showed that P and K content were significantly higher and Na content was significantly lower at P fertilizer dosage of 108 kg P_2O_5/ha compared to 72 kg P_2O_5/ha (*Table 2*). Phosphorous nutrient beside being important for photosynthetic activity, it also has an important role in increasing activity of

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nitrogenase enzyme (Marschner et al., 1996). However, leaf N content did not significantly differ between the two dosages of P fertilizer as indicted in *Table 2*. In contrast, proline content of leaf was lower at 108 kg P_2O_5/ha compared to 72 kg P_2O_5/ha (*Table 2*), and this fact is in accordance with Al-Karaki et al. (1996) that *Phaseolus vulgaris* L. and *P. acutifolius* accumulate lower proline at lower dosage of P application. Application of P fertilizer on black paper (*Paper nigrum*) reduce salt stress through increasing assimilation of N, P, K, Mg, Ca, Fe, Zn, Mn, and Cu (Cimrin et al., 2010). This result indicates that application of high P fertilizer dosage preventing chlorophyll damage, and it might be due to lower Na and higher K content, so that the adverse effect of Na can be prevented and hence the relatively higher CCI can be maintained.

CCI of the two genotypes tested were quite the same during germination stage (0-15 DAP), but the CCI of K-13 was higher than Anjasmoro after 15 DAP (*Fig. 4B*). Leaf of K-13 contained lower N, higher K and Na, but had no difference in K/Na ratio, and K-13 also had higher proline than Anjasmoro (*Table 2*). Adverse effect of higher Na in K-13 could be minimized by high K content. Increasing K uptake is a mechanism of crop tolerance to salt stress because K has a competitive property against Na in maintaining water status in the crop (Capula-Rodríguez et al., 2016). Proline is an organic substance in plants with a function to maintain turgor pressure of cells. This result indicates that higher K and proline content in K-13 line than in Anjasmoro might be responsible to higher CCI.

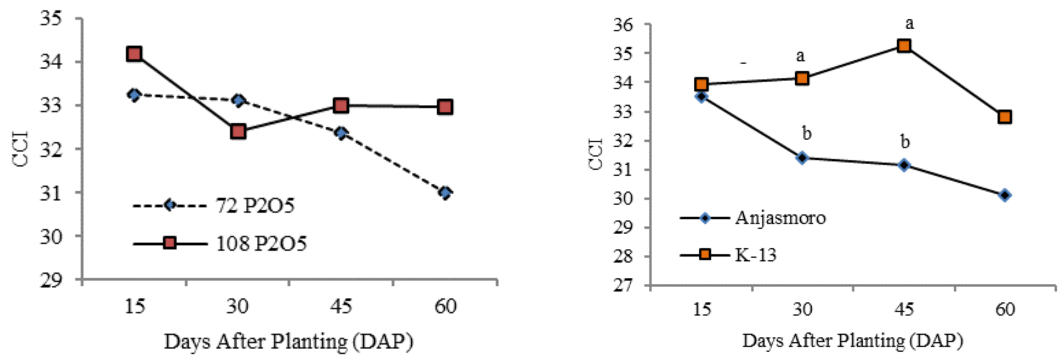

Figure 4. *Effect of different of P fertilization dosage (A) and different soybean genotype (B) on chlorophyll content index in saline soil. Tuban, second dry season 2018 (point in the graph with the same letter or without letter means no significant difference according to LSD 5%)*

Table 2. *N, P, K, Na content, K/Na ratio, and proline content of soybean leaf at 60 DAP at different P fertilizer rate and genotype in saline soil. Tuban, 2018*

Treatment	Proline (µg/g)	N (%)	P (%)	K (%)	Na (%)	K/Na ratio
P dosage						
• 72 kg/ha	119.5 a	1.51 a	0.14 b	1.33 b	0.054 a	24.8 b
• 108 kg/ha	70.0 b	1.49 a	0.17 a	1.52 a	0.047 b	32.7 a
Genotype						
• Anjasmoro	87.5 B	1.70 A	0.16 A	1.33 B	0.048 B	27.7 A
• K13	102.5 A	1.30 B	0.15 A	1.52 A	0.052 A	29.8 A

Numbers in the same column with the same letter means no significant difference according to LSD 5% test

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Yield and yield attributes

Phosphorus and genotype factors significantly affected yield and yield attributes, but there was no significant interaction effect between the factors (*Table 3*). Application of 108 kg P_2O_5/ha increased soybean grain yield by 77% compared to 72 kg P_2O_5/ha. The higher grain yield was in accordance with increasing number of filled pods, grain weight per plant, and a 100 seeds weight by 27%, 54%, and 25%, respectively. Phosphorus fertilization at dosage of 108 kg P_2O_5/ha increased P and K content, and hence improved soybean growth as indicated by high shoot biomass and CCI resulting in better pod setting and seed development.

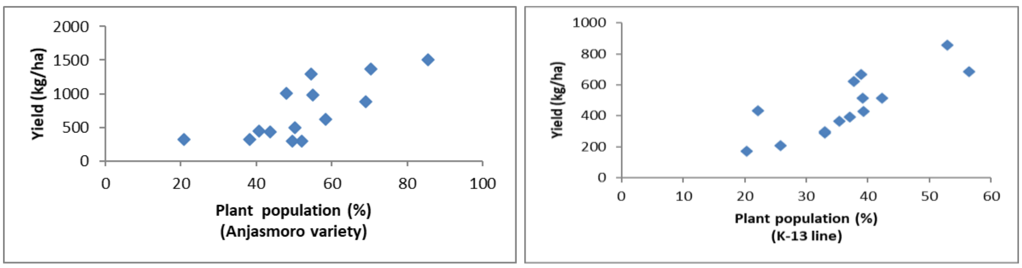
Both genotypes tested survive and complete their life cycle even under high salinity of soil and irrigation water stress. This indicates that the two genotypes tested (Anjasmoro and K-13) can tolerate soil salinity of 9-17 dS/m. Some other research studies also showed that these genotypes are tolerant to soil EC of 14.4 dS/m (Purwaningrahayu et al., 2015; Susanto et al., 2016; Taufiq et al., 2016; Putri et al., 2017, 2019).

Table 3. *Effect of P fertilization and genotype on yield and yield attributes of soybean in saline soil. Tuban, second dry season 2018*

Treatment	Number of filled pods/plant	Grain weight (g/plant)	Weight of a 100 seeds (g)	Grain yield (kg/ha)
P dosage				
• 72 kg/ha	23.1 b	4.38 b	8.74 b	430.52 b
• 108 kg/ha	29.5 a	6.77 a	11.00 a	763.46 a
Genotype				
• Anjasmoro	28.8 A	6.15 A	9.58 A	733.59 A
• K-13	23.7 B	5.00 A	9.15 A	460.39 b
CV (%)	27.34	30.78	13.54	20.72

Numbers in the same column with the same letter means no significant difference according to LSD 5% test

Soybean yield positively correlates with plant population (r = 0.75 and 0.81**, n = 28, respectively for Anjasmoro variety and K-13 line, at harvest, *Fig. 5A, B*). Plant stands during germination stage were relatively similar (73%) among the two P fertilization dosage (*Fig. 6A*). However, the final plant stand was 10% higher with P fertilization of 108 kg/ha P_2O_5 than 72 kg P_2O_5/ha (*Fig. 6B*). This indicates that higher dosage of P fertilization improves soybean survival under saline circumstance, and hence reduces the number of dead-plant.



(A) (B)

Figure 5. *Correlation between number of harvested-plant and soybean grain yield Anjasmoro variety (A) r = 0.75 and K-13 line (B) r = 0.81 in saline soil. Tuban, second dry season 2018*

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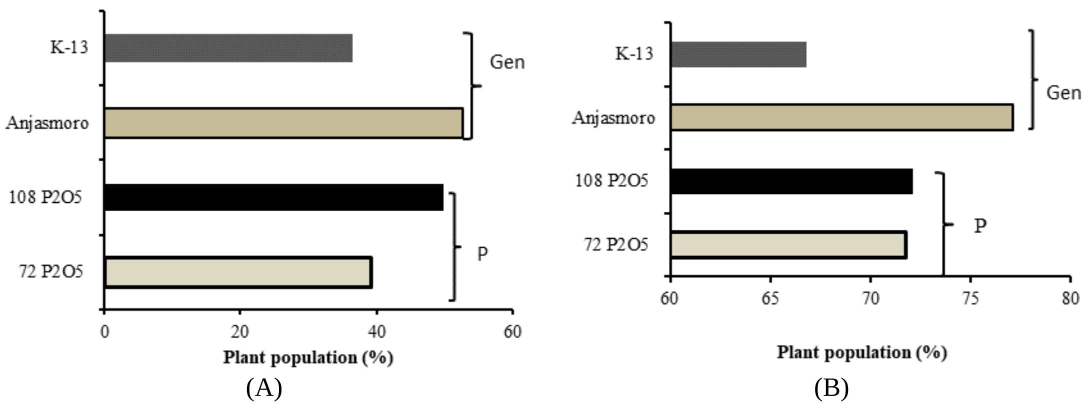

Figure 6. *Effect of P fertilization and soybean genotype on plant population percentage in germination stage (A) and at harvest (B) in saline soil. Tuban, second dry season 2018*

The two tested genotypes had different germination capability under high saline condition. Soil ECs during seedling stage was 9.0 dS/m, and germination percentage observed at 15 DAP of Anjasmoro variety was higher than that of K-13 line (*Fig. 6A*). Plant stands at harvest of Anjasmoro variety were also higher than that of K-13 (*Fig. 6B*). Grain weight per plant and 100 seeds of Anjasmoro variety did not differ from K-13, but Anjasmoro had filled pods 21.5% higher than K-13. So that Anjasmoro yielded 59.4% higher than K-13 (*Table 3*). Salt-tolerant genotypes has better germination capability and growth under saline condition than salt-sensitive genotypes (Putri et al., 2017a), and this phenomenon is related to the decreasing GA/ABA ratio (Shu et al., 2017). Based on plant population in early growth stage and at harvest, growth parameters, as well as grain yield, it seems that Anjasmoro variety is more tolerant to high soil salinity than K-13. However, soybean yield in this experiment is very low when compared with soybean productivity in optimal land. The yield potential of the Anjasmoro variety in optimal land is 2.03-2.25t/ha (Balitkabi, 2021). The average yield of soybean in saline soil/suboptimal land in this study can be categorized as moderate (430-760 kg/ha), from previous studies at ECs 8.19 dS/m Anjasmoro yield: 121.6 kg/ha, K-13 yield: 119.7 kg/ha; in saline soil at ECs 13.20 dS/m Anjasmoro yield: 77.4 kg/ha and K-13 yield 289.7 kg/ha; K-13 line yield: 800-1,090 kg/ha in saline soils at ECs 11-13 dS/m (Putri et al., 2017; Taufiq et al., 2017; Purwaningrahayu and Taufiq, 2018).

Conclusion

Plant growth during the vegetative and generative phases was severely hampered, with soil EC reaching 9.0-17.0 dS/m and EC of irrigation water reaching 6.0-16.4 dS/m. P fertilizer at a dose of 108 kg P_2O_5/ha in saline soil on tolerant soybean genotypes could improve tolerance by increasing P and K absorption and reducing Na uptake thereby reducing the number of dead plants. P fertilization at this dose also improved plant growth and seed development, thereby increasing the number of filled pods and yields. Based on seed germination, survival plant population and yield, the Anjasmoro variety was more saline tolerant than the K-13 line. The use of Anjasmoro variety and P fertilization as much as 108 kg/ha P_2O_5 could increase the growth and yield of soybeans in saline soil ECs up to 17 dS/m. It is recommended for future studies to repeat these treatments in other locations with different salinity levels in saline soil of Indonesia.

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