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Response of soybean genotypes to salinity in germination stage

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Abstract. *Putri PH, Susanto GWA, Artari R. 2017. Response of soybean genotypes to salinity in germination stage. Nusantara Bioscience 9: 133-137.* Salinity is one of the agricultural problem, especially in marginal land. The negative impact of salinity for plants such as delay germination time, growth inhibition, reduce nodules formation, decrease biomass accumulation, and reduce yield quantity and quality. Initial selection for salt resistance of soybean, conducted on 16 genotypes, using three levels of NaCl and three replications. Germination conducted for six days, in germinator which has been set at 25°C, with lighting. Mean Germination Time (MGT), Final Germination Percent (FGP), sprout length (root and overall) and weight (fresh and dry), seedling vigor index (length and weight) were observed. Data were analyzed using ANOVA, followed by LSD at 5%. Sixteen soybean genotypes show inhibition effect of salinity. The results showed that 125 mM NaCl does not inhibit germination but it causes abnormal growth at some genotypes. Number of normal germination decreased 20-30% at 125 mM NaCl. Sprouts total length declined by 17-58% at 75 mM and 45-78% at 125 mM NaCl. Root length decrease from 23.2-55.3% at 75 mM and 52.1-81.2% in 125 mM. Fresh weight diminish by 3.8-39.9% at 75 mM and 12.7-52.5% at 125 mM NaCl. Response of sixteen soybean genotypes into salinity stress is clearly visible at sprout length and fresh weight variable. Ichiyou was salt-sensitive based on all variable especially sprout length and fresh weight, while Baluran was salt-tolerant up to 125 mM NaCl based on sprout length and fresh weight.

Keywords: Germination, *Glycine max*, salinity, salinity stress

Abbreviations: DAS = Days After Sowing, FGP = Final Germination Percent, MGT = Mean Germination Time, SLVI = Seedling Length Vigor Index, SWVI = Seedling Weight Vigor Index

INTRODUCTION

Salinity becomes limiting factor for plants, particularly on marginal land. Salinity affects plants growth and development in three ways: inhibit plants water uptake, nutrient imbalance, and toxicity of specific ion (Kristiono et al. 2013). Effect of salinity on plants depends on growth stage, variety, and salinity level (Sobhanian et al. 2010; Kondetti et al. 2012; Agarwal et al. 2015).

Germination and early growth stages critically determine crops productivity, including soybean. Germination stage is known to be most sensitive to salinity. Therefore, this stage often used for selection of salinity tolerance in plants. Seeds with rapid and normal germination in saline conditions expected tolerant to salinity (Bybordi and Tabatabaei 2009). Essa (2002) argued that the ability to germinate in saline conditions indicate the genetic potential of saline tolerant.

In soybean, salinity is known to delay germination time (Xu et al. 2011; Taufiq and Purwaningrahayu 2013; Kandil et al. 2015). Soybean germination percentage decreases significantly with increasing levels of saline. Cokkizgin (2012) reported that increasing levels of NaCl from 0 to 180 mM reduces the germination of *Phaseolus* up to 50%, in line with Ahmadvand et al. (2012), Kondetti et al. (2012), Dhairyasheel and Sharad (2015), and Kandil et al. (2015) in soybeans.

Salinity stress affects the root and shoot length (Anitha and Usha 2012; Kondetti et al. 2012; Taufiq and Purwaningrahayu 2013), also decrease fresh and dry weight (Dolatabadian et al. 2011; Farhoudi and Tafti 2011; Anitha and Usha 2012; Kondetti et al. 2012). Cokkizgin (2012) reported that salinity reduces germination vigor index.

Research on Indonesian soybean response to salinity stress has been done in Wilis, Malabar, Sindoro, Lokon, Tidar, Slamet, Orba, Sinabung, Kawi, Kipas Putih and Jayawijaya. Early vegetative stage until harvest used to be an object of study (Lubis 2000; Sunarto 2001; Yuniati 2004; Farid 2006). However, the experimental study on response to salinity in soybean germination stage has not been done. The aim of this study was to evaluate the response of 16 soybean genotypes in germination stage as an initial information for soybean tolerance to salinity stress.

MATERIAL AND METHODS

Procedures

The study was conducted at Germplasm Laboratory; Indonesian Legume and Tuber Crops Research Institute (ILETRI), Malang, East Java, Indonesia; in 2015. Research material were 16 soybean genotypes: Detam 2, Detam 3, Detam 4, Pangrango, Ijen, Mahameru, Baluran, Petek, G100H, Kaba, Ichiyou, IAC100, Argopuro, Gumitir, Seulawah, and Merbabu. Completely randomized design with two factors and three replications was used as an experimental design. The first factor is soybean genotype and the second is saline level (NaCl concentration). The concentration of NaCl were 0 (control), 75 mM, and 125 mM. Pre-seed treatment was done by immersion in NaOCl for 5 minutes and then rinsed with distilled water. Fifty seeds were sown in petridish, using filter paper as a growth medium. NaCl solution (5 mL) with a predetermined concentration were given. Seeds were grown in germinator, at 25^{o}C, lighting condition, for 6 days (International seed association/ISTA with modification). Observations were carried out from 3 to 6 DAS. The observation variables were FGP (Equation 1), root length, total sprout length, fresh and dry weight of seedling, SLVI (Equation 2) and SWVI (Equation 3) (Mosavian and Eshraghi-Nejad 2013). Seeds germinate if the radicle appears along 2 mm. Sprouts with spiral hypocotyl and dwarf primary root categorized as abnormal sprout (Ahmadvand et al. 2012). Length (root and total), fresh weight and dry weight of seedling were calculated by taking 20 samples of the normal seedling. Dry weight was calculated after sprouts were dried in 60^{o}C, for 72 hours.

$$\text{Final Germination Percent} = \frac{\text{normal sprout at the last day of germination}}{\text{total seed}} \times 100\% \quad (1)$$

$$\text{seedling lenght vigour index} = FGP \times \text{total sprout lenght} \quad (2)$$

$$\text{seedling weight vigour index} = FGP \times \text{sprout dry weight} \quad (3)$$

Data analysis

Data were analyzed by ANOVA, followed by LSD 5%, using MSTAT 1.4 version.

RESULTS AND DISCUSSION

Materials consist of small-seeded soybean (Pangrango, Ichiyou, Petek, Seulawah, and Merbabu), also medium-large seeded (Detam 2, Detam 3, Detam 4, Ijen, Mahameru, Baluran, G100H, Kaba, IAC100, Argopuro, and Gumitir) (Balitkabi 2016). ANOVA results showed the significant effect of genotype, concentration and interaction factor into all of the variables, except dry weight (Table 1). Dry weight highly significant influenced by genotype (Pr = 0.00), significant by concentration (Pr = 0.03), but was not influenced by interaction of both factors (Pr = 0.24). The significant influence of interaction on observation variables (except dry weight) indicates that effect of genotypes and concentration factor are not independent.

Mean Germination Time (MGT)

Most of genotypes were germinate at 3 DAS, either in control or saline (75 and 125 mM NaCl). The differences between genotypes were not clearly visible under the saline condition on medium-large seeded genotypes although ANOVA results indicate that genotype and NaCl concentration affected the MGT significantly. Treatment with 75 mM NaCl delay Detam3 germination, while treatment with 125 mM NaCl delay Gumitir germination (Figure 1A).

Differences between genotypes seen in the small seeded group. Genotypes Seulawah and Merbabu delayed germination at 75 mM NaCl, whereas Ichiyou and Petek delayed germination at 125 mM NaCl (Figure 1B). Most of the genotype delayed germination in salinity stress though no more than one day. This shows that the materials are still able to cope salinity stress at 125 mM NaCl.

Final Germination Percent (FGP)

FGP measurement indicate values diversity between genotype and concentration. Salinity reduces FGP value on all genotypes except Detam 2, Detam 4, Ijen, and G100H. FGP decreased by 20-30% at 125 mM NaCl concentration. Gumitir and Seulawah also impaired FGP ± 20% at a concentration of 125 mM NaCl, although it has the power to grow less well in the control condition.

Final Germination Percent of Gumitir, Argopuro, IAC100, Detam 3, Ichiyou, and Seulawah start to decline at 75 mM NaCl. This indicates that those genotypes more sensitive than others in saline condition (Figure 2). Detam 2, Detam 4, Ijen, and G100H more tolerant since their FGP does not decline at 125 mM.

Sprout length

Root length reduction due to the enhancement of NaCl concentration can be an indicator of soybean salinity tolerance in germination stage. In general, sprout length of 16 soybean genotypes was decreased with increasing level of saline, show inhibition effect. Sprout length of G100H and Ichiyou significantly reduced in 75 mM NaCl, continued in 125 mM (Table 4). This result is in line with Agarwal et al. (2015), reported that salinity reduces shoot length and dry weight at different levels of salinity, according to soybean varieties.

Fresh and dry weight

Interaction factor has no significant effect on sprout dry weight based on ANOVA. Therefore, further test on dry weight variable was not taken. Fresh weight of G100H significantly declines from 12 g at control into 8.8 g at 75 mM and 7.2 g at 125 mM. Ijen, Mahameru, and Baluran more tolerant than other varieties since fresh weight at 75 mM and 125 mM were not significantly different compared to control (Table 5).

Vigor index

Seedling vigor index illustrates seed ability to germinate in suboptimal conditions, in this case, saline condition. SLVI value obtained by multiplying sprouts length and normal germination percentage, while SWVI value obtained from the average of dry weight and normal germination percentage.

In general, SLVI value of 16 soybean genotypes decreases with increasing concentrations of NaCl (Figure 3). SLVI value of Ichiyou (genotype code no. 11) decreased significantly at 75 mM (419.5) and 125 mM (169.1) compared with control (1229). Argopuro (genotype code no.13) decreases significantly from 1008 (control), became 636.2 (75 mM) and 364 (125 mM), Mahameru (genotype code no.6) and Baluran (genotype code no.7) reduces significantly at 125 mM NaCl than control, whereas 75 mM NaCl has no significant effect on both varieties. It seems that Mahameru and Baluran are more tolerant to salinity than others according to SLVI in saline condition.

There is no significant differences between concentrations for SWVI value except at Pangrango, Mahameru, Kaba, and Ichiyou. Application of 75 mM NaCl decreased Mahameru and Ichiyou SWVI value but does not affect Pangrango and Kaba. SWVI value of Pangrango and Kaba decreased at higher concentrations: 125 mM NaCl (Figure 4).

Table 1. ANOVA on FGP, MGT, sprout length, sprout weight and Vigor index of 16 soybean genotypes

Source of variance	Pr > F*							
	FGP	MGT	Root length	Sprout length	Fresh weight	Dry weight	SLVI	SWVI
Genotype	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Concentration	0.00	0.00	0.00	0.00	0.00	0.03	0.00	0.00
Interaction	0.00	0.00	0.00	0.00	0.00	0.24	0.00	0.00
CV (%)	13.74	5.27	15.14	17.41	11.55	22.38	20.82	20.99

Note: *level of significance 5%

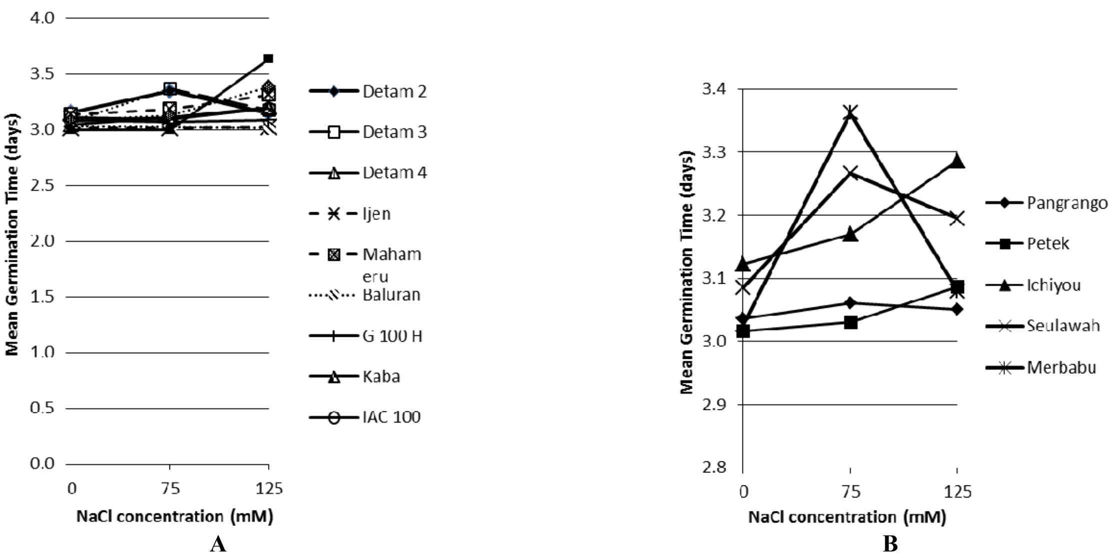

Figure 1. Mean germination time of 16 soybean genotypes at three level NaCl, ILETRI Germplasm Laboratory, 2015

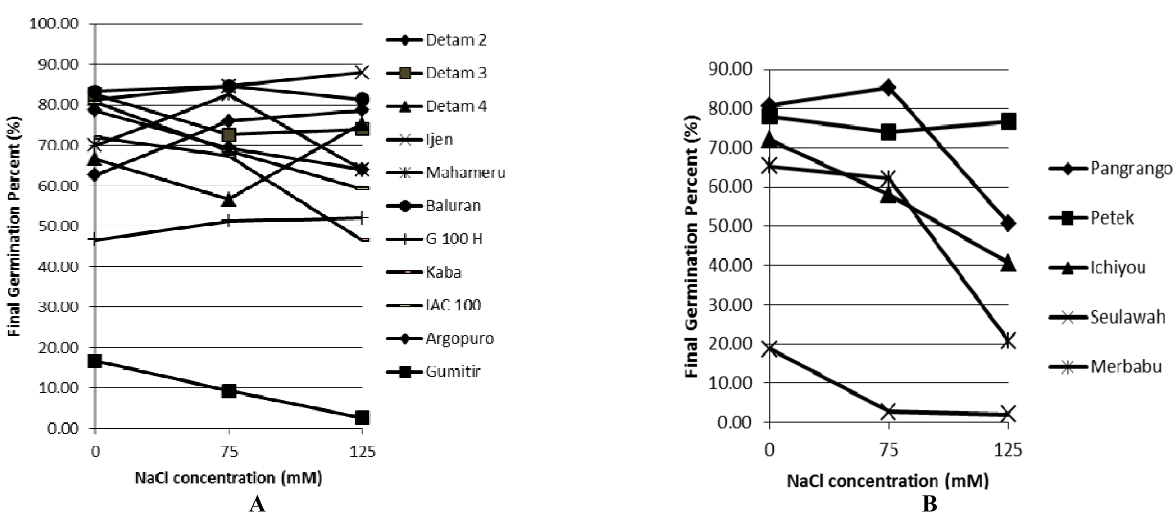

Figure 2. Final germination percent 16 soybean genotypes in three level of NaCl. ILETRI Germplasm Laboratory 2015

Table 4. Sprout length of 16 soybean genotypes at three levels of NaCl

Genotype	Total length (cm)						Root length (cm)					
	0		75 mM		125 mM		0		75 mM		125 mM	
Detam 2	10.7	c*	6.2	def	3.9	cdef	7.2	cd	5.4	bcd	2.7	cde
Detam 3	9.7	c	5.8	ef	3.5	defg	5.9	de	4.2	ef	2.0	f
Detam 4	11.3	bc	5.6	f	4.6	b	7.4	c	4.3	def	3.1	bc
Pangrango	9.9	c	6.5	cdef	3.9	bcde	6.8	cd	4.2	ef	2.8	cd
Ijen	10.3	c	7.0	bcde	3.4	defg	6.4	cd	3.8	f	1.7	f
Mahameru	10.5	c	7.8	ab	4.1	bcd	4.9	ef	3.8	f	2.0	ef
Baluran	9.9	c	8.2	ab	5.4	a	7.2	cd	5.0	cde	3.4	ab
Petek	11.7	bc	7.6	bc	4.0	bcd	7.3	cd	4.4	def	2.1	def
G100H	17.6	a	8.0	ab	4.6	bc	12.5	a	5.6	bc	3.0	bc
Kaba	10.5	c	5.9	ef	3.2	efg	6.8	cd	3.5	f	1.8	f
Ichiyou	17.4	a	7.2	bcd	4.0	bcd	9.7	b	4.4	def	2.3	def
IAC100	10.4	c	5.8	ef	3.0	g	6.9	cd	3.9	ef	1.9	f
Argopuro	12.8	bc	9.1	a	5.6	a	10.2	b	7.1	a	4.0	a
Gumitir	5.3	d	2.0	g	0.4	h	4.0	f	1.5	g	0.3	g
Seulawah	0.6	e	0.0	h	0.0	h	0.4	g	0.0	h	0.0	g
Merbabu	14.2	b	9.1	a	3.2	fg	10.2	b	6.3	ab	1.9	f
LSD	3.2		1.3		0.7		1.5		1.2		0.7	

Note: *similar notation on the same column showed no significant difference at 5% significance level

Table 5. Fresh weight of 16 soybean genotypes at three levels NaCl

Genotype	Fresh weight (g)					
	0		75 mM		125 mM	
Detam 2	8.1	de*	6.6	cd	6.5	cd
Detam 3	7.9	de	5.4	de	5.6	def
Detam 4	7.1	ef	4.9	ef	5.2	ef
Pangrango	8.6	cd	6.8	c	5.7	def
Ijen	4.5	hi	6.4	cd	5.6	def
Mahameru	9.6	bc	9.2	ab	8.1	b
Baluran	7.4	def	10.1	a	9.2	a
Petek	5.7	gh	6.3	cd	4.9	f
G100H	12.1	a	8.8	b	7.2	bc
Kaba	7.6	def	6.9	c	5.4	ef
Ichiyou	6.4	fg	3.9	f	3.6	g
IAC100	8.1	de	6.7	c	6.1	de
Argopuro	10.6	b	9.1	ab	8.2	b
Gumitir	3.8	i	2.0	g	0.4	h
Seulawah	0.8	j	0.0	h	0.0	h
Merbabu	7.6	def	6.7	c	3.6	g
LSD	1.41		1.22		0.98	

Note: *similar notation on the same row and column showed no significant difference at 5% significance level

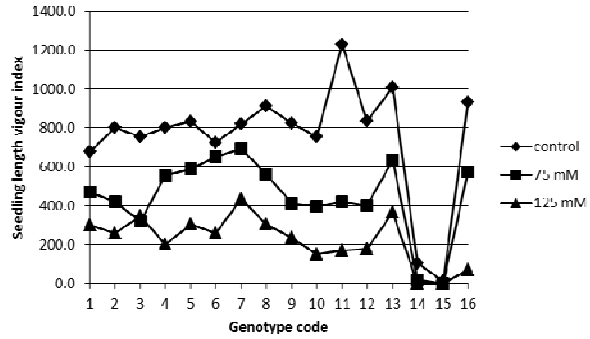

Figure 3. SLVI value of 16 soybean genotypes in three level of NaCl, ILETRI Germplasm Laboratory 2015.

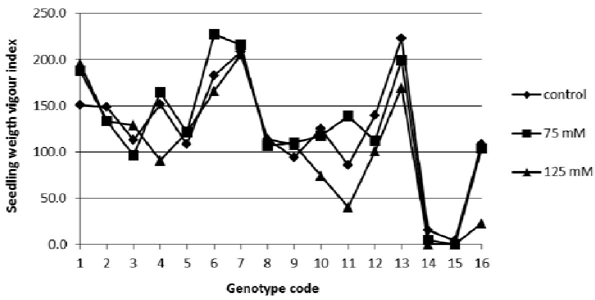

Figure 4. Seedling weight vigor index of 16 soybean genotypes in three level of NaCl, ILETRI Germplasm Laboratory 2015

Discussion

Seed viability, germination time, hypocotyl length and root dry weight variables are very sensitive to salinity stress (Dianawati et al. 2013; Taufiq and Purwaningrahayu 2013), so that used to be an observation variable in the evaluation of salinity response. Genotype without significant differences compared to control is potential to be developed as salt tolerant.

All of the genotypes does not lead to delay the germination either in 75 mM or 125 mM NaCl. Detam-3, Seulawah, Merbabu, Gumitir, Ichiyou, and Petek delayed germination but did not reach one day. Xu et al. (2011) reported that 100 mMolal.L^{-1} NaCl decreases MGT value. Farhoudi and Tafti (2011) and Taufiq and Purwaningrahayu (2013) reported that salinity delay germination in soybean. MGT is average time of germination. Delayed germination in saline conditions caused by changes in cells osmotic potential thereby inhibit water uptake in imbibition stage (Cokkizgin 2012; Kondetti et al. 2012). This experiment suggests that the materials able to germinate up to 125 mM NaCl.

FGP value in this experiment represents the number of normal seedlings of each genotype on a certain NaCl concentration. FGP value for Gumitir, Argopuro, IAC100, Detam 3, Ichiyou, and Seulawah decreased with increasing concentrations of NaCl. It means that increasing of NaCl concentration causing the decline of their normal seedling percentage. Number of normal germination decreased 20-30% at 125 mM NaCl. The results showed that 125 mM NaCl does not inhibit germination but it causes abnormal growth at some genotypes. Sprout abnormal growth may be caused by the toxic effects of Na^+, Cl^-, or reactive oxygen species (ROS). Subramanyam et al. (2012) reported that H_2O_2 is one of the ROS resulting in apoptosis, cell shrinkage, chromatin condensation and DNA fragmentation.

Salinity inhibits sprouts growth of 16 soybean genotypes significantly since sprout length and weight decreased with increasing concentrations of NaCl. Sprouts total length declined by 17-58% at 75 mM and 45-78% at 125 mM NaCl. Root length decrease from 23.2-55.3% at 75 mM and 52.1-81.2% in 125 mM. This experiment shows that root is more sensitive to salinity stress so that it can be used as one indicator of salinity tolerant. The growth of Detam 4, G100H, and Ichiyou inhibited >50% at 75 mM NaCl so it can be said salt-sensitive. Baluran is salt-tolerant due to growth inhibition <20% at 75 mM and <50% at 125 mM NaCl.

Fresh weight diminish by 3.8-39.9% at 75 mM and 12.7-52.5% at 125 mM NaCl. Detam 3, Detam 4, G100H and Ichiyou are salt-sensitive since fresh weight declined by 20% at 75 mM NaCl, whereas the Ijen and Baluran are salt-tolerant because there is no decrease in fresh weight either at 75 mM and 125 mM NaCl. Kandil et al. (2015) reported that increasing concentrations of NaCl can inhibit roots and stems growth also reduce seedling fresh and dry weight. Hypocotyl and root fresh weight reduced 48-56% at 80 mM NaCl compared with controls (Sobhanian et al. 2010). Salt stress also can reduce K^+ levels on dry matter (Farhoudi and Tafti 2011), This negative impact of salinity was due to ions toxic effects and inhibition of water uptake by potential osmotic changes so disturbed metabolism for growth (Dolatabadian et al. 2011).

Seedling vigor index has a linear function to a normal seedling percentage as well as the seedling length and dry weight. Ichiyou vigor index is low because it has a low percentage of normal seedling, sprout length and fresh weight. This is inline with Cokkizgin (2012) that reported salinity could reduce seedling vigor index in soybean.

The results of this study showed that soybean has a varying response to salinity. Each genotype shows specific to respond to salinity. Some genotypes can germinate in saline condition but face growth inhibition in the further development stage. Salt-tolerance genotypes have to well germinate and growth vigorously under saline condition. The response of sixteen soybean genotypes into salinity stress is clearly visible at sprout length and fresh weight variable. Ichiyou was salt-sensitive based on all variable especially sprout length and fresh weight, while Baluran was salt-tolerant up to 125 mM NaCl based on sprout length and fresh weight.

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