Biosaintifika 10 (2) (2018) 408-415

Biosaintifika
Journal of Biology & Biology Education

http://journal.unnes.ac.id/nju/index.php/biosaintifika

Salinity Tolerance of Mungbean Genotypes at Seedling Stage

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DOI: http://dx.doi.org/10.15294/biosaintifika.v10i2.13999

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History Article

Received 7 May 2018
Approved 11 July2018
Published 30 August 2018

Keywords

Germination; Salt; Seedling Vigor; *Vigna radiata*

Abstract

Salinity is a major abiotic stress limiting mungbean production worldwide including Indonesia. Since mungbean plant is very sensitive to salt condition, selection of salinity tolerant genotypes becomes important for mungbean improvement. The objective of this study was to evaluate the tolerance of eight mungbean genotypes to salinity at seedling stage under different levels. The experiment was arranged in a randomized complete block design with two factors (mungbean genotypes and salinity levels) and triplicates. Observation variables were germination percentage, vigor index, germination rate, hypocotyls length, epicotyls length, root length, number of root, seedling fresh weight, and seedling dry weight. The result showed that increasing level of salinity concentration inhibited the speed of germination, germination percentage, vigor index, normal seedling fresh weight, and number of lateral roots. Murai and Vima 1 were identified as tolerant genotypes, while Vima-2 and MLGV 0180 were identified as salinity sensitive genotypes at seedling stage. Currently, mungbean varieties with special characters, such as saline-tolerant is not yet available. The availability of saline-tolerant variety of mungbean is a cheaper and easier technology for farmers to anticipate the expansion of the saline area. The tolerant genotypes may be further tested at the later stage to obtain promising genotype tolerant to salinity that effectively assist mungbean breeding program.

How to Cite

Hapsari, R. T. & Trustinah. (2018). Salinity Tolerance of Mungbean Genotypes at Seedling Stage. *Biosaintifika: Journal of Biology & Biology Education*, 10(2), 408-415.

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p-ISSN 2085-191X
e-ISSN 2338-7610

INTRODUCTION

Mungbean is one of important cash crop legumes in Indonesia, especially in dry regions due to its short life (early maturity) and good adaptation in drought condition. In Indonesia, mungbean ranks third after soybean and groundnut as a strategic legumes crops. Mungbeans are rich with vegetable proteins and very popular as food raw material in Indonesian community. The Indonesian new release varieties (Vima 1, Vima 2 and Vima 3) have high protein content , i.e. 21-28% on dry basis (ILETRI 2016). Mungbean is mostly used as food such as porridge, flour products, beverage products, cakes, noodles, sprouts and a small portion of fodder. However, the yield of mungbean is still low. The average yield is approximately 1.6 tons of dry grains per hectare with an area of agricultural land decreased.

Based on Indonesian agricultural statistic report of food crops (2017), the mungbean harvested area in 2015 was 229.475 ha, which has decreased by 27.9% when compared to the harvested area in 2005 (318.337 ha). One of the causes of declining cultivation area is land degradation due to salinity (Las *et al.,* 2006). Currently, the statistics data of saline land in Indonesia is not available yet. However, the existence ofsaline soilshas been reported by several researchers, mainly located in coastal areas. Widjanarko *et al.* (2017) reported EC (Electrical Conductivity) soil in Banda Aceh various from 6.48 to 8.91 dS/m. Taufiq *et al.* (2016) reported that in coastal area located in Tuban and Lamongan district (East Java), EC soil has ranged from 4.4 to 8.0 dS/m. Sitorus (2012) reported that there was a changing in land use in Indramayu, i.e. from agricultural land into ponds. Marwanto *et al.* (2009) reported that thesalinity soil in Indramayu district was varied from 0.03-12.91 dS/m and it was classified as saline as effect of sea intrusion. Dachlan (2013) reported that in South Sulawesi, soil salinity has become important problem, especially in coastal areas such as in Districts of Jeneponto, Pangkep, Bantaeng, Selayar, and Barru. Moreover, recent global climate change has made this situation much worse.

Mungbean plant is a highly salt-sensitive crop (Rachmawatie and Nasir 2014). Salinity stress causes severe yield loss and affects the quality of mungbean (Saha *et al.,* 2010). Excessive accumulation of sodium ions in saline soils results in different physiological abnormalities and can reduces the grain yield up to 100% (Tavakkoli *et al.,* 2010; Hasanuzzaman *et al.,* 2012; Sehrawat *et al.,* 2015). Sehrawat *et al.* (2015) reported that high salinity (75 mM NaCl) in susceptible variety cause yield losses up to 100% in the dry season. Taufiq and Purwaningrahayu (2013) reported that salinity decreased yield and yield components of 10 varieties of mungbean up to 15-47% (ECw 4.0 dS/m; ECs 2.65 dS/m).

The most critical stage in seedling stage is seed germination (Kandil *et al.,* 2012; Sehrawat *et al.,* 2014, El Kafafi *et al.,* 2015, Trustinah *et al.,* 2016). Salinity stress during the entire life cycle of the crop cause considerable yield losses in mungbean (Sunil *et al.,* 2012). Salt stress at seedling stage reduces seed germination, fresh and dry biomass, shoot and root lengths, and seedling vigor of mungbean (Sehrawat *et al.,* 2014; Dutta and Bera 2014; Ghosh *et al.,* 2015). At saline condition, the seed germination was significantly delayed and there were large differences among genotypes (Hetharie, 2008; Kandil *et al.,* 2012, Taufiq dan Purwaningrahayu 2013; Sehrawat *et al.,* 2014, El Kafafi *et al.,* 2015, Trustinah *et al.,* 2016).

Selection of salinity tolerant genotypes becomes important for mungbean improvement. Currently, mungbean varieties with special characters, such as saline-tolerant has not yet been developed. The availability of saline-tolerant variety of mungbean is a cheaper and easier technology for farmers in order to anticipate the expansion of the saline area. The objective of this study was to evaluate the salinity tolerance of eight mungbean genotypes at seedling stage under different salinity levels.

METHODS

Plants Materials

A total of eight genotypes used for this experiment consisted of five improved varieties, two introducing genotypes and one local genotype derived from Indonesian Legumes and Tuber Crops Research Institute's (ILETRI) genebank, Indonesian Agency for Agricultural Research and Development (IAARD). List of mungbean genotypes used in this study is presented in Table 1.

Salinity assay under laboratory scale

This research was conducted at Seed Quality Testing Laboratory, Indonesian Legumes and Tuber Crops Research Institute (ILETRI) Malang, East Java. The experiment was arranged in a randomized complete block design with two factors and three replications. The first factor was eight mungbean genotypes which consisted of Vima 1, Vima 2, Vima 3, Murai, Kenari, MLGV 0180, MLGV 0589, and MLGV 0066. The se-

cond factor was four salinity levels consisting of four treatments using diluted sea water. Those four diluted sea water treatments were control (EC 0 dS/m), diluted by 10% (EC 5.74 dS/m), diluted by 15% (EC 8.15 dS/m), and diluted by 20% (EC 10.32 dS/m). Therefore, there were 32 different combinations of trials.

Table 1. Mungbean genotypes used for salinity tolerance evaluation *

Genotype	Origin	Remark
Vima 1	Indonesia	Improved variety
Vima 2	Indonesia	Improved variety
Vima 3	Indonesia	Improved variety
Murai	Indonesia	Improved variety
Kenari	Indonesia	Improved variety
MLGV 0066	India	Introducing genotype
MLGV 0180	Taiwan	Introducing genotype
MLGV 0589	Indonesia	Local

*The colection of ILETRI Genebank

Each experiment used 50 sterilized seeds, which germinated in plastic box. Seeds were surface sterilized by immersion for 2 minutes in sodium hypochlorite solution, then repeatedly washed with deionized water. To ensure the germinated seeds remain standing straight up, gauze was put inside the plastic box. Seeds were germinated in seed germinator (Seedburo Equipment Company), adjusted to 25 ± 1˚C in dark condition.

Data collection

The observation was carried out from one until seven days after sowing (DAS). Seeds were categorized as germinated, hard, or non-viable as described by International Seed Testing Association/ISTA (2014). The observation variables were speed of germination, vigor index, germination percentage, hypocotyls length, epicotyls length, root length, number of lateral roots, normal seedling fresh weight, and normal seedling dry weight. Hypocotyl length, epicotyls length, root length and number of lateral roots, were calculated by taking 25 samples of normal seedling. Normal seedling dry weight was calculated after sprouts were dried in 70˚C for 48 hours.

Data analysis

The data were statistically analyzed using PKBT-STAT 1.0 software (Center for Tropical Fruit Studies IPB 2007). The significant data based on the analysis variance were analyzed using Least Significant Different 5%.

RESULTS AND DISCUSSION

The result showed that different salinity stress levels affected all seedling characters, and each genotype had different responses to salt stress (Table 2). There was a significant interaction effect between mungbean genotypes and salinity levels on speed of germination, vigor index, germination, normal seedling fresh weight, normal seedling dry weight, and number of lateral roots.

The increasing salinity level was significantly reduced the speed of germination (Table 3). The speed of germination is one of the parameters that indicate growing strength of seed vigor which is more sensitive parameters than germination rate (Sadjad *et al.,* 1999; Sari *et al.,* 2013). Speed of germination shows the number of normal seedlings per day. High speed of germination reflects the vigor of seeds, since the seeds can germinate rapidly in a relatively short

Table 2. Analysis of variance on seedling characters

Characters	F test			
	Replicates	Genotypes (G)	Salinity levels (S)	G*S
Germination rate (%/etmal)	**	**	**	*
Vigor index (%)	**	**	**	*
Germination (%)	**	**	**	**
Normal seedling fresh weight (g)	ns	**	**	**
Normal seedling dry weight (g)	**	**	**	**
Root length (cm)	**	*	**	ns
Hypocotyl length (cm)	ns	**	**	ns
Epycotil length (cm)	ns	*	**	ns
Number of lateral roots	*	**	**	*

time. Based on Table 3, it clearly shows that increasing salinity level to 10%, 15% and 20% reduce speed of germination by 34%, 55%, and 69% respectively compared to control. Decrease percentage varied among the tested genotypes. This condition occurs due to the genotype response to salt stress, that is by delaying the time to germinate. This result is in accordance with those reported by Sehrawat *et al.* (2014) and Trustinah *et al*. (2016). Murai variety has the highest speed of germination, while Vima 3 and MLGV 0180 have the lowest speed of germination at 20% salinity stress level. Kandil *et al.* (2012) reported that saline condition reduces the ability of seed to absorb water causing rapid reduction in germination rate and induces many metabolic changes.

Similar to speed of germination, vigor index also decreased along with the increasing salinity stress level. Increasing salinity level to 10%, 15% and 20% reduce vigor index by 22%, 39%, and 72%, respectively compared to the control (Table 4). These results are in line with those reported by Sehrawat *et al.* (2014) who reported that vigor index decreased with increasing the salinity levels. Vigor index was observed from number of normal seedling on the first count, calculated on day 5 after planting. The seedling vigor determines the potential of seedling to grow under salinity stress environment. At 10% salinity level, five genotypes still had a vigor index over 70%. However, if salinity stress level was increased to 15%, only one genotype had a vigor index over 70% (Vima 1). Salinity stress at 20%, extremely reduced vigor index all of tested genotypes. Nevertheless, Vima-1, MLGV 0589, and Murai still showed good vigor index performance in 20% salt conditions compared to the other genotypes. On the contrary, MLGV 0180 extremely responded sensitive to salt stress. Sunil *et al.* (2012) reported that salt stress adversely affected the biometrics, morpho-physiological, biochemical and biophysical characters of mungbean. Furhermore, he was explained that salt stress reduced total chlorophyll contents, nitrate reductase activity, photosyntetic rate, transpiration rate, and stomatal conductance.

Table 3. Speed germination of eight mungbean genotypes in various salt levels

Genotypes	Speed of germination (%/etmal) in various salt levels			
	0%	10%	15%	20%
Vima 1	20.01^{a}	14.49^{cd}	11.11^{fg}	5.86^{jk}
Vima 2	18.62^{ab}	11.66^{d-g}	6.12^{jk}	2.75^{l-n}
Vima 3	19.04^{ab}	6.10^{jk}	4.74^{j-m}	1.78^{n}
Murai	18.67^{ab}	14.74^{c}	9.74^{gh}	6.03^{jk}
Kenari	14.11^{c-e}	9.26^{g-i}	6.61^{ij}	3.31^{k-n}
MLGV 0589	18.04^{ab}	13.77^{c-f}	10.09^{gh}	5.14^{j-l}
MLGV 0180	16.62^{bc}	11.39^{e-g}	6.65^{ij}	1.36^{n}
MLGV 0066	14.35^{cd}	10.71^{g}	7.51^{h-j}	2.05^{mn}

Note: value in the same column followed by the same letter were not significantly different based on LSD test at α 0.05

Table 4. Vigor index of eight mungbean genotypes in various salt levels

Genotypes	Vigor index (%) in various salt levels			
	0%	10%	15%	20%
Vima 1	94.00^{a}	72.67^{b-e}	77.33^{a-e}	38.67^{j-l}
Vima 2	92.67^{a}	42.00^{i-l}	31.33^{k-m}	11.33^{no}
Vima 3	91.33^{ab}	72.00^{b-f}	38.67^{j-l}	12.00^{m-o}
Murai	92.67^{a}	82.67^{a-c}	68.67^{c-h}	29.33^{l-n}
Kenari	76.00^{a-e}	52.67^{f-j}	38.00^{j-l}	16.00^{m-o}
MLGV 0589	86.67^{a-c}	80.67^{a-d}	62.00^{d-h}	30.00^{k-n}
MLGV 0180	84.67^{a-c}	72.67^{b-e}	49.33^{h-k}	0.67^{o}
MLGV 0066	70.00^{c-g}	60.67^{e-i}	51.33^{g-j}	11.33^{no}

Note: value in the same column followed by the same letter were not significantly different based on LSD test at α 0.05

Saline condition also reduced germination percentage. Germination index are shown in Figure 1. Almost all of genotypes had a significant reduction in germination parameters except Murai. At a rate of 10% salinity stress level, almost all genotypes had a germination percentage over 70% except Vima 2. Compared to other genotypes, it seems that Vima 2 was particularly susceptible to salinity in germination parameters. An increase on salt stress up to 15%, Murai, Vima 1 and MLGV 0589 were still had germination percentage more than 80%. Those genotypes also showed a not significantly different of germination percentage if compared with control. Based on the data in Table 5, it seems that salinity levels of 10% up to 15% were showed relatively less significant difference in germination response. However, if the salinity level was increased to 20%, Murai as the one which showed a not significantly different of germination percentage when compared with the control. This means that Murai was tolerant to salt stress at 20% diluted seawater or similar with EC 10.32 dS/m. This result is in agreement with Taufiq and Purwaningrahayu (2014) who reported that Murai showed 82% germination percentage at EC 13.1 dS/m. High accumulation of sodium and chloride ions produced an outside osmotic potential that avoids adequate water uptake or toxic effect of Na^+ and Cl^- ions in saline environment resulted in poor activation of the hydrolytic enzymes and further reduced the seed germination (Murillo-Amador *et al.,* 2002; Khajeh-Hoosseini *et al.,* 2003; Mohammed 2007).

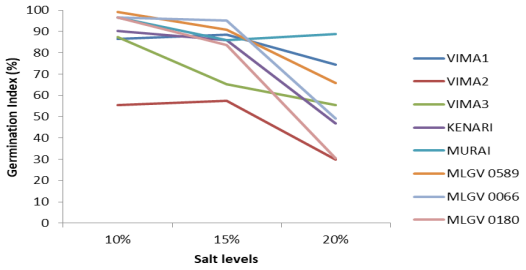

Figure 1. Average of germination index in various salt stress

Table 5. Germination of eight mungbean genotypes in various salt levels

Genotypes	Germination (%) in various salt levels			
	0%	10%	15%	20%
Vima 1	99.33^{a}	86.00^{a-e}	88.00^{a-e}	74.00^{d-f}
Vima 2	94.00^{a-c}	52.00^{gh}	54.00^{gh}	28.00^{i}
Vima 3	96.00^{ab}	84.00^{a-e}	62.67^{fg}	53.33^{gh}
Murai	94.67^{a-c}	91.33^{a-d}	81.33^{a-e}	84.00^{a-e}
Kenari	81.33^{a-e}	73.33^{d-f}	70.00^{e-g}	38.00^{hi}
MLGV 0589	93.33 $^{a-c}$	92.67^{a-c}	84.67^{a-e}	61.33^{fg}
MLGV 0180	94.00^{a-c}	90.67^{a-d}	78.67^{b-f}	28.67^{i}
MLGV 0066	81.33^{a-e}	78.67^{b-f}	77.33^{c-f}	40.00^{hi}

Note: value in the same column followed by the same letter were not significantly different based on LSD test at α 0.05

Table 6. Interaction between genotypes and salt levels on normal seedling fresh weight

Genotypes	Seedling freshweight (g) in various salt levels			
	0%	10%	15%	20%
Vima 1	30.01^{ab}	21.95^{c-g}	20.58^{c-h}	11.98^{l-o}
Vima 2	30.61^{ab}	11.20^{m-p}	12.98^{j-o}	6.85^{op}
Vima 3	24.87^{b-f}	18.88^{f-k}	12.78^{k-o}	9.59^{m-p}
Murai	29.68^{ab}	25.66^{b-e}	20.38^{d-h}	17.97^{g-l}
Kenari	26.29^{a-d}	22.14^{c-g}	19.09^{f-j}	9.05^{n-p}
MLGV 0589	17.50^{g-l}	22.11^{c-g}	15.58^{h-m}	12.09^{l-o}
MLGV 0180	32.15^{a}	26.68^{a-c}	19.58^{e-i}	5.67^{p}
MLGV 0066	14.89^{h-n}	15.25^{h-m}	14.11^{i-n}	5.36^{p}

Note: value in the same column followed by the same letter were not significantly different based on LSD test at α 0.05

Table 7. Interaction between genotypes and salt levels on normal seedling dry weight

Genotypes	Normal seedling dry weight (g) in various salt levels			
	0%	10%	15%	20%
Vima 1	1.17^{e-h}	1.29^{c-g}	1.45^{a-e}	1.34^{b-f}
Vima 2	1.63^{ab}	0.85^{h-l}	0.86^{h-l}	0.53^{lm}
Vima 3	1.24^{d-g}	0.99^{g-k}	0.84^{h-l}	0.80^{j-l}
Murai	1.26^{c-g}	1.46^{a-e}	1.37^{b-f}	1.55^{a-d}
Kenari	1.27^{c-g}	1.35^{b-f}	1.46^{a-e}	0.82^{i-l}
MLGV 0589	0.77^{j-m}	1.15^{e-i}	1.10^{f-j}	0.85^{h-l}
MLGV 0180	1.56^{a-d}	1.59^{a-c}	1.75^{a}	0.67^{k-m}
MLGV 0066	0.72^{k-m}	0.83^{h-l}	0.85^{h-l}	0.45^{m}

Note: value in the same column followed by the same letter were not significantly different based on LSD test at α 0.05

Table 8. Interaction between genotypes and salt levels on number of lateral roots

Genotypes	Number of lateral roots in various salt levels			
	0%	10%	15%	20%
Vima 1	20.29^{a}	11.93^{d-g}	9.31^{e-j}	8.76^{f-j}
Vima 2	18.95^{ab}	6.29^{j-l}	6.05^{j-l}	6.39^{j-l}
Vima 3	17.05^{a-c}	11.57^{d-g}	8.60^{g-k}	7.15^{h-l}
Murai	12.96^{c-f}	9.71^{e-j}	8.97^{f-j}	7.10^{h-l}
Kenari	17.07^{a-c}	11.88^{d-g}	9.77^{e-j}	5.87^{j-l}
MLGV 0589	13.22^{c-e}	14.88^{b-d}	11.21^{d-h}	8.72^{g-k}
MLGV 0180	18.99^{ab}	9.41^{e-j}	6.73^{i-l}	4.53^{kl}
MLGV 0066	10.81^{d-i}	7.26^{h-l}	6.39^{j-l}	3.65^{l}

Note: value in the same column followed by the same letter were not significantly different based on LSD test at α 0.05

Furthermore, normal seedling fresh weight was correspondingly influenced by salt stress. Normal seedling fresh weight was significantly decreased with increasing the salinity stress level (Table 6). Putri et al (2017) also reported similar results, that increasing of NaCl concentration causing the decline of their normal seedling percentage in soybean. In this experiment, mungbean sprouts that exposed to salt stress will showed abnormal growth, including delayed germination, shortened root, shortened shoot length, and yellowish-colored cotyledons. Moreover, in extreme conditions, susceptible genotypes were not be able to germinate at all. Normal seedling fresh weight had a linear function with germination percentage. Salt stress at 10% up to 15% level, were relatively less significant difference. Increasing salt stress up to 20% decreased normal seedling fresh weight by 38.13% compared to control. However, some genotypes such as Murai, MLGV 0589, and Vima 1 had the highest performance in 20% salt conditions, while MLGV 0180 and MLGV 0066 had the lowest fresh weight. On the contrary, the normal seedling dry weight was not continually depressed with the rising of salt levels (Table 7). Murai, Vima 1 and MLGV 0589 have an increased normal seedling dry weight as compared to the control although the results were not significant. Dutta and Bera (2014) also reported that root fresh and dry weight in some of the cases was noticed to increase under salinity.

The number of lateral roots was also affected by salt stress. Number of lateral roots decreased along with the increasing of salinity stress level. Increasing salinity level to 10%, 15% and 20% reduce number of lateral roots by 36%, 48%, and 60% respectively, compared to the control (Table 8). Reduction in number of lateral roots allegedly caused by osmotic stress due to osmotic changes outside the roots which will reduce the ability of the plant to absorb water. A decrease in root growth is in line with Sehrawat *et al.*

(2013); Rachmawatie and Nasir (2014); El Kafafi *et al.* (2015). In other commodities, Roslim *et al.* (2015) reported that the seedling root growth was the best parameter to study salt tolerance in rice. Salt stress caused imbalanced water in plant, low intra-cellular water potential and water scarcity around the root zone which resulted roots failed to absorb sufficient water and nutrients for adequate plant to growth normally (Mohammed 2007; Sunil *et al.,* 2012; Sehrawat *et al.,* 2015). The symptoms are almost similar to the drought stress where the roots of the plant lose the ability to access water.

Plant adaptation or tolerance to salinity stress involves complex physiological traits, metabolic pathways and molecular or gene networks (HanumanthaRao *et al.,* 2016). The mechanism of the mungbean to salt stress can be classified into two, namely the tolerance mechanism and avoidance mechanism (Levitt 1980). Physiological and biochemical mechanisms are required to maintain the viability of cell protoplasm, whereas the salt evasion mechanism involves physiological adaptation of plant structures to minimize the concentration of salt in cells or exclusion physiologically by root membranes (Koyro *et al.,* 2011). Munns and Tester (2008) stated that plant growth responds to salinity in two phases: (1) a rapid, osmotic phase that inhibits growth of young leaves, and (2) a slower, ionic phase that accelerates senescence of mature leaves. Plant adaptations to salinity are of three distinct types: (1) osmotic stress tolerance, (2) Na(+) or Cl(-) exclusion, and (3) the tolerance of tissue to accumulated Na(+) or Cl(-).

CONCLUSION

Increasing level of salinity concentration will decreasing the speed of germination, vigor index, germination, normal seedling fresh weight, and number of lateral roots. Murai and Vima 1 were identified as tolerant genotypes, while Vima 2 and MLGV 0180 were identified as salinity sensitive genotypes at seedling stage. The tolerant mungbean genotypes were able to germinate up to 20% salinity stress level or equivalent to EC 10.32 dS/m. In contrast, germination percentage will be reduced by half at 10% or equivalent to EC 5.74 dS/m on susceptible mungbean genotypes. This experiment suggested that the further research may use 20% salinity stress level or equivalent to EC 10.32 dS/m for screening mungbean genotypes tolerant to saline condition at seedling stage.

ACKNOWLEDGEMENT

The authors thank to Siti Mufidatunniswah and Nofadilah Qurrota Ayun for their involvement in conducting this project. We would like to thank Ayda Krisnawati for correction and discussion this paper. This work was supported by the Indonesian Agency for Agricultural Research and Development (IAARD), Ministry of Agriculture, Republic of Indonesia.

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