# TESTING OF BLACK SOYBEAN SEED RESPONSE TO SALINITY STRESS IN GERMINATION STAGE

### Halimursyadah\*, Hasanuddin and Munanzar Noorman

# Agrotechnology Department, Agricultural Faculty of Syiah Kuala University \* Corresponding Author: <u>rhalimursyadah@yahoo.com</u>

#### Abstract

The objective of this research is to know the response of black soybean seed to salinity stress in germination stage and also to identify the interaction between the two factors. This research has been conducted from April to August 2016 at the Laboratory of Seed Science and Technology of the Faculty of Agriculture, Syiah Kuala University. The experiment was designed using a Factorial Random Block Design. The first factor was the four national varieties of black soybeans (Detam 1, Detam 2, Mutiara 2 and Mutiara 3) and the second factor was the concentration of NaCl (0; 2; 4; 6; 8; and 10 g L-1). There are 24 treatment combinations each repeated three times. The parameters observed were viability (maximum growth potential, germination and time to reach 50% of total germination) and vigor of growing strength (vigor index, relative growth rate, synchrony growth, primary root length of normal seedling germination and dry weight of normal seedling germination. The study showed that Detam 2 were more tolerant than other varieties based on all viability and vigor parameters of growing strength observed. The increase in NaCl concentration at level 4 g L-1 had a very significant effect on the observed parameters indicated by the decrease of viability value and vigor of growing strength. There was a very significant interaction between varieties and concentration of NaCl to viability and vigor of soybean seedling growth that is at the maximum growth potential, primary root length of normal seedling germination and dry weight of normal seedling germination.

Keywords: salinity, varietas, viability and vigor, black soybean.

#### BACKGROUND

Demand for soybeans increases with population growth. The average annual soybean requirement is 2.3 million tons. Soybean production in 2014 and 2015 in Indonesia respectively consume as much as 954,997 tons and 982,967 tons of dry beans, with productivity reached 1.57 tons ha-1 in 2015 (Badan Pusat Statistik, 2016). In general there are two types of soybeans known in Indonesia, namely yellow soybeans and black soybeans. Black soybean has the advantage as raw material of healthy food and raw material of good quality soy sauce industry, besides it can also improve the quality of black color in soy sauce (Adie et al., 2009). The content of anthocyanin, isoflavones and mineral Fe black soybeans is higher than that of yellow soybean (Kuo et al., 2006). However, the productivity of black soybean at farmers' level is only around 1.1 tons ha-1 (Badan Litbang Pertanian, 2008). The national soybean area in 2000 of 824,485 hectares decreased to 567,624 hectares in 2012 and increased the area of soybean by 624,848 hectares in 2015. Despite increasing land area, it has not been able to supply soybean requirement of 2.3 million tons year-1 (Badan Pusat Statistik, 2016).

One effort that can be done to overcome this is to optimize the sub optimum land potential. Tidal land, saline, peat lands and lands near the mining area are sub optimum lands (Yuniati, 2004). Saline land is one of the land that can be used for soybean planting. Saline fields contain large amounts of dissolved NaCl salts which are abiotic stress factors that interfere with plant growth and production (Neumann, 1995). The salin categories, according

to Jones (2002) are divided into four levels: if the value of Electricity Supply (DHL) <1.2 dSm-1 includes non-saline; 1.2-2.4 dSm-1 including somewhat saline; 2.4-4.4 dSm-1 including medium salinity and 4.5-8.9 dSm-1 including high salinity.

Seeds grown in high-margin areas are very difficult and can't germinate, caused by water-absorption by seeds and poisoning by the salt-producing ions (Copeland, 1976). According to Sunarto (2001), efforts to overcome the conditions of saline can be achieved through the assembly of varieties that are tolerant to salinity or adapt the superior varieties that already exist in the saline conditions. Thus the use of tolerant varieties is the most effective way to harness the potential of saline land in an effort to increase national soybean production.

Based on the above description, it is necessary to have an early test to find out how big the influence of salinity to vigor growth strength of some varieties of black soybeans, so that finally obtained varieties are tolerant to high salinity.

#### **METHODS**

The research was conducted at the Laboratory of Seed Science and Technology Agrotechnology Department Faculty of Agriculture, Syiah Kuala University. Black soybean seeds of Detam 1, Detam 2, Mutiara 2 and Mutiara 3 varieties obtained from BALITKABI and BATAN, NaCl pro analysis, aquadest, stencil paper, plastics, oven, electricity conductivity meters, and germinator.

Sorting black soybean seeds of each variety that has been provided, taking into account the desired seed criteria, is physically looking good, pithy and not wrinkled. The seeds used are certified seeds that have high initial germination percentage, Detam 1 (86.66%), Detam 2 (98.66%), Mutiara 2 (94.6%) and Mutiara 3 (90.66%). The total required of seed is 1800 grain.

*Preparation of NaCl solution.* NaCl was weighed using an analytical scale, according to the treatment of 0 g L<sup>-1</sup>, 2 g L<sup>-1</sup>, 4 g L<sup>-1</sup>, 6 g L<sup>-1</sup>, 8 g L<sup>-1</sup> and 10 g L<sup>-1</sup>.

*Preparation of Planting Media*. The paper media substrate is moistened using a NaCl solution, according to the concentration set.

*Germination of seed.* Seed germination was carried out on sterilized paper media according to a NaCl concentration of 25 grains per unit, tested using the medium with a rolled-up paper test method. Then seeds are added by using germinator.

Statistical analysis. This study used a Completely Randomized Design 4x6 factorial pattern with two factors (varieties and NaCl concentration). Thus, there were 24 treatment combinations with 3 replications to obtain 72 experimental units.

### RESULT

## Effect of Variety on Viability and Vigor Black Soybean Seed

Effect of varieties very significant on maximum growth potential, germination, relative growth rate, synchrony growth, primary root length of normal seedling, and dry weight of normal seedling. Then, no significant effect on the vigor index and time to reach 50% of total germination. Value of viability and vigor parameters are presented in Table 1.

	Varietas					
Parameter	Detam 1	Detam 2	Mutiara 2	Mutiara 3		
	(V <sub>1</sub> )	(V <sub>2</sub> )	(V <sub>3</sub> )	(V4)		
Maximum potential growth (%)	94.00 a	99.33 c	95.56 ab	96.67 b		
Germination (%)	49.52 a	61.26 b	58.54 b	50.56 a		
Vigor Index (%)	9.07	10.05	9.20	8.94		
Relative growth rate (%)	43.52 a	56.29 b	53.97 b	43.98 a		
Synchrony growth (%)	21.35 a	28.39 b	22.65 a	21.36 a		
Time to reach 50% of total germination (day)	1.27	1.17	1.25	1.17		
Primary root length of normal seedling (cm)	18.66 a	19.26 b	19.60 b	19.59 b		
Dry weight of normal seedling (g)	1.40 bc	1.20 a	1.50 c	1.32 ab		

Tabel 1. Mean of maximum growth potential, germination, relative growth rate, synchrony growth, vigor index and time to reach 50% of total germination, primary root length of normal seedling, and dry weight of normal seedling in some black soybean varieties

Description: The numbers followed by the same letter on the same line show no significant difference at the 0.05 chance level (DMRT Test)

Tabel 1 showed that Detam 2 was the best variety, having high vigor compared to Detam 1, Mutiara 2, and Mutiara 3. Detam 2 variety produced the highest normal seedlings on several parameters observed. The Detam 1 variety is a low viability and vigor variety and can not afford normal germination. Detam 2 is a superior variety compared to some other varieties, heaviness and vigor. It is believed that the genetic characteristics of Detam 2 varieties are more tolerant of high salinity, so as to adapt to an environment less favorable for growth. In accordance with the opinion of Sadjad *et al.* (1999) each variety has different superior properties, genetic properties are the result of the arrangement of genes in the form of varieties that are not homogeneous. Genetic arrangement of a variety determines the character of the varieties. Copeland (1976) states, genetic factors Is one of the causes of seed vigor difference. High vigor that produce tolerant seeds can grow and develop in suboptimum land conditions, in addition to being stored for a long time. Seeds that have good vigor will have a high growth rate, so the seeds will quickly germinate in a short time. Conversely, seeds that have a bad vigor will affect the physiological and morphological crops produced in the field (Camargo and Vaughan, 1973).

## Effect of NaCl Concentration on Viability and Vigor Black Soybean Seed

NaCl concentration had significant effect on all observed parameters, including: maximum growth potential, germination, vigor index, relative growth rate, synchrony growth, and time to reach 50% of total germination, root length of the normal seedling and dry weight of normal seedling, presented in Table 2. The negative effect is, NaCl will decrease the percentage of normal seedlings on each of the observed benchmarks.

	NaCl Concentration					
Parameters	0 g L-1	2 g L-1	4 g L <sup>-1</sup>	6 g L-1	8 g L-1	10 g L <sup>-1</sup>
Maximum potential growth (%)	98.00 c	99.00 c	96.33abc	96.67 bc	94.00 a	94.33 ab
Germination (%)	74.93 d	58.62 c	55.59 c	50.10 b	49.65 b	40.93 a
Vigor index (%)	53.03 b	0.57 a	0.57 a	0.57 a	0.57 a	0.57 a
Relative growth rate (%)	85.99 d	54.80 c	50.44 b	40.04 b	38.43 b	26.93 a
Synchrony growth (%)	16.12 b	27.71 c	25.70 с	39.52 d	29.04 c	2.21 a
Time to reach 50% of total germination (day)	0.58 a	1.03 b	1.45 c	1.49 c	1.34 c	1.42 c
Primary root length of normal seedling (cm)	21.04 d	21.48 d	21.01 d	19.59 c	18.91 b	13.63 a
Dry weight of Normal seedling	1.74 c	1.39 b	1.31 b	1.25 b	1.42 b	1.02 a

Tabel 2. Mean of maximum growth potential, germination, relative growth rate, synchrony<br/>growth, vigor index, time to reach 50% of total germination, primary root length of<br/>normal seedling, and dry weight of normal seedling in some NaCl concentration

Description: The numbers followed by the same letter on the same line show no significant difference at the 0.05 chance level (DMRT Test); ( ): is the number before the arcsin transformation  $\sqrt{(\%)}$ 

Plants that live in saline conditions generally face two major problems. namely the negative water potential and high concentrations of sodium (Na<sup>+</sup>) and chloride (Cl<sup>-</sup>) ions. The negative water potential will spur water out of the tissue so that the plant loses the pressure of the turgor that causing the plant cells to become weak. Thus, shrinking the cytoplasm and the escape of the plasma membrane from the cell wall called plasmolysis.

Salinity greatly affects the germination of soybean seeds. Dianawati *et al.* (2013) states that. The higher the concentration of NaCl the germination of soybean seeds is decreasing. In accordance with the results of this study. The higher the concentration given, the decreasing germination rate. Increasing the NaCl concentration may inhibit the seeds imbibition process because salt solubility can decrease the osmotic pressure so that the seed can not absorb water from the growing environment, which is necessary to activate the enzyme for germination process. In addition to blocking the process of seed imbibition, plants will become dehydrated due to the high salinity of the soil. This condition causes plants to experience hyperosmotic pressure characterized by reduced turgor pressure and water loss from the tissues (Boudsocq and Lauriere, 2005). Lubis (2008) added that the increased concentration of NaCl causes increased Na<sup>+</sup> and Cl<sup>-</sup> ions absorbed into tissues that would inhibit metabolism in plants. In addition, high concentrations of salinity are capable of causing toxic effects to seeds that resulting in the germination of seeds inhibited, even able to kill seeds.

# Interaction between Varieties with NaCl Concentration on Viability and Vigor of Black Soybean Seed

There is a very significant interaction between varieties and NaCl concentration on the viability and vigor of black soybean seeds on the parameters of maximum growth potential, primary root length of normal seedling, and dry weight of normal seedling. The values are presented in Table 3.

	-			NaCl Care	antration.			
Parameter	Variety -	Naci Concentration						
		0 g L-1	2 g L-1	4 g L-1	6 g L-1	8 g L-1	10 g L-1	
Maximum potential growth (%)	Detam 1	98.67	100.00	94.67	94.67	86.67	89.33	
		ABc	Bc	ABbc	ABbc	Aa	Aab	
	Detam 2	100.00	100.00	100.00	100.00	97.33	98.67	
		Ba	Ba	Ba	Ba	Ba	Ba	
	Mutiara 2	94.60	96.00	97.33	98.67	93.33	93.33	
		Aab	Aab	ABab	Bb	Ba	ABab	
	Mutiara 3	98.67	100.00	93.33	93.33	98.67	96.00	
		ABbc	Bc	Aa	Aab	Bbc	Babc	
Primary root length	Detam 1	21.68	20.66	20.19	19.46	18.24	11.76	
		Be	Ade	Acd	Ac	Ab	Aa	
	Detam 2	20.87	21.74	21.13	19.13	18.89	13.83	
of normal		ABc	Bc	ABc	Ab	ABb	Ва	
seedling	Mutiara 2	20.51	21.79	21.47	19.87	18.93	15.00	
(cm)		Acd	Be	Bde	Abc	ABb	Са	
	Mutiara 3	21.13	21.72	21.24	19.92	19.60	13.94	
		ABc	Bc	Bc	Ab	Bb	BCa	
Dry weight of normal seedling (g)	Detam 1	2.37	1.31	1.27	1.11	1.36	1.00	
		Bc	Aab	Aab	Aab	ABb	Aa	
	Detam 2	1.37	1.26	1.24	1.34	1.07	0.90	
		Ab	Ab	Aab	ABb	Aab	Aa	
	Mutiara 2	1.54	1.69	1.53	1.51	1.64	1.13	
		Ab	Bb	Ab	Bb	Bb	Aa	
	Mutiara 3	1.68	1.30	1.22	1.05	1.62	1.07	
		Ab	Aab	Aab	Aa	Bb	Aa	

Table 3. Interaction between varieties with NaCl concentration to maximum growth potential, primary root length of normal seedling and dry weight of normal seedling of black soybean seed

Description: The numbers followed by the same letter (uppercase on the same column. lower case on the same line) show no significant difference at the 0.05 chance level (DMRT Test)

Table 3 shows that Detam 2 at NaCl concentration of 0 g L<sup>-1</sup>. 2 g L<sup>-1</sup>. 4 g L<sup>-1</sup>. 6 g L<sup>-1</sup>. 8 g L<sup>-1</sup> have the highest maximum growth potential, but not significantly different at NaCl concentration 10 g L<sup>-1</sup>. Maximum growth potential was lowest for the Detam 1 at NaCl concentration 8 g L<sup>-1</sup> were significantly different from Detam 2. Mutiara 2 and Mutiara 3 at NaCl concentration 8 g L<sup>-1</sup>. Based on primary root length of normal seedling, the longest root varieties contained in the treatment Detam 1 at NaCl concentration 0 g L-1 which is significantly different of Mutiara 2. In contrast to the Detam 2. Mutiara 2 and Mutiara 3 varieties seen decreased root length at NaCl concentration 6 g L<sup>-1</sup>. These results are consistent with the statement Bernstein and Kafkafi (2002). the accumulation of salts in the soil can damage to root growth due to an osmotic effect which causes the effect of water deficit or excess salinity ions. Wang and Yamauchi (2006) adding excess salt can affect orientation root cell growth (growth anisotropy). Change anisotropy of cell growth implies that NaCl can affect for the root growth. Interaction between varieties and NaCl concentration to primary root length of normal seedling can be seen in Table 3. The highest values found in Detam 1 varieties were significantly different from Detam 2. Mutiara 2 and Mutiara 3 at NaCl concentration of 0 g L<sup>-1</sup>. The lowest of dry weight of normal seedling is found in Detam 2. Dry weight of normal seedling has decreased with salinity treatment. The average value of interaction between varieties and NaCl concentration on dry weight of normal seedling can be seen in Table 3.

Follet *et al.* (1981) states that under saline conditions, water availability decreases but the rate of plant respiration tends to increase. This leads to a decrease in dry weight of normal seedlings. This result is consistent with the research of Neves *et al.* (2005) where

salinity stress can significantly reduce germination. root growth and biomass weight in response to the given NaCl concentration. The decrease in dry weight of plants due to salinity stress has also been reported in rice and wheat crops (Zeng *et al.* 2002; Hu *et al.* 2006).

### CONCLUSION

### Conclusion

Detam 2 is the most tolerant varieties of salinity stress compared to Detam 1, Mutiara 2 and Mutiara 3. The NaCl concentration begins to affect germination at 2 g L-1. The best interaction is in the treatment of Detam 2 varieties at a concentration of 6 g L-1 NaCl due to Detam 2 is still able to tolerate salinity stress to a concentration of 6 g L-1 NaCl.

#### REFERENCES

- Adie. M.M., Suharsono and Sudaryono. (2009). Prospek Kedelai Hitam Varietas Detam 1 dan Detam 2. Buletin Palawija. 18: 66-72.
- Badan Litbang Pertanian. (2008). Ketersediaan Teknologi dalam Mendukung Peningkatan Produksi Kedelai Menuju Swasembada. http://agri-research.or.id [4 Juni 2015].
- Badan Pusat Statistik. (2016). www.bps.go.id. [21 Februari 2016].
- Bernstein, N., and U. Kafkafi. (2002). Root Growth under Salinity Stress. p. 787-805. In Y. Waisel. A. Eshel. and U. Kafkafi (Eds). Plant Roots. The Hidden Half: 3d Edition. Marcel Dekker. New York.
- Boudsocq. M and Lauriere. C. (2005). Osmotic Signaling in Plants: Multiple Patways Mediated by Emerging Kinase Families. Plant Physiology. (38): 1185-1194.
- Camargo. C.P. and Vaughan. C.E. (1973). Effect of Seed Vigor On Field Performance and Yield of Grain Sorghum Sorghum bicolot- (L.) Moench.Proc. Assoc. Official Seed Analysts 63.135-147.
- Copeland. L. O. (1976). Principles of Seed Science and Technology. Burgers Pulb. Co. Minneapolis. Minnessota. 369 p.
- Dianawati, M.D., P. Handayani, Y. R. Matana, S. M. Belo. (2013). Pengaruh Salinitas Terhadap Viabilitas dan Vigor Benih Dua Varietas Kedelai (Glycine max. L.). Agrotop. 3 (2): 35-41.
- Follet. R. H., L.S. Murphy and R.L. Donahue. (1981). Fertilizer and soil amendments. Prentice Hall Inc. Englewood. New Jersey.
- Hu. YC, Z. Burucs. and U. Schmidhalter. (2006). Short-term Effect of Drought and Salinity on Growth and Mineral Elements in Wheat Seedlings. J. Plant Nutr (29): 2227-2243.
- Jones. J. B. 2002. Agronomic Handbook: Management of crops, Soil and Their fertility. CRC Press. New York. 450 pp.
- Kuo. L.C., W.Y. Cheng. R.Y. Wu. C.J. Huang. K.T. Lee. (2006). Hydrolysis of Black Soybean Isoflavone Glycosides by Bacillus subtilis natto. Appl. Microbiol.Biotechnol. 73:314-320.
- Lubis. M. S. (2008). Pertumbuhan dan Kandungan Protein Jagung di bawah Cekaman NaCl. Jurusan Pendidikan Biologi. Yogyakarta.
- Neumann. P.M. (1995). Inhibition of Root Growth by Salinity Stress: Toxicity or an Adaptive Biophysical Response in: Baluska. F. Ciamporova. M. and Gasparikova. O. Eds. Structure and Function of Roots. Kluwer Academic Publishers. 299-304.
- Neves. G. Y. S., P. C. Zonetti. M. L. L. Ferrarese. A. L. Braccini, and O. Ferrarese-Filho. (2005). Seed Germination and Seeding Growth of Soybean (Glycine max (L.) Merr) Under Salt Stress. Bioscience. J. 21 (1): 77-83.
- Sadjad, S., E. Murniati, dan S. Ilyas. (1999). Parameter Pengujian Vigor Benih dari Komparatif ke Simulatif. Grasindo. Jakarta. 185 p.
- Sunarto. (2001). Toleransi Kedelai terhadap Tanah Salin. Bul. Agron. 29 (1): 27-30.
- Wang. H., and A. Yamauchi. (2006). Growth and Function of Roots under Abiotic Stress in Soils. p. 271-319. In B. Huang (Ed). Plan-Environment Interaction. Tylor and Francis Group. Florida.

- Yuniati. R. (2004). Penapisan Galur Kedelai Glycine max (L.) Merrill Toleran Terhadap NaCl untuk Penanaman di Lahan Salin. Makara Sains, Vol. 8 No. 1. FMIPA. Universitas Indonesia. Depok.21-24.
- Zeng L. M.C. Shannon and C.M. Grieve. (2002). Evaluation of Salt Tolerance in Rice Genotypes by Multiple Agronomic Parameters. Euphytica (127): 235-245.