Salt Stess on Soybean (*Glycine max* L Merr): Improving Salt Stress Tolerance through Seed Priming

Maman Suryaman, Adam Saepudin, Dedi Natawijaya, and Darul Zumani

Abstract— Soybean seeds rapidly deteriorate or loss of viability and vigor, especially in stress conditions including by saline. This study was aimed to obtain the best seed viability and vigor of soybean treated by seed priming under salt stress. This study used a randomized completely block design with factorial pattern. First factor was the saline stress of NaCl concentration (C) which consisted of three levels (c0 = 0%, 0.5% = c1, c2 = 1%).Second factor was the treatment of seed priming (P) that consisted of 4 levels (p0 = hydropriming, p1 = osmopriming, p2 = matripriming, p3 = vitamin priming). The experiment was repeated three times. Data collected consisted of: germination capacity, germination rate, hypocotyl and epicotyl length, the weight of seedling, and the electrical conductivity. Data were analyzed by analysis of variance followed by Duncan's multiple range test at 5 percent. The results showed that osmopriming, matripriming, and vitamin priming improved total germination and germination rate of soybean seeds under salinity stress, while seed priming with hydropriming caused significantly the reduction of germination total and germination rate in salinity stress of 1 percent. Increased salinity stress from 0 to 1 percent caused a reduction in hypocotyl and epicotyl length, different with osmopriming, matripriming, and vitamin priming that produced hypocotyl and epicotyl longer than hydropriming. In all seed primings, increased salinity stress from 0 to 1 percent lowered the weight of seedlings, and most drastic reduction of seedling weight occurred in seeds treated with hydropriming. Among seed priming treatments, osmopriming, matripriming, and vitamin priming were more able to reduce membrane leakage compared to hydropriming as indicated by lower electrical conductivity rates contributing the increase in tolerance to salt stress and high in seed viability and vigor.

Key words — germination, salt stress tolerance, seed priming, electrical conductivity, soybean

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1 INTRODUCTION

Soybean is one of the important crop as a source of protein and as a component of fodder. In Indonesia, the demand of soybean for food is increasing every year and still not met by national production. Due to insufficient domestic production Indonesia every year import soybean. The government continue its effort to increase national production, such as by the expansion of planting areas, including utilizing marginal land.

Salinity is an abiotic stresses which cause serious environmental problems. Salinity causing disruption to the growth and productivity of plants [1]. This are caused by: (1) the potential low osmotic soil solution thus reducing the availability of water for the plants, and (2) an increase in the concentration of ions that are toxic to plants which spurred an imbalance in the metabolism of nutrients [2, 3]. The lowered osmotic potential causes a decrease in crop productivity due to reduced water uptake. In saline conditions, the plants require more energy to absorb water and maintain cell turgor. If the plant does not have sufficient energy, water uptake process and transpiration flow will be decreased allowing the growth and yield may also be disrupted. In addition, the plant will also undergo an imbalance of ions that can cause toxicity to plants [3].

Salinity stress affects almost all physiological and biochemical processes [4] as well as the stages of plant growth [5]. Germination and phase of seedlings is a most sensitive phase for abiotic stresses of most crops, including soybeans [6, 7], indicating the plant resistance to stress salinity can be evaluated in this phase [5]. Stress inhibits the germination process, reducing the germination rate and increase the heterogeneity of germination resulting in lower growth and yield [6]. The vigorous seed can grow and be productive in a variety of soil conditions, including sub-optimum conditions. The success of plants cultvation is highly dependent on the growth and development in the germination phase. Germination is a period that is very prone to stress, thus germination period require seed priming treatment to accelerate the germination period which expected to increase stress tolerance [8]. Seed priming treatment is intended to improve the performance of seeds, increase germination, improve the homogeneity and stimulate vegetative growth [6, 9], including the treatment of hydro priming, osmo priming, hormone priming, vitamin priming, and matriconditioning/matripriming [10, 11, 7, 9]. The aim of the study was to obtain the best seed viability and vigor of soybean treated by seed priming under salt stress. By the increased viability and vigor, it is expected to increase germination tolerance in an environmental salt stress.

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2 MATERIALS AND METHODS

The research was conducted at the laboratory of Agriculture Faculty, Siliwangi University Tasikmalaya West Java Indonesia in 2016 .

The materials needed in these experiments are: (1) PEG 6000; (2) soybean seeds; (3) husk, (4) ascorbic acid, (5) rice paper, (6) NaCl. The experiment was designed in randomized block design with factorial pattern. The first factor was level of saline stress in simulation of NaCl concentration (C) that consisted of three levels (c0 = 0%, 0.5% = c1, c2 = 1%) and second factor was seed priming treatment (P) that consisted of 4 levels (p0 = hydropriming, p1 = osmopriming, i2 = matripriming, i3 = vitamin priming). The experiment unit was repeated three times.

The NaCl solution of 0%, 0.5% and 1% (w/v) as a treatment of salinity stress were prepared. For the treatment of seed priming, matripriming was made from rice husk which is pulverized and then moistened with water and subsequently mixed with the seed until embedded in the seed coat evenly. Osmopriming prepared by dissolving PEG into the water until it reaches a concentration of 15%. Vitamin priming is prepared by dissolving ascorbic acid in water to form a solution with a concentration of 1%.

Seeds that have been treated with priming were then planted on germination media of rice paper arranged as many as five pieces after treating with salinity stress, and then put in germinator. Maintenance and observation was done until the trial ends. For the measurement of electrical conductivity, the seeds were treated with seed priming, after dried it immersed in NaCl solution according to treatment (0, 0,5, and 1%) for 24 hours at room temperature, then the immersion water was measured by means of electrical conductivity meters.

The main responses observed were: (1) germination percentage; (2) germination rate; (3) epicotyl length; (4) hypocotyl length; (5) electrical conductivity; (6) dry weight of normal seedling. To determine the level of tolerance shown by the variable responses observed, the data analysis was performed by univariate ANOVA and Duncan's Multiple Range Test at a probability level of 95% [12].

3 RESULTS AND DISCUSSION

Results of statistical analysis of seed germination capacity at first count showed that stress treatment significantly affected germination, while seed priming as well as the interaction between these two treatments did not give significant influence to the increase of germination. Table 1 appears that the increasing salinity stress from 0 to 1% reduced the number of seeds that germinated. According to Hassen *et al.* [13] increased stress salinity through the increase of NaCl concentration will result in the accumulation of ions which are toxins that damage the embryo and reduce the potential of osmotic which will inhibit or reduce the absorption of water into the seed resulting water is not sufficient to be able to activates hydrolytic enzymes at the beginning of the process of germination. This is ultimately led to the inhibition in germination process. Meanwhile, in among various of seed priming techniques no significant effect occurred to germination capacity. This indicates that all seed priming techniques have the similar effective use to improve the germination of soybean seed. This condition may be caused by metabolic activities happened during the seed priming process which led the seed ready to emerge radicula generating the seeds can germinate immediately [14].

Table. 1. Effect of seed priming on first count germination in salt stress conditions

Treatments	Germination capacity (%)
Salinity stress	
0.0 %	98.3a
0.5 %	96.8a
1.0 %	94.0b
Seed priming	
hidro priming	95.1a
osmo priming	96.4a
matri priming	97.5a
vitamin riming	96.4a

Note : The values following by same letters are not significantly different according to Duncan's multiple range test at 5% level.

Seed priming techniques and salt stress gave interaction effect significantly to germination total (Table 2).The whole seed priming treatments on conditions of without stress and stress of 0.5% did not show any significant difference to the total germination. This means that all the seed priming techniques applied can maintain germination capacity at higher level.

Table 2. Effect of seed priming on seed germination total in salt stress conditions (%)

Seed priming	Salinity stress 0.0 %	0.5 %	1.0 %
hydro priming	98.6 a A	98.0 a A	74.7 b B
osmo priming	100.0 a A	99.3a A	100.0a A
matri priming	99.3 a A	95.3a A	97.3a A
vitamin priming	100 a A	98.6a A	92.0a A

Note : The values following by same letters in the columns and the raws are not significantly different according to Duncan's multiple range test at 5% level.

Even salinity levels under stressful conditions of 1.0%, with osmopriming, matri priming, and vitamin priming, was able to cope stress disorder of treatment at a high germination rate and no significant different with no stress conditions. While, hydropriming caused a decrease in seed germination on seed experiencing salt stress conditions of 1.0%. This showed that the negative effects of salinity stress can be covered by seed priming treatments. This is also consistent with the opinion of Ansari and Sharif-Zadeh [6] that seed priming can overcome the negative effects of abiotic stress.

Seed priming treatment and salinity stress interactions significantly affected the rate of germination of soybean seed (Table 3).

Table 3. Effect of seed priming on seed germination rate in salt stress conditions (% etmal^{-1})

Seed priming	Salinity stress		
	0.0 %	0.5 %	1.0 %
hydro priming	8.5 a	8.4 a	7.9 b
	A	A	B
osmo priming	8.5 a	8.4a	8.4a
	A	A	A
matripriming	8.4 a	8.4a	8.4a
	A	A	A
vitamin	8.5 a	8.5a	8.5a
priming	A	A	A

Note : The values following by same letters in the columns and the raws are not significantly different according to Duncan's multiple range test at 5% level.

All seed priming techniques applied to soybean seed in the condition without stress and salinity stress of 0.5% showed no significant difference to the rate of germination. This illustrated that the hydropriming was able to accelerate the germination rate, the same as with other seed primings. However, the hydropriming technique, caused a decrease in germination rate at salinity stress of 1%, from 8.5 % etmal^{-1} to 7.9% etmal^{-1}. Seed priming by osmopriming, matripriming, and vitamin priming were able to overcome the adverse effects of salinity stress by revealing a high germination rate and did not vary with the condition without stress. Even seed priming using ascorbic acid (vitamin priming) showed the highest germination rate (8.5% etmal^{-1}) compared with other seed priming techniques. Ascorbic acid plays an important role in improving the tolerance of plants to abiotic stresses [1, 15]. Ascorbic acid include to non-enzymatic antioxidant that can counteract reactive oxygen species (ROS) produced by plants when undergoing stress. ROS is very unstable and highly reactive, thus it can easily react with other molecules, such as lipids, proteins, and DNA [15] which cause damage to cells and tissues of plants that can inhibit the growth or germination rate.

Treatment of salinity stress significantly affected the length of hypocotyl and epicotyl, while it, seed priming techniques affected significantly to epicotyl length, and non significantly to hypocotyl length. Similarly, the effect of interaction was not significant difference (Table 4).

The increased salinity stress undergone by soybean seed from 0.0% to 1.0% NaCl stress led epicotyl and hypocotyl growth further hampered allowing the size of both become shorter. Increased salinity levels will result in reduced levels of water uptake and impaired metabolic processes that impact on decreasing the activity of meristematic cells or cell elongation [16], thus into hypocotyl and epicotyl length reduced.

Table 4. Effect of seed priming on hypocotyl and epicotyl length in salt stress conditions

Treatments	Hypocotyl (cm)	Epicotyl (cm)
Salinity stress		
0.0 %	8.7 a	12.1 a
0.5 %	8.0 a	8.2 b
1.0 %	5.3 b	4.1 c
Seed priming		
hydro priming	6.6 a	6.7 c
osmo priming	8.3 a	8.5 ab
matri priming	69 a	9.5 a
vitaminpriming	7.6 a	7.9 b

Note : The values following by same letters in the columns and the raws are not significantly different according to Duncan's multiple range test at 5% level.

On the other hand, with increasing stress, growth to the top (epicotyl) tend to be more depressed than the growth downwards (hypocotyl). The same thing happened to Abdul Qados [17] that the height of pepper plants suffers from salinity stress. Research of Arif *et al*. [14] also reported that growth of plumula more depressed than radicle. This can occur as a result of osmotic adjustment quick to stress salinity, as one of the mechanisms of plant tolerance to salinity [18].

The seed priming treatment caused significant difference in hypocotyl length. Apparently all the seed priming techniques may improve metabolism during imbibition process which further triggered germination and can influence the increase in biomass accumulation. Meanwhile, hydro priming produced epicotyl shorter than the other seed priming techniques. Seed priming with osmopriming, matripriming, and vitamin priming seems to have advantages compared to hydropriming.

Table 5. Effect of seed priming on seedling weight in salt stress conditions (g)

Seed priming	Salinity stress		
	0.0 %	0.5 %	1.0 %
hydro	0.81 b	0.65 b	0,56 b
priming	A	B	B
osmo	0.86 ab	0.81a	0.60ab
priming	A	A	B
matri	0.87 ab	0.77a	0.67a
priming	A	B	C
vitamin	0.91 a	0.71ab	0,62ab
priming	A	B	B

Note : The values following by same letters in the columns and the raws are not significantly different according to Duncan's multiple range test at 5% level.

The seed priming and salinity stress gave interaction effect significantly to the weight of soybean seedling (Table 5). Increased salinity stress from 0.0% to 1.0% led to lowering weight of soybean seedling at various treatment of seed priming. The same is obtained from research [13] that an increase in salinity stress causes weight loss of chili seedling. Increased salinity stress resulted in increased absorption of Na^+ and Cl^- compared with no stress. Na^+ uptake in excess causes problems in the membrane, the enzyme activity is inhibited, and damage to the system of metabolism that causes disorganization in multiplication, elongation and cell structures [19]. Salinity stress also cause oxidative damage to the cells of plants due to reactive oxygen species that affect plant physiology and biochemistry process that can reduce crop yields [20].

Hydropriming produces the lowest seedling weight at varies levels of stress compared to other seed priming techniques. This indicates that among the seed priming techniques used, hydropriming was less able to inhibit or counteract the disruption caused by salinity stress. Research conducted by Abdallah *et al.* [21] showed that osmopriming with PEG solution was superior in improving germination percentage compared to hydropriming. Further Abdallah *et al.* [21] and Ghiyasi *et al.* [22] revealed that osmopriming affected the germination and seedling growth, root length and plumula, fresh and dry weight of seedling, even improved germination performance in stress conditions. Research by Ilyas [11] showed that the use of matripriming gave a positive effect on improvement of the percentage of germination and its emergence onto the surface of the soil and germination rate than without matripriming. Vitamin priming at 0.5 % and 1.0 % salinity was also higher in seedling weight resulted. This was the use of ascorbic acid as antioxidants play an important role in improving the tolerance of plants to salinity stress by way of counteracting reactive oxygen species [23, 24]. Ascorbic acid protects the metabolic processes of the derivatives of oxygen that is toxic to a various of enzyme activity, reduce oxidative damage, stabilize cell membranes, as well as increasing levels of auxin for stimulating the multiplication and extension of cells [16] which eventually increase the weight of the seedling.

The seed priming and salinity stress gave interaction effect significantly to the electrical conductivity (Table 6). Increased salinity levels from 0 to 1% enhanced the electrical conductivity of seeds in all seed priming treatments. This condition illustrates that increased levels of NaCl undergone by seeds will be responded by an increase in ROS. Enhanced level of ROS can cause damage to biomolecules such as lipids, proteins, and DNA [25, 26]. Further damage resulted in the inactivation of enzyme, inhibitation of protein synthesis, increased proteolityc degradation, DNA damage, enhanced membrane fluidity and permeability, and so forth ultimately resulting in cell death [25]. Decreased integrity of cell membranes due to stress is followed by increased permeability leading many substances such as sugars, amino acids, organic acids, and various mineral ions come out of the seeds and leached out into the germination medium [27], thus causing seedling weight to decrease. The more leakage the cell membrane the greater the electrical conductivity number, the lower the seed vigor [28]. Among seed priming treatments, osmopriming, matripriming, and vitamin priming were more able to counteract the effects of salinity stress than hydropriming as indicated by lower electrical conductivity rates.

Table 6.Effect of seed priming on electrical conductivity in salt stress conditions (μs cm^{-1} g^{-1})

Seed priming	Salinity stress 0.0 %	0.5 %	1.0 %
hydro priming	68.2 a A	170.8 c B	271,0 c C
osmo priming	48.9 a A	105.5 a B	160.1 a C
matri priming	64.9 a A	120.6 a B	197.6 b C
vitamin priming	58.7 a A	160.7 b B	251,6 c C

Note : The values following by same letters in the columns and the raws are not significantly different according to Duncan's multiple range test at 5% level.

Based on analysis of correlation between electrical conductivity (Table 6) and seedling weight (Table 5), there was significant negative correlation on all seed priming treatments (r = -0.99, -0.94, -0.99, and -0.98). The greater the number of electrical conductivity the more decreased seedling weight. The treatment of hydro priming has equation $Y = 718.10 – 816.98\ x$ ($R^2 = 0.98$), osmo priming with equation $Y = 391.12 – 378.35\ x$ ($R^2 = 0.88$), matripriming with $Y = 628.59 – 663.500\ x$ ($R^2 = 0.99$), and vitamin priming with $Y = 634.38 – 639.35\ x$ ($R^2 = 0.97$). The decrease of seed vigor as indicated by decreasing seedling weight was due to seed degradation or membrane leakage. Membrane degradation leads to increased permeability causing many substances or nutrient reserves turn out, energy for growth decreased, viability and vigor decreased and accelerate seed deterioration (29). However, membrane damage due to salinity stress in osmopriming treatment, matripriming and vitamin priming were less than that treated with hydropriming.

Generally from this research, seed treatment with osmopriming, matripriming and vitamin priming were more able to improve seed germination tolerance to salt stress condition compared to hydropriming contributing the increase in seed viability and vigor of soybean.

4 CONCLUCIONS

1. Seed priming by osmopriming, matripriming, and vitamin priming improved total of germination and rate of germination of soybean seeds under salinity stress up to a concentration of 1 percent. Hydropriming caused the lowering germination total and germination rate in salinity stress of 1 percent.

2. Increased salinity stress from 0 to 1 percent lead to the reduction in hypocotyl and epicotyl length. Osmopriming, matripriming, and vitamin priming produced hypocotyl and epicotyl longer than hydropriming.
3. Increased salinity stress from 0 to 1 percent lowered the weight of seedling. The most drastic seedling weight reduction occurred in seeds treated by hydropriming.
4. Increased levels of salinity caused greater membrane leakage as showed by large electrical conductivity rates. Osmopriming, matripriming and vitamin priming reduced membrane leakage compared to hydropriming leading to the improve of salt stress tolerance and high seed viability and vigor.

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