Evaluation of Resistance Improvement of Soybean *(Glycine Max* (L) Merr.) against Salinity using Mass Selection and Gene Expression of Salinity Tolerant

Nini Rahmawati[1], Rosmayati[1], Delvian[2], Mohammad Basyuni[2] and Hirosuke Oku[3]
[1]*Faculty of Agriculture, Universitas Sumatera Utara, Medan 20155, Sumatera Utara, Indonesia*
[2]*Faculty of Forestry, Universitas Sumatera Utara, Medan20155, Sumatera Utara, Indonesia*
[3]*Centers of Molecular Biosciences, University of the Ryukyus, Okinawa, Japan*

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Abstract: Saline soils are land that has not been utilized widely for the cultivation of soybean due to the toxic effects that interfere with the growth of the plant. This research aims to get the soybean genotypes that have salinity resistant through mass selection methods and gene expression testing of third generation of saline tolerant of the soybean genotypes that salinity resistant (F3). The research of mass selection conducted on research's land with salinity of land 5-6 mmhos/cm and molecular analysis carried out at the Center of Molecular Biosciences University of the Ryukyus, Japan. Molecular analysis of saline tolerant gene at the root of soybean selection results F3 and soybean grobogan varieties showed mRNA expression gene DREB5, GPRP3, P5CS, bZIP, ERF and NHX1 higher in selected soybean that salinity resistant F3 that was treated by salinity compared to the controls, while the level of gene expression GmCLC1 and PAP3 lower than the control. Comparison of gene expression levels in soybeans that given salinity stress show there has been an increased of expression genes that associated with the ability of adaptation of plants to salinity stress.

1 INTRODUCTION

Salinity is one of the important abiotic factors that limiting the production of soybean in the world. Reclamation of soil is not an economical option to increase soybean production that experiencing in salinity stress. Therefore, genetic improvement for salt tolerance is a more cost effective option. Conventional breeding has contributed significantly to enhancement of soybean production in the last 50 years. Through conventional breeding, it is easy to manipulate the inheritance of qualitative properties that are less sensitive to environment changes, but quantitative properties like yield or tolerance to abiotic stress were significantly influenced by the environment (Pathan *et al*., 2007).

Some plants develop mechanisms to cope with these stresses, in addition there are also being adapted. The majority of the cultivation of plants are vulnerable and could not survive in high salinity condition, or even survive, but with yields reduced. A study of the response plant to salinity is important in efforts to achieve effective plant filtering technique. Soybean varieties showed broad spectrum in its ability to tolerate salt. Filtering of soybean genotypes have been conducted to identify genetic properties that show a high tolerance to salt stress. Currently, the breeding is the main strategy to improve salt tolerance in soybean (Phang *et al*., 2009).

Plant breeding in the future will be further lead to the use of techniques and methodologies of molecular breeding using genetic markers. The use of "molecular breeding" has been promising simplicity of the constraints and challenges in plant breeding complexity. Selection indirectly using molecular markers that are bound to the desired properties has allowed individual studies on the related to the selection of the double properties and inaccuracy of measurement due to the expression of properties that caused by external factors of double genetic locus (Sudarmi, 2013).

Genes that induced by abiotic stresses such as high salinity have been found and provide an important opportunity to improve the tolerant

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properties to high salinity through genetic engineering approach. Target genes include genes that encode the enzymes that necessary for the biosynthesis of various osmoprotectant, enzymes that eliminated reactive oxygen species, end embryogenesis proteins (LEA), enzymes to detoxify and transcription factors (Santoso *et al*., 2012).

Molecular approach through screening is done at the plant to determine the genes that are play a role in the resistance mechanism (Turan *et al*., 2012; Ermawati, 2011).

The main mechanism for salt tolerance is to minimize salt that was taken up by the roots, split them at the level of cells and tissues so as do not reach concentrations that contain of toxic on the cytosol in the leaves. Candidate genes, among others for ion transport, osmoprotectant, and make plants grow faster in saline soil. A study of gene expression in the roots and leaves has been widely reviewed and various suggestions have been submitted to increase plant resistance in saline soils (Munns, 2005). Some researchers have reported several genes that associated with tolerance soybean against salinity stress, among others P5CS, GmCLC1, GmSOS1, GmNHX1, GmbZIP, GmDREB, GPRP3 (Phang *et al*., 2008; Peng *et al*., 2012; Lan *et al*., 2011; Celik and Atak, 2011; Gao *et al*., 2011; Zhang *et al*., 2009; Li *et al*., 2006; Liao *et al*., 2003; Sun *et al*., 2013).

2 MATERIALS AND METHODS

2.1 Study Area

Mass selection starts from elders to the fourth generation (F4) was conducted in the Paluh Merbau village, Percut Sei Tuan, Deli Serdang district with a height of ± 1.5 m above sea level and salinity levels 5-6 mmhos/cm that was conducted in May 2011 to the May 2013.

2.2 Procedures

Molecular analysis carried out in the greenhouse and laboratory of Center of Molecular Biosciences (COMB) University of the Ryukyus, Okinawa, Japan, from October to December 2012. The limit of selection that used was 10% of the plant population. And the heritability was analyzed using the following formula:

$$h^2 = \frac{\sigma^2 g}{\sigma^2 p} = \frac{\sigma^2 g}{\sigma^2 g + \sigma^2 e}$$

Criteria of heritability:
$h^2 > 0.5$: High
h^2 0,2 - 0,5 : Moderate
$h^2 < 0.2$: Low
(Stansfield, 1991).

Soybeans that will be used for molecular analysis planted in greenhouse in a tub of plastic for 14 days and were given the control treatment (without salinity stress) and the treatment of salinity stress given for 72 hours after 14 HST by using a commercial salt powder (Red Sea Salt, Houston, TX, USA) by DHL 5-6 mmhos/cm. Media salinity level is done every day by using a salinity refractometer S/Mill-E (Atago Co. Ltd., Tokyo, Japan). RNA isolation is done by using RNA easy Plant Mini Kit (Qiagen). Total RNA quantitative test using nanodrop, while the qualitative test using electrophoresis and agarose. The next stage is the synthesis of cDNA with Hexamer Random Method using High Capacity RNA-to-cDNA (Applied Biosystems). Primary will be used for the analysis of gene expression and housekeeping genes (for normalization of target genes) were 8 primers that designed using data from NCBI and software Genetyx (Table 1).

Table 1: Primary that Designed and Used for Gene Expressions Analysis.

Gene	Forward primer sequence [5'-3']	Reverse primer sequence [5'-3']
DREB5	GTGAGCGATGAC CAGGTTCATG	CATCCAAATC ATCCCACATGGG
GPRP3	CTGCTGCTGCTT ATGGTGCTCA	ATGCTTTCCA TGCTTGCCAAAC
P5CS	GGAAGTGCACAT ACTGATTCCG	TGGACCCCGA GCATGAATCCTG
BZIP	CACCAGTTGGTG ACTGTTCAGA	CCTTTACCAG CTTTCCCAGTTG
ERF3	GCTCCTGAGATC TCATCCATGC	GTCACCAGAT AGCAAGGATTCC
CLC1	TTACATGGGTGG AGCTGGCTGA	GCGAAGTCCT ACTTGTCTGAAG
NHX1	GCATCACGATAA CCACAGATCC	CATCAAACTT ACGCCACAAGCG
PAP3	GTCGGAGATGGT GGAAATCAAG	GCCATCATCA TTGCGATTCCAG
ACTI I	ATTTTGACTGAG CGTGGTTATTCC	GCTGTCTCCA GTTCTTGCTCGT

2.3 Data Analysis

Analysis of genes expression were using Real Timer-PCR that using Fast SYBR®GreenMaster Mix. RT-PCR result data were analyzed to determine the level of expression of each variety and treatment.

3 RESULT AND DISCUSSIONS

3.1 Heritability, Selection Limits and Selection Advancement of Salinity Resistant Soybean Selection

Selection of soybeans to obtain plants that have the potential to be developed as saline tolerant varieties showed the progress of selection is not yet stable, but observation variable of production character has high heritability values. Soybean production is determined by genetic factors, besides that, it also strongly influenced by environmental conditions, especially changes in soil salinity levels. Selection of salinity resistant soybean ranging from elders to the fourth generation showed the average of each observation variable of growth and production showed diverse results (Table 2 and Table 3). Growth and production of best soybean is reached on the second generation of selection. Soybean production per plant in the second generation reached 12.00 g. While on the third generation of plant production (weight of seeds per plant) reached its low point of 1.6 g per plant. The growth and production of soybean increased again in the fourth generation of selection, where production per plant was 10.55 g.

The highest soybean production was obtained in the second generation, namely 12.0 g/plant (Table 2). This is supported by the environmental conditions with the level of soil salinity is relatively stable. While in the third generation, there was a decrease of the production average due to changes in soil salinity is very high, reaching 10.36 mmhos/cm. Ghassemi-Golezani and Taifeh-Noori (2011) reported the production of soybeans that grown in soil with DHL 9 dS/m decreased by 354.55% compared to the DHL 0 dS/m.

Heritability value of the first generation to fourth generation showed a change (Table 4 and Table 5). Observation variables of production character such as harvest age, number of pods, number of containing pods, the number of empty pods, and the weight of seeds/plants that are tend to have high heritability values in each generation. Heritability is one of the most important considerations in plants evaluating, selection method and crossbreeding system. More specifically, heritability is part of the total variation in properties that caused by genetic differences among observed plants. Heritability is the ratio between the genetic variance to the phenotypic variance. Phenotypic variance is influenced by genetic and environmental factors. Heritability value of production characters at every stage of selection is likely to be stable and included in high or moderate criteria. This shows that genetic factors are more dominant to controlling the characters. Roy (2000) stated that the success of selection is determined by the existing of diversity that controlled by genetic factors, while Ceccarelli *et al.* (2007) suggested that selection on the stress environment conducted in the target environment so as to maximize the expression of genes that control the yield capability and plant adaptability. Marquez-Ortiz *et al.,* (1999) stated a low heritability value means that environmental factors have greater influence than genetic factor.

Table 2: The Average of Agronomic Observation Variable from Elders to Second Generation.

Variable Observation	Generation		
	Elders	First	Second
Plant Height (cm)	20.30	20.86	34.60
Number of Branches(branch)	1.60	1.80	5.40
Flowering Age (day)	29.80	29.12	30.50
Harvest Age (day)	68.00	86.53	84.90
Number of Pods (pod)	10.10	3.83	44.40
Containing Pod (pod)	9.80	3.64	42.00
Empty Pod (pod)	0.30	0.30	2.40
Seed/Plant Weight (g)	2.90	0.66	12.00

Table 3: The Average of Agronomic Observation Variable from Third to Fourth Generation and Optimal Condition.

Variable Observation	Generation		Optimal Condition
	Third	Fourth	
Plant Height (cm)	29.20	34.10	61.24
Number of Branches (branch)	3.90	4.00	2.90
Flowering Age (day)	29.00	29.20	35.00
Harvest Age (day)	72.90	73.15	76.12
Number of Pods (pod)	9.80	32.15	60.50
Containing Pod (pod)	8.60	27.7	53.15
Empty Pod (pod)	1.30	4.45	6.35
Seed/Plant Weight (g)	1.60	10.55	14.04

Selection limit and selection advancement of each stage of selection can be seen in Table 6. The highest selection limit was on the second and fourth generation, namely 10.16 g/plant, while the lowest selection limit was in the third generation, namely 0.21 g/plant. Selection progress also showed diverse results. Selection regress occurred from the second generation to the third generation, there was 2.38. Selection progress rebound in the second and fourth-generation, namely 3.57 and 3.93.

Changes in saline environments also causes the selection boundary to the character of production is not yet stable. The highest selection limit is achieved by the second generation, namely 10.16 and decreased to 0.21 in the third generation due to

increased soil salinity. Selection boundary on the fourth generation increased again to 10.16 and 1.69 for the selection progress. Selection progress in this research showed an increase in the third and fourth generation. Selection progress is a value which is a parameter of the success of the selection that we done. Sketchily, the value of the selection progress is the difference of the initial population and the further population that has experiencing selection (Idris et al., 2011). The high value of selection progress is a manifestation of the value of diversity additive in a population. The diversity of additive itself is a necessary component for recurrent selection (Sutoro, 2006).

Table 4: Heritability Value of the First Generation and Second Generation.

Variable Observation	First Generation		Second Generation	
	H	Criteria	h	Criteria
Plant Height (cm)	0.89	H	0.45	M
Number of Branches (branch)	0.00	L	0.00	L
Flowering Age (day)	0.00	L	0.05	L
Harvest Age (day)	0.39	M	0.00	L
Number of Pods (pod)	0.98	H	0.50	H
Containing Pod (pod)	0.97	H	0.50	H
Empty Pod (pod)	0.88	H	0.41	M
Seed/Plant Weight (g)	0.98	H	0.50	H

H = High, M = Medium, L = Low

Table 5: Heritability Value of the Third Generation and Fourth Generation.

Variable Observation	Third Generation		Fourth Generation	
	h	Criteria	h	Criteria
Plant Height (cm)	0.76	H	0.95	H
Number of Branches (branch)	0.00	L	0.20	L
Flowering Age (day)	0.26	M	0.13	L
Harvest Age (day)	0.81	H	0.52	H
Number of Pods (pod)	0.67	H	0.91	H
Containing Pod (pod)	0.68	H	0.93	H
Empty Pod (pod)	0.00	L	0.83	H
Seed/Plant Weight (g)	0.75	H	0.82	H

H = High, M = Medium, L = Low

Uncontrolled environmental conditions are one of the obstacles to assemble soybean that salinity resistant that has high yield capability. Ashraf (2004) stated that the direct selection in the field about quantitative properties which is tolerant to high salinity still difficult because of uncontrolled environmental factors. One approach to improve the efficiency of the breeding program is to adopt a new selection criterion based on the knowledge of physiological processes, which is the delimiter from production plants at the time of exposure of high salinity.

Table 6: Selection Limit and Selection Progress every Generation.

Generation	Selection limit Of Products/Plants	Selection Progress
Elders	2.37	-
First Generation	0.48	4.68
Second Generation	10.16	2.38
Third Generation	0.21	3.57
Fourth Generation	10.16	3.93

3.2 Molecular Analysis of Saline Tolerant Genes in Soybean Roots Resistant to Salinity

Strong interactions between agronomic properties which are morphological markers with environmental factors encourage the use of molecular breeding methodology using genetic markers to support the selection of soybean that salinity resistant. Salinity stress caused some changes in physiological processes, metabolism, and the expression of several genes that allegedly played an important role in the adaption response of plants to salinity stress. Santoso *et al.,* (2012) describes the genes that was induced by abiotic stresses such as high salinity have been found and provide an important opportunity to improve the properties of tolerant to high salinity through genetic engineering approach. Target genes include genes that encode the enzymes that necessary for the biosynthesis of various osmoprotectant, enzymes that eliminated the reactive oxygen species, late embryogenesis proteins (LEA), enzyme for detoxification and transcription factors. This research examined the expression of several genes that are responsive to salinity stress, namelyDehydration Responsive Element Binding Protein 5 (DREB5), Glycine and Proline Rich Proteins 3 (GPRP3), Δ1-Pyrroline-5-carboxylate synthetase (P5CS), bZIP Transcription Factor (ZIP), EREBP/AP2 Transcription Factor (ERF), Gm Chloride Channel 1 (GmCLC1), Gm putative Na+/ H+ antiporter (NHX1), and Purple Acid Phosphatases 3 (PAP3).

Molecular test of saline tolerant gene at the root of soybean selection result F3, soybean grobongan varieties and Burangrang varieties can be seen in Table 7, Figure 1 and Figure 2. In Table 7 and Figure

1, it can be seen that the mRNA expression of Dehydration Responsive Element Binding Protein 5 (DREB5) genes, Glycine and Proline Rich Proteins 3 (GPRP3), Δ1-Pyrroline-5-carboxylate synthetase (P5CS), bZIP Transcription Factor (ZIP), EREBP/AP2 Transcription Factor (ERF) and Gm putative Na+/H+ Antiporter (NHX1) higher (up-regulated) in selected soybean that salinity resistant F3that was given salinity treatment compared with the control treatment, while the level of gene expression of Gm Chloride Channel 1 (GmCLC1) and Purple Acid phosphatases 3 (PAP3) lower (down-regulated) than control treatment. P5CS genes showed the highest level of expression enhancement that is 1.75 fold in soybean that treated salinity.

Table 7: Level of mRNA Expression of Salinity Tolerant Genes (fold) on soybean genotypes that salinity resistant and Grobogan varieties on Control Treatment and Salinity Stress 5-6 mmhos/cm.

Gene	Soybean Result of Selection Salinity Resistant F3		Grobogan Varieties Soybean	
	Control	Salinity Stress	Control	Salinity Stress
DREB5	0.61	0.94	0.54	0.71
GPRP3	0.40	0.56	0.46	0.49
P5CS	0.76	1.75	1.09	1.30
BZIP	0.35	0.51	0.35	0.25
ERF3	0.59	0.92	0.60	0.68
CLC1	0.63	0.28	0.60	0.38
NHX1	0.44	0.54	0.60	0.38
PAP3	0.43	0.31	0.50	0.34

Shinozaki and Yamaguchi-Shinozaki (1997) also mentions that some of the genes that responsive to drought stress, high salinity, and cold temperatures at the level of transcription (mRNA) have been widely reported. The amount of mRNA of the gene that is responsive to salinity decreases if the stress on the plant is stopped. This is an evidence that these genes induced by salinity on plant growth environment.

Gene expression that induced by environmental stress will produce proteins that function as a signal conductor from the surface of plant cells into the cell, enzymes that involved in the biosynthesis of molecules that influence the defense mechanisms (such as proline, some types of carbohydrates and polyamine), or a transcription factor that activates the expression of genes that play a role in plant defense mechanisms against the stress. Cis and elements trans involved in gene expression that induced by stress has been widely analyzed in detail and carefully to ravel the mechanisms of plants in defend themselves against environmental stress. Dehydration-responsive element (DRE) is cis elements that contained in a promoter and co-regulate the expression of genes in times of drought stress, high salinity and cold temperatures. This element has a motive sequences A/GCCGAC that detected by DRE-binding protein (DREB) transcription factor (Hardiarto, 2010). One of the DREB gene subfamily is GmDREB5. This gene plays an important role in the soybean plant resistance to drought stress by recognizing the response of dehydration (Lan et al., 2011). The results of research showed the level of gene expression DREB 5 and GPRP3 gene (Glycine and Proline Rich Proteins 3) in third generation of selected soybean salinity increases with salinity stress (Figure 1). Penget al. (2012) also reported an increase in gene expression, particularly in soybean roots that induced by salinity stress. DREB5 and GPRP3 allegedly play an important role in mediating independent pathways of ABA (abscisic acid) of the salinity stress (Phang et al., 2008; Peng et al., 2012). Decrease in leaf water potential to stimulate the synthesis of ABA. ABA concentration in the crown would affect the expression of genes that determine the synthesis of proteins (including protein function and protein regulator). Functional protein that referred is among LEA proteins, proteinase, detoxifying enzymes, and synthesis regulator and osmotic controller enzymes osmotic, namelyproline, betaine and sugar. It appears that the rapid response of plants is closing of stomata. While the density of stomata allegedly changed after the plant experienced continual stress in a relatively long time (weekly to monthly) other changes in the crown, leaf senescence, changes in root growth, vernalization changes, when flowering and seed filling. There appears to be the link between a decrease of relative water content of leaves that followed by an increase in ABA with synthesis of proline as osmotic control compound (Shinozaki and Yamaguchi-Shinozaki, 1997; Passiora, 1996; Swasono, 2012).

MRNA expression of several genes in unselected soybean Grobongan varieties that treated salinity also showed an enhancement that is DREB5, P5CS and ERF3 compared with controls (Table 5 and Figure 2). P5CS genes showed the highest level of expression of 1.30 fold. While the level of gene expression GPRP3, ZIP, CLC1, NHX1 and PAP3 in soybean Grobongan varieties that are subjected lower salinity treatment than the control.

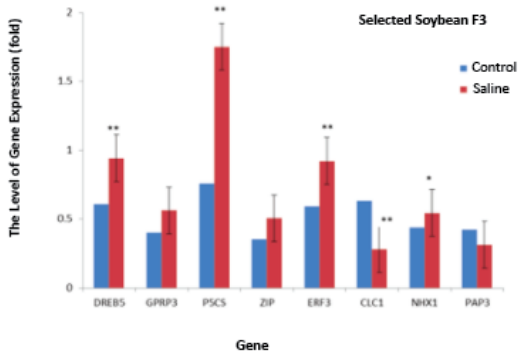

Figure 1: Level of Gene Expression in Selection Soybean that Salinity Resistant F3, Grobogan varieties (**t <0.001, *t> 0.05 Compared Control Using t test).

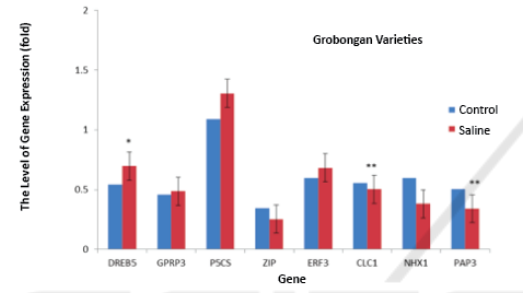

Figure 2: Level of Gene Expression in Soybean Grobogan Varieties (** t <0.001, * t> 0.05 Compared Control Using t test).

Proline is the main compounds that can protect the cells through stabilization of proteins and cell membranes. On a variety of plant species, found accumulation of proline in salinity stress. Proline accumulation is regulated by a balance between the synthesis and catabolism. Δ1-Pyrroline-5-Carboxylate Synthetase (P5CS) is a key enzyme in the mechanism of proline. , P5CS is primarily responsible genes in the biosynthesis of proline and proline decomposition by the proline dehydrogenase enzymes that is an enzyme in mitochondria and the other mechanisms that can increase the concentration of proline. The level of gene expression is very important to understand the mechanism of proline accumulation (Ashraf and Foolad 2007; Lutts et al., 1999). The results of research showed the expression of gene P5CS in selected soybean salinity F3 increases with salinity stress, as well as soybean Grobogan varieties despite an increase in that gene expression was lower than selected soybean salinity (Figure 1 and Figure 2). Celik and Atak's research (2011) also showed an increase in gene expression P5CS by increasing the salt concentration in the growing media, especially in soybeans that salinity tolerant.

BZIP gene expression Transcription Factor (ZIP) in selected soybean that salinity resistant also showed an increase in the presence of salinity stress, while in soybean Grobongan varieties and Burangrang decreased due to salinity stress. Gao et al. (2011) explained that over-expression of GmbZIP1increases the response of transgenic plant to forming of an independent ABA and triggering stomatal closure under stress conditions, thereby potentially increasing the tolerance to multiple abiotic stresses including high salt stress. So far, the relationship between salt stress and stomata is still largely unknown. In this research, it is known that GmbZIP protein play a positive role in stomatal closure as effect of salt induction. The results showed that GmbZIP can act as a positive regulator of the salinity response by controlling stomatal closure. Thus, under salinity stress, excess GmbZIP in transgenic plants may be able to prevent the entry of Na+ and Cl-, stomatal closure, and reduce membrane cell damage that caused by ions and so that increases tolerance to salt stress.

The results also showed gene expression EREBP/AP2 Transcription Factor 3 (ERF3) increases in selected soybean that salinity resistant that experiencing salinity, in soybean Grobogan varieties increased expression of the gene is not as high in selected soybean (Figure 1 and Figure 2). The same result has also been reported to Zhang et al., (2009) in the treatment of salt stress, mRNA GmERF3 accumulates after 5 hours of initiation and reached a peak after 10 hours of initiation. GmERF3 transgenic tobacco than given 200 mM NaCl stress is able to maintain the green leaves and roots that can grow well. Allegedly biotic and abiotic stresses, including salinity stress can regulate the expression of GmERF3 and abundance of transcriptional activator GmERF3 and most likely regulate transcription, up-regulate several genes that induced by stress, and their protein products contribute to increase resistance to stress conditions. In addition, the accumulation of soluble sugars and proline as osmolite, plays an important role in plants that exposed to stress.

In addition to increased osmolite, maintenance of ion homeostasis is an important strategy in plants to survive in salinity stress. This mechanism will prevent the toxicity effect that repercussions of ion poisoning which causes cytoplasmic organelle membrane damage. GmNHX1 and GmCLC1 are genes that regulate ion homeostasis in soybean (Li et al., 2006). The results of research showed that the salinity stress also increases the gene expression of

Gm Putative Na+/H+ antiporter (NHX1) in selected soybean that salinity resistant F3, while in soybean Grobogan varieties and Burangrang, gene expression is decreased in the presence of salt stress. It shows gene expression of NHX1 in selected soybean that salinity resistant F3 more resistant to salinity stress than soybean Grobogan varieties. Staal et al. research results (1991) also showed the activities of NHX tonoplast higher on Plantogomaritima NaCl tolerant compared to Plantogomaritima NaCl sensitive. Research Li et al., (2006) showed the transgenic cell vacuole of GmNHX1-YFPcontained Na+ accumulation, whereas in control YFD vacuoles is no accumulation of Na+. Allegedly ion transporter that located in the plasma membrane and tonoplast can help the release of ion from cells and ions compartment within the cell, thereby reducing the effects of ion poisoning.

GmCLC1 gene encodes a protein of Cl- to the vacuole, namely by transferring ions from the cytoplasm into the vacuole to reduce the toxic effects of salts (Li et al., 2006). GmCLC1 genes also play an important role to increase plant tolerance to salinity, reducing damage to the structure of the membrane, increasing osmotic adjustment and regulation of antioxidant enzymes in salinity stress conditions (Sun et al., 2013). Gene expression Gm Chloride Channel 1 (GmCLC1) in selected soybean that salinity resistant F3, soybean Grobogan varieties on NaCl stress conditions lower than those in the control treatment. Instead, the research of Sun et al. (2006) showed increased expression of GmCLC1 which increases the tolerance of transgenic plants Populusdeltoides × P. euramericana 'Nanlin 895' against salinity stress. This difference is expected because Cl compartment not only increased because GmCLC1 expression and the presence of other mechanism of Cl released that reduce toxic effects on plants.

Salinity stress not only induces the accumulation of proline, sugars and other osmolite, but it can also lead to increased production of reactive oxygen species (ROS) that can cause damage to lipid membranes, proteins and nucleic acids that can cause cell death. Plants develop enzymatic protection mechanisms that can be scavenged ROS and prevent the damaging effects of free radicals. Liao et al. (2003) stated that the GmPAP3 genes alleged related to soybean adaptation to NaCl stress through its involvement in the depuration of reactive oxygen species (ROS). The results showed GmPAP3 expression in selected soybean that salinity resistant lower than the treatment without salt stress, so it is necessary to put other efforts to help plants cope with oxidative stress that caused by ROS. Exogenous antioxidants applications such as ascorbic acid, salicylic acid, glutathione, tocoferol, ubiquinone, ubiquinol, and cysteine are expected to help soybean plants to overcome the problems of the free radicals. Research of Sitinjak et al. (2012) demonstrated the application of ascorbic acid at a dose of 500 ppm on soybean Grobogan varieties in saline soil with DHL 5-6 mmhos/cm that produce the highest production. Based on these results, in the third stage studies of ascorbic acid is applied to increase soybean resistance to salinity stress.

4 CONCLUSIONS

Salinity-resistant soybean selection shows success which is indicated by high and moderate heritability in production characters and increased selection progress. Molecular test of the third generation salinity tolerant gene shows the expression of DREB5, GPRP3, P5CS genes, bZIP Transcription Factor (ZIP), EREBP/AP2 Transcription Factor (ERF3) and Gm Putative Na^+/H^+ Antiporter (NHX1) were higher in selected F3 salinity-resistant soybeans that received salinity stress compared to control treatment, whereas GmCLC1 and PAP3 genes is lower than the control treatment. Increasing the heritability and expression of several salinity tolerant genes in salinity-resistant soybean related to their role in plant defense mechanisms against salinity stress shows the potential for selected soybeans to be developed as a salinity tolerant variety.

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