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Unravelling stress responsive NAC1 gene (OsNAC1) from potential Indonesia and Vietnam local varieties rice

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Abstract The wide coastal area of rice production is reached 76.94% which showed the percentage of areas with high salinity constraints in Indonesia and Vietnam. The result was approximately 200 sequences from all samples which was similar to Oryza sativa Japonica Group NAC domain containing protein 92 (LOC4336052) transcribed transcript variant X2, mRNA (XM_015778438.2) for percentage of 95 – 98%. The conclusion confirmed that OsNAC1 gene from eleven genotype was someone similar evident by Phylogenetic construction result.

Keywords: Salt Tolerance; *Oryza sativa*, OsNAc1, Indonesia, Vietnam

Introduction

The need for rice in Indonesia will continue to increase. Data shows rice production in 2022 for food consumption of the population is estimated at around 32.07 million tons, an increase of 718.03 thousand tons or 2.29 percent compared to rice production in 2021 which amounted to 31.36 million tons. Meanwhile, the per capita need for rice reaches 114.6 kg (BPS-Statistics Indonesia, 2023). So that production is the government's full priority to meet the needs of 273 million people. Even though North Sumatra is included in the top 10 in rice production, this production is still very low, namely 2004.45 and 2131.67 tons in the last two years (2022-2023).

Effective effort that can be done is extensification. But the obstacles for Indonesia as a maritime country is the extent of the coastal area which is synonymous with high salinity. Geographically, the proportion of Indonesia's sea area is recorded at 76.94% which has implications for the extent of the coastal area in Indonesia (Ramdhan and Arifin, 2018). Research on salinity levels in coastal areas in North Sumatra shows that the distribution of criteria for high

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salinity levels is 83.4% and medium is 16.6% with a low Na-dd of 0.15 me/100 (Prasetya, 2018).

Rice is a very sensitive plant to salinity stress. a salinity level of 6.0 dS/m (100mM NaCl) will reduce production by up to 60%. Salinity stress has an impact on metabolism including physiological, enzymatic (Phosphoenol pyruvate carboxylase and Ribulose bisphosphate carboxylase) and genetic expression caused by oxidative stress, ionic poisoning, metabolic disorders and genotoxicity (Reddy *et al.,* 2017). This situation triggers nutrient imbalance and Reactive Oxygen Species (ROS) in root such as hydrogen peroxide (H2O2), superoxide (O2) and hydroxide (OH). Consequently, it could reduce seed vigour, vegetative growth, flowering and fruit development (Yang *et al*., 2019). In the germination phase, stress causes a decrease in osmotic potential, a change in the orientation of protein synthesis, and damage to the embryo due to NaCl poisoning. Whereas in the generative phase it can cause sterility of rice panicles which leads to a decrease in production of up to 70%. In the aspect of soil fertility, an increase in Na+ will lead to a decrease in the availability of the elements Ca+, Mg2+, and K+ and a decrease in the mobility of P in the soil. so that salinity stress will have a significant negative impact causing death in plants. This circumstance could also lead to decreased yield parameters including grain weight, panicle number toward critical threshold.

Salinity tolerance mechanisms are grouped into ion exclusion and osmotic tolerance which involve the transport of Na and Cl ions in the roots, maintaining stomatal conduction for detoxification. Reactive Oxygen Species (ROS). Both mechanisms are regulated by the stress-responsive NAC 1 (POWER1) [5*(Hu et al*., 2006; Zhang *et al*., 2020). express genPOWER1 also shows increased resistance of transgenic plants in highly saline soil conditions (Rasel *et al.,* 2021). So far it has been reported that there are 75 gene families NAC predicted to be found in the rice genome associated with tolerance to cross-stress and drought stress. Transgenic rice containing gene fusionPOWER1 and GFP showed the ability of rice to last up to 12 days with a resistance percentage of up to 80%. So far in Indonesia, efforts have been made to explore and identify potential local rice that can withstand salinity stress. Genetic research that can be initiated is gene identification POWER1 along with cis-elements associated with salinity stress responsiveness of several potential local varieties in North Sumatra.

The NAC family of transcription factors (NAM, ATAF1/2, CUC2) is predicted to be present in quantities of approximately 117 and 151 in Arabidopsis and rice, respectively. This transcription factor is known to have a DNA binding domain located in the N-terminal part. Through research, it was found that overexpression of OsNAC2 can increase ABA production and inhibit the ABA catabolism process, thereby increasing the concentration of ABA in plants (Mao

et al., 2017). These findings were also supported by the increased expression of several genes in the SNAC Gene group (SNAC 1, SNAC2, SNAC6, etc.) after treatment with ABA. Furthermore, the expression of SNAC1, OsNAC5, and OsNAC6 was associated with an increase in the ability of transgenic rice to withstand salinity stress. Quantitative analysis of the expression of OsNAC45 genes, including OsCYP89G1, OsDREB1F, OsEREBP2, OsERF104, OsPM1, OsSAMDC2, and OsSIK1, also showed their association with tolerance to salinity stress(Tammi, 2022). SNAC1 was also reported to increase spikelet fertility, production and grain weight, as well as showing tolerance to stress due to drought and salinity stress (Ganie *et al*., 2019). Efforts to transform the SNAC1 gene in high-yielding rice varieties, such as BRRI Dhan-55, BRRI Dhan-56, and BRRI Dhan-49, were carried out to increase tolerance to stress through inserting the rd29A promoter. Therefore, SNAC1 is considered to have greater potential to be analysed as one of the genes that is potentially tolerant to salinity stress. The research finding was aimed to investigate on OsNAC1 from potential local variety from Indonesia and Vietnam.

Materials and methods

Sample collection

Sample varieties were previously collected from Balai Benih Induk (BBI), North Sumatera, Indonesia and Chau Thanh, Kien Giang Province, Vietnam. There were 5 Indonesia Local varieties from Indonesia along with 6 Vietnam Local varieties to be undergone stress test as shown in the table 1. There were 30 seeds for ever local variety rice.

Table 1. List local variety of vice from Indonesia and Vietnam

No	Variety name	Country of origin
1	ST24	Vietnam
2	4900	
3	ST25	
4	576	
5	OM18	
6	2517	
7	VAR I SP	Indonesia
8	VAR 2 SM	
9	VAR 3 AL	
10	VAR 4 KB	
11	VAR 5 PK	

These varieties have been suspected to be tolerant toward salinity stress due to their habitat and Primary prediction. Indonesian local varieties were transported to Vietnam prior to obtaining a phytosanitary certificate from the Quarantine centre in North Sumatera.

Salinity stess field test

The test of salinity stress was conducted in the greenhouse at Kien Giang University, Kien Giang Province Vietnam (105°14'33.21" E; 9°91'41.13" N) and was arranged in a completely randomized design. All the samples were tested under a NaCl concentration of 150 mM. Seeds were germinated on foam sheets floating on tap water. The treatments started from germination by soaking the germinated seeds in saline solution containing 150 mM NaCl. The parameters measured in this test were survival rate, shoot and root length for a period of 3weeks with one week interval after treatment.

Genome DNA extraction and amplification of NAC1 gene

DNA analysis was conducted in the Lab of molecular biology in Biotechnology and Food technology School, Can Tho University. DNA extraction was performed by following the protocol of Roger and Bendich, 1988 with some modifications. The leaf samples were cut into small pieces and put into the tube with 0.1g/tube. Soak both the tubes and sample grinder tools in liquid nitrogen for about 15 minutes. Grind the sample by machine: grind 3-4 times, each time 30 seconds. Add 1 ml extraction solution (10 ml EB + 7 µl b-Mercaptoethanol) and 50 µl of SDS 10% (w/v), and incubate in water at 65oC for 30 minutes. Centrifuge at 13000 rpm for 10 minutes. After that, 800 µl supernatant was transferred into a new microcentrifuge tube and mixed using isopropanol with equal volume. The mixture was incubated continuously at -20^{o}C for 2 hours and then centrifuged at 13000 rpm for 15 minutes. The supernatant was discarded and added 400 µL of THE 0.1X and 7 µl of RNAse, and incubated at 37^{o}C for 25 minutes. Add 800 µl of chloroform/isoamyl alcohol (24:1), and mix gently by inversion. Centrifuge at 13000 rpm for 10 minutes. Transfer 700 µl supernatant into a new microcentrifuge tube and add 1.4 ml of 96% cold ethanol mix and leave for 15 minutes at room temperature. Centrifuge at 13000 rpm for 10 minutes, discard the ethanol and keep the precipitate. Put 700 µl of 70% ethanol into the tube to wash the DNA pellet and mix gently by inversion. Centrifuge 13000 rpm for 10 minutes, remove ethanol and keep the precipitate (repeat this step twice). Allow the DNA to dry briefly at room temperature. Add 150 µl TE 0.1X and store at -20^{o}C.

NAC1 Gene from all samples was isolated by specific primer OsNac-F 5'-GTCGTCCGTCGTCCTCCCTC-3' and OsNac-R 5'-CTGCCGTCGGGTTAAAGAACTG-3' by following configuration (denaturation was carried at 95ºC for 5 minutes and annealing temperature was 56oC for 30 seconds. Extension was performed at 72ºC for 5 minutes). The amplification was carried out for 35 cycles. Upon the completion of the amplification process, the amplicon was visualized by using EtBr. Two directional sequencing was performed by utilizing a sequencing machine (Brand, USA).

Blast analysis and phylogenetic construction

Homology of OsNAC1 sequenced from rice sample was analysed using BLAST tools (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Phylogenetic tree was constructed using offline software Molecular Evolutionary Genetic Analysis (MEGA) Version 11. This analysis was carried by bootstrap method for 500 time multiplication thereby performing maximum likelihood tree.

Results

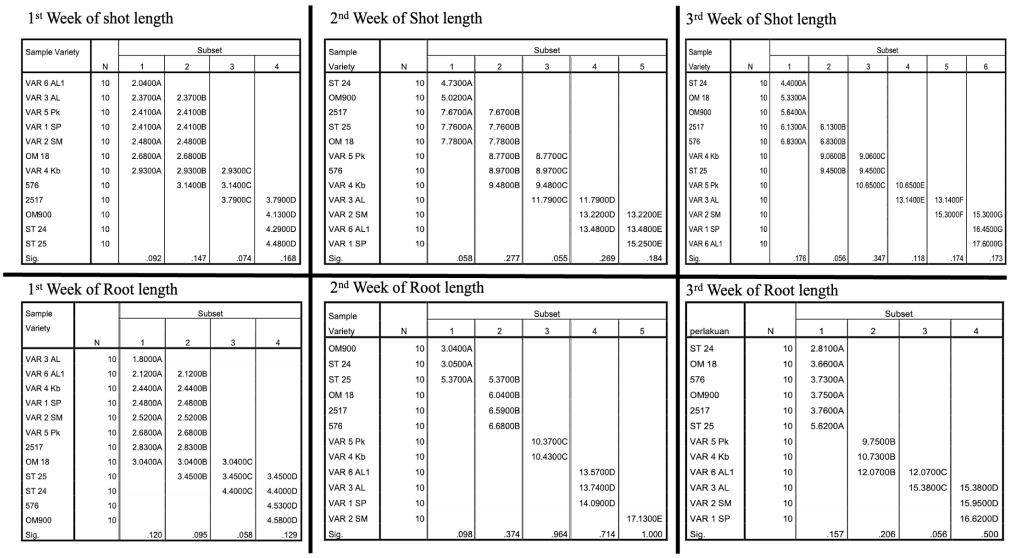
Salinity stress field test

Survival rate of tested rice varieties from Indonesia and Vietnam is shown in Table 2. Total sample grown for every sample was 30 seeds. Sample counted as a dead was indicated by the tissue colour changing into yellowish and brownish without showing a growing signal.

Table 2. Result of checking salt tolerance of 13-rice variety seedlings at 21 days after germination

No	Variety name	Total seedlings	Country of origin	Survival plant number	Survival rate (%)
1	ST24	30	Vietnam	14	46,67
2	4900			13	43,33
3	ST25			28	93,33
4	576			23	76,67
5	OM18			19	63,33
6	2517			20	66,67
7	VAR I SP		Indonesia	29	96,67
8	VAR 2 SM			29	96,67
9	VAR 3 AL			29	96,67
10	VAR 4 KB			25	83,33
11	VAR 5 PK			30	100,00
12	VAR 6 AL1			30	100,00

For the study, shoot and root lengths from three-week-old plants post-treatment were analysed using SPSS software with a completely randomized design, and the data were quantified using the DNMRT test, with results shown in Figure 1.



1st Week of shot length

Sample Variety	N	Subset 1	2	3	4
VAR 6 AL1	10	2.0400A			
VAR 3 AL	10	2.3700A	2.3700B		
VAR 5 Pk	10	2.4100A	2.4100B		
VAR 1 SP	10	2.4100A	2.4100B		
VAR 2 SM	10	2.4800A	2.4800B		
OM 18	10	2.6800A	2.6800B		
VAR 4 Kb	10	2.9300A	2.9300B	2.9300C	
576	10		3.1400B	3.1400C	
2517	10			3.7900C	3.7900D
OM900	10				4.1300D
ST 24	10				4.2900D
ST 25	10				4.4800D
Sig.		.092	.147	.074	.168

2nd Week of Shot length

Sample Variety	N	Subset 1	2	3	4	5
ST 24	10	4.7300A				
OM900	10	5.0200A				
2517	10	7.6700A	7.6700B			
ST 25	10	7.7600A	7.7600B			
OM 18	10	7.7800A	7.7800B			
VAR 5 Pk	10		8.7700B	8.7700C		
576	10		8.9700B	8.9700C		
VAR 4 Kb	10		9.4800B	9.4800C		
VAR 3 AL	10			11.7900C	11.7900D	
VAR 2 SM	10				13.2200D	13.2200E
VAR 6 AL1	10				13.4800D	13.4800E
VAR 1 SP	10					15.2500E
Sig.		.058	.277	.055	.269	.184

3rd Week of Shot length

Sample Variety	N	Subset 1	2	3	4	5	6
ST 24	10	4.4000A					
OM 18	10	5.3300A					
OM900	10	5.6400A					
2517	10	6.1300A	6.1300B				
576	10	6.8300A	6.8300B				
VAR 4 Kb	10		9.0600B	9.0600C			
ST 25	10		9.4500B	9.4500C			
VAR 5 Pk	10			10.6500C	10.6500E		
VAR 3 AL	10				13.1400E	13.1400F	
VAR 2 SM	10					15.3000F	15.3000G
VAR 1 SP	10						16.4500G
VAR 6 AL1	10						17.6000G
Sig.		.176	.056	.347	.118	.174	.173

1st Week of Root length

Sample Variety	N	Subset 1	2	3	4
VAR 3 AL	10	1.8000A			
VAR 6 AL1	10	2.1200A	2.1200B		
VAR 4 Kb	10	2.4400A	2.4400B		
VAR 1 SP	10	2.4800A	2.4800B		
VAR 2 SM	10	2.5200A	2.5200B		
VAR 5 Pk	10	2.6800A	2.6800B		
2517	10	2.8300A	2.8300B		
OM 18	10	3.0400A	3.0400B	3.0400C	
ST 25	10		3.4500B	3.4500C	3.4500D
ST 24	10			4.4000C	4.4000D
576	10				4.5300D
OM900	10				4.5800D
Sig.		.120	.095	.058	.129

2nd Week of Root length

Sample Variety	N	Subset 1	2	3	4	5
OM900	10	3.0400A				
ST 24	10	3.0500A				
ST 25	10	5.3700A	5.3700B			
OM 18	10		6.0400B			
2517	10		6.5900B			
576	10		6.6800B			
VAR 5 Pk	10			10.3700C		
VAR 4 Kb	10			10.4300C		
VAR 6 AL1	10				13.5700D	
VAR 3 AL	10				13.7400D	
VAR 1 SP	10				14.0900D	
VAR 2 SM	10					17.1300E
Sig.		.098	.374	.964	.714	1.000

3rd Week of Root length

perlakuan	N	Subset 1	2	3	4
ST 24	10	2.8100A			
OM 18	10	3.6600A			
576	10	3.7300A			
OM900	10	3.7500A			
2517	10	3.7600A			
ST 25	10	5.6200A			
VAR 5 Pk	10		9.7500B		
VAR 4 Kb	10		10.7300B		
VAR 6 AL1	10		12.0700B	12.0700C	
VAR 3 AL	10			15.3800C	15.3800D
VAR 2 SM	10				15.9500D
VAR 1 SP	10				16.6200D
Sig.		.157	.206	.056	.500

Figure 1. DNMRT root and shoot length for 1 – 3 week after treatment

DNA extraction and OsNAC1 amplification

Several local varieties of rice from both Indonesia and Vietnam have been reported as tolerant rice under salinity stress. Effective DNA extraction procedure should be conducted for obtaining good quality genome DNA to enhance potential for gaining OsNAC1 fragments. Amplification of potential salt tolerant gene resulted in partial fragment with product length about ± 200 bp (Figure 2).

Blast analysis

To ascertain the obtained sequence is OsNAC1 gene, we identified amplicon by performing two-direction sequencing. 11 rice accessions has successfully identified those sequences as shown in Tabel 3.

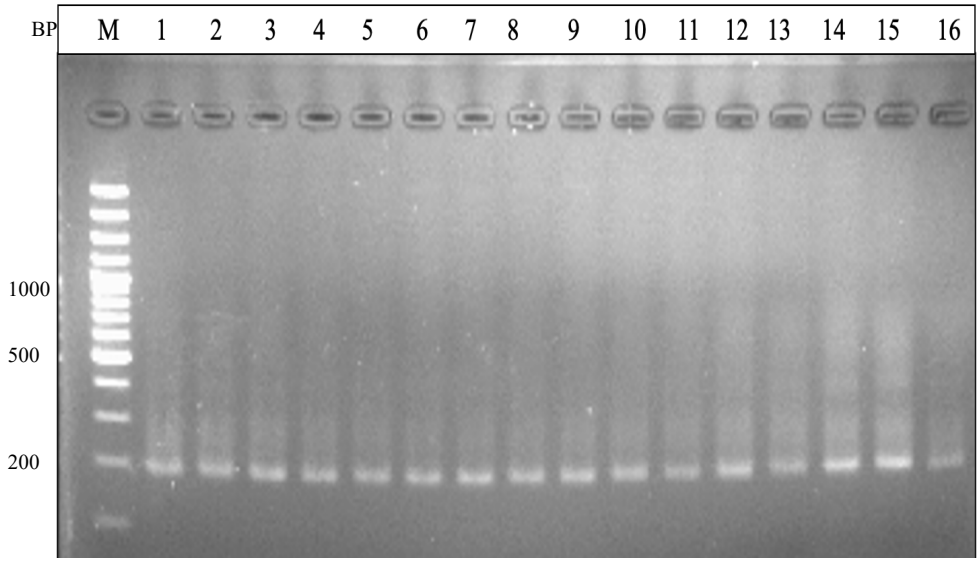


Figure 2. Amplification of OsNAC1 gene fragment (M: DNA Marker; 1,2: Var1 SP; 3,4: OM18; 5,6: VAR 4B; 7,8: VAR 2 SM; 9, 10: Var3 AL; 11, 12: 576; 13: 2517; 14: 4900; 15: Var 5PK; 16: ST25

Table 3. Sequence of OsNAC1 gene from local variety rice in Indonesia and Vietnam

No	Variety	Country of Origin	Sequence NAC1 Gene
1	ST24	Vietnam	GGNNAGGGCNNGCGGCGCGTCGACCGGCGACGACGGCGCACG TGACCTGCTTCTCCAACGCGCTGGAGGGCCAGTTCTTTAACCC GACGGCAGAGGGAGGGAANCAAGTGGGAGTGCNTCGNNCNN GTTNGNNNGTGTGATGGAAGGGGCGGNNNNNNNGGGGGG
2	4900		GGNNCGGCGCGCGGCGCGTCGACCGGCGACGACGGCGCACGT GACCRGCTTCTCCAACGCGCTGGAGGGCCAGTTCTTTAACCCG ACGGCAGAGGGAGGGAANCAAGATGGGNNTGCAATCAGNNC NGGTNNNAGGTGGATGGAACNGNNNGNNNNTCTGNNNGAGG NNNN
3	ST25		GGNNAGGGCNNGCGGCGCGTCGACCGGCGACGACGGCGCACG TGACCTGCTTCTCCAACGCGCTGGAGGGCCAGTTCTTTAACCC GACGGCAGAGGGAGGGAANCAAGTGGGAGTGCNTCGNNCNN GTTNGNNNGTGTGATGGAAGGGGCGGNNNNNNNGGGGGG

4	576		GNNNCNGACGANGCGGCGCGTCGACCGGCGACGACGGCGCAC GTGACCTGCTTCTCCAACGCGCTGGAGGGCCAGTTCTTTAACC CGACGGCAGAGNNAGGNTAACAAGATGGGGNATGCNTCAGCG TNNAGNTNGNNNNNNTGAATNTAGGGGGCGAA
5	OM18		CCAAACCCCGTTAAACGGCGCCGGCGTCGTCGACCCGGCGAC GACGGCGCACGTGACCTGCTTCTCCAACGCGCTGGAGGGCCA GTTCTTTAACCCGACGGCAGA
6	2517		TTTCCGGAACCGGNCGGCGCCGCGCCGTCGGACCCGVGCGAC GACGGCGCACGTGACCTGCTTCTCCAACGCGCTGGAGGGCCA GTTCTTTAACCCGACGGCAGAAGACAAGCGCCGGGGAGGGAA GGGATATGGGGGATACGGTTAGCATTAAGGTTGTTTGCGTGTA GAAAAANNGGGAAGAGGGAGGGGAGGGACGGAACGNGAACG AACAAAATNNNNNNGTCATGTAGNNTTNNNN
7	VAR I SP		CAATAACGTCAACGGCGCGGCGACGTCGACCCGGCGACGACG GCGCACGTGACCTGCTTCTCCAACGCGCTGGAGGGCCAGTTCT TTAACCCGACGGCAGAGTGAGGGAGCAATATGGGATTATACA ATCAGCGTCGAGGTTTGATGCGTGTAGACGAATCGGGGAAGG AGGGGAGGCGGCGGACGAAACAAAA
9	VAR 2 SM		GGGNNNGGACGGGCGGCGCGTCGACCGGCGACGACGGCGCAC GTGACCTGCTTCTCCAACGCGCTGGAAGGGCCAGTTCTTTAAC CCGACGGCAGANNNNAGGGAAGGAATATGGGNNGTGCAATC AGCNTCGAGNNTTGNGCGTGTGAGTCTGACGAAGGGGAAGGN AGGGAGGNGAGGGACAAAA
10	VAR 3 AL	Indonesia	GGTNCNGNCNNNCGGCGCGTCGACCGGCGACGACGGCGACGT GACCTGTTCTCCAACGCGCTGGAGGGCCAGTTCTTTAACCCGA CGGCAGAGTGAGGGAANGAAGARGGGRNGTGCANCAGNGTN AGGTTNTCT
11	VAR 4 KB		CCACCACGGTGCAACGGCGCGCACGCCGTCGTACCGCGCGAC GTACGAGCGCACGTGACCTGCTTCTCCAACGCGCTGGAGGGCC AGTTCTTTAACCCGACGGCAGA
12	VAR 5 PK		GGNNNGGNCNGGCGGCGACGTCGACCGGCGACGACGGCGCAC GTGACCTGCTTCTCCAACGCGCTGGAGGGCCAGTTCTTTAACC CGACGGCAGANNGAGGGAANNAAGATGGGCTGATCTCATTCG GNNNNGNNTNNTNNTTAGCGAGGGAANGNAGGGNAAACCCC CGGGGNNGNNNNGAGGGGGAGNN

Bioinformatic analysis

Comparative analysis of obtained sequence was performed by using Blast (https://blast.ncbi.nlm.nih.gov/Blast.cgi) thereby configurated it to moderately similar. Blast analysis is presented in Table 4 and the alignment along with the phylogenetic three are shown in Figure 3 and 4 respectively.

Table 4. Blast analysis of OsNAC1 gene from local variety rice in Indonesia and Vietnam

No	Rice Variety	Accession	Name	Query Covered (%)	Per. Identity (%)
1	ST24	XM_015778438.2	Predicted: *Oryza sativa japonica* Group NAC domain containing protein 92 (LOC4336052) transcribed transcript variant X2, mRNA	49	97,59
2	4900			51	95,56
3	ST25			50	97,59
4	576			50	97,59
5	OM18			84	98,88
6	2517			37	96,70
7	VAR I SP			45	97,75
8	VAR 2 SM			43	96,43
9	VAR 3 AL			58	95,18
10	VAR 4 KB			85	91,30
11	VAR 5 PK			42	97,59
12	VAR 6 AL1			58	95,18

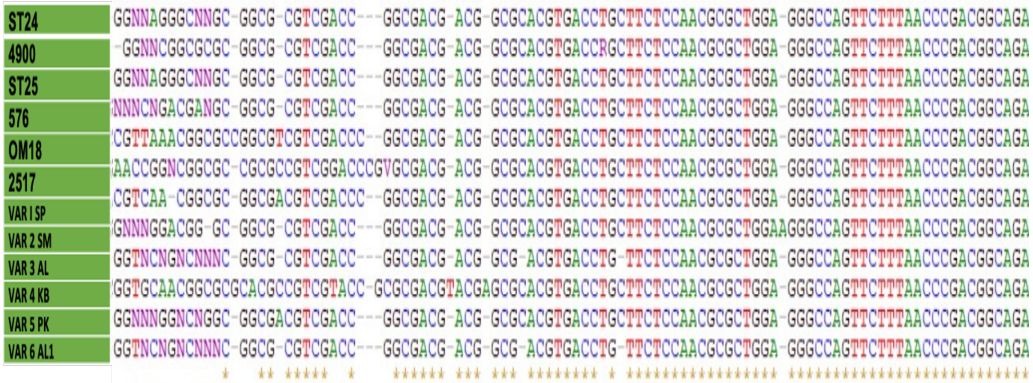


Figure 3. Multi-alignment of OsNAC1 Local variety rice from Indonesia and Vietnam

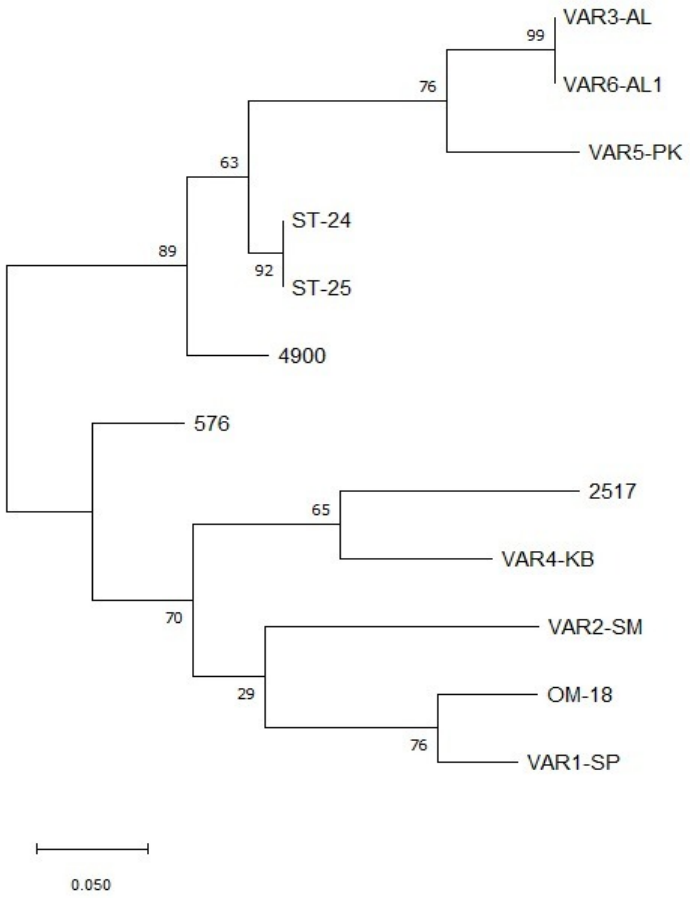


Figure 4. Phylogenetic tree OsNAC1 local variety rice from Indonesia and Vietnam

Discussion

It is crucial to understand the mechanisms for developing salinity-tolerant rice varieties before conducting DNA sample tests. Studying potential varieties for salinity stress tolerance requires a thorough understanding of these mechanisms (Shahzad *et al*., 2022). Research on stress-tolerant varieties has been ongoing to identify potential candidates, with wild rice being a valuable genetic resource for salinity tolerance(Le *et al*., 2021). Despite reduced growth under salinity stress, the survival rates in the study were notably high, ranging from 43.33% to 100%.

In Vietnam, 179 rice landraces were screened for stress tolerance using 21,623 SNPs, while Indonesia evaluated 25 rice genotypes under 6 g/L salinity during germination, identifying six tolerant genotypes (BDR *et al*., 2020; Siregar *et al.,* 2021). Another study tested ten brown rice varieties at four salinity levels, observing significant impacts on plant height, grain weight, and peroxidase activity (Siregar *et al*., 2021). Additionally, 116 Asian rice cultivars were categorized into tolerant, moderate, and sensitive groups based on morphological, physiological, and biochemical assessments. Salt-tolerant varieties included Pokkali (India), TCCP 266 and IR 45427 (Philippines), and Namyang 7 (Korea), evaluated using Na/K ratio, proline and sugar accumulation, and levels of malondialdehyde (MDA) and hydrogen peroxide (H2O2) (Alshiekheid *et al*., 2023).

For the study, shoot and root lengths from three-week-old plants post-treatment were analysed using SPSS software with a completely randomized design, and the data were quantified using the DNMRT test, with results shown in Figure 1.

It's been noted that genetic modifications driven by mutations have yielded a salt-tolerant rice variety called Khangdan Dotbien, achieved through the identification of potential genes using Marker Assisted Backcrossing (MABC) (Le Hung *et al*., 2012). This variety was primarily developed to enhance salt tolerance in Vietnamese rice. Assessments of salt tolerance in Vietnamese rice have also been carried out, considering parameters such as plant height, root length, emergence of new roots, and dry matter, under varying salinity levels (9.38, 12.5, and 15.63 dS/m). These evaluations reveal that heightened salinity diminishes plant height due to impeded cell division and elongation (Duy and Lien, 2022). Furthermore, rice has evolved several mechanisms to cope with salinity stress, including the production and storage of osmolytes for cell protection, maintenance of ion balance and distribution, utilization of antioxidants to detoxify Reactive Oxygen Species (ROS), and activation of programmed cell death.

Among rice varieties originating from Indonesia, VAR 5 PK exhibited the highest measurement. Indonesia's efforts in breeding for salinity tolerance initially focused on developing rice varieties for swampy areas where salinity poses a significant challenge. This endeavour resulted in the introduction of two rice varieties, Dendang and Lambur, in 1999 and 2001 respectively. In this study, we investigated dry land rice varieties due to their demonstrated resilience and tolerance to biotic and abiotic stresses, although there has been limited research concerning salt tolerance. Thus far, these varieties have displayed superior tolerance to salinity stress during the vegetative stage (Electrical Conductivity of 12 dS/m) (Hairmansis *et al*., 2017). In North Sumatra, several salt-tolerant

cultivars have been identified, such as Inpara 9, Lipigo 2, and Sigambiri Merah, which could prove valuable for breeding programs aimed at enhancing tolerance (Lubis *et al*., 2022). The results confirm that salt stress significantly reduces the growth rate of both shoot and root during the vegetative stage of plant development.

Figure 2 shows OsNAC1 gene has been succeed to isolate from 16 samples on Indonesia and Vietnam rice genotypes. the pair of specific primer is predicted to obtain product length of approximately 200 bp. this set of primer produced specific band projected to be OsNAC1 Gene. In terms of NAC (NAM, ATAF, and CUC) TFs, studies have demonstrated that their overexpression in genetically modified plants can enhance salinity tolerance across various plant species. For instance, transgenic rice plants engineered to overexpress either OsNAC6 or OsNAC1 have displayed robust tolerance to salt stress. OsNAC1 to OsNAC8, which encode proteins containing NAC domains in rice (*Oryza sativa* L.). Each of these OsNAC genes demonstrates specific expression patterns across different organ and It was discovered that the expression of OsNAC6 is triggered by cold, drought, high salinity, and the application of ABA. Amplifying the expression of the stress-responsive gene SNAC1 (STRESS-RESPONSIVE NAC1; also known as ONAC033 or AK104551) improves drought and salt tolerance in transgenic rice without impeding growth. Although SNAC1 shares similarity with OsNAC6, it is noteworthy that OsNAC6 is classified under the ATAF subgroup, whereas SNAC1 falls into the OsNAC3 subgroup. OsNAC6-over-expressing lines were tolerant to dehydration and high-salt stresses where it is also be called OsNAC1 Utilizing stress-inducible promoters, it is confirmed that enhance stress tolerance in rice (Nakashima *et al*., 2007).

According to analysis the sequence resembles to sequence of interest is Predicted: Oryza sativa Japonica Group NAC domain containing protein 92 (LOC4336052) transcribed transcript variant X2, mRNA (XM_015778438.2). The genome consisted of 3,475 genomic sequences, comprising 2,482 Bacterial Artificial Chromosomes (BACs), 901 P1-derived Artificial Chromosomes (PACs), 37 fosmids, 2 plasmids, 5 Polymerase Chain Reaction (PCR) products, 41 partial sequences from genomic clones, and 7 contigs from Syngenta (Kawahara *et al*., 2013). Similar percentage has proved to be high regardless query covered. It noticeable that OsNAC1 gene sequence obtained is quite short except genotype encoded OM18 and VAR 4 KB.

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