



Microsatellite Markers and Metabolite Profiles of Salt-Tolerant Rice: Inpari Unsoed 79 Agritan

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Abstract

Salinity is a challenge in crop production. High salinity affects soil osmotic pressure and the balance of nutrients that inhibit plant growth. In such case, utilization of salt-tolerant rice varieties could be an alternative. This study aims to identify microsatellite markers associated with salt tolerance, compare the Inpari Unsoed 79 Agritan variety with ten other rice genotypes based on microsatellite markers and determine the qualitative composition metabolites in Inpari Unsoed 79 Agritan associated with the plant response to salinity. This research was carried out at the Laboratory of Plant Breeding and Biotechnology Universitas Jenderal Soedirman and Indonesian Institute of Sciences, Bogor. This research used eleven rice varieties and ten microsatellite markers. The identification of microsatellite markers consisted of genomic DNA extraction, quantification and qualification of DNA, amplification of microsatellite DNA and data analysis. Metabolite profiling was conducted on Gas Chromatography-Mass Spectrometry (GC-MS) instrument. The results showed that microsatellite markers RM 241, RM 515, RM 519 and RM 528 differentiate the Inpari Unsoed 79 Agritan from the IR 29 genotype. Microsatellite markers RM 129 and RM 292 distinguished the Nona Bokra from the IR 29 genotypes. The genetic relationship of eleven rice genotypes resulted in two clusters. The GC-MS metabolite compounds in Inpari Unsoed 79 Agritan are β -Alanine and trimethylsilyl ester β -Alanine, a derivative compound of β -Alanine. These findings suggested that microsatellite markers RM 129, RM 292, RM 241, RM 515, RM 519 and RM 528 were associated with salt-tolerant in the seedling stage.

Keywords: cluster analysis; metabolic modelling; multi-omics analysis; salt stress; simple sequence repeats

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INTRODUCTION

Indonesia is the largest rice-producing country after China and India. Rice harvested area in 2019 covered 10.68 million hectares which decreased by 6.15% compared to 2018. Indonesian rice production in 2019 was 54.60

million tons, decreased by 4.60 million tons compared to 2018 (Badan Pusat Statistik, 2020). One of several efforts to increase rice production is to utilize the existing saline lands in Indonesia. In Indonesia, saline land is around 13.2 million hectares lands (Suhartini and Harjosudarmo, 2017). Saline land which can be

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utilized for agriculture is tidal swampland, paddy fields along the coast and dry land (Putri et al., 2017; Tolib et al., 2017). Salinity is an obstacle in sustainable agriculture that can inhibit rice growth from vegetative to generative stages (Hariadi et al., 2015). Plant breeding programs are needed to develop salt-tolerant rice varieties in order to support sustainable agriculture (Hairmansis et al., 2017).

Salinity is a problem in agriculture which classified as abiotic stress. It can be caused by the accumulation of high dissolved salts that damage agricultural soils, especially sodium chloride salts (NaCl) in soil and water (Hussain et al., 2019). Salinity has caused osmotic stress and ionic toxicity in plants (Yildiz et al., 2020). Osmotic stress at high salinity has affected the ability of plants to absorb water and nutrients (Shrivastava and Kumar, 2015). Root cells would be dehydrated at high salinity conditions, disrupted maintenance of turgor cells, wilting and then followed by plant death. The use of saline-resistant rice varieties can be an alternative to increase crop production and take advantage of the potential of saline land in Indonesia (Jalil et al., 2016).

The Inpari Unsoed 79 Agritan is an irrigated lowland rice variety resulting from a cross between the Cisadane and Atomita 2 varieties. Indonesian Ministry of Agriculture released this variety in 2014 with a unique trait such as resistance to high salinity in the seedling phase with a stress of 12 dS m⁻¹. Inpari Unsoed 79 Agritan also has a reasonably high yield potential of 8.2 tons ha⁻¹. Inpari Unsoed 79 Agritan has fairly fluffier rice texture, content of amylose of ± 22.6%, plant height of ± 105 cm and harvesting age of 109 days (Indonesian Ministry of Agriculture, 2014).

The DNA markers are DNA sequences at specific regions of the inherited genome which either encode or do not necessarily have any traits but are unaffected by the environment. Plant breeders highly regard DNA markers for their ability to precisely map different interacting genes linked to desirable agronomic traits such as abiotic stress tolerance (Akos et al., 2019). Unlike biochemical and morphological markers, DNA markers provide significantly high genetic polymorphisms and at the same time, permit analysis at any developmental stage (Dar et al., 2019).

Several types of molecular markers are commonly used for DNA analysis, such as Simple Sequence Repeats (SSR), Restriction Fragment Length Polymorphism (RFLP), Random Amplified Polymorphism DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP), Sequence-Tagged Site (STS), Sequence Characterized Amplified Region (SCAR) and Single Nucleotide Polymorphism (SNP) (Lestari et al., 2016). Microsatellite markers have been widely used to determine plant genetic diversity (Bernardi et al., 2016).

Identification of microsatellite markers or SSR has been widely used due to their high polymorphism, co-dominant and presence throughout the genome sequence (Rani and Sharma, 2019). Microsatellite markers were used to identify the major gene loci that play a role in salinity tolerance; hence, they can be used to develop new cultivars (Moniruzzaman et al., 2013). A molecular study of salt-tolerant markers managed to identify salt tolerance in the nursery phase of 30 rice varieties (Kumari et al., 2019). The study was able to differentiate rice varieties using 24 microsatellite marker primers. A total of six primers (RM 140, RM 1287, RM 3412, RM 10745, RM 10764 and RM 10772) produced polymorphic bands and can be used as salt-tolerant markers. Ali et al. (2014) explained that microsatellite markers RM 8094, RM 336 and RM 8046 are the most competent descriptors to screen the salt-tolerant genotypes with a higher polymorphic information content coupled with higher marker index value, significantly distinguished the salt-tolerant genotypes.

In contrast to characterization based on genotypic characters (molecular markers), characterization of a variety against salinity stress could be done by metabolite analysis. Physiological responses of plants experiencing salinity stress can be observed or traced starting from the molecular, metabolite, cellular, tissue, organ and organ systems levels (Gupta and Huang, 2014). Metabolite profiling is a valuable tool for investigating plant responses to the environment at the molecular level (Nam et al., 2015). The comprehensive, quantitative and qualitative measurement of cellular metabolites prepared from cells or tissues exposed to stress conditions can provide a broad overview of the biological responses of plants to stresses (Nam et al., 2015). Metabolites associated with

salinity stress are glutamate, aspartate, proline, valine, lactate, alanine, malate (Das et al., 2015).

The previous metabolomics research has been used to determine the response and content of metabolites in four rice plants (Sujala, MTU 2079, Nona Bokra and Bhunath) treated with NaCl. Metabolic analysis using GC-MS showed the presence of serotonin and gentisic acid compounds at high levels in saline-tolerant rice varieties (Gupta and De, 2017). The accumulated serotonin compounds play a role in Reactive Oxygen Species (ROS) to delay the aging of rice leaves (Kang et al., 2009). Gentisic acid compounds were detected in plants with high salinity resistance (Gupta and De, 2017) and acted as signalling molecules for defense response (Bellés et al., 1999).

Molecular and metabolomic identifications completed the characterization of Inpari Unsoed 79 Agritan rice plants, in addition to morphology and agronomy characterizations from the previous report (Suprayogi et al., 2012). The microsatellite markers used in this study are expected to be associated with salinity tolerance traits and able to differentiate salinity tolerance rice varieties. Metabolomics identification is expected to identify metabolites in rice and their contribution to salinity stress.

This study aims to identify salt tolerance microsatellite markers, analyze the relationship between the Inpari Unsoed 79 Agritan variety and ten comparison rice genotypes based on microsatellite markers and determine the qualitative composition metabolites in Inpari Unsoed 79 Agritan associated with the plant response to salinity.

MATERIALS AND METHOD

The research was conducted from August 2020 to April 2021 in Plant Breeding and Biotechnology Laboratory Faculty of Agriculture, Research Laboratory of Jenderal Soedirman University and Laboratory in Indonesian Institute of Sciences, Bogor. Eleven rice genotypes were tested: Inpari Unsoed 79 Agritan, Cisadane, Atomita II, Pelopor, Dendang, Lambur, Siak Raya, Unsoed 1, Unsoed Parimas, Nona Bokra (positive control) and IR 29 (negative control). Ten microsatellite markers were used: RM 129, RM 156, RM 241, RM 292, RM 336, RM 493, RM 515, RM 519, RM 528 and RM 1287 (Table 1).

Identification of microsatellite markers

The genomic DNA was extracted from the leaves of eleven rice genotypes (21 to 28 day-old) using Cetyl Trimethyl Ammonium Bromide (CTAB) method as described by Doyle and Doyle (1990) with modifications. The modified part was the volume of solution, the length of the centrifuge and incubation with water bath to optimize the process of DNA extraction. The quality of extracted DNA was checked using agarose (Nzytech) gel 1% and the quantity of DNA samples was measured with a spectrophotometer (Implen). The genomic DNA obtained was stored at -20°C in Tris-EDTA buffer.

The PCR-based amplification was carried out in a thermocycler machine (Applied Biosystems). The components of the PCR reaction consisted of 12.5 µl of PCR Mix (Thermo Fisher Scientific), 1 µl of forward primer, 1 µl of reverse primer, 1 µl of template DNA (50 ng) and 9.5 µl of free water nuclease (Thermo Fisher Scientific). The pre-denaturation was carried out at 95°C for 5 min, followed by PCR cycle, which consisted of denaturation at 95°C for 1 min, annealing at 53.6°C to 67°C at 1 min, extension at 72°C for 1 min with the total cycles of 35. The final extension was done at 72°C for 5 min. The specific annealing temperature of each primer used is presented in Table 1.

Amplification products were separated using agarose gels 2% with 100 bp DNA ladder (Bioline) as a size marker to determine the molecular size of amplicons. Electrophoresis was done at 50 V for 60 minutes in 1x Tris Borat EDTA (TBE) buffer. The agarose gels were visualized with UV Transilluminator.

Identification of metabolites

The metabolites were extracted following the method described by Roessner and Beckles (2012). The rice leaves were dried with liquid nitrogen and ground. The sample was put into a 2 ml Eppendorf tube; 500 µl of methanol, 20 µl of internal standard (sorbitol/valine/ribitol) were added and vortexed for 10 seconds. The mixture was shaken for 15 minutes at 70°C in a thermoshaker, then centrifuged for 15 minutes. The supernatant was transferred to a new tube. The pellet was added 500 µl of distilled water, vortexed and centrifuged for 15 minutes. The supernatant was transferred and combined

with the previous supernatant. The combined supernatant was added to 400 µl chloroform, mixed thoroughly and centrifuged. This step was repeated twice. The aliquots were obtained by cold vacuum and derivatized using methoxyamine hydrochloride and N-methyl-trimethylsilyl-trifluoroacetamide (MSTFA). Metabolites were analyzed with the GC-MS instrument (Roessner and Beckles, 2012). The results of the GC-MS analysis were in tabular form based on peak, retention time, area and similarity index (SI) of metabolites.

Data analysis

Molecular data were analyzed by scoring DNA bands in agarose gel representing an allele from each genotype. Genotype Nona Bokra was used as a positive control and was

given a score of 1, while genotypes that were not the same length as Nona Bokra were given a score of 0; therefore, the band results scoring were in the form of binary data. The binary data from the scores of the DNA bands were processed using Principal Component Analysis (PCA) analysis in XLSTAT 2020 software. PCA compressed data from high to low dimensions, making it easier to analyze agricultural data (Osawaru et al., 2015). The results would show the clustering of markers according to their ability in distinguishing salt tolerance varieties. Binary data were also used for phylogenetic analysis using Sequential Agglomerative Hierarchical and Nested (SAHN) - Unweighted Pair Group Method with Arithmetic (UPGMA) in NTSYS software version 2.1 (Rohlf, 2000).

Table 1. List of SSR primers

Primer		Sequence*	Annealing temperature (°C)
RM 129	Forward	TCTCTCCGGAGCCAAGGCGAGG	58.0
	Reverse	CGAGCCACGACGCGATGTACCC	
RM 156	Forward	GCCGCACCCTCACTCCCTCCTC	67.0
	Reverse	TCTTGCCGGAGCGCTTGAGGTG	
RM 241	Forward	GAGCCAAATAAGATCGCTGA	55.0
	Reverse	TGCAAGCAGCAGATTTAGTG	
RM 292	Forward	ACTGCTGTTGCGAAACGC	53.6
	Reverse	TGCAGCAAATCAAGCTGGAA	
RM 336	Forward	CTTACAGAGAAACGGCATCG	55.6
	Reverse	GCTGGTTTGTTCAGGTTTCG	
RM 493	Forward	TAGCTCCAACAGGATCGACC	55.0
	Reverse	GTACGTAAACGCGGAAGGTG	
RM 515	Forward	TAGGACGACCAAAGGGTGAG	59.0
	Reverse	TGGCCTGCTCTCTCTCTC	
RM 519	Forward	AGAGAGCCCCTAAATTTCCG	55.0
	Reverse	AGGTACGCTCACCTGTGGAC	
RM 528	Forward	GGCATCCAATTTTACCCCTC	55.0
	Reverse	AAATGGAGCATGGAGGTCAC	
RM 1287	Forward	GTGAAGAAAGCATGGTAAATG	55.0
	Reverse	CTCAGCTTGCTTGTGGTTAG	

Note: *Reference: www.gramene.org

RESULTS AND DISCUSSION

Identification of microsatellite markers

DNA amplification was performed using ten pairs of microsatellite primers, namely RM 129, RM 156, RM 241, RM 292, RM 336, RM 493, RM 515, RM 519, RM 528 and RM 1287. Each primer produced amplicons of varying specific sizes in each genotype tested.

Visualizations of PCR results with ten primary pairs used are presented in Figure 1.

Visualization of PCR results using RM 493 primer (Figure 1f) showed one genotype that did not produce bands, namely the Lambur genotype. Visualization of PCR results using RM 519 primer (Figure 1h) also showed one genotype that did not produce bands, namely the Unsoed Parimas genotype. The DNA band

appears because the DNA has been successfully amplified, while the absence of the DNA band was because the DNA was not amplified. The success of DNA amplification in the PCR process is influenced by the suitability of the primer with the template DNA. The mismatch of primers and template DNA is caused by differences in base pair arrangement, preventing the amplification

process (Sinaga et al., 2017). In addition to primer mismatch, the factors that influence the success of DNA amplification in the PCR process are deoxyribonucleotide triphosphate (dNTP), oligonucleotide primers, template DNA, buffer solution composition, number of reaction cycles, enzymes used and technical and non-technical factors such as contamination (Feranisa, 2016).

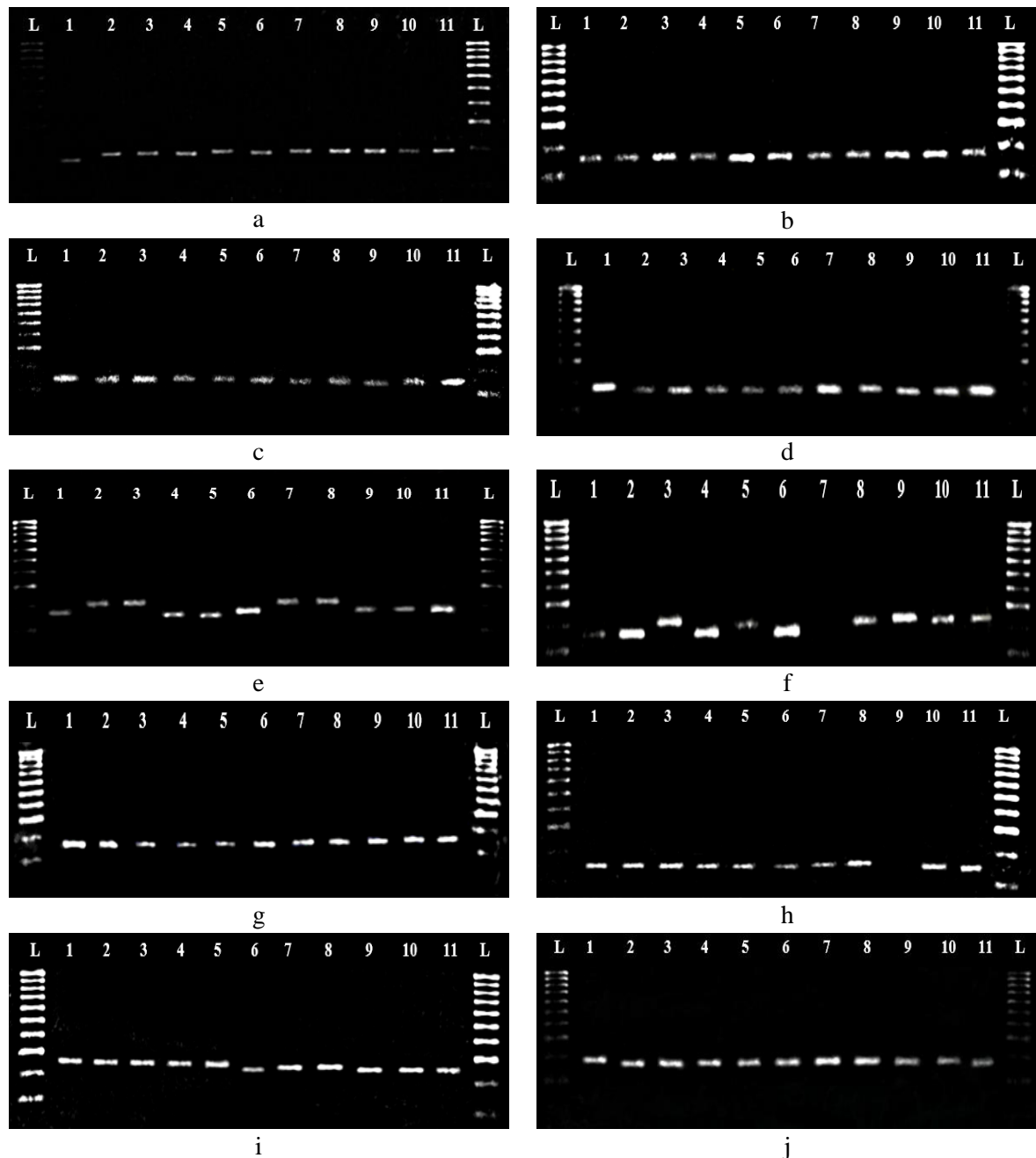


Figure 1. Visualization DNA result PCR (a) RM 129, (b) RM 156, (c) RM 241, (d) RM 292, (e) RM 336, (f) RM 493, (g) RM 515, (h) RM 519, (i) RM 528 and (j) RM 1287

Note: L = *Ladder*; 1 = Nona Bokra; 2 = Inpari Unsoed 79 Agritan; 3 = Cisadane; 4 = Atomita 2; 5 = Pelopor; 6 = Dendang; 7 = Lambur; 8 = Siak Raya; 9 = Unsoed Parimas; 10 = Unsoed 1; 11 = IR 29

The results of DNA amplification from ten pairs of primers showed a polymorphism pattern; it could be seen from the difference in the size of the DNA bands even though the distance was not too big. These results were in accordance with Sinaga et al. (2017), which stated that polymorphism patterns are formed from DNA bands of different sizes, so it is easy to observe the variations that exist from the observed sample. Differences in the primers caused the difference in band size attached to the plant

genome (Purnomo and Ferniah, 2018). Primers RM 336 and RM 493 showed bands with a high polymorphism pattern compared to other primers. This result supported the research of Rani and Sharma (2019), which stated that RM 493 and RM 336 have high levels of polymorphism, so they are more informative. They can be used to distinguish saline-tolerant genotypes. RM 336 can identify salt-tolerant gene in rice and can also be used as Marker Assisted Selection (MAS) in plant breeding (Moniruzzaman et al., 2013).

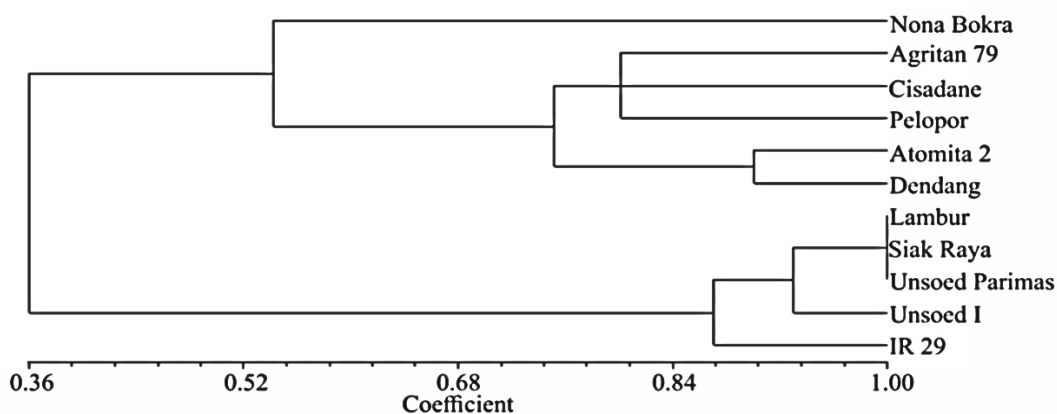


Figure 2. Phylogenetic tree of eleven genotypes rice based on scoring DNA bands

Phylogenetic analysis was performed to see the genetic relationship between eleven rice genotypes based on scoring with the NTSYS software. Phylogenetic results showed that the eleven rice genotypes were divided into two clusters at a similarity coefficient level of 0.52 (Figure 2). The first cluster consisted of genotype Nona Bokra, Inpari Unsoed 79 Agritan, Cisadane, Pelopor, Atomita 2 and Dendang. The second cluster consisted of Lambur, Siak Raya, Unsoed Parimas, Unsoed 1 and IR 29 genotypes.

The scoring values of the ten microsatellite primers classified saline tolerant and saline sensitive genotypes as indicating that the genotypes of Nona Bokra, Inpari Unsoed 79 Agritan, Cisadane, Pelopor, Atomita 2 and Dendang were in one cluster. The Nona Bokra genotype is a variety that has a high level of tolerance to saline stress, as indicated in Wang et al. (2012) study, which described Nona Bokra as control positive for saline tolerant genotype. Nona Bokra increased the concentration of K^+ ions and decreased the concentration of Na^+ ions during saline stress. The IR 29 genotype

in the second cluster can be said to be a saline sensitive cluster. The IR 29 genotype was used as a negative control because it is susceptible to saline on a scale of 6 out of 9 (scale 9 = almost dead or dead plants) (Khush and Virk, 2005). The IR 29 genotype in the 12 dS m^{-1} EC treatment showed a score of 9 which means it is susceptible to saline stress (Mohammadi-Nejad et al., 2008).

Identification of metabolites

The results of the GC-MS analysis of the Inpari Unsoed 79 Agritan genotype were presented in the form of a chromatogram (Figure 3). The identified metabolites were derivative compounds as the result of derivatization reactions. The metabolites of the Inpari Unsoed 79 Agritan genotype from the GC-MS are presented in Table 2. The metabolite compound (+) Chiro-Inositol TMS Ester has a high similarity index of 91 at a retention time of 22.476.

(+) Chiro-Inositol TMS Ester is a derivative compound of Chiro Inositol. Chiro Inositol compound is another name for Inositol (CID 892), which has the chemical formula $C_6H_{12}O_6$ and

a molecular weight of 180.6 g mol^{-1} . Inositol plays a role in maintaining osmotic balance and transporting Na^+ ions from roots to plant shoots (Cotsaftis et al., 2011). Himabindu et al.

(2016) stated that halophytic plants could survive in high salinity conditions by accumulating osmoprotectant compounds such as inositol, glycine betaine and proline.

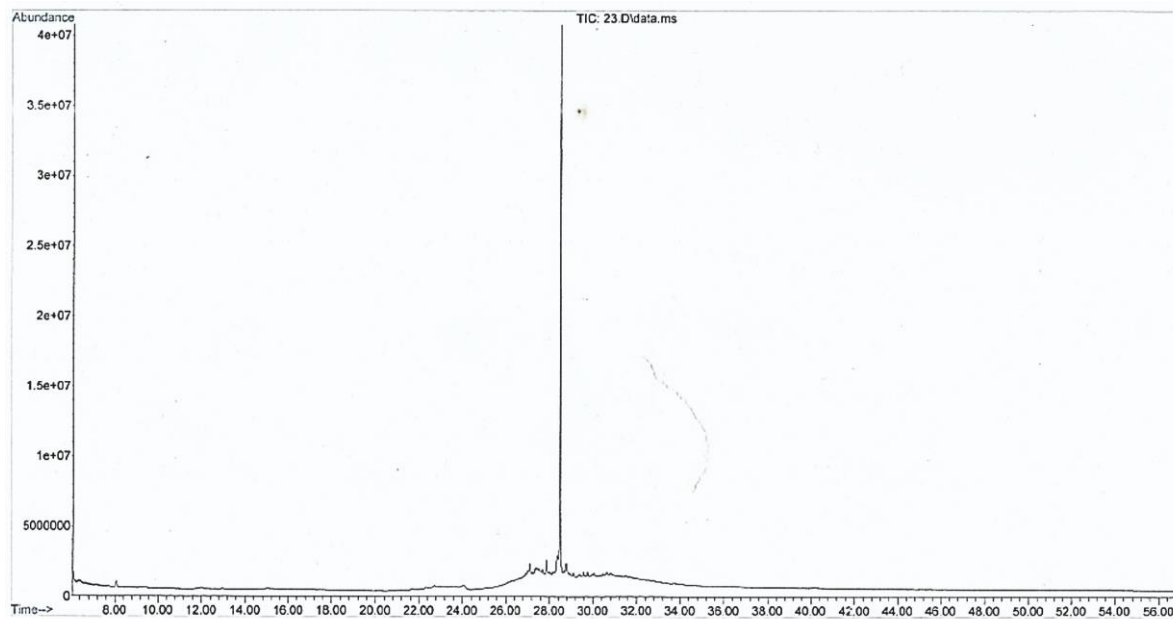


Figure 3. Chromatogram of Inpari Unsoed 79 Agritan

Table 2. Metabolite compounds of Inpari Unsoed 79 Agritan genotype

Retention time	Chemical compounds	Similarity index
27.393	β -Alanine, trimethylsilyl ester	55
27.862	β -Alanine, trimethylsilyl ester	38
28.476	(+) Chiro-Inositol, trimethylsilyl ester	91

β -alanine trimethylsilyl ester (CID 568356) is a derivative compound of Alanine. β -alanine trimethylsilyl ester has the chemical formula of $\text{C}_9\text{H}_{23}\text{NO}_2\text{Si}_2$ and a molecular weight of $233.45 \text{ g mol}^{-1}$. The metabolite of β -Alanine affects saline tolerance because it is positively correlated with Na^+ ions (Hill et al., 2013). β -Alanine is a primary metabolite belonging to the amino acid group, which serves as a precursor of β -alanine betaine. The synthesis process involves S-adenosyl-L-methionine (SAM)-dependent enzymatic N-methylation to produce β -alanine betaine (Parthasarathy et al., 2019). β -Alanine Betaine compounds play a role in osmoprotectants in saline tolerant plants (halophytes) (Jiménez-Arias et al., 2021). The analysis metabolomics did not use the Nona Bokra genotype as a positive control because it focused on the Inpari Unsoed 79 Agritan and mother plant, namely Atomita 2 and Cisadane.

CONCLUSIONS

This research suggested that microsatellite markers for identifying salt-tolerant genes were RM 129, RM 292, RM 241, RM 515, RM 519 and RM 528. Inpari Unsoed 79 Agritan falls into cluster with Nona Bokra, Cisadane, Pelopor, Atomita 2 and Dendang. Metabolite compound found in genotype Inpari Unsoed 79 Agritan is β -alanine trimethylsilyl ester that plays an important role in salinity tolerance. Further research is important to determine the association of microsatellite markers with salinity-associated genes and specific metabolites produced in saline conditions.

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REFERENCES

- Akos, I. S., Yusop, M. R., Ismail, M. R., Ramlee, S. I., Shamsudin, N. A. A., Ramli, A. B., Haliru, B. S., Ismai'la, M., & Chukwu, S. C. (2019). A review on gene pyramiding of agronomic, biotic and abiotic traits in rice variety development. *International Journal of Applied Biology*, 3(2), 65–96. Retrieved from <https://journal.unhas.ac.id/index.php/ijoab/article/view/7818>
- Ali, M. N., Yeasmin, L., Gantait, S., Goswami, R., & Chakraborty, S. (2014). Screening of rice landraces for salinity tolerance at seedling stage through morphological and molecular markers. *Physiology and Molecular Biology of Plants*, 20(4), 411–423. <https://doi.org/10.1007/s12298-014-0250-6>
- BPS - Statistics Indonesia. (2020). *2019 harvested area and production of paddy in Indonesia*. Retrieved from <https://www.bps.go.id/publication/2020/12/01/21930121d1e4d09459f7e195/luas-panen-dan-produksi-padi-di-indonesia-2019.html>
- Bellés, J. M., Garro, R., Fayos, J., Navarro, P., Primo, J., & Conejero, V. (1999). Gentisic acid as a pathogen-inducible signal, additional to salicylic acid for activation of plant defenses in tomato. *Molecular Plant-Microbe Interactions*, 12(3), 227–235. <https://doi.org/10.1094/MPMI.1999.12.3.227>
- Bernardi, J., Colli, L., Ughini, V., & Busconi, M. (2016). *Use of microsatellites to study agricultural biodiversity and food traceability*. In (Ed.), *Microsatellite Markers*. London, United Kingdom: IntechOpen. <https://doi.org/10.5772/64863>
- Cotsaftis, O., Plett, D., Johnson, A. A. T., Walia, H., Wilson, C., Ismail, A. M., Close, T. J., Tester, M., & Baumann, U. (2011). Root-specific transcript profiling of contrasting rice genotypes in response to salinity stress. *Molecular Plant*, 4(1), 25–41. <https://doi.org/10.1093/mp/ssq056>
- Dar, A. A., Mahajan, R., & Sharma, S. (2019). Molecular markers for characterization and conservation of plant genetic resources. *Indian Journal of Agricultural Sciences*, 89(11), 1755–1763. Retrieved from https://www.researchgate.net/publication/337339423_Molecular_markers_for_characterization_and_conservation_of_plant_genetic_resources
- Das, P., Nutan, K. K., Singla-Pareek, S. L., & Pareek, A. (2015). Understanding salinity responses and adopting 'omics-based' approaches to generate salinity tolerant cultivars of rice. *Frontiers in Plant Science*, 6, 712. <https://doi.org/10.3389/fpls.2015.00712>
- Doyle, J. J., & Doyle, J. L. (1990). Isolation of plant DNA from fresh tissue. *Focus*, 12(1), 13–15. Retrieved from <https://www.scienceopen.com/document?vid=46e6093b-769a-467f-be1a-fd0c2ecfa9c0>
- Feranisa, A. (2016). *Komparasi antara Polymerase Chain Reaction (PCR) dan loopmediated isothermal amplification (Lamp) dalam diagnosis molekuler. ODONTO: Dental Journal*, 3(2), 145–151. <https://doi.org/10.30659/odj.3.2.145-151>
- Gupta, B., & Huang, B. (2014). Mechanism of salinity tolerance in plants: Physiological, biochemical, and molecular characterization. *International Journal of Genomics*, 2014, 701596. <https://doi.org/10.1155/2014/701596>
- Gupta, P., & De, B. (2017). Metabolomics analysis of rice responses to salinity stress revealed elevation of serotonin, and gentisic acid levels in leaves of tolerant varieties. *Plant Signaling and Behavior*, 12(7), e1335845. <https://doi.org/10.1080/15592324.2017.1335845>
- Hairmansis, A., Nafisah, N., & Jamil, A. (2017). Towards developing salinity tolerant rice adaptable for coastal regions in Indonesia. *KnE Life Sciences*, 2(6), 72–79. <http://dx.doi.org/10.18502/cls.v2i6.1021>
- Hariadi, Y. C., Nurhayati, A. Y., Soeparjono, S., & Arif, I. (2015). Screening six varieties of rice (*Oryza sativa*) for salinity tolerance. *Procedia Environmental Sciences*, 28, 78–87. <https://doi.org/10.1016/j.proenv.2015.07.012>
- Hill, C. B., Jha, D., Bacic, A., Tester, M., & Roessner, U. (2013). Characterization of ion

- contents and metabolic responses to salt stress of different arabidopsis AtHKT1;1 genotypes and their parental strains. *Molecular Plant*, 6(2), 350–368. <https://doi.org/10.1093/mp/sss125>
- Himabindu, Y., Chakradhar, T., Reddy, M. C., Kanygin, A., Redding, K. E., & Chandrasekhar, T. (2016). Salt-tolerant genes from halophytes are potential key players of salt tolerance in glycophytes. *Environmental and Experimental Botany*, 124, 39–63. <https://doi.org/10.1016/j.envexpbot.2015.11.010>
- Hussain, S., Shaikat, M., Ashraf, M., Zhu, C., Jin, Q., & Zhang, J. (2019). *Salinity stress in arid and semi-arid climates: Effects and management in field crops*. In (Ed.), *Climate Change and Agriculture*. London, United Kingdom: IntechOpen. <https://doi.org/10.5772/intechopen.87982>
- Indonesian Ministry of Agriculture. (2014). *Varietas Inpari Unsoed 79 Agritan*. Ministry of Agriculture, Indonesia. Retrieved from <http://www.litbang.pertanian.go.id/varietas/1082/>
- Jalil, M., Sakdiah, H., Deviana, E., & Akbar, I. (2016). Pertumbuhan dan produksi beberapa varietas padi (*Oryza sativa* L.) pada berbagai tingkat salinitas. *Jurnal Agrotek Lestari*, 2(2), 63–74. Retrieved from <http://jurnal.utu.ac.id/jagrotek/article/view/597>
- Jiménez-Arias, D., García-Machado, F. J., Morales-Sierra, S., García-García, A. L., Herrera, A. J., Valdés, F., Luis, J. C., & Borges, A. A. (2021). A beginner's guide to osmoprotection by biostimulants. *Plants*, 10(2), 363. <https://doi.org/10.3390/plants10020363>
- Kang, K., Park, S., Kim, Y. S., Lee, S., & Back, K. (2009). Biosynthesis and biotechnological production of serotonin derivatives. *Applied Microbiology and Biotechnology*, 83(1), 27–34. <https://doi.org/10.1007/s00253-009-1956-1>
- Khush, G. S., & Virk., P. S. (2005). *IR varieties and their impact*. Los Baños, Philippines: International Rice Research Institute. Retrieved from http://books.irri.org/9712202062_content.pdf
- Kumari, R., Kumar, P., Sharma, V. K., & Kumar, H. (2019). Seedling stage salt stress response specific characterization of genetic polymorphism and validation of SSR markers in rice. *Physiology and Molecular Biology of Plants*, 25(2), 407–419. <https://doi.org/10.1007/s12298-018-0623-3>
- Lestari, P., Risliawati, A., & Koh, H. J. (2016). Identifikasi dan aplikasi marka berbasis PCR untuk identifikasi varietas padi dengan palatabilitas tinggi. *Jurnal AgroBiogen*, 8(2), 69–77. <https://doi.org/10.21082/jbio.v8n2.2012.p69-77>
- Mohammadi-Nejad, G., Arzani, A., Reza, A. M., Singh, R. K., & Gregorio, G. B. (2008). Assessment of rice genotypes for salt tolerance using microsatellite markers associated with the saltol QTL. *African Journal of Biotechnology*, 7(6), 730–736. Retrieved from <https://www.ajol.info/index.php/ajb/article/view/58504>
- Moniruzzaman, M., Alam, M. S., Rashid, J. A., Begum, S. N., & Islam, M. M. (2013). Marker-assisted backcrossing for identification of salt tolerant rice lines. *International Journal of Agricultural Research, Innovation and Technology*, 2(2), 1–8. <https://doi.org/10.3329/ijarit.v2i2.14008>
- Nam, M. H., Bang, E., Kwon, T. Y., Kim, Y., Kim, E. H., Cho, K., Park, W. J., Kim, B. G., & Yoon, I. S. (2015). Metabolite profiling of diverse rice germplasm and identification of conserved metabolic markers of rice roots in response to long-term mild salinity stress. *International Journal of Molecular Sciences*, 16(9), 21959–21974. <https://doi.org/10.3390/ijms160921959>
- Osawaru, M., Ogwu, M. C., & Imarhiagbe, O. (2015). Principal Component Analysis (PCA) as an ideal tool for analysing on-farm research data. *Biological and Environmental Sciences Journal for the Tropics*, 12(1), 514–522. Retrieved from https://www.researchgate.net/publication/286620136_PRINCIPAL_COMPONENT_ANALYSIS_PCA_AS_AN_IDEAL_TOOL_FOR_ANALYSING_ON-FARM_RESEARCH_DATA
- Parthasarathy, A., Savka, M. A., & Hudson, A. O. (2019). The synthesis and role of β -alanine

- in plants. *Frontiers in Plant Science*, 10, 921. <https://doi.org/10.3389/fpls.2019.00921>
- Purnomo, E., & Ferniah, R. S. (2018). Polimorfisme cabai rawit dan cabai gendot dengan penanda RAPD (Random Amplified Polymorphic DNA) menggunakan primer OPA-8. *Berkala Bioteknologi*, 1(1), 1–5. Retrieved from <https://ejournal2.undip.ac.id/index.php/bb/article/viewFile/2176/1379>
- Putri, P. H., Susanto, G. W. A., & Taufiq, A. (2017). Toleransi genotipe kedelai terhadap salinitas soybean genotype tolerance to salinity stress. *Penelitian Pertanian Tanaman Pangan*, 1(3), 233–242. Retrieved from https://www.researchgate.net/publication/323938007_Evaluasi_Ketahanan_Sumber_Daya_Genetik_Kedelai_terhadap_Cekaman_Salinitas/fulltext/5ab3b284a6fdcc1bc0c2f3bf/Evaluasi-Ketahanan-Sumber-Daya-Genetik-Kedelai-terhadap-Cekaman-Salinitas.pdf
- Rani, B., & Sharma, V. K. (2019). Microsatellite (SSR) markers assisted characterization of rice (*Oryza sativa* L.) genotypes in relation to salt tolerance. *Indian Journal of Biotechnology*, 18(2), 151–163. Retrieved from <http://nopr.niscair.res.in/handle/123456789/49661>
- Roessner, U., & Beckles, D. M. (2012). Metabolomics for salinity research. In: Shabala, S., Cuin, T. (eds) *Plant Salt Tolerance. Methods in Molecular Biology (Methods and Protocols)*, vol 913. Totowa, NJ: Humana Press. https://doi.org/10.1007/978-1-61779-986-0_13
- Rohlf, F. J. (2000). *NTSYS-pc: Numerical taxonomy and multivariate analysis system version 2.1*. Setauket, New York: Exeter Software. Retrieved from https://www.researchgate.net/publication/246982444_NTSYS-pc_-_Numerical_Taxonomy_and_Multivariate_Analysis_System
- Shrivastava, P., & Kumar, R. (2015). Soil salinity: A serious environmental issue and plant growth promoting bacteria as one of the tools for its alleviation. *Saudi Journal of Biological Sciences*, 22(2), 123–131. <https://doi.org/10.1016/j.sjbs.2014.12.001>
- Sinaga, A., Putri, L. A. P., & Bangun, M. K. (2017). Analisis pola pita andaliman (*Zanthoxylum acanthopodium* D.C) berdasarkan primer Opd 03, Opd 20, Opc 07, Opm 20, Opn 09. *Jurnal Agroekoteknologi*, 5(1), 55–64. Retrieved from <https://talenta.usu.ac.id/joa/article/view/2289>
- Suhartini, T., & Harjosudarmo, T. Z. P. (2017). Toleransi plasma nutfah padi lokal terhadap salinitas. *Buletin Plasma Nutfah*, 23(1), 51–58. <http://dx.doi.org/10.21082/blpn.v23n1.2017.p51-58>
- Suprayogi, Susanti, D., & Putranto, A. S. D. (2012). Keragaan agronomik galur-galur padi salin unsoed pada lahan non salin. *Prosiding Seminar Nasional Pengembangan Sumber Daya Pedesaan dan Kearifan Lokal Berkelanjutan II*, 232–238. Retrieved from <http://jurnal.lppm.unsoed.ac.id/ojs/index.php/Prosiding/article/viewFile/255/254>
- Tolib, R., Kusmiyati, F., & Lukiwati, D. R. (2017). Pengaruh sistem tanam dan pupuk organik terhadap karakter agronomi turi dan rumput benggala pada tanah salin. *Journal of Agro Complex*, 1(2), 57–64. <https://doi.org/10.14710/joac.1.2.57-64>
- Wang, Z., Chen, Z., Cheng, J., Lai, Y., Wang, J., Bao, Y., Huang, J., & Zhang, H. (2012). QTL analysis of Na⁺ and K⁺ concentrations in roots and shoots under different levels of NaCl stress in rice (*Oryza sativa* L.). *PLoS ONE*, 7(12), e51202. <https://doi.org/10.1371/journal.pone.0051202>
- Yildiz, M., Poyraz, İ., Çavdar, A., Özgen, Y., & Beyaz, R. (2020). Plant responses to salt stress. In (Ed.), *Plant Breeding - Current and Future Views*. London, United Kingdom: IntechOpen. <https://doi.org/10.5772/intechopen.93920>