

Molecular Profiles of Five Salinity-Resistant Soybean [*Glycine max* (L.) Merr.] CultivarsJuwarno^{1*}, Hartanto Nugroho², Triani Hardiyati¹, Alice Yuniaty¹¹Fakultas Biologi Universitas Jenderal Soedirman, Purwokerto, Indonesia²Fakultas Biologi Universitas Gadjah Mada, Yogyakarta, Indonesia

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ABSTRACT. In this study, the molecular profiles of five soybean cultivars (Burangrang, Gema, Grobogan, Panderman, and Sinabung) exhibiting salinity resistance were elucidated. The DNA profiles of the five cultivars were found to differ based on simple sequence repeat (SSR), insertion-deletion polymorphism (InDel)-QS080465, and sequence characterized amplified region (SCAR)-QS08064 markers. Three distinct SSR profiles—Satt-243, Satt-294, and Satt-308—and the SCAR-QS08064 marker were only observed in the Grobogan cultivar, whereas the InDel-QS080465 marker was only successfully amplified from the Burangrang, Gema, and Grobogan cultivars. The results indicate that the Grobogan cultivar is the most tolerant soybean cultivar, followed by the Burangrang and Gema cultivars. Results were consistent with those from genetic similarity analysis, which showed that Grobogan is genetically more similar to Burangrang and Gema compared to Sinabung and Panderman. In conclusion, the five soybean cultivars have different molecular profiles that are related to their resistance to salinity. SSR markers, InDel QS080465-152, and SCAR QS08064-383 are molecular markers specific to salinity-resistant cultivars.

Keywords: InDel, salinity, SCAR, soybean, SSR

INTRODUCTION

Soybean [*Glycine max* (L.) Merr.] is a highly nutritious food item that contains the protein, fat, minerals, and vitamins needed to fulfil human dietary requirements. Soybean is a good source of dietary protein as it does not contain cholesterol (Ghassemi-Golezani, & Minoo, 2011). However, according to the Central Bureau of Statistics (2014), Indonesia's soybean production is low. In 2012, 783,158 and 847,000 tons of soybean was produced. These production volumes are far below the required volume of 1.96 million tons. Therefore, soybean has to be imported currently to meet domestic demand.

The low volume of soybean production in Indonesia is due to a reduction in agricultural land area because of changes in land-use. This problem can be overcome by using coastal land. However, the soil in coastal areas has high porosity, low nutrient content (Gunawan, 2009), and is highly saline (Atabayeva et al., 2013). In particular, high salinity levels can be toxic and disrupt soybean growth (Farid & Sjahril, 2006).

The development of salinity-tolerant soybean cultivars is part of the current efforts to increase soybean production in Indonesia. Not all soybean cultivars can grow well and produce high yields when grown on coastal land with high salinity. Previous research has found that salinity-tolerant soybean is characterized by high fresh and dry weights,

chlorophyll a and b contents, and proline content (Saad-Allah, 2015; Khan, Hakeem, Siddiqi, & Ahmad, 2013; Sofalian, Miandoab, Asghari, Sedghi, & Eshghi, 2013; Kondetti, Narendra, Apte, & Ganpat, 2012; El-Rodeny & El-Okkiah, 2012; Amirjani, 2010). Other characteristics of salinity-tolerant soybean include long roots, many broad leaves, and a tall plant height (Saad-Allah, 2015; Kondetti et al., 2012; Farid & Sjahril, 2006). These characteristics have also been observed in other salinity-tolerant plants, such as *Lathyrus sativus* L., *Pisum sativum* L., and *Brassica napus* L. (Baniaghil, Arzanesh, Ghorbanli, & Shahbazi, 2013).

In this study, the molecular features of five soybean cultivars (Burangrang, Gema, Grobogan, Panderman, and Sinabung) were characterized using simple sequence repeat (SSR), insertion-deletion polymorphism (InDel)-QS080465, and sequence characterized amplified region (SCAR)-08064 markers. These cultivars are well-known for their salinity tolerance (Farid & Sjahril, 2006). Nevertheless, no study to date has determined the molecular characteristics of salinity-tolerant soybean cultivars.

The use of molecular markers can facilitate plant breeding programs (Darmawan, Hartati, Setiawan, Heliyanto, & Sudarsono, 2011). Various molecular markers are available for plant genetic studies, such as random amplified polymorphic DNA (RAPD) (Khan

et al., 2013), SSR, InDel, and SCAR markers (Guan et al., 2014). SSR markers, also known as microsatellites, are commonly used to elucidate plant genetic characteristics (Nuraida, 2012). This technique allows for a more accurate read of DNA fragments (accuracy of up to 1 bp). SSR markers can be easily amplified using polymerase chain reaction (PCR) because they are widely distributed in the genome (Chaerani, Utami, Hidayatun, Abdullah, & Suprihatno, 2014). Moreover, SSR markers can be reliably used in genetic analyses as they exhibit high levels of allelic variation and are highly reproducible.

Salinity-tolerant soybean cultivars have highly polymorphic DNA, based on RAPD marker analysis (Khan et al., 2013). SSR markers are also reported to be codominant markers that can be applied practically to support plant breeding programs (Darmawan et al., 2011). SSR marker analysis allows plant breeders to select resistance genes in plants based on differences in DNA sequences between susceptible and resistant individuals (Lukman, Afifuddin, & Hoerussalam, 2013). SSR markers have been shown to reliably differentiate salinity-tolerant from susceptible Tiefeng 8 soybean cultivars (Guan et al., 2014). Notably, the InDel-QS080645 and SCAR-QS08064 markers are useful for characterizing salinity-tolerant soybean cultivars (Guan et al., 2014). The selection efficiency of the QS080465 locus is 94.3%, whereas that of the SCAR-QS08064 locus is 80.0%. Salinity-tolerant soybean cultivars have a 383-bp SCAR-QS08064 marker. Therefore, the SSR, InDel-QS080645, and SCAR-QS08064 markers should reliably characterize the molecular features of the Burangrang, Gema, Grobogan, Panderman, and Sinabung soybean cultivars.

This study had two aims. The first objective was to elucidate the molecular characteristics of the Burangrang, Gema, Grobogan, Panderman, and Sinabung soybean cultivars. The second objective was to identify the markers related to salinity tolerance.

EXPERIMENTAL SECTION

Research Location and Period

This study was conducted at the experimental farm and laboratory of the Tropical Horticulture Study Centre at IPB University (Bogor, Indonesia) by members of the Genetic Laboratory of Biology at Jenderal Soedirman University (Purwokerto, Indonesia). The study took place from June to September 2017.

Materials

Five soybean cultivars, Burangrang, Gema, Grobogan, Panderman, and Sinabung, were studied. The soybean seeds were obtained from Balitkabi Malang, East Java. The experimental materials for DNA analysis were cetyltrimethylammonium bromide (CTAB) buffer, TE buffer, agarose, ethidium bromide, primer pairs (Table 1), and MyTag PCR mix. The

equipment included a thermocycler, water bath, micro pipette, and electrophoresis device.

DNA Isolation

Soybean genomic DNA was isolated using a modified CTAB method (Doyle & Doyle, 1990). DNA was isolated as follows. CTAB buffer was first incubated in a water bath at 65 °C for 30 min. Soybean leaf samples (~0.5 g each) were washed clean, sprayed with 70% alcohol, dried on tissue paper, and finely ground in 1.500 µL of CTAB solution using a mortar and pestle. For each sample, the crushed leaves were placed in a microcentrifuge tube, 15 µL of β-mercaptoethanol was added, and the mixture was incubated in a water bath at 65 °C for 1 h and slowly flipped every 10 min. The mixture was then centrifuged at 11,269 × g for 20 min. The supernatant was transferred to a new microcentrifuge tube, and 800 µL of chloroform and isoamyl alcohol were added in a 24:1 ratio. The mixture was homogenized by flipping it repeatedly and slowly, and subsequently centrifuged at 11,269 × g and 4 °C for 20 min. The supernatant was transferred to a new microcentrifuge tube, and 1/10 the supernatant volume of 7.5 M ammonium acetate was added with 2/3 the total volume (supernatant + ammonium acetate) of absolute ethanol. The mixture was homogenized by slowly flipping the tube and centrifuged at 9,469 × g for 5 min. The supernatant was removed, and the DNA was washed with 750 µL of 70% ethanol and centrifuged again at 9,469 × g for 5 min. The supernatant was removed again, and the DNA pellet was dried. After it had dried, the DNA pellet was dissolved in 100 µL of 1× TE buffer and stored at 4 °C.

Amplification of DNA fragments using PCR

SSR profiles of the five soybean cultivars were produced with PCR using nine SSR primer pairs (Table 1). The PCR reaction was performed in a total reaction volume of 10 µL, which consisted of 5 µL of 2 × GoTaq Green PCR mix, 50 ng of DNA template, 0.25 µL of forward and reverse primers (10 µM), and nuclease-free water in a 1× reaction. The amplification began with pre-denaturation at 94 °C for 5 min, followed by 35 cycles consisting of denaturation at 94 °C for 1 min, primer annealing at 54 °C for 1 min, and primer extension at 72 °C for 1 min 30 s. The final extension step was performed at 72 °C for 5 min. At the end of each cycle, the thermocycler was held at 10 °C for 1 min.

Specific InDel and SCAR markers of the five soybean cultivars were amplified using InDel-QS080465 and SCAR-QS08064 primer pairs. The DNA ladder marker used to detect InDel QS080465 and SCAR QS08064 was 100 bp. The PCR reaction took place in a total reaction volume of 12.5 µL consisting of 6.25 µL of MyTaq PCR mix, 50 ng of DNA template, 1 µL of forward and reverse primers (10 µM) (Table 1), and ion-free water

Table 1. SSR, InDel, and SCAR Primer Sequences for Molecular Analysis of the Five Soybean Cultivars (Lestari et al., 2016)

Primer Name	Sequence	
	Forward 5' - 3'	Reverse 5' - 3'
SSR primer		
Satt-009	CCAACTTGAAATTACTAGA	CTTACTAGCGTATTAACCCCTT
Satt-114	GGGTTATCCTCCCCAATA	ATATGGGATGATAAGGTGAAA
Satt-177	CGTTTCATTCCCATGCCAATA	CCCGCATCTTTTTTCAACCAC
Satt-147	CCATCCCTTCCTCCAAATAGAT	CTTCCACACCCTAGTTTAGTGACAA
Satt-242	GCGTTGATCAGGTCGATTTTATTGT	GCGAGTGCCAACTAACTACTTTTATGA
Satt-243	GCGCATTGCACATTAGGTTTTCTGTT	GCGTAAGATCACGCCATTATTTAAGA
Satt-294	GCGGGTCAAATGCAAATTATTTT	GCGCTCAGTGTGAAAGTTGTTTCTAT
Satt-308	GCGTTAAGGTTGGCAGGGTGGGAAGTG	GCGCAGCTTTATACAAAAATCAACAA
Satt-414	GCGTATTCCTAGTCACATGCTATTTCA	GCGTCATAATAATGCTAGAACATAAA
SCAR-QS08064	ACGTAAGTGGTTGAAGGCGTT	GGGCAAGGGATATGAAAA
InDel- QS080465	ACTCAAGAGCAACTCACAAC	GCTAACGACTACCTCAATGC

in a 1 × reaction. The amplification process was as follows. The pre-denaturation stage was conducted at 95 °C for 5 min, followed by 35 cycles consisting of denaturation at 95 °C for 15 s, primer annealing at 55 °C for 15 s, and primer extension at 72 °C for 10 s. The final extension step was performed at 72 °C for 7 min.

The PCR products of the SSR markers were electrophoresed in 1.5% agarose gel, whereas those of the InDel-QS080465 and SCAR-QS08064 markers were electrophoresed in 2% agarose gel. PCR products were visualized using a UV transilluminator, and the gels were photographed using a digital camera.

Data Analysis

Salinity tolerance in each cultivar was characterized descriptively by comparing the presence and size of each marker among cultivars (Table 2). Based on band patterns, SSR, InDel-QS080465, and SCAR-08064 markers were scored in a binary fashion as 1 and 0, denoting presence and absence, respectively (Table 2). The binary data were then used to build a phenogram to classify the five cultivars and analyse the genetic distance among the cultivars. A phylogram representing the five soybean cultivars was constructed based on their molecular characteristics using Phylogenetic Analysis Using Parsimony (PAUP) 4.0 software (Swofford, 2001).

RESULTS AND DISCUSSION

The SSR primers used were able to amplify SSR markers from all soybean cultivars. The SSR marker alleles ranged from 100 to 750 bp in size. Two InDel marker alleles with lengths of 148 and 152 bp were amplified only from three cultivars (Burangrang,

Gema, and Grobogan). An ~380-bp SCAR marker allele was only obtained from the Grobogan cultivar. All alleles and their presence and absence in each cultivar are listed in Table 2. Of interest, only Grobogan had the specific SSR marker Satt-294_150 (Table 2). Grobogan is a salinity-tolerant cultivar (Farid & Sjahril, 2006; Juwarno, 2019). Therefore, Satt-294_150 may be a specific molecular marker for salinity resistance in soybean cultivars. Satt-294_150 may be cosegregated with the salt-tolerance gene in Grobogan. Guan et al. (2014) reported that an SSR marker that is positively associated with salinity tolerance in a soybean Tiefeng 8 variety is cosegregated with the salt-tolerance gene. However, the SSR alleles found in this study differ from those in Guan et al. (2014). In the present study, SSR Satt serial markers were used, whereas Guan et al. (2014) used SSR serial alleles in the Barcsoy3_3 locus. Nevertheless, there is some evidence to indicate that SSR markers are related to salinity tolerance in soybean cultivars.

The InDel-QS080465 markers were only amplified from the Burangrang, Gema, and Grobogan cultivars (Table 2), with sizes of approximately 148 and 152 bp. The sizes fall into the expected range, as using the same primer, Guan et al. (2014) also obtained a 148-bp InDel-QS080465 allele from 23 soybean accessions and a 152-bp InDel-QS080465 allele from soybean Tiefeng 8 varieties. InDel QS080465 was only present in the Burangrang, Gema, and Grobogan cultivars. Therefore, the primer used was only able to amplify the marker from those three cultivars. Complementary sequences may be absent in the Panderman and Sinabung cultivars. Hence, the primer could not be used to amplify InDel markers from the latter two cultivars.

Table 2. SSR, SCAR, and InDel marker alleles and their presence (1) and absence (0) in five soybean cultivars

No	Allele	Cultivar					Previous Publications Guan et al. (2018)		
		Pan	Bur	Gro	Sin	Gem	SSR	SCAR	InDel
1	Satt-114_750	0	1	0	0	0			
2	Satt-114_150	0	0	0	0	1			
3	Satt-114_100	1	1	1	1	0			
4	Satt-414_260	1	0	0	1	0			
5	Satt-414_250	0	1	1	0	1			
6	Satt-242_150	1	1	1	1	0			
7	Satt-242_100	0	0	0	0	1			
8	Satt-177_250	0	1	0	0	0			
9	Satt-177_150	1	1	1	1	0	Positiv ely related to salt- toleran ce charac ter		
10	Satt-243_260	1	0	0	1	0			
11	Satt-243_250	0	1	1	0	0			
12	Satt-243_150	0	1	0	0	0			
13	Satt-147_160	0	0	1	1	0		263/383*	148**
14	Satt-147_150	1	1	0	0	0			152***
15	Satt-308_750	1	1	0	1	1			
16	Satt-308_500	1	0	0	1	0			
17	Satt-308_250	1	0	0	1	0			
18	Satt-294_270	0	0	0	1	0			
19	Satt-294_260	1	1	1	0	0			
20	Satt-294_150	0	0	1	0	0			
21	Satt-009_250	1	0	0	1	0			
22	Satt-009_150	0	1	1	0	1			
23	SCAR_380	0	0	1	0	0			
24	InDel_148	0	1	0	0	1			
25	InDel_152	0	0	1	0	0			

Note: * 383 positively related to salt-tolerance

** 148 present in some salt-tolerance cultivar

*** 152 positively related to salt-tolerance

The suffix in the allele name indicates allele size in bp. Based on Guan et al. (2018), all SSR markers found, 383-bp SCAR markers, and InDel_152 are positively associated with salt tolerance, whereas InDel_148 can be found in some salt-tolerant cultivars. Pan, Panderman; Bur, Burangrang; Gro, Grobogan; Sin, Sinabung; Gem, Gema.

InDel QS080465-148 was found in the Burangrang and Gema cultivars, whereas InDel QS080465_152 was amplified from the Grobogan cultivar. Grobogan, Burangrang, and Gema are salinity-tolerant soybean cultivars (Farid & Sjahlil, 2006). Therefore, InDel QS080465 can be a molecular marker for salinity-tolerant cultivars. InDel QS080465_152 was only present in the Grobogan cultivar, whereas InDel QS080465_148 was present in the Burangrang and Gema cultivars. According to the results, we assume that the Grobogan cultivar is more salt tolerant than the Burangrang and Gema cultivars. Similarly, Guan et al. (2014) reported that InDel QS080465-148 was present in only 2 of 23

soybean varieties, whereas InDel QS080465-152 was present in 22 salinity-resistant accessions.

Even though Burangrang and Gema have the InDel-QS080465-148 marker, it is likely that both exhibit salinity tolerance for two reasons. First, the InDel-QS080465-148 marker has only been found in salinity-tolerant accessions (Guan et al., 2014). Second, Burangrang and Gema exhibit morphological traits that are specific to salinity-resistant soybean, such as those seen in the Grobogan cultivar (Kondetti et al., 2012; Saad-Allah, 2015; Juwarno, 2019).

SCAR-QS08064 markers with an allele size of 380 bp were only successfully amplified from the

salinity-tolerant Grobogan cultivar (**Table 2**; Farid & Sjahril, 2006). A similarly sized marker (383 bp) was obtained from a salinity-tolerant accession (Guan et al., 2014). Therefore, SCAR QS08064-380 is likely a molecular indicator of salinity tolerance. The SCAR-QS08064-383 allele is 80% related to salinity resistance (Guan et al., 2014). Grobogan also exhibits morphological traits indicative of salinity tolerance (Juwarno, 2019; Kondetti et al., 2012; Saad-Allah, 2015).

Based on the three types of markers used, Grobogan is most tolerant to salinity, followed by Burangrang and Gema, whereas Sinabung and Panderman are sensitive to salinity. Grobogan has a unique molecular profile (Satt-294_150, InDel QS080465-152, and SCAR QS08064-380) compared to the other cultivars and morphological (Farid & Sjahril, 2006; Kondetti et al., 2012; Saad-Allah, 2015) and molecular (Guan et al., 2014) traits characteristic of salinity tolerance.

Genetic relationships among the five soybean cultivars were examined through molecular similarity analysis (**Figure 1**). A phenogram illustrating the genetic relationships among the five soybean cultivars was constructed based on the binary data summarized in **Table 2** (**Figure 1**). The tree obtained had a retention index (RI) of 0.7500 and a consistency index (CI) of 0.8846. The RI and CI values are considered high because they are close to 1, which indicates low homoplasy and a reliable tree (Lipscomb, 1998; Ucu, 2016).

A homoplastic case is one in which two or more different lineages have highly similar characteristics

as a result of convergence (Klingenberg & Gidaszewski, 2010), i.e., these similar characteristics evolved independently (Grandjean et al., 2017). According to the tree topology (**Figure 1**), Grobogan is most closely related to Burangrang, and both are grouped with Gema, indicating that Grobogan and Burangrang are genetically similar and can be considered sister cultivars. As Grobogan is salinity tolerant, Burangrang should be the next-most salinity tolerant cultivar, followed by Gema. Panderman is the basal cultivar and was assumed have the most primitive characteristics. Accordingly, this cultivar is sensitive to salinity. Based on the phenogram, the soybean cultivars can be divided into two different groups. Group I consists of Grobogan, Burangrang, and Gema, which are salinity tolerant, whereas Group II consists of Sinabung and Panderman, which are sensitive to salinity. The classification shown in **Figure 1** is consistent with that based on morphological, anatomical, physiological, and production characteristics analysed using PAUP (Juwarno, 2019).

The results based on molecular data obtained in this study support those based on morphological data in previous studies (Farid & Sjahril, 2006; Kondetti et al., 2012; Saad-Allah, 2015); namely, Grobogan, Burangrang, and Gema are salinity-tolerant cultivars. We found that Grobogan is the most tolerant cultivar, as indicated by the presence of SCAR QS08064_380 and InDel QS080465_150. On the other hand, Sinabung and Panderman are more sensitive cultivars as they do not have those specific SCAR and InDel markers.

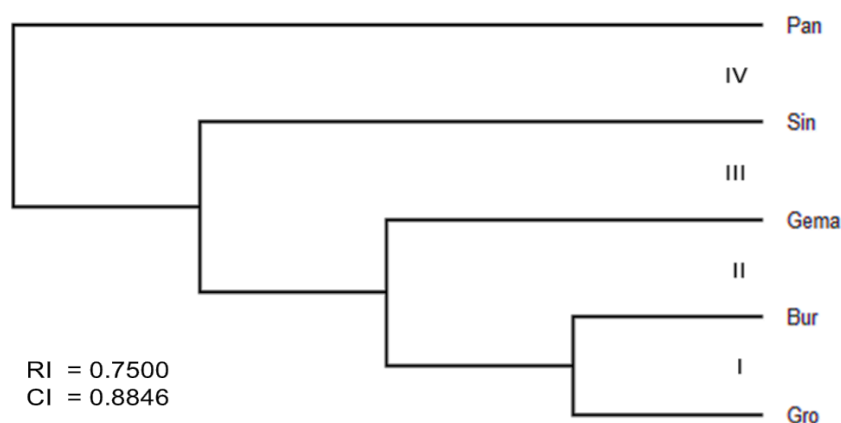


Figure 1. Genetic relationships of five soybean cultivars based on SSR, InDel, and SCAR markers.

Note: RI = Retention Index, CI = Consistency Index, Gro = Grobogan, Bur = Burangrang, Gema = Gema, Sin = Sinabung, Pan = Panderman

CONCLUSIONS

The five soybean cultivars investigated here have different genetic profiles. The Satt-294-150, InDel-QS080465_150, and SCAR-QS08064_380 markers are positively associated with salinity-tolerance characteristics. Grobogan is more tolerant to salinity than Burangrang and Gema, whereas Sinabung and

Panderman are more sensitive. SSR, InDel, and SCAR markers are reliable molecular indicators of salinity tolerance in soybean cultivars.

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