

Response of Indonesian Eggplants due to Nematode Attack and Genetic Diversity Revealed by SSR Marker

Aida Ainurrachmah^{1,2,*}, Taryono^{1,2} and Siwi Indarti³

¹Departement of Agronomy, Universitas Gadjah Mada, Indonesia

² Agrotechnology Innovation Center, Universitas Gadjah Mada, Indonesia

³ Department of Plant Protection, Universitas Gadjah Mada, Indonesia

Corresponding author. Email: <u>ainurrachmahaida@gmail.com</u>

ABSTRACT

Eggplant (*Solanum* sp.) is one of the most important solanaceous vegetable crop plants. Root-knot nematode (*Meloidogyne incognita*-RN) is one of the biotic factors and becoming a serious problem, affecting the root system and physiological activities, resulting in reduced plant growth and yield of eggplant. This study aims to evaluate morphological response of 24 eggplant accessions from Gene Bank of PIAT UGM collection to nematode infection at the nursery phase and to determine the diversity between solanaceae accessions from different regions. Collection and characterization of genetic resources and local cultivars are required for the improvement of new varieties. The response of morphological plants is done by looking at the morphology condition of plant and susceptible index value due to nematode infection. SL- TE 42 and SL-TE 28 had the highest average susceptible index, namely 2.15 and 2.45. Accessions SL-TE 590 (*S. aculeatissimum*), SL-TE 74 (*S. aculeatissimum*), and SL-TE 589 (*S. torvum*) had the lowest susceptible index values, namely 0.37; 0.36, and 0.79. PCoA analysis differentiated accessions in to four main clusters with wild type cultivars (SL-TE 74, SL-TE 589, and SL-TE590) grouped in cluster I and local cultivars in cluster II, III, and IV. Accessions SL- TE 74, SL-TE 589, and SL-TE590 showed the highest percentage of polymorphic loci, respectively, 17.57%, 20.95%, and 20.95%. The results revealed that there were 68% of the total genetic diversity within the population, and 32% among population.

Keywords: Eggplant, Solanum, Meloidogyne incognita, SSR marker.

1. INTRODUCTION

Eggplant is a vegetable that is used for its fruit. Eggplant belongs to the Solanaceae family, consisting of approximately 3,000 species spread across 90 countries [1]. Eggplant is one of the vegetables that contain high antioxidants, about 68.88 (µmol TE/g FW) because of its high phenolic content [2]. Eggplant also contains important nutrients because of its composition in phytochemicals, especially minerals such as P, K, Ca, and Mg [3]. Pointing from the economic and nutritional importance of eggplant, eggplant breeding is still very limited. The use of exotic germplasm in breeding programs can be very relevant for crop improvement.

One of the biotic stresses of eggplant is root knot nematode caused by nematodes (*Meloydogine spp.*). *Meloydogine spp.* is a nematode that causes disease in vegetables and causes a decrease in plant productivity. Nematodes caused significant yield losses around 42-54% in vegetables and 30-60% in eggplant. In India, nematodes caused 16.67% of eggplant yield losses [4]. Nematodes are the main disease affecting eggplant fields in Kurdistan and Iraq. Yield losses due to nematodes are estimated at 50% or worth 100 billion dollar [5].

Meloydogine spp. is a biotropic parasite that has produced cell wall-destroying enzymes to successfully infect plants. Root-knot nematode infection begins with the secretion of enzymes into the host plant. In the root meristem zone, stage 2 juvenile nematodes infect and actively attack the host. Nematodes migrate intercellularly to root shoots and then to vascular cylinders. After finding the feeding site, gall formation will occur. Gall formation will inhibit the absorption and transport of water and essential substances to plants. This affects the efficiency of the root system which affects the physiological activities in plant growth. If infection occurs at an early stage of plant growth, rootknot nematodes can directly cause host death [6].

Plant resistance to nematodes can be a mechanism of resistance before or after infection [7]. Resistance before infection occurs on the root surface or around the root area (rhizosphere). Root exudates produced by plants can attract or repel nematodes. The mechanism of resistance after infection can affect physiological processes in the roots, such as preventing the nematode feeding process, preventing the formation of feed sites, inhibiting nematode development, and inhibiting nematode reproduction [8].

Resistant cultivars are considered as the most effective, environmentally safe, and ecofriendly approach [9]. To assemble nematode-resistant varieties of eggplant, plants with superior genes are needed to be used as donor parents. Knowledge of genetic diversity in germplasm collections is helpful in making decisions about breeding strategies for use in current and future breeding programs [10]. The study of genetic diversity also makes it possible to select genetically different parents to obtain the desired recombinant in eggplant segregation generation. This study was conducted with the aim of knowing the genetic diversity of eggplant germplasm and morphological responses of eggplant to nematode attack. Genetic resources can be evaluated morphologically in the seedling stage at the screenhouse and molecularly characterized by using DNA marker techniques.

2. MATERIAL AND METHODS

2.1. Plant material

Twenty-four eggplant accessions were studied for genetic variability by molecular analysis and root- knot nematode resistance at the seedling stage through field. Among these accessions, 20 belonged to *Solanum melongena*, two land races belonged to *Solanum aculeatissimum*, and one each accession belonged to *Solanum ferox* and *Solanum torvum*. The accessions were collected from different regions of Indonesia and procured from Agrotechnology Innovation Centre, Universitas Gadjah Mada (AIC- UGM), Indonesia (Table 1). The field evaluation experiment was conducted at the greenhouse of AIC- UGM, and DNA extraction and molecular studies were carried out in the Laboratory at the Department of Agronomy, Faculty of Agriculture, Universitas Gadjah Mada, Indonesia.

No	Eggplant accessions	Species	Province of origin in Indonesia
1	SL-TE 45	S. melongena	Yogyakarta
2	SL-TE 579	S. melongena	Yogyakarta
3	SL-TE 187	S. ferox	West Borneo
4	SL-TE 28	S. melongena	East Java
5	SL-TE 74	S. aculeatissimum	Yogyakarta
6	SL-TE 580	S. melongena	Yogyakarta
7	SL-TE 581	S. melongena	Yogyakarta
8	SL-TE 582	S. melongena	Yogyakarta
9	SL-TE 25	S. melongena	Yogyakarta
10	SL-TE 589	S. torvum	Yogyakarta
11	SL-TE 590	S. aculeatissimum	Yogyakarta
12	SL-TE 13	S. melongena	Yogyakarta
13	SL-TE 583	S. melongena	Yogyakarta

Table 1. Eggplant accessions used in the study.

2.2. Eggplant accession assay for resistance to nematodes

Evaluation was carried out in a completely randomized design (CRD) with three replications. Nematode isolates were established as a single mass for pure cultures (*M. incognita*) and inoculated into eggplant seedlings at the two-true-leaf-stage or 3 weeks after germination. The eggplant seedlings were transplanted into 120 cm \times 55 cm \times 10 cm rectangular plant pot trays containing sterilized sand. One mL of nematode suspension containing 100 stage 2 juvenile was inoculated into the roots of each plant by three injection points.

The level of resistance to stress is calculated based on the value of the stress sensitivity index. The sensitivity index was calculated using the formula of Fischer and Maurer, 1978 [11], as follows:

Stress Sensitivity Index (S) = (1 - (Y/Yp))

$$(1 - (X/Xp))$$

Susceptible index = Yp - Y

Note:

Y = average values for one examined genotype in stress condition

Yp = average values for one examined genotype in nonstress condition

X = average values for all examined genotypes in stress condition

Xp = average values for all examined genotypes in nonstress condition



2.3. SSR marker analysis

DNA was extracted from 100 mg of fresh leaf tissue by using a Geneaid kit by Geneaid Biotech Ltd, Taiwan. Fifteen types of SSR primers were used to determine genetic variability of eggplant (Table 2). The PCR conditions were as follows: 5 min at 95 °C as the initial denaturation step, 1 min at 94 °C, 1 min at 47.3 °C, 1 min 30 s at 72 °C (33 cycles), 7 min at 72 °C, and a final cycle at 12 °C for an indefinite period. The PCR amplification for SSR was carried out in a 10 µl volume comprising 5 µl of master mix (2.25 mM MgCl, 0.2 mM dNTPs, 0.5 U Taq DNA polymerase, and 109 PCR buffer), 2 µl of DNA (25 ng/µl), 0.5 µl of primers, and 3.5 µl of nuclease-free water. The results of SSR amplification were electrophoresed in 2% agarose gel in $1 \times$ TAE and visualized by staining with fluoresafe DNA. The DNA ladder size marker was used to estimate the size of the amplification results.

3. RESULT AND DISCUSSION

Based on plant growth parameters data, plants that were not inoculated and inoculated by nematode were decrease in growth in almost all accessions. In the fresh weight of plant, SL-TE 28 and SL-TE 42 has the largest decrease, 86.39% and 82.76%, respectively. On the other hand, SL-TE 590, SL-TE 580, SL-TE 588 has the lowest declines, 4.13%, 9.65%, and 10.47, respectively. The fresh weight plant of SL-TE 589 has decrease about 23.26%, while SL-TE 74 decrease about 33.97% (Table 3 and 4).

Based on the root fresh weight data, SL-TE 46 and SL-TE 47 have the largest decrease, which were 29.38% and 29.05%, respectively. On the other hand, SL-TE 590 and SL-TE 581 are the lowest declines, which were 5.34% and 4.94%, respectively. Accession SL-TE 589 decrease in fresh weight of roots by 26.00%, while SL-TE 74 decreased by 20.15%.

Marker	Primer ID	Primer Sequence	Reference
CCD	EN (114	F: AGCCTAAACTTGGTT GGT TTTTGC	11 2019 [12]
SSK	EM114	R: GAAGCTTT AAGAGCC TTCTATGCAG	Hoque, 2018 [12]
SSR EM120		F: GGA T C A A C T G A A G A G C T G G T G G T T	II
		R: C A G A G C T T C A A T G T T C C A T T T C A CA	Hoque, 2018 [12]
CCD	EDGCD 92	F: AC A T G C C A C T C A T G T T G G T G	Heave 2018 [12]
35K	EFSSK 02	R: CTTC A G C C A T G G A C C A C A T T	Hoque, 2018 [12]
CCD	CCD01	F: GTGACTACGGTT TCA CT GGT	II
55K	SIISSKUI	R: GATGACGA CGACGATAATAGA	Hoque, 2018 [12]
CCD		F: AAT G A G T C A G A A A C C A C G C C	II
55K	SIIISSK04	R: CGTT T A A C C T T T G G C T C G G A A	Hoque, 2018 [12]
CCD	EM125	F: ATCCTGTTGCTGCTCATTTTCCTC	Mamma 2012 [12]
35K	ENTISS	R: AGGAGGATCCAAGAGGTTTGTTGA	verilla, 2012 [15]
SSR	EM140	F: CCAAAACAATTTCCAGTGACTGTGC	Mamma 2012 [12]
		R: GACCAGAATGCCCCTCAAATTAAA	verilla, 2012 [15]
SSR EM	EM145	F: CAGTGCTACATAAATTGAGACAAGAGG	Varma 2012 [12]
	Elvi145	R: GGAGGTACAACGGATTTTCATATGGT	verilla, 2012 [15]
CCD	EM155	F: CAAAAGATAAAAAGCTGCCGGATG	Mamma 2012 [12]
SSK		R: CATGCGTGAGTTTTGGAGAGAGAG	verilla, 2012 [15]
CCD	Cam	F: GCGTACCAATTCTAACCACAAG	Cromozio et el 2010 [14]
SSK	CSIII4	R: GTAATCCGCTTCCCATTTCTC	Gramazio et al., 2019 [14]
CCD	Csm27	F: TGTTTGGAGGTGAGGGAAAG	Cromagia et al. 2010 [14]
35K		R: TCCAACTCACCGGAAAAATC	Gramazio et al., 2019 [14]
CCD	Ema11f04	F: ACCCCCAAATCAAATCATTTACCC	Cromagia et al. 2010 [14]
SSK	Emel1104	R: GGCATGGTTAGGGTTTTTAGCGTT	Gramazio et al., 2019 [14]
CCD	EM 117	F: GAT CAT CAC TGG TTT GGG CTA CAA	A damiii at al. 2012 [15]
55K	ENI 117	R: AGG GGA GAG GAA ACT TGA TTG GAC	Adeniji et al., 2012 [15]
CCD	EM 122	F: GCG GAT CAC CTG CAG TTA CAT TAC	Adamiii at al. 2012 [15]
SSR	EM 155	R: TCC TTT GAC CTA TAG TGG CAC GTA GT	Adeniji et al., 2012 [15]

Table 2. SSR Marker.

	Root fr	esh weight	Plant fresh weight Plant height		height	Volume of Root		
Accession	control	inoculated	control	inoculated	control	inoculated	control	inoculated
SL-TE 590	0.79±0.01b	0.74±0.18a-b	1.72±0.036ef	1.65±0.1b	15.4±0.61a-c	14.12±2.46a-c	0.64±0.02b	0.55±0.04b
SL-TE 587	0.29±0.01g-h	0.27±0.23b	1.53±0.29ef	0.58±0.15b	6.92±1.07j-k	6.12±0.08f-i	0.25±0.02h-	0.32±0.24b
SI -TE 584	0 32+0 04f-h	0.21+0.04b	2 33+0 62c-f	1 61+0 35b	12 1+0 52c-g	$10.01+0.44c-\sigma$	1 0 3+0 02f-i	0 26+0 06b
GL TE 504	0.32±0.041 1	0.21 ± 0.040	2.55±0.020 1	0.41.01	12:1±0:520 g	10.01±0.440 g	0.9±0.021 1	0.25±0.000
SL-TE 581	0.27 ± 0.02 g-h	0.26±0.34b	1.61 ± 0.29 ef	0.41 ± 0.6	4.5±0.25 k	3.36±0.14 h-1	0.2 ± 0.1	0.27 ± 0.3 b
SL-1E 582	0.63±0.02 b- c	0.59±0.15 a-b	2.27±0.54 c- f	0.74±0.16 b	10.72±1.6 d- i	8.79±0.3 d-g	0.59±0.01b- c	0.59±0.17 h
SL-TE 586	0.355+0.11d-	0.3+0.32 b	- 3.52+0.58 b-	0.87 ± 0.45	9.28+1.63 f-i	6.2+0.0 f-i	- 0.29+0.1 σ-	0.34 ± 0.32
	h		d	b			i	b
SL-TE 580	0.34±0.14 e-	0.3±0.08 b	2.03±0.34 d-	1.83±0.28	7.66±1.52 i-	9.19±0.61 c-	0.31±0.12	0.25±0.06
	h		f	b	k	g	e-i	b
SL-TE 579	0.22±0.06 h	0.18±0.11 b	1.9±0.54 d-f	0.72±0.1 b	8.18±1.64 h- k	5.51±0.44 g- i	0.18±0.06 i	0.49±0.53 b
SL-TE 583	0.59±0.015b-	0.53±0.16	2.15±0.51 c-	1.49±0.25	14.13±0.23	12.04±0.37	0.54±0.02b-	0.51±0.05
	e	a-b	f	b	a-d	b-e	е	b
SL-TE 589	0.4±0.04 c-h	0.3±0.11 b	0.65±0.2 f	0.5±0.01 b	8±1.8 h-k	5.18±0.07 g- i	0.47±0.05b- h	0.4±0.1 b
SL-TE 13	0.55±0.06 b-	0.47±0.02	2.9±0.39 b-e	1.68±0.11	13.29±1.16	13.36±1.07	0.56±0.02b-	0.45±0.03
	f	a-b		b	b-e	a-d	с	b
SL-TE 15	0.42±0.14 c- h	0.27±0.14 b	2.33±0.19 c- f	0.8±0.01 b	11.3±1.61 d- i	11.79±0.58 b-e	0.37±0.11 c-i	0.29±0.09 b
SL-TE 18	0.31±0.14 f- h	0.19±0.14 b	2.68±0.55 b- e	0.68±0.19 b	11.72±0.67c- h	9.44±1.05 c- g	0.32±0.087 d-i	0.25±0.15 b
SL-TE 187	1.28±0.11 a	0.94±0.06 a	6.36±0.51 a	4.49±2.46 a	16.02±0.76a- b	12.75±0.35 a-e	1.27±0.09 a	1.25±0.22 a
SL-TE 25	0.4±0.01 c-h	0.31±0.06 b	2.02±0.51 d- f	1.21±0.44 b	8.37±0.11 g- i	7.86±1.02e-h	0.38±0.01 c-i	0.37±0.04 b
SL-TE 28	0.35±0.02 d- h	0.33±0.32 b	3.05±0.93 b- e	0.42±0.3 b	9.7±1.27 e-j	2.57±2.74 i	0.31±0.02 e-i	0.31±0.36 b
SL-TE 42	0.6±0.17 b-d	0.22±0.06 b	4.38±0.5 b	0.76±0.04 b	10.83±2.06 d-i	6.52±0.48 f-i	0.53±0.16 b-f	0.25±0.04 b
SL-TE 44	0.6±0.03 b-d	0.48±0.34	2.57±0.62 c-	0.94±0.21	9.253±1.45	8.73±0.58d-	0.55±0.01	0.5±0.26 b
QL TE 45	0.52.0.04	ab	e	b	t-j	g	b-d	0.47.0.01
SL-1E 45	0.52±0.04 c- g	0.51±0.06 a-b	3.86±0.37 b- c	1.67±0.44 b	10.76±0.44 d-i	9.66±1.58 c- g	0.48±0.01 b-h	0.47±0.01 b
SL-TE 46	0.43±0.05 c-	0.3±0.12 b	2.22±0.45 c-	1.23±0.13	9.3±0.35 f-j	8.93±0.47d-	0.4±0.07 c-i	0.31±0.06
	h		f	b	, i i i i i i i i i i i i i i i i i i i	g		b
SL-TE 47	0.51±0.03 c-	0.36±0.11a-	2.66±0.57 b-	1.38±0.18	12.24±0.41	10.94±1.75	0.49±0.03	0.4±0.07 b
SL-TE 74	$g_{0.45+0.11c}$	0 0 36+0 16 b	c 2 37+1 14 c	U 1 57+0 18	17.75 ± 0.25	U-1 17 27+0 80 a	0-g 0.47+0.13	0 30+0 15
SL-1E /4	0.45±0.11 ℃- h	0.30±0.10 D	∠.ɔ/±1.14 C- f	n.37±0.18 b	17.75±0.25 a	1/.2/±0.09 a	0.47±0.15 b-h	0.39±0.13 b
SL-TE 585	0.4±0.07 c-h	0.32±0.15 b	1.89±0.14 d-	1.45±0.73	8.98±0.88 f-j	9.23±1.56 c-	0.38 ± 0.08	0.4±0.17 b
			f	b		g	c-i	
SL-TE 588	0.59±0.01 b-	0.5±0.06 a-	2.84±0.73 b-	2.55±0.22	16.2±1.25 a-	16.44±1.5 a-	0.56 ± 0.02	0.51 ± 0.02
	e	b	e	a-b	b	b	b-c	b

Table 3. Response of eggplant accession to *Meloidogyne incognita* on plant growth parameters.

Note: the mean \pm standard deviation followed by different letters indicates a significant difference based on the Bonferroni LSD statistical test at a 95% confidence level.

Table 4. Percentage reduction in plant performance

Accessions	Root Fresh Weight (%)	Plant Fresh Weight (%)	Plant Height (%)	Root Volume (%)
SL-TE 590	5.34	4.13	8.33	14.84
SL-TE 587	7.82	62.25	11.56	-28
SL-TE 584	35.26	30.63	17.27	13.33
SL-TE 581	4.94	74.6	25.33	-36.67
SL-TE 582	5.64	67.52	18	1.12
SL-TE 586	12.42	75.47	33.15	-16.57
SL-TE 580	12.75	9.65	-19.96	20.43
SL-TE 579	19.39	62.22	32.64	-25.93
SL-TE 583	11.1	30.65	14.81	4.94
SL-TE 589	26	23.26	35.31	15.36
SL-TE 13	14.59	41.96	-0.51	18.93
SL-TE 15	38.48	65.5	-4.34	20
SL-TE 18	38.84	74.79	19.45	22.92
SL-TE 187	26.47	29.4	20.43	1.88
SL-TE 25	21.96	39.95	6.06	2.61
SL-TE 28	5.33	86.39	73.59	1.08
SL-TE 42	63.76	82.76	39.84	53.77
SL-TE 44	18.61	63.41	5.66	9.09
SL-TE 45	6.67	56.64	10.19	1.39
SL-TE 46	29.38	44.76	4	21.88
SL-TE 47	29.05	48.34	10.67	18.24
SL-TE 74	20.15	33.97	2.7	15.71
SL-TE 585	19.15	23.81	-2.74	-6.9
SL-TE 588	15.34	10.47	-1.48	9.82

The level of plant resistance to biotic stress can be determined based on the calculation of the stress sensitivity index on several vegetative growth variables. The value of the stress sensitivity index is the relative value of the decrease in the value of various variables observed under stress conditions compared to non-stress conditions (control). The tolerance level of each variety to biotic stress was determined by referring to the average value of the Stress Sensitivity Index of all observed variables. Accessions were classified as resistant if S < 0.5, including moderately resistant groups if $0.5 \leq S \geq 1$, and vulnerable groups if S > 1 [11].

Based on plant fresh weight data, the susceptible index of 24 eggplant accessions were obtained: SL-TE 42 and SL-TE 28 had the highest average susceptible index, 3.62 and 2.64, which means that when grown under normal conditions and in nematode-infected conditions, the accessions experienced a significant decrease in performance (Table 5). Accessions SL-TE 590 (S. *aculeatissimum*), SL-TE 74 (S. *aculeatissimum*), and SL-TE 589 (S. *torvum*) had the low susceptible index values, 0.07, 0.81, and 0.15 which indicated that the plant's performance when grown under normal conditions

Table 5. Susceptibility index and stress sensitivity index of
24 accessions of eggplant to Meloidogyne incognita
infection based on morphological variables.

Accessions	Susceptible Index and Stress						
	Sensitivity Index Based on						
	Biomass Plant Fresh Weight						
	Susceptible	Status					
SI TE 570		Index	~				
SL-TE 579	1.18	1.26	S				
SL-TE 581	1.2	1.51	S				
SL-TE 586	2.65	1.52	S				
SL-TE 28	2.64	1.74	S				
SL-TE 18	2.01	1.51	S				
SL-TE 15	1.52	1.32	S				
SL-TE 587	0.95	1.26	S				
SL-TE 585	0.45	0.48	R				
SL-TE 45	2.18	1.14	S				
SL-TE 580	0.2	0.19	R				
SL-TE 582	1.53	1.36	S				
SL-TE 13	1.21	0.85	S				
SL-TE 588	0.3	0.21	R				
SL-TE 583	0.66	0.62	SR				
SL-TE 46	0.99	0.9	S				
SL-TE 47	1.29	0.98	S				
SL-TE 590	0.07	0.08	R				
SL-TE 25	0.81	0.81	SR				
SL-TE 42	3.62	1.67	S				
SL-TE 44	1.63	1.28	S				
SL-TE 589	0.15	0.47	R				
SL-TE 584	0.71	0.62	SR				
SL-TE 187	1.87	0.59	S				
SL-TE 74	0.81	0.69	SR				

Note: S=Susceptible, R= Resistance, SR=Slightly Resistance

and conditions infected with nematodes, did not decrease a lot. The SL-TE 588 and SL-TE 580 accessions, which are species of Solanum melongena, showed a fairly good susceptibility index value. Both have very low susceptible index values, namely 0.30 and 0.20.

Based on the calculation of the stress sensitivity index (SSI) (Table 5), the highest index values based on plant fresh weight were the accessions of SL-TE 42, SL-TE 28, and SL-TE 18 with a value of 1.67; 1.74, and 1.51, while the lowest stress sensitivity index values were accessions of SL-TE 580 and SL-TE 588 with values of 0.19 and 0.21 (Table 4). The SL-TE 590 and SL-TE 74 also have a fairly low SSI value of 0.08 and 0.69. However, SL-TE 589 has a fairly high stress sensitivity index value of 0.47.

 Table 6. Polymorphic information content

Primer	DNA Band	PIC
SSR_114	8	0.21
SSR_120	18	0.16
SSR_82	11	0.31
SSR_01	18	0.21
SSR_04	10	0.28
SSR_135	3	0.35
SSR_140	13	0.23
SSR_145	11	0.18
SSR_155	16	0.17
SSR_117	8	0.24
SSR_133	9	0.19
SSR_CS04	9	0.25
SSR_27	7	0.24
SSR_FO4	7	0.12

According to Botstein et al. (1980) [16], molecular markers can be categorized into three categories based on their PIC values. The marker will be included in the less informative category if the PIC value < 0.25, the category quite informative if 0.25 < PIC < 0.5, and the very informative category if the PIC value> 0.5. The 14 SSR markers used in this study, the markers SSR_120 and SSR_01 produced the most alleles, 18 alleles (Table 6). The marker that produced the fewest alleles was the SSR_135 marker with only 3 alleles. The results of the analysis using the iMEC device show that the values of the Polymorphic Information Content (PIC) markers SSR_120 and SSR_135 are 0.16 and 0.21. The SSR_135 marker has the largest PIC value of 0.35, while the SSR FO4 marker has the smallest PIC value of 0.12. It can be concluded, SSR_135 is quite informative.

Expected heterozygosity (HE) or genetic diversity is the expected heterozygosity probability of individuals at related loci in a multilocus system (for all loci analysed). In other words, HE was determined from all individuals who were heterozygous for each randomly selected locus. It is often calculated based on the determination of the square root of the frequency of the zero (recessive) allele [17].

The results of the analysis of the expected heterozygosity and the percentage of polymorphic loci (PPL) of 24 accessions showed that the highest PPL and heterozygosity values were SL-TE 589 and SL-TE 590 accessions with PPL and heterozygosity values respectively 20.95% and 0.087 (Table 7). Accessions that had the lowest PPL and heterozygosity values were SL-TE 42 and SL-TE 584 with PPL and heterozygosity values being 9.46% and 0.039, respectively.

Table 7. Expected heterozygosity and the percentage of	
polymorphic loci (PPL) of 24 accessions.	

Population	PPL	Expected Heterozygosity
SL-TE 42	9.46%	0.039
SL-TE 18	10.81%	0.045
SL-TE 46	10.81%	0.045
SL-TE 582	12.84%	0.053
SL-TE 585	11.49%	0.048
SL-TE 28	13.51%	0.056
SL-TE 25	11.49%	0.048
SL-TE 580	14.86%	0.062
SL-TE 44	14.19%	0.059
SL-TE 586	13.51%	0.056
SL-TE 587	14.86%	0.062
SL-TE 47	12.16%	0.050
SL-TE 187	19.59%	0.081
SL-TE 583	14.19%	0.059
SL-TE 581	15.54%	0.064
SL-TE 13	14.19%	0.059
SL-TE 588	16.22%	0.067
SL-TE 579	14.86%	0.062
SL-TE 45	11.49%	0.048
SL-TE 584	9.46%	0.039
SL-TE 15	14.19%	0.059
SL-TE 74	17.57%	0.073
SL-TE 589	20.95%	0.087
SL-TE 590	20.95%	0.087

The results of the analysis of molecular variance (AMOVA) showed that the diversity between populations was significantly different, which means that there was a genetic diversity between accessions, which was 32% (Table 8). The genetic diversity in the population is 68%, which means that there is diversity between individuals in the population. However, based on the results of this AMOVA, the value of diversity between populations is smaller than the value of genetic diversity within the population. PCoA analysis differentiated accessions in to four main clusters with wild type cultivars (SL-TE 74, SL-TE 589, and SL-TE590) grouped in cluster I and local cultivars in cluster II, III, and IV (Fig.1).

These results reveal that the genotypes taken in the study are genetically diverse. The genetically divergent varieties identified in the present study can be utilized in the hybridization programmes. Majority of the cultivated varieties did not cluster in one cluster. The genetic diversity of eggplant accession reported in this study will be useful when planning future crosses amongst these varieties.

Table 8. Analysis of molecular variance

Source	df	SS	MS	Est. Var.	%	P- value
Among Pop	23	195.986	8.521	1.664	32%	0.001
Within Pop	48	169.333	3.528	3.528	68%	
Total	71	365.319		5.192	100%	





Figure 1. Principal Coordinates Analysis

4. CONCLUSION

SL-TE 44 and SL-TE 28 had the highest average susceptible index by plant fresh weight, 3.62 and 2.64 which means those accession is susceptible. Accessions SL-TE 590 (*S. aculeatissimum*), SL-TE 74 (*S. aculeatissimum*), and SL-TE 589 (*S. torvum*) had the lowest susceptible index values, 0.37; 0.36, and 0.79 which means those are resistance to nematode. Accessions SL-TE 74, SL-TE 589, and SL-TE590 showed the highest percentage of polymorphic loci, respectively, 17.57%, 20.95%, and 20.95%. The results revealed that there were 68% of the total genetic diversity within the population, and 32% among population.

ACKNOWLEDGMENTS

The author would like to gratefully acknowledge Agrotechnology Innovation Centre -Universitas Gadjah Mada, Indonesia for providing eggplant breeding material. This research was funded by Program Penelitian Kompetitf Nasional Category Penelitian Dasar by Direktorat Riset dan Pengabdian Masyarakat Direktorat Jenderal Riset dan Pengembangan Kementerian Riset, Teknologi, dan Pendidikan Tinggi, Indonesia. Thank you very much to Veronica Inggrit Hapsari who help the author.

REFERENCES

- [1] Vorontsova M S and Knapp S 2012 A new species of solanum (solanaceae) from South Africa related to the cultivated eggplant PhytoKeys 8 pp 1–11
- [2] Zambrano-Moreno E L, Chávez-Jáuregui R N, Plaza M L, Wessel-Beaver L 2015 Phenolic content and antioxidant capacity in organically and conventionally grown eggplant (Solanum melongena) fruits following thermal processing Food Science and Technology 35 pp 414-420
- [3] Gramazio P, Prohens J, Plazas M, Mangini G, Herraizi F, Garcia-Fortea 2015 Genomic tools for the enhancement of vegetable crops: a case in

eggplant Not. Bot. Hort. Agrobot. Cluj. 46 pp 1-13

- [4] Zhou X, Liu J, Bao S, Yang Y and Zhuang Y 2018 Molecular cloning and characterization of a wild eggplant Solanum aculeatissimum NBS-LRR gene, involved in plant resistance to *Meloidogyne incognita* Int. J. Mol. Sci. 19 pp 583-595
- [5] Ismael J H S, Sulaiman S M, Muhidden A, Faraj S M, Abdul-Khaliq H 2017 Control of root-knot nematode of eggplant and its effect on plant growth and yield under plastic house condition Int. J. For. Hort. 2 pp 28-34
- [6] Hafiza N, Khandaker M M and Mat N 2016 Occurance and control of root knot nematode in crops: A review Australian Journal of Crop Science 10 pp 1649-54
- Huang J S 2001 Plant Patogenesis and Resistance; Biochemistry and Physiology of Plant– Microbe Interactions (London: Kluwer Academic Publisher)
- [8] Trudgill D L 1991 Resistance to and tolerance of plant parasitic nematodes in plants Annual Review of Phytopathology 29 pp 167-192
- [9] Devran Z, Burcu B, Ayse T and Fatmana D 2013 Comparison of PCR-based molecular markers for identification of Mi gene Acta Agric. Scand. Sec. B Soil Plant Sci. 63 pp 395-402
- [10] Begum F, Islam A K M A, Golam R M, Mian M A K and Hossain M M 2013 Morphological diversity of eggplant (Solanum melongena) in Bangladesh Emir. J. Food Agric. 25 pp 45-51
- [11] Fischer R A and Maurer R 1978 Drought Resistance in Spring Wheat Cultivars: I.Grain Yield Responses. Australian Journal of Agriculture Research. 29 pp 897–912.
- [12] Hoque M E and Tasrin K 2018 Molecular characterization and DNA fingerprinting of some local eggplant genotypes and its wild relatives International Journal of Environment, Agriculture and Biotechnology (IJEAB) 3 pp 1562-70
- [13] Verma M, Rathi S, Munshi A D, Kumar A, Arya L, Bhat K V and Kumar R 2012 Genetic diversity of Indian brinjal revealed by RAPD and SSR markers Indian J. Hort. 69 pp 517-522
- [14] Adeniji O T, Kusolwa P, Reuben S O W M and Deo P 2012 Molecular diversity among seven Solanum (eggplant and relatives) species assessed by simple sequence repeats (SSRs)



markers African Journal of Biotechnology 11 pp. 15643-15653

- [15] Gramazio P, Chatziefstratiou E, Petropoulos C, Chioti V, Mylona P, Kapotis G, Vilanova S, Prohens J, and Papasotiropoulos V 2019 Multilevel characterization of eggplant accessions from greek islands and the mainland contributes to the enhancement and conservation of this germplasm and reveals a large diversity and signatures of di_erentiation between both origins Agronomy 9 pp 887-907
- [16] Botstein D, White R L, Skolnick M, Davis R W 1980 Construction of a genetic linkage map in man using restriction fragment length polymorphisms American Journal of Human Genetics 32 pp 314–331.
- [17] Chesnokov Y V and Artemyeva A M 2015 Evaluation of the measure of polymorphism information of genetic diversity Agricultural Biology 50 pp 571-578