AGRIVITA
Journal of Agricultural Science

Genetic Diversity Analysis in 27 Tomato Accessions Using Morphological and Molecular Markers

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ARTICLE INFO

Keywords:
Genetic diversity
Morphology
RAPD marker
Tomato
UPGMA

Article History:
Received: November 25, 2015
Accepted: May 6, 2016

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ABSTRACT

Genetic diversity is the most important aspect in tomato breeding activities. Better assessment on the diversity of the collected accessions will come up with better result of the cultivar development. This study aimed at analyzing the genetic diversity of 27 tomato accessions by morphological and molecular markers. Twenty seven accessions collected from various regions of Indonesia were planted in the field and evaluated for their morphological traits, and RAPD analyzed for their molecular markers. The UPGMA clustering analyzes, elaborating the combination of morphological and molecular data, indicated that the tomato accessions could be grouped into 5 major groups with 70 % genetic similarity levels. Current study indicated that although many accessions came from different locations, they congregated into the same group. Cherry, Kudamati 1 and Lombok 3 were the farthest genetic distant accessions to the others. Those three genotypes will be the most valuable accessions, when they were crossed with other accessions, for designing a prospective breeding program in the future.

INTRODUCTION

Tomato is one of the most important vegetables in Indonesia in addition to potatoes, peppers and onions. Tomato is consumed both in fresh and processed fruit. The use of special varieties for processing tomatoes, including tomato juice, sauce, puree, paste, and dried tomato, are more than for fresh consumption. Tomatoes have significantly high nutritional value, such as a source of vitamin C, vitamin A and antioxidants (Li, 2008). Tomato production in the year of 2014 was as many as 915,987 t with national total planting area of tomato in 2014 was about 59,008 ha (Direktorat Jenderal Hortikultura, 2015).

Indonesia is not the tomato's center of origin (Bai & Lindhout, 2007; Redden et al., 2015), however there are high variation of tomatoes found in traditional market indicating high genetic diversity. Institutionally, currently, there are 46 accessions of tomato collected by Indonesian Vegetables Research Institute in Lembang, Bandung, from various regions in Indonesia plus 16 strains introduced from AVRDC (ASEAN Vegetable Research Development Center) Taiwan and 2 strains of Rembang. Other local tomato landraces were also collected by the Plant Genetics and Breeding Division of the Department of Agronomy and Horticulture, Bogor Agricultural University, and had been used for various studies, such as on fruit crack resistance (Wahyuni, Yunianti, Syukur, Witono, & Aisyah, 2014). However, there are many other local tomatoes that have not been registered in the Indonesian tomato germplasm. Current efforts in tomato breeding program focused on discovering and utilizing the gene for the trait most important for human being by exploring tomato germplasms (Bai & Lindhout, 2007). Knowledge on germplasm diversity is very important for conservation purposes and plant breeding activities in order to develop varieties and to improve production and productivity (Poczai, Varga, Bell, & Hyvönen, 2011; Herison, Handajaningsih, Fahrurrozi, & Rustikawati, 2017).

ISSN: 0126-0537 Accredited by DIKTI Decree No: 60/E/KPT/2016

Cite this as: Herison, C., Sutjahjo, S. H., Sulastrini, I., Rustikawati, & Marwiyah, S. (2018). Genetic diversity analysis in 27 tomato accessions using morphological and molecular markers. AGRIVITA Journal of Agricultural Science, 40(1), 36–44. http://doi.org/10.17503/agrivita.v40i1.726

The diversity of tomatoes can be evaluated by morphological and molecular traits. Morphological traits showed significant differences between seasons and genotypes, indicating a differential genotypic variability and crop growth conditions (Mekhlouf et al., 2006). Molecular markers that frequently used to assess genetic diversity are random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP) and simple sequence repeat (SSR) markers. RAPD markers are more effective and efficient to determine the recombinant variability than morphological markers in assessing genetic diversity (Paul, Bandyophadyay, Acharyya, & Raychaudhuri, 2010; Hapsoro, Warganegara, Utomo, Sriyani, & Yusnita). RAPD markers also become a potential tool to identify genetic differences among varieties (Biswas, Akhond, Al-Amin, Khatun, & Kabir, 2009). Only in a few cases morphological characteristics can be attributed to local adaptation and appropriate genetic variations (Reck-Kortmann, Rodrigues, Ruas, & Freitas, 2013). Dendrogram generated by RAPD markers are good enough in featuring geographical genotypic (Pu et al., 2009). The purpose of this study was to analyze the genetic diversity of 27 accessions of tomato and their genetic relationship based on molecular markers and morphological traits.

MATERIALS AND METHODS

The research was conducted during March to November 2013. The Field experiment to access morphological traits was conducted at Leuwikopo Field Research Station, and molecular analysis was carried out in the Plant Molecular Laboratory of the Department of Agronomy and Horticulture, the Faculty of Agriculture, Bogor Agricultural University.

Morphological Analysis

Twenty five tomato accessions collected from nine province of Indonesia and two commercial varieties (Table 1) were germinated on soaked paper tissues for 3 days and then sown on 72 cell trays containing mix media of manure and soil, 1:1 (v/v). At four weeks after sowing, the seedlings were then transplanted onto field beds. Ten plants of each genotype, randomly sampled, were measured and thoroughly observed for their quantitative and qualitative morphological traits. The quantitative measurement included plant height, number of leaves, age to flowering, number of flower per inflorescence, number of inflorescences, number of fruit per inflorescence, number of fruit per plant, fruit weight per plant and percent fruit crack. The qualitative traits were characterized following the Descriptor for Tomato (IPGRI, 1996), i.e. young plant stem color, mature plant stem color, young fruit color, mature fruit color, fruit shape, cross sectional fruit type, type of fruit tip, growth type, leaf stand and resistance to bacterial wilt (Table 2).

Molecular Analysis

Five seedlings of two weeks old of each genotype were randomly sampled for their DNA polymorphism profiling. Six polymorphic random primers (Table 3) previously were selected from 40 primers used in this study. The primers, belong to OPERON primers, derived the RAPD loci.

Table 1. Accession name and region/province where the accession being collected

No	Name	Region/Province	No	Name	Region/Province
1	Kudamati 1	Ambon	15	Kefaminano 14	East Nusa Tenggara
2	Lombok 1	West Nusa Tenggara	16	Aceh 3	Aceh
3	Lombok 2	West Nusa Tenggara	17	Aceh 5	Aceh
4	Lombok 3	West Nusa Tenggara	18	Situbondo Bulat Kecil	East Java
5	Lombok 4	West Nusa Tenggara	19	Situbondo Gelombang	East Java
6	Makassar 1	Makassar	20	Cherry	East Nusa Tenggara
7	Makassar 3	Makassar	21	Tanah Datar	West Sumatera
8	Makassar 4	Makassar	22	Kemir	commercial variety
9	Kefaminano 3	East Nusa Tenggara	23	Meranti 1	Riau
10	Kefaminano 6	East Nusa Tenggara	24	Meranti 2	Riau
11	Kefaminano 7	East Nusa Tenggara	25	Bajawa Ngada Flores	East Nusa Tenggara
12	Kefaminano 9	East Nusa Tenggara	26	Gondol Lonjong	commercial variety
13	Kefaminano 12	East Nusa Tenggara	27	Kali Acai Abepura	Jayapura
14	Aceh 1	Aceh			

Table 2. Quantitative and qualitative morphological characters and sub characters

Character	Sub character	Total
Qualitative		
young stem color	green, purple	2
leaf stand	erect, horizontal, droopy	3
plant growth type	determinate, semi determinate, indeterminate	3
old stem color	green, brown	2
resistance to bacterial wilt	very resistance, resistance, medium resistance, medium susceptible, susceptible, very susceptible	6
young fruit color	light green, green	2
mature fruit color	pink, orange, red	3
Fruit shape	long, round, flatten	3
fruit cross-sectional	round, angular	2
fruit blossom end shape	indented, flat, pointed	3
Fruit crack	crack, not crack	2
Quantitative		
Plant height (cm)	dwarf (40-60), medium (60-80), high (> 80)	3
number of leaves	low (9-12), medium (12-15), high (> 15)	3
number of flower inflorescence	low (5-16), medium (16-28), high (> 28)	3
number of flower per inflorescence	low (5-8), medium (8-11), high (> 11)	3
total number of fruit per plant	low (15-24), medium (24-34), high (> 34)	3
total fruit weight per plant (g)	low (80-244), medium (245-409), high (> 409)	3
days to flower (day)	early (18-23), medium (24-29), late (> 29)	3
crack (%)	small (0-16), medium (16-33), large (> 33)	3
	Total	**55**

Table 3. Random primer information and distribution

Primer pair code	Sequence	Annealing Tm (^oC)	Marker size ranged (bp)	Total marker	Polymorphic markers
OPH19	CTGACCAGCC	33.6	300-1700	12	11
OPH5	AGTCGTCCCC	36.2	250-1600	10	6
OPH13	GACGCCACAC	34.6	250-2000	14	7
OPE19	ACGGCGTATG	36.2	200-1900	14	11
OPE1	CCCAAGGTCC	37.3	300-1700	17	14
OPE7	AGATGCAGCC	33.6	400-1700	13	11
Total				**80**	**60**

DNA Isolation

Genomic DNAs were extracted by grinding the young seedling leaves of each accession following the method of Herison, Winarsih, Handayaningsih, & Rustikawati (2012) and Naz, Zafrullah, Shahzadhi, & Munir (2013) with modification. About 0.2 g of a composite of 5 sample seedlings were grinded in 700 ml CTAB added with 0.001 g polyvinyl pyrolidone. The solution was placed in a 2 ml plastic tube and incubated in a water bath of 65 ^oC for 30 minutes. Then, a 700 ml CIA was added into the tube, vortexed thoroughly, and centrifuged at 4 °C 12,000 rpm for 12 minutes. The supernatant was moved into a new tube and added with 1000 µl absolute ethanol. The solution was centrifuged at 4 ^oC 12,000 rpm for 6 minutes. The liquid phase was removed, and the pellet was vacuum dried for 30 minutes. DNA pellets were suspended in aqua bides 100 µl. The purity and concentration of genomic DNAs were measured with a Perkin Elmer spectrophotometer.

DNA Amplification

The RAPD loci were amplified by a polymerase chain reaction (PCR) in a 25 ml assay mixture of a KAPA 2G PCR Kit. The kit consisting of 5 µl buffer A, 0.5 µl 10 mM dNTP, 1.25 µl primer, 0.1 µl KAPA 2G Fast DNA Polymerase, 2.5 µl DNA template with a concentration of 10-25 ng and distilled water to the final volume of 25 µl. The PCR program used was 5 minutes pre-denaturation at 94 °C; 45 cycles of 55 seconds at 94 °C denaturation, 30 seconds annealing at temperatures Tm-4 (4 °C below the melting point of the primer), 1 minute elongation at a temperature of 72 °C; and stop the PCR cycle at a temperature of 72 °C for 10 minutes.

Data Analysis

Quantitative morphological data were converted into categorical data based on the difference between the lowest and the highest value measured divided by number of classes determined. Each category was then presumed as a sub character representing one locus. The observed values were matched to that category. The fit value was then scored as 1 to the specified sub character and scored as 0 to other sub characters. Similarly did with the qualitative data. The observed variation of qualitative trait was justified as the sub characters. The existence bands obtained with different RAPD primers were scored as present (1) or absent (0) band (Herison, Winarsih, Handayaningsih, & Rustikawati, 2012) for all of the accessions under the study. Genetic similarities were computed for genetic diversity assessment and cluster analysis. All data collected, quantitative and qualitative morphological and RAPD data, in the binary format were combined to calculate pairwise similarity coefficient by the SIMQUAL method in NTSYS-pc version 2.01. A phylogenetic analysis with UPGMA using NTSYS-PC software was performed based on a similarity coefficient matrix (Rohlf, 2000).

RESULTS AND DISCUSSION

Morphological Analysis

Measurement and observation on morphological trait revealed 21 characters consisting of 61 sub character (Table 2) with polymorphic rate of 93.75 %. High variation was noticeable from the visual appearance of either vegetative or generative traits both quantitatively and qualitatively. This tremendous variation, attributable to genetic diversity, might be contributed by local adaptation to specific geographical condition for centuries. This results was in harmony with Wahyuni, Yunianti, Syukur, Witono, & Aisyah (2014) that there were differences among Indonesian local landraces of tomato in term of morphological traits such as fruit length, fruit diameter, fruit flesh thickness, locule number, time to harvest, number of fruit per plant, yield per plant and fruit crack. High diversity of quantitative traits was also demonstrated by local tomato landraces of Western Tigray, Northern Ethiopia (Chernet, Belew, & Abay, 2014) and regionally adapted processing tomato in North America (Merk et al., 2012). High diversity among genotypes was a great value for breeders to develop varieties suitable to the market through hybridization of any pair genotypes with most beneficial traits. However, the breeders have to specify their objectives to make best use of genotypes where the traits were highly divergent.

Molecular Analysis

Results of the primer selection indicated that there were 6 primers showing most polymorphic bands, i.e. OPH19, OPH5, OPH13, OPE19, OPE1 and OPE7 (Table 3) and representatively shown in Fig. 1. DNA amplification on all accessions in the study produced a binary score of 80 alleles, averaging of 13.3 allele per loci with 75 % polymorphic levels. The diversity coefficient value was in line with the percentage of polymorphic loci, i.e., the higher the rate of polymorphic loci, the higher the diversity coefficient arose in a population (Kristamtini, Taryono, Basunanda, & Murti, 2014).

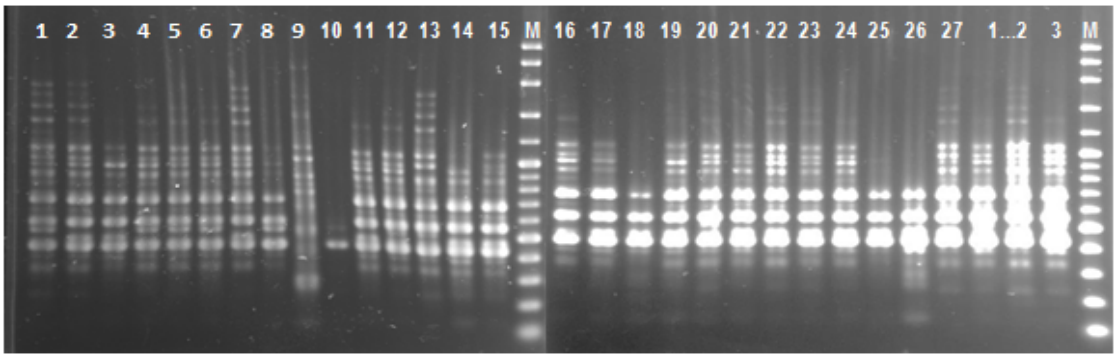

Fig. 1. A representative RAPD amplification profiling by OPE7 primer on 27 tomato accessions. The consecutive number denoted the accessions number following Table 1. The last three lanes on the most right hand side of the figure was the profile revealed by OPE19. M indicated the standard marker

The highest polymorphism was obtained in OPH19 primer. Polymorphic DNA showed the level of molecular variation among accessions. The amount of alleles per locus was highly dependent on the accession diversity tested and the characteristics of the markers used (Kristamtini, Taryono, Basunanda, & Murti, 2014). RAPD markers produced in this study were high density in most of accessions. Some loci seemed to be the position of the genes in common of Lycopersicum family due to their existence in almost all accessions like OPE7 550 bp and 700 bp. Other markers were polymorphic attributing the genetic diversity. Higher polymorphism value was also obtained by Comlekcioglu, Simsek, Boncuk, & Aka-Kacar (2010) 25-80 % by 50 primer RAPD markers when assessing tomato genetic diversity. With different random primer, OPA, Tabassum, Sony, Bhajan, & Islam (2013) obtained higher polymorphism level of 94.168 %.

Similar matrix was calculated using similarity coefficient based on polymorphic bands, and the dendrogram was established through UPGMA cluster analysis (Rohlf, 2000). Naz, Zafrullah, Shahzadhi, & Munir (2013), with the use of RAPD markers, found that genetic diversity value at 25 tomato accessions was 75.6 %. In addition, with the use of RAPD primer of OPB18, the work of Sharifova, Mehdiyeva, Theodorikas, & Roubos (2013) showed high genetic diversity in cultivated tomatoes, up to 82.3 %.

In this study, phylogenetic analysis of 27 tomato genotypes, based on morphological and molecular characters, indicated the genetic diversity was in the range of 7.4 % to 61.4 % (genetic similarity value of 39.6 to 92.6 %). A high genetic similarity value was also obtained by Aida & Eltayeb (2015), that the genetic similarity value in common tomato genotypes was 88 % based on RAPD markers. Naz, Zafrullah, Shahzadhi, & Munir (2013), with RAPD of only 24.4 % polymorphic markers, obtained the genetic similarity on 25 tomato accessions were average of 75.6 %.

Dendrogram generated from UPGMA based analysis on 27 tomato accessions revealed 5 groups at the similarity level of 70 % with a value of r = 0.92, meaning that the dendrogram was fit to illustrate the grouping of 27 tomato accessions (Fig. 2). The accessions clustered into 5 groups might be due to both geographic and genetic factors. The former factors, however, could not be used as the index of genetic diversity (Merk et al., 2012; Kaur, Cogan, Forster, & Paull, 2014).

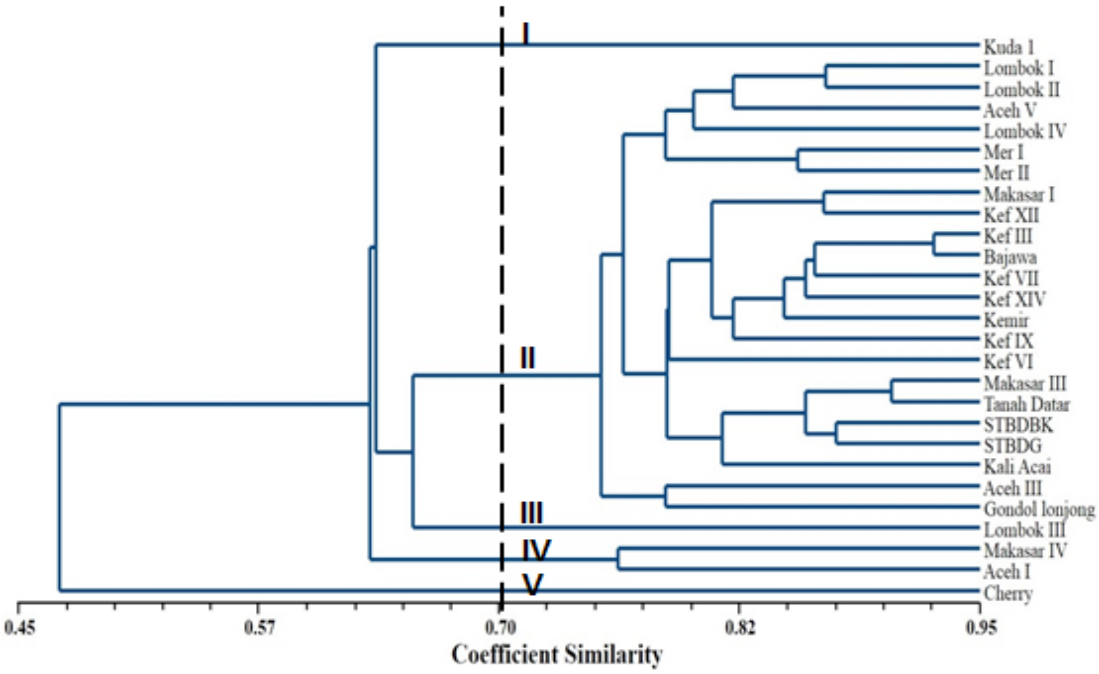

Fig. 2. Dendrogram generated by UPGMA analyses on 27 tomato accessions based on morphological and RAPD markers

The first group consisted of only one accession, Kudamati 1. The second group comprised of 3 sub cluster with genetic similarity value within the group of 74.6 %. The first sub cluster was made from 6 accessions, namely 'Lombok 1', 'Lombok 2', 'Lombok 4', 'Aceh 5', 'Meranti 1' and 'Meranti 2'. This sub cluster had similarity on morphological markers of number of fruit per plant, the number of inflorescence, cross-sectional plane of fruit, fruit crack tolerance, young and old stem color. The second sub cluster comprised of 14 accessions with genetic similarity within the group of 70.8 % to 92.5 %. They were 'Makasar 1', 'Makasar 3', 'Kefaminano 3', 'Kefaminano 6', 'Kefaminano 7', 'Kefaminano 9', 'Kefaminano 12', 'Kefaminano 14', 'Bajawa', 'Kemir', 'Tanah Datar', 'Situbondo Bulat Kecil', 'Situbondo Gelombang' and 'Kali Acai'. Those 14 accessions possessed the similarity in young and old stem color. Local tomatoes of Bajawa and Kefaminano 3 have the highest genetic similarity value, 92.6 %. The third sub cluster contained two accessions, 'Aceh 3' and 'Gondol Lonjong 2' with 78.7 % genetic similarity. Those two accessions had similarity on the number of flower per plant, total fruit per plant, flowers per inflorescence, and flower inflorescence, fruit shape, the cross-sectional plane of fruit, fruit crack tolerance, the color of the young fruit, the color of ripening fruit, young and old stem color.

The third group consisted of 1 accession, namely 'Lombok 3'. The fourth group contained two accessions, 'Makassar 4' and 'Aceh 1', with 76.2 % genetic similarity. They had similarity on fruit weight per plant, plant height, the number of flowers per inflorescence, leaves, fruit per plant, and flowers per plant, fruit tip, fruit crack tolerance, leaf stand, young and old stem color. The fifth group contained only one member, 'Cherry'. 'Cherry' tomato has the highest genetic differences value with 'Kali Acai', up to 60.4 %. This genotype also had far genetic relationship to other accessions.

Tomato yield per plant was determined by the number of inflorescence, the number of flowers per inflorescence, percent fruit formation, and weight per fruit. According to Wahyuni, Yunianti, Syukur, Witono, & Aisyah (2014) the yield and plant height is the main variable distinguishing between genotypes. However, in this study the second and fourth group showed no differences in those characters. The 22 accessions in group two and four had moderate diversity values (27-42 %), and 2 accessions, 'Kefaminano 3' and 'Bajawa' showed very low genetic differences (< 10%). Accessions showing low genetic diversity meaning that they were genetically almost identical or duplication, might be derived from population with genetically very close to one another, although they were collected from different region (Tasma, Warsun, Satyawan, Syafaruddin, & Martono, 2013; Chernet, Belew, & Abay, 2014). On the other hand, accessions of 'Makassar 4' and 'Aceh 3' had a high diversity values with other accessions from the same region. 'Makassar 4', 'Makassar 1 and 'Makassar 3', although they came from the same area, they had genetic diversity value of 34.5 % to 47.9 %. Similarly, were 'Aceh 1' and 'Aceh 3', possessing genetic diversity value up to 36.8 %. Relatively high genetic diversity among accession within one region might be due the geographical variation within region, besides natural genetic differences. Ganesamurthy, Ecknitha, & Elangovan (2010) found that high diversity might be caused by differences in ecotypes (origin location accession). There was a correlation between geographic and genetic diversity; and geographic diversity, however, had to be excluded to access an index of genetic diversity.

Tomatoes cultivar development, until recently, have been focused on elevating yield with the addition of specific character suitable to farmers such as earliness, resistance to diseases, and tolerance to adverse environmental conditions, or to consumers such as high nutritive value, good appearance, fruit uniformity and suitable for processing machinery. Breeding strategy to develop such desired cultivars always starts with creating genetic recombination generated from accessions with the farther genetic distance (Hapsoro, Warganegara, Utomo, Sriyani, & Yusnita). Many efforts have been done to study genetic relationship elaborating as many as observable characters. Relationship among accession could be explored by quantitative morphological characters (Chernet, Belew, & Abay, 2014). With the improvement of DNA technology, assessment of genetic relationship have been done by many molecular markers (Biswas, Akhond, Al-Amin, Khatun, & Kabir, 2009; Chen et al., 2009; Pu et al., 2009; Comlekcioglu, Simsek, Boncuk, & Aka-Kacar, 2010; Meng, Xu, Huang, & Li, 2010; Reck-Kortmann, Rodrigues, Ruas, & Freitas, 2013; Sharifova, Mehdiyeva, Theodorikas, & Roubos, 2013; Tabassum, Sony,

Bhajan, & Islam, 2013; Tasma, Warsun, Satyawan, Syafaruddin, & Martono, 2013; Aida & Eltayeb, 2015) Other researchers used the combination of both morphology and molecular makers (Paul, Bandyophadyay, Acharyya, & Raychaudhuri, 2010; Naz, Zafrullah, Shahzadhi, & Munir, 2013).

In this study, qualitative and quantitative morphological characters in combination with RAPD markers were used to discover genetic relationship among accession collected from 9 different regions/ provinces. Accessions of 'Cherry', 'Lombok 3' and 'Kudamati 1' were the accessions which had great genetic distance to other accessions. 'Kudamati 1' was resistance to bacterial wilt and fruit crack. Although it was highly susceptible bacterial wilt and fruit crack, 'Lombok 3' possessed high yield components. While 'Cherry', even though somewhat susceptible to bacterial wilt, it was tolerant to fruit crack and had great number of fruit per inflorescence. In accordance with the goal of plant breeding, accessions having a high genetic distances could be used as parental crossings with accessions having other superior properties. For instance, 'Kudamati 1' can be used as one of the parental cross to develop new tomato varieties high yielding, resistance to bacterial wilt and tolerance fruit crack.

CONCLUSION AND SUGGESTION

The 27 tomato accessions collected from 9 provinces in Indonesia could be grouped into 5 major clusters based on morphological and RAPD markers. The genetic diversity presented for the tomatoes species will be useful for tomato breeding programs. Further works have to be conducted to exploit prospective accessions to build breeding populations to develop new high yielding tomato varieties which have superiority on resistance to bacterial wilt and tolerance to fruit crack.

ACKNOWLEDGEMENT

This research was funded by National Agricultural Collaborative Partnership Research and Development program of Indonesian Agency for Agricultural Research and Development (IAARD) Ministry of Agriculture the Republic of Indonesia. The authors thanked to Syafei, Eriana and Yudiansyah who helped for the field and laboratory works.

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