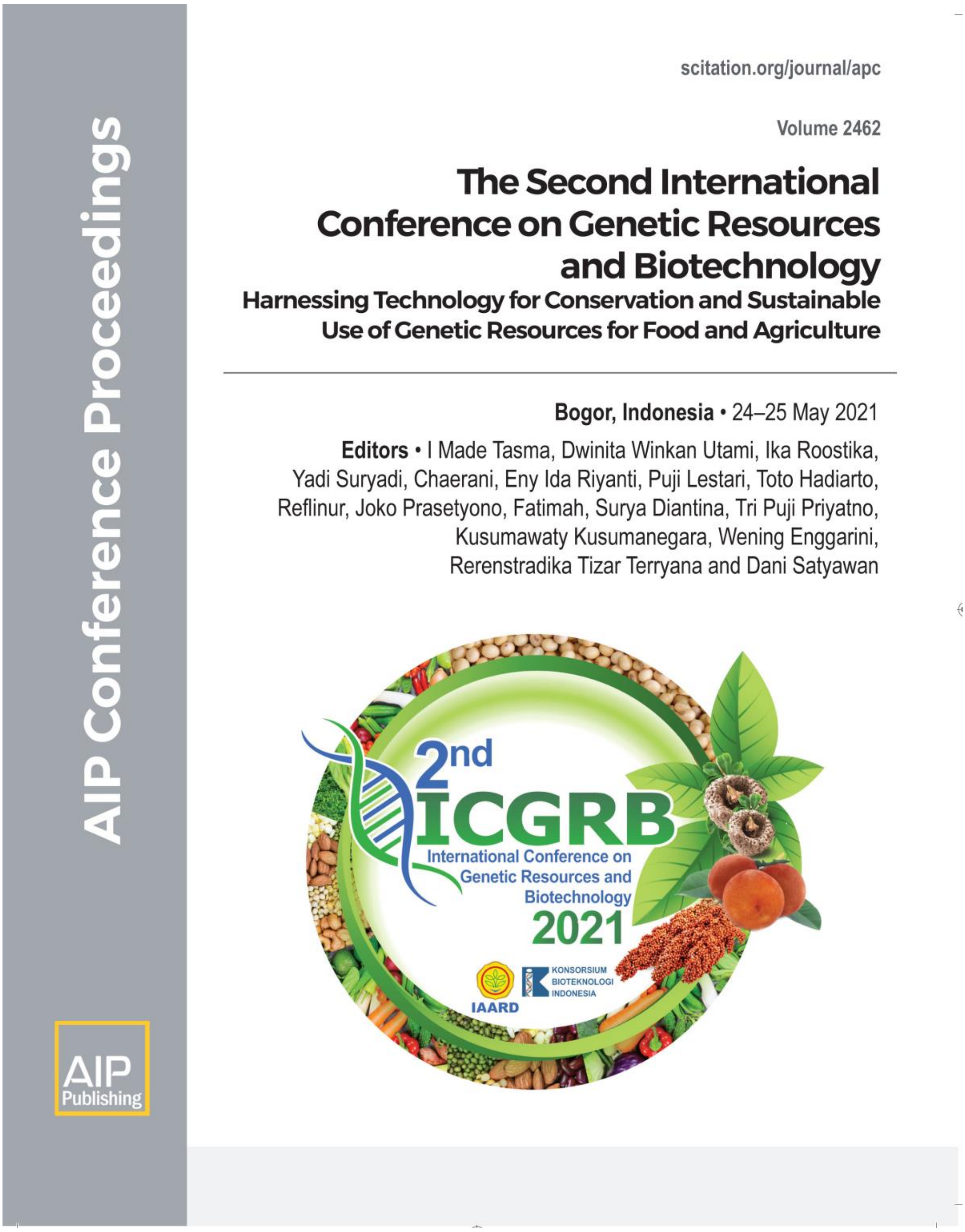

January 2022

THE SECOND INTERNATIONAL CONFERENCE ON GENETIC RESOURCES AND BIOTECHNOLOGY: Harnessing Technology for Conservation and Sustainable Use of Genetic Resources for Food and Agriculture

Committees: The Second International Conference on Genetic Resources and Biotechnology

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Preface: The Second International Conference on Genetic Resources and Biotechnology

The Second International Conference on Genetic Resources and Biotechnology, which is the continuation of the first event held in 2018, focuses on topics related to advances in biotechnology to create more opportunities for effective conservation and sustainable utilization of genetic resources for food and agriculture. This year conference's theme is Harnessing Technology for Conservation and Sustainable Use of Genetic Resources for Food and Agriculture. The conference was organized by Indonesian Agency for Agricultural Research and Development (IAARD), Ministry of Agriculture, Indonesia, in collaboration with Indonesian Biotechnology Consortium and held on 24^{th}-25^{th} of May 2021virtually due to the pandemic of COVID-19.

The conference aims to share and exchange current scientific information and technological developments on biotechnology and their applications for conservation and sustainable use of genetic, to encourage and promote quality, efficiency, and modernization of management and utilization of genetic resources, and to facilitate national and international collaboration among participants. There are five scopes discussed in this conference. They are effective management of conservation and sustainable use of genetic resources for food and agriculture, application of genomics and molecular markers for genetic resource conservation and crop adaptation to climate change, application of innovative crop improvement techniques for conservation and sustainable use of plant genetic resources for food and agriculture, plant cell and tissue culture for conservation and effective utilization of genetic resources, and the use of microbial genetic resources as biological control agents of agricultural pests and diseases, and for soil bioremediation.

Five speakers from the United States of America, Japan, India and Indonesia were invited to discuss about their expertise and knowledge on relevant subjects in the plenary sessions. This conference was attended by more than 100 participants including 75 presenters and 44 listeners worldwide. They came from diverse governmental, private, or academic institutions and also scientific communities. The presented materials have undergone peer review processes and only qualified papers were selected. Furthermore, all papers were subjected to double blind peer-review and expected to meet the scientific criteria of significance and academic excellence to be published in a conference proceedings indexed in a well-known, reputable service.

We would like to express our sincere gratitude to our speakers, presenters and all participants for their contributions in this conference. We would also like to express our appreciation for the generosity of our sponsors that support this conference: PT CropLife, PT ITS Science Indonesia, PT Fajar Mas Murni and PT Prima Instrument Analitika. Lastly, special thanks to all committee members for their exceptional work and contributions in the conference and publication.

Chair of Organizing Committee

Dr. Toto Hadiarto

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Hybrid purity assessment in F_1 hybrids segregating for phytophthora root rot resistance genes of chili pepper (*Capsicum annuum* L.)

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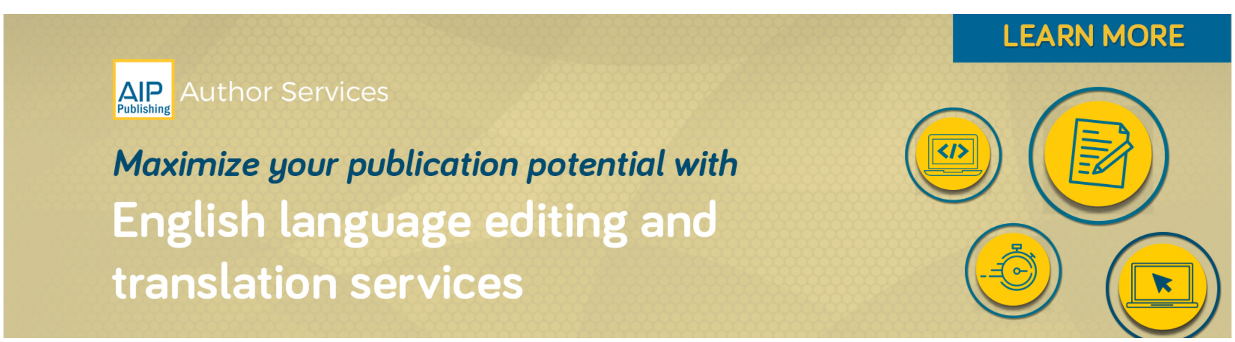

Hybrid Purity Assessment in F_1 Hybrids Segregating for Phytophthora Root Rot Resistance Genes of Chili Pepper (*Capsicum annuum* L.)

Fatimah[1,a)], Reflinur[1], Joko Prasetiyono[1], Wartono[1], Kristianto Nugroho[1], Rinda Kirana[2], Dani Satyawan[1], Rerenstradika Tizar Terryana[1], Aqwin Polosoro[1], Puji Lestari[3] and I Made Tasma[1]

[1]*Indonesian Center for Agricultural Biotechnology and Genetic Resources Research and Development, IAARD, Jln. Tentara Pelajar No. 3A, Bogor 16111, West Java, Indonesia*
[2]*Indonesian Vegetables Research Institute, IAARD, Jln. Raya Tangkuban Parahu No. 517, Lembang, West Bandung 40391, West Java, Indonesia*
[3]*Indonesian Center for Rice Research, IAARD, Jln. Raya No. 9, Sukamandi, Subang 41256, West Java, Indonesia*

[a)]Corresponding author: fatimahsuw@gmail.com

Abstract. Root rot caused by *Phytophthora capsici* is the most devastating disease in Indonesia causing up to 100% yield loss. Breeding resistant cultivars is the most effective strategy to reduce losses caused by this disease. This study aimed to (1) determine the parentage confirmation and genetic relatedness of the parents, (2) genotype two F_1 populations using a subset of six markers, and (3) measure the horticultural characteristics of two F_1 populations. Tanjung-2 and Ciko varieties were used as recurrent parent (RP) in a cross with donor parent (DP) CM334 having Phytophthora root rot resistance gene. Molecular analysis of the parents was done using 33 markers linked to the resistance genes of which 17 markers demonstrated clear polymorphism. The phylogenetic tree classified the parents into two groups, i.e. group 1 consisted of two RPs (Ciko and Tanjung-2) and group 2 consisted of CM334. Clustering analysis clearly revealed that genetic materials used in this study were not closely related. Genotyping using six polymorphic markers showed that the marker-hybrid purity index ranged from 47.17 to 100% for F_1 Tanjung-2/CM334 and 80 to 100% for F_1 Ciko/CM334. The selected F_1 was self-hybridized to develop F_2 population. Horticulture characterization showed that branch number per plant, fruit diameter, fruit weight, and total fruit weight per plant of F_1 Ciko/CM334 were higher than F_1 Tanjung-2/CM334. Meanwhile, for plant height, canopy diameter, flowering time, and harvesting time the F_1 Tanjung-2/CM334 demonstrated better performances than the F_1 Ciko/CM334. The populations developed from this study can be used in genetic linkage mapping of genes conferring resistance to *P. capsici*.

INTRODUCTION

Chili pepper (*Capsicum annuum* L.) is one of the vegetable crops with high economic value and highly consumed in the world, including in Indonesia [1]. Data from the Central Bureau of Statistics and the Directorate General of Horticulture [1] shows that national chili productivity in 2015–2016 decreased by 0.18%. One of the factors causing a decrease in chili productivity is susceptibility to pests and diseases.

One of the limiting factors for chili pepper production in Indonesia is root rot disease caused by *Phytophthora capsici* Leon. The pathogen of this disease can infect chili pepper plants at any growth and developmental stages, causing damping off, root rot, stem rot, collar rot, fruit rot, and foliar blight. *P. capsici* root rot (PRR) is the most devastating disease of chili pepper, causing up to 100% yield losses under warm (25–28°C), humid environmental conditions [2]. This disease syndrome depends on the host species, the point of infection and is also influenced by environmental conditions. Even further, the severity of this disease is influenced by plant stage, adult plants are generally more resistant than seedlings or young fruit [3].

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Currently, farmers generally control Phytophthora diseases chemically, by irrigation management, crop rotation, and fungicide applications [4]. However, overuse of fungicide not only lead to increased production costs, but also possess health risks to farmers and consumers, as well as environmental harms. Fungicide is applied to minimize yield loss because once the pathogen attacks the crop, it will be difficult to control the disease [5]. *P. capsici* can also easily move in a quick manner from one farm to another [6]. Climate change phenomenon such as floods and storms can cause new initiations to make the disease even more widespread [7].

Indonesian Vegetables Research Institute (IVEGRI) has released several superior chili pepper varieties, such as Tanjung-2 and Ciko which have yield potential above 12 t/ha and 20.5 t/ha, respectively. Tanjung-2 variety was released in year 2001, while Ciko in year 2011. Tanjung-2 is classified as an early maturing variety, first harvest starting at 70 days after planting (DAP), simultaneous fruit ripeness, short harvest period, and radiant red fruit colour which gave a more attractive red colour for pasta products. Tanjung-2 variety is relatively tolerant of thrips (*Thrips parvispinus*), aphids (*Myzus persicae*), and white fly (*Bemisia tabaci*), but susceptible to PRR [8].

Utilization of molecular marker technology has been recognized as a powerful tool for breeding applications. To date, several molecular markers associated with resistance to PRR in chili pepper which can be used for more rapid selection have been reported [9–11]. Major quantitative trait locus (QTL) for resistance to PRR was identified in chromosome 5 [12–14] and chromosome 10 [10]. Several studies have identified and transferred these QTLs into elite varieties and molecular markers were identified for MAS applications [14–18].

Due to the complex pathosystem of *P. capsici* [19] and largely absent of genetic resistance sources in the most affected crop plants (pepper, eggplant, and cucurbits) [20], the incidence of PRR has been increasing. Therefore, the use of resistant varieties is an option to overcome the problem of the disease. This study aimed to (1) determine the parentage confirmation and genetic relatedness of the parents, (2) genotype two F_1 populations using a subset of six markers, and (3) measure the horticultural characteristics of two F_1 populations.

MATERIALS AND METHODS

This research was conducted in a glasshouse of Indonesian Center for Agricultural Biotechnology and Genetic Resources Research and Development (ICABIOGRAD) in year 2020 to 2021.

Plant Materials and Breeding Scheme

Tanjung-2 and Ciko varieties were used as recurrent parents (RPs) crossed with Criollo de Morelos-334 (CM334) as a donor parent (DP) having for resistance to *P. capsici*. This latter genotype was introduced from Mexico. while Jalapeno was used as susceptible control for Phytophthora disease resistance assay. The F_2 population development scheme for Tanjung-2 and Ciko crossed with CM334 to obtain F_1 seeds is shown in Fig. 1. The selected F_1 was then self-hybridized to develop F_2 population.

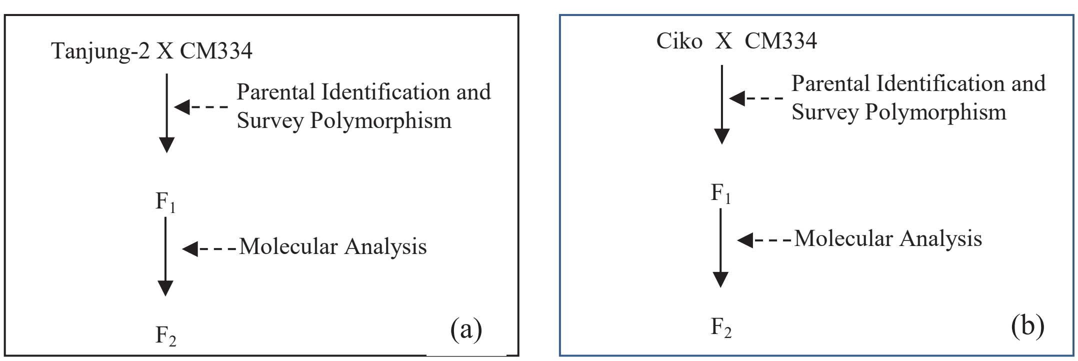

FIGURE 1. F_2 population breeding scheme for the development of chili pepper lines resistance to *Phytophthora capsici*. (a) Tanjung-2/CM334. (b) Ciko/CM334.

Molecular Analysis

Total genomic DNA was extracted using Dellaporta *et al*. [21] method. The molecular analysis was divided into two steps: 1) parental identification, survey polymorphism, and genetic relatedness and 2) genotyping of F_1 hybrid chili pepper. The parental polymorphism was surveyed by using 33 molecular markers near the region linked to PRR resistance genes (Table 1). The markers showing clear polymorphisms were selected for further genotyping studies. PCR was performed in a total volume of 20 µl containing 50 ng of DNA template, 10 pmole of each primer, 1.5 mM of $MgCl_2$, 0.2 mM of dNTP, and 1U of Taq polymerase. The PCR amplification was generated using a PCR machine (Bio-Rad T100^{TM} Thermal Cycler) by following PCR conditions: (i) an initial denaturation step at 94°C for 2 min, (ii) 30 cycles at 94°C for 45 sec, at 55°C for 45 sec, at 72°C for 1 min, and (iii) a final extension step at 72°C for 5 min. Amplified products was separated by electrophoresis in 8% polyacrylamide gel at 100 volts (C.B.S. Scientific) or in agarose gel, observed with ethidium bromide or silver staining, and photographed under ultraviolet light using a gel documentation system (Bio-Rad ChemiDoc^{TM} EQ Imaging System).

TABLE 1. Molecular markers linked to genes or QTLs resistant to *Phytophthora capsici* used in this study.

Resistance locus	Marker or gene	Marker Type[a]	Repeat Motif	Reference
Phyto5.2- LG3	CAMS152	SSR	(ac)15a(ta)8	[15]
	CAMS612	SSR	(taa)16	[15]
	CAMS011	SSR	(ca)3(ac)11a(ta)3	[15]
	CAMS390	SSR	(ag)19	[15]
Phyto5.2-LG15	CAMS362	SSR	(tc)9	[15]
	CAMS051	SSR	(gt)3a(ta)4(tg)11	[22]
	CAMS163	SSR	(at)7(gt)16	[22]
	CAMS211	SSR	(tg)7	[15]
	CAMS420	SSR	(tc)5ca(tc)4	[22]
	CAMS319	SSR	(tc)20	[15]
	CAMS839	SSR	(tct)16	[15]
	CAMS134	SSR	(ac)18	[15]
Phyto5.2	P5-SNAP	SNAP	-	[23]
	Phyto5.2.	SCAR	-	[23]
CaPhyto	CAeMS-068	SSR	(cct)5ctt(cct)3	[22]
	CaDMR1-dCAPS	dCAPS	-	[24]
	NBS1-CAPS	CAPS	-	[16]
	ZL6726	SSR		[9]
	ZL6970	SSR		[9]
	ge26-90pmH0045C	SSR	(ac)19a(ta)9g(ga)3	[25]
	CA524065	SSR	(ct)14(ca)9ga (ca)4ga(ca)4	[9]
Genetic variation	EPMS331	SSR	(ca)10	[26, 27]
	EPMS335	SSR	(acat)3(at)17	[26, 27]
	EPMS404	SSR	(ctt)12	[26, 27]
	EPMS441	SSR	(ag)11	[26, 27]
	GPMS112	SSR	(at)8(gt)19	[26, 27]
	GPMS117	SSR	(ta)25(ga)14	[26, 27]
	GPMS194	SSR	(ta)17(ga)12	[26, 27]
	GPMS197	SSR	(ga)3(tat)16	[26, 27]
	GPMS29	SSR	(gt)15(ggt)7(gtt)2	[26, 27]
	Hpms1-1	SSR	(ca)12(ta)4	[28]
PhR10	P52-11-21	SSR		[10]
	P52-11-41	SSR		[10]

SSR = simple sequence repeat, SNAP = single nucleotide amplified polymorphism, SCAR = sequence characterized amplified region, dCAPS = derived cleaved amplified polymorphic sequence, CAPS = cleaved amplified polymorphic sequence.

Horticultural Characterization

The horticultural characteristics observed in this study included plant height, stem diameter, canopy diameter, branch number per plant, harvest time, fruit length, fruit diameter, fruit number per plant, fruit weight per plant, and total fruit weight.

Data Analysis

In parental polymorphism analyses, the DNA profiles between two parents were scored visually by comparing the observed DNA bands to a standard DNA ladder. The parameters used to determine the marker characteristics were calculated using PowerMarker software. Dendrogram was generated using NTSYS-pc software. The polymorphism analysis of the F_1 hybrid was carried out by scoring DNA bands as an "A" (when allele was derived from male parent), as a "B" (when allele was derived from female parent), and as an "H" (when the alleles were derived from both parents, hybrid). Purity index for each marker was calculated using scored data by applying the following Bohra *et al*. formula [29]: the number of true hybrids containing alleles of both parents divided by the total number of hybrid seeds tested. The horticulture characteristics of each tested line were analyzed using Microsoft Excel software.

RESULTS AND DISCUSSIONS

Parental Polymorphism and Genetic Relatedness

Among 33 markers surveyed surrounding the region linked with resistance to PRR disease, 17 polymorphic markers showed clear polymorphism and the remaining markers showed either monomorphic (14 markers) or no amplification products (2 markers). These 17 markers were able to be measured in four varieties for genetic relatedness analysis: Tanjung-2 and Ciko as RP, CM334 as DP for source of resistance gene, and Jalapeno as a susceptible check. The marker characteristic data are presented in Table 2.

TABLE 2. Molecular characteristics of SSR markers used for parental polymorphism and genetic relatedness analyses of four chili pepper genotypes used in this study.

Marker	Major allele frequency	Allele number	Allele size range (bp)	Gene diversity	Heterozygosity	PIC value
EPMS331	0.500	2	73–101	0.500	0.000	0.375
EPMS335	0.750	2	250–296	0.375	0.000	0.305
EPMS441	0.750	2	104–128	0.375	0.000	0.305
GPMS197	0.500	3	276–400	0.625	0.000	0.555
Hpms1-1	0.750	2	258–275	0.375	0.000	0.305
P52-11-21	0.750	2	125–150	0.375	0.000	0.305
P52-11-41	0.500	3	300–400	0.625	0.000	0.555
ZL6726	0.875	2	152–195	0.219	0.250	0.195
CA524065	0.750	2	230–250	0.375	0.000	0.305
CAMS152	0.750	2	180–190	0.375	0.000	0.305
CAMS612	0.750	2	170–180	0.375	0.000	0.305
CAMS011	0.875	2	150–170	0.219	0.250	0.195
CAMS390	0.500	3	160–170	0.625	0.000	0.555
CAMS051	0.500	3	150–170	0.625	0.000	0.555
CAMS319	0.625	3	160–200	0.531	0.250	0.468
CAMS839	0.500	3	180–210	0.625	0.000	0.555
CAMS134	0.750	2	220–235	0.375	0.000	0.305
Total		40				
Average	0.669	2.353		0.447	0.044	0.379

PIC = polymorphism information content.

The genetic variation analysis revealed that there were 40 total alleles observed with the average number of alleles per locus was 2.3 and ranged from 2 to 3 alleles per marker locus. Gene diversity value ranged from as low as 0.219 to as high as 0.625 with the average of 0.447 and heterozygosity (H_o) value ranged from 0.00 to 0.25 with the average of 0.044. Gene diversity or expected heterozygosity (H_e) is the probability that two alleles are randomly selected from a population [30]. The polymorphism information content (PIC) values ranged from 0.195 to 0.55 with average of 0.379 (Table 1). PIC values provide information about the polymorphism of a marker. The presence of 40 alleles in the four varieties indicates low genetic diversity and low PIC value within the markers tested. The low PIC values were also due to the number of genotypes tested was only four.

The genetic relatedness among genotypes tested was performed using cluster analysis to determine the similarity coefficient matrices calculated from molecular markers to generate a dendrogram. When a cut-off value of 0.57 was used among the four chili pepper genotypes tested, the genotypes were clustered into two groups (Fig. 2). Group 1 consisted of two RP genotypes (Ciko and Tanjung-2) clustered together with Jalapeno, a widely chili pepper genotype that has been used as susceptible check variety. Group 2 consisted a single genotype CM334 known as resistance check and has been used as a DP in this study. The clustering analysis clearly revealed that the breeding materials used in this study were not closely related. If breeders want to increase the probability of introducing several resistance genes/alleles into their breeding material, it would be prudent to introduce resistance genes from the accessions that are genetically distant [27].

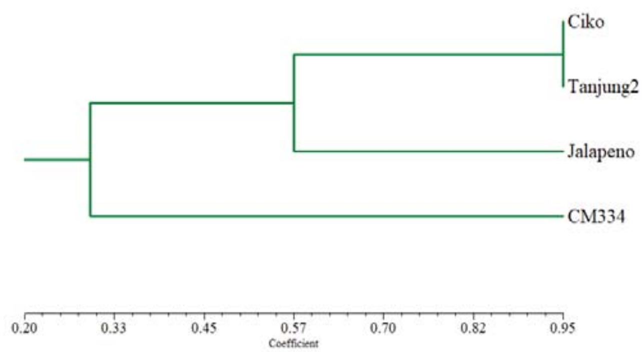

FIGURE 2. Genetic relatedness among the three parental chili pepper genotypes together with a control variety resulted from analysis using 17 SSR markers.

Genotyping of F_1 Hybrid Chili Pepper

Among the 17 polymorphic markers used, 6 markers were found to be suitable for further used in this study and the remaining 11 markers loci showed either large number of nonessential genotypes or no amplification products at all based on the analysis results using more than 25% of F_1 plants tested. To identify a set of informative markers that could be used for F_1 hybrid genotyping of, minimum number of SSR marker was determined based on the parentage confirmation and amplification pattern (Fig. 3). A subset of six markers can distinguish both of the parental alleles as well as the susceptible check allele with high quality banding patterns. The six selected markers were then used to verify the F_1 hybrid. From the six markers, five markers were previously reported [15] linked to *Phyto5.2* resistance locus. This marker was located on LG3 and LG15 on chromosome 5. Other markers included P52-11-21 linked to PRR resistance gene [10]. PRR resistance was reported to be controlled by a single dominant gene, *PhR10*, that was mapped to an interval of 16.39 Mb at the end of the long arm of chromosome 10 [10].

The frequency of tested hybrids showing banding patterns typical of both parental alleles was dependent upon the markers under analysis. The marker-hybrid purity index varied between markers, it ranged from 47.17% (P52-11-21) to 100% (CAMS319 and CAMS839) for F_1 Tanjung-2/CM334 (Table 3), and from 80% (CAMS134) to 100% (CAMS390, CAMS051, CAMS319, CAMS839, and P52-11-21) for F_1 Ciko/CM334 (Table 4). Based on this analysis, F_1 Ciko/CM334 showed higher rates of purity compared to that of F_1 Tanjung-2/CM334. These selected heterozygote lines were candidates for developing F_2 population.

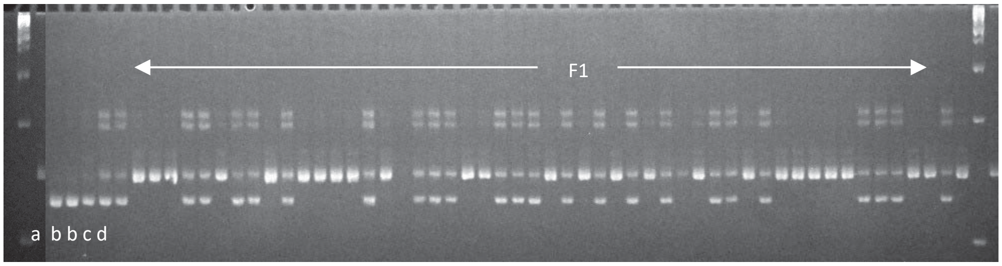

FIGURE 3. Genotyping of F_1 hybrid Tanjung-2/CM334 using SSR marker P52-11-21 electrophoresed in 8% polyacrylamide gel electrophoresis. a = CM334, b = Tanjung-2, c = Jalapeno, d = F_1 Tanjung-2/CM334.

TABLE 3. Hybrid purity index of different SSR markers used for genotyping F_1 hybrid of Tanjung-2/CM334.

Marker	Number of individuals (%)				Purity of designated hybrid (%)
	Lacking amplified product	Amplified product	Having allele from parent A	Having allele from parent B	
CAMS390	1.89	98.11	0.00	47.17	50.94
CAMS051	0.00	100.00	37.74	1.89	60.38
CAMS319	0.00	100.00	0.00	0.00	100.00
CAMS134	24.53	75.47	16.98	0.00	58.49
CAMS839	0.00	100.00	0.00	0.00	100.00
P52-11-21	1.89	98.11	50.94	0.00	47.17

TABLE 4. Hybrid purity index of different SSR markers used for genotyping F_1 hybrid of Ciko/CM334.

Marker	Number of individuals (%)				Purity of designated hybrid (%)
	Lacking amplified product	Amplified product	Having allele from parent A	Having allele from parent B	
CAMS390	0.00	0.00	0.00	100.00	100.,00
CAMS051	0.00	100.00	0.00	0.00	100.00
CAMS319	0.00	100.00	0.00	0.00	100.00
CAMS134	20.00	80.00	0.00	0.00	80.00
CAMS839	0.00	100.00	0.00	0.00	100.00
P52-11-21	0.00	100.00	0.00	0.00	100.00

The F_1 hybrids contain DNA from both parents and SSR markers identified both male and female parent-specific markers allowing differentiation of true hybrids from selfed individuals and outcrossed individuals with foreign pollen. Presence of non-essential genotypes observed among the hybrid seedlings established the importance of hybrid purity tests and those false hybrids could be removed at the early stage of multiplication. The SSR marker information through this study will be of immense help to select appropriate marker combinations and assess hybrid purity of the clones. Other possible applications of hybrid purity assessment include development of genetic linkage maps, quantitative analysis of economically important traits, and marker assisted selection where true and pure hybrids are essential [31].

Horticultural Characterization

The F_1 hybrids of Tanjung-2/CM334 generally have a tall growth habit (82 cm) with wide canopy diameter (78 cm) compared to Tanjung-2 (50 cm and 70 cm, respectively) and CM334 (68 cm and 45 cm, respectively). Meanwhile, F_1 Ciko/CM334 were similar to Ciko with a compact growth habit. CM334 had the highest branch number per plant (9) and stem diameter (10 mm), while the branch number per plant of Tanjung-2 and Ciko varieties were similar (6). Nevertheless, the stem diameter of Ciko was higher (9 mm) than that of Tanjung-2 variety

(8 mm). The stem diameter of F_1 Tanjung-2/CM334 and F_1 Ciko/CM334 were similar (8.8 mm). However, the branch number per plant of F_1 Ciko/CM334 was higher (8.4) than that of F_1 Tanjung-2/CM334 (5.4). Comparison between parents and the hybrids revealed that Ciko was the earliest to flower and to harvest (70 and 84 days after sowing/DAS, respectively) followed by F_1 Ciko/CM334 (74.8 and 93.6 DAS), Tanjung-2 (74 and 103 DAS), CM334 (78 and 105 DAS), and F_1 Tanjung-2/CM334 (96.6 and 133 DAS, respectively) (Table 5).

The fruit length and the fruit weight of Tanjung-2 and Ciko varieties were similar (10 cm and 10 g). Nevertheless, the fruit diameter of Tanjung-2 was higher (19.3 mm) than Ciko (18.2 mm), while CM334 had tiny fruits and less weight (3.8 cm and 3 g) with wide fruit diameter (13.3 mm). The fruit length of F_1 Tanjung-2/CM334 dan F_1 Ciko/CM334 were similar (8.5 cm). Nevertheless, the fruit diameter and weight of F_1 Ciko/CM334 was higher (19.5 mm and 9.2 g) than that of F_1 Tanjung-2/CM334 (15.5 mm and 7.2 g). The observations of the yield components of average total fruit number and fruit weight revealed that the highest number and weight of total fruit was obtained from Ciko (21.4 fruits and 209.1 g), while CM334 had low number and fruit weight (6.3 fruits and 10 g). The average total fruit number of Tanjung-2, F_1 Tanjung-2/CM334, and F_1 Ciko/CM334 were similar (11 fruits). However, the average total fruit weight of Tanjung-2 was higher (112.9 g) compared to that of F_1 Ciko/CM334 (107.14 g) and F_1 Tanjung-2/CM334 (81.9 g) (Table 5, Fig. 4).

TABLE 5. The average of horticulture characteristics of chili pepper varieties in Tanjung-2, Ciko, CM334, F_1 Tanjung-2/CM334, and F_1 Ciko/CM334.

Characteristics	Tanjung-2	Ciko	CM334	F_1 Tanjung-2/CM334	F_1 Ciko/CM334
Plant height (cm)	50.0±1.53	50.0±2.14	68.3±5.77	82.0±12.04	56.4±5.41
Canopy diameter (cm)	70.0±4.18	37.0±3.56	45.7±5.13	78.0±6.71	49.8±7.50
Stem diameter (mm)	8.0±0.27	9.0±0.35	10.0±0.00	8.8±0.76	8.8±1.10
Branch number per plant	6.0±1.20	6.0±0.51	9.0±1.73	5.4±0.55	8.4±1.67
Flowering time (days)	74.0±5.82	70.0±0.40	78.0±0.00	96.6±13.05	74.8±1.79
Harvesting time (days)	103.0±0.5	84.0±0.00	105.0±0.00	133.0±17.36	93.6±3.58
Fruit length (cm)	10.1±2.01	10.6±1.46	3.8±0.61	8.5±0.68	8.5±1.06
Fruit diameter (mm)	19.3±2.34	18.2±1.71	13.3±2.03	15.5±1.74	19.5±1.98
Fruit weight (g)	10.5±2.67	10.9±2.16	3.0±1.28	7.2±1.39	9.23±1.70
Total fruit number per plant	11.3±2.6	21.4±3.8	6.3±4.16	11.4±3.97	11.6±19.15
Total fruit weight per plant (g)	112.9±16.79	209.1±21.5	19.0±9.02	81.95±28.12	107.14±29.20

FIGURE 4. Variations of chili pepper fruits and seeds performances observed from the parents, F_1 hybrid Ciko/CM334, and F_1 hybrid Tanjung-2/CM334 progenies. (a) From left to right: CM334, Ciko variety, and F_1 Ciko/CM334. (b) From left to right: CM334, Tanjung-2 variety, and F_1 Tanjung-2/CM334.

CONCLUSION

Parentage and genetic relatedness analyses of the parental chili pepper genotypes demonstrated that the parents were classified into two groups, i.e. group 1 consisted of two RPs (Ciko and Tanjung-2) and group 2 consisted only one DP of CM334. Genotyping two F_1 populations developed from these parents using a subset of six markers resulted higher marker-hybrid purity index in F_1 Ciko/CM334 than that in F_1 Tanjung-2/CM334. The horticultural characteristics of the two F_1 populations revealed that the fruit diameter, fruit weight, and total fruit weight per plant of F_1 Ciko/CM334 were higher than that of F_1 Tanjung-2/CM334. Hybrid purity assessment has confirmed the specific true and pure hybrid identification. The subset of six SSR markers could be used to select the true hybrids of the F_1 progenies. The populations developed from this study can be used in genetic mapping of genes resistance to PRR.

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