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MOLECULAR DIVERSITY IN POPULATIONS OF CHILI (*CAPSICUM ANNUUM* L.)

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SUMMARY

Chili (*Capsicum annuum* L.) is a self-pollinated crop, with natural cross-pollination occurring below 4%–5%. It intends to have low heterosis. Developing cross-pollination in chili currently receives much attention to achieve diversity in trait improvement. Double-crossing becomes one of the alternatives to achieving this goal. In this study, three different parental chili genotypes (K, B, and T) gained crossing, with four populations (S2 K, F3 KB, F2 BTKB, and F2 KBBT) developed. Using 11 selected sequence-related amplified polymorphism (SRAP) combination markers that target Open Reading Frame (ORF) regions assessed molecular diversity in these chili populations. Results revealed the possibility of identifying diversity using SRAP markers based on primer profile information. The iMEC analysis showed high values of PIC (0.3381), discriminant power (0.882), and mean polymorphic value (97.88%). The highest similarity emerged between the populations BTKB and KBBT as the reciprocal. Then, the smallest similarity appeared between K and the double cross. Compared with the self-pollinated genotype, SRAP primers discovered that double crosses provided more variation based on Shannon's index (I) and percentage of polymorphic loci (PPL). The genetic distance denotes maternal inheritance or extraneous involvement in progeny. However, multiple-parent hybridization authenticated the boost in genetic diversity.

Keywords: Interspecific hybridization, chili hybrid, segregation, diversity of hybrid chili, reciprocal, separated clustering

Key findings: Eleven selected SRAP marker combinations can detect genetic diversity in the chili (*Capsicum annuum* L.) hybrid populations. The double cross population also has the potential to address the uniformity problem in the chili hybrids.

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INTRODUCTION

Hybridization and mutation are vital tools mostly used to create variation and diversity among crop plants. In chili (*Capsicum annuum* L.), hybridization can also enhance genetic diversity, using its impact for improvement (Gurung *et al*., 2020). One of Indonesia's primary vegetables, it has a high economic value, with most farmers cultivating hybrid cultivars (Syukur *et al*., 2010). Chili household consumption reached 596,140 t in 2021, an increase of 8.49% (46,670 t) compared with 2020 consumption (Irjayanti *et al*., 2021). A breeding program can optimize chili production by creating new genotypes and improving the

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desirable yield-related traits. Capsicum species have perfect flowers where male and female reproductive structures come in a single flower, which performs self-fertilization as their primary mating system (Rêgo *et al*., 2012; Deka *et al*., 2016). Pure lines mostly lack favorable traits; thus, F_1 hybrid chilies are the most commonly cultivated. Accordingly, crossing multi-parent proves advantageous, besides providing a broad genetic base (Pathy *et al*., 2019) and preventing the further narrowing of the genetic base (Cooper *et al*., 2001) in chili populations.

Assembling parental lines from diverse genetic populations provides the basis to address the issue of genetic uniformity, which mostly becomes vulnerable to pests and diseases (Pathy *et al*., 2018). Broadening the genetic base may be necessary for maintaining, even increasing, the current levels of diversity, complementing new crop improvements, such as, hybrid breeding (Cooper *et al*., 2001; Yeh *et al*., 2016). Double cross is a technique to combine desired traits in genotypes, requiring four parents (Lebenzon, 1988). The progeny derived from hybrid parents generates more genetic variations. Hence, using cultivars with a broad genetic base to produce hybrid progeny helps sustainable chili production (Pathy *et al*., 2018). For the breeding program, progeny variation should be examined, with these populations used in producing promising new lines that can serve as candidates for new cultivars as pure lines (open-pollinated cultivars) and as parental genotypes in the production of the hybrids.

Previous studies characterized the genetic variation in chili using morphological, biochemical, and molecular markers. Molecular markers are preferred over traditional phenotypic and biochemical markers because they provide more polymorphisms and preciseness and are unaffected by environmental factors (Massa *et al*., 2001). Sequence-related amplified polymorphism (SRAP) is a PCR-based dominant marker technique. Known as better than common dominant markers like RAPD, AFLP, and ISSR markers, it offers the advantages, such as simplicity, low cost, versatility, and moderate codominance (Li and Quiros, 2001; Yi *et al*., 2021). The principle of SRAP focuses more on amplified coding region of open reading frames (ORF) of the genome, including two primers, a forward primer targeting GC-rich sequence on exons to ORF regions, and an AATT sequence in reverse primer to aim at AT-rich regions frequently on promoters, introns, and spacers (Li and Quiros, 2001; Robarts and Wolfe, 2014). However, SRAP markers cannot discriminate heterozygosity and consider the data scored dominant. Using SRAP has successfully assessed patterns of genetic variability in *Capsicum frutescens* (Hadthamard *et al*., 2021), elephant grass (Xie *et al*., 2009), rice (Dai *et al*., 2012), banana and plantain (Youssef *et al*., 2011), and grape (Gou *et al*., 2012).

Assessment of genetic diversity in chili single-cross and double-cross segregated populations using SRAP markers remains limited. Hence, this study is interested in the pattern of grouping hybrid chili populations and their reciprocity and diversity. The latest study aimed to assess the values of genetic diversity and genetic distance in chili hybrid populations, comparing the estimated variance within and among the populations and identifying the variation in chili single-cross and double-cross hybrids.

MATERIALS AND METHODS

Plant material and procedure

Three chilies (*Capsicum annuum* L.) parental cultivars, i.e., Cabai Besar (B), Cabai Keriting Cipanas (K), and Cabai TM999 (T), were crossed to generate single-cross and double-cross populations. The double cross came from crossing F_1 KB (Cabai Keriting × Cabai Besar) to F_1 BT (Cabai Besar × Cabai TM999) and its reciprocal. TM999 is a commercial hybrid. The current research involves 22 populations of self-pollinated Keriting Cipanas (S_2 K), 23 third filial populations of a single cross (F_3 KB), and 24 and 23-second filial populations of the double cross (F_2 BTKB and F_2 KBBT), respectively. All the selected samples underwent molecular diversity analysis. The experiments began from November 2020 until March 2022, with all the breeding materials cultivated in the experimental field at Banguntapan, Yogyakarta, Indonesia. The molecular marker analysis ensued at the Laboratory of Genetic and Plant Breeding, Faculty of Agriculture, Universitas Gadjah Mada, Indonesia.

DNA extraction

Total DNA extracted from 100 mg of young leaves of each sample employed the modified method of Doyle and Doyle (1990). A pellet of DNA dissolved in 100 µL ddH_2O proceeded to

mix for quantitative evaluation using a spectrometer (GeneQuant 1300).

The procedure of the SRAP marker system

Thirty-five primer combinations gain evaluation, with 11 SRAP primer combinations selected (Table 1). The selected primer combinations applied to DNA samples of the 92 lines produced the polymorphic and some codominant bands. For amplification preparation, each 10 µL of PCR components consisted of 2.5 µL template DNA, 6 µL GoTaq® Green Master Mix 1x (Promega), 1.5 µL Nuclease-Free Water (Promega), and 0.5 µL each primer forward and reverse. Protocol of PCR cycle supported by T100™ thermal cycler (Bio-Rad) was used. Afterward, pre-denaturation at 94°C for 5 min followed, with the first five cycles programmed as 94°C for one minute, 35°C for one minute, 72°C for one minute; then 35 cycles consisted at 94°C for one minute, 50°C for one minute, 72°C for one minute; and ended by a final extension at 72°C for eight minutes. The amplified products were separated by 1.5% agarose gel with FloroSafe DNA staining, with the gel electrophoresis run at 400 v and 100 mA for 75 min and then visualized under UV light.

Statistical analysis

The distribution of amplified DNA bands was converted into binary data, present (1) or absent (0), with the data analysis using iMEC, an online marker efficiency calculator (Amiryousefi *et al*., 2018) that provides heterozygosity index (H), polymorphic information content (PIC), effective multiplex ratio (E), marker index (MI), discriminating power (D), resolving power (R), an additional total number of bands (TNB), number of polymorphic bands (NPB), and percentage of polymorphic bands (PPB) to elucidate information of polymorphism degree and profile of the primers. Further, the NTSYS-pc software package estimated relationships at the germplasm level, with the genetic similarity coefficient based on Dice, evaluated to construct an Unweighted Pair Group Method with Arithmetic Mean (UPGMA) dendrogram to determine the genetic relationship. Genetic distance, genetic identity, analysis of molecular variance (AMOVA), diversity parameters, and principal coordinate analysis (PCoA) used theGenAlEx 6.5 (Peakall and Smouse, 2012).

RESULTS

Profile information of SRAP

The well-established variation within the number of bands found at each locus exhibits that the SRAP markers include a high average polymorphism value (97.88%) (Table 2). Confirmed profile information of 11 primer combinations based on iMEC revealed that the SRAP primers ably peruse and distinguish each chili genotype. A high average PIC value (0.3381) close to the maximum value (0.5) indicates a high level of polymorphism, such as the percentage of polymorphic bands (PPB). The effective multiplex ratio (E) value of 11 primers had a wide range (3.4673 – 8.8804) with a mean of 6.2905. The mean value of resolving power (R) was 6.6047, while the discriminating power (D) at 0.882. These two parameters describe the ability of markers to distinguish the chili genotypes. The mean heterozygosity (H) value at 0.4214 indicated the genetic variation among the 92 chili lines was relatively high.

Genetic similarity according to Nei's coefficient

Two populations (F2 KBBT and F2 BTKB) with near genetic distance have high genetic identity values (Table 3). Chili hybrid populations F2 KBBT and F2 BTKB have the closest genetic distance (0.050) with the highest value of genetic identity (0.952). These values indicated these populations were close together and exhibited a more relevant similarity. The maximum distance emerged between KBBT and K (0.092), followed by BTKB and K (0.091), which also have smaller genetic identities, i.e., 0.912 and 0.913, respectively. These two chili populations of the double cross (F2 KBBT and F2 BTKB) indicated the lowest similarity to population K due to two earlier generations. Population KB as the female parent for hybrid KBBT has a closer genetic distance (0.055) compared with BTKB (0.066), with the same also illustrated by the value of the genetic identity of KB - KBBT (0.947), which was smaller than KB - BTKB (0.936). The K was most relative to KB (0.083); thus, K was closest to KB based on genetic identity (0.921).

Table 1. SRAP primers used in the study.

Marker combinations	Sequence 5'- 3'
me2-em4	F: TGAGTCCAAACCGGAGC, R: GACTGCGTACGAATTTGA
me4-em4	F: TGAGTCCAAACCGGACC, R: GACTGCGTACGAATTTGA
me5-em4	F: TGAGTCCAAACCGGAAG, R: GACTGCGTACGAATTTGA
me5-em5	F: TGAGTCCAAACCGGAAG, R: GACTGCGTACGAATTAAC
me6-em6	F: TGAGTCCAAACCGGACA, R: GACTGCGTACGAATTGCA
me7-em4	F: TGAGTCCAAACCGGACG, R: GACTGCGTACGAATTTGA
me8-em4	F: TGAGTCCAAACCGGACT, R: GACTGCGTACGAATTTGA
me8-em7	F: TGAGTCCAAACCGGACT, R: GACTGCGTACGAATTCAA
me9-em4	F: TGAGTCCAAACCGGAGG, R: GACTGCGTACGAATTTGA
me10-em4	F: TGAGTCCAAACCGGAAA, R: GACTGCGTACGAATTTGA
me10-em5	F: TGAGTCCAAACCGGAAA, R: GACTGCGTACGAATTAAC

Table 2. SRAP primer profile of the whole chili population using iMEC.

Primer combination	Size (bp)	TNB	NPB	PPB (%)	PIC	E	MI	R	H	D
Me2-em4	1500-100	18	18	100	0.3431	5.2826	0.0013	5.5652	0.4146	0.9139
Me4-em4	690-110	14	13	92.85	0.3043	7.2391	0.0028	6.6956	0.4994	0.732
Me5-em4	1490-100	20	20	100	0.3215	7.3043	0.0018	9.2173	0.4636	0.8667
Me5-em5	700-105	12	12	100	0.3073	5.3152	0.0023	3.8043	0.4934	0.804
Me6-em6	1400-100	18	17	94.44	0.3306	5.9782	0.0016	4.0869	0.4436	0.8898
Me7-em4	1500-110	22	22	100	0.371	4.7934	0.0008	5.5	0.3408	0.9526
Me8-em4	1400-115	23	22	95.65	0.3483	6.4021	0.0012	7.1956	0.4017	0.9226
Me8-em7	2000-110	27	27	100	0.3488	7.4782	0.0012	10.1304	0.4005	0.9233
Me9-em4	1200-110	31	31	100	0.3455	8.8804	0.0012	11.5869	0.4088	0.918
Me10-em4	1000-100	16	15	93.75	0.3075	7.0543	0.0023	4.8913	0.4930	0.8057
Me10-em5	1450-110	21	21	100	0.391	3.4673	0.0004	3.9782	0.2757	0.9728
Mean		20.18	19.18	97.88	0.3381	6.2905	0.0015	6.6047	0.4214	0.882

Note: TNB: total of number bands; NBP: number of polymorphic bands; PPB: percentage of polymorphic bands; PIC: polymorphic information content; E: effective multiplex ratio; MI: marker index; R: resolving power; H: expected heterozygosity; D: discriminating power.

Table 3. Nei's unbiased measures of genetic identity and genetic distance among the populations.

Populations	KBBT	BTKB	KB	K
KBBT		0.952	0.947	0.912
BTKB	0.050		0.936	0.913
KB	0.055	0.066		0.921
K	0.092	0.091	0.083	

Note: Nei's genetic identity (above diagonal) and genetic distance (below diagonal).

Comparison of diversity pattern

Analysis of molecular variance (AMOVA) showed that the variation within the chili population (69%) was higher than among the populations (31%) (Table 4). The excellent distribution of variation within the population resulted from the contribution of each parental allele inherited during hybridization. Hybrid populations (F2 KBBT, F2 BTKB, and F3 KB) will form the diversity inherited from the parents because most populations were a segregation population (F_2 and F_3). The low percentage of variation (PV) among the populations (31%) was due to less diversity between parental and progeny populations.

The Shannon's index (I) and percentage of polymorphic loci (PPL) parameters elucidated that the BTKB population has more variation than KBBT, KB, and K (Table 5). Shannon index can reveal diversity by being interpreted through other parameters, including PPL. High PPL values indicate abundant diversity. For the values of I corresponding to PPL, BTKB has the highest scores of I and PPL, with K the lowest.

The dendrogram visualizes four chili populations in two major clusters—cluster I has K, while cluster II includes three populations KB, KBBT, and BTKB. The least coefficient of similarity (0.51) appeared between the K and the other three populations, causing the separation of the K cluster. Population KB, a direct parent, joined KBBT and BTKB in the same major cluster. Each chili sample assembled in its population, and no mixing occurred. The double cross populations (KBBT and BTKB), as reciprocals separated in the sub-sub-cluster, demonstrated a massive coefficient of similarity. The most significant coefficient (0.89) came from the samples KBBT 24_2 and KBBT 24_5.

Table 4. Analysis of molecular variance (AMOVA) based on the SRAP markers inferred four chili populations.

SV	df	SS	MS	Est. Var	PV (%)
Among population	3	670.058	223.353	8.870	31
Within population	88	1707.899	19.408	19.408	69
Total	91	2377.957		28.278	100

Note: SV: source of variance; df: degree of freedom; SS: sum of the square; MS: mean square; Est. Var: estimated variance; PV (%): percentage of variance.

Table 5. Genetic diversity of four chili populations.

Populations	N	Na	Ne	I	PPL (%)
KBBT	23	1.369	1.288	0.267	64.86
BTKB	24	1.437	1.301	0.284	68.47
KB	23	1.167	1.292	0.261	54.50
K	22	1.104	1.212	0.203	53.60

Note: N: sample size; Na: number of different alleles; Ne: number of effective alleles; I: Shannon's information index; PPL: percentage of polymorphic loci.

DISCUSSION

SRAP markers used precisely assessed the diversity among the 92 chili accessions. SRAP amplifies the open reading frame (ORF) region, and the advantage of SRAP consists of forward and reverse primers that can be combined randomly (Li and Quiros, 2001). For the primer selection criteria, the primer combination must be a polymorphic marker capable of recognizing the four chili populations (K, KB, BTKB, and KBBT). Fundamental to recognize the primer profile information utilizing iMEC to determine the capacity of the selected primer combinations in distinguishing chili diversity (Amiryousefi *et al*. 2018). Eleven SRAP primer combinations out of 35 primers gained selection for analyzing 92 chili accessions. Relevant to iMEC data, 11 combinations of SRAP amplify a wide range of fragments starting from 100 to 2000 bp (Table 2). Each primer combination amplifies the bands by varying amounts. The total number of bands (TNB) values ranged from 12 to 31 bands. The number of amplified bands per primer in populations dominated by hybrids (BTKB, KBBT, and KB) was more numerous; thus, the average percentage of polymorphic bands in 11 SRAP combinations was high (97.88%).

The high degree of polymorphism was indicated by analyzing hybrids with a high level of diversity. The high mean value of PIC described the diversity of the hybrid population. The highest value of PIC was presented by me10-em5 (0.391), whereas the lowest was me4-em4 (0.3043). Similarly, in other cases, the PIC value of the SRAP marker displayed moderately high in F_1 of *Streptocarpus* × *hybridus* V. and *Toona ciliate* (0.407 and 0.41), respectively (Li *et al*., 2015; Harta *et al*., 2020). It reflects SRAP as a dominant marker capable of detecting diversity. The PIC parameter depends on the number of alleles identified and the frequency of allele distribution (Botstein *et al*., 1980). A dominant marker with equal distribution of alleles in the population has a higher value, with a maximum of 0.5 (Chesnokov and Artemyeva, 2015). In hybrid progeny, it authenticates that high PIC can identify and specify genetic diversity.

The values of the effective multiple ratios (E) corresponded to the number of polymorphic loci. In this study, the average E value was 6.2905, with the highest in me9-em4 (8.8804) and the lowest in me10-em5 (3.4673). A higher value of E refers to a more efficient primer system (Chesnokov and Artemyeva, 2015). The results of the marker index (MI) parameters were low, with the highest value of 0.0028 in me4-em4. In contrast, a higher MI value (2.314) using the same analysis on prairie grass Yi *et al*. (2021), which may be due to the influence of the higher values of E and H_{av} (Powell *et al*., 1996). The higher value of MI, the more information obtained from primers regarding the plant material (Medhi *et al*., 2014). Higher values of E and MI indicate that the primers were appropriate for analyzing interspecific and intraspecific genetic diversity in mung beans (Singh *et al*., 2014).

Resolving power (R) is the foremost vital parameter to decide the discriminatory efficiency of the primers in the hybrid derivates of *Eleusine coracana* (Venkatesan *et al*., 2021). The average R was 6.6047, lower than 10.74 in the Moroccan argan tree (Pakhrou *et al*., 2020). However, in this study, the R-value has a wide range of 3.8043 (me5-em5) to 11.5869 (me9-em4). A high R-value shows that the various primer combinations can distinguish the cultivars. Expected heterozygosity (H) is a common statistic for evaluating genetic variation within populations (Harris and DeGiorgio, 2017) and describes the proportion of heterozygous genotypes expected under Hardy-Weinberg equilibrium (Nei, 1973). The average value of R was 0.4214, with the highest value of 0.4994 (me4-em4) and the lowest of 0.2757 (me10-em5). The ability of the various primer combinations (me10-em5) was inadequate for analyzing hybrid populations with high heterozygosity.

Discriminating power (D) is the probability that two random individuals have different banding patterns and are recognizable from one another (Amiryousefi *et al*., 2018). The highest D value was 0.9728 (me10-em5), whereas the lowest was 0.732 (me4-em4), with an average of 0.882. The more locus patterns per primer, the value of D will reach a maximum (1.0). The mean value of 0.882 from 11 primer combinations indicates that SRAP was efficient in distinguishing two random individuals. According to iMEC parameter values, including PIC, effective multiplex ratio, marker index, resolving power, expected heterozygosity, and discriminating power, the 11 primer combinations were competent in identifying genetic diversity in the chili hybrid populations.

Using the allelic similarities between the populations and the average over several loci estimates the genetic identity based on genotypic distribution, with the accumulated alleles per locus as an approximation for the genetic distance (Nei, 1972). Beaumont *et al*. (1998) explained genetic distance as a measure of the evolutionary divergence between copies of homologous genes and the mutation that has occurred since a common ancestor of homologous genes. The chili double cross populations (KBBT and BTKB) revealed the closest genetic distance (0.050), having these populations composed of the same genetic material due to F_2 and its reciprocal. Unequal contribution of cytoplasmic determinants from male and female gametes to the zygote and segregation and recombination of parental traits lead to dissimilarities in each individual (Satyavathi *et al*., 2016). When individual chromosomes are toward gametes, alleles of different genes are mixed and matched concerning one another. KB-KBBT (0.055) and KB - BTKB (0.066) have the closest genetic distance compared with K - KBBT (0.092) and K - BTKB (0.091). KB is a direct parent of KBBT, and BTKB also inherits half of the genetic material by giving a more significant similarity. Thus, the genetic distance formed gets closer. The genetic distance of K and KB is also close (0.083). K is a quarter of the composition of the double cross parent, and the distance between K and the double cross progeny populations is considerable.

Genetic distance (D*)* is linear to the number of subpopulations (Nei, 1972). If the number of subpopulations is linearly related to the geographic area where the experimental materials are located, then D becomes a linear function of the region. Thus, one can assume that crosses of individuals originating in an area will have a relatively close genetic distance, hence the explanation of why the crossing between chili genotypes has a small genetic distance in this study. The current study observed the genetic distance of KB is closer to KBBT than BTKB. Several factors can satisfy this possibility, e.g., extra-chromosomal DNA transfer occurs into the nucleus, maternal inheritance, and extra-chromosomal DNA exists through extraction. Kirkpatrick and Lande (1989) and Park *et al*. (2021) described the similarity between parental and progeny as caused by Mendelian inheritance; in some cases, mothers have the strongest influence through chloroplast and mitochondrial genome express non-Mendelian pattern on the progeny. Matsuo *et al*. (2005) stated that DNA conduct from plastids to the nucleus was progressing, and in any case, effective gene conduct was rare. Moreover, the plant nuclear genome is in equilibrium between frequent integration and rapid elimination of the chloroplast genome.

In this study, variation within the population (69%) was exceeding among the populations (31%). The expansive variation within the population occurred due to segregation in the hybrid populations. Benowicz *et al*. (2020) mentioned that segregation variation relates to the increment in variance due to the segregation of alleles in a second filial generation (F_2). The creation of F_2 generations based on broad crosses may alter the quality of relations among the traits and generate novel trait combinations. The role of hybridization expanded genetic variation and new gene combinations to aid the development and acquisition of novel adjustments (Rieseberg, 2003).

Low variance among the populations (31%) occurred, as expected since it compares populations still related to each other. Diploid organisms obtain one allele from each parent randomly. The transfer of genetic material through inherited alleles causes similarities between parents and progeny; thus, variation between populations is low. Rieseberg (2003) stated speciation of diploid hybrid helps the formation of a fit hybrid genotype, and the process prevents gene flow. Gene flow manifests mediated by reproduction and vertical gene transfer from parent to progeny (Choudhuri, 2014) and against local differentiation (Slatkin, 1987).

Nei (1975) claimed that Shannon's index value is tough to interpret. Hence, it requires to be related to other parameters like PPL. Study results further revealed that the BTKB population heads the diversity. Descendant of the double cross has the potential to receive numerous allele combinations. Else parameter that supports the high diversity of BTKB is the number of different alleles (Na) which showed the highest value (1.437). The BTKB is a diploid organism that receives two alleles per locus randomly and the parent with diverse traits, which enables BTKB to receive more diverse alleles, confirmed with the highest percentage of polymorphic loci (68.47%). Three parental genotypes will give more accessibility to genetic material compared with two parents. Instead of utilizing four parents, it uses three parental genotypes, which also explains that the level of diversity in KBBT (I = 0.267) was not significantly different from KB (I = 0.261). However, hybrid populations (BTKB, KBBT, and KB) appear more diverse than self-pollinated (S2 K). Hybrid descendants result from segregation that encourages diversity when non-sister chromatids provide a new combination of genes through crossing over. In agreement with Pathy *et al*. (2018), the progenies of double cross reflect more segregation and recombination than single-cross hybrids. As Osabe *et al*. (2012) mentioned, blending genomes from diverse species gives genome variation and new opportunities to broaden the gene redundancy, which may cover recessive deleterious alleles by dominant ones.

There was increased genetic variability within the F_2 generation due to additional recombination in meiosis (Couto *et al*., 2019). Homologous chromosomes in the first meiosis form a tetrad and then exchange segments of alleles, also known as recombination, cross over randomly happens on every chromosome resulting in different gene combinations. It explains the difference between progenies from their biological siblings (Mercier *et al*., 2015). The genetic distance of BTKB and KBBT was the closest among others, however, referring to the visual of the dendrogram shows that the populations BTKB and KBBT were separated into sub-sub-classes. The clustering results revealed that the four chili populations further divide into two major clusters (I and II). Cluster I only consisted of S2 K, and cluster II included F3 KB and two sub-clusters, F2 BTKB

and F2 KBBT. The coefficient of similarity listed on the dendrogram describes that S2 K has the least similarity (0.51) to the three populations (KB, BTKB, and KBBT). Consequently, S2 K group moved into different classes. The mean coefficient of similarity was quite high (0.70), which was related to the value of the genetic distance between populations close to each other. Genetic distance also responds to the fact that F3 KB merges with double-crosses (BTKB and KBBT) in the dendrogram rather than F3 KB combining the parent (S2 K). If one assumes that each descendant received the same proportion of genetic material from each parent, then each parent contributes one fourth of the total genetic material of the progeny. Zhang *et al*. (2015a) claimed one double cross population from four inbred lines A, B, C, and D as parents, which are homozygous. There may be up to four alleles at each locus. Zhang *et al.* (2015b) added that when no distortion occurs, the four genotypes will form the Mendelian ratio 1:1:1:1. Since Cabai Besar (B) contributes half of the complete genetic information of progeny and Keriting Cipanas (K) only contributes a quarter of the genetic material, then K has a small coefficient of similarity with other populations (KB, BTKB, and KBBT). The dendrogram exhibits that KBBT 24_2 and KBBT 24_5 have a tight similarity coefficient (0.89). Hence, the possibility that the diversity of KBBT and KB based on Shannon's index was not significantly different.

CONCLUSIONS

Utilizing SRAP markers to distinguish the genetic diversity of chili (*Capsicum annuum* L.) hybrid populations is the appropriate alternative; SRAP markers can partition each population and describe each population's diversity as expected. According to the value of genetic distance and genetic identity, KBBT and BTKB have prominent similarities. KB was the female parent for KBBT and has a closer genetic distance compared with BTKB. Investigation of genetic diversity using SRAP markers showed that double-cross populations had the maximum sufficient genetic diversity, followed by a single cross and self-pollinated. Speciation helps to maximize the formation of the unique characteristic of each population from interspecific hybridization for a subsequent breeding program.

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