

Phenotypic variability evaluation and genetic variation in F2 intraspecific hybrids of cucumber (*Cucumis sativus* L.) using retrotransposon-based markers

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Abstract. Setiawan AB, Zahidah QA, Kaltsum DN, Purwantoro A. 2023. Phenotypic variability evaluation and genetic variation in F2 intraspecific hybrids of cucumber (*Cucumis sativus* L.) using retrotransposon-based markers. *Biodiversitas* 24: 2596-2604. Cucumber (*Cucumis sativus* L.) has a narrow genetic base compared to other *Cucumis* species. The genetic base of the cucumber can be improved by crossing two distantly related cultivars from different geographical locations. This study aimed to evaluate phenotypic variability and investigate genetic variation of F2 cucumber population using two retrotransposon-based markers, namely Inter Retrotransposon Amplified Polymorphism (IRAP) and Inter-SINE Amplified Polymorphism (ISAP). Two parental lines and the selected 59 genotypes from F2 population were subjected to PCR analysis for DNA profiling. The phenotypic variation of F2 genotypes revealed significant diversity in fruit weight, fruit diameter, fruit radian, pedicel length, fruit length, and fruit ratio (length-diameter). The genetic variation of the F2 hybrids was successfully detected and discriminated by IRAP and ISAP markers. Both IRAP and ISAP markers showed high heterozygosity ranged from 0.4021 to 0.4997, with an average of 0.4649 and moderate polymorphic information content ranged from 0.3489 to 0.3929 with an average of 0.3648. Cluster analysis showed that the F2 population and parental lines were grouped into two major clusters with a similarity coefficient ranged from 0.67 to 0.96. These findings imply that the F2 population is highly segregated and can be utilized to create new cultivars of cucumber.

Keywords: *Cucumis sativus*, genetic variation, IRAP marker, ISAP marker, phenotypic variation

INTRODUCTION

Cucumber (*Cucumis sativus* L., $2n = 2x = 14$ chromosomes) is an annual crop that is one of the most valuable species in the genus *Cucumis* (Wibowo et al. 2018; Setiawan et al. 2020a; Sharma et al. 2020). Despite its economic importance, cucumber has a narrow genetic base according to molecular genetics and cytogenetics constitution (Staub et al. 2005; Wibowo et al. 2018). Genetic improvement of cucumber can be improved through intraspecific hybridization between two cultivars of diverse origin and/or interspecific hybridization with its closely related species, transfer gene via agrobacterium mediated transformation, and genome editing such as CRISPR/Cas 9 (Chen et al. 1997, 2002, 2003; Nie et al. 2015; Chandrasekaran et al. 2016; Wang et al. 2017; Xin et al. 2022). However, Interspecific hybridization has reproductive barriers that prevent mating and fertilization as well as post-fertilization problems (Kuligowska et al. 2015). Moreover, transfer gene and genome editing require a well-established laboratory and are time- and money-consuming. The most effective and simple way for plant breeders to create new cultivars with desired features in cucumber and other plant species has been proven via intraspecific hybridization (Choi et al. 2018; Sáez et al. 2021; Liu et al. 2022).

Hybridization and recombination are required to acquire segregating materials for use in plant selection

procedures, resulting in new varieties with improved yields and resistance to biotic and abiotic stresses. Hybridization can result in an F1 offspring with a strong heterotic impact, allowing transgressive variants to emerge and be chosen in segregating generations (Cazzola et al. 2020). Transposable elements are abundant in plant genomes and have an impact on genome structure, evolution, function, and genetic diversity because of insertion and deletion events (Moghaddam et al. 2014; Seibt et al. 2016; Setiawan et al. 2020c; Li et al. 2022a). The cucumber genome size ranged from 232.5 Mb to 251.1 Mb depending on accessions, with Long Terminal Repeat (LTR) retrotransposons accounting for 32.5-38.5% of these genomes (Huang et al. 2009; Li et al. 2022b). There are two types of retrotransposons, namely LTR-Retrotransposon families, such as Ty1/Copia and Ty3/Gypsy; and Non-LTR Retrotransposon families, such as Long Interspersed Nuclear Element (LINE) and Short Interspersed Nuclear Element (SINE) (Schulman et al. 2012; Elbarbary et al. 2016; Orozco-Arias et al. 2019). Kalendar and Schulman (2006) and Wenke et al. (2015) have developed retrotransposon-based markers namely Inter-Retrotransposon Amplified Polymorphism (IRAP) and Inter-SINE Amplified Polymorphism (ISAP). Both IRAP and ISAP have been used as molecular markers in cucumber, melon, and other plant species to assess genetic diversity in plant germplasm and to validate the genuine hybrids from intergeneric crossing in a segregated

population (Seibt et al. 2012; Wenke et al. 2015; Diekmann et al. 2017; Fatmawati et al. 2021; Sormin et al. 2021).

Transgressive segregation can be evaluated using phenotypic and genotypic traits of a segregated population. In previous trials, the F1 generation resulted from crossing two diverse cucumber origins, namely Metavy x Shiroibo Fushinari (SF) exhibited transgressive segregation in fruit length, with the F1 plants having longer fruit lengths ranging from 35 to 40 cm compared to the Metavy and SF, which had fruit lengths of 23.5 and 27.5 cm, respectively (Nurhidayah 2021). However, the genetic variation in a segregated F2 population has not been evaluated yet. Therefore, selection and evaluation in the F2 generation are critical, and a cautious examination employing not only phenotypic features, but also genotypic variables using molecular markers is essential. The use of IRAP and ISAP markers in genotyping of a segregated cucumber population is poorly reported. This study aimed to evaluate the phenotypic variability and investigate genetic variation of F2 cucumber population using IRAP and ISAP markers.

MATERIALS AND METHODS

Plant materials

Two parental lines including Metavy (commercial variety) from Indonesia and Shiroibo Fushinari (JP. No. 215589) from Japan, and the selected 59 genotypes from the F2 population were used in this study. The Shiroibo Fushinari (SF) was cucumber landrace retrieved from The National Agricultural Research Organization (NARO, Japan). The plants were grown and maintained in the Experimental Research Station Banguntapan, Yogyakarta, Indonesia from October to December 2021.

Phenotypic traits observation

The phenotypic traits variation was observed using yield component variables (Ene et al. 2016). The following variables were measured in the performance of two parental lines and the selected 59 genotypes from the F2 population after harvesting: fruit length, fruit diameter, fruit ratio (length-diameter), fruit radian, fruit weight, and pedicel length.

Genomic DNA isolation and quantification

Total DNA was extracted from young leaves of cucumber genotypes using the CTAB (hexadecyltrimethylammonium bromide) extraction method described by Setiawan et al. (2020b). The 0.5 g of young leaves were ground in 500 µL of a preheated CTAB isolation buffer, then the samples were incubated at 60°C for 30 minutes. The samples were purified twice using 200 µL of 24:1 Chloroform:Isoamyl Alcohol (CIAA) mixed gently but thoroughly and centrifuged at 1600x g for 15 minutes. Then, 1/10 volume of 3M sodium acetate and 2/3 volume of cold isopropanol were added sequentially into aqueous phase of each sample, followed by 1 hour of DNA precipitation at -30°C and centrifugation at 500x g for 2 minutes. The samples were air dried after being washed twice with 600 µL of DNA wash buffer (60 mM potassium acetate, 10 mM Tris-HCl pH 7.5, 60% ethanol). The DNA was re-suspended in 100 µL of TE buffer (10 mM Tris-HCl pH 7.4, 1 mM EDTA) and treated with RNase A to remove the RNA contamination. DNA concentration and purity were measured using GeneQuant 1300 spectrophotometer. DNA was homogenized at a final concentration of 50 ng/µL. Finally, the DNA quality was checked using gel electrophoresis in 1.5% agarose.

PCR amplification

For PCR assay, 2.5 µL of 50 ng/µL DNA was added into reaction mixture that consists of 5 µL of 1X GoTaq® Green Master Mix (Promega, USA), 1 µL of 0.2 µM primers, and 1.5 µL of nuclease-free water in a 10 µL reaction volume. PCR amplification is conducted by T100™ thermal cycler (Bio-Rad, USA), the amplification conditions consisted of pre-denaturation at 94°C for 5 min, 35 cycles of denaturation at 94°C for 30 sec, annealing temperature as specified in Table 1 for 30 sec, with an extension at 72°C for 90 sec, and the final extension at 72°C for 7 min. The amplified products of the PCR were analyzed by 1.5% agarose gel (w/v) electrophoresis using the florosafe DNA staining. Gel documentation was visualized using UV transilluminator and captured using Nikon D5000 Series.

Table 1. The list of IRAP and ISAP primers used in this study

Primer code	Marker type	Primer combination	Sequence (5'-3')	Annealing temperature (°C)	Reference
K1	ISAP	So1s-IIIaF	CCTATGTGGTTTGCAGC	40.0	Seibt et al. (2012)
		So1s-IVR	CCCTTTGGATCAATCACAGC		
K2		CsSINE1-01	GTCCNGTGAGATTAGTHGAGGTG	40.0	Sormin et al. (2021)
		CsSINE1-02	GACAYBCAAATGTTGTAGGGTCAG		
K3		CsSINE1-02	GACAYBCAAATGTTGTAGGGTCAG	40.6	Sormin et al. (2021)
		CsSINE1-03	TTACCACTAGGNCAACCCANGATGG		
K4		CsSINE1-01	GTCCNGTGAGATTAGTHGAGGTG	41.9	Sormin et al. (2021)
		CsSINE1-03	TTACCACTAGGNCAACCCANGATGG		
K5	IRAP	RTvr1-01F	CCTATGTGGAGAATAGGCCCAA	40.6	Fatmawati et al. (2021)
		RTvr1-02R	CATAGGAAGRGAGCCCAATACC		
K6		PvRT01 Ye R	AACCAAACTGGCCCTAAAGT	40.6	Fatmawati (2022)
		PvRT01 Ye F	GCAACTACCTCGAAGCACTCCTTTGA		
K7		PvRT3 F	AMCCATGATTCGTYCCACAAGA	40.0	Fatmawati (2022)
		PvRT1 R	GGGCCTTCTACATTACTYWTGGAC		

Data analysis

The data of phenotypic traits were subjected to Analysis of Variance (ANOVA) for Randomized Complete Blok Design (RCBD) using R software. The data were clustered based on the algorithm of Unweighted Pair Group Method with Arithmetic mean (UPGMA) and Principal Component Analysis (PCA) using R software. The molecular data were transformed into binary data. DNA bands were scored as present (1) or absent (0). The data were analyzed using iMEC (online marker efficiency calculator) (Amiryousefi et al. 2018). To evaluate the polymorphism degree and the profile information of the primers, the parameters Total Amplified Loci (TAL), Total Polymorphic Loci (TPL), Percentage of Polymorphic Loci (PPL), Heterozygosity (H), Polymorphic Information Content (PIC), Effective multiplex ratio (E), Marker Index (MI), Discriminating power (D), and Resolving power (R) were analyzed. The NTSYS-PC program with dice coefficient similarity (Rohlf 2009) and the GenAlEx software version 6.5 (Peakall and Smouse 2012) were used to do the clustering and Principal Coordinate Analysis (PCoA) of the IRAP and ISAP bands, respectively.

RESULTS AND DISCUSSION

Phenotypic traits variation in F2 population of cucumber

The F2 population derived from hybridization of diverse origins i.e., Metavy (commercial variety) x Shiroibo Fushinari (Japan landrace) were grown and evaluated for phenotypic trait variation. Analysis of variance revealed significant differences among cucumber genotypes for the phenotypic traits measured, except the fruit length (Table 2). The selected genotypes of the F2 population had fruit length varied from 22.3 to 35 cm, while the Metavy and SF had fruit length of 26.6 and 24.5 cm, respectively. The female and male parent fruit diameters were 0.54 and 0.35 cm, respectively, with fruit ratios of 48.99 and 68.85. The fruit diameter, fruit ratio, and fruit radian of selected genotypes from the F2 population varied from 0.35 to 0.56 cm, 40.08 to 78.65, and 0.18 to 0.27 cm, respectively. The Metavy and SF had fruit weight of 477.9 and 194.3 g, while the selected genotypes of the F2 population had fruit weight that varied from 134 to 556 g. These results suggested that transgressive segregation occurred in the F2 population, similar to the previous study (Cazzola et al. 2020). Genetic variation in segregated population of cucumber plays a significant role in cucumber improvement programs. Behera et al. (2022) and Yang et al. (2022) were able to select superior lines of cucumber with the desired traits and identify specific genes responsible for these traits using F2 population. Genetic variation analysis gives information on the population's genetic variety and genetic structure, as well as a platform for selecting superior genotypes to create new cultivars in crop improvement breeding programs (Verma et al. 2019). Cucumber has a narrow genetic base (Staub et al. 2005) and the popularity of a few modified cucumber varieties among farmers leads to a small genetic basis of crop species, resulting in increased sensitivity of

crops to different abiotic and biotic stress effects and yield losses. Therefore, using a broad useful genetic base such as landrace in breeding programs can play an important role in increasing resistance to different abiotic and biotic stress damages and improving yield potential of cucumber.

The PCA biplot showed the phenotypic traits profiles of the F2 genotypes and the results indicated a correlation between traits with genotypes. The PCA analysis showed that the first principal component accounted for 45.7% of total variance, while the second component accounted for 32.1% of total variation in the population (Figure 1A). A heatmap is a two-dimensional visual representation of data that uses color variations from colors to darker intensities to signify distinct values. In this study, a clustered heatmap was created to determine the overall performance of the six observable traits across F2 genotypes with two parental lines. Each column represents a unique characteristic, and each row represents a measurement of that trait. Therefore, the heatmap generated two dendrograms: one vertical, indicating the F2 genotypes and parental lines, and one horizontal, reflecting the attributes that triggered the diffusion. The UPGMA based heatmap-dendrogram analysis revealed that the selected F2 genotypes of cucumber was grouped into four major clusters according to phenotypic traits for fruit characters (Figure 1B). Another vertical dendrogram showed three significant groups (Figure 1B). Group one associated with Fruit Weight (FW), Fruit Diameter (FD), Fruit Radian (FR), group two associated with Pedicel Length (FPL), and group three associated with Fruit Length (FL), and Fruit ratio (Length-Diameter) (FLD). These results suggest that the F2 population had phenotypic variation for the fruit characters and some of the selected genotypes exhibit transgressive segregation in fruit weight and fruit length. The F2 genotype numbers 34, 45, 39, 56, 55, and 4 are promising lines with high potential yield. Similar study has been reported in cucumber, melon, and rice in which high phenotypic variation is commonly occurred in a segregated population (Kozik et al. 2013; Amanullah et al. 2022; Huang et al. 2022; Lv et al. 2022; Zhu et al. 2022). Fruit weight is an important character for determining cucumber yield. Singh and Dhillon (2022) reported that the fruit character including fruit weight and length had medium heritability and direct positive effect on cucumber yield. Therefore, this trait can be used for selection criteria in advanced generation.

Table 2. ANOVA of two parental lines and the selected 59 genotypes from the F2 population based on phenotypic traits

Characteristic	Sum of square	Mean of square	F value	Pr (>F)
Fruit length	15.7	7.857	0.542	0.585
Fruit diameter	0.16644	0.08322	16.740	2.6e-06*
Fruit ratio (length-diameter)	2025	1012.5	16.965	1.92e-06*
Fruit radian	0.03886	0.019431	15.31	5.42e-06*
Fruit weight	451984	225992	11.079	9.82e-05*
Pedicel length	26.34	13.170	20.769	2.19e-07*

Note: *significant at 1%

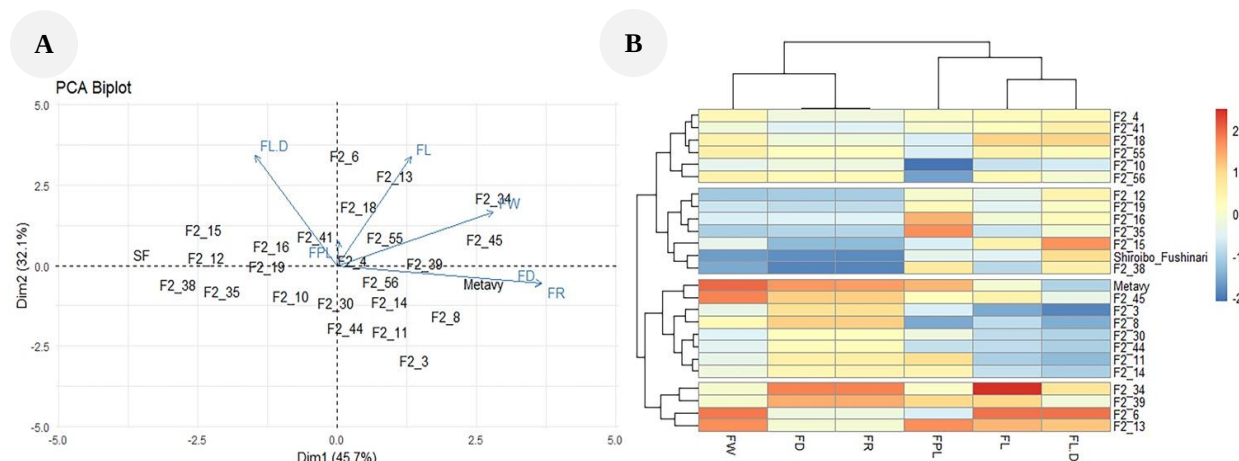


Figure 1. Phenotypic trait relationships among F2 genotypes of cucumber studied based on: A. Principal component analysis, B. Cluster analysis using UPGMA. Note: FW: Fruit Weight, FD: Fruit Diameter, FR: Fruit Radian, FPL: Pedicel Length, FL: Fruit Length, FLD: Fruit ratio (Length-Diameter)

DNA profiles of F2 cucumber genotypes amplified by IRAP and ISAP markers

Four ISAP and three IRAP primer combinations successfully amplified DNA from all cucumber genotypes used in this study. Both markers can clearly distinguish the genetic constitutions of all progenies resulting from parental hybridization of diverse origins caused by a genetic recombination event in the F2 cucumber population. The K7 IRAP primer effectively amplified DNA from both parents as well as the selected 59 genotypes of the F2 population (Figure 2A). This primer produced unique amplicons for Metavy at 210 bp, 620 bp, 690 bp, and 1010 bp, however, no particular allele amplified on Shiroibo Fushinari except at 290 bp, which was present in Metavy. They are three types of genetic constitution of the F2 population amplified by K7 IRAP primer, i.e.: i) some of the F2 genotypes resembled Metavy's genetic make-up, displaying the presence of alleles from female parents, ii) The F2 genotypes that only had the allele at 290 bp similar to the male parent, and iii) genotypes that had the combination alleles from both parental lines. The F2 genotypes 2, 3, 6, and 8 had unique alleles acquired from the female parent, whereas genotypes 13, 14, 18, 19, and 20 reflected the genetic structure of the male parent, with a DNA amplicon of just 290 bp. Based on this DNA profile, the ratio of F2 genotypes that followed Metavy, SF, and the combination of both parents was 11:10:38, with 64.41% of the selected genotypes from the F2 population having the combination allele of their parents.

Based on the K4 ISAP marker, the DNA band amplified of the F2 population has a distinct inheritance allele of Metavy, Shiroibo Fushinari, or a mixture of both parents (Figure 2B). The band size amplified on the K4 ISAP marker ranged from 130 to 590 bp (Table 3). Shiroibo Fushinari had a particular allele at 320 bp, while Metavy had a specific allele at 490 bp. Genotype numbers 2 and 3 have a particular inheritance allele of Metavy, the F2 genotype numbers 13, 14, 16, 17, and 18 had a specific allele inherited from Shiroibo Fushinari, whereas, genotype

numbers 4, 19, and 23 had an allele inherited from both parents. Based on DNA profile, the ratio of F2 genotypes that followed Metavy, SF, and the combination of both parents was 16:7:36, with 61.02% of the selected genotypes from the F2 population having the combination allele of their parents. In addition, some of the F2 genotypes had a deleted allele at 130 bp including genotype numbers 1, 52, 53, 54, and 59. These results suggest that the retrotransposon-based marker can be used to detect the inheritance of alleles into progenies from their progenitor. Previous study reported that the IRAP and ISAP markers has been used also to investigate the genuine hybrids resulted from intergeneric hybridization (Fatmawati et al. 2021), to evaluate genetic diversity in *Cucumis* species (Sormin et al. 2021) and potato (Diekmann et al. 2017), and to detect the somaclonal variation in date palm (Mirani et al. 2020). In this study, both IRAP and ISAP amplified a specific DNA amplicon in both male and female parents and that was inherited into their progenies. Mirani et al. (2020) and Fatmawati et al. (2021) reported that IRAP marker used in their study amplified a unique DNA band that can be used to confirm the successful of intergeneric hybridization in mungbean and differentiate the somaclonal variation in date palm. In addition, our study also confirmed that ISAP marker used in this study exhibited a high degree of polymorphism similar to the previous results reported by Diekmann et al. (2017) and Sormin et al. (2021) and can be used to evaluate the genetic variation in the segregated population of cucumber.

IRAP and ISAP polymorphism among cucumber genotypes

The retrotransposon-based markers (IRAP and ISAP) used in this study successfully investigated the polymorphism among F2 genotypes of cucumber. These primers generated DNA amplicons ranged from 50 to 1510 bp with an average of 7.00 and 9.00 amplicons/primers for ISAP and IRAP markers, respectively (Table 3). The K1 ISAP primer had the highest number of TAL and produced 11 amplified bands, while K3 primer had the lowest number of TAL

with 2 amplicons. The average percentage of polymorphic locus from 7 primer combinations is 80.86%. The heterozygosity (H) and polymorphism information index (PIC) were used to determine the degree of polymorphism (PIC). Heterozygosity is a strategy for determining genetic variability that provides information about an individual's probability of heterozygosity in all analyzed loci (Nei and Li 1979; Aziz and Tahir 2022). Heterozygosity (H) values ranged from 0.4021 to 0.4997, with an average H value of 0.4649. The K4 primer has the highest heterozygosity (0.4997) and the lowest (K1) (0.4021). The average PIC value from 7 primer combinations is 0.3648, with the greatest PIC value of 0.3929 (K6) and the lowest PIC value of 0.3501 (K1), meaning that the PIC value was regarded as highly informative (Eltaher et al. 2018; Fatmawati et al.

2021). The PIC value of the IRAP and ISAP markers was likewise coequal, as established by previous studies, with the moderate IRAP marker producing a PIC value ranging from 0.20 to 0.50 (Zein et al. 2010; Cheraghi et al. 2018; Li et al. 2020; Fatmawati et al. 2021; Sormin et al. 2021). These results also supported that the IRAP and ISAP is dominant markers. Alikhani et al. (2014) reported that molecular markers are dominant if the $PIC \leq 0.5$ and evenly distributed within the population. PIC values represent a marker's capacity to create polymorphisms within populations based on discovered alleles and dispersed frequency (Botstein et al. 1980). These results suggest that the F2 genotypes of cucumber have high genetic variation based on IRAP and ISAP markers.

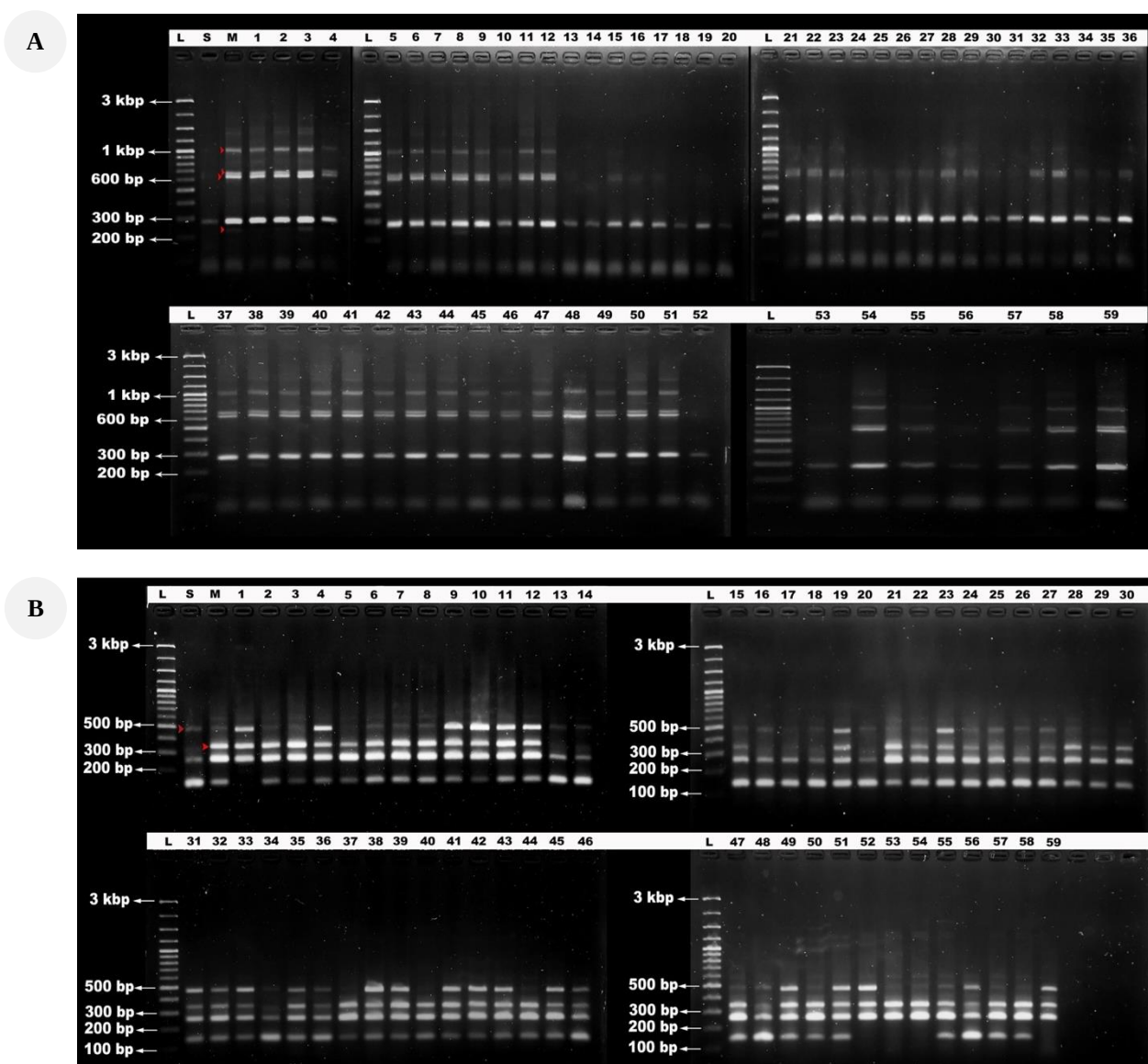


Figure 2. DNA profile of two parental lines and 59 of the selected genotypes from the F2 population based on IRAP and ISAP markers. A. Amplification of DNA among cucumber genotypes using K7 IRAP primers. B. Amplification of DNA among cucumber genotypes using K4 ISAP primers. L: DNA Ladder, S: Shiroibo Fushinari (male parent), M: Metavy (female parent), and the numbers above the well depict the F2 progeny lines. The red arrows represent the amplified DNA at a certain size that is presented in female parent and is transmitted to F2 progenies

The average effective multiplex ratio (E) is 4.8416, with a maximum E value of 7.8545 (K1) and a minimum E value of 0.9273 (K1) (K3). The average MI is 0.0049, with a maximum MI of 0.0054 (K7) and a minimum MI of 0.0042 (K3). The higher MI the better quality of molecular marker. The average discriminating power (D) from seven primer combinations is 0.6409, with the highest and lowest discriminating powers (K3) and 0.4803 (K6), respectively. Discrimination power is used to demonstrate the ability of two random individuals with diverse patterns to identify each other. The average resolving power (R) is 5.3455, with the greatest being 8.2182 (K5) and the lowest being 1.4545. (K3). Resolving power is used to find markers with the capability to evaluate genetic variation. It also implies that a highly effective multiple ratio value indicates increased primer efficiency. The MI evaluates the frequency of use of the marker systems. MI is derived from the polymorphism information content and effective multiple ratios of each individual (Chesnokov and Artemyeva 2015). These results suggest that the ISAP and IRAP markers used in this study can be utilized for investigating DNA profiling and the genetic variation in the F2 population of cucumber.

Cluster analysis of the F2 population based on IRAP and ISAP markers

Clustering analysis was performed on the F2 population of cucumber using IRAP and ISAP markers to investigate the genetic variation in this population. The cluster analysis was conducted using Unweighted Paired Group Method with Arithmetic mean (UPGMA) based on dice coefficient

similarity (Figure 3A). The coefficient similarity of the F2 population is in the range of 0.58-0.96 with an average of 0.77. The cluster analysis showed that the F2 population and parental lines were grouped into two major clusters. The first cluster consisted of Shiroibo Fushinari (male parent) and genotypes 14, 20, 18, 13, 17, 16, and 26, while the second cluster comprised two sub-cluster including Metavy and progenies resembled genotype of female parent i.e., genotype 1, 9, 10, 39, 2, 8, 6, 7, 11, 41, 12, 3, 43, 50, 58, 42, 44, 5, 21, 37, 40, 45, 47, 48, 54, and 55; and the progenies inherited the alleles from both parents. The PCoA analysis result coincided with the UPGMA clustering result, which showed that the F2 population was divided into two large clusters, one of which resembled the genotype of the female parent and the other arising from recombination of two parental lines (Figure 3B). The PCoA analysis showed that the first coordinate accounted for 29.78% of total variance while the second coordinate accounted for 8.82% of total variation in the F2 population. These results suggest that the IRAP and ISAP markers can be utilized to investigate the genetic constitution of F2 population in cucumbers based on their inheritance pattern of DNA fragments. These results were supported by the use of IRAP markers to differentiate the banana accessions and grouped them based on their genome constitution (A or B genome) (Teo et al. 2005). A similar study revealed that utilizing the IRAP marker, off-type accession of date palm propagated by tissue culture can be clearly distinguished from the population and grouped independently (Mirani et al. 2020).

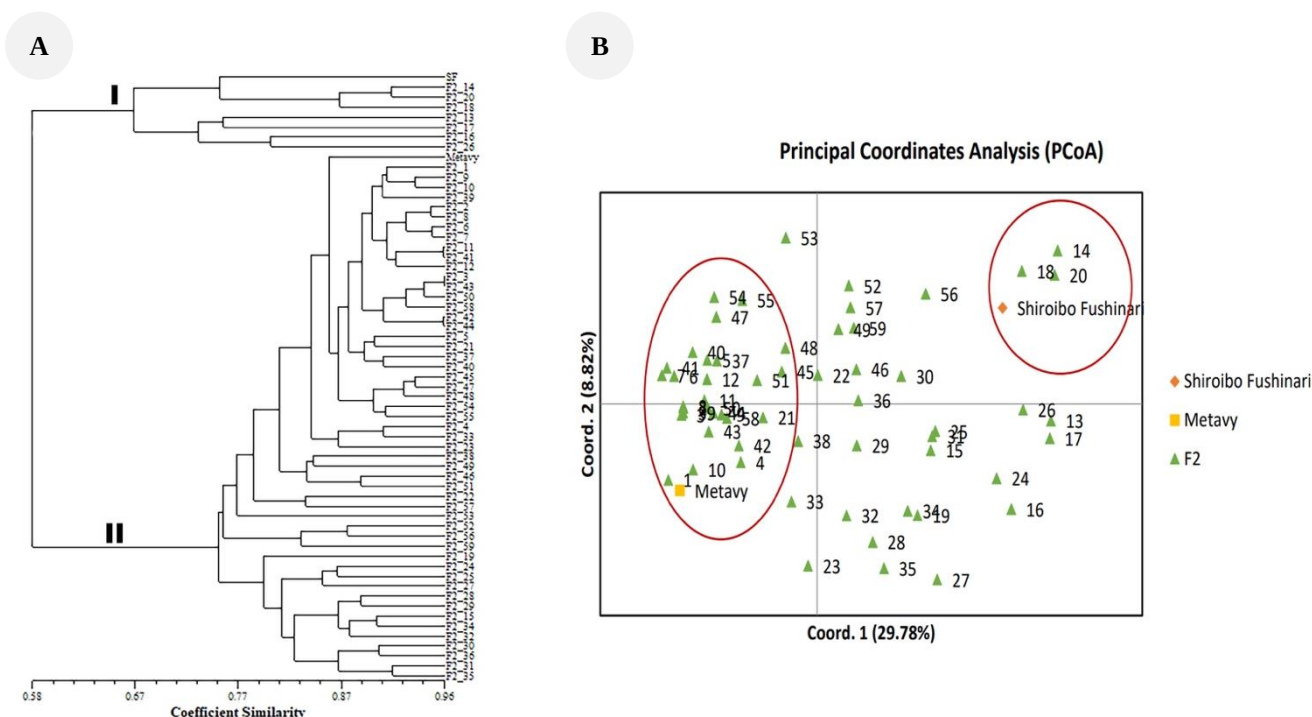


Figure 3. The cluster analysis of F2 59 cucumber genotypes based on IRAP and ISAP markers. A. dendrogram constructed by UPGMA. B. Principal Coordinate Analysis

Table 3. Polymorphism information among cucumber genotypes based on IRAP and ISAP markers

Primer combination	Band size (bp)	TAL	TPL	PPL	H	PIC	E	MI	D	R
K1	50-1510	11	10	91.00	0.4084	0.3904	7.8545	0.0053	0.4905	5.8909
K2	50-600	10	9	90.00	0.4993	0.3491	5.1818	0.0047	0.7319	7.1273
K3	180-490	2	1	50.00	0.4974	0.3501	0.9273	0.0042	0.7873	1.4545
K4	130-590	5	4	80.00	0.4997	0.3489	2.5636	0.0047	0.7380	4.0364
K5	105-1100	10	8	80.00	0.4899	0.3537	5.7091	0.0051	0.6745	8.2182
K6	50-1050	9	9	100.00	0.4021	0.3929	6.4909	0.0053	0.4803	5.0182
K7	50-1500	8	6	75.00	0.4577	0.3690	5.1636	0.0054	0.5839	5.6727
Average of ISAP marker		7.00	6.00	77.75	0.4762	0.3596	4.1318	0.0047	0.6869	4.6273
Average of IRAP marker		9.00	7.67	85.00	0.4499	0.3719	5.7879	0.0052	0.5796	6.3030
Overall average		7.86	6.71	80.86	0.4649	0.3648	4.8416	0.00490	0.6409	5.3455
Total		55	47	566	3.2545	2.5539	33.8909	0.0346	4.4864	31.4182

Note: TAL: Total Amplified Loci, TPL: Total Polymorphic Loci, PPL: Percentage of Polymorphic Loci, H: Heterozygosity, PIC: Polymorphic Information Content, E: Effective multiplex ratio, MI: Marker Index, D: Discriminating power, R: Resolving power

Several cucumber progeny lines from the F₂ population exhibited great variation in the fruit characters. In addition, the DNA profile of the F₂ population revealed genetic variation based on IRAP and ISAP markers. These findings imply that the F₂ population is highly segregated and can be utilized to create new cultivars of cucumber by selecting superior line in advanced generation.

In conclusion, the phenotypic trait evaluation and the retrotransposon-based markers confirmed the existence of phenotypic and genetic variation in the F₂ population of cucumber derived from hybridization of diverse origins. The genetic variation of the F₂ hybrids was successfully detected and discriminated by IRAP and ISAP markers. The IRAP markers showed a higher degree of polymorphism (85%) than ISAP markers (77.75%) in all tested cucumber populations. Cluster analysis showed that the F₂ population and parental lines were grouped into two major clusters with the similarity coefficient ranged from 0.67 to 0.96. The first cluster consisted of Shiroibo Fushinari (male parent) and genotypes 14, 20, 18, 13, 17, 16, and 26, while the second cluster comprised two sub-cluster including Metavy and progenies that resembled genotype of female parent i.e., genotype 1, 9, 10, 39, 2, 8, 6, 7, 11, 41, 12, 3, 43, 50, 58, 42, 44, 5, 21, 37, 40, 45, 47, 48, 54, and 55; and the progenies inherited the alleles from both parents.

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