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Full Length Research Paper

Comparison of effectiveness of ISSR and RAPD markers in genetic characterization of seized marijuana (*Cannabis sativa* L.) in Turkey

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This paper presents results from comparison of effectiveness of Inter Simple Sequence Repeats (ISSR), Randomly Amplified Polymorphic DNA (RAPD), and ISSR and RAPD (ISSR-RAPD) markers together in characterization of *Cannabis* accessions. The plant material used was common accessions of psychoactive *Cannabis* samples (a total of 17 accessions), which were used in discriminating drug type *Cannabis* from hemp type *Cannabis* via ISSR and characterization of *Cannabis* samples via RAPD. Data were analyzed via cluster and principal coordinate analyses (PCoA). Discriminating powers of ISSR and RAPD markers on the seized *Cannabis* accessions were evaluated by utilising polymorphism information content, resolving power and marker index (MI). The PCoA of ISSR and ISSR-RAPD markers data produced similar results. Average resolving power and MI values of ISSR assay found to be slightly higher than those of RAPD assay. Consequently, ISSR markers would be a better choice compared to RAPD markers in characterization of *Cannabis* accessions.

Key words: *Cannabis sativa* L., individualization of seized marijuana, inter simple sequence repeat (ISSR), randomly amplified polymorphic DNA (RAPD), principal coordinate analysis, cluster analysis.

INTRODUCTION

Cannabis sativa L. is thought to have originated from the Central Asia region and has since been distributed worldwide by humans (Small and Cronquist, 1976). It is a plant that provides food and oil from its seeds, fibre for rope, fabric from its stems, and psychoactive drugs from its flowers and leaves. Hemp seeds oil can also be used for fuel and as raw material for plastics (Ranalli and Venturi, 2004) as well as feed for livestock or as a fertilizer (Karus and Vogt, 2004).

Beside the economical properties mentioned above, some varieties of *Cannabis* have psychoactive potency as well. *Cannabis* plants that contain low Δ^9-tetrahydrocannabinol (THC), a low THC: Cannabidiol (CBD) ratio and are cultivated for fibre and/or achenes (e.g. seeds) are called hemp. On the other hand, *Cannabis* plants that have high THC content, high THC: CBD ratio are used for their psychoactive potency known as marijuana (Alghanim and Almirall, 2003). For hemp, EU has assigned the upper levels of THC and THC: CBD ratio as 0.2 and 2%, respectively.

In many countries, including Turkey, possession and cultivation of *Cannabis* was either ceased or limited because of its potential use as a drug. When samples of suspect materials are recovered, they must be tested for the presence of controlled substances (eg. marijuana). In addition to the identification of marijuana samples, it is desirable but difficult to link individual growers and distributors to specific illicit field and greenhouse operations. Molecular genetics may offer solution in identification and individualization via investigating the genetic relatedness between individuals/populations.

Jagadish et al. (1996) were able to distinguish between the samples from distinct sources in a randomly amplified polymorphic DNA (RAPD) assay conducted with 51 *C. sativa* samples. Genetic analysis used in combinations of

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RAPD and restriction fragment length polymorphism (RFLP) methods were also found to be useful in distinguishing between drug type, fibre type and intermediate drug type strains (Shirota et al., 1998). Hakki et al. (2003) used RAPD and amplified fragment length polymorphisms (AFLP) markers to fingerprint the 18 different *Cannabis* individuals from five different locations representing 3 geographical regions of Turkey. It was reported that it was possible to discriminate illegal, potent marijuana cultivars from hemp plants by using AFLP markers (Datwyler and Weiblen, 2006). In a preliminary work conducted with three strains of *C. sativa* from different sources, Kojoma et al. (2002) reported that different samples were identified by means of inter simple sequence repeat (ISSR). In a study by using ISSRs, marijuana (*C. sativa* L.) was separated efficiently from hemp (Hakki et al., 2007). In a recent study by using RAPD markers, it is reported that *Cannabis* samples seized from 29 different locations of Turkey were separated according to geographical regions (Pinarkara et al., 2009).

Gillan et al. (1995) reported the differentiation of *C. sativa* samples with the use of RAPDs when HPLC analysis was inefficient. Faeti et al. (1996) assessed genetic diversity of *C. sativa* cultivars/accessions (from 5 European countries, and one accession from Korea) by using RAPD markers and high levels of polymorphism were reported. In a study of genetic structure and degree of variability of six *C. sativa* L. varieties via RAPD markers, it was reported that 5 varieties were properly identified with the scored loci (Forapani et al., 2001). Hsieh et al. (2003) investigated the usage of short tandem repeat (STR) loci in identification of *Cannabis* samples and predicting their genetic relationship. Alghanim and Almirall (2003) were developed STR markers for *Cannabis*. They reported that STR markers were very effective in uniquely identifying 27 profiles of the *Cannabis* samples tested and useful for DNA typing and genetic relatedness analyses. Mendoza et al. (2009) attempted to individualize cannabis samples using single reaction sixplex STR markers. However, they reported that it is difficult to distinguish marijuana and hemp samples on the basis of this system since they are usually similar genetically. Gilmore and Peakall (2003) isolated microsatellite markers, which have utility for characterizing genetic diversity in cultivated and naturalized *Cannabis* populations. Gilmore et al. (2003) reported that STR markers are capable of discriminating among individuals and varieties of *Cannabis*.

RAPD markers were used to individualize Palo Verde tree in a criminal case (Yoon, 1993) and strawberry in a civil case (Congiu et al., 2000). In both cases the method has been accepted in court, although, in the Palo Verde tree case the statistical significance was not used since the representative population consists of too few samples. Congiu et al. (2000) employed RAPD markers for individualization of strawberry because of its two main advantages: it allows random sampling of markers over whole genomic DNA and does not require any previous information on the genome of the organism under investigation. RAPD and ISSR markers were utilized for comparative analysis of genetic diversity in blackgram genotypes (Souframanien and Gopalakrishna, 2004).

The objective of this study was to compare effectiveness of ISSR, RAPD, and ISSR and RAPD (ISSR-RAPD) markers together in characterization of *Cannabis* accessions. In addition, discriminating powers of ISSR and RAPD markers on the seized *Cannabis* accessions were also evaluated.

MATERIALS AND METHODS

Plant materials

In this study, a common sample of seized psychoactive *Cannabis* sample (a total of 17 accessions) which were used in discriminating drug type *Cannabis* from hemp type *Cannabis* via ISSR markers by Hakki et al. (2007) and in characterization of *Cannabis* samples via RAPD markers by Pinarkara et al. (2009) were used. These referred *Cannabis* samples were seized from different locations representing geographically distinct and problematic areas of Turkey. All the information relevant to the seized samples used in this study was given at Table 1. Ten seeds were planted from each accession to produce material for DNA extraction. Plants were grown in a fully automated greenhouse.

DNA extraction from leaf

Leaves collected from three-week-old seedlings were shock-frozen in liquid nitrogen and stored at -80°C until DNA isolations were performed. DNAs were extracted individually from a total of 170 samples. Total DNAs of the samples were extracted using a standard 2X CTAB protocol with minor modifications (Rogers and Bendich, 1988). For each accession, 100 mg of leaf sample from 10 different plants were used and DNAs were isolated individually. After concentrations were determined by an Eppendorf BioPhotometer, sample DNAs were diluted to the working concentration of 20 ng/µL.

ISSR assay

In this study, 50 ISSR primers were used for initial screening. Out of the 50 primers, 17 of them that gave the most informative patterns (in terms of repeatability, scorability and the ability to distinguish between accessions) were selected for identification. The selected 17 primers were 14–23-mers based on various di- tri- or pentanucleotide repeats. They were anchored at the 5`end or 3`end, by zero nucleotide or by one to three partially degenerated selective nucleotides.

Each reaction contained 10 mM Tris-HCl (pH 8.8); 50 mM KCl; 0.8% Nonidet P40; 200 mM of each of the dNTPs; 0.5 µM primer; 20 ng DNA template and 0.4 units of Taq DNA Polymerase (Fermentas Life Sciences) in a final reaction volume of 25 µl. After a pre-denaturation step of 3 min at 94°C, amplification reactions were cycled 35 times at 94°C for 1 min, at annealing temperature (Table 2) for 50 s and 72°C for 1 min in Eppendorf Mastercycler gradient thermocycler. A final extension was allowed for 10 min at 72°C. Upon completion of the reaction, amplified products were loaded onto a 2.0% agarose/1x Tris-Borate EDTA gel and

Table 1. *Cannabis* accessions used in the study and their origin.

Sample ID[a]	Paralel ID[b]	Settlement seized[c]	Geographical Region
1	3102	Tekirdag	Marmara
2	04-62927/5432 ND	Tekirdag	Marmara
3	05/002598/315	Edirne	Marmara
4	04/409/5782	Susehri ili, Sivas	Central Anatolia
5	058576/5057	Tekirdag	Marmara
6	065364/5645	IST CS/Golcuk	Marmara
7	4243-2	Ferizli	Marmara
8	4243	Ferizli	Marmara
9	2075/1	Salihli	Aegean
10	758/9	İzmir DGM	Aegean
11	847/1-C-1	Denizli	Aegean
12	677/2	Didim	Aegean
13	315/2	Aydin	Aegean
14	676/2 A	Didim	Aegean
15	04 4047	Osmaniye	Mediterranean
16	AT 05/1458	Kadirli	Mediterranean
17	AT 05/678	G. Antep	South Eastern Anatolia

[a] Sample name used in this study; [b]File code in seized samples; [c]The region where sample was seized.

Table 2. ISSR primers used in the study and the number and the type of fragments they amplified (melting temperature, base pair, GC content and annealing temperature were given in Hakki et al. (2007)).

Primer	Primer sequence	NSB	NPB	PPB	PIC	MRP	RP	MI
ISSR 1	$(AGC)^6$-G	9	9	100.00	0.30	0.43	3.88	2.70
ISSR 2	$(ACC)^6$-G	7	6	85.71	0.38	0.59	3.53	1.93
ISSR 3	$(AGC)^6$-C	9	5	55.56	0.31	0.42	2.12	0.85
ISSR 4	$(CA)^{10}$-C	5	4	80.00	0.33	0.53	2.12	1.04
ISSR 5	$(GA)^9$-C	8	8	100.00	0.31	0.47	3.76	2.44
ISSR 6	GT-$(CAC)^7$	8	7	87.50	0.29	0.44	3.10	1.78
ISSR 7	$(AG)^9$-C	6	2	33.33	0.16	0.18	0.35	0.11
ISSR 8	$(AC)^9$-G	6	6	100.00	0.30	0.43	2.59	1.79
ISSR 9	$(AC)^8$-CG	12	12	100.00	0.20	0.27	3.29	2.42
ISSR 10	$(AC)^8$-CC/T	11	8	72.73	0.31	0.43	3.41	1.80
ISSR 12	$(GACAC)^4$	9	9	100.00	0.17	0.22	2.00	1.55
ISSR 13	$(CA)^6$-A/GG	9	8	88.89	0.26	0.37	2.94	1.87
ISSR 14	$(CA)^6$-RY	10	6	60.00	0.22	0.25	1.53	0.77
ISSR 15	$(CA)^8$-AG	4	4	100.00	0.23	0.29	1.18	0.93
ISSR 16	$(CA)^8$-GC	10	7	70.00	0.36	0.52	3.65	1.76
ISSR 17	CAG-$(CA)^8$	3	1	33.33	0.11	0.12	0.11	0.04
ISSR 18	CGT-$(CA)^8$	3	1	33.33	0.11	0.12	0.11	0.04
	Total	129	103					

NSB: Number of scored band; NPB: Number of polymorphic band; PPB: Percentage of polymorphic band; PIC: Polymorphism information contents; MRP: Mean resolving power; RP: Resolving power; MI: Marker index.

electrophoresed at 4 V/cm. $MgCl_2$ used at final concentration of 2.0 mM was generally found to generate bands of high intensity with minimum background. However, in the PCR of two primers (ISSR 9 and 10), 2.5 mM $MgCl_2$ concentration was found to produce better banding pattern. Thus, conditions were optimised for every primer.

Template DNA concentration was also found to influence band

Table 3. RAPD primers used in the study, number and the type of fragments they amplified (melting temperature, base pair, GC content and annealing temperature were given in Pinarkara et al. (2009)).

Primer	Primer sequence	NSB	NPB	PPB	PIC	MRP	RP	MI
RAPD L2	5'- GTT TCG CTC C -3'	12	11	91.67	0.32	0.47	5.18	3.26
RAPD L3	5'- GTA GAC CCG T -3'	6	4	66.67	0.23	0.26	1.06	0.61
RAPD L4	5'- AAG AGC CCG T -3'	10	6	60.00	0.42	0.65	3.88	1.51
RAPD L5	5'- AAC GCG CCG T -3'	13	10	76.92	0.28	0.38	3.76	2.15
RAPD L6	5'- CCC GTC AGC A -3'	6	3	50.00	0.35	0.47	1.41	0.52
RAPD B1	5'- CCC GCC GTT G -3'	23	18	78.26	0.24	0.35	6.24	3.42
RAPD B2	5'- TGC GCC CTT C -3'	7	3	42.86	0.24	0.39	1.18	0.31
RAPD B3	5'- GAT GAC CGC C -3'	5	2	40.00	0.28	0.35	0.71	0.23
RAPD B4	5'- CTC ACC GTC C -3'	8	7	87.50	0.14	0.15	1.06	0.85
RAPD B5	5'- GAC GGA TCA G -3'	14	7	50.00	0.31	0.47	3.29	1.08
RAPD B6	5'- CCG ATA TCC C -3'	9	5	55.56	0.32	0.49	2.47	0.89
RAPD B7	5'- TTG GTA CCC C -3'	3	2	66.67	0.25	0.29	0.59	0.33
RAPD B8	5'- ACG GTA CCA G -3'	7	3	42.86	0.23	0.27	0.82	0.29
RAPD B9	5'- CCA GCG TAT T -3'	9	9	100.00	0.29	0.42	3.76	2.63
RAPD B10	5'- CTA CTG CGC T -3'	6	4	66.67	0.30	0.38	1.53	0.79
RAPD B11	5'- CCT CTG ACT G -3'	2	1	50.00	0.29	0.35	0.35	0.15
RAPD B12.2	5'- TCC GAT GCT G -3'	3	1	33.33	0.29	0.35	0.35	0.10
RAPD B13	5'- TTC AGG GTG G -3'	3	2	66.67	0.20	0.24	0.47	0.27
RAPD B14	5'- TCC TGG TCC C -3'	6	2	33.33	0.39	0.59	1.18	0.26
RAPD B16	5'- AGT CGG GTG G -3'	9	7	77.78	0.30	0.45	3.18	1.60
RAPD B17	5'- GTC GTT CCT G -3'	11	11	100.00	0.30	0.43	4.71	3.28
RAPD B18	5'- GAG TCA GCA G -3'	8	5	62.50	0.20	0.24	1.18	0.62
	Total	180	123					

NSB: Number of scored band; NPB: Number of polymorphic band; PPB: Percentage of polymorphic band; PIC: Polymorphism information contents; MRP: Mean resolving power; RP: Resolving power; MI: Marker index.

intensity.

RAPD assay

In this study, 22 arbitrary RAPD primers that gave the most informative patterns were selected for identification (Table 3). Each reaction contained 2.5 mM $MgCl_2$; 10 mM Tris-HCl (PH 8.8); 50 mM KCl; 0.8% Nonidet P40; 200 mM of each of the dNTPs; 0.5 µM primer; 20 ng DNA template and 0.3 units of Taq DNA Polymerase (Bioron) in a final reaction volume of 25 µl. After a pre-denaturation step of 3 min at 94°C, amplification reactions were optimised for every individual primer and optimisation started by cycling the reaction 45 times at 94°C for 1 min, at annealing temperature for 50 s and 72°C for 1 minute in Eppendorf Mastercycler gradient thermocycler. A final extension was allowed for 10 min at 72°C. Upon completion of the reaction, amplified products were loaded onto a 2.0% agarose/1x Tris-Borate EDTA gel and electrophoresed at 4 V/cm.

In both ISSR and RAPD assays, every accession was represented by a total of ten individual plants. The resultant products were run in agarose gel and evaluated. Amplifications were repeated at least twice (in different time periods) for each primer, using the same reagents and procedure.

Data collection and polymorphism information contents

Each DNA fragment generated was treated as a separate character and scored as a discrete variable, using 1 to indicate presence, and 0 for absence both for ISSR and RAPD markers. Accordingly, rectangular binary data matrices were obtained for ISSR and RAPD markers.

Primer banding characteristics such as number of scored bands (NSB), number of polymorphic band (NPB), and percentage of polymorphic bands (PPB) were obtained. Polymorphism information content (PIC_i) of a band was calculated according to Anderson et al. (1993) as follow:

$$\mathrm{PIC}_i = 1 - \sum_j f_{ij}^2$$

where f_{ij} is the frequency of the j^{th} pattern of the i^{th} band (note that dominant markers have two patterns for a band as being present and absent). Then, the PIC of each primer was calculated as:

$$\mathrm{PIC} = 1/n \sum_{i=1}^{n} \mathrm{PIC}_i$$

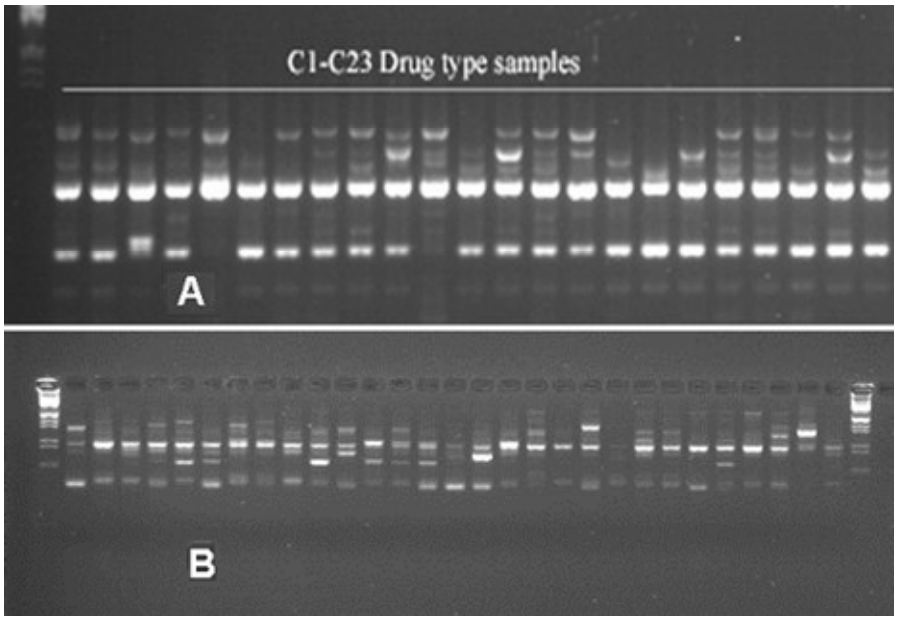

Figure 1. A representative agarose gel where PCR products of ISSR (A: amplified by use of ISSR 6 primer) and RAPD (B: amplified by use of RAPD L2 primer) markers (modified from Hakki et al. (2007) and Pinarkara et al. (2009), respectively).

where *n* is the NPB for that primer. Informativeness of a band (BI_i) was calculated as:

$$BI_i = 1 - \left(2 \times \left|0.5 - p\right|\right)$$

where *p* is the proportion of the 17 accessions containing the band. Then, the resolving power (RP) of each primer was calculated as:

$$RP = \sum_{i=1}^{n} BI_i$$

where *n* is the NPB for that primer (Prevost and Wilkinson, 1999). Further, we calculated mean resolving power for each primer as:

$$MRP = 1/n \sum_{i} BI_i$$

Following Milbourne et al. (1997), marker index (MI) was calculated as product of PIC_i and effective multiplex ratio (EMR), which is defined as the product of the fraction of polymorphic loci and the number of polymorphic loci. In addition, relationships between PIC, MRP, RP, and MI were evaluated.

Statistical analysis

Rectangular binary data matrices of ISSR and RAPD markers were used for statistical analyses. Further, ISSR and RAPD rectangular binary data matrices were combined (ISSR-RAPD) to facilitate an analysis of combined ISSR and RAPD markers together as well. Pair-wise similarity matrices were generated using simple matching similarity coefficient by means of SIMQUAL procedure of NTSYS-pc version 2.1 (Rohlf, 2000) statistical package and principal coordinate analyses (PCoA) were performed using a batch mode of NTSYS-pc for ISSR, RAPD and ISSR-RAPD markers sets. Cluster analysis was performed by means of SAHN procedure of NTSYS-pc via unweighted pair-group method using arithmetic average (UPGMA) to develop dendrograms.

Comparisons (ISSR vs. RAPD, ISSR vs. ISSR-RAPD, and RAPD vs. ISSR-RAPD) were made on the graphs obtained from result of PCoA and on dendrograms that obtained from results of cluster analysis. In addition, matrix comparisons of Mantel Z test (Mantel, 1967), for the correspondence of the similarity matrices (ISSR vs RAPD, ISSR vs ISSR-RAPD, and RAPD vs ISSR-RAPD), were performed by means of MXCOMP procedure of NTSYS-pc for the null hypothesis that there is no association between these similarity matrices. To obtain significance level, 5000 permutations were performed.

RESULTS AND DISCUSSION

ISSR and RAPD assays amplification and polymorphism information contents

The ISSR and RAPD markers used allowed reproducible and informative polymorphisms (Figure 1). Detailed information was given in Hakki et al. (2007) and Pinarkara et al. (2009). In the common set (17 accessions) used in this study, 17 ISSR primers produced 103 polymorphic bands, while 22 RAPD primers produced 123 polymorphic bands. For each ISSR

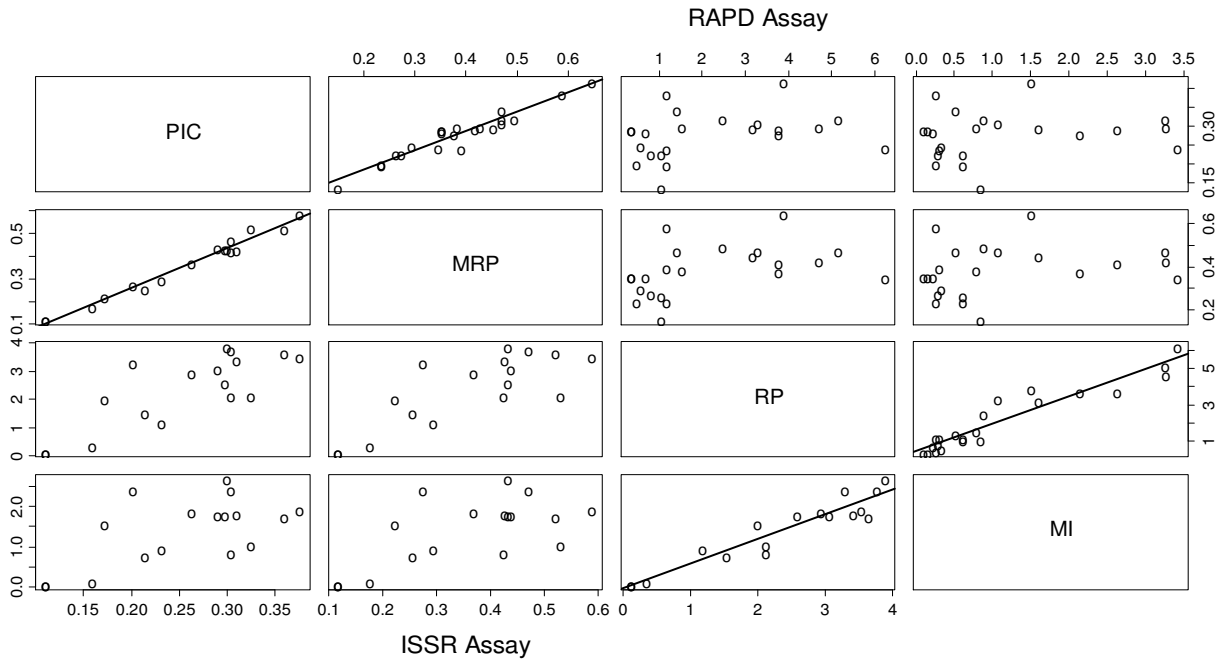

Figure 2. Scatter matrix plot of PIC, MRP, RP, and MI for RAPD assay (above diagonal) and ISSR assay (below diagonal).

and RAPD primers NSB, NPB, PPB, PIC, RP, MRP and MI were given at Tables 2 and 3, respectively. ISSR primers produced minimum 1, maximum 12, and in average 6.06 bands while RAPD primers produced minimum 1, maximum 18, and in average 5.59 bands. Average PIC and MRP values of all primers of ISSR assay (0.25 and 0.36, respectively) and RAPD assay (0.28 and 0.38, respectively) were similar. However, average RP and MI values of all primers were found to be slightly higher in ISSR assay (2.33 and 1.40, respectively) than those of RAPD assay (2.20 and 1.14, respectively). We note that these two measurements (RP and MI) are functions of distribution and number of alleles (bands) within the sampled genotypes, although there are small differences in the derivations of their formulas. A particular band would have optimal discriminating power when it is scored in the 50% of the genotypes. Given the bands discriminating powers, a primer's discriminating power increases with the increasing number of bands of that primer.

There were strong linear relationships between MI and RP both in ISSR and RAPD assays (r = 0.95 and r = 0.95, respectively). Similarly, strong linear relationships were observed between PIC and MRP both in ISSR and RAPD assays (r = 0.99 and r = 0.96, respectively). Regression equations for these linear relationships and coefficient of determinations (R^2) were:

$\mathrm{MRP} = -0.09 + 1.76 \times \mathrm{PIC}$ (R^2 = 0.97) for ISSR assay;
$\mathrm{MRP} = -0.12 + 1.78 \times \mathrm{PIC}$ (R^2 = 0.91) for RAPD assay;
$\mathrm{MI} = -0.04 + 0.62 \times \mathrm{RP}$ (R^2 = 0.89) for ISSR assay, and
$\mathrm{MI} = -0.17 + 0.60 \times \mathrm{RP}$ (R^2 = 0.90) for RAPD assay.

To facilitate visual comparisons, scatter matrix plot of PIC, MRP, RP, and MI for ISSR and RAPD assays were shown in Figure 2.

Statistical analysis of genotypic data

Results from PCoA of ISSR markers showed that *Cannabis* accessions 1, 2, 3, and 9 can be grouped together (group 1) and can be separated from the rest by using the PCo axis 1 (marked with dotted ellipse in Figure 3A). Group 1 accessions were clustered together in the dendrogram obtained from cluster analysis via UPGMA with a dissimilarity coefficient of 0.25 (marked with dotted rectangular in Figure 3B). Accession 8 was attached to this main branch with a dissimilarity coefficient of 0.29. This accession took place between the group 1 and the rest in the graph of PCoA according to Pco axis 1 (Figure 3A). Another main branch in the dendrogram was made up of accessions 11, 7, 16, 13, 15, 4, and 12 (marked with broken line in Figure 3B).

Results from PCoA of RAPD markers showed no clear separation of *Cannabis* accessions (Figure 3C). However, when the figure evaluated further, it was seen that group 1 accessions of ISSR markers analysis took place on a strip of PCo axis 2 (strip between the dotted lines in Figure 3C). Group1 accessions were clustered

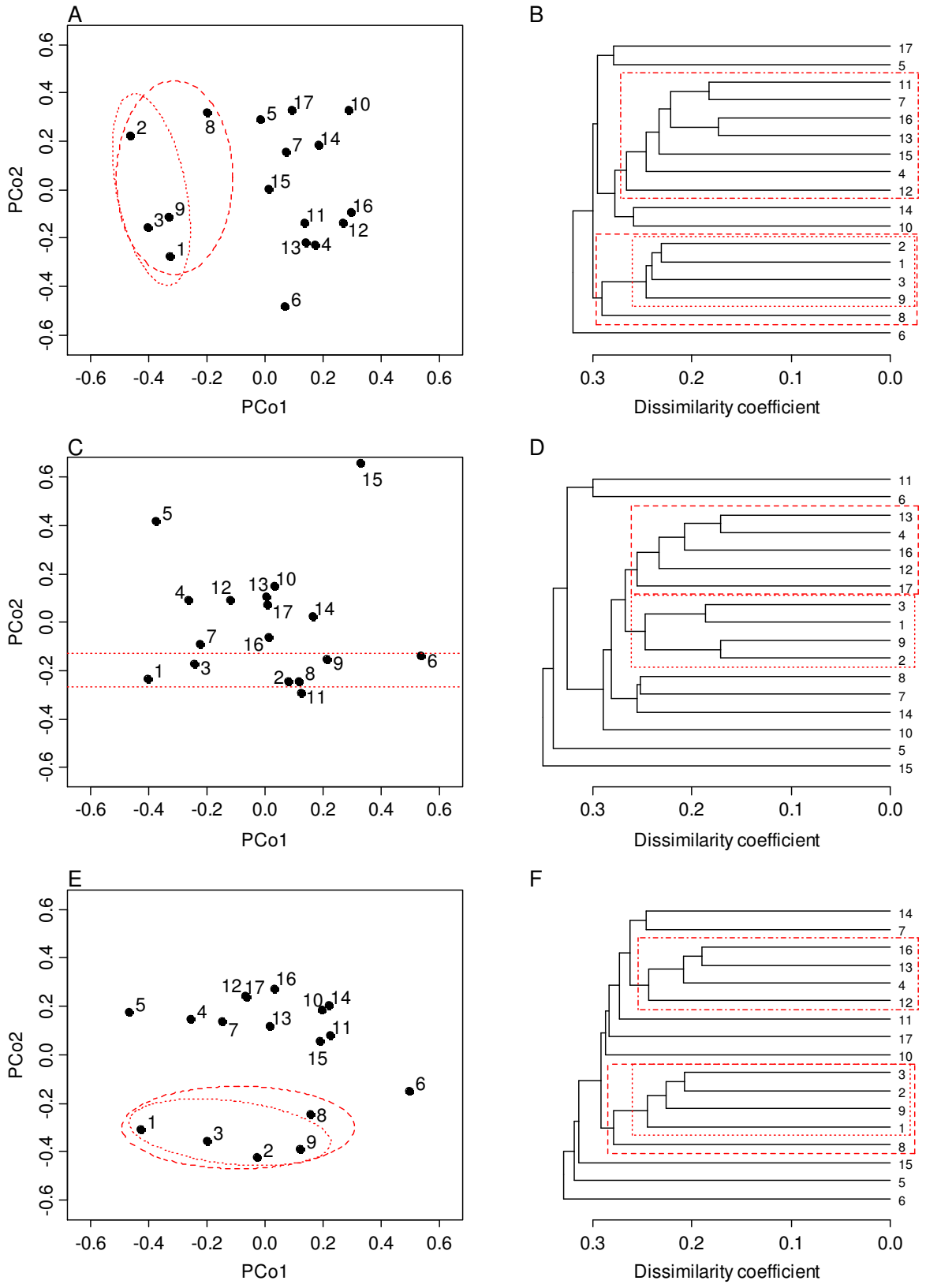

Figure 3. A) Graph of PCo1 vs. PCo2 for ISSR data, B) Dendrogram of ISSR data, C) Graph of PCo1 vs. PCo2 for RAPD data, D) Dendrogram of RAPD data, E) Graph of PCo1 vs. PCo2 for ISSR and RAPD data combination and F) Dendrogram of ISSR and RAPD data combination.

together in the dendrogram obtained from cluster analysis of RAPD markers via UPGMA with a dissimilarity coefficient of 0.25 (marked with dotted rectangular in Figure 3D). Another main branch, group 2, was composed of accessions 13, 4, 16, 12, and 17 with a dissimilarity coefficient of 0.26 (marked with dashed rectangular in Figure 3D).

Results from PCoA of accessions by using ISSR-RAPD markers together showed that *Cannabis* accessions can be separated into two main groups by using PCo axis 2 (Figure 3E). Here, group 1 accessions were clustered together and can be separated from others by PCo axis 2 (circled with dotted ellipse in Figure 3E). Accession 8, which lied between the group 1 and the rest in the graph of PCoA of ISSR markers analysis according to Pco axis 1, took a closer place to group 1 in this analysis (circled with dashed ellipse in Figure 3E). Group 1 accessions were clustered together in the dendrogram obtained from cluster analysis of ISSR-RAPD markers together via UPGMA with a dissimilarity coefficient of 0.25 (highlighted with dotted rectangular in Figure 3F). As in ISSR markers analysis, accession 8 was attached to this main branch with a dissimilarity coefficient of 0.28 (marked with dashed rectangular in Figure 3F). Except accession 17, group 2 accessions of RAPD marker analysis were clustered together in the cluster analysis of ISSR-RAPD markers together via UPGMA with a dissimilarity coefficient of 0.25 (highlighted with broken-line rectangular in Figure 3F).

Results from similarity matrices comparisons via Mantel Z test showed that there was a very weak correlation between the similarity matrices of ISSR and RAPD (r = 0.30, $P < 0.03$), a weak correlation between the similarity matrices of ISSR and ISSR-RAPD (r = 0.70, $P < 0.001$) and a good correlation between the similarity matrices of RAPD and ISSR-RAPD (r = 0.88, $P < 0.001$). Having a good correlation between the similarity matrices of RAPD and ISSR-RAPD markers was concordant with the results of analyses of RAPD and ISSR-RAPD markers both via PCoA (group 1 accessions were separated by PCo axis 2) and UPGMA (group 1 and group 2 accessions (except accessions 17) were clustered together). On the other hand, having a weak correlation between the similarity matrices of ISSR and RAPD markers was reflected to the results of analyses of RAPD and ISSR markers both via PCoA (group 1 accessions were separated by PCo axis 1 in ISSR markers while they were separated by PCo axis 2 in RAPD markers) and UPGMA (group 1 and group 2 accessions (group 2 accessions did not cluster together in ISSR but in RAPD while group 1 accessions clustered together in both marker systems). Here, we note that Rohlf and Fisher (1968) showed that most cophenetic correlations found are statistically significant. We also note that strength of a correlation coefficient of Mantel Z test is interpreted differently from strength of a correlation coefficient of two random variables.

Intention was comparing results presented in this study with similar attempt made in the analysis of *Cannabis* accessions using ISSR and RAPD markers for characterization purpose. However, we did not come across any ISSR, RAPD markers effectiveness comparison study on this plant. However, there are studies in other plant species and they were included here. Behera et al. (2008) analyzed genetic relatedness of 38 morphologically and geographically distinct bitter gourd accessions by using ISSR, RAPD and AFLP markers and reported that RAPD and ISSR markers were not able to uniquely discriminate all the bitter gourd accessions examined while AFLP was discriminatory. Average MI values derived from the three different marker systems found to be differed dramatically, indicating that they vary in their discriminatory power (AFLP > ISSR > RAPD). Thimmappaiah et al. (2009) utilized ISSR, RAPD and ISSR-RAPD combination to characterize cashew germplasm accessions and reported that ISSR-RAPD combination discriminate better. They also reported a matrix correlation of 0.4176 for ISSR and RAPD markers. Kafkas et al. (2006) reported a matrix correlation of 0.58 between ISSR and RAPD markers, in a study of detecting DNA polymorphism and genetic diversity in a wide pistachio germplasm using ISSR, RAPD and AFLP markers. In a diversity study on cultivated tea clones and wild tea using ISSR and RAPD markers, Lai et al. (2001) reported a matrix correlation of 0.811 between ISSR and RAPD and also reported that ISSR-RAPD combination gave similar results to ISSR results. In a study with Calamagrostis porterii populations, Esselman et al. (1999) reported that ISSR markers detected more diversity than RAPD markers. Mattioni et al. (2002) utilized ISSR and RAPD markers to characterize 3 Chilean Nothofagus species. They reported a matrix correlation of 0.95 between similarity matrices of ISSR and RAPD and a similar result for UPGMA and PCoA. Patzak (2001) reported a matrix correlation of 0.96 between the similarity matrices of ISSR and RAPD markers in a characterization study based on 5 Czech and 5 foreign hop varieties.

Conclusion

The PCoA of ISSR and ISSR-RAPD markers data produced similar results. There was no clear separation of *Cannabis* accessions in the PCoA of RAPD data. Average resolving power and MI values of ISSR assay found to be slightly higher than those of RAPD assay, although average PIC of all primers for both assays was very similar.

It is shown that ISSRs which have similar properties to RAPDs can make substantial contribution to molecular diversity analysis. Further, due to their similar properties, they can be used simultaneously. Both marker systems can be economically used in laboratories, which have basic molecular analysis equipments. Since high

repeatability of ISSRs (compared to RAPDs), they may find further usage especially with organisms that do not have adequate genomic information for locus specific genotyping.

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