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Research paper

Assessment of genetic diversity in different types of tobacco using molecular markers

Mostafa Mehdizadeh[1], Mohammad Mohsenzadeh Golfazani[2*], and Habibollah Samizadeh Lahiji[3]

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[1]Researcher, Plant Breeding, Faculty of Agricultural Sciences, University of Guilan, Rasht, Iran.
[2]Associate Professor, Department of Agricultural Biotechnology, Faculty of Agricultural Sciences, University of Guilan, Rasht, Iran.
[3]Professor, Department of Agricultural Biotechnology, Faculty of Agricultural Sciences, University of Guilan, Rasht, Iran.
[*]Corresponding author; Email: mohsenzadeh.mohamad@guilan.ac.ir

Abstract
Information about the amount of genetic diversity in the germplasm and genetic relations of genotypes is essential for analyzing and designing breeding programs and could be used for assisting in genetic identification and improvement of the germplasm. In this research, the genetic diversity of 48 genotypes of the flue-cured, burley, and oriental types of tobacco was investigated by 12 ISSR, 10 SSR, and five IRAP primers. The maximum number of bands was observed in the UBC825, RTR-10, and mixed primer UBC817 + UBC826 with 17 bands, UBC817 with 16 bands, and RTR-8 with 15 bands. UBC824 with 10 and UBC823 with 11 bands showed the minimum number of bands among ISSR and IRAP primers, and PT30044 and PT30046 primers with two alleles showed the minimum number of alleles among SSR markers. In ISSR and IRAP, the observed percentage of polymorphism ranged from 76.92% for RTR-1 and RTR-7 to 94.11% for RTR-10, and in SSR markers, it was 100%. The average polymorphism percentage was 90.7%, which indicated suitable genetic diversity among the tobacco genotypes. The polymorphism information content ranged from 0.31 to 0.5 with an average of 0.42. The average diversity of Nei and Shannon indices were 0.39 and 0.58, respectively. Cluster analysis by the UPGMA method classified the 48 tobacco genotypes into five groups, containing 5, 12, 10, 6, and 15 genotypes, respectively. Principal coordinate analysis showed that the 14 first components could explain 51.49% of the total variance. The primers used in this study had high efficiency, which can be used to study the genetic diversity in tobacco.

Keywords: burley, flue-cured, IRAP, oriental, SSR

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Introduction

For thousands of years, tobacco (*Nicotiana tabacum* L.) has been cultivated as a medicinal and commercial product in most regions worldwide (Flavell *et al*. 1992). Understanding plant genetic diversity facilitates the use of genetic resources in breeding programs. Today, DNA molecular markers not only reveal differences in coding sequences but also reveal differences between non-coding sequences of the genome. Studying ISSR regions is an important method that quickly shows the genetic diversity of individuals in a species. Inter-

simple sequence repeat (ISSR)-PCR is a technique involving microsatellite sequences as primers in a polymerase chain reaction to generate multilocus markers. It is a simple and quick method that combines most of the advantages of microsatellites (SSRs) and amplified fragment length polymorphism (AFLP) to the universality of random amplified polymorphic DNA (RAPD) (Pradeep Reddy *et al*. 2002). Also, the ISSR marker requires little template DNA and has higher primer length and Mendelian behavior (Ammiraju *et al*. 2001). ISSR markers have been used to study the genetic diversity of important crops such as wheat (Nagaoka and Ogihara 1997), rice (Blair *et al*. 1999), olive (Nezamivand Chegini *et al*. 2016), maize (Kantety *et al*. 1995), and bean (Mohsenzadeh Golfazani *et al*. 2021). Del Piano *et al.* (2000), Yang *et al.* (2005), Yang (2007), Xiao and Yang (2007), Samizade Lahiji *et al.* (2013), and Mohsenzadeh Golfazani *et al.* (2012) indicated that ISSRs are quick, simple, and inexpensive in assessing the genetic diversity in tobacco.

Retrotransposons are evolutionarily widespread genetic elements in the genome of eukaryotes. Unlike transposons, these elements insert their copies into new genomic regions through sequential transcription, reverse transcription, and eventual production of new cDNA, and the first copy will remain in its place (Wicker *et al.* 2007). All retrotransposons are bounded by long terminal repeats (LTRs). Two amplification-based marker methods based on the position of given LTRs within the genome have been developed. The IRAP (Inter-retrotransposon amplified polymorphism) markers are generated by the proximity of two LTRs using outward-facing primers annealing to LTR target sequences. In REMAP (retrotransposon-microsatellite amplified polymorphism), amplification between retrotransposons proximal to simple sequence repeats (microsatellites) produces the marker bands (Kalendar *et al.* 1999).

The present study reports on the use of ISSR markers for assessing the genetic diversity and relationships among 48 genotypes of flue-cured, burley, and oriental tobacco cultivars for future breeding purposes in Iran.

Materials and Methods

In this research, 48 tobacco genotypes were selected from the Tobacco Research Center of Guilan, including 28 burley genotypes, 12 oriental genotypes, and eight flue-cured genotypes (Table 1) to determine their genetic diversity. DNA was extracted according to Doyle and Doyle (1987)**.** The quality and quantity of DNA samples were determined by the agarose gel electrophoresis and spectrophotometer.

Table 1. Characteristics of the studied tobacco genotypes in the current study.

No.	Name	Type	Origin	No.	Name	Type	Origin
1	Burley-TN86	Burley	USA	25	Burley1	Burley	USA
2	Burley-A1	"	France	26	Burley151	"	USA
3	Burley-B.B16E	"	France	27	Burley-white-Iv-GeeI	"	USA
4	Burley-B.B163	"	France	28	Burley7	"	USA
5	Burley-Bursanica	"	Germany	29	B.12-2	Oriental	Zimbabwe
6	Burley-	"	Germany	30	B140-1	"	Zimbabwe
7	Shiro-enshu201	"	Japan	31	Basma178-2	"	Iran
8	Burley-keruzing	"	Germany	32	Basma-seres31	"	Belgium
9	Burley-R219	"	Germany	33	Ch.T.269-12D	"	Iran
10	Bursana-R220	"	Germany	34	D-566	"	USA
11	Bursanica	"	Germany	35	H.169	"	USA
12	By.14	"	USA	36	B.18i-8	"	Zimbabwe
13	Burley-TMV2	"	USA	37	Ch.T.269-12E	"	Iran
14	Burley-TMV3	"	USA	38	Ch.T.283-8	"	Iran
15	Burley-TMV4	"	USA	39	Ch.T.273-3B	"	Iran
16	Burley-WR.14	"	Belgium	40	Hiks426	"	Japan
17	Burley-B.5	"	Germany	41	Florida513	Flue-	USA
18	Burley-Pr-144	"	Germany	42	Badisher-Geudert-	"	Germany
19	Burley-Ree103	"	Belgium	43	Geudert-Heimer	"	Germany
20	Burley-Semperant	"	Belgium	44	Havana142	"	USA
21	Burlina	"	Germany	45	Holandicher-zigareten	"	Germany
22	Badisher-Burley-E	"	Germany	46	H-254-DRP4	"	Germany
23	Banket-A1	"	Zimbabwe	47	Havana307	"	USA
24	BB16A	"	France	48	Cu357	"	USA

Amplification of the genomic DNA for ISSR and IRAP primers (Table 2) was performed according to Yang *et al.* (2007) and for SSR (Table 3) according to Moon *et al.* (2009). The PCR reaction was done in a 10-microliter volume, including template DNA with the concentration of 30 to 30-40 ng, 0.6 mM dNTP, 0.5 mM $MgCl_2$, 1X PCR buffer, and 1 U DNA *Tag* polymerase enzyme (Samizade Lahiji *et al.* 2013).

Amplification of the genomic DNA by ISSR and IRAP primers was performed in a thermocycler (Biometra) programmed for a first denaturation step of 4 minutes at 94 ^{o}C, followed by 35 cycles of 94 ^{o}C for 40 seconds, 49-68 ^{o}C (varied for each primer according to Tables 2 and 3) for 40 seconds, 72 ^{o}C for 2 minutes, and final expansion at 72 ^{o}C for 5 minutes and then held at 4 ^{o}C. The amplified products were separated by electrophoresis in 1.5% (w/v) agarose gels for ISSR and IRAP primers and in 3% (w/v) agarose gels for SSR primers. Visualization of products in the gel was done by ethidium bromide staining and photographing under UV light. The banding pattern was rated

Table 2. The ISSR and IRAP primers were used for genetic diversity analysis of tobacco genotypes.

Primer	No.	Name	Ta	5’ - 3’
ISSR	1	UBC811	51	GAGAGAGAGAGAGAGAC
	2	UBC812	51	GAGAGAGAGAGAGAGAA
	3	UBC813	51	CTCTCTCTCTCTCTCTT
	4	UBC816	49	CTCTCTCTCTCTCTCTA
	5	UBC817	51	CTCTCTCTCTCTCTCTG
	6	UBC823	55	CACACACACACACACAT
	7	UBC824	55	CACACACACACACACAA
	8	UBC825	52	TCTCTCTCTCTCTCTCC
	9	UBC826	53	TCTCTCTCTCTCTCTCG
	10	UBC873	56	ACACACACACACACACT
	11	UBC811+UBC812	51	GAGAGAGAGAGAGAGAC + GAGAGAGAGAGAGAGAA
	12	UBC817+UBC826	52	CTCTCTCTCTCTCTCTG + TCTCTCTCTCTCTCTCG
IRAP	13	RTR-1	61	TATGCTGACCAAGGTGGTAC
	14	RTR-2	66	AAGTTGTCTGAGGCTTATGTGACTT
	15	RTR-7	61	TCCGCTGTGCAGTAGTGTTTAGTG
	16	RTR-8	64	CTTACCTCTCCCATACATCACCA
	17	RTR-10	62	AACGTGTTAATGTCGCTTTGTC

Ta: Annealing temperature

Table 3. Specifications of SSR primers used in this research.

No.	Name	Forward_Primer (5’- 3’)	Reverse_Primer (5’- 3’)	Repeat	Size	Ta
1	PT30001	TCATTTCGGGTTGAGTACCTTT	CATATGCTTCGGGAGATTGA	TA	187	55
2	PT30008	CGTTGCTTAGTCTCGCACTG	GGTTGATCCGACACTATTACGA	TA	192	56
3	PT30014	TGCCGTGTAAATTTCATTTGG	AGGATTCCTAACGTGTATTATGTTCT	TA	205	53
4	PT30021	CATTTGAACATGGTTGGCTG	CTCAACTCTCGTCGCTCTTG	TA	224	52
5	PT30024	GTAGATGGGAGAGCCACGTC	AAAGGAGGTAAATTGCAGCG	TA	169	51
6	PT30028	AAACTTGAAGCAGAGACGGC	GCACATGCGGATCTTGATTT	CA	171	55
7	PT30034	GACGAAACTGAGGATATTCCAAA	TGGAAACAAAGCCATTACCC	TAA	216	56
8	PT30036	GACCATGAGAGAACAAACCCA	GAAATCAAATCCAACGGCAC	GA	213	52
9	PT30044	TTAGCGGGAATGTAGAAGCG	GCGGTGGAAAGAGCTTCAG	GA	206	52
10	PT30046	GATAGGTAGATTATCCTCTGCAACA	GGTGCTAGCAACATCATCAAA	TA	187	56

Ta: Annealing temperature

based on the presence or lack of the bands, respectively (one and zero). The resulting data were entered in Excel software as a 48 × 263 matrix for further analyses.

A simple matching similarity matrix was constructed from the ISSR, IRAP, and SSR data, which was used for grouping the tobacco genotypes by the UPGMA clustering method. Also, principle coordinate analysis was carried out by Genestat Ver.12 to verify if the primers were able to cover a large part of the chromosomal regions of the tobacco genome in this study.

The percentage of polymorphic bands was calculated for each primer. The polymorphism information content (PIC) of a marker was calculated by the following equation),

$$PIC= 1 - \sum p_i^2$$

where pi is the frequency of the ith allele.

The number of effective alleles (Zhu *et al.* 1998), Shannon index (Shannon 1948), and genetic diversity of Nei (1972) were calculated by POPGEN ver. 1.31.

Results and Discussion

The 27 used ISSR, IRAP, and SSR primers amplified 263 bands, of which 228 bands were polymorphic (Figures 1 and 2, Table 4). UBC825, RTR-10, and mixed primers of UBC817 + UBC826 with 17 bands, UBC817 with 16 bands, and RTR-10 with 15 bands, had the highest number of amplified bands, and primer UBC824 with 10 bands, and primer UBC823 with 11 bands had the lowest number of bands among ISSR and IRAP primers. PT30044 and PT30046 had the lowest number of bands among SSR primers with two bands (Table 4). The percent of polymorphic bands varied from 76.42% for RTR-7 and RTR-1 to 94.11% for RTR-10 for ISSR and IRAP primers, however, it was 100% for the SSR primer. The average percent of polymorphic bands was 90.7% which shows the existence of proper genetic diversity among the tobacco genotypes (Table 4).

Mohsenzadeh Golfazani *et al.* (2012) obtained 147 polymorphic bands in tobacco (*Nicotiana tabacum*) using 12 ISSR primers, three retrotransposon primers, and a combined primer. Among them, TOS-2 with 17 bands, UBC 811, UBC 814, and TOS-1 with 16 bands had the highest number of bands, and UBC 825 and TOS-3 with six bands had the lowest number of bands. In their study, the percent of polymorphic bands varied from 61.5% for primers UBC 825 and TOS-3 to 94.11% for primer TOS-2 with an average of 77.36%. Our results agree with those of Mohsenzadeh Golfazani *et al.* (2012), possibly because of the similarities of ISSR primers used in both research.

Denduangboripant *et al.* (2010) amplified the genomic DNA of 66 tobacco varieties with 20 ISSR primers, which produced 128 bands. The bands for each primer varied from 1 to 11 with an average of 5. Five primers developed repeatable polymorphic bands and the others developed faint bands. The

Table 4. Primers, percentage of polymorphism, number of the bands, and the number of their polymorphic bands.

	Primer	Number of bands	Polymorphic bands	Percentage of polymorphism
ISSR	UBC811	13	12	92.3
	UBC812	13	12	92.3
	UBC813	12	10	83.33
	UBC816	12	10	83.33
	UBC817	16	14	87.5
	UBC823	11	10	90.9
	UBC824	10	8	80
	UBC825	17	14	82.35
	UBC826	14	11	78.57
	UBC873	14	11	78.58
	UBC811 + UBC812	13	12	92.3
	UBC817 + UBC826	17	15	88.23
Mean		162	139	85.8
IRAP	RTR-1	13	10	76.92
	RTR-2	13	11	84.61
	RTR-7	13	10	76.92
	RTR-8	15	13	86.66
	RTR-10	17	16	94.11
Mean		71	60	83.8
SSR	PT30001	3	3	100
	PT30008	3	3	100
	PT30014	4	4	100
	PT30021	4	4	100
	PT30024	3	3	100
	PT30028	3	3	100
	PT30034	3	3	100
	PT30036	3	3	100
	PT30044	2	2	100
	PT30046	2	2	100
Mean		30	30	100

differences between different studies are common due to the use of different primers and populations.

PIC varied from 0.31 to 0.5 with an average of 0.42. The highest PIC was observed in PT30046 (0.5), followed by

PT30044 (0.49), RTR010 (0.48), PT 30034 (0.48), UBC811 + UBC812 (0.47), RTR-8(0.46), and UBC825 (0.46). This indicates the efficiency of these primers in differentiating the varieties studied in this research.

Among ISSR primers, UBC812 + UBC811, UBC823, and UBC826 + UBC817 had the highest amount of PIC, and primers UBC811 and UBC816 had the lowest PIC (Table 5). Among IRAP primers, RTR-10 and RTR-8 showed the highest PIC and RTR-7 sowed the lowest PIC. Among SSR primers, PT30046, PT30044, and PT30034 had the highest PIC and PT30021 and PT30014 had the lowest PIC.

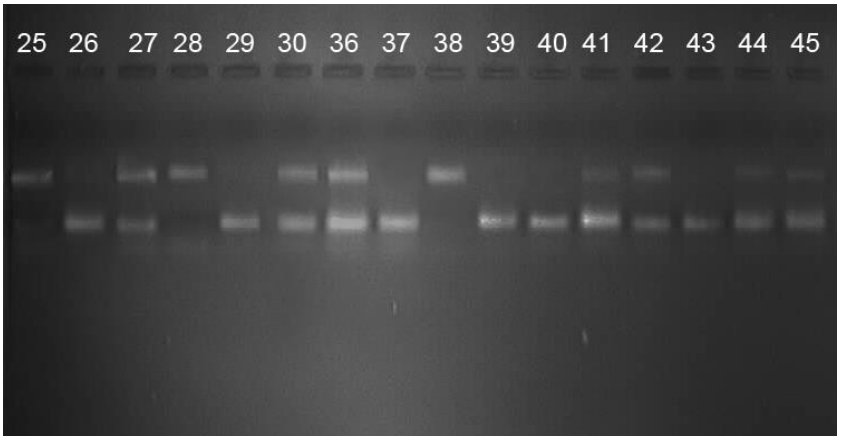

Figure 1. SSR amplification profile of 16 tobacco varieties by primer PT30044.

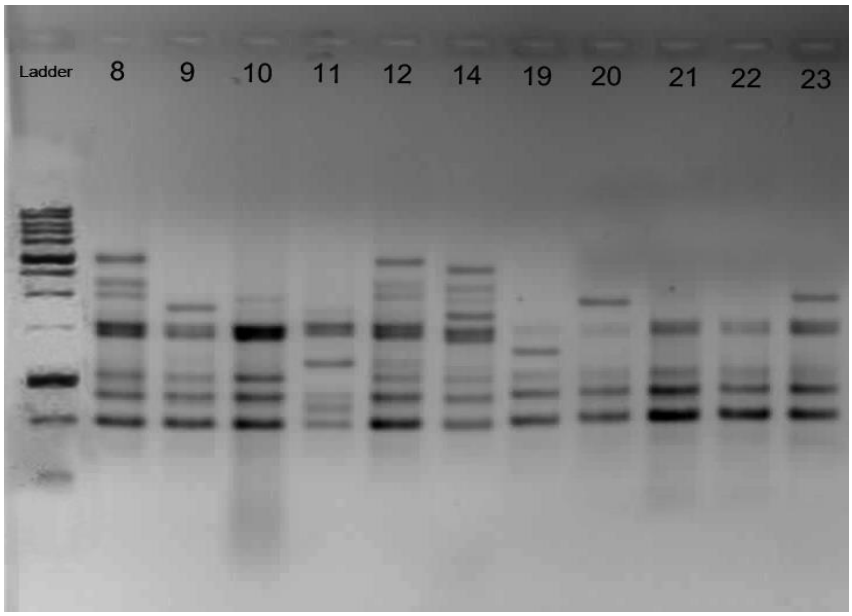

Figure 2. ISSR amplification profile of 11 tobacco varieties by primer UBC812.

Table 5. Polymorphism and diversity parameters for SSR and ISSR markers in the studied wheat genotypes.

No. of primers	Primer name	PIC	Shannon's index (H)	Nei genetic diversity (I)	Number of effective alleles (A)
1	UBC811	0.33	0.68	0.48	1.95
2	UBC812	0.40	0.59	0.40	1.73
3	UBC813	0.42	0.60	0.42	1.74
4	UBC816	0.36	0.55	0.37	1.67
5	UBC817	0.37	0.58	0.40	1.71
6	UBC823	0.46	0.58	0.40	1.70
7	UBC824	0.42	0.50	0.32	1.52
8	UBC825	0.41	0.65	0.46	1.87
9	UBC826	0.41	0.61	0.42	1.75
10	UBC876	0.44	0.63	0.44	1.82
11	UBC811+ UBC812	0.47	0.57	0.38	1.64
12	UBC817+ UBC826	0.46	0.60	0.41	1.74
13	RTR-1	0.43	0.58	0.40	1.70
14	RTR-2	0.42	0.59	0.41	1.72
15	RTR-7	0.40	0.64	0.45	1.85
16	RTR-8	0.46	0.62	0.43	1.77
17	RTR-10	0.48	0.62	0.42	1.75
18	PT30001	0.42	0.46	0.29	1.41
19	PT30008	0.42	0.45	0.29	1.45
20	PT30014	0.36	0.43	0.26	1.36
21	PT30021	0.31	0.41	0.26	1.42
22	PT30024	0.44	0.56	0.37	1.62
23	PT30028	0.44	0.50	0.32	1.48
24	PT30034	0.48	0.62	0.43	1.75
25	PT30036	0.44	0.58	0.40	1.70
26	PT30044	0.49	0.60	0.41	1.71
27	PT30046	0.50	0.58	0.40	1.66
Mean		0.42	0.57	0.39	1.67

Davalieva *et al.* (2010) used 30 SSR primers to study the genetic diversity of 10 tobacco varieties from Maghdonia and they reported a PIC between 0 to 0.76 with an average of 0.39. They concluded that the markers used to evaluate the Macedonian collection were appropriate and powerful tools for identifying desirable genotypes in Macedonian tobacco breeding programs. Siva Raju *et al.* (2008) used 9 paired AFLP primers to assess the genetic diversity among 54 cultivated varieties and three introduced germplasm of tobacco. All primers had high PIC, ranging from 0.72 to 0.87. Therefore, they concluded that all primers in their

research can be useful in assessing the genetic diversity of tobacco.

The number of effective alleles varied from 1.36 to 1.95 with an average of 1.67 (Table 5). Number of effective alleles was high for UBC811, UBC825, RTR-7, UBC8736, RTR-8, PT30034, UBC826, and RTR-10 primers (Figure 3). Therefore, they have the potential to be used in the studies for assessing the genetic diversity of tobacco.

One of the important indices for assessing the genetic diversity among different genotypes and populations is the Nei's index (Nei 1972). Based on the Nei index, the amount of genetic diversity varied from 0.26 to 0.45 with an average of 0.39. The highest amount of genetic diversity was shown for RTR-7, RTR-8, RTR-10, and PT30034, respectively.

The Shannon coefficient is an indicator of polymorphism (Shannon 1948). In this study, the average Shannon coefficient was 0.58 and varied from 0.41 to 0.68, which is an indicator of the existence of average diversity among the tobacco genotypes in this study. UBC811, UBC825, RTR-7, UBC8736, RTR-8, PT30034, and RTR-10 had the highest Shannon coefficient. These primers seem suitable for explaining genetic diversity in the population. The PT30014 and PT30021 primers had the lowest Shannon index (Table 5). In a study with different tobacco cultivars, the PIC, Shannon's index, and Nei's index were reported as 0.36, 0.4, and 0.72, respectively (He *et al.* 2020).

The dendrogram from the UPGMA clustering method using the simple matching coefficient of similarity is shown in Figure 4. At the cut-off point of 0.49 simple matching similarity coefficient, 48 tobacco genotypes were classified into five groups, which comprised 5, 12, 10, 6, and 15 genotypes, respectively.

Marker-based grouping was somewhat consistent with the origin of the genotypes, such that all five genotypes from Iran, including Basma178-2, Ch.T.269-120, Ch.T.269-12E, Ch.T.283-8, and Ch.T.273-3B, were placed in the 5th group. The reason should be that these five genotypes were all Eastern type. Of four genotypes from France, Burley-A1, Burley-BB16E, and Burley-BB163 were placed in the 1st group but the other genotype (B.B.163) was placed in the 3rd group. Of four genotypes from Belgium, three genotypes, Burley-WR-14, Burley-Ree103, and Burley-Semperant, were placed in the 2nd group and Basma-Seres31 was placed in the 4th group, which could be because the three genotypes were Burley type and Basma-Seres31 was an oriental type tobacco.

In classifying 49 Flue-cured tobacco genotypes, Mohsenzadeh *et al.* (2012) used 16 ISSR and retrotransposon primers with a UPGMA clustering algorithm. The genotypes

were classified into five groups. Grouping based on DNA markers was almost consistent with their geographical origin, such that Bergerac-c, Virgin, and Badisher Geudert from Germany were placed in the same group, and genotypes Tirtash-33 and Tirtash-4 from Iran were located together in another group. In another study, Edrisi *et al.* (2012) assessed the genetic diversity of 40 tobacco genotypes by using 12 ISSR primers. The clustering algorithm based on the UPGMA method placed the genotypes in 9 groups, indicating the existence of genetic diversity among the tobacco genotypes.

Yang *et al.* (2007) divided 118 tobacco genotypes into seven clusters. They used 29 ISSR markers and 5 IRAP markers. The first group had three subgroups which were comprised of Burley and wild-type genotypes. Most of the sun-cured genotypes were distributed in the 2nd and 3rd groups. The 4th, 5th, and 7th groups contained the flue-cured tobbaccos.

In a study using 12 ISSR primers, 89 tobacco genotypes were classified into five groups by the UPGMA algorithm (Samizade Lahiji *et al*. 2013). Also, Hassani-Tesie *et al.* (2014) evaluated the genetic diversity of 45 genotypes of tobacco, including Burley, flue-cured, and oriental types, using 12 ISSR primers. Cluster analysis using the UPGMA method divided the genotypes into six groups. The 1st and 3rd group consisted of Burley type, the 2nd group included flue-cured genotypes, and the groups 4, 5, and 6 comprised of the oriental type genotypes.

In another study, Hassani *et al.* (2016) used 20 combinations of IRAP and REMAP

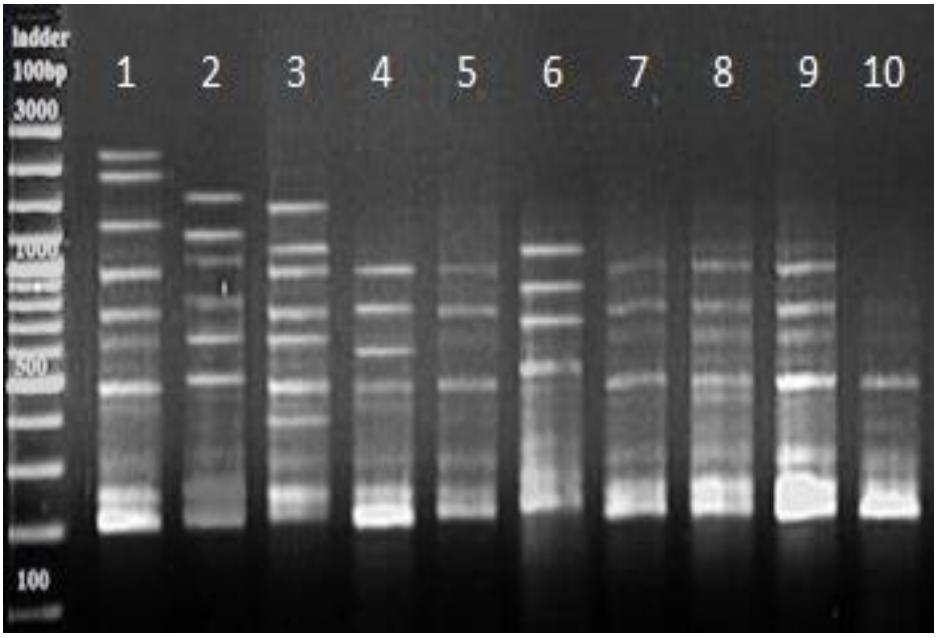

Figure 3. IRAP amplification profile of 11 tobacco varieties by primer RTR-7.

primers, of which five IRAP primers and nine REMAP primers were capable of producing banding patterns. In total, they reproduced 188 loci, 153 of which were polymorphic. UPGMA cluster analysis divided the 45 studied genotypes into five groups. Again, Burley, flue-cured, and oriental types were grouped in separate clusters. Their results indicated the usefulness of IRAP and REMAP markers for studying the genetic diversity of among different tobacco types.

Table 6 shows the distribution of 48 tobacco genotypes in the five clusters in our study. Genotypes from different groups may be used as parents in the crossbreeding programs of tobacco to produce desirable offspring (Brummer 1999). Nevertheless, genetic distance is not the only influential factor in identifying suitable parents for producing hybrid plants (Azizi *et al.* 2012). Other factors such as combining ability and genetic distance based on morphological traits should also be considered (Brummer *et al.* 1995; Brummer 1999). There have been

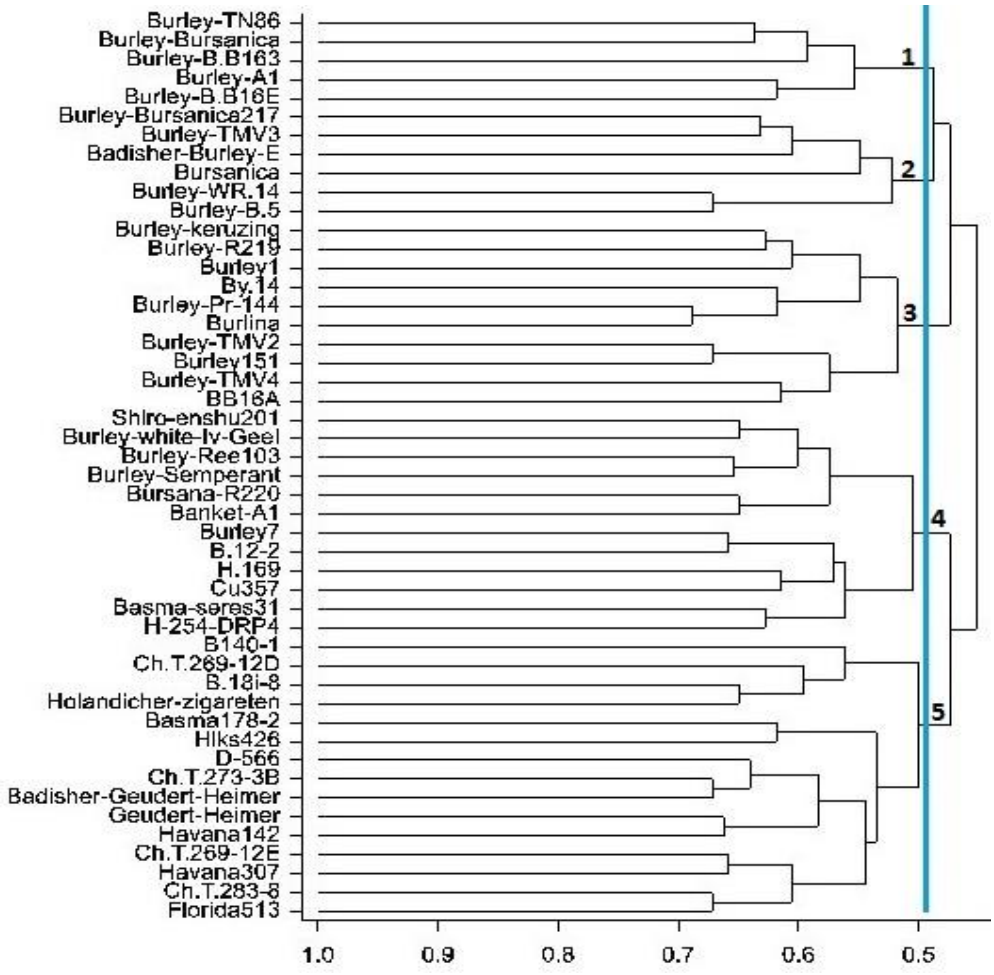

Figure 4. The dendrogram based on the UPGMA method and simple matching similarity matrix for 48 genotypes of tobacco under study.

Table 6. Groups of tobacco genotypes based on cluster analysis.

Group 1	Group 2	Group 3	Group 4	Group 5
Burley-TN86	Burley-Bursanica217	Burley-keruzing	Shiro-enshu201	B140-1
Burley-Bursanica	Burley-TMV3	Burley-R219	Burley-white-Iv-GeeI	Ch.T.269-12D
Burley-B.B163	Badisher-Burley-E	Burley1	Burley-Ree103	B.18i-8
Burley-A1	Bursanica	By.14	Burley-Semperant	Holandicher-zigareten
Burley-B.B16E	Burley-WR.14	Burley-Pr-144	Bursana-R220	Basma178-2
	Burley-B.5	Burlina	Banket-A1	Hiks426
		Burley-TMV2	Burley7	D-566
		Burley151	B.12-2	Ch.T.273-3B
		Burley-TMV4	H.169	Badisher-Geudert-
		BB16A	Cu357	Geudert-Heimer
			Basma-seres31	Ch.T.269-12E
			H-254-DRP4	Havana142
				Havana307
				Ch.T.283-8
				Florida513

contradictory results about the use of genetic diversity based on molecular markers for selecting parents of hybrid plants (Liu *et al.* 1999). This issue can be caused by three factors: the lack of linkage of the genes of interest to the markers, the incompatibility of the individuals selected for crossing, and the errors related to the molecular markers (Emel 2010). However, molecular markers can be used in breeding programs to identify the most diverse genotypes as parents of synthetic cultivars and hybrids (Azizi *et al.* 2012; Dadras *et al.* 2014; Hoshyardel *et al.* 2016; Ghafari Azar *et al.* 2019; Karim *et al.* 2019; Salehian *et al*. 2021).

Principal coordinate analysis (PCO) has been widely used to study genetic diversity based on qualitative traits and allows further interpretation of the relationship among individuals. The principal coordinate analysis showed that the first 14 vectors were able to explain only 51.49% of the total variance (Table 7). The first two vectors together accounted for 11.04% of the total variance. Therefore, PCO could not be used to group the tobacco individuals in our study. Nevertheless, the small share of the total variance by different eigenvalues indicated that the markers were scattered in different locations of the tobacco genome and covered more chromosomes to efficiently determine the genetic diversity among tobacco genotypes (Mohsenzadeh Golfazani *et al.* 2012).

Conclusion

In this study, the use of different genetic diversity indices such as the number of effective alleles, Ni‘s index, Shannon index, and PIC for primers UBC825, UBC876,

RTR-8, RTR-10, and PT30034 showed that these primers properly differentiated the tobacco genotypes under study. Therefore, these primers may be used efficiently to study the genetic diversity of tobacco. Cluster analysis showed that the grouping of genotypes with molecular data was to some extent consistent with their geographical origin. The genotypes from the cluster with the highest genetic distance can be used in the crossbreeding programs of tobaccos. In the principal coordinate analysis, a large number of components accounted for a small percentage of the total variation. Therefore, the primers used were correctly selected and covered larger chromosomal regions in the genome. As a result, they were able to separate tobacco genotypes well from each other.

Conflict of Interest

The authors declare that they have no conflict of interest with any people or organization concerning the subject matter discussed in the manuscript.

Table 7. Eigenvalues, variances, and cumulative variances obtained from the principal coordinate analysis.

No. of coordinates	Eigenvalue	Variance (%)	Cumulative variance (%)
1	1.24	6.1	6.1
2	1.01	4.94	11.04
3	0.88	4.33	15.37
4	0.81	4.01	19.38
5	0.77	3.78	23.16
6	0.74	3.66	26.82
7	0.74	3.64	30.46
8	0.69	3.38	33.84
9	0.67	3.33	37.17
10	0.64	3.17	40.34
11	0.60	2.98	43.32
12	0.57	2.82	46.14
13	0.55	2.71	48.85
14	0.53	2.64	51.49

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بررسی تنوع ژنتیکی انواع مختلف توتون از طریق نشانگرهای مولکولی

مصطفی مهدی زاده[1]، محمد محسن زاده گلفزانی[2*] و حبیب ا... سمیع زاده لاهیجی[3]

۱- محقق اصلاح نباتات، دانشکده علوم کشاورزی، دانشگاه گیلان، رشت
۲- دانشیار گروه بیوتکنولوژی کشاورزی، دانشکده علوم کشاورزی، دانشگاه گیلان، رشت
۳- استاد گروه بیوتکنولوژی کشاورزی، دانشکده علوم کشاورزی، دانشگاه گیلان، رشت
مسئول مکاتبه؛ Email: mohsenzadeh.mohamad@guilan.ac.ir

چکیده

اطلاعات مربوط به میزان تنوع ژنتیکی در ژرم‌پلاسم و روابط ژنتیکی بین ژنوتیپ‌ها برای بررسی و طراحی برنامه‌های به‌نژادی مهم می‌باشد و می‌تواند برای کمک به شناسایی و بهبود ژنتیکی ژرم‌پلاسم به‌کار رود. در این تحقیق، تنوع ژنتیکی ۴۸ ژنوتیپ توتون از تیپ‌های گرمخانه‌ای، بارلی و شرقی توسط ۱۲ نشانگر ISSR، ۱۰ نشانگر SSR و ۵ نشانگر IRAP بررسی شد. آغازگرهای UBC825، RTR-10 و آغازگر ترکیبی UBC817 + UBC826 با تعداد ۱۷ نوار و بعد از آن، آغازگر UBC817 با ۱۶ نوار و RTR-8 با ۱۵ نوار بیشترین تعداد نوار و آغازگر UBC824 با ۱۰ و UBC823 با ۱۱ نوار کمترین تعداد نوار در بین آغازگرهای ISSR و IRAP را داشتند. همچنین آغازگرهای PT30044 و PT30046 با ۲ نوار از کمترین تعداد نوار در بین آغازگرهای SSR برخوردار بودند. درصد چندشکلی به دست آمده از ۷۶/۹۲ درصد برای RTR-1 و RTR-7 تا ۹۴/۱۱ درصد برای RTR-10 در آغازگرهای ISSR و IRAP متغیر بود و چندشکلی در آغازگرهای SSR صد درصد به دست آمد. میانگین چندشکلی ۹۰/۷ درصد، وجود تنوع ژنتیکی مناسب بین ژنوتیپ‌های توتون را نشان می‌دهد. محتوای اطلاعات چندشکلی در این تحقیق بین ۰/۳۱ تا ۰/۵ و میانگین محتوای اطلاعات چندشکلی ۰/۴۲ بود. میانگین تنوع ژنی نی و شاخص شانون به ترتیب ۰/۳۹ و ۰/۵۸ به دست آمد. تجزیه خوشه‌ای به روش UPGMA، ژنوتیپ‌های توتون مورد بررسی را در پنج گروه قرار داد که به ترتیب شامل ۵، ۱۲، ۱۰، ۶ و ۱۵ ژنوتیپ شدند. بر اساس تجزیه به بردارهای اصلی ۱۴ مؤلفه اول ۵۱/۴۹ درصد از واریانس کل را توجیه کردند. پرایمرهای مورد استفاده در این مطالعه کارایی بالایی داشتند که می‌توان از آن‌ها برای بررسی تنوع ژنتیکی در توتون استفاده کرد.

واژه‌های کلیدی: بارلی، شرقی، گرمخانه‌ای، IRAP، ISSR، SSR