

Genetic diversity analysis of some species in Brassicaceae family with ISSR markers

Fatma Gül Maraş-Vanlıoğlu^{1,*}, Hümeysra Yaman¹, Fatma Kayaçetin²

¹Biotechnology Research Center, Field Crops Central Research Institute, Republic of Turkey Ministry of Agriculture and Forestry, 06170 Yenimahalle, Ankara, Turkey.

²Department of Breeding and Genetics, Field Crops Central Research Institute, Republic of Turkey Ministry of Agriculture and Forestry, 06170 Yenimahalle, Ankara, Turkey.

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*Corresponding Author

Tel.: +90 551 233 0330
E-mail:
fatmagul.maras@tarimorman.gov.tr

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Abstract

Brassicaceae is one of the biggest family which have thousands of species all around the world. In order to use wild mustard in a breeding process, their genetic kinship levels must be defined. Inter simple sequence repeats (ISSRs) are one of the common markers to evaluate genetic diversity. Here, 28 mustard genotypes representing four taxa, 17 of *Brassica juncea*, 2 of *B. nigra*, 2 of *B. rapa*, and 7 of *B. arvensis*, were investigated with seven ISSR primers. Totally, 160 bands were scored out of which 88.75% showed polymorphism. The polymorphism information content (PIC) varied from 0.25 to 0.40. The average heterozygosity (H_{av}), multiplex ratio (MR), marker index (MI), and resolving power (R_p) were calculated as 0.33, 9.07, 2.99, and 8.29, respectively. STRUCTURE (v. 2.3.4) analysis unraveled two subpopulations ($K=2$). The dendrogram, constructed based on Jaccard similarity coefficient using the Unweighted Pair Group Average (UPGMA), in which, the first branch consisted of *B. juncea*, *B. nigra* and *B. rapa*, and the second branch consisted of *B. arvensis*, supported the results of STRUCTURE analysis. Additionally, principal component analysis (PCA) analysis supported the dendrogram and clearly separated the four taxa. This study showed that ISSRs would be useful to determine the genetic diversity in the Brassicaceae family.

Introduction

In the world, there are about 372 genera and 4060 species of Brassicaceae family. Turkey has great biodiversity and the Irano-Turanian region is the possible origin district of this family (Karl & Koch, 2013). This region consists of approximately 900 species of Brassicaceae family, and Turkey has more than 606 species on its own and out of which 226 endemics (Koch et al., 2017; Mohammadin et al., 2017).

Investigation of Brassica genus was shown that it has a very long evolutionary process. The genus firstly took place in domestication as vegetables, and then as edible oilseed crops (Prakash et al., 2009; Kaur et al., 2014). It has three diploid species – *B. rapa* ($2n=20$, AA genome), *B. nigra* ($2n=16$, BB genome), and *B. oleracea* ($2n=18$, CC genome), and three amphidiploid species – *B. juncea* ($2n=36$, AABB genome), *B. carinata* ($2n=34$,

BBCC genome), and *B. napus* ($2n=38$, AACC genome) (Jiang et al., 2015). The relationship among these species was explained a long time ago (U, 1935). Furthermore, *B. arvensis* (*Sinapis arvensis*) has a close chloroplast and nuclear genome homology with *B. nigra* (Pankin & Khavkin, 2011; Prakash et al., 2009). Each species has been undergone different domestication process for different purposes like root vegetables (turnip), green leaves (Chinese cabbage), floral parts (broccoli, cauliflower), or oilseeds (Baker et al., 2017).

In the world, the total production of Brassicas is 71.3 M tonnes and China is at the first rank with the 33.9 M tonnes in 2016. The second country is India with 8.7 M tonnes production and followed by Russia with 3.1 M tonnes production. Brassicas are also cultivated in Turkey and the production is about 790 kilotonnes (FAOSTAT, 2018). Additionally, the production of oilseed brassicas is at the second rank among vegetable oils

(Banuelos et al., 2013). These show how important to develop new varieties that will be adapted better and give a higher yield. To achieve that, the gene pool should be increased. For this purpose, wild mustard like *B. arvensis* should be included in breeding programs. For these wild species, in addition to the morphological observations and yield parameters, a molecular analysis should be done to determine the genetic relationship among breeding materials. After the discovery of molecular marker techniques, they have been used for genetic relationships among plants during crop improvement programs (Varshney et al., 2005). Thanks to genetic data obtained by molecular markers, not only developing new varieties could take a shorter time but also more accurate data could be obtained.

One of the molecular markers which is fast, affordable, highly discriminative, and confidential is inter simple sequence repeats (ISSRs) (Safari et al., 2013). These markers recognize short DNA fragment repeats (2-6 bp) throughout the whole genome. Since ISSR loci have a high polymorphism ratio, a lot of alleles can be observed (Moghaddam et al., 2009). Because of that ISSRs are ideal tools to determine the similarity and differences among genotypes (Abdelmigid, 2012). With the help of statistical analysis of ISSR markers, the similarity ratio among targeted species can easily be brought out.

ISSR markers have been used for genetic diversity analysis and molecular characterization of different plants like bread wheat (El-Sherbeny et al., 2020), taramira (Zafar-Pashanezhad et al., 2019), ginger (Baruah et al., 2019), kewda (Nasim et al., 2020), cassava (Afonso et al., 2019), flax (Ahmed et al., 2019), asparagus (Chen et al., 2020), and anise (Akçali-Giachino, 2020). Besides, ISSR markers were used for genetic diversity analysis and molecular characterization of *Brassica* by different researchers (Kalia et al., 2017; Kaur et al., 2014; Koch et al., 2017; Kong et al., 2011; Mohammadin et al., 2017; Takahashi et al., 2019; Verma et al., 2016).

In this study, 28 *Brassica* genotypes consist of four different taxa were investigated for the genetic diversity analysis through ISSR markers. It was aimed to determine the genetic relationship between wild and cultivated *Brassica* species for directing future breeding programs. This method was chosen since it is effective, highly polymorphic, affordable, and rapid.

Materials and Methods

Plant material

The plants used in this study were obtained from the Central Research Institute for Field Crops (CRIFIC) and U.S. Department of Agriculture - Agricultural Research Services (USDA-ARS). Twenty-eight genotypes were chosen to represent four *Brassica* taxa; 17 of *B. juncea*, 2 of *B. nigra*, 2 of *B. rapa*, and 7 of *B. arvensis*.

All four taxa obtained were shown in Table 1. Young leaves of one to five plants were taken to represent each population and stored in silica gel.

Table 1. List of *Brassica* taxa and their locations.

No	Species	Locality
1	<i>Brassica juncea</i>	İzmir
2		Turkey
3		Tekirdağ
4		Tekirdağ
5		Kırklareli
6		Edirne
7		Tekirdağ
8		India
9		Pakistan
10		China
11		China
12		Russia
13		Russia
14		Germany
15		United States
16		India
17		Konya
18	<i>Brassica nigra</i>	Turkey
19		Turkey
20	<i>Brassica rapa</i>	Tekirdağ
21		Turkey
22	<i>Brassica arvensis</i>	Kırşehir
23		Tokat
24		Ankara
25		Tekirdağ
26		Şanlıurfa
27		Ankara
28		Ankara

DNA extraction and polymerase chain reaction

The DNA extraction of plants was made by using a DNeasy Plant Mini Kit from Qiagen (Hilden, Germany). The quality and quantity of samples were determined in the 1% agarose gel. PCR amplifications were done by following the instructions of the manufacturer (Jena Bioscience®) with some modifications; in a total volume of 20 µl master mix (5 x Red Load Taq Master); containing 20-50 ng of genomic DNA and 1µM primer. PCR reactions were started with an initial denaturation of 5 min at 94°C; followed by 35 cycles of 1 min at 94°C, 1 min at 52°C (annealing) and 1.5 min at 72°C (extension), with a final step at 72°C for 7 min. PCR products were separated by 2.5% agarose gel electrophoresis with 1 x TBE buffer and stained with ethidium bromide. The gels were visualized by using VILBER Quantum-ST4 1100/26MX Imaging Cabinet (Vilber Smart Imaging, Marne-la-Vallée, France).

Data analysis of ISSR

Seven primers formed reliable polymorphic bands out of 78 ISSR primers were chosen for ISSR screening (Table 2). The GC ratio of the primers was between 44.4-56%.

Resolving power (R_p) of each ISSR primer was calculated as $R_p = \sum I_b$ in which the value of I_b (band informativeness) is determined as $1 - (2 \times |0.5 - p|)$ and p is the proportion of accessions containing the band (Prevost & Wilkinson, 1999).

The polymorphism information content (PIC) of an ISSR locus was calculated as $PIC = 2p \times (1 - p)$, where p is the proportion of the accessions with a band and $(1 - p)$ is the proportion of the accessions without a band (Roldan-Ruiz et al., 2000). Average heterozygosity (H_{av}) was calculated by taking the average of PIC values obtained for all the markers used in and the formula is as below:

$$H_{av} = \sum [2p \times (1 - p)] / N$$

Multiplex ratio (MR) was obtained by the multiplication of the mean value of fragments amplified by genotypes to a specific marker (n) with polymorphic band ratio (β) as declared by (Kumar et al., 2014; Powell et al., 1996).

$$MR = n \times \beta$$

Marker index (MI) was estimated by the multiplication of H_{av} with MR (Powell et al., 1996).

$$MI = H_{av} \times MR$$

According to the fragment sizes, bands were recorded as present (1) and absent (0). Clustering analysis (CA) and principal component analysis (PCA) were made by using NTSYSpC ver. 2.1. program of IBM PC (Rohlf, 2000). A Mantel test was applied to DICE, simple matching (SM), and Jaccard similarity coefficients (Mantel, 1967). Unweighted Pair Group Average (UPGMA) clustering was made by using Jaccard similarity coefficient for CA. PCA was obtained by applying the following steps: a distance matrix between

the samples was calculated, the matrix of distances was double-centered, and finally, for the plot, the double-centered matrix was factored (Rohlf, 2000).

The population structure was investigated using STRUCTURE software (v. 2.3.4) that separates groups according to allelic frequencies (Pritchard et al., 2000). The hypothetical number of subpopulations (K) was set as a continuous series of K from 1 to 10 in 10 independent runs. A burn-in period of 100,000 steps was followed by 100,000 Monte Carlo Markov Chain (MCMC) iterations (Evanno et al., 2005). The highest ΔK values were calculated through STRUCTURE HARVESTER to unravel the final population structure (Earl & vonHoldt, 2012). The subpopulation number was obtained from the STRUCTURE program by using the K values. The F_{st} value for the differentiation of population and expected heterozygosity in the same cluster was calculated with the STRUCTURE.

Results

In this study, seven primers were chosen out of 78 ISSR oligonucleotide primers to find out the genetic relationship among 28 *Brassica* genotypes. Totally, 142 polymorphic, 5 monomorphic, and 13 unique bands were acquired. The bands which were common for more than 95% genotypes were called monomorphic. The highest number of polymorphic bands was derived from UBC 827 with 26 bands and the average frequency per primer was 20.29. The polymorphism ratio changed between 65.2-100% and the average was found as 88.75%. The highest polymorphism ratio was obtained from UBC 827 with 100%. The band length scored from primers ranged approximately from 120 base pairs (bp) to 2500bp. The PIC value was ranged between 0.25 and 0.4 (Table 2). The R_p value was between 5.36 and 14.57 for each primer and the mean value for R_p was calculated as 8.29. The average heterozygosity (H_{av}) obtained from all over the primers was 0.33 and the MR value was recorded as 9.07. Finally, the MI value which shows the efficiency of a marker was 2.99. Mantel test (Mantel, 1967) was performed to determine the 'goodness of fit' for the UPGMA dendrograms and the maximum r-value was obtained by Jaccard similarity coefficient as 0.98264.

Table 2. List of the information of ISSR primers used for the 28 accessions of *Brassica* taxa. R=A, G; Y=C, T.

Primers	Sequence 5'→3'	Tm (°C)	GC (%)	Size (bp) min-max	# of poly. bands	# of mono. bands	# of unique bands	Polymorphism rate (%)	PIC	R_p
UBC814	(CT) ₈ A	46	47	220-2.500	17	-	1	94.4	0.34	6.86
UBC826	(AC) ₈ C	55	52.9	120-1.200	21	1	-	95.5	0.36	7.86
UBC827	(AC) ₈ G	55	52.9	150-1.300	26	-	-	100	0.40	14.57
UBC830	(TG) ₈ G	55	52.9	120-1.350	23	2	2	85.2	0.32	7.36
UBC834	(AG) ₈ YT	49-52	44.4-50	190-1.000	15	1	1	88.2	0.32	5.36
UBC835	(AG) ₈ YC	50-54	50-56	140-1200	25	-	2	92.6	0.34	10.71
UBC845	(CT) ₈ RG	48-52	50-56	230-2000	15	1	7	65.2	0.25	5.36
Total					142	5	13			
Average					20.29			88.75	0.33	8.29

According to the scoring result, *B. juncea* (10) originated from China has the highest number and ratio of the polymorphic locus with a value of 87 and 61.3%, respectively. Otherwise, *B. rapa* (21) originated from Turkey gave the lowest number and ratio of the polymorphic locus with a value of 51 and 35.9%, respectively.

With respect to the dendrogram generated by Jaccard similarity coefficient (Figure 1), the samples from *Brassica* taxa were collected in two main groups (at 30% similarity level). The first group included *B. arvensis* accessions collected from the different regions of Turkey showed genetic similarity with the ratio of 48-56%. The second group consisted of 21 genotypes; 17 of *B. juncea*, 2 of *B. nigra*, and 2 of *B. rapa*. The genetic similarity ratio among this group ranged from 36.5% to 99%. The second group also had 2 subclusters. One of these subclusters consisted of 2 *B. rapa* accessions originated from Turkey, the other consisted of *B. juncea* and *B. nigra* samples. Moreover, *B. nigra* originated from Turkey, and *B. juncea* originated from a different region of the world that occurred in two separate clusters.

According to the clustering analysis, the similarity level of *B. juncea* (2) and *B. juncea* (3) is 99%. *B. juncea* (2) was obtained from USDA-ARS and only information about this accession was that the origin was Turkey. On the other hand, it is known that *B. juncea* (3) was collected from Tekirdağ, a city in the northwest of Turkey. This suggested that *B. juncea* (2) might have been collected from Tekirdağ. The second-high similarity level was observed between *B. juncea* (4) and *B. juncea* (5) with a value of 97%, which are from Tekirdağ and Kırklareli, respectively. The other sample in the same cluster was *B. juncea* (6) from Edirne. All of these accessions were located on the northwest side of Turkey between the Marmara and the western Black Sea. *B. juncea* (12) and *B. juncea* (13) originated from

Russia were similar to each other at a 92% similarity level.

The total number of bands (TNB) was 121 and 113 out of seven ISSR primers in *B. juncea* and *B. arvensis* accessions, respectively. The average number of bands per primer is 17.29 (*B. juncea*) and 16.14 (*B. arvensis*). The maximum number of amplified products was obtained from UBC 827 for both *B. juncea* and *B. arvensis* with the number of 23 and 21, respectively. The minimum number of bands was obtained as 11 from UBC 845 for *B. juncea* and 14 from UBC 834 for *B. arvensis*. The resolving power (*R_p*) of the primers used in *B. juncea* was observed between 2.6 for both UBC 834 and UBC 845 and 8.1 for UBC 827. For the *B. arvensis*, *R_p* ranged from 4.9 for UBC 830 and 10 for UBC 827. The number of private bands (NPB) obtained with primers is 9 which is from 6 out of the 7 ISSR primers. The NPB observed in all accessions of *B. nigra* was 3 that was absent in all accessions of *B. juncea*, *B. rapa*, and *B. arvensis*. NPBs were observed in all accessions of *B. rapa* was 2 that was absent from the rest. Finally, there were 4 private bands in all accessions of *B. arvensis*. The *PIC* value was obtained between 0.14 and 0.26 for *B. juncea*, the mean was 0.18, while it was between 0.21 and 0.39 for *B. arvensis* and its mean was 0.31 (Table 3). Since the number of *B. nigra* and *B. rapa* accessions is low, specific *PIC* value for these species was not included.

To investigate more, 2- dimensional PCA was computed based on the ISSR band pattern using the J similarity coefficient. (Figure 2). The first three eigenvectors occurred 47.01% of the total variance (29.29% the first vector, 9.54% the second vector, and 8.18% the third vector). The results were in line with cluster analysis and formed 4 groups as *B. juncea*, *B. rapa*, *B. nigra*, and *B. arvensis*. *B. juncea* (16) and *B. juncea* (17) had a high dissimilarity ratio with respect to the rest of *B. juncea* accessions according to Jaccard

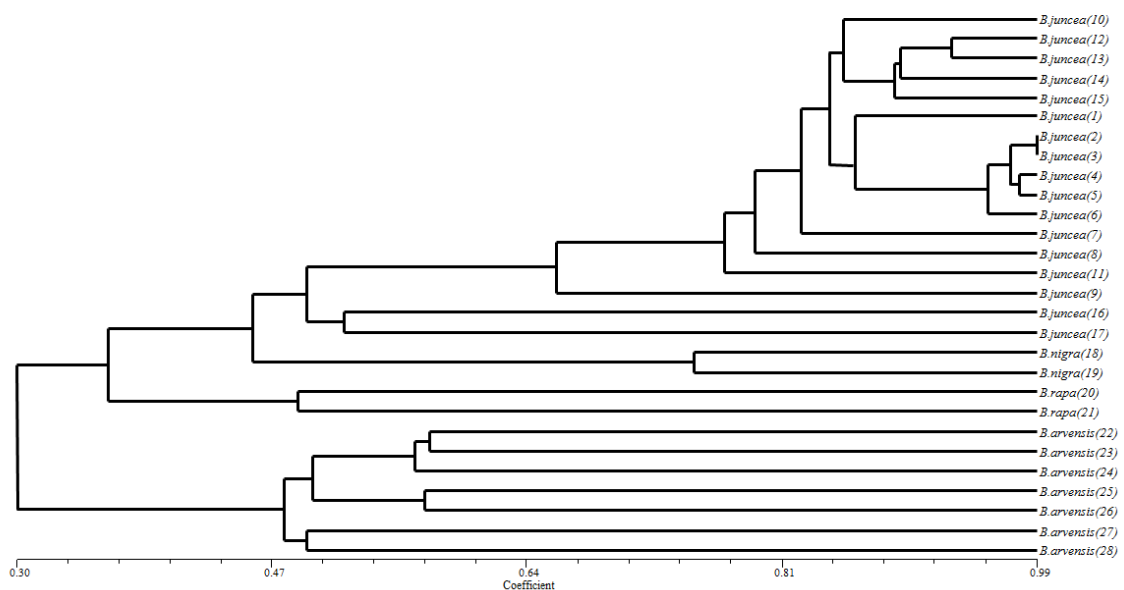


Figure 1. UPGMA-based cluster analysis of Brassicaceae family with 28 populations.

similarity coefficient index and this situation was confirmed by PCA. As expected, similar taxa were placed closer to each other.

Table 3. List of the information of ISSR primers for *B. juncea* and *B. arvensis*. Total number of bands (TNB), resolving power (*Rp*), number of private bands (NPB), and polymorphism information content (*PIC*). R= A, G; Y=C, T.

Amphidiploid lines of <i>B. juncea</i> (2n=4x=36)					
Primer	TNB	Polymorphism rate (%)	<i>Rp</i>	NPB	<i>PIC</i>
UBC814	14	92.9	3.4	-	0.20
UBC826	22	77.3	4.1	-	0.15
UBC827	23	87	8.1	-	0.26
UBC830	21	61.9	3.9	-	0.14
UBC834	13	69.2	2.6	-	0.15
UBC835	17	88.2	4.4	-	0.19
UBC845	11	90.9	2.6	-	0.19
Total	121		29.1	-	
Average	17.3	80.2	4.2		0.18

Diploid lines of <i>B. arvensis</i> (2n=2x=18)					
Primer	TNB	Polymorphism rate (%)	<i>Rp</i>	NPB	<i>PIC</i>
UBC814	12	91.7	5.6	-	0.39
UBC826	17	76.5	6.9	1	0.28
UBC827	21	95.2	10	1	0.34
UBC830	16	62.5	4.6	1	0.21
UBC834	14	85.7	7.7	-	0.36
UBC835	17	70.6	6.9	1	0.26
UBC845	16	93.8	7.7	-	0.34
Total	113		49.4	4	
Average	16.1	82.3	7.1		0.31

STRUCTURE (v. 2.3.4) analysis was used to analyze population structure. K values for sub-populations were determined as between 1 – 10 and the peaks were detected at K = 2 (Figure 3b) according to the

computational result of ΔK . At K = 2, the first cluster (Figure 3a, red) contains all *B. arvensis* accessions and the second cluster (Figure 3a, green) contains the 12 of 17 *B. juncea* accessions. The rest is in the transition between the two subpopulations (Figure 3a). These are compatible with the dendrogram (Figure 1) obtained from UPGMA method. The mean expected heterozygosity and *Fst* values were calculated as 0.2308 and 0.4212, respectively. These high values are the indicator of high heterozygosity among the genotypes.

Discussion

For breeding programs, obtaining of the targeted characters highly depends on having a big genetic pool. At this point, wild relatives of cultivated species have a great importance. These wild relatives can be used as a parent on the breeding process (Lara-Fioreze et al., 2013). Because of that, the main target of this study was to determine the genetic relationship between wild and cultivated *Brassica* species for directing future breeding programs.

There is a lot of research investigating the relationships in the Brassicaceae family by using microsatellite markers (El-Esawi et al., 2016; Singh et al., 2018; Thakur et al., 2017b). Also, there are a lot of studies in which specifically ISSR markers used to determine genetic relationships in the Brassicaceae family (Khalil & El-Zayat, 2019; Safari et al., 2013; Shen et al., 2016; Wang et al., 2017).

In this study using ISSR markers, the rate of polymorphism was obtained as 88.75% and the number of polymorphic bands per primer was 20.29. In another study conducted in *B. juncea*, polymorphism rate and the number of polymorphic bands per primer were 91.2% and 15.73, respectively (Gupta et al., 2014).

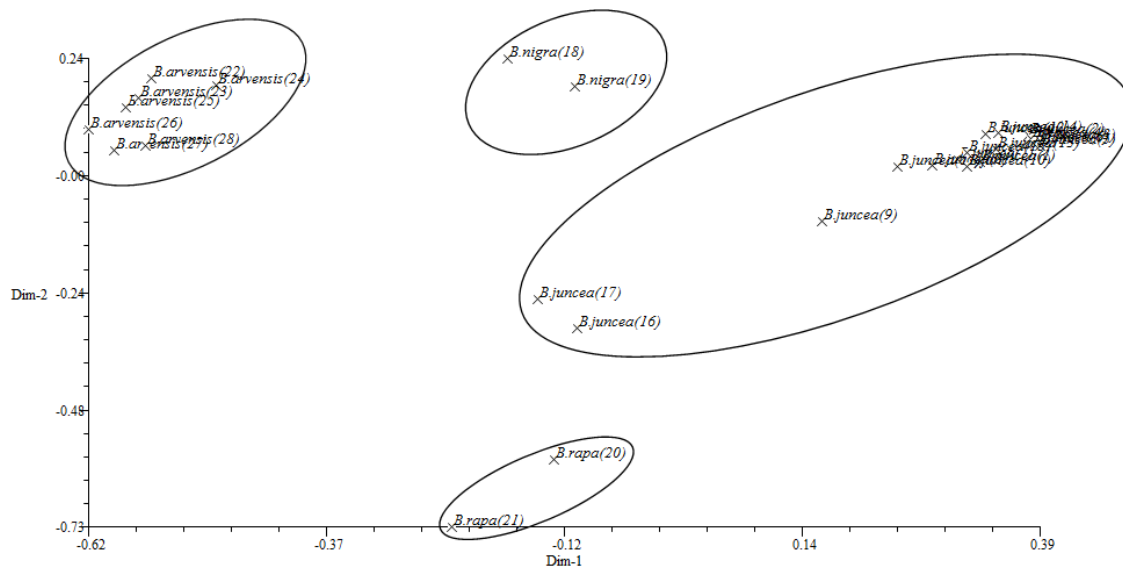


Figure 2. Two-dimensional PCA of 4 Brassica taxa with 28 accessions.

Furthermore, Abdelmigid et al. (2012) showed that the polymorphism rate was 87% and the number of polymorphic bands per primer was 13.4 in *B. napus*. The polymorphism rate of both studies was similar but the polymorphic bands per primer were higher in our study which could be because of different populations and markers.

Gohel and Mehta (2014) determined the genetic diversity among the 20 Indian mustard (*Brassica juncea*) genotypes grown in the northern states of India with ISSR primers. The dendrogram, which they drew according to Jaccard similarity index, was divided into two major branches and their similarities ranged from 47.8% to 100%. Here, we found that the similarity level among Indian origin *B. juncea* was determined as 50.6% and they divided into two different clusters. Similarly, Yadav and Rana worked with 30 Indian mustard genotypes in 2012, and found that the range of similarity was changing between 50% and 100%.

According to the triangle of U theory, *B. juncea* ($2n = 36$) is a hybrid of *B. nigra* ($2n = 16$) and *B. rapa* ($2n = 20$), which is closer to *B. nigra* in terms of genetic distance. Based on the results obtained in the current study, *B. nigra* was similar to *B. juncea* in the dendrogram. Similarly, in two different studies, it was found that *B. juncea* was closer to *B. nigra* more than *B. rapa* (Kaur et al., 2014; Thakur et al., 2017b).

For *B. nigra*, *B. rapa*, and *B. arvensis*, private ISSR bands were obtained. There are three, two, and four private bands for *B. nigra*, *B. rapa*, and *B. arvensis* accessions, respectively. These new markers could be used as a molecular tool to discriminate each species from the rest after testing these markers with more accessions from each species. A similar pattern had been described earlier to discriminate *Brachypodium distachyon* and *Brachypodium hybridum* species from each other (Contreras et al., 2017).

Additionally, a total of 13 unique bands were obtained from 5 of the 7 ISSR primers used in the study. These bands have great potential because they can be converted into Sequence Tagged Site (STS) or Sequence Characterized Amplified Regions (SCARs) (Gupta et al., 2014). Moreover, unique bands can be used to separate the cultivars from each other without the need for field trials (Fernández et al., 2002). In other words, unique bands obtained in this study can be used to obtain a genotype-specific profile. For example, in the current study, 5 of the 13 unique bands obtained from the ISSR marker screening belong to *B. rapa* (20), while 3 unique bands belong to *B. arvensis* (26) and 2 unique bands belong to *B. juncea* (9). Similarly, in a study conducted in 23 *B. juncea* accessions, originated from North India with 15 RAPD markers, 21 genetic specific unique bands

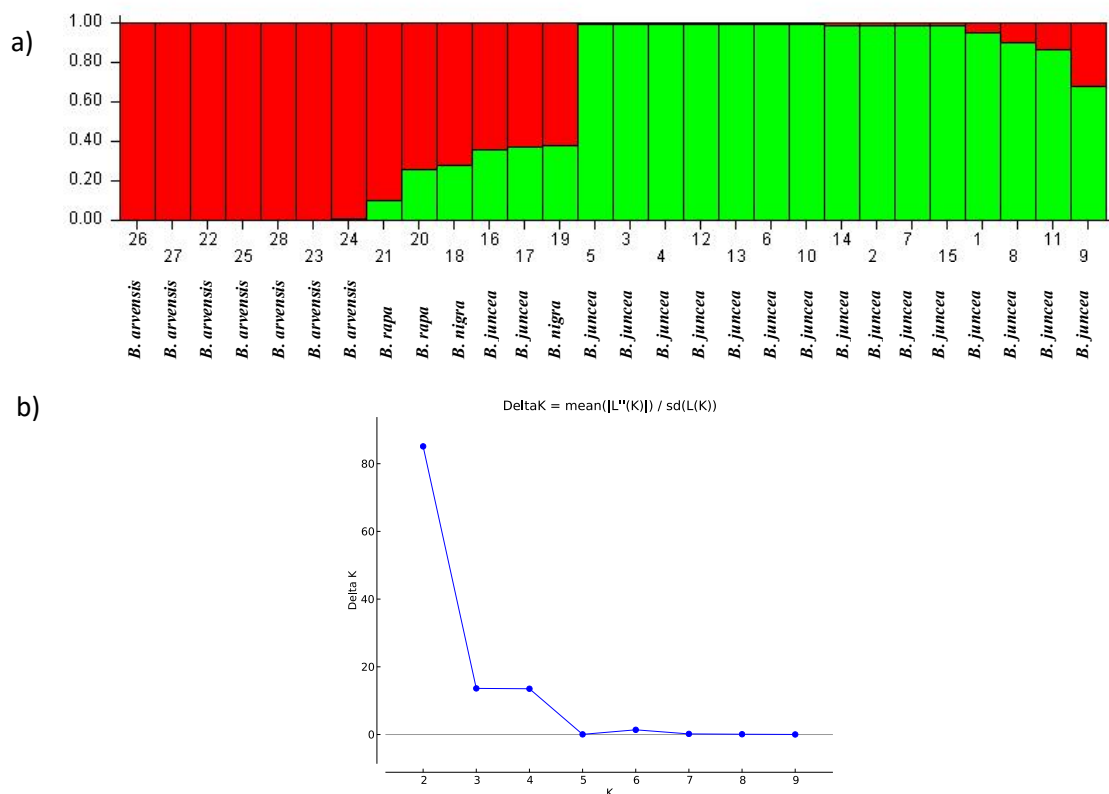


Figure 3. (a) Population structure of 28 Brassica genotypes using 160 loci based on ISSR scoring at $K = 2$. Each accession is indicated by a vertical line and each color represents a different cluster. (b) ΔK is computed between $K = 1 - 10$ and the peak value was obtained at $K = 2$.

were obtained from 12 RAPD markers (Gupta et al., 2014).

To understand the effectiveness of ISSR markers used, *Hav*, *MR*, *MI*, and *Rp* values were calculated. In this study, the *PIC* value was ranged between 0.25 and 0.4, and the *Hav* calculated from *PIC* value was 0.33 which is supported by Mahjoob et al. (2016). There are other studies for different plants like *Tribulus terrestris* (Sarwat et al., 2008), anise (Akçali-Giachino, 2020), and taramira (Zafar-Pashanezhad et al., 2019) and the value obtained for *Hav* are pretty much similar. The presence of higher *MR* value is always desirable for genetic diversity analysis. Here, we calculated *MR* value as 9.07 which was higher than the published other studies related to not only Brassicas but also other species (Afonso et al., 2019; Ahmed et al., 2019; Kalita et al., 2007). This shows the efficiency of the ISSR markers for the Brassicaceae family. Higher *MI* value (2.99) was obtained in comparison to Kalita et al. (2007). They also evaluated 28 Brassica accessions using 7 ISSR primers. However, Mahjoob et al. (2016) also revealed a higher *MI* value (6.4) than our study using 13 ISSR markers for 35 Brassica genera. The difference between *MI* values with respect to previous studies could be because of different accessions and primers used.

Rp is a parameter to determine the ability of a marker to separate the genotypes in the population. The *Rp* value higher than 1.50 points out a highly polymorphic character (Thakur et al., 2017a). The higher value of *Rp* is correlated with the number of fragments obtained from the marker. The reason for that is the formula of *Rp* is based on the cumulative I_b values. Our results for *Rp* ranges between 5.36 (UBC834 - UBC845) and 14.57 (UBC827). The mean value for overall markers was 8.29, which was really high with respect to previous studies (Gupta et al., 2014; Kalita et al., 2007; Singh et al., 2018; Teklewold & Becker, 2006; Thakur et al., 2017a), at which different species of Brassica genus were investigated.

The marker effectiveness was evaluated not only for all accessions but also specific to *B. juncea* and *B. arvensis* taxa. It was seen that ISSR markers used in the current study worked more effectively for *B. arvensis* (*Rp* = 7.1) than *B. juncea* (*Rp* = 4.2). This result is also supported by 4 NPBs for *B. arvensis* which can be used efficiently for the future analysis.

The population differentiation value (*Fst*) ranges between 0 and 1. A higher value means higher genetic differentiation between populations (Thakur et al., 2017a). Greater *Fst* value than 0.15 is an indicator of significant genetic differentiation (Frankham et al., 2010). In the present study, mean *Fst* value was computed as 0.4212 which was higher than previous studies (Chen et al., 2020; Ciancaleoni et al., 2018; Yousef et al., 2018) or similar to some of them (Sun et al., 2018; Tian et al., 2017). Likewise, the high value of expected heterozygosity is desirable for high genetic variation in the population. In previous studies, the mean value of expected heterozygosity was recorded as

0.088 (Takahashi et al., 2019) and 0.2883 (Wang et al., 2017). In the current study, the mean value of expected heterozygosity is higher than or similar to former studies which could be because of the substantial genetic diversity in the population.

ISSR technique is a combination of the benefits of AFLP and universality of RAPD. ISSRs are promising markers because of its longer primers (16-25 mers) with respect to RAPD primers (10-mers) that provides high annealing temperature (45-60°C) and reproducibility (Pradeep Reddy et al., 2002; Tarikahya-Hacıoğlu, 2016). Mahjoob et al. (2016) compared different markers like ISSR, IRAP and REMAP for evaluation of genetic diversity in *Brassica sp.* and it was shown that the ISSR markers were the most effective one for genetic diversity among Brassicaceae family.

Overall, this study showed that an acceptable level of analysis for genetic diversity of Brassica genus can be applied via ISSR markers and this data can be used to improve new *Brassica* species through a breeding program.

Conclusion

Brassica genus is an important crop since it is used not only in industry but also as vegetables. To increase the gene pool and to target the specific desired traits for future breeding programs, using wild relatives is so crucial. For this reason, identifying the genetic relationship among taxa would be very enlightening to breeders. Here, genetic diversity of four major taxa of Brassica genus was investigated by using ISSR primers. In the present study, a high proportion of interspecific and intraspecific variation was observed. This can be useful for selecting the parental lines to create a roadmap to develop new mustard varieties.

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