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Genetic Characterization of *Brassica oleracea* L. var. *acephala* DC cultivars from Bosnia and Herzegovina

Jasmin Šutković^{1*}, Petar Glamočlija², Lutvija Karić², Ahmet Yildirim¹

¹ Department of Genetics and Bioengineering, International University of Sarajevo, Sarajevo, BiH

² Faculty of Agricultural and Food Sciences, University of Sarajevo, BiH

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Abstract

Fifteen *Brassica oleracea* L. var. *acephala* DC accessions were collected and evaluated for their genetic diversity and molecular characteristics using 10 previously developed and used codominant SSR markers. The SSR loci used revealed a total of 51 alleles. The domestic kale varieties were collected locally and the hybrids kales were found in markets. The averages of total heterozygosity (H) and polymorphic information content (PIC) was 0.6774 and 0.6201, respectively. The average number of alleles was 4.2 per locus, where 8 of 10 markers showed high polymorphic information content (PIC), being more than 0.5. The genetic relationships among the populations revealed by Unweighted Pair Group Method with Arithmetic mean (UPGMA), showed a clear clustering into three distinct groups. As expected, all local varieties were separated within two separated clusters, whereas only two domestic varieties shared one cluster with the group of hybrids, indicating similar genetic background shared by domestic and hybrid varieties. These results indicate and confirm the regional and physiological differences between the kale varieties and confirm the SSR marker efficiency for genetic characterization assays.

*Correspondence

E-mail: jsutkovic@ius.edu.ba

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Introduction

The species *Brassica oleracea* L. ($2n = 2x = 18$) is an extraordinary vegetable, which includes different cultivars, each one with a slightly different morphology and domestication history: kales (var.

acephala); Chinese broccoli (var. *alboglabra*); cauliflowers (var. *botrytis*); cabbages (var. *capitata*); Brussels sprouts (var. *gemmifera*); kohlrabies (var. *gongylodes*); broccoli (var. *italica*); and savoy cabbages (var. *sabauda*) (Lotti et al., 2018). *B.*

oleracea wild kale relatives originated from the Mediterranean area or in the European northwest region 2000 BC through a domestication process (Acikgoz, 2011; Balkaya and Yanmaz, 2005).

Kales, Cabbage, and other Brassicas are at present grown extensively throughout all parts of the world. For example, in China the annual planting area is nearly 2.67 million hm² (Wei Y et al., 2019), in India, it is being grown over an area of 400 thousand hectares (Gorka et al., 2018). The annual average world production of cabbage is estimated to be about 70,644,191 tons. The major cabbage producing countries are China, India, Russian Federation, Japan, and the Republic of Korea, where globally the cabbage production was 71,048,011 tons on average, with the average productivity of about 29.23 t/ha. In Bosnia and Herzegovina, in the period 2001-2017, the Brassica variety production was on average 67450 tons (Faostat, 2019).

Further, kale and cabbage are essential vegetable crops as being very beneficial for human consumption. Therefore, it is a central food source containing many bioactive compounds (Singh et al., 2006; Volden et al., 2008). Kales are known as “powerhouse” vegetables because they are strongly associated with reduced risk of heart disease and other non-communicable diseases. This crop is ranked 15th on the list of “powerhouse” fruits and vegetables (a 100g serving provides $\geq 10\%$ of the daily value of 17 essential nutrients) (Siva et al., 2019). As one of the members of perennial plants, *Brassica oleracea* has a lifespan of up to 10 years or longer (Di Noia, 2014).

Bosnia and Herzegovina (BiH) belongs to the top European biodiversity countries. Due to the political and economic isolation in the past, many traditional crop varieties remained genetically authentic. Two seed genetic banks exist currently in BiH, where more than 1100 accessions are conserved (Duric and Golub, 2019). Some of domestic varieties are used in agricultural production, belonging to the autochthonous group, mainly found in Herzegovina region, located in the south-west region of BiH (FAO, 2012). Due to a high number of kale varieties in this region, preservation of genetic resources as a mandatory measure for diversity conservation is

required. The production of kale in Bosnia and Herzegovina, particularly in Herzegovina, is not of great commercial importance, but it is grown as a very common vegetable in almost every family farm (Sefo et al., 2010). Indeed, through governmental, regional, local and agricultural associations, the conservation of local kale varieties is partially carried out, but not efficiently. Therefore, there is a need for efficient species management and reliable conservation, which can be achieved and supported through genetic diversity assessment by using molecular DNA markers.

The morphological assessment studies for the trait analysis have been already conducted (Quamruzzaman et al., 2007; Yousef et al., 2015; Zhu et al., 2018). However, due to the effects of environmental variation, the morphological approach is limited and it is time-consuming. Molecular markers are able to overcome these limitations and serve as a powerful and reliable tool for assessing genetic diversity and relationships among species (Izzah et al., 2013). Therefore, various molecular markers, such as RAPD, RFLP, ISSR, AFLP, SSR and EST-SSR offer efficient assessment analysis of genetic diversity in diverse plant species (Sun et al., 2001; Kumar et al., 2009). Among all mentioned molecular markers, microsatellites or commonly known as Simple Sequence Repeat (SSR) are most frequently used (Varshney et al., 2005; Kandemir et al., 2012).

Simple sequence repeats (SSRs) are motives that occur randomly in the coding and non-coding part of the genome (Varshney et al., 2005). SSR markers result in highly polymorphic, abundant and reliable results. Further, compared to other DNA markers, the utilization of SSR markers represents a cheap procedure for genetic assessment of plant species, being highly reproducible and abundant DNA system marker (X. Zhang et al., 2015; Izzah et al., 2013; Guo-Liang, 2013; Jones et al., 1997; Powell et al., 1996). Several genetic diversity and relationship studies were conducted on *Brassica oleracea* L. var. *acephala* species, using different sets of SSR markers, as in Italy, China, Ireland, Spain (El-Esawi et al., 2016; Lotti et al., 2018; Tortosa et al., 2017; Zhu et al., 2018) and one central European based accession study (Christensen et al., 2011). However, no similar studies were done with genotypes from

Bosnia and Herzegovina. Therefore, in the present study, the aim is to genetically characterize 15 kale varieties found in Bosnia and Herzegovina, using 10 codominant SSR markers.

Material and methods

DNA extraction

In total 505 kale individuals, from 15 *Brassica oleracea* L var. *acephala* varieties, were used in this study (Table 1). All the analyzed populations, except domestic varieties, are currently commercially available, being very specific, very widespread among agricultural producers. Domestic varieties were collected from farmers as seeds or as fresh, fully grown plant, and stored in -20°C until DNA isolation.

The genomic DNA was isolated from dry seeds and fresh leaves. Plant leaves for the DNA extraction were collected from randomly selected plants on each analyzed site. After harvesting, the seeds and leaves were pulverized with liquid nitrogen using mortar and pestle. The DNA material was isolated according to the CTAB (Cetyltrimethyl ammonium bromide) protocol (Porebski et al., 1997), with a slight modification. The DNA quality was analyzed on 1% agarose gel and quantified using a Multiscan GO spectrophotometer (Thermo Fisher Scientific, USA) and stored in 100µl aliquots in 1 X TE buffer

at -20°C until PCR amplification.

SSR analysis

The PCR reactions were conducted in the total volume of 25 µl, containing the following mix: 2 mM of MgCl₂, 0.2 mM of dNTPs, 1 x PCR buffer, 0.2µl of DNA Polymerase from *Thermus aquaticus* (5 units/µL) and 30-50 ng of template DNA. The PCR protocol was optimized to include two phases. The first phase included the denaturation step at 95 °C for 5 min, following the touchdown (TD) profile for 20 cycles, with 95 °C denaturation for 45s, reducing the annealing temperature for each primer by - 0.7 °C per cycle for 45s, followed by the extension stage at 72 °C for 1 min (Table 2). The final PCR phase included 15 cycles in total, with 95 °C denaturation for 45s, with 54°C annealing temperature for 45s, with extension at 72°C for 45s (El-Esawi et al., 2016). A final extension step with 72 °C for 5 min was introduced to complete the PCR protocol. For the verification of the expected band size, according the M50pz DNA Ladder - MR20 from BLIRT, the PCR fragments were separated on 3% agarose gel for 60 minutes at 80V in 1 x TE buffer, previously stained with Ethidium bromide (10 mg/mL Sigma). Gel DNA fragments were photographed and documented using Gel documentation system (Chemi Doc XRS System) from Biorad.

Table 1. Kale cultivars used in this study

No	Name of variety	Code	Sample origin	No. of Samples
1	Mostar, Blagaj 1	MB1	Domestic, BiH	30
2	Mostar, Blagaj 2	MB2	Domestic, BiH	30
3	Mostar, Kočine 1	MK1	Domestic, BiH	35
4	Mostar, Kočine 2	MK2	Domestic, BiH	30
5	Stolac, Dubrave 1	SD1	Domestic, BiH	35
6	Stolac, Dubrave 2	SD2	Domestic, BiH	30
7	Stolac, Baljici	SB	Domestic, BiH	35
8	Stolac, Obradovici	SO	Domestic, BiH	20
9	Stolac, Rivine	SR	Domestic, BiH	35
10	Stolac, Gorica	SG	Domestic, BiH	30
11	Mostar, City	MC	Domestic, BiH	35
12	<i>Brassica oleracea</i> L. var. <i>meddullosa</i>	BM	Netherland	40
13	<i>Brassica oleracea</i> L. <i>Italica</i> group - Bonanza F1	BB	Italy	40
14	<i>Brassica oleracea</i> L. Red Russian	RR	Russia	40
15	<i>Brassica oleracea</i> L. Nero Di Toscana	NT	Italy	40

Data analysis

For the analysis of SSR loci, all the amplifications were scored as either present (1) or ab-sent (0). Further, to analyze the marker banding scheme, we obtained the number of scored bands (NTB), number of polymorphic bands (NPB) and percentage of polymorphic bands (PPB). The polymorphic information content (PIC) and heterozygosity (H) were assessed to discover the discriminatory power of each SSR marker, and all calculations were done using the online Gene-Calc program (Bińkowski and Miks, 2018). The polymorphic information content was calculated based on the following formula:

$$PIC = 1 - \sum_{i=1}^l p_i^2 - \sum_{i=1}^{l-1} \sum_{j=i+1}^l 2p_i^2 p_j^2$$

where p_i and p_j represents the population frequency of the i^{th} and j^{th} allele (Lemos et al. 2019).

For the heterozygosity genetic variation calculation, the following formula was used:

$$H = 1 - \sum_{i=1}^l p_i^2$$

The indication of high heterozygosity values means that the crops have evolved through a long-term natural selection for adaptation or through historic mixing of strains of different populations. In contrary, if the populations were isolated over a longer period of time, losing their unexploited genetic potential, a low level of heterozygosis will be indicated (Liu, 1998).

For the analysis of polymorphic band fractions and number of polymorphic bands, the effective multiplex ratio (EMR) was calculated using formula:

$$EMR = n \times \beta$$

where,

- n is the average number of fragments amplified by accession to a specific marker (multiplex ratio).
- β is estimated from the number of polymorphic loci (NPB) and the number of non-polymorphic loci (NMB); calculated by the following formula:

$$\beta = NPB / (NPB + NMB) \text{ (Kumar et al., 2009).}$$

Further, the resolving power (RP), assesses the marker combination discriminatory potential of the marker combination (Souza et al., 2019). The RP

Table 2. SSR primer sequences

	SSR primers	Sequence of primer (5'—3')	Expected (bp)
1	F-OL10-A03a	CTGGTTTTCTCCTTCATCAG	50–160 ^a
	R-OL10-A03a	CTGTGTAGCTTTTAGTCTTT	
2	F-OL10-F11a	TTTGGAACGTCCGTAGAAGG	64–240 ^a
	R-OL10-F11a	CAGCTGACTTCGAAAGGTCC	
3	F-OL10-H02	AACAGGAAGAAACGACGAGG	98–260 ^a
	R-OL10-H02	AGAGAGCCATGAGAAGCACC	
4	F-OL11-H02	TCTTCAGGGTTTCCAACGAC	96–250 ^a
	R-OL11-H02	AGGCTCCTTCATTTGATCCC	
5	F-OL12-E03	CTTGAAGAGCTTCCGACACC	160–190 ^a
	R-OL12-E03	GACGGCTAACAGTGGTGGAC	
6	F-OL11-G11	GTTGCGGGCGAAACAGAGAAG	70–210 ^a
	R-OL11-G11	GAGTAGGCGATCAAACCGAG	
7	F-OL12-F11	AAGGACTCATCGTGCAATCC	100–276 ^a
	R-OL12-F11	GTGTCAGTGGCTACAGAGAC	
8	SSR1F	CCTTCCTAGCCACACACCTC	150 ^b
	SSR1R	GAAGGAATAACCGGCAGCTA	
9	EPMS650F	AGAGGGAAGGGTTATTTGCC	250 ^c
	EPMS650R	CATGGGTGAGGGTACATGGT	
10	EPMS709F	ACGCCGAGGACTATGATGAC	262–280 ^d
	EPMS709R	TTCTTCATCCTCAGCGTGTG	

^a El-Esawi MA et al.,2016, ^b Kong et al.,2011, ^c Portis et al., 2007, ^d Tsaballa et al.,2015

was calculated based on the following formula:

$$RP = \sum Ib$$

- Ib represents the informative fragments, with a scale of 0/1, by the following formula;

$$Ib = 1 - (2 \times |0.5 - pi|),$$

where **pi** is the proportion of accessions containing the i^{th} band (Prevost and Wilkinson, 1999).

For the detection of polymorphic loci among the genotypes, stability and informativeness of each marker, the marker index (MI) was calculated based on the following formula:

$$MI = EMR \times PIC \text{ (Powel et al., 1996).}$$

The phylogenetic tree was constructed by using hierarchical clustering with UPGMA (Unweighted pair group method with arithmetic mean) analysis, where the genetic distance was measured based on Jaccard's dissimilarity index (Real and Vargas, 1996). The resulting tree was bootstrapped with 1000 replicates to obtain the best confidence (Felsenstein, 1985). For the complete data analysis, the DARwin 6.0.15 software was used, as described by Karić et al. (2019).

Results and Discussion

Combining 10 SSR markers in this study, we amplified in a total of 51 bands (Table 3). All the markers showed clear and observable bands, as shown with marker G11 (Figure 1). The resulted, rooted Phylogenetic UPGMA dendrogram is made of three groups (A, B and C). Group A shares one common ancestor, made of Stolac Baljici and Mostar city kales. Group B is divided into two sub-clusters. The first sub-cluster in group B is made of varieties from Stolac, Rivine and Gorica, sharing common origin with all hybrid kales, from the second out group, but clearly separated from all remaining accessions. The third group C is made of two sub-clusters, Dubrave kales (1 and 2) with Blagaj 2 in one sub-cluster, sharing a common ancestor with kales from Mostar, Blagaj 1 and Koćine 2 in the second sub-group (Figure 2).

In addition, for each marker, the Number of scored bands; Polymorphic bands; Monomorphic bands; Percentage of polymorphic band; Effective multiplex ratio; Marker index and Resolving power were calculated, as given in Table 3.

The highest PIC value was observed in marker 11-H02, with 0.797 and the lowest in marker EPMS790 with 0.337, with an average PIC of 0.6201. The

Table 3. Genetic diversity estimators for each marker in *Brassica oleracea* varieties

PRIMERS	H	PIC	NSB	PB	MB	PBB %	EMR	MI	RP
10-F11	0.653	0.588	3	3	0	100.0	1.5	0.9	1.0
10-H02	0.817	0.785	10	9	1	90.00	3.2	2.5	0.8
11-H02	0.809	0.797	7	7	0	100.0	2.2	1.8	0.6
A03	0.620	0.541	3	1	2	33.30	0.7	0.4	1.4
E03	0.817	0.747	7	6	1	85.70	2.8	2.1	0.9
12-F10	0.674	0.629	4	3	1	75.00	1.2	0.7	0.8
EPMS650	0.788	0.746	5	3	2	60.00	1.8	1.4	1.2
EPMS790	0.430	0.337	2	2	0	100.0	1.1	0.4	1.1
G11	0.552	0.511	7	6	1	85.70	4.0	2.0	1.3
SSR1	0.614	0.536	3	2	1	66.60	1.4	0.7	1.1
Mean	0.6774	0.6201	5.1	4.2	0.9	79.63	1.99	1.28	1.02

NSB: Number of Scored Bands; PB: Polymorphic Bands; MB: Monomorphic Bands; PPB: Percentage of Polymorphic Band; EMR: Effective Multiplex Ratio; MI: Marker Index; RP: Resolving power

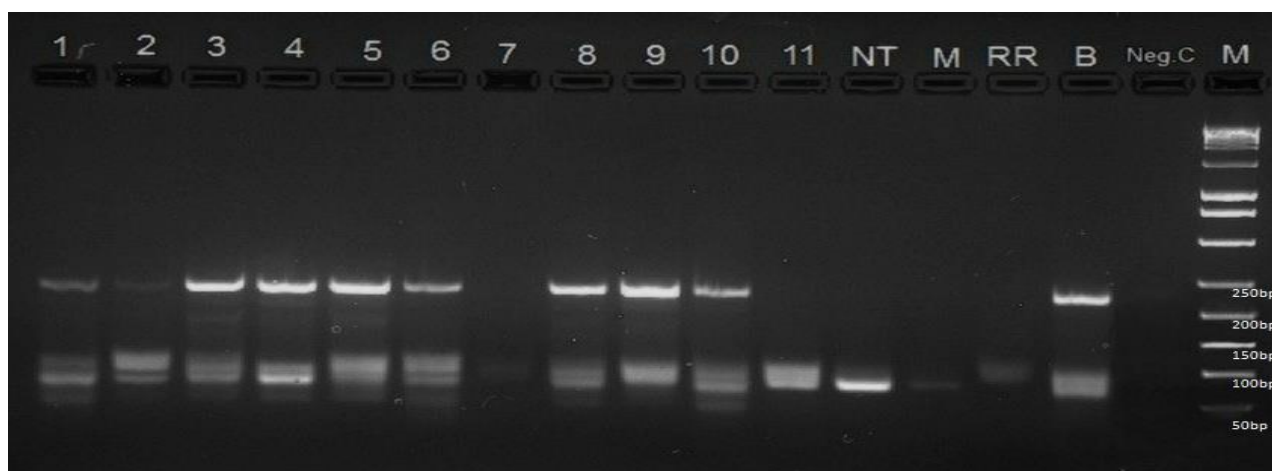


Figure 1. SSR profile of different genotypes generated by marker G11 on 3% agarose gel. From left to right: kale varieties, Neg. C as a negative control and M (M50pz DNA Ladder-M20-Blirt)

heterozygosity (H) values ranged from 0.430 to 0.817. The lowest H value (0.430) was observed in marker EPMS790, where markers EO3 and 10H02 with 0.817, show the highest observed heterozygosity value.

All markers resulted in minimum 33.3 % polymorphic bands, while the highest polymorphic behavior was observed in three markers, 11-H02, 10F11 and EPMS790, with 100.0%. The highest effective multiplex ratio (EMR) in this study is shown in marker G11 with a value of 4.0, and the lowest EMR ratio is observed in markers A03, with 0.7, with an average EMR of 1.99 per marker.

Further, the assessment of the discriminatory power of the given SSR marker combination, referring to the resolving power – RP, were in the range of 0.6–1.4, with an average of 1.02.

Marker A03 showed the highest RP value with 1.4, and the lowest was 0.6 with 11H02 marker. To check the total utility of each marker, the marker index (MI) factor was observed, as a product of polymorphic information content and effective multiplex ratio (Varshney et al., 2007). Highest MI (2.5) is observed in 10-H02 and the lowest was with A03 marker (0.4). A high marker index indicates that the marker system used is useful for the chosen genetic population (Ghanbari et al., 2019).

Principle coordinate analysis (PCoA) was performed in order to further assess the genetic relationships among the kale varieties by using 10 SSR markers (Sun et al., 2001). PCoA shows the hybrid kale populations RR, BB, BR and NT are separated into one cluster, together with domestic kales SR and SG. The remaining domestic kales were separated into two more distinct clusters, where SB and MC domestic kales are clearly apart populations, as seen in Figure 3.

This study involved the genetics characterization analysis of 15 different kale varieties, from BiH, evaluated by using 10 different markers. The genetic diversity of 11 domestic kales of known origin was compared to 4 hybrids, showing sufficient SSR dissimilarity characteristics and reflecting significant genetic diversity among kale varieties used in this study. All samples had higher polymorphic information content values than 0.337. The average value of the total heterozygosity (H) was 0.6774 and for PIC 0.601, values that are significantly higher if compared to similar researches (0.577, 0.249, 0.25, 0.338 and 0.294) (El-Esawi et al., 2016; Hintum et al., 2007; Watson-Jones et al., 2006). The PIC value ranged from 0 to 1, with values closer to 0 indicating no allelic variation, and values closer to 1 indicating

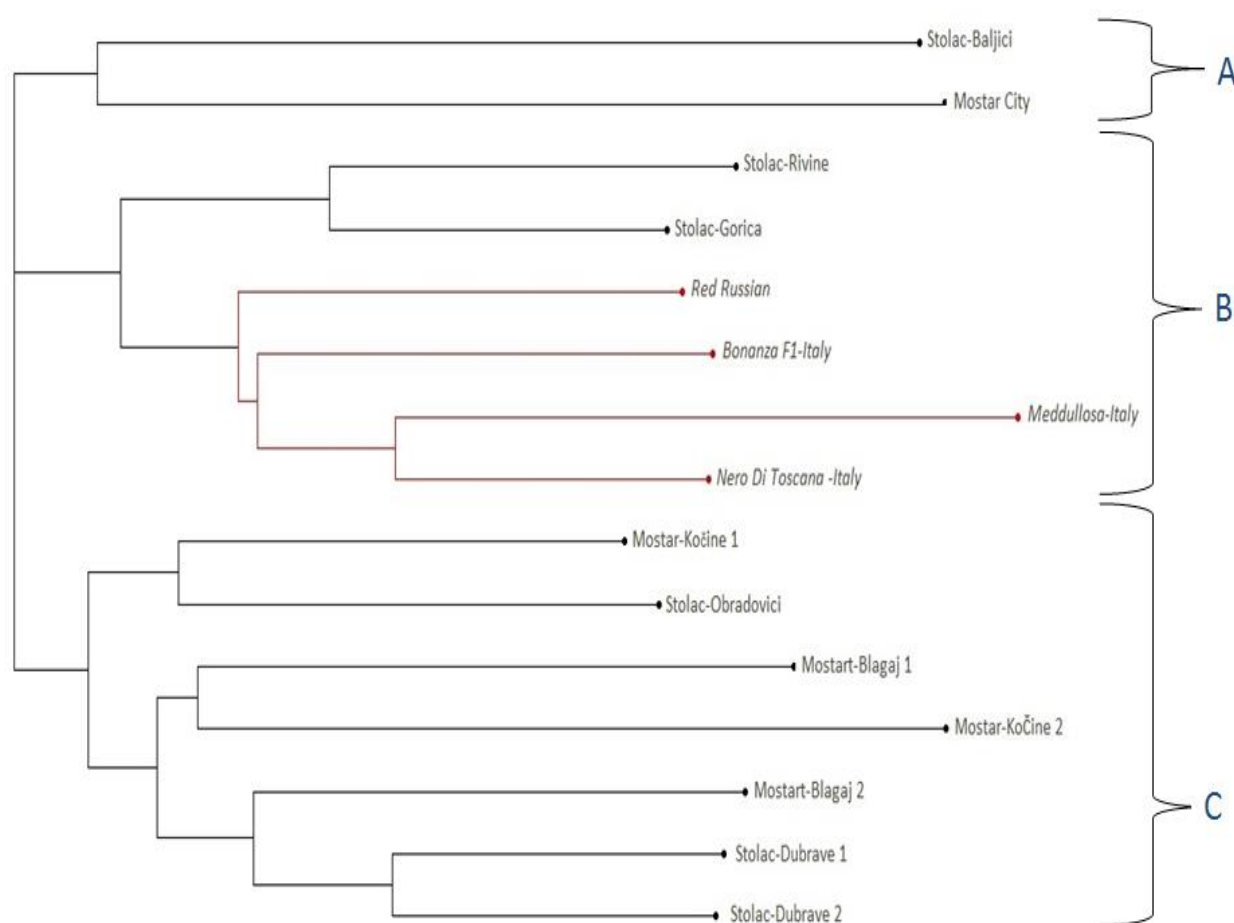


Figure 2. Dendrogram of local and hybrid kale cultivars based on genetic distance obtained from 10 SSR markers revealed by UPGMA method. The branch lengths are based on the distance values computed using Jaccard's coefficient of Darwin software. The large parentheses to the right side labeled with A-C are the major clusters, while all inner parenthesis showing sub-clusters within the respective.

that the genotype has only new allele (Guo and Elston, 1999). It is reported that high diversity of locus is demonstrated in PIC value greater than 0.5 (Alhasnawi et al., 2019). Two of the SSR markers (10H02 and G11) have high RP values (from 0.6 to 1.4), hereof being the most informative markers for distinguishing these kale genotypes in our study.

However, the resolving power provides no information on the ability of a marker to reflect the genetic relationships of a group of genotypes under the study (Prevost and Wilkinson, 1999). Genetic variation using 10 SSRs loci resulted in a total of 51 alleles, out of which 42 bands (80.7%) were polymorphic, with a mean of 4.2 alleles per locus. Further, an average effective multiplex ratio of 1.99 was obtained, as the average number of amplified bands per marker was 35 for all cultivars, ranging

from 16 to 54, confirming high polymorphic information potential. These results indicate that most of our markers are sufficient for the analysis intraspecific genetic diversity, especially marker G11, as a potent marker for fine dissection of the intraspecific relationship in kale varieties, as shown in a previous study (El-Esawi et al., 2016).

The phylogenetic tree resulted in expected evolution pattern, where most of domestic kale varieties originated from the same region sharing the same ancestor, hereof being genetically very similar. In contrary, all the hybrid kales are placed clearly in the same group, sharing common ancestor with only two domestic cultivars from Stolac (Rivine and Gorica), indicating a slight possibility that a common genetically similar sort, was used as a variety for the creation of these hybrids.

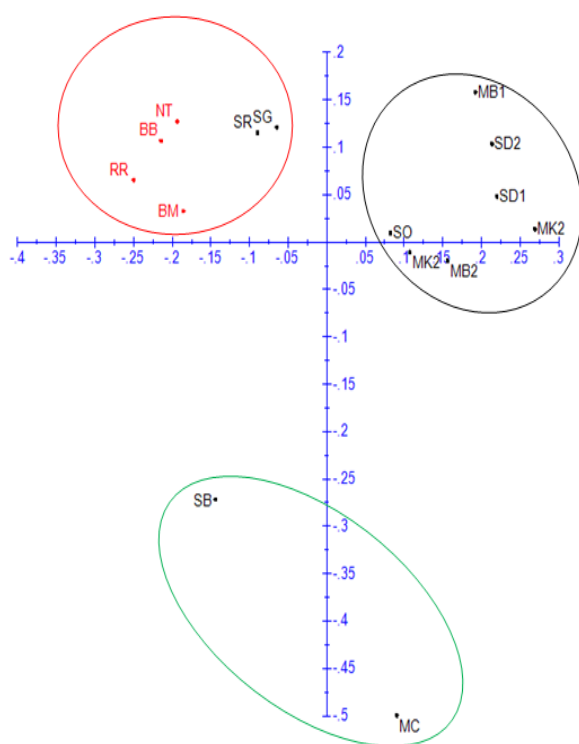


Figure 3. Principal coordinates analysis (PCoA) of *Brassica oleracea* var. *acephala* populations: Association among the 15 accessions, based on Jaccard's genetic distance. MB1= Mostar, Blagaj 1, MB2= Mostar, Blagaj 2, MK2= Mostar, Kočine 1, SD1= Stolac, Dubrave 1, SD2= Stolac, Dubrave 2. SB = Stolac, Baljici. SO = Stolac, Obradovici, SR = Stolac, Rivine, SG = Stolac, Gorica, MC = Mostar, City, BM= *B. Oleracea* L. var. *meddullosa*, BB = *B. Oleracea* L. *Italica* - Bonanza F1, RR = *B. Oleracea* L. *Red Russian*, NT = *B. Oleracea* L. *Nero Di Toscana*

The dendrogram generated using UPGMA cluster analysis, grouped 15 genotypes into three clusters with Jaccard's dissimilarity index ranging from 0.259 to 0.83 (Table S1), with threshold equality of 100%. Through the UPGMA hierarchical clustering and PCoA together, the genetic relationships among individual accessions was strongly assessed. All these analyses suggested the occurrence of a distinct group (Stolac Baljici and Mostar City) might be due to close geographical stratification. However, Stolac Baljici and Mostar City kales share common genetic evolution path with all the other accessions but being farthest away from other accessions. For an additional verification, PCoA analysis is in line with the phylogenetic tree. The PCoA is consistent, with two observable clusters, domestic accessions in one and the hybrids in the second cluster (Figure 1 and Figure 2).

This study confirmed that the applied SSR marker set is effectively used to estimate genetic distances among genotypes, as shown in several previous studies (El-Esawi et al., 2016; Raza et al., 2019). Among all markers, only marker EPMS790 showed lower values than 0.5 for H and PIC. We revealed that 9 of 10 SSR markers had a PIC ≥ 0.5 , confirming the codominant markers potential for polymorphism detection in *Brassica oleracea* L. var. *acephala* cultivars. However, to have a stronger understanding of the presence of genetic variability in *Brassica oleracea* L. var. *acephala* cultivars, the number of molecular markers should be higher and hereof lead to more efficient utilization of existing variation of *Brassica* crops in Bosnia and Herzegovina.

Conclusion

Brassica oleracea L. var. *acephala* DC cultivars are globally used as one of the essential crops in the human diet, in agriculture production, horticulture, animal fodder, condiments, oil and biofuel production, as being very rich in mustard oils, vitamins and minerals. Bosnia and Herzegovina (B&H) through centuries bears the tradition to grow and use *Brassica oleracea* L. var. *acephala* DC cultivars as crops, especially in the country southern region, Herzegovina. Through the evaluation of Polymorphic Information Content and Heterozygosity analysis, by using 10 codominant SSR markers, we concluded that B&H varieties are genetically diverse in comparison to hybrids. The phylogenetic dendrogram joined the domestic kales within two clusters, clearly separating them from hybrid kales. The genetic markers were tested for efficiency through several genetic estimators, where 9 of 10 markers showed sufficient polymorphism and discriminatory power.

Acknowledgement

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Conflict of interest

Authors declare no conflict of interest.

Supplementary Material

Table S1: Jaccard's dissimilarity index

	MB1	MB2	MK1	MK2	SD1	SD2	SB	SO	SR	SG	MC	BM	BB	RR
MB2	0.499													
MK1	0.476	0.494												
MK2	0.551	0.531	0.547											
SD1	0.378	0.476	0.453	0.528										
SD2	0.378	0.475	0.452	0.527	0.259									
SB	0.654	0.672	0.594	0.724	0.630	0.630								
SO	0.476	0.495	0.333	0.547	0.453	0.452	0.594							
SR	0.575	0.594	0.516	0.646	0.552	0.551	0.657	0.516						
SG	0.547	0.565	0.487	0.617	0.523	0.523	0.628	0.487	0.294					
MC	0.731	0.750	0.671	0.802	0.708	0.707	0.736	0.671	0.735	0.706				
BM	0.692	0.710	0.632	0.762	0.668	0.668	0.773	0.632	0.559	0.530	0.851			
BB	0.569	0.588	0.509	0.640	0.546	0.545	0.651	0.509	0.436	0.407	0.728	0.486		
RR	0.569	0.588	0.510	0.640	0.546	0.545	0.651	0.510	0.436	0.408	0.729	0.370	0.364	
NT	0.576	0.595	0.517	0.647	0.553	0.552	0.658	0.517	0.443	0.415	0.736	0.480	0.371	0.358

MB1 = Mostar, Blagaj 1, MB2 = Mostar, Blagaj 2, MK2 = Mostar, Kočine 1, SD1 = Stolac, Dubrave 1, SD2 = Stolac, Dubrave 2. SB = Stolac, Baljici. SO = Stolac, Obradovici, SR = Stolac, Rivine, SG = Stolac, Gorica, MC = Mostar, City, BM = *B. Oleracea* L. var. *meddullosa*, BB = *B. Oleracea* L. *Italica* - Bonanza F1, RR = *B. Oleracea* L. *Red Russian*, NT = *B. Oleracea* L. *Nero Di Tosca*

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