OCL 2023, 30, 18

https://doi.org/10.1051/ocl/2023019

OCL Oilseeds & fats Crops and Lipids

Available online at:
www.ocl-journal.org

RESEARCH ARTICLE OPEN ACCESS

Genetic relationship and diversity among some Moroccan and introduced rapeseed (*Brassica napus* L.) varieties as revealed by molecular markers☆

Karim Houmanat[1], Abdelghani Nabloussi[1], Yousra Rhazlaoui[1,2], Hakima Bahri[2], Mohamed El Fechtali[1] and Jamal Charafi[1,*]

[1] Research Unit of Plant Breeding and Plant Genetic Resources Conservation, Regional Agricultural Research Center of Meknes, National Institute of Agricultural Research, Avenue Ennasr, PO. Box 415, 10090 Rabat, Morocco
[2] Department of Plant Production, National School of Agriculture of Meknes, PO. Box S/40, 50000 Meknes, Morocco

Received 26 March 2023 – Accepted 23 July 2023

Abstract – Rapeseed (*Brassica napus* L.) crop can be a lever for the development of oilseed sector in Morocco due to its adaptation to local conditions and its major economic and food importance. Genetic diversity and selection of valuable crossing parents are the key to successful breeding and improvement of this crop. In this regard, genetic variation within the existing germplasm must be explored and characterized. Therefore, the present study was carried out to investigate the genetic diversity among 22 varieties from Morocco as well as other origins, using twenty ISSR primers. The selected primers have generated a total of 319 markers. Polymorphic amplified bands varied from 8 to18, with an average of 13 per primer. The diversity index (PIC value) ranged from 0.295 to 0.509, with a mean value of 0.37 per primer, indicating a good genetic diversity level for the primers used. The average similarity coefficient was 0.31, fluctuating between 0.176 and 0.456, and the pairwise comparison of the studied varieties showed a great discriminating power of primers and a large genetic diversity among accessions. A total of eight ISSR primers could be identified as key to rapeseed varietal determination. Hierarchical classification allowed identifying three groups with some phylogeographic structuring. This is the first report of molecular characterization of rapeseed germplasm in Morocco and Africa. The obtained results have important implications for management of this germplasm to conserve the existing genetic diversity and use it properly in breeding programs in Morocco as well as in other Mediterranean and African countries.

Keywords: *Brassica napus* L. / crossbreeding / genetic diversity / ISSR markers / Moroccan germplasm

Résumé – Relation génétique et diversité entre des variétés marocaines et introduites de colza (*Brassica napus* L.) révélées par des marqueurs moléculaires. La culture du colza (*Brassica napus* L.) peut être un levier pour le développement de la filière oléagineuse au Maroc en raison de son adaptation aux conditions locales et de son importance économique et alimentaire majeure. La sélection est la clé du succès de cette culture et le choix judicieux des parents de croisement est une condition préalable à la réussite du programme de sélection et au développement de nouvelles variétés plus performantes que les anciennes. À cet égard, la variation génétique au sein du matériel génétique existant doit être explorée et caractérisée. Par conséquent, la présente étude a été réalisée pour étudier la diversité génétique parmi 22 variétés du Maroc ainsi que d'autres origines, en utilisant vingt amorces ISSR. Les bandes polymorphes amplifiées variaient de 8 à 18, avec une moyenne de 13 par amorce. L'indice de diversité (PIC) variait de 0,295 à 0,509, avec une valeur moyenne de 0,37 par amorce, indiquant un bon niveau de diversité génétique pour les amorces utilisées. Le coefficient de similarité moyen était de 0,31, fluctuant entre 0,176 et 0,456, et la comparaison par paires des variétés étudiées a montré un grand pouvoir discriminant des amorces et une grande diversité génétique entre les accessions. Huit amorces ISSR ont pu être identifiées comme clé de détermination variétale chez le colza. La classification hiérarchique a permis d'identifier trois groupes avec

☆ Contribution to the Topical Issue "Rapeseed / Colza".

*Correspondence: jamal.charafi@inra.ma

une certaine structuration phylogéographique. Il s'agit du premier rapport de caractérisation moléculaire du germoplasme de colza au Maroc et en Afrique. Les résultats obtenus ont des implications importantes pour la gestion de ce matériel génétique afin de conserver la diversité génétique existante et de l'utiliser correctement dans les programmes de sélection au Maroc ainsi que dans d'autres pays méditerranéens et africains.

Mots clés : *Brassica napus* L. / croisement / diversité génétique / marqueurs ISSR / germoplasme marocain

Highlights

- Genetic diversity assessment and selection of appropriate crossing parents are the key to successful breeding program.
- First molecular study of rapeseed genetic diversity in Moroccan and Africa.
- Hierarchical classification allowed identifying four groups with some phylogeographic structuring.
- Some Moroccan varieties are genetically close to some introduced varieties.
- Identification of eight ISSR primers as key to varietal determination and authentication in rapeseed.

1 Introduction

Over the world, the Brassicaceae family has an important diversity of plant species including more than 3700 species and 338 genera. *Brassica* species have many uses such as oil, forage, green manure, biodiesel, and other uses (Sharma *et al.*, 2022). Various species of *Brassica* have been domesticated in different parts of the world depending on ecology and human needs. Generally, *Brassica* constitute a potential pool of genetic material with many desirable agronomic and horticultural traits (Sharma *et al.*, 2022).

Oilseed *Brassica* crops collectively contribute about 15% of the world's total supply of vegetable oils and became the third-leading source of edible oil in the world after soybean and palm (McVetty et al., 2016; Guirrou *et al.*, 2023). Rapeseed (*Brassica napus* L.) is the most important oilseed *Brassica* species, which is a profitable and evolving crop thanks to the dynamism and relevance of scientific and agronomic research, mainly in genetic breeding, improvement of crop management and molecular biology.

Rapeseed is a crop that presents a broad adaptation to different environmental conditions (climate, soil, etc.). The main objective of the rapeseed-breeding program is the development of highly productive and stable varieties with high seed oil and good oil quality. Throughout the past few decades, rapeseed (*Brassica napus* L.) has known an intensified varietal selection based on agronomic and economic traits, which led to a reduced genetic base. Crossing is actually the main strategy adopted to create and broaden the existing genetic diversity and to develop the desired varieties. Nevertheless, the judicious choice of crossbreeding parents remains a big challenge for successful selection program and development of new varieties that perform better than the old ones. Traditionally, parents are selected based on their phenotypic attributes; however, the morphological features do not give the real level of genetic diversity among rapeseed germplasm. Since their domestication, oilseed *Brassica* species have undergone progressive transformation. Efforts to breed *B. napus* have primarily focused on improving seed yield, oil quality, as well as disease resistance, abiotic stress tolerance, and herbicide resistance (Ton *et al.*, 2020).

The revolution in plant biotechnology, including molecular markers, genomic tools, and genetic mapping, has made it possible to understand the complex genetic makeup and genes function (Ton *et al.*, 2020). Plant breeders rely on a diversity of crop genetic resources, breeding tools and methods to incorporate genetic diversity into commercial cultivars or to develop new cultivars with improved agronomic characteristics, such as high yield, tolerance to biotic and abiotic stresses, and high nutritional quality (Swarup *et al.*, 2021).

Molecular markers are very powerful DNA-based biotechnology tools that can reveal genetic polymorphism not influenced by the environment conditions and, thus, present a great potential to compensate for the low discriminative power of morphological traits. Molecular markers allow identifying and characterizing plant genotypes through direct access to genetic information. Analysis by molecular markers enables choosing parental types for mapping populations, marker-assisted selection, and crossing schemes. Thus, diversity analysis using molecular markers help breeders to improve crop species (Paul *et al.*, 2020). Inter simple sequence repeat (ISSR) are generally very effective in genetic diversity studies because of their longer primers compared with random amplified polymorphic DNA (RAPD) primers (Pradeep Reddy *et al.*, 2002; Tarıkahya-Hacıoğlu, 2016). Likewise, by comparing different markers, such as ISSR, Interretrotransposon amplified polymorphism (IRAP), and retrotransposon microsatellite amplified polymorphism (REMAP), for the assessment of genetic diversity within the Brassicaceae family, it was found that ISSR markers were the most efficient (Mahjoob *et al.*, 2016). In fact, this technique combines the advantages of the Amplified Fragment Length Polymorphism (AFLP) and the universality of the RAPD (Maras-vanlioğlu *et al.*, 2020).

In Morocco, the development of oilseed crops, such as rapeseed, can reduce import dependence, improve the trade balance and strengthen the economic activity of this vital sector. Rapeseed area has increased from nearly 500 ha in 2012/2013 to around 10 000 ha in 2020/2021, ensuring a production of about 9000 tons (DDFP, 2022). Nevertheless, the new agricultural strategy "Green Generation" has just strengthened the sector to reach 30 000 ha by 2030. One of the main production basins for oilseed crops in Morocco is the region of Fez-Meknes.

Rapeseed-breeding program conducted in this country relies on hybridization and selection of promising lines, where

Table 1. Origin, type, pedigree and some phenotypic characters of the 22 rapeseed varieties studied.

Rapeseed varieties	Origin	Type/genetic structure	Pedigree	Phenotypic description
ADILA	Morocco	Synthetic Variety	Intercrossing of four inbred lines: INRA-L615/ INRA-L455 + INRA-L315 + INRA-L515	Leaves quite developed with medium serration, strong lobes, and an intermediate green blade. Main stem of medium height. Pale-yellow flowers. Long beaked siliques (Guirrou *et al.*, 2023).
LILA	Morocco	Synthetic Variety	Intercrossing of four inbred lines: INRA-L455/ INRA-L615 + INRA-L315 + INRA-L515	Leaves quite developed with strong serration, very strong lobes, and a light-green blade. Main stem of medium height. Pale-yellow flowers. Long beaked siliques (Guirrou *et al.*, 2023).
MOUFIDA	Morocco	Pure line	Cross between two introduced varieties: Westar/ Optima	Medium plant height. Developed leaves with medium serration, strong lobes, and a medium green blade. Flowers of bright-yellow color. Siliques with long beak (Guirrou *et al.*, 2023).
NARJISSE	Morocco	Pure line	Individual plant selection from an open-pollinated population	High plant with developed leaves, strong lobes, and a blade with a bright-green color. Mid-early flowering and high branching (Guirrou *et al.*, 2023).
CZ-H2	Morocco	Pure line	.....	.....
BARAKA	Morocco	Pure line	Cross between two inbred lines: INRA-L115/ INRA-L455	Very high plant with developed leaves, strong lobes, and a blade of light-green color. Late flowering and high branching(Guirrou *et al.*, 2023).
NAP9	Australia	Pure line	.....	.....
NAP10	Australia	Pure line	.....	.....
AUP1	Pakistan	Pure line	.....	.....
AUP3	Pakistan	Pure line	.....	.....
AUP4	Pakistan	Pure line	.....	.....
AUP13	Pakistan	Pure line	.....	.....
AUP16	Pakistan	Pure line	.....	.....
AUP17	Pakistan	Pure line	.....	.....
ALBA	Unknown	Pure line	.....	.....
KABEL	Spain	Pure line	.....	.....
LUCIA	Spain	Pure line	.....	.....
LYSIDE	Danemark	Pure line	.....	.....
MACRO	Germany	Pure line	.....	.....
SEVEN	Unknown	Pure line	.....	.....
JURA	France	Pure line	.....	
TRAPER	Germany	Hybrid Variety	.....	.....

Table 2. List of 20 different ISSR primers and annealing temperatures used in the molecular characterization of 22 rapeseed varieties.

ISSR Primers	Type of repetition	Sequence (5'-3')	Annealing temperature (°C)
F1 [a,b]	Di	$(CA)_6AT$	43
F2 [a,b]	Di	$(CA)_6GC$	58
F3 [b]	Di	(CA)6	43
F4 [a,b]	Tri	$(AGC)_4CT$	58
F8 [a,b]	Di	$(AG)_8CC$	56
F9[b]	Di	$(AG)_8CG$	43
F11[a]	Di	$(CA)_8AC$	56
F16 [a,b]	Di	$(GT)_8CG$	58
IMA-5-Z[b]	Di	$(CA)_8GT$	56
IMA9Z [a,b]	Di	$(GA)_8CG$	56
IMA834z [a]	Di	$(AG)_8CTT$	50
IMA8Z	Di	$(GA)_8GT$	50
IMA834-2[b]	Di	$(AG)_8YT$	50
UBC808-2[b]	Di	$(AG)_8C$	50
UBC810[b]	Di	$(GA)_8T$	50
UBC850[b]	Di	$(GT)_8TYC$	50
UBC856[b]	Tri	(GGAGA)3	56
IMA12-2 [a,b]	Di	$(CA)_8TC$	56
ISS F1[b]	Di	$(AG)_8TA$	50
ISSR1[b]	Di	$(AG)_8CA$	56

[a] Set of 8 primers allowing the distinction of the 22 genotypes.
[b] 17 primers to distinguish the three groups A, B and C in the dendrogram.

crossbreeding parents' choice is based on phenotypic evaluation under field conditions. Consequently, the environmental conditions largely affect the selection process.

Therefore, the objective of this research is to characterize the genetic diversity among a set of rapeseed varieties, using the ISSR genetic markers, and to identify the level of genetic phylogeographic structure. Knowledge of genetic distances among the genotypes studied and identification of homogeneous genetic pools will be helpful for the ongoing rapeseed-breeding program in the selection of genetically distant and complementary breeding parents.

2 Materials and methods

2.1 Plant material

The plant material used in this study consisted of 22 rapeseed varieties of which six are Moroccan while sixteen are introduced from different countries in the world. The Moroccan varieties were developed by the National Institute of Agronomic Research (INRA-Morocco) and registered in the Official Catalogue as high-performing inbred lines and synthetic varieties in terms of seed yield and oil content (Guirrou *et al.*, 2023). Table 1 presents the list of the varieties studied, their origins, their types, their pedigree, and their phenotypic description. Information on pedigree and phenotypic description is only available for the six Moroccan varieties.

2.2 DNA isolation

Genomic DNA of each variety was extracted from a sample of 40 mg of lyophilized young leaves using the Cetyl Trimethyl Ammonium Bromide (CTAB) modified method (Saghai-Maroof *et al.*, 1984). The extracted DNA was then quantified using a spectrophotometer (Bio-drop Touch Spectrophotometer, USA).

2.3 Amplification of DNA using ISSR markers

A set of 32 ISSR primers was tested on a single genotype and through the PCR-gradient (Eppendorf Mastercycler Gradient, Germany) to adjust the annealing temperature of each primer and to select primers that regenerate very clear and reproducible profiles. Finally, twenty primers were selected due to their molecular profiles with the determination of the optimum hybridization temperature for each primer (Tab. 2).

The mixture for DNA amplification was prepared in a final volume of 25 µl containing 20 ng/µl of DNA, 1x Taq buffer, 1.5 mM MgCl2, 0.2 mM of dNTP, 5 µM of ISSR primer, and 1U of Taq polymerase. The PCR amplifications were carried out in a 96-well thermal cycler (Eppendorf model, Mastercycler gradient) according to the following program: Denaturation step at 94 °C for 5 min, followed by 35 cycles with a denaturation step at 94 °C for 1 min, hybridization at a primer specific temperature (Tab. 2) for 1 min, and an

elongation step at 72 °C for 1 min. Amplification is sealed off by an elongation step at 72 °C for 10 min.

Electrophoresis of PCR products was performed on a 2% agarose gel prepared with a 1x TBE buffer and at 4 V/cm voltage. A 1 kb plus-ladder (invitrogen) served as the size marker. The revelation was carried out using ethidium bromide (0.5 μg/mL) for 30–45 min. Agarose gels are photographed by a UV imaging system.

2.4 Scoring data and analysis

The size of the obtained bands was calculated using the Mesurim Pro software v3.4 (Madre, 2013) based on an algorithm produced by the bands of the size marker. The data obtained was transformed into a binary matrix (0–1) with only clear bands being considered.

Genetic relationships among the varieties were studied based on a similarity matrix using all polymorphic bands. The genetic distances were estimated by the Simple Matching coefficient (Sokal and Michener, 1958). The histogram of pairwise comparison among genotypes based on the number of allelic differences was established.

The polymorphism information content (PIC) related to the genetic diversity for each primer used was determined according to Anderson *et al.* (1993) as follows:

$$PICi = 1 - \sum_j f_{ij}^2,$$

where f_{ij} is the frequency of the pattern j in the band i.

Since dominant markers have two models for a group: present and absent. Then, the PIC of each primer was calculated as follows:

$$PIC = \frac{1}{n}\sum_{i=1}^{n} PICi.$$

The Effective multiple ratio (EMR) was obtained according to Powell *et al.* (1996) as follows:

$$EMR = np(np/n),$$

where np is the number of polymorphic loci and n is the total number of loci.

Moreover, the Marker index (MI) was estimated according to Chesnokov and Artemyeva (2015) as the product of PIC value and EMR value:

$$MI = PIC \times EMR.$$

The Marker index (MI) is a statistical parameter used to estimate the total utility of the maker system.

Script AMaCAID "Accurate Marker Choice for Accession Identification and Discrimination" (Caroli *et al.*, 2011) designed for execution in R program was used to identify the minimum number of markers necessary to distinguish the different varieties studied and to define a set of markers maximizing the number of varieties distinguished.

Finally, a dendrogram was drawn based on the unweighted pair group method with arithmetic averages (UPGMA) algorithm using the NTSYS-pc program version 2.11a (Rohlf, 2002).

3 Results and discussion

3.1 Polymorphism and diversity parameters

Among the 32 ISSR primers tested using the annealing temperature gradient and revelation on 2% agarose gel, 20 primers (62.5%) generated readable and reproducible profiles (Fig. 1). The annealing temperatures of the retained primers varied between 43 °C and 58 °C. In a similar study on genetic characterization of rapeseed genotypes, the hybridization temperature of ISSR markers ranged from 45 to 60 °C (Tarıkahya-Hacıoğlu, 2016). The size of the bands generated by these 20 primers is highly variable, oscillating between 137 bp for UBC856 and 1329 bp for F4 (Tab. 3). According to Zietkiewicz *et al.* (1994), the ISSR technique generates amplified DNA fragments ranging from 200 to 2500 bp; however, more recent studies reported DNA fragment sizes ranging from 150 to 1100 bp (Sica *et al.*, 2005), 140 to 1500 bp (Nagaraju *et al.*, 2002), and 250 to 2800 bp (Pradeep *et al.*, 2005).

These ISSR primers revealed a total of 319 markers and a number of bands ranging from 11 bands for the IMA9Z primer to 22 bands generated by F8 primer, with an overall average of 16 fragments per primer (Tab. 3). The total polymorphism rate of the 20 primers is 81.5%. This number reflects the high level of polymorphism among the evaluated varieties revealed by the set of selected primers. This value is very close to that reported by Abdelmigid (2012) (87%) and higher than that reported by Paul *et al.* (2020) who found a polymorphism percentage of 71.5% in their rapeseed genetic diversity characterization using ISSR markers. When compared with other species, the percentage of polymorphic bands revealed by ISSR primers is higher in *Asparagus acutifolius* L. (100%) (Sica *et al.*, 2005) and *Lupinus* spp. (99%) (Talhinhas *et al.*, 2003), and comparable in *Oryza sativa* (80.9%) (Nagaraju *et al.*, 2002).

The number of ISSR markers generated is positively correlated with the number of primers used. However, this number can be greatly influenced by the plant species and the nature of the migration gel used (Wiesner and Wiesnerová, 2003).

The polymorphism information content (PIC) values indicate the ability of each primer to distinguish among varieties. All the primers have generated very polymorphic profiles, with an index varying between a minimum value of 0.295 for F11 and a maximum value of 0.509 for IMA834-2, with an average of 0.37. This is in agreement with Safari *et al.* (2013) who found a PIC of 0.346 for RAPD and 0.35 for ISSR markers in their study on rapeseed genetic diversity. However, our values are higher than those of Maras-vanlioğlu *et al.* (2020) who reported PIC values generated by the ISSR primers varying between 0.14 and 0.26, with an average of 0.18 in their study on rapeseed genetic assessment. However, in our study, most primers have PIC values around 0.4, indicating the high discriminating power of the used primers.

The analysis AMaCAID-script showed that among the 20 ISSR primers used, a number of eight primers "IMA12-2; F1; F2; F4; F16; IMA9Z; F8 and IMA834Z" were suitably able to distinguish all the rapeseed varieties studied. Thus, among the 136 bands generated by these 8 primers, only 9 markers (IMA12-2-1036; F1-415; F1-366; F2-691; F4-451; F16-719;

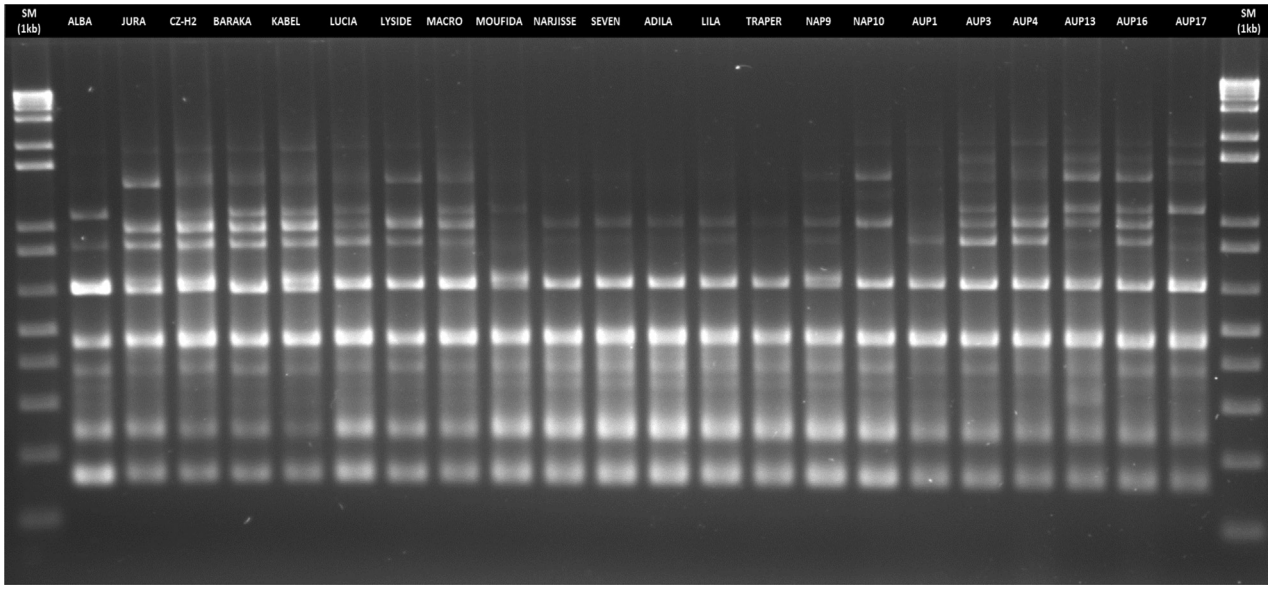

Fig. 1. ISSR genomic profile of the 22 rapeseed varieties generated by the primer IMA834-2.

Table 3. ISSR primers and amplification results of 20 ISSR primers used for the molecular characterization of 22 rapeseed varieties.

Primer	AT (°C)	SB	TNB	NPB	NMB	PPB	EMR	MI	PIC
F1	43	184-1122	19	18	1	94.74	17.05	6.41	0.38
F2	58	192-808	14	9	5	64.29	5.79	2.07	0.36
F3	43	270-934	15	11	4	73.33	8.07	3.4	0.42
F4	58	167-1329	20	16	4	80	12.8	4.57	0.36
F8	56	161-1249	22	18	4	81.82	14.73	6.08	0.41
F9	43	237-765	13	9	4	69.23	6.23	2.43	0.39
F11	56	176-718	17	16	1	94.12	15.06	4.44	0.3
F16	58	238-1220	18	14	4	77.78	10.89	3.82	0.35
IMA-5-Z	56	171-966	18	16	2	88.89	14.22	4.72	0.33
IMA9Z	56	185-726	11	11	0	100	11	3.7	0.34
IMA834z	50	175-911	16	13	3	81.25	10.56	4.54	0.43
IMA8Z	50	166-935	15	14	1	93.33	13.07	4.9	0.38
IMA834-2	50	175-1122	15	10	5	66.67	6.67	3.39	0.51
UBC808-2	50	193-984	19	11	8	57.89	6.37	2.28	0.36
UBC810	50	190-929	16	13	3	81.25	10.56	3.35	0.32
UBC850	50	265-1311	14	11	3	78.57	8.64	2.78	0.32
UBC856	56	137-985	15	14	1	93.33	13.07	4.48	0.34
IMA12-2	56	210-1085	16	15	1	93.75	14.06	5.23	0.37
ISS F1	50	299-1213	14	13	1	92.86	12.07	3.69	0.31
ISSR1	56	183-717	12	8	4	66.67	5.33	2.13	0.4
Average	52.25	–	15.95	13	2.95	81.4885	10.812	3.9205	0.369

AT: annealing temperatures; SB: size of bands (bp); TNB: total number of bands; NPB: number of polymorphic bands; NMB: number of monomorphic bands; PPB: percentage of polymorphic bands; EMR: effective multiple ratio; MI: marker index; PIC: polymorphic information content.

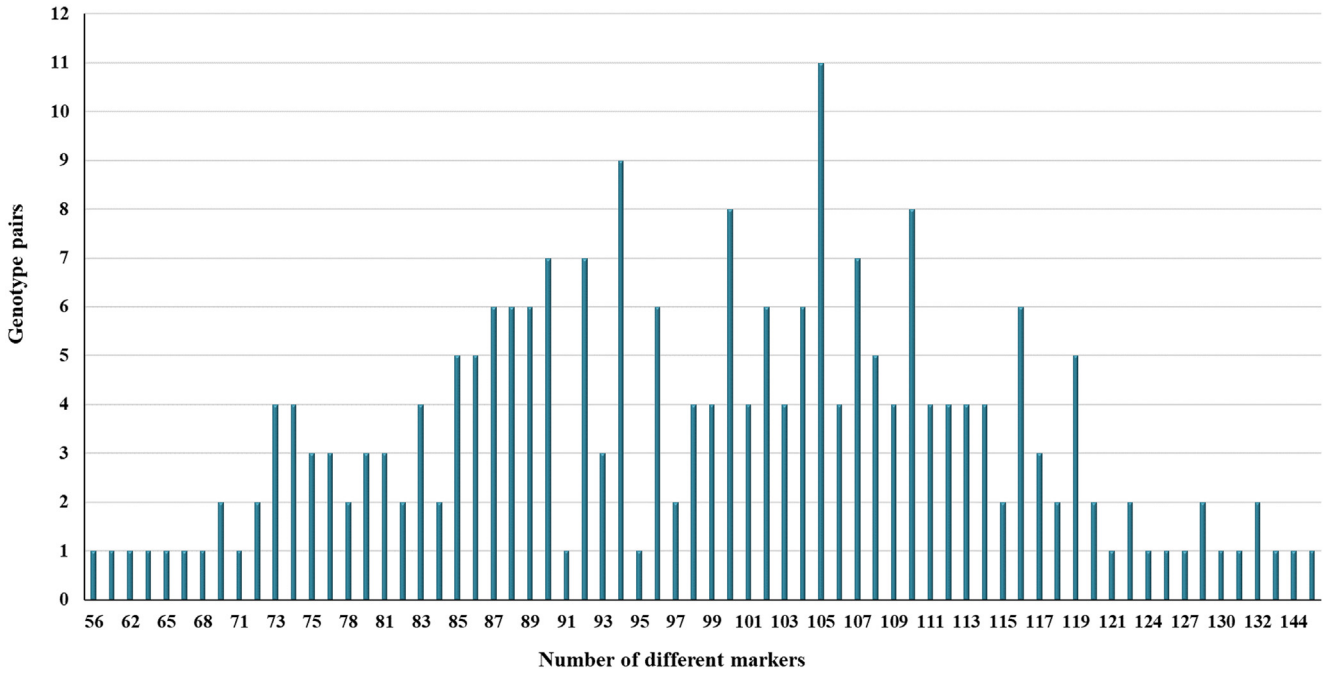

Fig. 2. Frequency distribution of genetic dissimilarity for all pairwise combinations among 22 rapeseed varieties.

IMA9Z-403; F8-559 and IMA834Z-414) can distinguish the 22 varieties of rapeseed. The 8 primers will facilitate future studies in rapeseed genetic diversity using a minimum number of primers. These primers can be used particularly for varietal authentication and fingerprinting.

The pairwise comparison of the 22 varieties according to the Simple Matching coefficient showed distances ranging from a minimum of 0.176 for (Seven/Narjisse) to a maximum of 0.456 for (AUP4/Adila), with an average of 0.31. This range of variation is relatively smaller, compared with that reported by Safari *et al.* (2013) who found distances varying between 0.26 and 0.95. In addition, based on 15 ISSR primers, Abdelmigid (2012) indicated that within 10 accessions of rapeseed from different countries, the highest genetic similarity was 0.72 while the lowest one was 0.41. These differences might be attributed to the nature/type of accessions investigated, the number of these accessions and their multiple geographical origins.

The average similarity among the 22 varieties herein studied is 69% and the pairwise comparison shows that all varieties are genetically distinct by a minimum of 56 markers in the case of (Seven/Narjisse) and a maximum of 145 markers in the case of (AUP4/Adila) (Fig. 2). This clearly indicates the high level of genetic diversity among the varieties studied. Similar previous studies in rapeseed showed an average level of similarity of 67.3% using AFLP markers (Yu *et al.*, 2007) and 64% using RAPD markers (Ana *et al.*, 2009). In another work on canola, the percentage of similarity among 10 genotypes of different geographical origins, ranged from 0.47 to 0.73 using a combination of ISSR and RAPD markers (Abdelmigid, 2012).

3.2 Cluster analysis

The dendrogram obtained by analysis of the 319 ISSR markers has allowed dividing the varieties into four distinct groups at a similarity index of 69% (Fig. 3).

The group A contains a mixture of ten Moroccan and introduced varieties. The group B includes all varieties of Pakistani origin. The group C contains the Moroccan varieties Adila and Lila, the Australian varieties Nap 9 and Nap 10, and the German hybrid Traper. However, the variety Alba is very distinct from all the rest of genotypes and consequently classified as a single branch (Group D). Among the 20 primers used, 17 primers were sufficient to distinguish the three groups A, B and C having a number of markers of 160, 127 and 231, respectively. Group D is distinguished with a single genotype. However, no markers are specific to the different groups.

The dendrogram clearly separated the varieties according to their origins, indicating that there was an association between genetic similarity and geographic distance. Similar results were reported by Paul *et al.*, 2020 in their work on rapeseed germplasm characterization through ISSR markers and isozymes markers. However, our study shows that the Moroccan varieties are genetically close to a number of introduced varieties, which would indicate that they might derive from the same pedigree or belong to close genetic backgrounds. This is plausible since rapeseed remains a species characterized by a limited genetic diversity in comparison with other agricultural species (Nabloussi, 2015). As a result, the genetic progress achieved for this crop during the last 15–20 years is also limited. Hence the usefulness and interest of identifying genetically distant genotypes to use as crossing parents to broaden genetic diversity and enhance genetic progress in future selected varieties. This strategy is relevant for rapeseed which is, in fact, a partially allogamous plant, with 1/3 cross-pollination and 2/3 self-pollination (Nabloussi, 2015).

A concrete example that shows how effective the used markers are in structuring genotypes is the composition of group C. It turns out that this group, established by a very small coefficient of similarity, brought together pure lines of

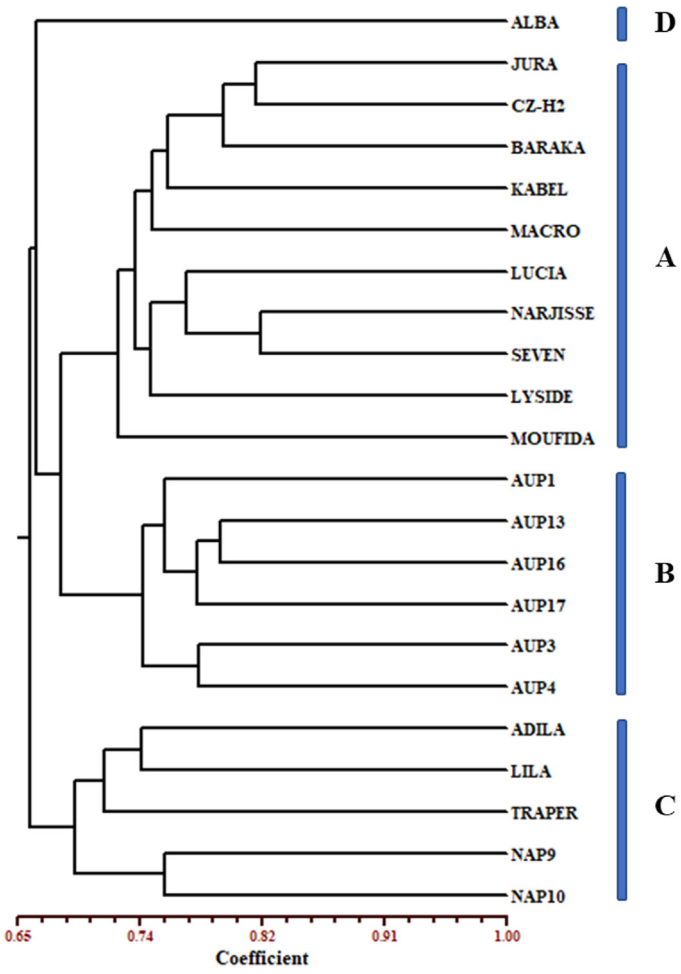

Fig. 3. Dendrogram generated for 22 rapeseed genotypes by ISSR markers using genetic distance and the UPGMA method.

Australian origin, Moroccan synthetic varieties and a German hybrid variety.

4 Conclusion

This study has confirmed that ISSR markers perform well for analyzing genetic diversity in rapeseed. The selected primers were found to be effective in discriminating the 22 varieties investigated, revealing a high genetic variability. Eight ISSR primers were identified as a key to rapeseed varietal determination and authentication. There was a phylogeographic structuring the analyzed varieties as their separation was done generally according to their geographical origins. However, we found that some Moroccan varieties are genetically close to a number of introduced varieties, which would indicate that these Moroccan and foreign varieties would have in their pedigree parents with a close genetic background. Our findings provide important clues in understanding the relationship between the rapeseed varieties studied, which will contribute to the success of breeding program by targeting crosses between genotypes belonging to genetically distinct groups and benefiting from heterosis effect. This will enable expanding the genetic variability and developing high-performing rapeseed cultivars for the development of the Moroccan oilseed sector.

Conflicts of interest

Authors declare they have no conflicts of interest.

Authors contributions

Karim Houmanat: Writing-original draft, data curation, formal analysis. Abdelghani Nabloussi: Conceptualization, validation, supervision, writing-review and editing. Yousra Rhazlaoui: Investigation, writing-original draft, formal analysis. Hakima Bahri: Supervision, writing-review and editing. Mohamed El Fechtali: Investigation. Jamal Charafi: Methodology, supervision, data curation, formal analysis, writing-review and editing.

References

Abdelmigid HM. 2012. Efficiency of random amplified polymorphic DNA (RAPD) and inter-simple sequence repeats (ISSR) markers for genotype fingerprinting and genetic diversity studies in canola. *Afr J Biotechnol* 11(24): 6409–6419.

Ana MJ, Ankica KS, Dejana SP, Radovan M, Nikola H. 2009. Phenotypic and molecular evaluation of genetic diversity of rapeseed (*Brassica napus* L.) genotypes. *Afr J Biotechnol* 8(19).

Anderson A, Churchill GA, Autrique JE, Tanksley SD, Sorrells ME. 1993. Optimizing parental selection for genetic linkage maps. *Genome* 36(1): 181–186.

Caroli S, Santoni S, Ronfort J. 2011. AMaCAID: a useful tool for accurate marker choice for accession identification and discrimination. *Mol Ecol Resour* 11(4): 733–738.

Chesnokov YV, Artemyeva AM. 2015. Evaluation of the measure of polymorphism information of genetic diversity. *Agric Biol* 50(5): 571–578.

DDFP. 2022. Statistiques sur les cultures oléagineuses au Maroc. Direction de Développement des Filières de Production (DDFP), Ministère de l'Agriculture, de la Pêche Maritime, du Développement Rural et des Eaux et Forêts.

Guirrou I, El Harrak A, El Antari A, *et al.* 2023. Bioactive compounds assessment in six Moroccan rapeseed (*Brassica napus* L.) varieties grown in two contrasting environments. *Agronomy* 13(2): 460.

Madre JF. 2013. Logiciel mesurim Pro. Ver. 3,4. Académie d'Améins (accessed on 29 May 2022).

Mahjoob B, Zarini HN, Hashemi SH, Shamasbi FV. 2016. Comparison of ISSR, IRAP and REMAP markers for assessing genetic diversity in different species of *Brassica* spp. *Russian J Genet* 52(12): 1272–1281.

Maras-vanlioğlu FG, Yaman H, Kayaçetin F. 2020. Genetic diversity analysis of some species in Brassicaceae family with ISSR markers. *Biotechnol Stud* 29(1): 38–46.

McVetty PB, Mietkiewska E, Omonov T, Curtis J, Taylor DC, Weselake RJ. 2016. *Brassica* spp. oils. Industrial oil crops. AOCS Press, pp. 113–156.

Nabloussi A. 2015. Amélioration génétique du colza : enjeux et réalisations pour un développement durable de la filière. ISBN: 978-9954-593-27-1. Rabat, Morocco : INRA.

Nagaraju J, Kathirvel M, Kumar RR, Siddiq EA, Hasnain SE. 2002. Genetic analysis of traditional and evolved Basmati and non-basmati rice varieties by using fluorescence-based ISSR-PCR and SSR markers. *Proc Nat Acad Sci* 99(9): 5836–5841.

Paul M, Islam T, Hoque MI, Sarker RH. 2020. Analysis of genetic diversity in oilseed *Brassica* germplasm through ISSR markers and isozyme profiling. *Bangladesh J Bot* 49(1): 147–158.

Powell W, Morgante M, Andre C, *et al.* 1996. The comparison of RFLP, RAPD, AFLP and SSR (microsatellite) markers for germplasm analysis. *Mol Breed* 2(3): 225–238.

Pradeep AR, Chatterjee SN, Nair CV. 2005. Genetic differentiation induced by selection in an inbred population of the silkworm Bombyx mori, revealed by RAPD and ISSR marker systems. *J Appl Genet* 46(3): 291.

Pradeep Reddy M, Sarla N, Siddiq EA. 2002. Inter simple sequence repeat (ISSR) polymorphism and its application in plant breeding. *Euphytica* 128(1): 9–17.

Rohlf FJ. 2002. NTSYS-pc Numerical Taxonomy System, ver. 2.1. Setauket, New York: Exeter Publishing Ltd.

Safari S, Mehrabi AA, Safari Z. 2013. Efficiency of RAPD and ISSR markers in assessment of genetic diversity in *Brassica napus* genotypes. *Int J Agric Crop Sci (IJACS)* 5(3): 273–279.

Saghai-Maroof MA, Soliman KM, Jorgensen RA, Allard R. 1984. Ribosomal DNA spacer-length polymorphisms in barley: Mendelian inheritance, chromosomal location, and population dynamics. *Proc Nat Acad Sci* 81(24): 8014–8018.

Sharma HK, Kumar A, Singh VV, *et al.* 2022. Genetic resources of brassicas. *Cash Crops: Genetic Diversity, Erosion, Conservation and Utilization*: 285–337.

Sica M, Gamba G, Montieri S, Gaudio L, Aceto S. 2005. ISSR markers show differentiation among Italian populations of *Asparagus acutifolius* L. *BMC Genet* 6(1): 1–7.

Sokal RR, Michener CD. 1958. A statistical method for evaluating systematic relationships. *Univ Kansas Sci Bull* 38: 1409–1438.

Swarup S, Cargill EJ, Crosby K, Flagel L, Kniskern J, Glenn KC. 2021. Genetic diversity is indispensable for plant breeding to improve crops. *Crop Sci* 61(2): 839–852.

Talhinhas P, Neves-Martins J, Leitao J. 2003. AFLP, ISSR and RAPD markers reveal high levels of genetic diversity among *Lupinus* spp. *Plant Breed* 122(6): 507–510.

Tarıkahya-Hacıoğlu B. 2016. Molecular diversity of the wild Crambe (Brassicaceae) taxa in Turkey detected by intersimple sequence repeats (ISSRs). *Ind Crops Prod* 80: 214–219.

Ton LB, Neik TX, Batley J. 2020. The use of genetic and gene technologies in shaping modern rapeseed cultivars (*Brassica napus* L.). *Genes* 11(10): 1161.

Wiesner I, Wiesnerová D. 2003. Effect of resolving medium and staining procedure on inter-simple-sequence-repeat (ISSR) patterns in cultivated flax germplasm. *Genetic Resour Crop Evol* 50(8): 849–853.

Yu C, Leišová L, Kučera V, *et al.* 2007. Assessment of genetic diversity of yellow-seeded rapeseed (*Brassica napus* L.) accessions by AFLP markers. *Czech J Genet Plant Breed* 43(3): 105.

Zietkiewicz E, Rafalski A, Labuda D. 1994. Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. *Genomics* 20(2): 176–183.

Cite this article as: Houmanat K, Nabloussi A, Rhazlaoui Y, Bahri H, El Fechtali M, Charafi J. 2023. Genetic relationship and diversity among some Moroccan and introduced rapeseed (*Brassica napus* L.) varieties as revealed by molecular markers. *OCL* 30: 18.