International Journal of Agriculture and Environmental Research

ISSN: 2455-6939

Volume:05, Issue:01 "January-February 2019"

MOLECULAR CHARACTERIZATION OF SOME *BRASSICA* SPECIES

[1]R. M. A. Khalil, [2]Mohamed Abd. S. El zayat

[1,2] Plant Genetic resources Dept. Desert Research center. Matariya, Cairo, Egypt

ABSTRACT

Nineteen ISSR were used to investigate polymorphism among five Brassica species. ISSR revealed 326 total bands the highest (89% polymorphism). 30 SRAP primer pairs revealed 480 bands with polymorphism (93%). ISSR and SRAP, both were effective in studying genetic diversity in Brassica. Based on similarity matrix of overall analysis (ISSR and SRAP), the dendrogram was constructed and separated the five Brassica into two main clusters. Results showed that the length of the MYB28 gene for glucosinolates production at the fragment of 1257 bp. This information can be used in the future for breeding and improvement programs and helps in national conservation programs.

Keywords: Brassica, ISSR, SRAP, glucosinolates and MYB28gene.

INTRODUCTION

Brassica genus (*Brassicaceae*) contains proteins, vitamins, minerals, glucosinolates and phenols. Glucosinolates contribute to reducing the risk of cancer **(Xu *et al*. 2008).** That activity is mainly related to the presence of isothiocyanates and nitralates. Most species are of great economic importance since most of them are edible, a source of high-value edible oil, fodder, Which helps to protect the food supply by expanding the range of genes available in the face of the challenges of the lack of agricultural production, and is of great medical importance such as: white mustard, black, brown, yellow, cabbage and broccoli help in the treatment of chronic, cough, asthma, chest pains, heart disease, headache, compresses the pain of rheumatism, joints, diuresis, stimulates the metabolism, clears the appetite, treats tumors, regulates blood sugar and prevents cerebral palsy.

Molecular markers are effective tools for revealing genetic diversity, inter-sequence simple repeat (ISSR), is useful for detecting genetic polymorphisms among genotypes by generating a large number of markers that target multiple microsatellite loci distributed across the genome **(Godwin *et al.*, 1997).** ISSR is a technique that overcomes most of other techniques limitations. This technology has been used to DNA fingerprint a wide range of species of medicinal plants,

ISSN: 2455-6939

Volume:05, Issue:01 "January-February 2019"

phylogeny and the geographic origin of some plant species as *Brassica napus* **(Harper *et al.* 2012)**.

Sequence-related amplified polymorphism (SRAP) is a new and useful molecular marker system for tagging and mapping in *B. oleracea* **(Li and Quiros, 2001)**. The SRAP system is easy to perform, gives stable and moderate yields and a high proportion of co-dominance, and ultimately simplifies the cloning and sequencing of target segments. The SRAP technique is an effective tool for constructing genetic and linkage maps **(Yuan *et al*., 2015),** carrying out comparative genomics and analyzing genetic diversity because sequences are amplified against open reading frames, and sequences near the centromeres and telomeres of genomes are only rarely amplified. The SRAP technique has been applied successfully in studies of sweet potato, rape, and Chinese cabbage and brocoli (**Riaz *et al*., 2001**; **Wu *et al*., 2009**; **Yu *et al*., 2011).**

Although there are many genetic studies related to plant traits, there are few in terms of identifying genes and their characterization, especially specific of Brassica for the main active substance such as glucosinolates .There is a great challenge to be addressed in the inventory of genetic variation that helps us to improve crops. Glucosinolates, in turn, are resistant to pests and microbes, as well as the industrial significance of brassica in the work of bombs in wars, thus preserving genetic diversity in gene banks and characterizing them and defining the important genes that we can transfer to important crops to be used as food and source of important effective substances. (MYB28) gene was found to participate in regulating the aliphatic GSL biosynthesis glucosinolates and have been successfully identified in several plant species including *Arabidopsis thaliana* **(Sønderby *et al*., 2010)**, *Brassica juncea* **(Augustine *et al*., 2013)**, and *B. rapa* **(Kim *et al*., 2013)**.

The objectives of this study were to investigate the level of genetic diversity and relationships in Brassica by molecular markers and provide valuable information for detecting glucosinolate gene.

MATERIALS AND METHODS

Five Brassica species were collected from the Gene Bank of North Sinai Station, Desert Research, Egypt during February 2015-2017, as shown in (Fig 1and Table 1).

ISSN: 2455-6939

Volume:05, Issue:01 "January-February 2019"

Fig (1): The five Brassica species collected from the Gene Bank of North Sinai Station, Desert Research, Egypt

Table (1): Taxa of the five studied Brassica species

No	Scientific name
1	*Brassica tournefortii*
2	*Brassica nigra* L.
3	*Brassica rapa* L.
4	*Brassica juncea* L.
5	*Brassica* carentia

DNA extraction

A total of 25 young fresh leaves from each of the 20 annual ryegrass plants were randomly collected and extracted using the DNeasy Plant Mini kit (Qiagen Inc, Valencia, CA, USA). The quality and concentration of the DNA were confirmed by electrophoresis on 0.8% agarose gels with the standardized lambda DNA size markers.

ISSR analysis

Finally, 15 primers (Life Technologies) (Table2) were selected for ISSR analysis. The PCR amplification comprised a total volume of 20 mL containing 3 mL 20 ng/mL DNA, 0.2 mL Taq DNA polymerase, 1.4 mL 2.5 mM dNTPs, 3 mL 10 mM primer, 1.4 mL 25 mM Mg , 2.5 mL 10X buffer, and 8.3 mL ddHO. PCR amplification was carried out on the Eppendorf PCR instrument and the reaction program comprised an initial 5 min at 94°C; 35 cycles of 45 s at 94°C, 45 s annealing at 50°C and a 90-s extension at 72°C; ending with a final extension of 5 min at 72°C and storage at 4°C. Amplified products were electrophoresed on 6% denaturing

ISSN: 2455-6939

Volume:05, Issue:01 "January-February 2019"

polyacrylamide gel, which was silver stained and photographed gels and visualized with the Gel Documentation System(Bio-Rad® Gel Doc-2001) (Germany).

Table (2): ISSR primer sequences

Primer name	Sequence	Primer name	Sequence
HB01	(CAA) 5	844B	(CT) 8 GC
HB02	(CAG) 5	HB9	(GT) 6 GG
HB04	(GACA) 4	HB10	(GA) 6 CC
17898A	(CA) 6 AC	HB11	(GT) 6 CC
17898B	(CA) 6 GG	HB12	(CAC) 3 GC
17899A	(CA) 6 AG	HB14	(CTC) 3 GC
17899B	(CA) 6 GG	HB15	(GTG) 3 GC
844A	(CT) 8 AC	814	(CT) 8TG
807	(AGA) 4GT	HB13	GAC)$_3$ GC
HB8	(GA)6 GG		

SRAP fingerprinting

Thirty selected pairs of primers (Life Technologies) (Table3) from 60 different primer combinations were used for detecting polymorphism in open reading frames (ORFs). Each 20-µLPCR mixture consisted of 1. 0 U Taq DNA polymerase, 6X PCR buffer, 0.6 mM dNTP, 0.35 µM primer, 1.5 mM Mg, and 25-200 ng template DNA. Thermal cycling (Biometra T1 Thermocycle) started with 5 min at 94°C for initial denaturing, and 5 cycles of 30 s at 94°C, 30 s at 35°C, and 45 s at 72°C, followed by 40 cycles of 30 s at 94°C, 30 s at 4°C and 45 s at 72°C. The last cycle was followed by a 7-min extension at 72°C. Amplified products were analyzed on 2% (w/v) gels and visualized with the Gel Documentation System (Bio-Rad® Gel Doc-2001) (Germany).

ISSN: 2455-6939

Volume:05, Issue:01 "January-February 2019"

Table (3): The forward and reverse SRAP primer in this study

Primer name	Forward primers	Primer name	Reverse primers
Me1	TGAGTCCAAACCGGATA	Em1	GACTGCGTACGAATTAAT
Me2	TGAGTCCAAACCGGAGC	Em2	GACTGCGTACGAATTTGC
Me3	TGAGTCCAAACCGGAAT	Em3	GACTGCGTACGAATTGAC
Me4	TGAGTCCAAACCGGACC	Em4	GACTGCGTACGAATTTGA
Me5	TGAGTCCAAACCGGAAG	Em5	GACTGCGTACGAATTAAC
Me6	TGAGTCCAAACCGGACA	Em6	GACTGCGTACGAATTGCA
Me7	TGAGTCCAAACCGGACG	Em7	GACTGCGTACGAATTCAA
Me8	TGAGTCCAAACCGGACT	Em8	GACTGCGTACGAATTCTG
Me9	TGAGTCCAAACCGGAGG	Em9	GACTGCGTACGAATTCAG
Me10	TGAGTCCAAACCGGAAA	Em10	GACTGCGTACGAATTCAT
Me11	TGAGTCCAAACCGGAAC	Em11	GACTGCGTACGAATTCTA
Me12	TGAGTCCAAACCGGAGA	Em12	GACTGCGTACGAATTCTC
DN06	TGAGTCCAAACCGGTAA	Em13	GACTGCGTACGAATTCTT
DN07	TGAGTCCAAACCGGTCC	Em14	GACTGCGTACGAATTGAT
DN08	TGAGTCCAAACCGGTGC	Em15	GACTGCGTACGAATTGTC
DN09	TGAGTCCAAACCGGTCA	Em16	GACTGCGTACGAATTCGA
DN10	TGAGTCCAAACCGGGCT	Em 17	GACTGCGTACGAATTAGC
DN11	TGAGTCCAAACCGGTAG	Em 18	GACTGCGTACGAATTGAG
DN12	TGAGTCCAAACCGGTGT	Em 19	GACTGCGTACGAATTGCC

Statistical analysis

Similarity matrix was developed by the statistical package for social science program SPSS based on combined analysis of overall molecular and biochemical markers.

Detection of gene Polymerase Chain Reaction (PCR) and Sequencing

PCR was conducted using forward and reverse primers, which were designed based on the sequence of MYb28 obtained from the Gen Bank (BLAST). The sequences of the primers were 5'-GGGACCATCACACAATTCATTTCTC-3' (forward) and 5'- ' (reverse).A mixture of 20

ISSN: 2455-6939

Volume:05, Issue:01 "January-February 2019"

µL solution consisting of 10x Ex Taq polymerase buffer, 2 mM MgCl2, 200 uM dNTPs, 25 pmol primers, 1U Taq polymerase and distilled water was used for each PCR reaction. The PCR program was set on 94ºC for 2 minutes preheating continued with 35 cycles consisting of 1 minute denaturation at 94ºC, 1 minutes annealing at 56ºC, and 90 seconds extension at 72 ºC. The PCR product was visualized on 1,5% agarose gel and subjected to 100 volts for 1hr and then photographed using UV gel documentation system, UVP corporation- UK.

Purification of PCR Product and Sequencing:

PCR products were purified using High Pure PCR Product purification Kit (Roche) and sequenced (MWG, Germany).

RESULTS AND DISCUSSION

ISSR analysis

Nineteen preselected ISSR primers were used in the present investigation to study the genetic relationships among the five studied Brassica species as shown in Fig (2) and (Table 4) .326 total bands, 36 monomorphic and 290 polymorphic distinct bands (89% polymorphism) were generated by the nineteen ISSR primers. The results showed that 17898B, 17899A, HB1, HB4, HB9, HB11 and HB15 primers were highly polymorphic (100% polymorphism), while HB13 generated the lowest polymorphism (74%). These results agreed with the results obtained by Khalil **(**2010**)** who used fifteen preselected ISSR primers to identify eleven species of *Brassicacea* and produced72 polymorphic distinct fragments (56.2% of polymorphism). Those results gave another dimension to detect the genetic variability in accurate differentiations and demonstrated significant levels of variation. Moreover, Santos *et al.*, (2016) studied ISSR primers and were capable of detecting genetic polymorphism among *Mimosa caesalpiniaefolia*. A total of 7 ISSR primers generated 52.7% polymorphism.

ISSN: 2455-6939

Volume:05, Issue:01 "January-February 2019"

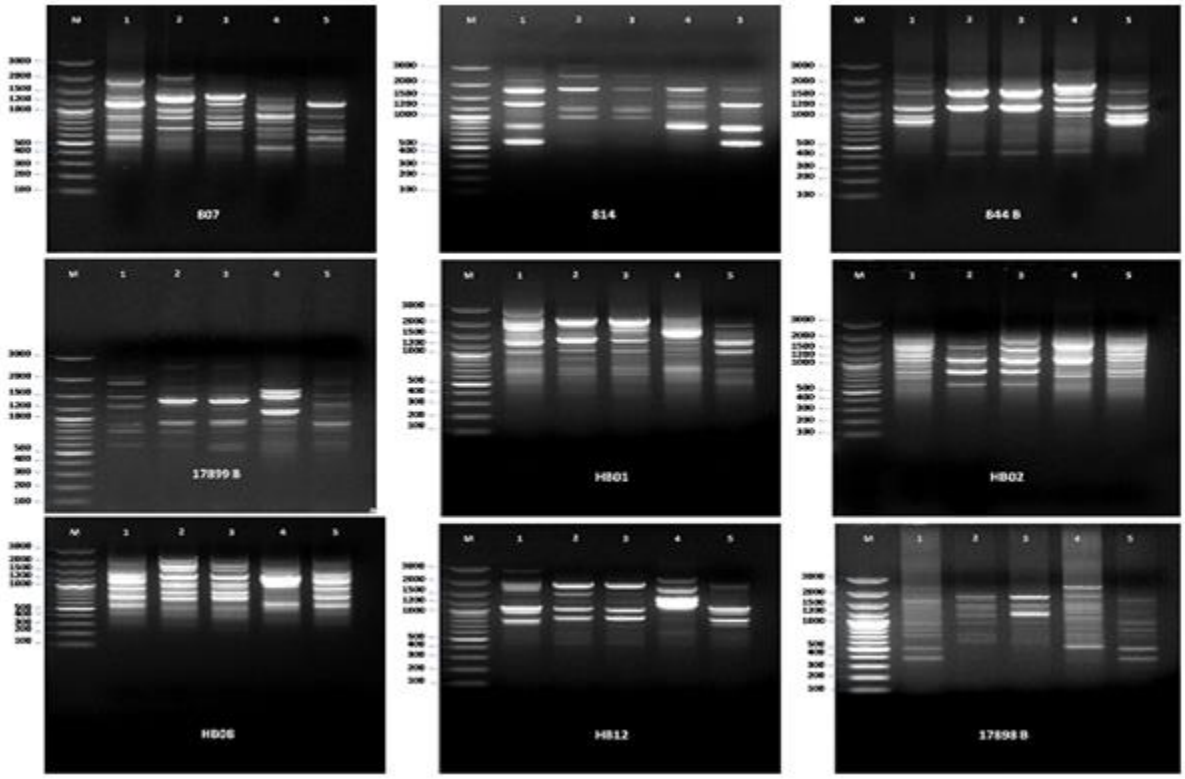


Fig (2): ISSR primers of five Brassica species*. **(M) = Maker, *(*Brassica tournefortii* (Gouam)) = 1, (*Brassica nigra* L.) =2, (*Brassica rapa* L.) = 3, (*Brassica juncea* L.) = 4, (*Brassica carentia*) = 5.

ISSN: 2455-6939

Volume:05, Issue:01 "January-February 2019"

Table (4): Primer codes, length range (bp), monomorphic bands, polymorphic bands, total amplified bands and polymorphism percentages of nineteen ISSR primers among five Brassica species.

	Primer code	Monomorphic bands	Polymorphic bands	Unique bands	Total amplified bands	Polymorphism percentages
1	807	2	18	7	20	90%
2	814	1	15	7	16	94%
3	844A	3	12	4	15	80%
4	844B	1	14	5	15	93%
5	17898A	1	14	5	15	93%
6	17898B	0	17	3	17	100%
7	17899A	0	13	3	13	100%
8	17899B	2	15	6	17	88%
9	HB1	0	13	3	13	100%
10	HB2	5	9	0	14	64%
11	HB4	0	20	7	20	100%
12	HB8	1	14	2	16	88%
13	HB9	0	8	1	8	100%
14	HB10	2	24	9	26	92%
15	HB11	0	14	1	14	100%

ISSN: 2455-6939

Volume:05, Issue:01 "January-February 2019"

16	HB12	2	15	4	17	88%
17	HB13	6	17	3	23	74%
18	HB14	1	13	6	14	93%
19	HB15	0	21	7	21	100%
	Total	27	286	83	329	87%

SRAP analysis

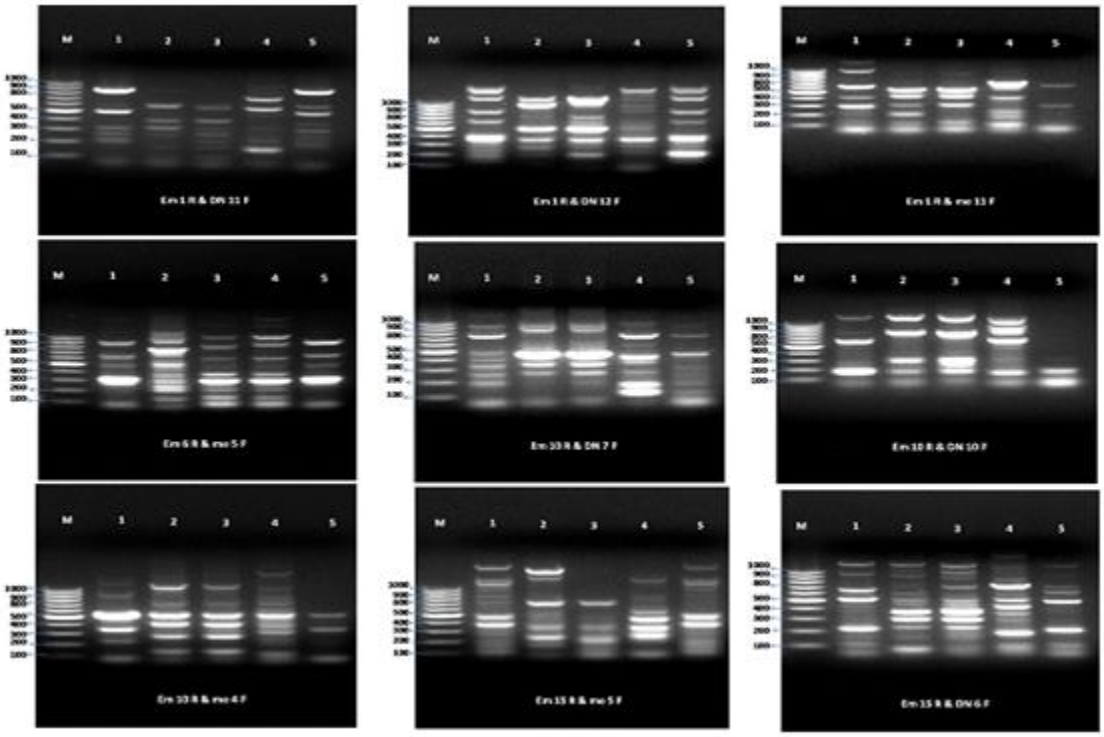
Thirty pairs of preselected SRAP primers were used in the present investigation to study the genetic relationships among the five studied species of Brassica as shown in Fig (3) and (Table 5 and 6). 480 total bands, 32 monomorphic bands and 448 polymorphic distinct bands (93% polymorphism) were revealed by the SRAP primers. The results showed that EM1RXme8F, EM6RXme5F, Em6RXme11F, EM10RXDN6F, EM10RXDN7F, Em10RXDN12F, Em13RXDN12F, Em17RXDN12F and Em18RXDN11F primer pairs were highly polymorphic (100% polymorphism). On the other hand, EM6RXDm8F primer pair revealed the lowest polymorphism (75%). Polymorphic information content (PIC) and Marker indexes (MI) were equal between ISSR and SRAP markers (Table 6). These results agreed with the results obtained by **Ahmad *et al*., (2014)** who studied the genetic diversity and relationships among *B. napus* accessions using SRAP markers, which preferentially amplify open reading frames. Using 20 SRAP primers, a total of 60 spring-type *B. napus* accessions revealed 162 polymorphic fragments. Cluster analysis displayed five major groups. The clustering pattern mostly supported their respective pedigree and characteristic traits. Additionally, **Yu (2014)** used SRAP and ISSR markers to assess the genetic diversity within and among 15 natural populations of *Stipa bungeana* from the Loess Plateau of China. Using 15 SRAP primers, 504 (99.80%) polymorphic loci were detected, and 372 polymorphic loci (96.12%) were identified using 15 ISSR primers. The results showed that genetic variation within populations is lower than that among populations. The results demonstrated that both SRAP and ISSR markers are effective and reliable for assessing the genetic diversity of *S. bungeana*. In addition, these data inform conservation and breeding strategies for *S. bungeana*. Besides, **Ma *et al*., (2015)** detected the genetic relationships of different *Cotoneaster schantungensis* natural populations and genetic diversity analysis based on SRAP markers. Twelve pairs primers were selected out, 93 bands were amplified, of which 91 bands (97.85%) were polymorphic. **Guenni *et al*., (2016)** used SRAP markers preferentially to amplify open reading frames and to study the genetic diversity of

ISSN: 2455-6939

Volume:05, Issue:01 "January-February 2019"

43Tunisian pistachio accessions. Using seven SRAP primer pairs, a total of 78 markers revealed (95.12%) that will help to design future conservation and breeding strategies concerning this species.

Fig (3): SRAP primers among five Brassica species*. **(M) = Maker, *(*Brassica tournefortii* (Gouam)) = 1, (*Brassica nigra* L.) =2, (*Brassica rapa* L.) = 3, (*Brassica juncea* L.) = 4, (*Brassica carentia*) = 5.



ISSN: 2455-6939

Volume:05, Issue:01 "January-February 2019"

Table (5): Primer codes, length range (bp), monomorphic bands, polymorphic bands, total amplified bands and polymorphism percentages of 29 SRAP

NO	Primer code	Monomorphic bands	Total Polymorphic bands	Unique bands	Total amplified Bands	Polymorphism percentages
1	Em1RXDN11F	1	12	3	16	94%
2	Em1RXDN12F	1	10	9	20	95%
3	EM1RXme8F	0	7	6	13	100%
4	EM1RXme11F	1	12	7	13	93%
5	EM2RXme12F	1	10	7	11	90%
6	EM6RXDm8F	3	9	3	12	75%
7	EM6RXme5F	2	17	7	19	100%
8	Em6RXme11F	0	18	4	18	100%
9	EM8RXme1F	1	8	1	9	88%
10	EM9RXme1F	1	16	5	17	94%
11	EM10RXDN6F	0	17	3	17	100%
12	EM10RXDN7F	0	20	6	20	100%
13	EM10RXDN8F	3	10	6	13	77%
14	Em10RXDN10F	2	10	4	12	83%
15	Em10RXDN12F	0	17	4	17	100%
16	Em10RXme4F	1	16	5	17	94%
17	Em12RXDN10F	1	15	8	16	94%
18	Em12RXDN12F	1	17	3	18	94%
19	Em13RXDN12F	0	14	4	14	100%

ISSN: 2455-6939

Volume:05, Issue:01 "January-February 2019"

20	Em15RXDN6F	2	19	5	21	90%
21	Em15RXme10F	3	24	0	27	89%
22	Em15RXDN7F	1	18	3	19	95%
23	Em15RXDN9F	1	8	9	9	88%
24	Em15RXme11F	1	8	1	9	88%
25	Em17RXDN10F	0	18	6	18	100%
26	Em17RXDN12F	0	12	3	12	100%
27	Em18RXDN11F	0	10	5	10	100%
28	Em18RXDN12F	2	10	1	12	83%
29	Em20RXDN10F	1	16	1	17	94%
	Total	**30**	**398**	**129**	**485**	**82%**

Table (6): Relative efficiency of molecular markers in determining polymorphism among five Brassica

Parameters for marker efficiency	**ISSR**	**SRAP**
Number of individuals	19	30
Total number of bands (*L*)	326	480
Polymorphic bands (p)	290	448
Number of loci/assay unit (*nu*)	17.1	16
Total number of effective alleles (*Ne*)	1213	1975.8
Average number of polymorphic bands/assay unit(*np*/*U*)	15.26	14.93
Polymorphic information content (PIC)	0.97	0.97
Fraction of polymorphic loci (β)	0.88	0.93
Assay efficiency index (*Ai*)	63.8	65.8
Effective multiples ratio (*E*)	15.26	14.93
Marker index (MI) = H_{av} × MR	14.82	14.62

ISSN: 2455-6939

Volume:05, Issue:01 "January-February 2019"

Genetic and Phylogenetic Relationships

The generated ISSR and SRAP profiles were further used to assess similarities among the studied cultivars. The estimation of genetic distance was accomplished using a computer software (NTSYS-PC version 1.8) based on the formula originally proposed by Nei and Li (1979). The results are presented in (Table 7). Genetic distances ranged from (.385 to .762) in ISSR and SRAP Marker. The lowest genetic distance was found between *Brassica tournefortii* and *B.rapa*. The highest genetic distances w ere registered between *B.nigra* and *B.rapa*. The genetic distances were supported by the resulting dendrogram (Figure 4). Dendrogram revealed two main clusters, the first cluster splatted into two sub-clusteres included *B.tournefortii* and *B.rapa*. The second cluster included *B. nigra* and *B.rapa* in one subcluster and *B. juncea* in anotherThe use of ISSR and SRAP marker is st rongly supported by many studies dealing with the usefulness of such markers for investigating Brassica diversity. The current research is one of the attempts to use molecular markers in investigating the genetic relationships among various local genotypes geranium grown in desert Egypt.

Table (7): Comparison by similarity of relationships Brassica spp. genotypes as revealed by ISSR, SRAP marker systems

	Matrix File Input			
Case	*Brassica tournefortii*	*Brassica nigra*	*Brassica rapa*	*Brassica juncea*
Brassica tournefortii				
Brassica nigra	.386			
Brassica rapa	.385	.762		
Brassica juncea	.462	.457	.480	
Brassica carentia	.675	.393	.400	.445

ISSN: 2455-6939

Volume:05, Issue:01 "January-February 2019"

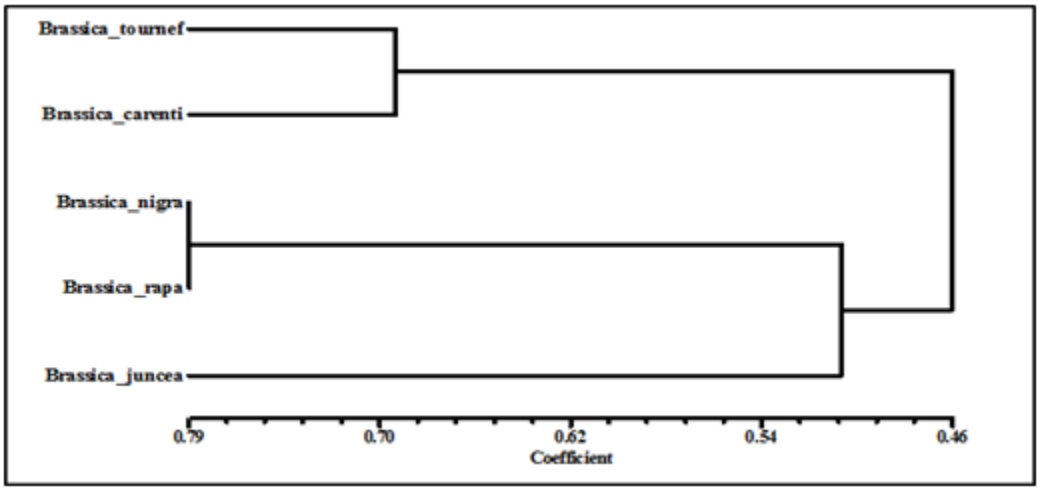


Fig. (4): An unweighted pair-group method with arithmetic averages (UPGMA) dendrogram of genetic relationships among five genotypes of Brassica obtained using ISSR and SRAP combined data.

MYB28 gene detection:

The PCR product using specific primer of MYB28 gene indicated that the appearance of one band for each with fragment size 1257 bp as shown in (Fig. 4 and 5). These results agreed with the results obtain **Ling *et al.*, (2017)** who isolated and characterized MYB28 gene from *B.oleraceae.*

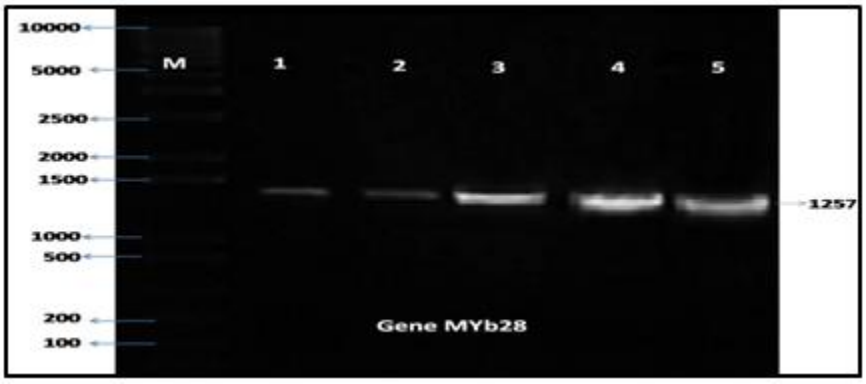


Fig. (6): PCR product of MYB28 gene from the five studied Brassica species

Analysis of the MYB28 gene Nucleotide sequencea lignment:

Sequencing and BLAST analysis showed that the length of TDC fragment shares high homology with the other known MYB28 as shown in (Fig. 7 and 8). Homology search results in Gene Bank

ISSN: 2455-6939

Volume:05, Issue:01 "January-February 2019"

(NCBI) showed that MYB28 nucleotide had high identity to other plants such as *B. oleraceae* (94% identities, accession number (AB702694.1) and *Raphanus sativus* (89% identities, accession number XM018625413.1).The highest identities was 99% for *B.rapa*.

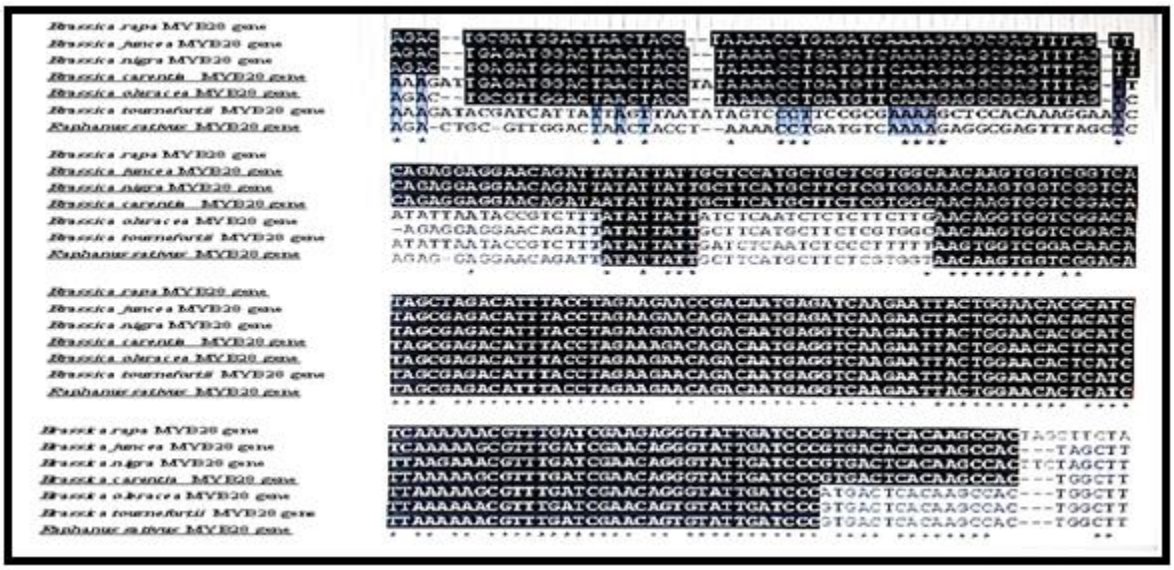


Fig. (7): Sequence alignment of MYB28 gene among Brassica five species.

Description	Max score	Total score	Query cover	E value	Ident	Accession
Brassica tournefortii MYB28 gene	379	847	89%	0.0	90%	
Brassica nigra MYB28 gene	442	1158	94%	0.0	96%	
Brassica rapa MYB28 gene	1399	1886	99%	0.0	99%	
Brassica juncea MYB28 gene	1284	1755	97%	0.0	98%	
Brassica carentia MYB28 gene	1276	1723	94%	0.0	95%	
Brassica oleracea MYB28 gene	399	838	92%	0.0	94%	AB702694.1
Raphanus sativus MYB28 gene	240	245	86%	0.0	89%	XM_018625413.1

Fig. (8): NCBI- BLAST analysis of MYB28 gene homology among Brassica five species.

Phylogenetic Analysis of DNA Sequence of TDC gene:

Phylogenetic analysis was done by aligning DNA sequences using software ClustalX software to construct a phylogenetic tree (Fig. 9). MYB28 gene among Brassica five species. *Brassica tournefortii*, *B. oleraceae* *B.juncea* , *B.rapa* and *Raphanus sativus* were grouped together and *B. nigra and B.crentia* were closer and group in another cluster**.**

ISSN: 2455-6939

Volume:05, Issue:01 "January-February 2019"

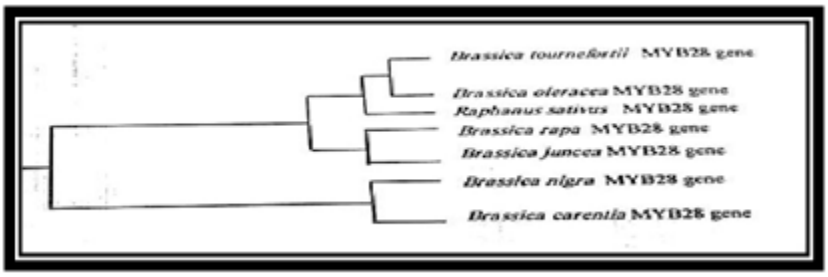


Fig. (9): Phylogenetic tree of the TDC gene sequences in *zygophyllaceae* genotypes:

CONCLUSION

ISSR and SRAP used effectively to estimate genetic diversity among investigated five species of Brassica and may help further in breeding programmers. Glucosinolates gene explorations are required to have better understanding of the presence of genetic variability in Brassica for improvement of other relative important crops.

REFERENCES

Harper A.L., Trick M., Higgins J., Fraser F., Clissold L., Wells R., Hattori C., Werner P., Bancroft I. (2012): Associative transcriptomics of traits in the polyploidy crop species *Brassica napus.* Nature Biotechnology, 30: 798–802.

Xu, A., C. Ma, E. Xiao, J. Quan, C. Ma, G. Tian, J. Tu, T. Fu and G. Zhang, 2008: Genetic diversity of *Brassica juncea* from western China. Acta Agronomica Sinica, 34:754−763.

Godwin I. D., Aitken EA and Smith LW (1997). Application of inter simple sequence repeat (ISSR) markers to plant genetics. Electrophoresis 18: 1524-1528.

Li G and Quiros CF (2001). Sequence-related amplified polymorphism (SRAP), a new marker system based on a simple PCR reaction: its application to mapping and gene tagging in *Brassica*. Theoretical and Applied Genetics, 103: 455-461.

Riaz, A. Li. G.; Quresh, Z. and Swati M.S. (2001). Genetic diversity of oilseed *Brassica napus* inbred lines based on sequence-related amplified polymorphism and its relation to hybrid performance. Plant Breeding, 120: 411-415.

Yu H.F.; Zhao, Z.Q.; Sheng, X.G. and Wang J.S. (2011). Evaluation of genetic diversity in self-incompatible broccoli DH lines assessed by SRAP markers. African Journal of Biotechnology. 10: 12561-12566.

Wu, X. M.; Chen, B. Y.; Lu, G. and Wang, H. Z. (2009). Genetic diversity in oil and vegetable mustard (*Brassica juncea*) landraces revealed by SRAP markers. Genetic Resource Crop Evolution, 56: 1011-1022

Yuan, J. J.; Liu, H. C.; Song, S. W.; Sun, G. W. and Chen, R. Y. (2015). SRAP markers for flower stalk color in Chinese kale. The Journal of Animal and Plant Sciences, 25: 55-58.

Sønderby, I. E. ; Burow M; Rowe, H. C. and Kliebenstein, D. J. (2010). A complex interplay of three R2R3 MYB transcription factors determines the profile of aliphatic glucosinolates in *Arabidopsis.* Plant Physiology. 153: 348-363.

Kim YB, Li X, Kim SJ, Kim HH (2013). MYB transcription factors regulate glucosinolate biosynthesis in different organs of Chinese cabbage (*Brassica rapa* ssp. *pekinensis*). Molecules 18: 8682-8695.

Augustine R, Majee M, Gershenzon J and Bisht NC (2013). Four genes encoding MYB28, a *Brassica napus* accessions using SRAP molecular markers .Plant Genetic Resources Characterization and Utilization, 12(1): 14–21.

Ma, Y.; Qu, S.; Xu, X.; Liang, T. and Zang, D. (2015). Genetic diversity analysis of *Cotoneaster schantungensis* using SRAP marker. American Journal of Plant Sciences, 6: 2860-2866.

Yu, J.; Jing, Z. B. and Cheng, J. M. (2014).Genetic diversity and population structure of *Stipa bungeana*, an endemic species in Loess Plateau of China, revealed using combined ISSR and SRAP markers. Genetics and Molecular Research 13 (1): 1097-1108.

Ling Y., Hancai C., Bihao C., Jianjun L. and Guoju C. (2017). Molecular Characterization of MYB28 Involved in aliphatic gucosinolate biosynthesis in chinese kale (*Brassica oleracea*var. *alboglabra* Bailey). Frontier Plant Science, 3: 263–270.

