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Original Research

Evaluation of the Genetic Relationships of Some Endangered Tunisian Peas Adapted to Arid Regions and Turkish Accessions Revealed by Inter Simple Sequence Repeat (ISSR) Markers

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Abstract

The study of crop genetic diversity has received attention in recent decades to develop new varieties adapted to harsh environmental conditions. Pea (*Pisum sativum* L.) is one of the main sources of legumes due to its higher protein content. In the present investigation, 25 accessions of Turkish and Tunisian peas were characterized by fifteen ISSR markers in order to preserve this germplasm and develop new high-performance varieties. Means of diversity indices, Polymorphism information content (PIC), resolving power, marker index, major allele frequency and Shannon index were 0.70, 5.56, 3.20, 0.89 and 0.35, respectively. The analysis revealed a higher polymorphism (84.15%). The coefficient of similarity Jaccard varied from 0.45 to 1 exhibiting a greater genetic variety. The patterns detected by the cluster analysis, divided the 25 pea genotypes into three main groups. These findings were in agreement with the population structure which divided accessions into three main populations. Therefore, this paper has clearly shown the usefulness of the ISSR markers to provide a great degree of polymorphism for peas. The information collected from this work can help pea breeders to implement a selection program that improves the distribution of this crop in Turkey and in the arid regions of Southern Tunisia.

Keywords: ISSR, genetic diversity, *Pisum sativum,* population structure, arid region, Turkey

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Introduction

Pisum sativum L. plant belonging to *Fabaceae* family, *Papilionoideae* subfamily has been used throughout the world for food, and feed purposes for centuries. It is a diploid genetic plant model with large genome size (5000 Mbp) susceptible to be studied by functional genomics [1]. Pea worldwide production is around 20.7 Mt, and it is regarded as the third most momentous crop after chickpeas and dry beans [2]. Pea grains are an excellent source of starch (50%), fibers, minerals, protein (23-25%), and vitamins [3].

Because of its universal appeal, extensive distribution and adaptability, it is cultivated in Ethiopia, Western and Southern Asia, the Mediterranean region, Southeastern Turkey and Northern Syria [4]. Nowadays, the knowledge of the genetic variety and population structure is useful in the assessment of the management of genetic material and in the breeding experiments as well, especially after the introduction of Frankel and Brown's basic collection concept [5]. In Turkey as well in Tunisia, the pea is cultivated in highly varied topography and climate regions. Pea in these areas has greater adaptability, due to its nutritional value and excellent growth habitat [4, 6]. The breeding of peas for varietal development in the Turkish and southern Tunisian regions did not give much momentum due to a tight genetic base of the cultured genetic pool within the genetic material adapted to the regions.

Under the Farmers' and Plant Breeders Rights Act, it is necessary to preserve the identification of varieties. As a result, a lot of researchers have focused their studies on the genetic diversity assessment in pea based on biochemical and morphological markers [7, 8]. These descriptors cannot distinguish these varieties because they are unstable and largely influenced by the environmental variations [9, 10]. Whereas to more accurately measure the genetic variation, molecular tools are the best alternatives [11].

PCR and non-PCR molecular markers have been extensively utilized due to their easy characterization and identification of genetic resources. In addition, they are polymorphic and not influenced by climatic and morphological variations [12]. According to UPOV guidelines, a set of DNA fingerprinting methods is very practical for distinguishing similar morphological cultivars precisely, distinctly and rapidly, such as Amplified Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP), Simple Sequence Repeat (SSR), Inter Simple Sequence Repeat (ISSR), Single-Nucleotide Polymorphism (SNP), and Sequence-Related Amplified Polymorphism (SRAP) markers. Previous studies about genetic diversity analysis in pea germplasm have been evaluated using a lot of DNA fingerprinting methods, such as SRAP [13], RAPD [14], SSRs [15, 16], and SNP [17]. Among these molecular tools, Inter Simple Sequence Repeat markers (ISSR) have been widely required for crop improvement [18]. ISSR is a dominant marker composed of a group of two to four nucleotide residues which form a single primer that amplifies the target region between two series microsatellite sequences [19]. It has proven to be an excellent tool for identifying and evaluating of genetic diversity, genome mapping and genetic tagging in a wide variety of cultivated species [20].

Also, detection of a high level of polymorphism has been reported even among closely related plant genetics germplasm [21]. The ISSR fingerprint technology for cultivar identification has been used in several cultures, viz. Pea [22], chickpea "*Cicer arietinum*" [23], Jasmine "*Jasminum* sp." [24], and Jute "*Corchorus* sp."[25].

Hence, this investigation was undertaken to assess the genetic relationship of Turkish and Tunisian pea accessions employing ISSR markers.

Experimental

Plant Material

This work was conducted out at the Biotechnology Research Center, Adana, Turkey. A collection of twenty-five Turkish and Tunisian pea accessions was used in this work to study the genetic diversity among them. The names of the accessions and their characteristics were presented in Table 1. The 14 Turkish and 11 Tunisian pea accessions were collected from different eco-geographical regions of Turkey and from the arid region of southern Tunisia, respectively. For the Turkish accessions, samples were provided by the Cumhuriyet University, while the Tunisian pea accessions samples were taken from the Arid Region Gene Bank held in the Arid Land Institute of Medenine (Tunisia). The seeds were washed with tap water for about 10 minutes, soaked in 70% (v/v) of ethanol for 2 minutes, and then immersed in 10% (w/v) sodium hypochlorite solution with 1-2 drops of Tween-20 for 20 minutes. Afterward, they were washed with sterile water. After surface sterilization, the seeds of *Pisum sativum* were cropped and propagated on MS medium [26] in a growth chamber at 25±2ºC for 2 weeks, until the DNA extraction.

DNA Isolation

Two-week pea leaves were sampled from the selected genotypes, deposited in liquid nitrogen and straight away stored at -80ºC until DNA extraction. The DNA was isolated from young seed leaves of five individuals from each accession, using the CTAB method [27]. DNA quality check and quantification were analyzed using a Nanodrop (ND 100) Spectrophotometer, and then were further diluted to a final concentration of 10ng/µL for ISSR-PCR analysis.

ISSR Analysis

To study the evaluation of the relationship between the Turkish and Tunisian pea accessions, a set of

Table 1. List of the accessions studied and collection sites of the pea germplasm.

Code	Accession	Collection site (Town, Province, Country)
P1	P1001	KsarJawamaa Benikdech, Medenine, Tunisia
P2	P3001	Ksar Hallouf Benikdech, Medenine, Tunisia
P3	P5001	Ksar Hallouf Benikdech, Medenine, Tunisia
P4	P6001	Ksar Hallouf Benikdech, Medenine; Tunisia
P5	P6003	Ksar Hallouf Benikdech, Medenine; Tunisia
P6	P7001	Mareth, Gabes, Tunisia
P7	P7002	Mareth, Gabes, Tunisia
P8	P9001	Mareth, Gabes, Tunisia
P9	P9002	Mareth, Gabes, Tunisia
P10	P9003	Mareth, Gabes, Tunisia
P11	P1002	Mareth, Gabes, Tunisia
TK4	TK4	Cumhuriyet Üniversitesi, Turkiye
T106	106	Manisa, Turkiye
T52	52	1995/10, Turkiye
TJOF	JOF	Variety, Turkiye
T57	57	Tekirdağ, Turkiye
TA10	A10	Konya, Turkiye
TK1	TK1	Cumhuriyet Üniversitesi, Turkiye
T49	49	1980/09, Turkiye
TK3	TK3	Cumhuriyet Üniversitesi, Turkiye
T50	50	Muğla, Turkiye
T58	58	Tokat, Turkiye
T100	100	Bursa, Turkiye
T109	109	(not applicable (e.g. breeding material) or unknown), Turkiye
TK7	TK7	Cumhuriyet Üniversitesi, Turkiye

25 ISSR markers acquired from British Columbia University (UBC) in Canada, were used for molecular analysis. Their characteristics are reported in Table 2. The PCR amplification mixture was carried out in a volume of 25µL containing 3µL of genomic DNA (10 ng/µL), 12.5 µL 2x PCR Master Mix (Fermentas K0171, Waltham, MA, USA), 0.1µL of 1 U Taq DNA polymerase (Fermentas EP0402), 0.5 µL of ISSR primer (25 mM), 0.25µL of MgCl2 (1mM) and 8.65µL of sterilized distilled water. The amplification was performed on the Eppendorf Master Cycler Gradient (Hauppauge, NY, USA) using a program consisting of an initial denaturation step of 3 min at 95ºC, then 35 cycles of 45s at 95ºC, an annealing step for 1 min at 55ºC, and 45s at 72ºC, followed by 7 min elongation step at 72ºC, and then maintenance at 4ºC. The products amplified by PCR were separated on a 1.5% agarose gel prepared in a TAE buffer for 3 h at 90 V, with a DNA molecular weight marker Gene Ruler 1kb Plus DNA Ladder (Fermentas). The results were viewed under UV light after staining with ethidium bromide and documented for further analysis. Only markers producing consistent and reproducible bands were chosen for data generation.

Data Analysis

The ISSR profiles were scored manually to form a binary matrix by‘1’ for presence and ‘0’ for absence. Only the clearest, strongest and reproducible bands were scored. For each ISSR marker, POPGENE 1.32 software was used to calculate the observed (na) and the effective number of alleles (ne), the Nei’s genetic diversity (h) and Shannon’s information index (I). PIC values for each locus were measured by using the following equation PIC = 1 –Σ pi^2, (where pi^2 is the

Table 2. Inter -simple sequence repeat markers selected to evaluate the genetic variability among Pea genotypes.

No	Primer name	Primer Sequence (5_3)	Tm (°C)
1	UBC807	AGAGAGAGAGAGAGAGT	55
2	UBC808	AGAGAGAGAGAGAGAGC	55
3	UBC810	GAGAGAGAGAGAGAGAT	55
4	UBC811	GAGAGAGAGAGAGAGAC	55
5	UBC812	GAGAGAGAGAGAGAGAA	55
6	UBC813	CTCTCTCTCTCTCTCTT	55
7	UBC814	CTCTCTCTCTCTCTCTA	55
8	UBC815	CTCTCTCTCTCTCTCTG	55
9	UBC816	CACACACACACACACAT	55
10	UBC817	CACACACACACACACAA	55
11	UBC819	GTGTGTGTGTGTGTGTA	55
12	UBC820	GTGTGTGTGTGTGTGTC	55
13	UBC823	TCTCTCTCTCTCTCTCC	55
14	UBC824	TCTCTCTCTCTCTCTCG	55
15	UBC825	ACACACACACACACACT	55
16	UBC827	ACACACACACACACACG	55
17	UBC828	TGTGTGTGTGTGTGTGA	55
18	UBC834	AGAGAGAGAGAGAGAGYT*	55
19	UBC835	AGAGAGAGAGAGAGAGYC*	55
20	UBC843	CTCTCTCTCTCTCTCTRA**	55
21	UBC844	CTCTCTCTCTCTCTCTRC**	55
22	UBC845	CTCTCTCTCTCTCTCTRG**	55
23	UBC846	CACACACACACACACART**	55
24	UBC847	CACACACACACACACARC**	55
25	UBC850	GTGTGTGTGTGTGTGTYC*	55

* Y and ** R are single letter abbreviations for mixed base positions. Y is presenting C or T, R is presenting A or G.

frequency of the i^{th} allele). Moreover, we calculated also the ISSR primers index (ISPI), the resolving (Rp) and the mean resolving power (mean Rp), the Multiplex Ratio (MR), the effective multiplex ratio (EMR) and the marker index (MI) as proposed respectively by the following authors [28-30]. Genetic similarities were calculated by Jaccard's Similarity Coefficient. The principal coordinates (PCoA) and cluster analysis was constructed by using the PASTv3.12 software [31]. For the cluster analysis, we used the Unweighted Pair Group Method with Arithmetic Average (UPGMA) [32].

For the structure population, STRUCTURE 3.2.1 software used a model-based Bayesian approach that applies Markov Chain Monte Carlo (MCMC) estimation to determine the number of the sub-populations in a core collection [33]. The models supposed of K values were set from one to ten, with 20 independent runs each, with a burn-in and MCMC of 50,000 runs [34].

Results

Genetic Polymorphism of ISSR Markers

ISSR markers were a molecular tool that has been widely used to assess the genetic variability of pea genetic material. In our paper, to screen the polymorphism of ISSRs, a total of 25 ISSR primers were employed for revealing the inter-population genetic diversity between the collected samples. Of these, 15 successful primers were found to amplify products reproducibly and showed clearly polymorphic patterns based on 25 accessions. These results were

summarized in Table 3. A set of 82 alleles were detected, including 69 polymorphs. The size of the amplified fragment ranged from 120 to 600 bp. Therefore, the greatest variation in the number of alleles per locus was observed in the primer UBC817 (190-600 pb) with a value of 11 alleles and the lowest was for primers UBC815 (290-490 pb), UBC812 (150-400 pb), and UBC818 (220-420 pb) with value of 3 alleles per locus. Based on these results, the percentage of polymorphism among these accessions had a value of 84.15%. Among the ISSR markers, 6 markers (UBC810, UBC811, UBC815, UBC817, UBC823, and UBC828) revealed 100% polymorphism. Similarly, the allele frequency ranged from 0.60 to 0.96 with a mean of 0.89 per primer. In addition, the polymorphic information content (PIC) of loci varied from 0.38 (UBC815) to 0.87 (UBC817), with an average of 0.7, on which four inter-simple sequence repeats markers (UBC807, UBC808, UBC817, and UBC828) had PIC values greater than 0.8. Furthermore, the greatest value of Shannon information index was recorded in the UBC810 primer with a value of 0.55. This suggests that the germplasm used in the present study was highly variable. Out of 15 ISSR markers, the marker UBC817 exhibited the highest primer index (ISPI = 9.58) and for the greatest mean Rp was presented by UBC812 (mean Rp = 1.87). The mean value for the observed and effective number of alleles (na and ne), and Nei's gene diversity (h) was 1.79, 1.39, and 0.23, respectively which indicate that *Pisum sativum* had an important genetic diversity. Moreover, the marker index, the multiplex ratio, and the effective multiplex ratio per ISSR markers had the following values 3.05, 5.47, and 4.4 respectively. All of these indexes indicated the high discrimination ability of the inter-simple sequence repeats to detect the polymorphism in the collected accessions.

Principal Coordinate Analysis

The two-dimensional diagram of the principal coordinate analysis (PCoA) generated a total variation of 48.74%; the contribution of the two first principal coordinates was 29.11 and 19.63% of the total molecular variance, respectively. As shown in the PCoA plot in Fig. 1, the T50 accession was the most distant genotype from the others. The Tunisian accessions except P4

Table 3. The ISSR markers used and their allele polymorphism in the diversity study of *Pisum sativum.*

SSR	Band size (pb)	TB	PB	FP	PIC	MAF	na	ne	h	I	ISPI	Rp	Mean Rp
UBC807	120-600	7	6	85.71	0.81	0.96	1.86	1.44	0.28	0.43	4.87	7.20	1.03
UBC808	120-550	6	4	66.67	0.81	0.96	1.66	1.46	0.26	0.39	3.25	8.64	1.44
UBC810	100-600	6	6	100.00	0.79	0.88	2.00	1.67	0.37	0.55	4.73	6.88	1.15
UBC811	120-600	7	7	100.00	0.72	0.60	2.00	1.23	0.14	0.20	5.07	4.56	0.65
UBC812	150-400	3	1	33.33	0.67	0.96	1.33	1.40	0.23	0.36	0.67	5.60	1.87
UBC815	290-490	3	3	100.00	0.38	0.68	2.00	1.48	0.29	0.43	1.13	1.76	0.59
UBC816	200-420	4	3	75.00	0.70	0.96	1.75	1.58	0.32	0.48	2.11	4.56	1.14
UBC817	190-600	11	11	100.00	0.87	0.76	2.00	1.58	0.31	0.44	9.58	8.96	0.81
UBC818	220-420	3	2	66.67	0.65	0.96	1.66	1.50	0.31	0.48	1.29	4.24	1.41
UBC823	200-500	4	4	100.00	0.68	0.92	2.00	1.04	0.04	0.08	2.74	5.12	1.28
UBC825	220-460	4	2	50.00	0.55	0.96	1.50	1.37	0.24	0.39	1.11	4.08	1.02
UBC828	200-900	9	9	100.00	0.81	0.92	2.00	1.24	0.16	0.26	7.28	6.40	0.71
UBC834	120-500	6	5	83.33	0.68	0.96	1.83	1.53	0.28	0.39	3.38	4.08	0.68
UBC835	140-460	5	3	60.00	0.80	0.96	1.50	1.14	0.11	0.21	2.39	8.48	1.70
UBC850	200-500	4	3	75.00	0.51	0.88	1.75	1.14	0.11	0.21	1.54	2.88	0.72
Range/ Mean		82	69	84.15	0.70	0.89	1.79	1.39	0.23	0.35	3.41	5.56	1.08
Mean PIC	MR = TB/TP				EMR = MR×FP						MI = EMR×Mean PIC		
0.70	5.47				4.60						3.20		

TB: total number of bands scored, PB: number of polymorphic bands, FP: Fraction of polymorphism, PIC: polymorphic information content, MAF: major allele frequency, na: observed number of alleles, ne: effective number of alleles, h: Nei's gene diversity, I: Shannon's information index, TP: total number of primers, ISIP: ISSR primer index, Rp: Resolving power, MR: multiplex ratio, EMR: effective multiplex ratio, MI: marker index

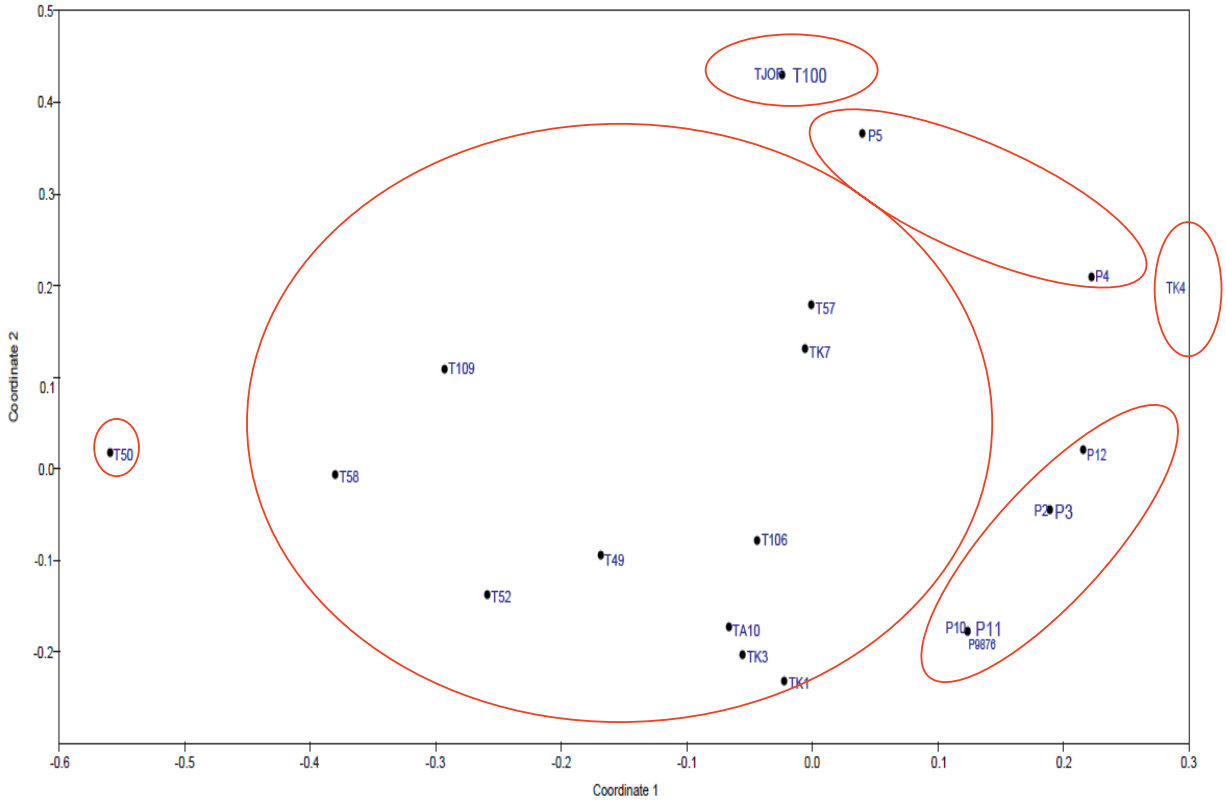

Fig 1. Two-dimensional principal coordinate analysis (PCoA) illustrating the relationship among 25 accessions of Pea.

and P5 were placed in the plot area close to each other, which indicated a low significant diversity between these accessions. For the other Turkish accessions, with the exception of TK4, T100 and TJOF were grouped together in this analysis. This result showed that there is an association between geographical distribution and accession of pea.

Cluster Analysis

In this study, the similarity degree between the 25 Tunisian and Turkish accessions using the Nei-Li similarity coefficient ranged from 0.45 to 1 with a mean of 0.72, which proves a great diversity present in the collection. At a similarity value of 0.45, the dendrogram obtained (Fig. 2) showed that the accessions were divided into two main clusters containing of 1 and 24 genotypes. The first cluster contain only one accession T50 failing to form a cluster. The second cluster carried the largest number of accessions. However, at around 0.52 similarity level, it was further divided into two subclusters. The first one grouped the two Tunisian accessions P5 and P4 together while the second comprised a maximum of 22 accessions which was also subdivided into three other subclusters IA, IB, and IC. IA consisted of 3 accessions (T100, TJOF, and TK4), whereas subcluster IB consisted of 6 Turkish accessions (TK7, T109, T52, T58, T87, and T106) and finally, IC consisted with the remaining accessions which also subdivided into subcluster containing the Tunisian and Turkish accessions separately. Strict correlations were instead observed among Tunisian accessions. The most closely related accessions were P2 and P3.

Population Structure

The analysis of the population structure was carried out to classify the 25 accessions into subpopulations using 15 markers. The result recorded a clear sharp peak at the optimal value of K = 3, which then divided the accessions into three subpopulations (Fig. 3). The subpopulation 1 comprised 2 accessions, while the second and the third contained 13 and 9 accessions, respectively in which some accessions were found as admixtures. All the Turkish accessions were attributed to a distinct population (subpopulation 2), while the Tunisian accessions were divided into two subpopulations (1 and 3). Each accession was marked with a vertical bar, separated in different colors. These different colors represented a different gene pool, which showed the potentially increasing proportion of individuals for each gene pool like as the result showed the admixture between the first and the third subpopulation that contained only the Tunisian accessions. These findings confirmed that the geographical origin represent a major factor of distinction between the studied accessions. Structure analysis was in agreement with the results obtained from PCoA and UPGMA dendrogram.

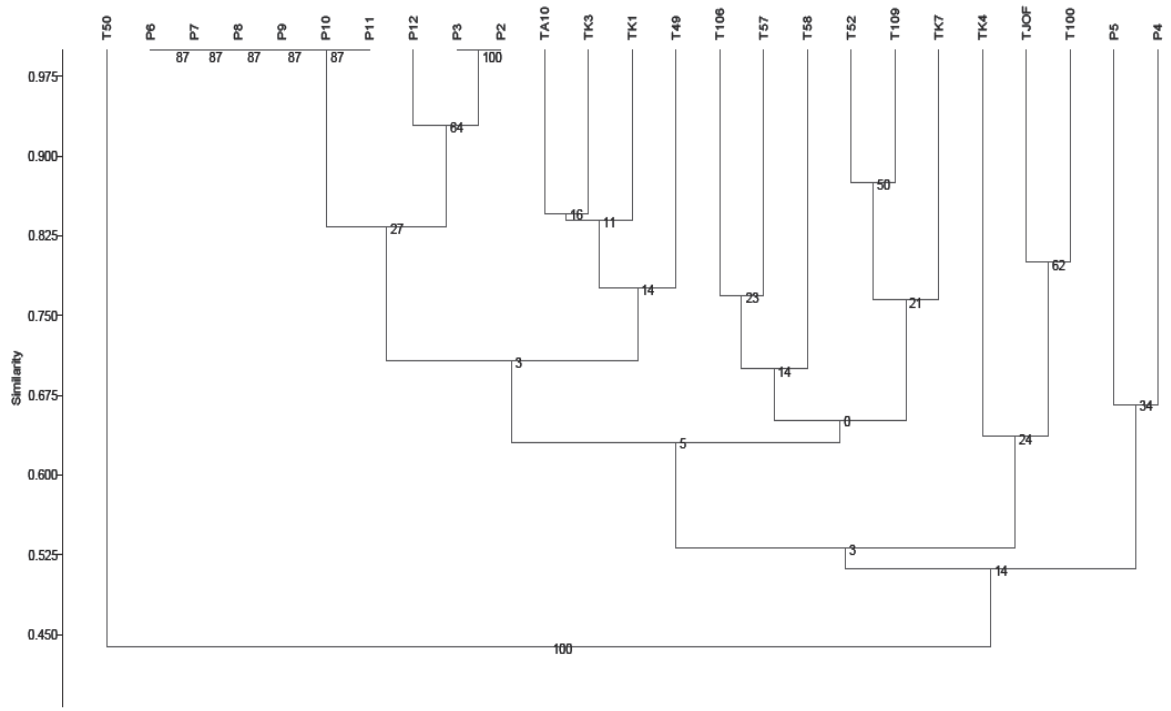

Fig 2. Dendrogram (UPGMA) of the pea genotypes based on genetic distances calculated with the genetic similarity coefficient matrices. The numbers near nodes represent the percentage of time when the node occurred among 10,000 bootstraps.

Discussion

In recent years, previous studies linked to the assessment of the genetic diversity and population structure of Mediterranean pea varieties have been reported by Loridon et al. [35] and Negisho et al. [36]. Various molecular markers have been used to evaluate DNA polymorphism in peas; it depends on the relationship between the collections and the type of cultivar. Among these, the markers most often used by the researchers are the SSRs [15, 37], but also SRAP [13] and ISSR [22] were employed because of their presumed reliability greater than RAPD and their ease of use greater than AFLP. In this investigation, we focus on the use of ISSR markers, due to their ability applied to the identification of genetic material of the

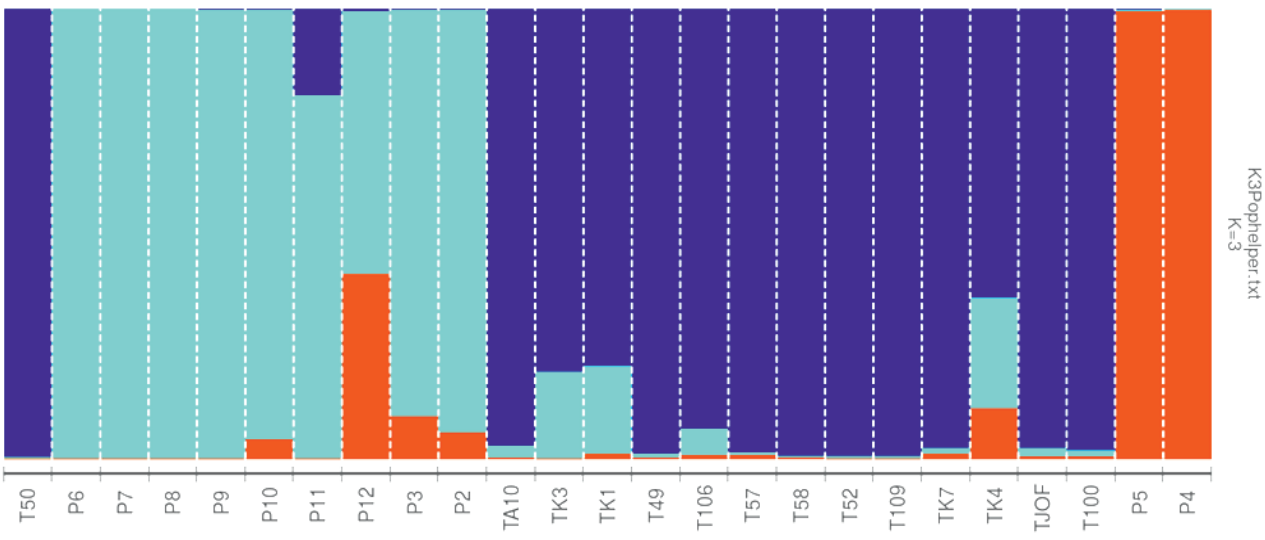

Fig 3. Structure plot of 25 pea accessions based on ISSR markers for K = 3. The length of the colored portion in each sample based on a membership threshold score corresponds to one of the subgroups.

Pisum genus.

The twenty-four Turkish and Tunisian pea accessions were amplified 25 ISSR markers. It has been found that the average number of alleles per primer has the value of 5.85. This result was greater compared to SSR (3.42) and RAPD markers (3.45) [16, 38] and lower than iPBS-retrotransposon (8.83) and SRAP markers (9.96) [4, 39]. The percentage of polymorphism varied from 33% for primer UBC812 to 100% for primers UBC810, UBC811 UBC815, UBC817, and UBC828. This result was higher compared to RAPD 59%, and SSR 81.92% [40, 41]. As mentioned previously, the high results of the PIC and the Mean RP given by these markers were higher than those reported by Kapila et al. [42] in the Indian pea genotypes using ISSR markers and showed a mean resolution power of 4, 50. While, they were lower to those obtained in other studies with ISSRs, such as those of Pandian et al. [43] in sorghum "*Sorghumsp.*", Basahi et al. [44] and Subhojit et al. [45] in faba bean "*Vicia faba*" and lentil "*Lens culinaris"*, and Dagnew et al. [46] in *Phaseolus vulgaris*. These results showed that the greatest degree of polymorphism among the 24 accessions proved the capacity of the ISSR marker to study the evaluation of genetic variability between close genotypes of identical or different geographic origin.

In order to acquire a complete analysis of the genetic relationships between pea accessions, an UPGMA cluster analysis based on the Jaccard similarity coefficient was performed. This coefficient (0.45) was lower than that reported by Nisar et al. [47] and Choudhury et al. [48] who founded an average of 0.52 and 0.71, respectively. From the outcome of our investigation it was possible to conclude that the accessions were isolated according to their geographical origin. Sharma et al. [49] divided Pea germplasm into two groups according to their origin. However, another study found by Baloch et al. [4] indicated that there was a lack of correlation between the genotypes of Turkish peas and their source of collection. The grouping of genotypes was not only influenced just by geographical origin; there were various factors that can affect such as the morphological and physiological characteristics, the selection method and also the type of markers [50, 51]. Summing up the results, it can be concluded the efficiency and reliability of the ISSR marker in designing a breeding program for crop improvement. PCoA was largely congruent with the assignments generated by cluster analysis. In fact, there was a clear differentiation between the Turkish and Tunisian pea accessions. Similar results were obtained on faba beans and peas from different countries by Asfaw et al. [52] and Mohamed et al. [15]. Population structure analysis has divided our genetic material into three subpopulations, which was in agreement with the phylogenetic tree. These results were in good agreement with other study where the population structure showed a convergence with the UPGMA cluster analysis [53]. Sharma et al. [49] investigated pea accessions from India and recorded clustering into two subgroups according to the geographical origin. In contrast to our results, in the literature, others research revealed that there was no link between the geographic origin and the genotypes of different crop such as garlic (*Allium sativum* L.), and barley (*Hordeum vulgare* L.) [54, 55].

In fact, it would be difficult to obtain a large genetic diversity in a few places where these crops were widely used for multiple functions. According to these results, this could be interesting when designing future studies on diversity, conservation and strategies for building basic collections on accessions of Mediterranean peas. Moreover, it has been reported by Govindaraj et al. [56] to conserve plant genetic material; we should maintain the high level of genetic variability between plant species. This result indicates that the conservation of seeds is desirable in these regions. For this reason, we suggest keeping these accessions with excellent specific characters for the selection program. In addition, crossing between Tunisian and Turkish accessions is also necessary to improve genetic diversity within the accessions and for subsequent reproduction.

Conclusions

Climate change has affected the development and growth of pea over the past 20 years. Therefore, we need to identify the most likely impacts of global warming in order to implement practical and effective countermeasures to ensure agrifood sustainability for future generations. By selecting the best crops, cultivars and cropping systems, farmers are able to adapt to changes in the environment. The best methods for the future must therefore be identified through studies to assess the genetic diversity of the local gene pool.

From the research that has been carried out, it is possible to conclude that exist a great genetic diversity between Turkish and Tunisian pea accessions by using ISSR markers. The results of our research are quite convincing, due to the high level of PIC, Rp and MI that prove the ability of these markers to assess the relationships among those accessions and marker-assisted breeding programs. In our future research, we intend to focus on the exploitation of these markers to help pea breeders to improve Mediterranean peas with improved nutritional and physiological traits adapting them to different climates all over the world and especially for the stressful conditions in the arid regions.

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Conflict of Interests

The authors declare no conflicts of interest.

References

1. SATO S., ISOBE S., TABATA S. Structural analyses of the genomes in legumes. Curr. Opin. Plant Biol., **13**, 146, **2010**.
2. Food and Agriculture Organization (FAO) of the United Nations, Rome, **2017**.
3. MAHESH C., TARTE G., ASHOK K., NILESH C. Studies on the effect of pH and temperature on the activity of esterase enzyme isolated from *Pisum sativum*. Asian J Biomed Pharm Sci., **3**, 2231, **2014**.
4. BALOCH F.S., ALSALEH A., SAENZ DE MIERA L.S., HATIPOGLU R., ÇIFTÇI V., KARAKVOY T., YILDIZ M., OZKAN H. DNA based iPBS-retrotransposon markers for investigating the population structure of pea (*Pisum sativum*) germplasm from Turkey. Biochem Syst Ecol., **61**, 244, **2015**.
5. FRANKEL O.H., BROWN A.H.D. Plant genetic resources today: a critical appraisal. In: Holden JHW, Williams JT (eds) Crop genetic resources: conservation and evaluation. George Allen and Unwin, London, UK, 249, **1984**.
6. LOUMEREM M., MOUSSA M., BELLACHHEB C. La collecte et l'étude de la diversité génétique des espèces cultivées aux aménagements hydrauliques dans les régions arides tunisiennes. In: Revue des Régions Arides, Actes de Séminaire International, Aridoculture et Cultures Oasiennes., **2004**.
7. BEN MANSOUR-GUEDDES S., SAIDANA-NAIJA D., FLAMINI G., CHERAIEF I., BRAHAM M. Assessment of the Climatic Condition's Impact on Volatiles, Polyphenols and Mineral Contents in Tunisian Olive Tree (*Olea europaea* L.). Pol. J. Environ. Stud., **31** (1), 219, **2022**.
8. YEHMED J., TLAHIG S., AYEB N., MOHAMED A., YAHIA H., DBARA M, MOHAMED LOUMEREM M. Agro-Morphological, Yield Components and Nutritional Quality Attributes of *Vicia faba* L. var. Minor Cropped in Tunisian Arid Regions. Pol. J. Environ. Stud., **31**(1), 929, **2021**.
9. ZAMEER M., SHAFIQUE S., SHAFIQUE S., TAHIR U., ZAHRA N., RASHID B., AFZAAL R. Physiological and Biochemical Response of Transgenic Cotton Plants to Drought Stress., Pol. J. Environ. Stud., **31** (4), 3909, **2022**.
10. OZKAN U., BENLIOGLU B., KAHRAMANOGULLARI C.T. A Comparison of Germination Responses on Italian Ryegrass (diploid vs tetraploid) Seeds to Interactive Effects of Salinity and Temperature. Pol. J. Environ. Stud., **31** (5), 4229, **2022**.
11. PIETRAS M., RUDAWSKA M., ISZKUŁO G., KUJAWA A., LESKI T. Distribution and Molecular Characterization of an Alien Fungus, Clathrus archeri, in Poland. Pol. J. Environ. Stud., **25** (3), 1197, **2016**.
12. NYARUMBU T.O., KASEKE T., GOBVU V., MURUNGWENI C., MASHINGAIDZE A.B., CHIKWAMBI Z. Phenotypic and genetic characterization revealed the existence of several biotypes within the Neorautanenia brachypus (Harms) C.A. wild accessions in South East Lowveld, Zimbabwe. BMC Ecol., **19** (1), 13, **2019**.
13. KOLE P.R., SHARMA M.K., KUMAR S., KUMAR A., SINGH S., SENGAR R.S. Assessment of Genetic Diversity in Indian Cultivated Pea (*Pisum sativum* L) by Using SRAP Markers. Int. J. Plant Res., **28** (3), 84, **2015**.
14. THAKUR B., SHARMA S.K., SHARMA I., SHARMA P., DEEPIKA., ZARGAR S.M. Diversity analysis of pea genotypes using RAPD markers. *Legume Res.,* **41**, 196, **2018**.
15. MOHAMED A., GARCÍA-MARTÍNEZ S., LOUMEREM M., CARBONELL P., RUIZ J.J., BOUBAKER M. Assessment of genetic diversity among local pea (*Pisum sativum* L.) accessions cultivated in the arid regions of Southern Tunisia using agro-morphological and SSR molecular markers. Genet Resour Crop Evol., **66**, 1189,**2019**.
16. HANCI F. Genetic variability in peas (*Pisum sativum* L.) from Turkey assessed with molecular and morphological markers. Folia Hort., **31**(1), 101, **2019**.
17. DUARTE J., RIVIÈRE N., BARANGER A., AUBERT G., BURSTIN J., CORNET L., LAVAUD C., LEJEUNE-HE´NAUT I., MARTINANT J.P., PICHON J.P., PILETNAYEL M.L., BOUTET G. Transcriptome sequencing for high throughput SNP development and genetic mapping in Pea. BMC Genom., **15**, 126, **2014**.
18. DHYANI P., BAHUKHANDI A., JUGRAN A.K., INDRA D., BHATT., RANBEER S., RAWAL., PANDE V. Inter Simple Sequence Repeat (ISSR) markers based genetic characterization of selected Delicious group of apple cultivars. Int. J. Adv. Res., **3** (2), 591, **2015**.
19. JATIN K., VEENA A. Analysis of genetic diversity and population genetic structure in *Simarouba glauca* DC. (an important bio-energy crop) employing ISSR and SRAP markers. Ind Crops Prod., **100**, 198, **2017**.
20. YHAN W., LI J., ZHENG D., FRIEDMAN C., WANG H. Analysis of genetic population structure and diversity in *Mallotus oblongifolius* using ISSR and SRAP markers. Peer J., **21** (7), 7173, **2019**.
21. ALANSI S., TARROUM M., AL-QURAINY F., KHAN S., NADEEM M. Use of ISSR markers to assess the genetic diversity in wild medicinal *Ziziphus spina-christi* (L.) Willd. collected from different regions of Saudi Arabia. Biotechnol. Biotechnol. Equip., **30** (5), 942, **2016**.
22. SAMIRA A., OSMAN., HODA B., ALI M. Genetic relationship of some *Pisum sativum* subspecies using different molecular markers. Jordan J. Biol. Sci., **14** (1), 65, **2021**.
23. SAMEER S., BHAGYAWANT., GUPTA N., GAUTAM A., CHATURVEDI S.K., SHRIVASTAVA N. Molecular Diversity Assessment in Chickpea through RAPD and ISSR Markers. World. J. Agric Res., **3** (6), 192, **2015**.
24. GHEHSAREH G., SALEHI M.H., KHOSH-KHUI M., NIAZI A. Application of ISSR markers to analyze molecular relationships in Iranian Jasmine (*Jasminum spp.*) accessions. Mol Biotechnol., **57**, 65, **2015**.
25. NAG S., MITRA J., SATYA P., KAR C., KARMAKAR P.G. Genetic diversity assessment in jute (*Corchorus*

species) utilizing inter simple sequence repeat and simple sequence repeat markers. Indian J Biotechnol., **17**, 316, **2018**.
26. MURASHIGE T., SKOOG F. A Revised Medium for Rapid Growth and Bioassays with Tobacco Tissue Cultures. Physiol Plantarum., **15**, 473, **1962**.
27. DOYLE J.J., DOYLE J.L. Isolation of plant DNA from fresh tissue. Focus., **12**, 13, **1990**.
28. ANDERSON J.A., CHURCHILL G.A., AUTRIQUE J.E., TANKSLEY S.D., SORRELLS M.E. Optimizing parental selection for genetic linkage maps. Genome., **36**, 181, **1993**.
29. POWELL W., MORGANTE M., ANDRE C., HANAFEY M., VOGEL J., TINGEY S., RAFALSKI A. The comparison of RFLP, RAPD, AFLP and SSR (microsatellite) markers for germplasm analysis. Mol Breed., **2**, 225, **1996**.
30. RAJWADE A.V., ARORA R.S., KADOO N.Y., HARSULKAR A.M., GHORPADE P.B., GUPTA V.S. Relatedness of Indian flax genotypes (*Linum usitatissimum* L.): an inter-simple sequence repeat (ISSR) primer assay. Mol Biotechnol., **45**, 161, **2010**.
31. HAMMER., HARPER D.A.T., RYAN P.D. PAST: paleontological statistics software package for education and data analysis. Palaeontologia Electronica., **4** (1), 9, **2001**.
32. NEI M., LI W.H. Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc Natl Acad Sci USA., **76**, 5269, **1979**.
33. PRITCHARD J.K., STEPHENS M., DONNELLY P. Inference of population structure using multi locus genotype data. Genetics., **155**, 945, **2000**.
34. EVANNO G., REGNAUT S., GOUDET J. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Mol Ecol., **14**, 2611, **2005**.
35. LORIDON K., MCPHEE K., MORIN J., DUBREUIL P., PILET-NAYEL M.L., AUBERT G., RAMEAU C., BARANGER A., COYNE C., LEJEUNE-HENAUT I., BURSTIN J. Microsatellite marker polymorphism and mapping in pea (*Pisum sativum* L), Theor. Appl. Genet., **111**, 1022, **2005**.
36. NEGISHO K., TESHOME A., KENENI G. Genetic Diversity in Ethiopian Field Pea (*Pisum sativum* L.) Germplasm Collections as Revealed by SSR Markers. Ethiop. J. Agric. Sci., **27**(3), 33, **2017**.
37. SINGH S., SINGH B., SHARMA V.R, KUMAR M., SIROHI U. Assessment of Genetic Diversity and Population Structure in Pea (*Pisum sativum* L.) Germplasm based on Morphological Traits and SSR Markers. Legume Res., **45**(6), 683, **2021**.
38. IQBAL A., BHATTACHARYYA U., AKHTAR R., DASGUPTA T. Genetic diversity computation in sesame genotypes using morphological traits and genic SSR markers. Indian J. Genet., **78**(3), 348, **2018**.
39. GUINDON M.F., MARTIN E., ZAYAS A., COINTRY E., CRAVERO V. Evaluation of SRAP markers for mapping of *Pisum sativum* L. Crop. Breed. Appl. Biotechnol., **16**, 3, **2016**.
40. TAHIR N.A., HAMAKAREEM H.F., HAMA-AMIN B.O. Differentiate of ten pea (*Pisum sativum* L.) cultivars by RAPD markers and seeds storage proteins. JJAS., **11**, 95, **2016**.
41. TAHIR N.A.R., LATEEF D.D., OMER D.A., KAREEM S.H.S., AHMAD D.A., KHAL L.H. Genetic Diversity and Structure Analysis of Pea Grown in Iraq Using Microsatellite Markers. Jordan J. Biol. Sci., **11**, 201, **2017**.
42. KAPILA K., NARYAL S., DHIMAN K.C. Analysis of genetic diversity among garden- and field-pea genotypes of higher Indian. J. Plant Biochem. Biotechnol., **21** (2), 286, **2012**.
43. PANDIAN S., SATISH L., SHILPHA J., RAMESH M. Genetic Diversity Analysis Reveals Strong Population Structure in Sorghum Germplasm Collection. Proc. Natl. Acad. Sci., India, Sect. B Biol. Sci., **2019**.
44. Basahi M. ISSR-based analysis of genetic diversity among sorghum landraces growing in some parts of Saudi Arabia and Yemen. C. R. Biol., **338** (11), 723, **2015**.
45. SUBHOJIT D., PRASOONPAL G., MAYANK K., KUMAR S. Genetic Diversity Analysis of Lentil (*Lens culinaris Medik*) Cultivars Using Inter Simple Sequence Repeats Markers. Mol. Plant Breed., **7** (23), 1, **2016**.
46. DAGNEW K., HAILESELASSIE T., FEYISSA T. Genetic diversity study of common bean (*Phaseolus vulgaris* L.) germplasm from Ethiopia using inter simple sequence repeat (ISSR) markers. Afr. J. Biotechnol., **13** (31), 3638, **2014**.
47. NISAR M., KHAN A., WADOOD S.F., SHAH A.A., HANCI F. Molecular characterization of edible pea through EST-SSR markers. Turk. J. Bot., **41**, 338, **2017**.
48. CHOUDHURY P.R., TANVEER H., DIXIT G.P. Identification and detection of genetic relatedness among important varieties of pea (*Pisum sativum* L.) grown in India. Genetica., **130**, 183, **2007**.
49. SHARMA H., SHARMA P., SHARMA R. Evaluation of Genetic Relatedness Among Temperate Pome Fruit Crops of Family Rosaceae Using Arbitrary Oligonucleotide Markers. Proc. Natl. Acad. Sci., India, Sect. B Biol. Sci., **88**,191, **2018**.
50. ABEL T., TOMAS B, DAGNE K., GELETA M. Assessment of genetic diversity in Ethiopian field pea (*Pisum sativum* L.) accessions with newly developed EST-SSR markers. BMC Genetics., **16**, 102, **2015**.
51. GANGADHAR B.H.M., RAGHVENDRA K.M. Genetic diversity study of Ethiopian Faba bean (*Vicia faba* L.) varieties based on phenotypic traits and inter simple sequence repeat (ISSR) markers. Eur Acad Res., **2**, 1346, **2016**.
52. ASFAW B.M., DAGNE K., WAKAYO G.K., KEMAL S.A., MULETA K.T. Genetic diversity study of Ethiopian faba bean (*Vicia faba* L) varieties based on phenotypic traits and inter simple sequence repeat (ISSR) markers. Afric. J Biotechnol., **17** (13), 433, **2018**.
53. AHMAD S., SINGH M., LAMB-PALMER N.D., LEFSRUD M., SINGH J. Assessment of genetic diversity in 35 *Pisum sativum* accessions using microsatellite markers. Can J Plant Sci., **92**, 1075, **2012**.
54. HOOGERHEIDE E.S.S., AZEVEDO FILHO J.A., VENCOVSKY R., ZUCCHI M.I., ZAGO B.W., PINHEIRO B.J. Genetic variability of garlic accessions as revealed by agro-morphological traits evaluated under different environments. Genet. Mol. Res., **16** (2), 1, **2017**.
55. TEKLEMARIAM S.S., BAYISSA K.N., MATROS A., PILLEN K., ORDON F., WEHNER G. The genetic diversity of Ethiopian barley genotypes in relation to their geographical origin. PLoS ONE., **17** (5), **2022**.
56. GOVINDARAJ M., VETRIVENTHAN M., SRINIVASAN M. Importance of Genetic Diversity Assessment in Crop Plants and Its Recent Advances: An Overview of Its Analytical Perspectives. Genet. Res., **14**, **2015**.