

## Article

# Exploring Genetic Variability among and within Hail Tomato Landraces Based on Sequence-Related Amplified Polymorphism Markers

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**Abstract:** Landraces are valuable sources of genetic characteristics, which are of plant breeders' interest to include in breeding programs for crop improvement. We assess the inter- and intra-genetic variability among 96 accessions representing three Hail tomato landrace using DNA-based marker sequence-related amplified polymorphism (SRAP). Seven SRAP primer combinations generated 55 alleles with a polymorphism of 100%, and an average of 7.86 polymorphic alleles per pair of primers. All primers showed an average of 0.68 polymorphic information content (PIC) value and discrimination power (DP) of 14.29. Principal coordinate analysis (PCoA) confirmed the clustering produced via the UPGMA similarity dendrogram allowed for the grouping of the 96 accessions according to its gene bank accession numbers and showed relatively good separation between landraces. A similarity value ranged from 0.04 to 1.0 among accessions of Hail 747, 0.05 to 1.0 in Hail 1072, and from 0.16 to 0.92 in Hail 548. These results showed the landraces harbor a wide range of genetic diversity at both inter and intra-variation levels. AMOVA showed that most of the genetic variation was because of differences within populations (87%). Tomato Hail landraces have well-differentiated genetic populations and admixtures, where Hail 747 formed their separate group, and both Hail 548 and Hail 1072 were admixed, and some accessions showed more diversity pattern. We have to take the SRAP technique's effectiveness in the study of genetic variability among and within landraces into consideration in the tomato-breeding programs through marker-assessed selection.

**Keywords:** landraces; *Solanum lycopersicum*; SRAP; AMOVA



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## 1. Introduction

Tomato hybrids are the primary source of tomato cultivation. These resources have narrow genetic bases because of several population bottlenecks in the natural, artificial selection and forms of founder events during the evolution and domestication of modern cultivars [1]. Zsögön et al. [2] stated that although the yield of tomato genotypes is increased by breeding and domestication, it was accompanied by a loss of genetic diversity and reduced nutritional value taste. Hence, researchers have suggested that wild tomato varieties should enrich the cultivated tomato varieties' genetic base. Farmers still grow tomato landraces on small farms because of the quality and particular demand of some consumers. These landraces are valuable sources of genetic characteristics, which are of plant breeders' interest to include in breeding programs for crop improvement. Conti et al. [3] reported that Italian local tomato varieties exposed to water stress showed a valuable reservoir of genetic biodiversity with more water stress-resistance, which opens research toward understanding the mechanisms of response to drought in these local

varieties. The fruits of Mediterranean long shelf-life tomatoes landraces remain sound up to 6–12 months after harvest, and most of them have been selected under drought and showed stress-tolerant [4].

Moreover, Scarano et al. [5] identified a new landrace that can produce good yield with higher nutritional values and tolerate a high temperature. Comparing these landraces to modern hybrids, they usually observe a higher molecular diversity level [6]. Bota et al. [7] observed variation within a traditional long-storage tomato from the Mediterranean Islands of Mallorca, and the accessions are consistent with a genetically variable population or landrace group. They will help prevent this local landrace group's genetic erosion and make up a resource for future investigation and breeding.

Although a set of morphological characteristics historically diagnoses a variety, these morphological descriptors rarely quantify genotypic distinction because environmental factors can alter the quantitative characteristics [8]. DNA-based marker technologies have become the choice to analyze genetic basis and variability, since they provide sufficient information, are highly efficient, and are not sensitive to environmental factors. They use these markers for identification, registration of a plant variety, monitoring seed purity, and authenticity with high accuracy, high reliability, and low cost. Various DNA-based marker systems have been used to study genetic diversity and phylogenetic relationships among tomato genotypes, including SRAP [9–14].

Sequence-related amplified polymorphism (SRAP) is an efficient and straightforward marker technique system for genetic diversity characterization because it possesses high reproducibility and discriminatory power, discloses many codominant markers, and targets open reading frames [15]. AlShaye et al. [16] used SRAP markers to assess genetic variability in the Saudi tomato germplasm. SRAP markers discriminated the landraces in groups almost corresponding to their geographical origin. They suggested using these markers to manage tomato ex-situ collections for breeding programs and direct use in quality markets. Saudi plant genetic resources (seed bank) have preserved and maintained a Saudi crop landrace collection, including tomato seeds. In this context, the evaluation of Saudi tomato landraces seems to be a suitable model to analyze the variation in tomato landraces. This study analyzes the inter and intra-genetic variability among Hail tomato landrace using DNA-based markers SRAP. The results will help understand the genetic diversity and population structure of tomato populations and the conservation of tomato landraces in various Saudi Arabia regions.

## 2. Materials and Methods

Hail governorate is a large agricultural area (total area >103,000 km<sup>2</sup>) in the northern part of Saudi Arabia (27°31' N, 41°41' E, 992 m.a.s.l.). Farmers practice the cultivation of tomatoes and other horticultural crops. Besides commercial cultivars, three different tomato landraces are still grown in the area. The Saudi plant genetic resources gene banks' primary goal is to conserve plant germplasm, mainly local and land-races genotypes. The plant production department at King Saud University has cooperated with the gene bank for a long time to characterize and evaluate germplasms. The Hail landraces have been under evaluation for ten years and have been introduced in breeding programs to select varieties with advanced tolerance for heat, drought, salinity, and disease. Our study is a part of an MSc. research to study the Hail landraces' inter- and intra-genetic variability. Out of over 100 accessions for each landrace, we randomly selected 32 (labeled and numbered from 1 to 32), which were subsequently evaluated at the molecular level using seven SRAP markers. One accession from young expanding leaves (two-week old), representing a single plant per each accession, was collected, dipped in liquid nitrogen, and stored in –80 °C till DNA extraction. The leaves were ground into fine powder using a mortar and pestle. According to the manufacturer's instructions, genomic DNA was extracted using a Promega Wizard Genomic DNA Purification Kit (Promega, Madison, WI, USA). Agarose gel electrophoresis and spectrophotometry detected DNA quantification using a NanoDrop

2000 (Fisher Scientific, Waltham, MA, USA). Then, DNA was diluted with Tris-EDTA and fixed at 50 ng/ $\mu$ L. SRAP-PCR amplification was performed according to [15].

SRAP markers are based on two-primer amplification. The primers comprise the core sequences, which are 13 to 14 bases long, where the first 10 or 11 bases starting at the 5' end, are sequences of no specific constitution ("filler" sequences), followed by the sequence CCGG in the forward primer and AATT in the reverse primer. Three selective nucleotides follow the core at the 3' end. The filler sequences of the forward and reverse primers must differ from each other. For the first five cycles, the annealing temperature is set at 35 °C. The following 35 cycles are run at 50 °C [15].

Seven SRAP primer combinations were used to analyze the genetic variability (Supplementary Table S1). For each PCR reaction, the 20  $\mu$ L reaction volume contained 1 $\times$  GoTaq Green Master Mix (Promega Corporation, Madison, WI, USA), 0.1  $\mu$ M each forward and reverse primers, 50 ng DNA template, and nuclease-free water to complete the volume to 20  $\mu$ L. The thermal cycler profile for the PCR amplification set is: denaturation at 94 °C for 5 min, followed by five cycles of denaturation at 94 °C for 1 min, annealing at 35 °C for 1 min, and elongation at 72 °C for 1 min. For the remaining 30 cycles, the annealing temperature increased to 50 °C for 1 min, with the last elongation step at 72 °C for 7 min. Amplified products from the SRAP reactions separated by horizontal gel electrophoresis unit using 1.5% agarose gel in 1  $\times$  TBE buffer and stained with acridine orange (10 mg/mL) run was performed at 80 volts for 60 min. DNA ladder 100 base pairs (bp) molecular size ladder was used, and the gel was photographed by Gel Documentation System.

Each primer's electrophoretic patterns scored as band present (1) or band absent (0). An example of electrophoretic pattern using Primer SRAP1 is presented in Supplementary Figure S1. Pair-wise comparisons between individuals were made to calculate the Jaccard (1908) coefficient of genetic similarity [17]. The cophenetic correlation coefficient was used to measure the goodness-of-fit between the cophenetic matrix and the original similarity matrix using PAST (v 3.15) software [18]. Cluster analysis was performed to produce a dendrogram constructed using the unweighted pair group method with an arithmetical average (UPGMA) using PAST (v.3.15) software [18]. Principal coordinate analysis (PCoA) based on the genetic distances' matrix was used to produce a coordinate plot in which points in the Cartesian plane represent accessions or populations. Similar to the cluster analysis, this analysis allows clear visualization of the genetic diversity level of accessions. The polymorphism information content (PIC) for each primer was calculated to estimate its allelic variation:

$$PIC = 1 - \sum_{j=1}^n P_{ij}^2 \quad (1)$$

where  $P_{ij}$  is the frequency of the  $i$ th allele for marker  $j$  and the summation extends over  $n$  alleles, calculated for each SRAP marker according to [19]. Discrimination power (DP) was calculated by dividing the number of polymorphic alleles amplified for each primer by the total number of polymorphic alleles obtained [20]. The binary matrix of presence/absence was used for further analysis with GenAlEx 6.503 complement for MSExcel [21]. Percentage of polymorphic loci (% P) and private alleles per population were calculated using different statistical parameters such as: the total number of different alleles ( $N_a$ ); the total number of effective alleles ( $N_e$ ) =  $1/(p^2 + q^2)$ ; Shannon's information index ( $I$ ) is  $-1 \times (p \times \ln(p) + q \times \ln(q))$ ; expected heterozygosity ( $H_e$ ) =  $(2 \times p \times q)$ ; where  $p$  is the allele present and  $q$  is the absent of the allele. Analysis of molecular variance (AMOVA) was performed using GenAlEx 6.503 complement for MSExcel [21] based on 999 permutations. The AMOVA was performed by partitioning genetic variation among and within the population. Genetic differentiation between populations was determined using PhiPT.

### 3. Results

The genetic variability of 96 accessions of three Hail tomato landraces, using seven SRAP primer combinations, was analyzed. These primer combinations generated 55 scoreable alleles, polymorphic, with an average of 7.86 polymorphic alleles per pair of primers. The

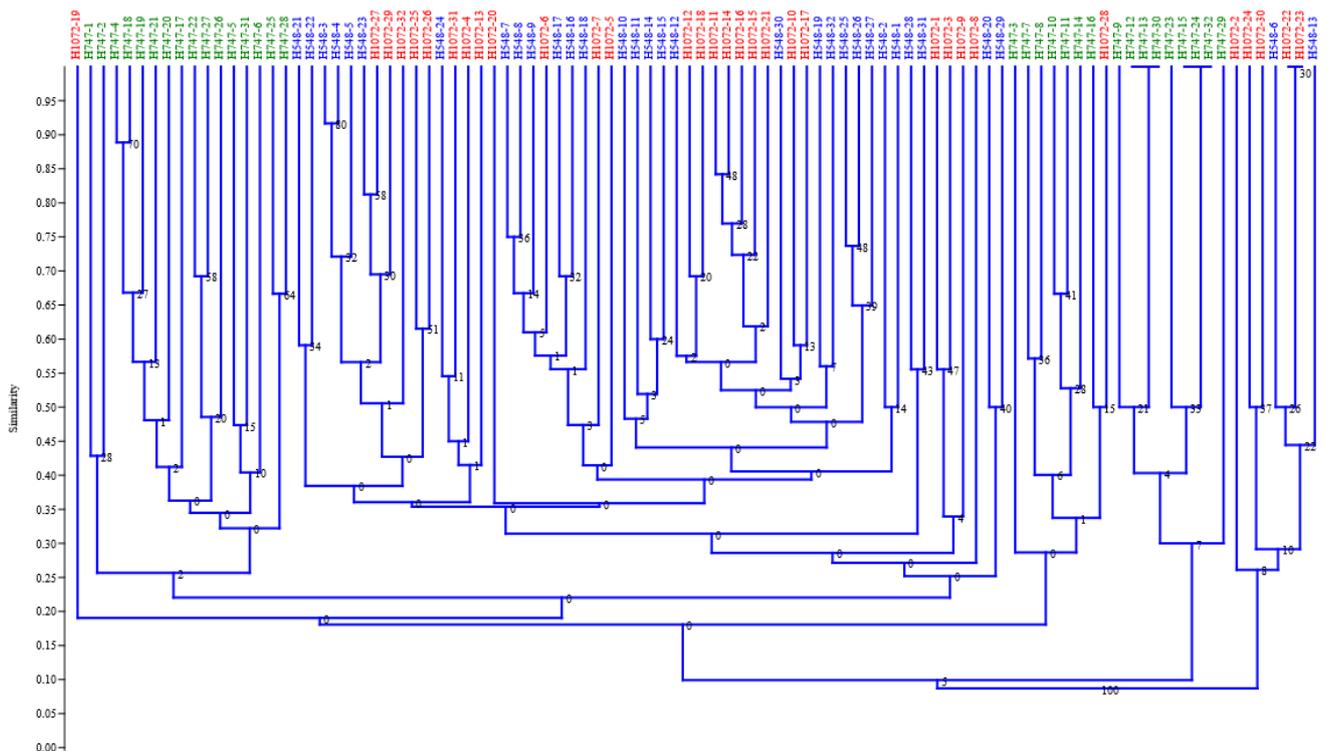
number of alleles scored per primer combination ranged from three for primer SRAP3 to 10 for primer SRAP5. Results in Table 1 present the major characteristics of these primers across the accessions. The primers SRAP2, SRAP4, and SRAP7 scored the maximum number of polymorphic bands with values 187, 153, and 222 bands/primers, respectively, while SRAP3 produced the minimum number (75) of bands. All primers generated in total 1027 bands across the accessions and performed polymorphic patterns with a value of 100%. The primers' average PIC was 0.68, with values ranging from 0.17 for SRAP3 to 0.82 for SRAP2. The (DP) of primers ranged from 5.45 for primer SRAP3 to 18.18 for the primer combination SRAP5 with an overall average of 14.29. All, except SRAP5 primers, over exceeded the overall means of the PIC and DP values.

**Table 1.** Features of SRAP amplified products used to analyze genetic diversity in Hail tomato accessions.

Primers Name	Primer Sequence 5'-3'	# Alleles	# Bands	Polymorphism%	PIC	DP
SRAP1	F-TGAGTCCAAACCGGAGA R-GACTGCGTACGAATTGAA	9	121	100	0.73	16.36
SRAP2	F-TGAGTCCAAACCGGAGA R-GACTGCGTACGAATTTGC	8	187	100	0.82	14.55
SRAP3	F-TGAGTCCAAACCGGACC R-GACTGCGTACGAATTATG	3	75	100	0.17	5.45
SRAP4	F-TGAGTCCAAACCGGACT R-GACTGCGTACGAATTGAC	8	153	100	0.76	14.55
SRAP5	F-TGAGTCCAAACCGGTAA R-GACTGCGTACGAATTACG	10	122	100	0.77	18.18
SRAP6	F-TGAGTCCAAACCGGTAA R-GACTGCGTACGAATTCAA	9	147	100	0.79	16.36
SRAP7	F-TGAGTCCAAACCGGAGA R-GACTGCGTACGAATTTGA	8	222	100	0.72	14.55
	Total	55	1027	—	—	—
	Min	3	75	100	0.17	5.45
	Max	10	222	100	0.82	18.18
	Mean	7.86	146.71	100	0.68	14.29

# Number of alleles, # Bands = number of bands across all accessions, PIC = Polymorphism information content, DP = discrimination power.

Jaccard's similarity index ranged from 0.0 to 100% among accessions, with an average value of 0.24 (Supplementary Table S2). This value was used to group the accessions into five main clusters presented in the UPGMA dendrogram (Figure 1). Supplementary Table S3 shows the distribution and frequency of accessions in each cluster. The first cluster compassed 16 accessions of Hail 747 with a similarity index ranging from 0.26 to 0.89. Thirty out of 32 of Hail 548 accessions aggregated to form the second cluster with 25 accessions from Hail 1072 with frequencies of 55% and 45%, respectively. The similarity index in this cluster ranged from 0.25 to 0.91. Seven accessions from Hail 747 and one accession from Hail 1072 formed the third cluster with a similarity index ranging from 0.28 to 0.66. The fourth cluster compassed only Hail 747 accessions, and the similarity value among accessions ranged from 0.30 to 1.0. Seven accessions from Hail 548 (2 accessions) and five accessions from Hail 1072 aggregated to form the fifth cluster with a similarity index ranging from 0.26 to 1.0. One accession from Hail 1072 failed to aggregate and individually separated.



**Figure 1.** Unweighted pair group method with an arithmetical average UPGMA dendrogram generated by Sequence-related amplified polymorphism SRAP markers, showing the relationships among tomato Hail accessions from Saudi Arabia, based on Jaccard's coefficient. Numbers at the branches show bootstrap values, computed from 100 replications of the data.

Principal coordinate analysis (PCoA) was performed, and the latent roots (Eigenvalues) for powerful PCs ranged from 6.65 (PC1) to 1.11 (PC8). The first three PCs used for constructing a scatter plot explained 33.17 percent of the total variance. The first PC contributed 16.19 percent and separated all accessions of Hail 747 from accessions of Hail 548 and Hail 1072 (Supplementary Figure S2). This analysis supported the separation of the accessions as a dendrogram, where one group consisted mainly of all accessions from Hail 747 and in the other most admixtures appear between the two landraces, Hail 548 and Hail 1072. In summary, the phylogenetic tree and PCA strongly supported that tomato Hail landraces have well-differentiated genetic populations and admixtures.

Genetic diversity indices of the Hail tomato landraces analyzed in this study are presented in Table 2. The average number of different Alleles ( $N_a$ ) ranged from 1.558 for Hail 1072 to 1.791 for Hail 548. Hail 548 recorded the highest number of effective alleles (1.485), the highest Shannon index (0.442), expected heterozygosity (0.291), and polymorphic percentage of 88.37%. However, Hail 747 recorded the lowest number of effective alleles (1.344), the lowest Shannon index, and expected heterozygosity with 0.346 and 0.217, respectively. Three private alleles, two were in Hail 1072, and one reported in Hail 548, and no private alleles were recorded in Hail 747.

**Table 2.** Genetic diversity parameters for Hail tomato landraces using seven SRAP primer combinations.

Population		N	Na	Ne	I	He	Polymorphism%	Number of Private Bands
Hail 548	Mean	32	1.791	1.485	0.442	0.291	88.37%	1.0
	SE		0.091	0.051	0.034	0.025		
Hail 747	Mean	32	1.698	1.344	0.346	0.217	83.72%	0.0
	SE		0.108	0.050	0.034	0.025		
Hail 1072	Mean	32	1.558	1.414	0.367	0.242	76.74%	2.0
	SE		0.126	0.059	0.040	0.029		
Mean			1.682	1.287	0.385	0.250	82.95%	
SE			0.063	0.025	0.021	0.015		

Na = No. of Different Alleles, Ne = No. of Effective Alleles =  $1/(p^2 + q^2)$ , I = Shannon's Information Index =  $-1 \times (p \times \ln(p) + q \times \ln(q))$ , He = Expected Heterozygosity =  $2 \times p \times q$ .

The analysis of Molecular Variance (AMOVA) revealed 87% within and 13% among the population variations (Table 3). Population differentiation and genetic structure were highly significant for all variances (PhiPT = 0.133,  $p < 0.001$ ). A lower value recorded between Hail 747 and Hail 1072 (0.094), and the highest was between Hail 548 and Hail 1072. Wide genetic variability within the landraces was detected.

**Table 3.** Summary of analysis of molecular variance (AMOVA) (A) and population differentiation index (B).

(A) Source	df	SS	MS	Est. Var.	%
Among landraces	2	65.354	32.677	0.848	13%
Within accessions	93	515.813	5.546	5.546	87%
Total	95	581.167		6.394	100%

(B) Pair-wise Population PhiPT Values		
	Hail 548	Hail 747
Hail 747	0.114 **	0.000
Hail1072	0.179 **	0.094 **
Mean PhiPT	0.133	$p < 0.001$

\*\* significant at  $p < 0.01$ , P = Probability, for PhiPT, is based on standard permutation across the full dataset.

The number of alleles detected varied between 39 for Hail 747 to 43 for both Hail 548 and Hail 1072. The primer combinations SRAP5 in Hail 1072 recorded the highest number of alleles (10) and the average of 6.14 alleles recorded in both Hail 548 and Hail 1072 and 5.5 alleles in Hail 747. Polymorphism information content (PIC) value averaged 0.7 in Hail 548 and 0.6 in Hail 747 and Hail 1072. Primer combination SRAP2 recorded the highest PIC value in all landraces (0.8 in Hail 548 and 0.7 in Hail 747 and Hail 1072). The primer combination SRAP6 recorded the highest (DP) among accessions of Hail 548 and Hail 747 with 20.93 and 17.9, while SRAP5 revealed the highest (DP) in Hail 1072 with the 23.2-value (Table 4).

The genetic similarity values among accessions ranged from 0.16 to 0.92 (average 0.36) for Hail 548, 0.04 to 1.0 (average of 0.26) for Hail 747, and 0.05 to 1.0 (average of 0.30) for Hail 1072 (Supplementary Tables S6, S9 and S11). Accessions for each landrace were aggregated in four major groups at the mean of their similarity indices (Supplementary Figures S3, S5 and S7). The principal coordinate analysis was also used to concentrate the variations on a different axis. The first two coordinates were used to construct the scatter plots and explained 38.88%, 48.47%, and 42.21% of the total variation in Hail 548, Hail 747, and Hail 1072, respectively (Supplementary Tables S8, S10 and S12 and Supplementary Figures S4, S6 and S8). Genetic similarity among landraces and accessions within landraces also showed that similarity among landrace accessions is higher than among landraces. The maximum similarity was reported among accessions within Hail 548 (0.36) and (0.31)

within Hail 1072, and the lowest was for Hail 747 (0.27). The lowest similarity among landraces was reported for Hail 548 and Hail 1072 with Hail 747 with a value of 0.15 (Supplementary Table S7).

**Table 4.** Features of SRAP amplified products used for analysis of genetic diversity in Hail tomato landraces.

Primer	Hail 548				Hail 747				Hail 1072			
	#Alleles	#Bands	PIC	DP	#Alleles	#Bands	PIC	DP	#Alleles	#Bands	PIC	DP
SRAP1	8	66	0.79	18.600	4	21	0.46	10.26	5	34	0.72	11.63
SRAP2	8	79	0.84	18.60	7	46	0.79	17.95	7	62	0.78	16.28
SRAP3	3	34	0.47	6.98	3	21	0.54	7.69	3	20	0.27	6.98
SRAP4	7	63	0.78	16.28	7	34	0.71	17.95	7	56	0.74	16.28
SRAP5	4	44	0.53	9.30	6	25	0.71	15.38	10	53	0.79	23.26
SRAP6	9	65	0.84	20.93	7	40	0.78	17.95	4	42	0.56	9.30
SRAP7	4	82	0.68	9.30	5	58	0.62	12.82	7	82	0.74	16.28
Total	43	433	—	—	39	245	—	—	43	349	—	—
Min	3	34	0.47	6.98	3	21	0.46	7.69	3	20	0.27	6.98
Max	9	82	0.84	20.93	7	58	0.79	17.95	10	82	0.79	23.26
Mean	6.144	61.86	0.70	14.29	5.57	35.00	0.66	14.28	6.14	49.86	0.66	14.28

PIC = polymorphism information content, DP = Discrimination power. # Number of alleles, # Bands = number of bands across all accessions.

#### 4. Discussion

The importance of tomato landraces as a valuable source of genetic variability for the improvement of cultivars or the production of new varieties is well documented. The genetic variation must be assessed to understand its potential use in the breeding program. DNA-based molecular markers, including SRAP, have been used in studying the genetic variability in cultivated, wild relatives and landraces of tomato [9–11].

In this study, SRAP markers showed a magnificent performance in the amplification of Hail tomato landraces. The polymorphism (%), PIC, and DP were higher than the results of several SRAP marker research works [9–11,16,22], using other molecular markers, AFLP [23], ISSR [12–14,24], and RAPD markers [25,26]. Kaushal et al. [27] evaluated 25 tomato genotypes with 20 microsatellite markers and recorded a PIC value between 0.0 to 0.54 with an average of 0.27. An average of 0.63 was reported by Marin- Montes et al. [28] when evaluating the genetic diversity of 26 accessions of native Mexican tomatoes with 18 SSR molecular markers. The higher PIC values reported in this study confirmed that the selection of primers performed well in assessing genetic diversity patterns and helped in grouping-related accessions, and thus can be applied for germplasm characterization studies. It was assumed that markers with PICs of over 0.5 are efficient in genotype discrimination and useful for measuring polymorphism at a locus [29]. Botstein et al. [30] stated that a PIC value > 0.5 accounts for a highly informative marker, 0.5 > PIC > 0.25 for an informative marker, and PIC < 0.25 for a slightly informative marker. This wide variation in the results reported referred to the number of landraces used, DNA fragmentation method, and vast geographical distribution of the genotypes' origins.

The narrow genetic differences between used accessions could be behind the lower values of polymorphism and PIC values, and the moderate values could be due to the narrow genetic base of the tomato cultivars and to highly informative markers used in this study [30]. The high percentage of polymorphism (100%), combined with a high number of polymorphic alleles generated per pair of primers (7.86), could be explained by both wide range of genetic diversity and geographical collection area, and by the efficiency of SRAP markers to reach sufficient polymorphism which targeted open reading frames as functional regions of the tomato genome [15]. It can be successfully used for determining genetic diversity and population structure. Although the number of screened accessions

(96) representing three landraces was small, we found an average of 7.86 polymorphic alleles, which exceeded that reported in other studies [9–11,16,22].

Mazzucato et al. [6] stated that a relatively small number of diverse landraces from relatively small geographical regions could encompass the same amount of genetic diversity shown by vast collections of varieties. We reported overall genetic diversity values ranging from 0.0 to 1.0, with an average of 0.24. UPGMA similarity dendrogram with a high cophenetic correlation coefficient ( $r = 0.84$ ) based on SRAP polymorphisms allowed for the grouping of 96 accessions of the three landraces into five main clusters—their gene bank accession numbers—showing a relatively good separation between landraces. The intra-genetic similarity was also shown in a wide range. For example, the range from 0.28 to 0.66 was recorded in one cluster and from 0.26 to 1.0 in another cluster. The principal coordinate analysis confirmed the clustering produced via the UPGMA dendrogram, showing that tomato Hail landraces have well-differentiated genetic populations and admixtures, where Hail 747 formed their separate group in principal coordinate analysis and both Hail 548 and Hail 1072 admixed, and some accessions showed more diversity pattern. Tomato landraces have been reported to contain higher genetic and phenotypic variability than commercial cultivars and hybrids. Several studies have analyzed the population structure for various tomato landraces, breeding materials, and commercial cultivars and concluded that landraces, in most cases, aggregated to form separated clusters because of their high genetic diversity. Park et al. [23] found that UPGMA clustering and PCA grouped the most modern hybrid cultivars in one cluster, whereas cultivars released before 1970 in the USA were grouped in another cluster, and others grouped related to their pedigree. These results coincided with [31], who reported RFLP and RAPD alleles distinguished old cultivars, modern cultivars, South American regional cultivars, and wild *L. esculentum* var *cerasiforme*, and *L. cheesmanii* accessions. They also explained that low genetic distance from old cultivars to wild types might result from the inter-crossing of these materials with wilder germplasm. The introgression of wild germplasm into modern cultivars increased genetic diversity within commercial cultivars.

It was reported that the level of polymorphism and genetic diversity is monitored by different factors, including the mating system, the history of domestication, collection size, and type of collection (wild type, varieties, breeding lines, landraces). Alvarez et al. [32] found that the self-compatible species (*L. esculentum*, *L. cheesmanii*, *L. parviflorum*, and *L. chmielewskii*) had a lower gene diversity than the outcrossing species, reflecting their autogamous mode of reproduction. Mazzucato et al. [6] reported a lower level of genetic diversity in the cultivated tomato gene pool than in other self-pollinating species; however, when several wild accessions entered the collection, high numbers of alleles per polymorphic SSR locus were obtained. Corrado et al. [33] reported that the SNP set could distinguish landraces from commercial varieties, and admixture between landraces and cultivars is low because of the farmer selection for quality traits.

Tomato landraces harbor a higher number of minor-alleles and a more robust population structure than contemporary varieties, which explained a strong divergent or directional selection operating on many traits during adaptation to local conditions and practices. Recently, Gonias et al. [34] found that UPGMA clustering confirmed in the PCoA supported discriminating landraces from hybrids and modern varieties. These results agreed with [35], who found that Brazilian landraces fell within one group, whereas the commercial cultivars and three hybrids were in four groups. However, Sacco et al. [36] reported a low level of genetic diversity in landraces than contemporary cultivars because of the varied breeding programs that the two groups followed. The contemporary germplasm undergoes a long time crossing with wild relatives, leads to broadening the genetic diversity in contemporary germplasm concerning landrace germplasm. Farmers usually collect seeds from the best fruits rather than the best productive genotype to maintain excellent quality. These conflicting results are probably because of differences in the germplasm collection and molecular markers sampled for the analysis. Castellana et al. [37] found that Umbrian landraces are highly differentiated compared to commercial varieties, and

the subpopulation structure within landraces could result from selection, the historicity of cultivated varieties, and adaptation to the environment. The low admixture between commercial varieties and Umbrian local landraces could be because of farmers' historical cultivation and manufacturing traditions. Cattáneo et al. [38] confirmed the 'Platense' tomato landrace as a highly diverse resource, with some of its subpopulations keeping most of the total diversity; the seed exchange makes it difficult to identify clear genetic boundaries among tomato landrace.

Different molecular markers also studied genetic analysis among tomato genotypes collected from different origins and showed variable results. Zhou et al. [39] found that SSR molecular markers grouped the cultivated and introgression lines in one cluster, and the wild type comprised the other seven clusters. These results also coincided with that of [40], who reported that tomato accessions clustered based on RAPD and SSR markers according to their types were cultivated varieties in one cluster and the wild varieties into the second cluster. Sharifova et al. [14] showed that the geographical origin does not influence the clusters, where accessions from different regions might have a similar genetic background. However, accessions with the same or adjacent geographic origin cluster together, accessions from different regions were also closely related regardless of their geographic origin [25]. However, high genetic variation in the tomato germplasm studied and the tomato landraces grouping was in concordance with their geographical distribution areas [24]. UPGMA clustered a collection of determinate and indeterminate cultivars of tomato from India's different geographical locations into five groups, with the USA cultivars forming a distinct group. Clustering was consistent with the known information regarding the geographical location and growth habit [41]. Nine tomato cultivars (*L. esculentum* L.), one tomatillo (*P. philadelphica*), and one cherry tomato (*S. lycopersicum* var. *cerasiforme*) accessions collected from different Egypt regions were characterized using RAPD and ISSR markers. UPGMA cluster analysis placed all tomato accessions and cultivars into a single group, while the tomatillo and cherry tomato accessions were placed in a second group [42].

Genetic diversity parameters and AMOVA showed that most of the genetic variation was due to the differences within populations (87%), while the variability among populations had a significantly lower contribution (13%). Population differentiation and genetic structure were highly significant for all variances ( $\Phi_{PT} = 0.133$ ,  $p < 0.001$ ). The difference between the number of alleles in each locus and the number of effective loci shows the presence of two rare alleles in Hail 1072 accessions. Rare alleles are those that have low frequency and occur in one or more populations. These alleles could be used to identify populations using a combination of some genetic loci [43]. Three private alleles were obtained, which could be explained by slippage and proofreading errors during DNA replication that primarily changes the arrangement of DNA nucleotides [44]. The expected heterozygosity ( $H_e$ ) and Shannon's Information Index ( $I$ ) are considered a significant parameter in genetic diversity studies since  $H_e$  estimates genetic diversity within and between accessions, while Shannon information index is for genetic diversity within and between populations, regardless of the number of accessions accessed [45]. The expected heterozygosity reported in this study was higher than that obtained in [37], which recorded 0.111, while Mazzucato et al. [6] reported 0.41 and [23] reported 0.689. These differences could be linked to various marker techniques used in assessing various positions of the genome.

The results revealed that most of the genetic variation was contributed to within rather than among landrace variability.

The importance of within genotypes or population diversity is reported in many studies. Mansour et al. [46] compared three markers in the assessment of genetic diversity in tomatoes. AMOVA analysis showed that all markers were high within cultivars' genetic variability. Gonias et al. [34] stated that the loss of allelic diversity in landraces was lower than that of commercial variety, losing expected heterozygosity, because breeding was relatively small for comparisons between landraces and modern varieties and higher for

comparisons between modern varieties and hybrids. However, landraces have not undergone systematic breeding selection, where individuals within a population share common phenotypic and agronomic traits and contain a high degree of heterozygous alleles.

## 5. Conclusions

Our results show that Hail tomato landraces are unique genetic resources, beneficial for in situ conservation of agro-biodiversity. The high genetic variation among Hail tomato landraces serves as a rich germplasm source with potential breeding programs. The dramatic biodiversity loss and climate changes highlighted the urgent need to characterize and preserve landrace genetic variation and rescue our kingdom gene pool's wealth. The SRAP technique's effectiveness in studying genetic variability among and within landraces must be considered in tomato-breeding programs through marker-assessed selection.

**Supplementary Materials:** The following are available online at <https://www.mdpi.com/1424-2818/13/3/135/s1>, Table S1. SRAP primers sequences used in PCR amplification; Table S2. Jaccard's similarity coefficient among the three tomato Hail landrace accessions based on SRAP data scored; Table S3. Distribution of accessions Hail tomato landraces frequency and the percentage into the main clusters; Table S4. Eigenvalue, percent, and cumulative variance of the first seven principal coordinates (PCoA) analysis based on SRAP molecular markers; Table S5. The top-loading accessions in the total variance for the first two coordinates contribution; Table S6. Jaccard's similarity coefficient among Hail 548 landrace accessions based on SRAP data scored; Table S7. Jaccard similarity index among landraces and accessions with landraces of Hail Tomato landraces; Table S8. Eigenvalue, percent, and cumulative variance of the first seven principal coordinates (PCoA) analysis based on SRAP molecular markers for Hail 548 accessions; Table S9. Jaccard's similarity coefficient among Hail 747 landrace accessions based on SRAP data scored; Table S10. Eigenvalue, percent, and cumulative variance of the first seven principal coordinates (PCoA) analysis based on SRAP molecular markers for Hail 747 accessions; Table S11. Jaccard's similarity coefficient among Hail 1072 landrace accessions based on SRAP data scored; Table S12. Eigenvalue, percent, and cumulative variance of the first seven principal coordinates (PCoA) analysis based on SRAP molecular markers for Hail 1072 accessions; Figure S1. SRAP electrophoretic pattern of Hail tomato accessions obtained by primer SRAP1. Figure S2. Principal coordinate analysis (PCoA) from 96 accessions of Hail landraces (Hail 548, Hail 747, and Hail 1072) assessed with 7 SRAP primer combinations. PCoA ordination results are shown with 95% confidence ellipses (coordinate 1 explained 16.19% and Coordinate 2 explained 10.32); Figure S3. Phenogram of Hail 548 accessions based on SRAP data constructed according to Jaccard's (1908) genetic similarity matrix and clustered with the UPGMA method. Each arm of the tree corresponds to the scaled genetic similarity; Figure S4. Principal coordinate analysis (PCoA) from 32 accessions of Hail 548 landraces assessed with 7 SRAP primer combinations. PCoA ordination results are shown with 95% confidence ellipses; Figure S5. Phenogram of Hail 747 accessions based on SRAP data constructed according to Jaccard's (1908) genetic similarity matrix and clustered with the UPGMA method. Each arm of the tree corresponds to the scaled genetic similarity; Figure S6. Principal coordinate analysis (PCoA) from 32 accessions of Hail 747 landraces assessed with 7 SRAP primer combinations. PCoA ordination results are shown with 95% confidence ellipses; Figure S7. Phenogram of Hail 1072 accessions based on SRAP data constructed according to Jaccard's (1908) genetic similarity matrix and clustered with the UPGMA method. Each arm of the tree corresponds to the scaled genetic similarity; Figure S8. Principal coordinate analysis (PCoA) from 32 accessions of Hail 1072 landraces assessed with 7 SRAP primer combinations. PCoA ordination results are shown with 95% confidence ellipses.

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