Print ISSN: 0375-9237
Online ISSN: 2357-0350

EGYPTIAN JOURNAL OF BOTANY (EJBO)

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PUBLISHED BY
THE EGYPTIAN
BOTANICAL SOCIETY

Analysis of genetic diversity and relationships among fifteen Egyptian garlic genotypes using SCoT and SRAP markers

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Garlic holds significant economic relevance due to its medicinal and dietary value. The propagation of garlic through clonal propagation poses challenges to breeding programs, as it reduces genetic diversity. Therefore, it is imperative to identify both the morphological and molecular differences among garlic genotypes cultivated in Egypt. In this investigation, fifteen garlic genotypes were collected from different regions of Delta and cultivated in new reclaimed sandy loam soil over two seasons (2022 and 2023). The selection of garlic clones and strains with genetic traits that facilitate adaptation to fluctuating and unpredictable environmental conditions is of utmost importance. Also protecting agricultural genetic resources is a paramount responsibility. Molecular genetic markers have emerged as a crucial contemporary method for assessing genetic diversity and enhancing agricultural yield in breeding programs. In this study, SRAP (sequence-related amplified polymorphism) and SCoT (start codon targeted) markers were employed to evaluate the genetic variation and relationships among the garlic genotypes. Results of the morphological analysis revealed significant variations in all the investigated traits. Genotypes 1 and 7 exhibited superiorities over other genotypes in certain morphological features, such as bulb weight, bulb height, and yield. Furthermore, the SRAP and SCoT markers demonstrated a high level of genetic variation among the garlic genotypes. Notably, several positive and negative markers associated with bulb weight and yield traits were identified using the SRAP and SCoT markers. At the molecular marker level, our findings indicated that the G2 genotype displayed superior yields and weights compared to its original genotype, G5, and exhibited genetic differentiation and independence in a distinct clade. This suggests that new garlic varieties can be genetically enhanced by leveraging the genes present in this genotype. The utilization of markers was found to generate valuable information on genetic diversity that can be utilized by garlic breeders. The results of this investigation demonstrated a high level of genetic variation between the fifteen Egyptian garlic genotypes when assessed using SRAP and SCoT markers. This information will be valuable for garlic breeders in understanding and utilizing genetic diversity effectively.

Keywords: *Allium sativum* L.; molecular markers; genetic diversity analysis

ARTICLE HISTORY
Submitted: April 05, 2024
Accepted: August 07, 2024

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DOI: 10.21608/ejbo.2024.281414.2794

EDITED BY: R. Gaafar

INTRODUCTION

Garlic (*Allium sativum* L.) is an annual plant that has been cultivated for approximately 5,000 years in the Middle East. It is widely grown and consumed worldwide due to its edible and medicinal properties (Li et al., 2022). Garlic belongs to the Liliaceae family and is commonly found in Asia, Europe, and America. This genus comprises over seven hundred species, including *A. cepa* L., *A. sativum* L., *A. chinense, A. rubellum*, and numerous other species from various countries. Garlic thrives in cold regions and produces bulbs consisting of multiple cloves, with each stem containing 4–40 cloves. The bulbs can grow to a size of 2.5–7 cm and are accompanied by long, flat, and folded leaves (Kurnia et al., 2021).

Garlic is typically a diploid species that spreads vegetatively, allowing for easy exchange of garlic clones among farmers and collectors. Over time, these clones have given rise to several cultivars due to their gradual adaptation to diverse agroclimatic conditions. Presently, clonal lineages display a remarkable range and a diversity of phenotypes, encompassing traits such as bulb weight, clove count, coat layer, leaf length and width, growth behaviour, stress tolerance, leaf count, and bolting tendency (Chen et al., 2014).

The vegetative propagation method employed in garlic cultivation results in low genetic diversity. Vegetatively propagated crops possess relatively stable genetic profiles, making the development of new varieties highly challenging and primarily reliant on rare spontaneous mutations (El-Fiki and Adly, 2020). According to Volk and Stern (2009), variations in garlic can be observed in plant height, bulb thickness, number of cloves, dry matter content, and chemical composition. Early identification of garlic germplasms primarily relied on morphological characteristics, which were heavily influenced by environmental conditions and local habitats in cultivated regions. However, the abundance of garlic's genomic resources, its environmental resilience, and the availability of non-tissue-specific DNA markers have proven advantageous in investigating its biodiversity, facilitating the identification and evaluation of its genetic diversity (Li et al., 2022).

Genotype characterization plays a crucial role in enhancing breeding efficiency and enabling direct

selection based on desirable traits and genes (Khatab et al., 2021; Adly et al., 2023). Molecular markers offer a means to achieve these objectives, as they expedite the emergence of new genotypes possessing desired traits compared to conventional crop selection techniques, especially when these traits are difficult to measure (Al-Ghamedi et al., 2023). One such molecular marker system, known as sequence-related amplified polymorphism (SRAP), has been developed for the selective amplification of open reading frames. The variations detected by SRAP markers are primarily attributed to differences in promoters, introns, and spacers among species and individuals. SRAP markers are highly informative and reproducible, making them a valuable tool for assessing genetic diversity in various plants. They have been successfully employed in genetic diversity studies, gene tagging, map construction, and phylogenetic investigation (Xie et al., 2015; Zaki et al., 2023; El-Badan et al., 2024).

The majority of start codon targeted (SCoT) markers were repeatable; yet there were several anomalies that suggested primer length and annealing temperature are not the only variables that affect repeatability. The PCR amplification patterns of SCoT markers revealed dominant markers like RAPD markers. This approach is suggested, particularly in labs that favor agarose gel electrophoresis, to be probably utilized in conjunction with these markers for applications including bulked segregant analysis, genetic analysis, and quantitative trait locus mapping (El-Fiki and Adly, 2020).

The main objective of this investigation was to analyse the genetic diversity and relationships among fifteen Egyptian garlic genotypes using SRAP and SCoT markers to develop strategies for conservation and sustainable utilization.

MATERIALS AND METHODS

Plant Material

Fifteen garlic genotypes were used as plant materials in this investigation. Geographically referenced events were obtained using Google Earth version 9.3.25.5. The geospatial points with species names were saved in a CSV matrix and are depicted in Figure 1 and Table 1. The main garlic genotypes used in this investigation, G2 and G3, are newly developed genotypes currently under registration and belong to Dr. Essam M. Helmy and Entsar I. Ragheb, Assistant Professors of Vegetable Breeding, Department of Vegetable Crops, Alexandria University, Egypt. Thirteen commercial cultivars were sourced from farmers who grew foreign garlic germplasm in the Delta region at 31°02’00”N and planted in 2022 and 2023. The newly reclaimed longitude was 28°26’00”E.

Design and Method of Morphological Characterization

From September 2021 to May 2023, the experiment was conducted, and samples were taken from ripe garlic of all genotypes. The samples were then carried in the open air in the field. The fifteen garlic genotypes (G1, G2, G3, G4, G5, G6, G7, G8, G9, G10, G11, G12, G13, G14, and G15) were grown in fifteen blocks, with each group representing a genotype. Samples from each group were cultivated in a Randomized Complete Block Design (RCBD) with four replicates. To prepare the soil for planting, compost was added using a plow. The ground was sketched with lines spaced 20 cm apart, and the plants were spaced 15 cm apart. The seed furrow was made at a depth of 5 cm. Once the plants had completely dried, morphological readings were taken. Morphological measurements included bulb weight, weight loss, bulb height, bulb diameter, number of rows, number of cloves, bark color, and dry weight for each plant. The mean values were determined from these measurements to assess the differences among the groups. Data were subjected to statistical analysis by the method of LSD tests as reported by Gomez and Gomez (1984). All statistical analysis was performed with SAS computer software.

Molecular Characterization Method

DNA Extraction: Genomic DNA was manually extracted from young leaves of the fifteen genotypes using the cetyltrimethylammonium bromide procedure (CTAB) method (Aboul-Maaty and Oraby, 2019). The quality and quantity of DNA were assessed spectrophotometrically using the Bio-Rad SmartSpec 3000 UV/Vis Spectrophotometer and 0.8% agarose gel electrophoresis. The DNA concentration was adjusted to 10 ng/µL.

PCR Amplification and Purification: Eight sequence-related amplified polymorphism (SRAP) primers (Li and Quiros, 2001) and ten start codons targeted (SCoT) primers (created by Willowfort, UK) were used for PCR amplification. All PCR reactions were performed in a total volume of 25 µL in 96-well plates using a labCycler thermocycler (SensoQuest, Germany). The PCR reaction mixture contained 12.5 µL of COSMO PCR RED Master Mix DNA Polymerase (WF10203001 Co., Ltd.), 2.0 µL of genomic DNA (20

ng/µL), 2.0 µL of each primer (10 M), and 6.5 µL of distilled water to adjust the final volume of the PCR product. The SRAP-PCR program consisted of an initial denaturation at 94°C for 5 minutes, followed by five cycles of denaturation at 94°C for 30 seconds, annealing at 36°C for 45 seconds, and extension at 72°C for 90 seconds. This was followed by thirty cycles of denaturation at 94°C for 30 seconds, annealing at 47°C for 45 seconds, and extension at 72°C for 90 seconds, with a final extension at 72°C for 7 minutes, as described by Chen et al. (2014). The SCoT-PCR program included an initial denaturation at 94°C for 3 minutes, followed by thirty-five cycles of denaturation at 94°C for 1 minute, annealing at primer-specific annealing temperature for 1 minute, and extension at 72°C for 2 minutes. After the last cycle, a final step of 5 minutes at 72°C was included to allow complete extension of all amplified fragments, and the samples were then held at 4°C (Collard and Mackill, 2009). The amplification products were separated by one-hour electrophoresis at 80 V on a 2% agarose gel and visualized using ethidium bromide (10 mg/mL) in 1x TAE buffer. After electrophoresis, the DNA profiles were visualized on a UV transilluminator in a gel documentation system and photographed for analysis. The DNA marker used in this investigation was BERUS 100 bp DNA Ladder (WF10407001 Co., Ltd.).

Allele Scoring and Diversity Analysis: The gel images were analyzed using EgyGene GelAnalyzer4 (Ahmed, 2021) to determine the molecular sizes of the amplified fragments. The amplified fragments were scored as present (1) or absent (0). PIC values for SRAP markers were calculated using the online polymorphic information content and heterozygosity calculator (https://irscope.shinyapps.io/iMEC/). PIC values for SCoT markers were determined using the online program for calculating polymorphic information content and heterozygosity (https://gene-calc.pl/). Similarities were estimated using Jaccard's coefficient. The similarity coefficient and genetic distance were analyzed according to the method described by Nei (1973). Cluster analysis was carried out using NTSYS-pc software with the UPGMA algorithm (Rohlf, 2000).

RESULTS

Morphological Characteristics

The morphological characteristics of the fifteen genotypes used in this investigation, including bulb weight, bulb height, bulb diameter, number of rows, number of cloves, and dry weight as well as bark color, were examined, and significant differences were found among these genotypes (Table 2). The highest values for bulb weight were recorded in genotypes G7 and G1 (68.5 g and 60.2 g, respectively). Genotypes G6 and G1 had the highest fresh weight of cloves, measuring 6.5 g and 4.7 g, respectively. In terms of bulb height, genotypes G7 and G1 exhibited the highest values (2.7 cm and 2.5 cm, respectively). Additionally, G2 and G6 had the largest bulb diameters, measuring 5.3 cm and 5.1 cm, respectively. Genotype G2 had the highest number of rows (3 rows/bulb), while genotypes G5 and G2 had the highest number of cloves/bulb (21 and 19, respectively). Regarding the dry matter content per 100 g of fresh weight, genotypes G7, G9, G14, and G10 recorded the highest values (76.1 g, 72.3 g, 72 g, and 70.0 g, respectively). Analysis of variance (ANOVA) indicates the presence of significant differences in several traits such as clove weight, bulb height, and number of rows as well as some highly significant traits such as bulb weight, bulb diameter, number of cloves, and dry weight.

Molecular Data Analysis

SRAP Amplification and Its Cluster Analysis: A total of 101 bands were generated using ten SRAP primers, with an average of 12.6 bands per primer (Figure 2 and Table 3). The mean effective number of alleles, Nei's gene diversity (H), polymorphic information content (PIC), and polymorphic loci percentage (P%) were calculated for all SRAP primer combinations (Figure 2 and Table 3). The ME3-EM2 primer combination produced the highest values for these parameters (1.98, 0.495, 0.372, and 95.5%, respectively). The ME1-EM2 primer combination had values of 1.404, 0.288, 0.247, and 100% for the same parameters. The amplified bands ranged in size from 120 bp to 1550 bp. The effective number of alleles and genetic diversity values indicated high genetic diversity among the 15 garlic genotypes, with the effective number of alleles ranging from 1.404 to 1.98 and genetic diversity ranging from 0.495 to 0.288. The similarity coefficients ranged from 0.24 to 0.84 among the fifteen garlic genotypes, with a mean similarity coefficient of 0.51 and genetic distances ranging from 0.37 to 0.85. A dendrogram based on the SRAP molecular markers categorized the fifteen garlic accessions into four groups: group I (6 accessions), group II (6 accessions), group III (2 accessions), and group IV (1 accession). Groups I, II, and III clustered together with group IV at a Nei's coefficient of 0.42.

Figure 1. A diagram illustrating the sites where samples from various governorates were collected.

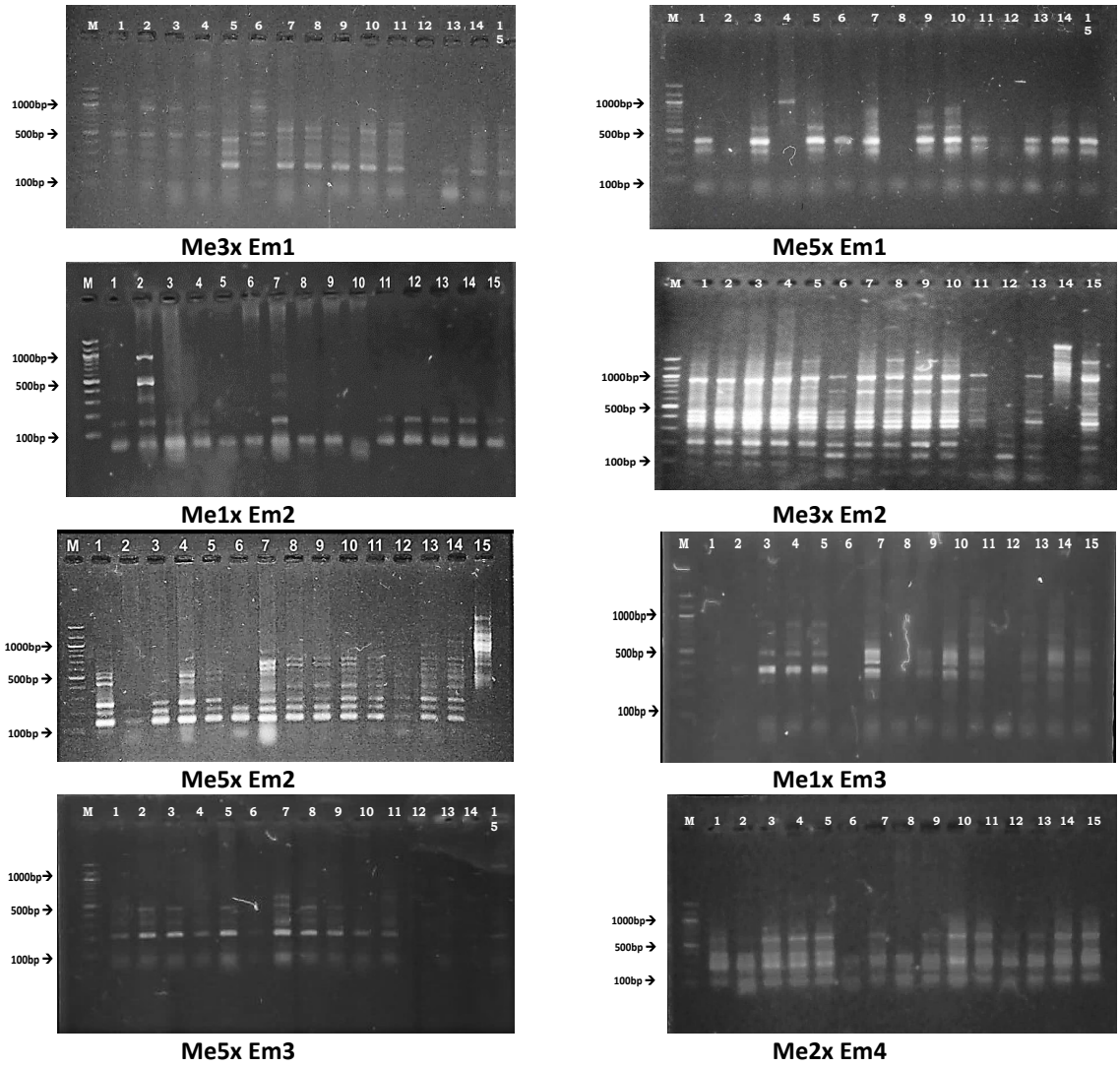

Figure 2. SRAP profiles obtained from 15 garlic genotypes amplified by eight primers, M = 100 bp ladder size marker.

Table 1. Geographic origin of collected garlic seeds.

Genotype number	Source of collection		Location coordinates
	Country	Province	
G1	Abu El Matamer	Alexandria	30.908411°N 30.148487°E
G2	Amreya		31.104538°N 29.766226°E
G3	Amreya		31.104538°N 29.766226°E
G4	Sugar beet area		30.777147°N 29.648186°E
G5	Al-Busaili	Al-Bahira	31.330873°N 30.394491°E
G6	Imam Malik village		26.507349°N 20.239225°E
G7	Sadat City	Al- Menoufia	30.370101°N 30.394691°E
G8	Al-Khatahtbeh		30.362483°N 30.808311°E
G9	Burgash	Giza	30.165773°N 31.022663°E
G10	Zawyat Razin	Al- Menoufia	30.414293°N 30.837564°E
G11	Natrn Valley	Al-Bahira	30.435124°N 29.596179°E
G12	Saba Pasha area	Alexandria	31.237005°N 29.953583°E
G13	Natrn Valley	Al-Bahira	30.435124°N 29.596179°E
G14	Quwaysna	Al- Menoufia	30.562258°N 31.148127°E
G15	Damlo village, Benha	Al-Qaliubiya	30.446841°N 31.180304°E

Table 2. The recorded morphological traits of the plants under investigation after drying the plants completely.

Genotype number	Bulb weight/g	Clove weight/g	Bulb height/cm	Bulb diameter /cm	Number of rows	Number of cloves/bulb	Dry weight /g	Shell colour
G1	60.2 a±12.4	4.7 ab±0.32	2.5 a±0.07	4.8 ab±0.09	2.0 b±0.02	12 bc±0.017	6.0 ab±0.06	White
G2	44.5 b±9.2	3.4 b±0.41	2.1 ab±0.03	5.3 a±0.04	3.0 a±0.02	21 a±0.12	5.9 b±0.11	White
G3	27.9 c±11.5	1.7 c±0.29	1.9 b±0.08	3.6 c±0.13	2.0 b±0.07	16 ab±0.09	5.6 b±0.11	White
G4	34.4 c±14.6	2.5 bc±0.34	2.1 ab±0.13	4.0 c±0.07	2.0 b±0.07	18 a±0.09	6.3 a±0.09	White
G5	23.3 cd±8.3	2.5 bc±0.37	1.6 b±0.04	3.3 c±0.03	1.0 c±0.03	19 a±0.14	6.0 ab±0.14	White
G6	38.2 bc±7.3	6.5 a±0.27	2.1 ab±0.06	5.1 a±0.08	1.0 c±0.02	7 e±0.06	4.5 c±0.10	Crimson
G7	68.5 a±9.8	2.9 bc±0.13	2.7 a±0.03	4.7 b±0.16	2.0 b±0.02	17 ab±0.08	7.6 a±0.08	Crimson
G8	25.7 c±7.1	3.2 bc±0.28	1.8 b±0.02	4.6 b±0.21	2.0 b±0.06	12 bc±0.10	4.0 d±0.12	White
G9	33.6 c±6.9	2.6 bc±0.26	1.5 bc±0.02	4.2 bc±0.08	2.0 b±0.11	13 bc±0.09	5.2 cd±0.20	Crimson
G10	34.0 c±9.2	2.5 bc±0.25	1.8 b±0.05	4.5 b±0.03	2.0 b±0.03	15 b±0.11	5.5 c±0.18	Crimson
G11	21.9 cd±5.9	1.3 c±0.21	1.7 b±0.02	4.3 b±0.06	1.0 c±0.02	13 bc±0.06	5.2 cd±0.09	White
G12	45.6 b±8.8	3.7 b±0.2.1	2.3 ab±0.02	3.9 c±0.02	1.0 c±0.02	14 b±0.09	6.1 ab±0.17	Crimson
G13	29.6 c±7.2	3.6 b±0.26	2.3 ab±0.06	4.1 bc±0.06	1.0 c±0.04	8 d±0.11	6.0 ab±0.14	Crimson
G14	36.1 bc±8.1	1.5 c±0.13	1.4 bc±0.02	5.1 a±0.03	2.0 b±0.02	13 bc±0.07	4.2 d±0.19	Crimson
G15	5.3 cd±5.9	3.4 b±0.41	2.2 ab±0.06	5.3 a±0.04	2 b±0.11	14 b±0.09	6.1 ab±0.12	Crimson
LSD $_{P<5\%}$	16.4**	2.7*	0.82*	0.64**	1,0*	4.2**	0.8**	

The means which have at least one common letter do not have significant difference based on LSD test at 5% level.

Table 3. Amplification results and polymorphism information obtained with the SRAP primer combinations: primer code (PC), size ranges (SR), amplified bands (AB), number of polymorphic loci (NPL), polymorphism percentage (P%), polymorphic information content value (PIC), Nei's gene diversity (H), and effective number of alleles (Ne).

No.	PC	Seq.	SR (bp)	AB	NPL	P%	PIC	H	Ne
1.	Me1 x Em2	F 5´-TGGAGTCCAAACCGGATA3´	130-1000	8	4	100	0.247	0.288	1.404
		R 5´-GACTGCGTAVGAATTTGC-3´							
2.	Me1 x Em3	F 5´-TGGAGTCCAAACCGGATA3´	300-1200	10	6	100	0.325	0.408	1.689
		R 5´-GACTGCGTACGAATTGAC3´							
3.	Me2 x Em4	F 5´-TGAGTCCAAACCGGAGC-3´	120-550	10	7	100	0.354	0.460	1.851
		R 5´-GACTGCGTACGAATTTGA-3´							
4.	Me3 x Em1	F 5´-TGAGTCCAAACCGGAAT-3´	180-1490	12	10	100	0.343	0.440	1.785
		R 5´-GACTGCGTACGAATTAAT-3´							
5.	Me3 x Em2	F 5´-TGAGTCCAAACCGGAAT-3´	180-1450	22	18	95.5	0.372	0.495	1.980
		R 5´-GACTGCGTAVGAATTTGC-3´							
6.	Me5 x Em1	F 5´-TGAGTCCAAACCGGAAG-3´	300-1000	10	5	100	0.321	0.403	1.675
		R 5´-GACTGCGTACGAATTAAT-3´							
7.	Me5 x Em2	F 5´-TGAGTCCAAACCGGAAG-3´	150-1550	18	12	100	0.341	0.436	1.773
		R 5´-GACTGCGTAVGAATTTGC-3´							
8.	Me5 x Em3	F 5´-TGAGTCCAAACCGGAAG-3´	200-600	11	8	100	0.340	0.436	1.773
		R5´-GACTGCGTACGAATTGAC-3´							
		Total		101					
		Average		12.6	8.75	99.4	0.3304	0.42	1.741

SCoT Amplification and Its Cluster Analysis: Using ten SCoT primers, a total of 152 bands were generated, with an average of 15.2 bands per primer (Figure 3 and Table 4). The number of bands per primer ranged from 12 to 20, and the number of polymorphic bands varied from 10 to 19. Primer SCoT-15 produced the highest number of products (20 bands), while primer SCoT-21 detected the lowest number (11 bands). The percentage of polymorphism per primer ranged from 78.57% to 100%, with an average of 93.04%. The polymorphic information content (PIC) values ranged from 0.44 to 0.49, with an average of 0.4858, indicating high informativeness of the SCoT primers. The size of the amplified bands varied from 120 bp to 2020 bp (Figure 3 and Table 4). The UPGMA dendrogram based on SCoT data categorized the 15 garlic genotypes into four groups: group I (5 accessions), group II (4 accessions), group III (5 accessions), and group IV (1 accession). Groups I, II, and III clustered together with group IV at a Nei's coefficient of 0.42.

Molecular Markers for Economic Traits: This investigation aimed to identify and analyse molecular markers associated with bulb weight and yield, which are economically significant traits for farmers. Two molecular marker techniques, sequence-related amplified polymorphism (SRAP) and start codon targeted (SCoT) markers, were employed to identify positive and negative markers linked to these traits. Additionally, a combination cluster analysis using the Jaccard similarity coefficient was conducted to estimate genetic relationships among the accessions. The SRAP markers revealed three positive markers, Me3 x Em1 (200 bp) and Me3 x Em2 (530 bp and 210 bp), as well as two negative markers, Me5 x Em2 (590 bp) and Me5 x Em3 (250 bp), associated with bulb weight and yield traits. Similarly, the SCoT markers identified five positive markers: SCoT-5 (520 bp and 400 bp), SCoT-18 (980 bp and 910 bp), and SCoT-28 (1180 bp), along with four negative markers: SCoT-3 (1150 bp), SCoT-13 (870 bp), and SCoT-19 (920 bp and 1000 bp).

Combination Cluster Analysis: To assess the genetic relationships among the accessions, the Jaccard similarity coefficient was used to analyse the marker data. The resulting dendrogram, constructed using the combined SRAP and SCoT genetic similarity coefficients, is presented in Table 5 and Figure 4. The analysis revealed genetic similarities ranging from 0.44 (between G2 and G6) to 0.83 (between G8 and G9). Notably, certain morphological features corresponded to the genetic relatedness determined by the pooled SRAP and SCoT markers. For instance, the distinction between bolting and non-bolting accessions was clear. Additionally, agromorphological characteristics, such as dry weight and shell colour clustered together in the first group, comprise accessions G1, G2, G3, and G5. Furthermore, the phylogenetic trees (Figure 5) indicated the presence of purple garlic genotypes in the second and third groups. The UPGMA clustering results based on the combination of both molecular markers closely resembled those obtained from the SCoT analysis. The accessions were classified into three groups, with eight accessions in group I, five accessions in group II, and one accession in group III. Although the accessions clustered together, the genetic similarity values varied when assessed using different approaches. For example, the genetic similarity between G2 and G6 was 0.38 based on the SCoT molecular markers, while it increased to 0.44 when considering the combination of molecular markers. Similarly, the genetic similarity between G4 and G12 was 0.24 based on the SRAP molecular markers, but it increased to 0.47 when using the combination of molecular markers.

In conclusion, this investigation successfully identified several positive and negative molecular markers associated with bulb weight and yield traits in garlic cultivars. The combination cluster analysis using SRAP and SCoT markers provided valuable insights into the genetic relationships among the accessions. These findings contribute to a better understanding of the genetic basis of economically important traits in garlic and have implications for breeders and farmers seeking to improve crop productivity.

Principal Component Analysis: Principal component analysis (PCA) was applied to the combined raw data of SRAP and SCoT matrix using PASTv4.03 for Windows, and the contribution rates of the first three principal coordinates were 32.9%, 30.1%, and 6.1%. Figure 5 shows that the 15 genotypes fell into three distinct groups. PCA Group 1 comprised 5 genotypes, PCA Group 2 included 4 genotypes, PCA Group 3 included 5 genotypes, and PCA Group 4 included 1 genotype, generally consistent with the results of UPGMA. PCA was also applied to the grades data from the agromorphological traits matrix using PASTv4.03 for Windows, and the contribution rates of the first three principal coordinates were 32.8%, 30.2%, and 11.5% (Figure 6).

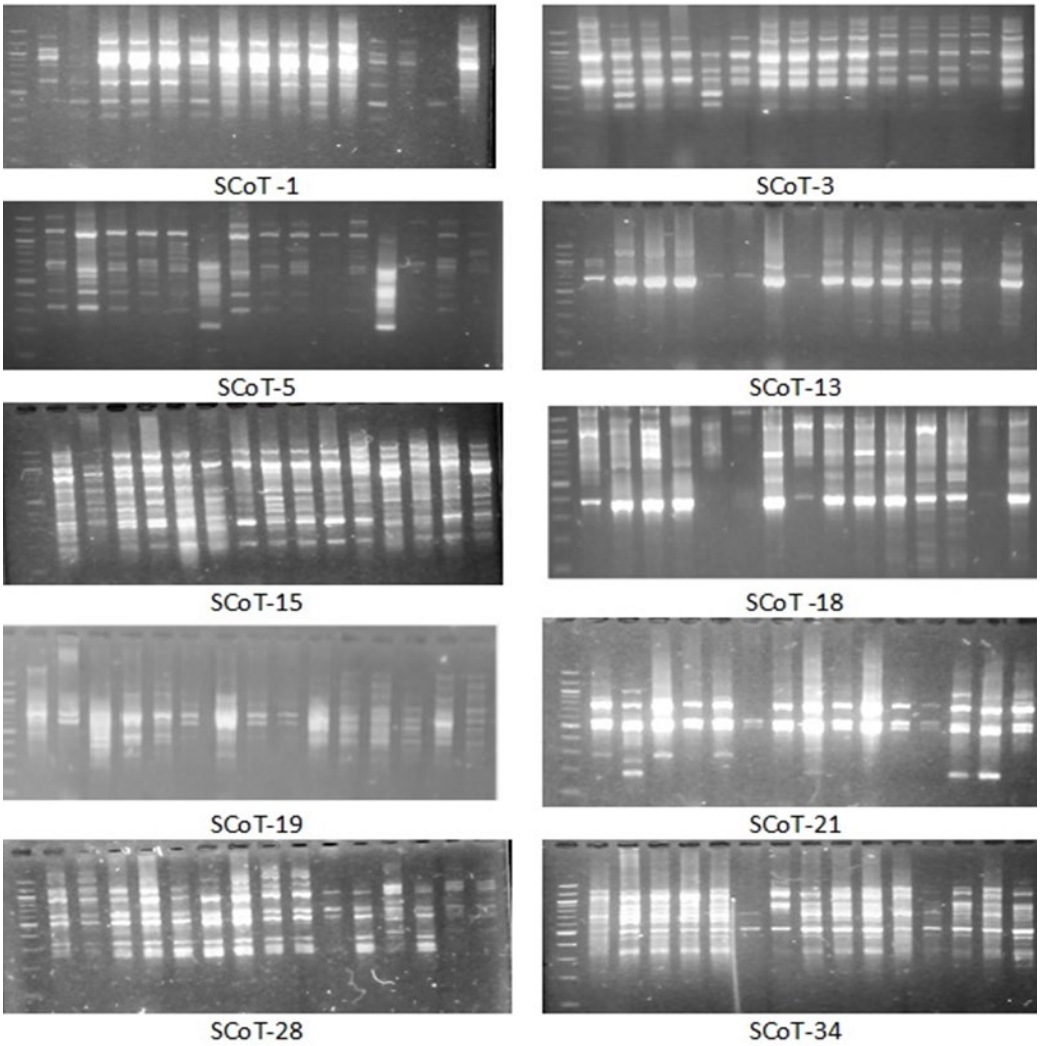

Figure 3. SCoT profiles obtained from 15 garlic genotypes amplified by 10 primers, M = 100 bp ladder size marker.

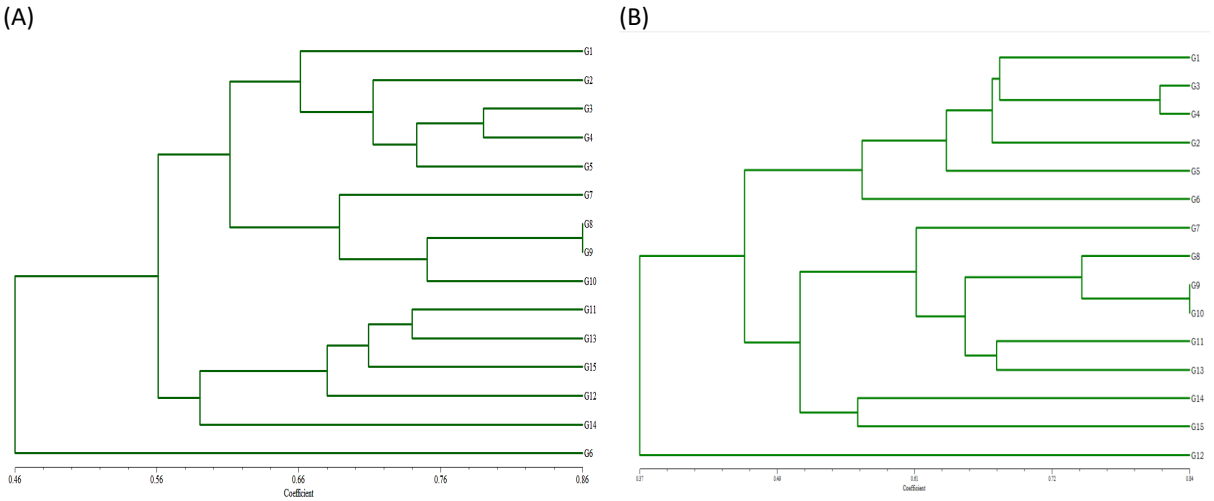

Figure 4. Dendrogram for the 15 garlic genotypes constructed from (a) SCoT and (b) SRAP data using UPGMA and similarity matrix computed.

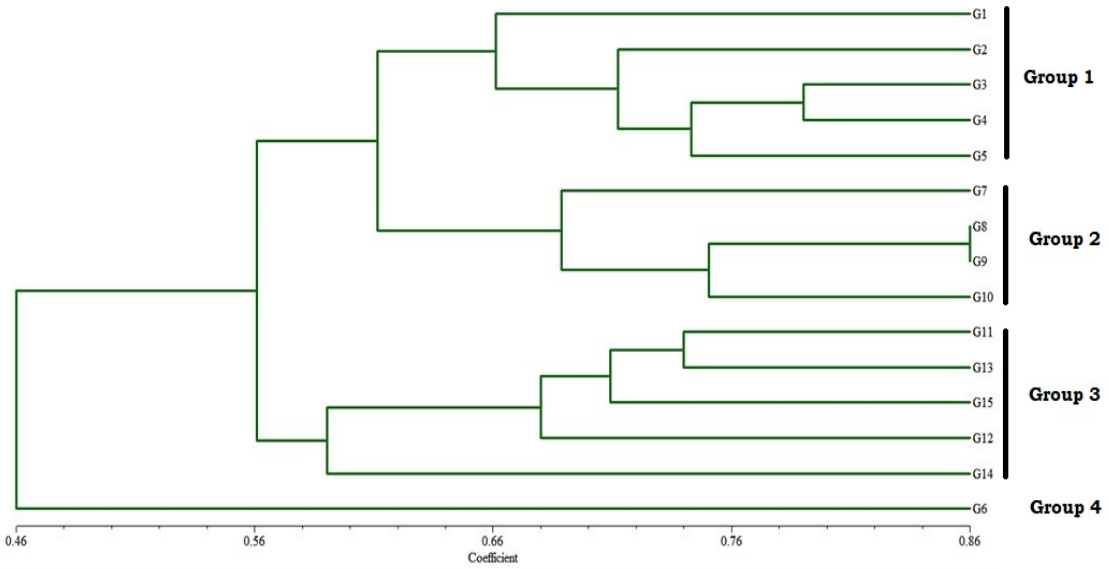

Figure 5. UPGAM dendrogram among the 15 genotypes of garlic used based on Nei's coefficients using SRAP and SCoT combination markers.

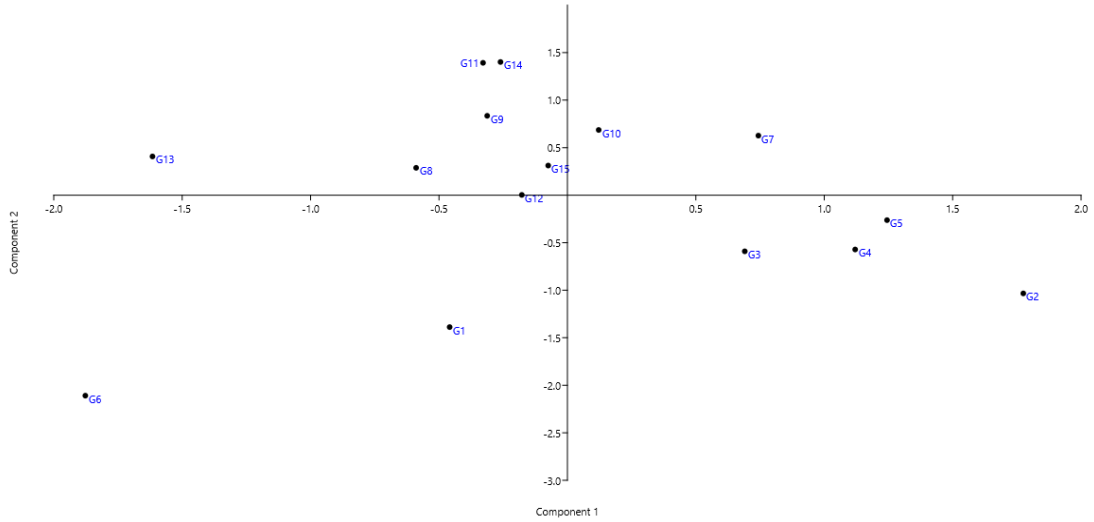

Figure 6. Relationships among principal component analyses based on combined markers and the 8 morphological traits used for 15 genotypes. Four groups were defined based on combined markers: I, II, III, and IV. Group I include 5 genotypes, group II includes 4 genotypes, group III includes 5 genotypes, and group IV includes 1 genotype. The first and the second components account for 32.8% and 30.2% of the total variation, respectively. The numbers correspond to the sample numbers given in Table 1. Percentages in parentheses are the variance of each component.

DISCUSSION

The extensive domestication, selection, adaptability to local climate, agricultural fields, and consumer preferences have led to alterations in several genotypes of garlic. While latitude remains unchanged, other physiological factors such as temperature and light vary with elevation. The evaluation of germplasm resources is crucial for utilizing garlic cultivar diversity effectively, as it serves as a significant foundation for breeding new varieties (Kamenetsky, 2007). It is important to assess genetic resources to ensure their efficient utilization for breeding purposes. Garlic, being a clonally propagated crop, exhibits high levels of uniformity and reproducibility. However, the limited exposure to natural variation in garlic may explain the nonsignificant correlation between genotypes and year for agricultural traits ($p < 0.01$, $p < 0.05$), as reported by Benke et al. (2020). The results of the

Table 4. SCoT primer names (PN), sequences, size ranges (SR), amplified bands (AB), number of polymorphic loci (NPL), unique bands (UB), polymorphism percentage (%), and polymorphic information content (PIC).

No.	PN	Sequence (5'-3')	SR (bp)	AB	NPL	P %	PIC	H	Ne
1	SCot -1	CAACAATGGCTACCACCA	330-1430	13	13	100	0.490	0.49	1.947
2	SCot -3	CAACAATGGCTACCACCG	320-1950	19	17	89.47	0.499	0.65	2.842
3	SCot -5	CAACAATGGCTACCACGA	200-1530	17	17	100	0.484	0.48	1.928
4	SCot -13	ACGACATGGCGACCATCG	270-1580	12	11	100	0.440	0.44	1.8
5	SCot -15	ACGACATGGCGACCGCGA	190-1600	20	19	100	0.492	0.49	1.964
6	SCot -18	ACCATGGCTACCACCGCC	120-1560	61	15	100	0.49	0.49	1.965
7	SCot -19	ACCATGGCTACCACCGGC	220-2020	15	12	93.33	0.498	0.5	1.993
8	SCot -21	ACGACATGGCGACCCACA	165-1280	12	10	83.33	0.489	0.49	1.96
9	SCot -28	CCATGGCTACCACCGCCA	230-1570	14	11	78.57	0.494	0.5	1.99
10	SCot -34	ACCATGGCTACCACCGCA	250-1620	14	10	85.71	0.478	0.48	1.92
	Total			197	135				
	Average			19.7	13.5	93.04	0.485	0.50	2.0309

Table 5. Similarity matrix among 15 garlic genotypes as revealed by combination cluster between SRAP and SCoT markers.

Genotypes	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12	G13	G14	G15
G1	1.00														
G2	0.67	1.00													
G3	0.71	0.74	1.00												
G4	0.65	0.69	0.80	1.00											
G5	0.64	0.63	0.73	0.71	1.00										
G6	0.48	**0.44**	0.50	0.47	0.52	1.00									
G7	0.60	0.59	0.63	0.59	0.59	0.51	1.00								
G8	0.60	0.58	0.58	0.54	0.64	0.56	0.69	1.00							
G9	0.59	0.56	0.55	0.55	0.60	0.56	0.67	**0.83**	1.00						
G10	0.58	0.52	0.57	0.52	0.62	0.52	0.65	0.74	0.80	1.00					
G11	0.54	0.41	0.52	0.54	0.52	0.45	0.59	0.59	0.62	0.64	1.00				
G12	0.54	0.50	0.53	0.47	0.48	0.44	0.51	0.50	0.52	0.52	0.61	1.00			
G13	0.54	0.44	0.51	0.51	0.49	0.45	0.56	0.60	0.63	0.66	0.72	0.66	1.00		
G14	0.53	0.48	0.51	0.51	0.48	0.37	0.48	0.54	0.52	0.57	0.57	0.54	0.61	1.00	
G15	0.56	0.46	0.54	0.49	0.49	0.37	0.54	0.57	0.60	0.58	0.64	0.61	0.65	0.59	1.00

investigation revealed significant genetic diversity among the investigated genotypes at the molecular level, as determined by SRAP and SCoT markers. The SRAP marker showed 100% polymorphism, while the SCoT marker exhibited 93.04% polymorphism. Genetic similarity ranged from a minimum of 0.24 (between G4 and G12), indicating a distant genetic relationship, to a maximum of 0.84 (between G9 and G10), representing the closest genetic relationship. These genotypes originated from Burgash and Zawyat Razin in Giza and Al- Menoufia governorates, respectively. Similar results were obtained using the SCoT marker, with a minimum genetic similarity of 0.38 (between G2 and G6) and a maximum of 0.86 (between G8 and G9). These genotypes originated from Al-Khatahtbeh, Burgash, and Giza. The close genetic relationship observed between genotypes from nearby locations suggests that their similarities may be attributed to shared cultivation practices in similar climates and terrains, as discussed in Chen et al. (2013).

The Polymorphic Information Content (PIC) values for SRAP and SCoT markers were 0.3304 and 0.485, respectively, and the genetic similarity rates ranged from 0.24 to 0.84 for SRAP and 0.38 to 0.86 for SCoT (Tables 3 and 4). Similar results regarding genetic diversity in garlic genotypes have been reported in previous studies that used EST and SSR markers (Zhao et al., 2011; Chand et al., 2015; Kumar et al., 2019; Barboza et al., 2020). These findings are consistent with El-Sayed et al. (2021), who demonstrated genetic similarity ranging from 45% to

95% using SRAP and 57% to 89% using SCoT markers in Egyptian garlic. SRAP and SCoT markers have proven effective in fingerprinting and investigating genetic diversity in garlic (Chen et al., 2013; El-Sayed et al., 2021).

Previous investigation by Li et al. (2007) has also shown high genetic diversity among garlic germplasms. In our investigation, SRAP markers revealed significant allelic variation among the Egyptian garlic germplasms (Balady and Chinese). Additionally, the SRAP cluster analysis indicated a correlation between garlic ecotype and latitude; these results were consistent with Chen et al. 2013. Using SRAP and SCoT markers, we found that genotypes G1, G2, G3, and G4 were genetically close to G5. However, using the SRAP technique, genotypes G3 and G4 were found to be the closest to each other, forming a clade with G1, and all were close to G2, belonging to G5. Conversely, the SCoT results showed that genotypes G3 and G4 were the closest, closely followed by G2, and all were close to G5, with G1 being the most distant. Genotype G2 exhibited greater productivity than the G5 parent but lower productivity than the G1 parent (Table 2). This difference is attributed to the genetic uniqueness of genotype G2 as it belongs to a distinct clade. The clustering of accessions from the same geographic areas using SRAP and SCoT markers suggests the regular exchange of garlic types among various provinces and regions, particularly for a vegetatively propagated crop like garlic.

By analyzing the molecular markers (SCoT and SRAP), the characteristics of bulb head weight as a productive trait can be traced by examining the sequences of the obtained bands to identify the responsible genes (Li et al., 2007). Because molecular markers are a more reliable technique of assessing genetic diversity than agromorphological features, it would be beneficial to create an appropriate link between the two approaches to help plant breeding. Our results using SRAP and SCoT markers indicated that garlic had a lot of genetic variety according to Chen et al. (2012a). We also discovered a link between specific agromorphological features and DNA markers. Cluster analysis using allele frequency data from eight SRAP and 10 SCoT loci divided the accessions into three groups. Principal components analysis (PCA) generated groupings that were congruent with the cluster analysis and demonstrated that these groups had distinct agromorphological features.

CONCLUSION

The present investigation contributes valuable primary data on the collection and preservation of genetic germplasm of Egyptian garlic. The investigation involved the comprehensive morphological and molecular characterization of 15 garlic genotypes obtained from diverse locations across Egypt. This investigation serves as a fundamental step towards facilitating the transfer of garlic germplasm between regions and enables the efficient characterization of extensive garlic collections.

Utilization of SRAP and SCoT markers proved highly effective in distinguishing between different garlic germplasms and played a pivotal role in determining their genetic relationships. These markers have the potential to be further developed for the purpose of assessing the genetic relationships among various garlic genotypes. The correlation between garlic ecotype and latitude analysis revealed genetic similarities ranging from 0.44 (between G2 and G6) to 0.83 (between G8 and G9). Notably, certain morphological features corresponded to the genetic relatedness determined by the pooled SRAP and SCoT markers. Notably, certain morphological features corresponded to the genetic relatedness determined by the pooled SRAP and SCoT markers.

In summary, this investigation has significantly advanced our understanding of Egyptian garlic germplasm, shedding light on its collection and preservation. The findings highlight the importance of morphological and molecular characterization in facilitating the transfer and characterization of garlic germplasm. Moreover, the successful application of SRAP and SCoT markers in discerning genetic variations and relationships among garlic genotypes underscores their utility in future genetic studies.

ABBREVIATIONS

SCoT: start codon targeted marker
SRAP: sequence-related amplified polymorphism marker

ACKNOWLEDGEMENT

The authors would like to express thanks to the Academy of Scientific Research and Technology (ASRT) for funding the national breeding project namely “Towards Breeding of Promising Hybrids and Cultivars of Some Strategic Vegetable Crops,” which was carried out at the National Research Centre in cooperation with the Universities of Alexandria, Al-

Arish, and El-Fayoum. Also, the authors would like to express their appreciation to Dr. Essam M. Helmy and Entsar I. Ragheb, Assistant Professors of Vegetable Breeding, Department of Vegetable Crops, Alexandria University, Egypt. They are breeder rights owners who supplied the garlic genotypes of G2 and G3 used in this investigation. All other genotypes were collected and conserved by first author Prof. Dr. Ahmed AbdAlla Glala at Horticultural Crop Technology Department, Agricultural and Biological Institute, National Research Centre, Dokki, Egypt.

FUNDING

This investigation was funded through the national breeding proeject namely "Towards Breeding of Promising Hybrids and Cultivars of Some Strategic Vegetable Crops," Academy of Scientific Research and Technology (ASRT).

REFERENCES

Aboul-Maaty, N. A. F., and Oraby, H. A. S. (2019). Extraction of high-quality genomic DNA from different plant orders applying a modified CTAB-based method. *Bulletin of the National Research Centre*, *43*(1), 1-10. https://doi.org/10.1186/s42269-019-0066-1.

Adly, W., Abdelkader, H., Mohamed, M., EL-Denary, M., Sayed, E., Fouad, A. (2023). Development of SSR Markers to Characterize Potato (Solanum tuberosum L.) Somaclones with Improved Starch Accumulation. Egyptian Journal of Botany, 63(3), 1173-1185. doi: 10.21608/ejbo.2023.212700.2341

Ahmed, N. E. (2021). EgyGene GelAnalyzer4: a powerful image analysis software for one-dimensional gel electrophoresis. Journal of Genetic Engineering and Biotechnology, 19, 1-7. https://doi.org/10.1186/s43141-020-00114-x

Al-Ghamedi, K., Alaraidh, I., Afzal, M., Mahdhi, M., Al-Faifi, Z., Oteef, M. D. and Khemira, H. (2023). Assessment of genetic diversity of local coffee populations in southwestern Saudi Arabia using SRAP markers. *Agronomy, 13*(2), 302. https://doi.org/10.3390/agronomy13020302

Barboza, K., Salinas, M. C., Acuña, C. V., Bannoud, F., Beretta, V., Garcia-Lampasona, S., and Cavagnaro, P. F. (2020). Assessment of genetic diversity and population structure in garlic (*Allium sativum* L.) germplasm collection varying in bulb content of pyruvate, phenolics, and solids. *Scientia Horticulturae*, *261*, 108900. https://doi.org/10.1016/j.scienta.2019.108900.

Benke, A. P., Nair, A., Krishna, R., Anandhan, S., Mahajan, V., and Singh, M. (2020). Molecular screening of Indian garlic genotypes (*Allium sativum* L.) for bolting using DNA-based Bltm markers. *Vegetable Science*, *47*(01), 116-120.

Chand, S. K., Nanda, S., Rout, E., and Joshi, R. K. (2015). Mining, characterization, and validation of EST-derived microsatellites from the transcriptome database of Allium sativum L. *Bioinformation*, *11*(3), 145. https://doi.org/10.6026%2F97320630011145

Chen, S., Chen, W., Shen, X., Yang, Y., Qi, F., Liu, Y., and Meng, H. (2014). Analysis of the genetic diversity of garlic (Allium sativum L.) by simple sequence repeat and inter simple sequence repeat analysis and agro-morphological traits. *Biochemical Systematics and Ecology, 55*, 260-267. https://doi.org/10.1016/j.bse.2014.03.021.

Chen, SX. J.; Q. Zhou; Y. X. Chen; Du. J. N. Chang and H. W. Meng (2013). Analysis of the genetic diversity of garlic (*Allium sativum* L.) germplasm by SRAP. Biochem. Syst. Ecol., 2013; 50:139- 146. https://doi.org/10.1016/j.bse.2013.03.004.

Collard, B. C., and Mackill, D. J. (2009). Start codon targeted (SCoT) polymorphism: a simple, novel DNA marker technique for generating gene-targeted markers in plants. *Plant molecular biology reporter*, *27*, 86-93. https://doi.org/10.1007/s11105-008-0060-5

CoStat Software (1998). Microcomputer program analysis, CoHort software, Version 6.303, Monterey, CA, USA.

El-Badan, G., Abbas, N., Taia, W., Hassan, A., El-Sadek, L. (2024). Molecular Techniques to Ascertain the Genetic Strategies in Some Malvaceae s.l. Species. Egyptian Journal of Botany, 64(2), 709-726. doi: 10.21608/ejbo.2024.257350.2624

El-Fiki, A., and Adly, M. (2020). Morphological, molecular, and organ sulphur compounds characterization in irradiated garlic (*Allium sativum*) by GC–MS and SCot markers. Journal of Radiation Research and Applied Sciences, 13(1), 61-70. https://doi.org/10.1080/16878507.2019.1697079

El-Sayed, M. A., Merghany, M. M., Samy, M. M., Osman, E. A., and Kabil, F. F. (2021). Induction of new clones of Egyptian garlic (Allium sativum cv. Elbalady) by using chemical mutagens and somatic embryogenesis. Natural Volatiles and Essential Oils Journal (NVEO), 12650-12674. https://www.nveo.org/index.php/journal/issue/view/30

Gomez, K. A. and A. A. Gomez. 1984. Statistical procedures for agriculture research. International Rice Research institute. Textbook (2 ED.): 84-297. https://pdf.usaid.gov/pdf_docs/pnaar208.pdf

Kamenetsky, R. (2007). Garlic: botany and horticulture. *HORTICULTURAL REVIEWS-WESTPORT THEN NEW YORK-*, *33*, 123. https://doi.org/10.1002/9780470168011.ch2

Khatab, I., El-Mouhamady, A., Mariey, S. (2021). Comprehensive Selection Criteria for High-Yielding Bread Wheat (Triticum aestivum L.) Hybrids under Salinity Stress. Egyptian Journal of Botany, 61(3), 709-730. doi: 10.21608/ejbo.2021.65333.1637

Kumar, M., Rakesh Sharma, V., Kumar, V., Sirohi, U., Chaudhary, V., Sharma, S. and Sharma, S. (2019).

Genetic diversity and population structure analysis of Indian garlic (*Allium sativum* L.) collection using SSR markers. *Physiology and molecular biology of plants*, *25*, 377-386. https://doi.org/10.1007/s12298-018-0628-y

Kurnia, D., Ajiati, D., Heliawati, L., and Sumiarsa, D. (2021). Antioxidant properties and structure-antioxidant activity relationship of Allium species leaves. Molecules, 26(23), 7175. https://doi.org/10.3390/molecules26237175

Li, G., and Quiros, C. F. (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. https://doi.org/10.1007/s001220100570

Li, H.Z., Yin, Y.P., Zhang, C.Q., Zhang, M., Li, J.M. (2007). Evaluation of application of SRAP on analysis of genetic diversity in germplasms of *Allium fistulosum* L. Acta Hort. Sinica 34 (4), 929–934. https://www.ahs.ac.cn/EN/Y2007/V34/I4/929

Li, X., Qiao, L., Chen, B., Zheng, Y., Zhi, C., Zhang, S., and Cheng, Z. (2022). SSR markers development and their application in genetic diversity evaluation of garlic (*Allium sativum*) germplasm. Plant Diversity, 44(5), 481-491. https://doi.org/10.1016/j.pld.2021.08.001

Zhao, W. G., Chung, J. W., Lee, G. A., Ma, K. H., Kim, H. H., Kim, K. T., and Park, Y. J. (2011). Molecular genetic diversity and population structure of a selected core set in garlic and its relatives using novel SSR markers. *Plant Breeding*, *130*(1), 46-54. https://doi.org/10.1111/j.1439-0523.2010.01805.x.

Nei, M. (1973). Analysis of gene diversity in subdivided populations. *Proceedings of the national academy of sciences*, *70*(12), 3321-3323. https://doi.org/10.1073/pnas.70.12.3321

Rohlf, F. (2000). NTSYSpc: numerical taxonomy and multivariate system. Version 2.1 Exeter software. *Applied Biostatics Inc., New York*.

Volk, G. M., and Stern, D. (2009). Phenotypic characteristics of ten garlic cultivars grown at different North American locations. Hort. Science, *44*(5), 1238-1247. https://doi.org/10.21273/HORTSCI.44.5.1238

Voytas, D. (2000). Agarose gel electrophoresis. *Current Protocols in Molecular Biology*, *51*(1), 2-5. https://doi.org/10.1002/0471142727.mb0205as51

Xie, Q., Liu, Z.H., Wang, S. H. and Li, Z.Q. (2015). Genetic diversity and phylogenetic relationships among five endemic Pinus taxa (*Pinaceae*) of China as revealed by SRAP markers. *Biochemical Systematics and Ecology, 62*, 115-120. https://doi.org/10.1016/j.bse.2015.08.005

Zaki, H., El-Tayeh, N., Abo El Hagag, F., Youssef, N. (2023). Correlation between Genetic- physiological Traits of Egyptian Lupin (Lupinus termis) Induced by Drought Stress. Egyptian Journal of Botany, 63(3), 1141-1154. doi: 10.21608/ejbo.2023.191189.2245