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Molecular genetic assessment of some potato (*solanum tuberosum*) cultivars.

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Abstract
Background: Potato (*Solanum tuberosum* L.) is one of the most important food crops globally and in Egypt. Understanding the genetic diversity among cultivars is crucial for enhancing yield, stress tolerance, and disease resistance. Molecular markers such as SCoT and ISSR provide reliable tools for assessing this diversity at the DNA level. **Methods:** Five potato cultivars (Caruso, Diamant, Lady Rositta, Santana, and Spunta) commonly grown in Egypt were analysed using six SCoT and five ISSR primers. DNA was extracted from young leaves, amplified via PCR, and the resulting bands were analysed to determine polymorphism rates and genetic similarity. **Results:** The SCoT markers generated 18 bands, with 50% polymorphism, while ISSR primers yielded 22 bands with 45.45% polymorphism. Combined analysis revealed a total of 40 bands, 19 of which were polymorphic, resulting in an overall polymorphism rate of 47.5%. The genetic similarity ranged from 0.784 to 0.909, and unique bands specific to certain cultivars were identified. **Conclusion:** The use of SCoT and ISSR markers effectively distinguished genetic variation among the studied cultivars. These findings support their application in cultivar identification and breeding programs to enhance potato genetic improvement in Egypt.

Keywords: Potato (*Solanum tuberosum*), genetic diversity, SCoT markers, ISSR markers, molecular characterization.

1. Introduction

The potato *Solanum tuberosum* L., a member of the Solanaceae family, is one of the world's most significant vegetable crops, with an annual production of around 385 million tons. This tetraploid plant has a basic chromosomal number of 12 (x = 12). In Egypt, potato cultivation holds a strategic economic position, as the country is considered one of the leading producers and exporters of potatoes in the region [1]. Understanding the genetic diversity of local cultivars is particularly vital considering challenges such as climate change, emerging diseases, and evolving consumer demands [2]. Traditional methods of cultivar evaluation, although valuable, are often influenced by environmental conditions and developmental stages, limiting their reliability [3]. In contrast, molecular marker technologies, such as ISSR and SCoT, offer environment-independent, precise tools for characterizing genetic variation at the DNA level [4]. These tools not only accelerate breeding programs but also provide critical insights necessary for the conservation of genetic resources and the development of improved cultivars with enhanced yield, quality, and stress tolerance traits [2].

Potatoes rank as the third most significant food crop in the world and are the main non-grain food crop. They play a significant role in food security and in raising farmers' earnings. The crop is well-adapted to varied environmental circumstances [5] and is rich in important nutrients [6]. In many potato-producing nations, tubers are stored at cold temperatures (approximately 4°C) after harvest to maintain a year-round supply for both industrial processing and consumer markets [7].

Various methodologies have been applied to evaluate potato cultivars, including morphological, physiological, and biochemical examinations, along with DNA markers. For instance, Simple Sequence Repeats (SSRs) were applied in France [8], microsatellites in Brazil [9], and RAPD analysis in Pakistan [10]. Molecular markers are strong tools for analysing genetic diversity, enabling breeders to select desirable features that lead to enhanced agricultural output and quality. These markers, such as ISSR and RAPD, have shown efficiency in measuring genetic diversity across multiple plant species [11], [12], [13]. Among these, Start Codon Targeted (SCoT) markers have shown advantages over other dominant DNA marker systems due to their higher polymorphism rates and superior resolution, as demonstrated in multiple studies, including those by Mohamed et al. in apricot strains [14], Abd El-Aziz et al. in tomato [15], Abd El-Hadi et al. in squash [16], Awad et al. in local apricot lines [17], Safaa et al. in deciduous rootstocks [18], and Abd El-Aziz et al. in apricot rootstocks [19].

The SCoT technique has been effectively utilized for cultivar identification and genetic diversity analysis across numerous plant species, including crops like barley [20] and fruit trees such as mango [21], grapes [22], date palm [23], and pomegranate [24]. In the case of pomegranate, numerous DNA markers have been applied to examine genetic variation and relationships, including ISSR [25], AFLP [26], and SCoT [27].

The primary objective of this study is to evaluate the molecular genetic diversity and genetic relationships among five commercially cultivated potato (*Solanum tuberosum* L.) cultivars in Egypt

(Caruso, Diamant, Lady Rositta, Santana, and Spunta), utilizing Start Codon Targeted (SCoT) and Inter Simple Sequence Repeat (ISSR) molecular markers. A total of six SCoT and five ISSR primers were employed. Construct a dendrogram to illustrate the genetic relationships among the tested cultivars. Identify unique molecular markers that can be used for cultivar identification and future breeding programs.

2. Materials and Methods

Plant Materials

This study was carried out in the Department of Genetics and Genetic Engineering Labs, Faculty of Agriculture, Banha University, Egypt. Five cultivars of potato (*Solanum tuberosum* L.) that are commonly cultivated in Egypt were selected for genetic analysis. These cultivars were obtained from the Dakahlia Agricultural Development Company and included the following:

1. Caruso
2. Diamant
3. Lady Rositta
4. Santana
5. Spunta

Molecular Genetic Markers

The aim was to assess the genetic diversity among these cultivars using ISSR and SCoT molecular marker techniques. These marker systems have proven effective for detecting polymorphism and evaluating genetic relationships in various plant species [4], [3], and [12].

DNA Isolation

Genomic DNA was extracted from freshly harvested young leaves using the DNeasy Plant Mini Kit (Bio Basic). The quality and purity of the DNA were assessed by measuring absorbance ratios (A260/A280) with a UV spectrophotometer. DNA samples with A260/A280 values ranging from 1.8 to 2.0 were considered pure. Additionally, the integrity of the extracted DNA was verified through electrophoresis on a 1% agarose gel stained with ethidium bromide [3].

Polymerase Chain Reaction (PCR)

The extracted genomic DNA served as a template for PCR amplification with six SCoT primers (Table 1) and five ISSR primers (Table 2) to assess the genetic diversity among the five potato cultivars. The primers used were purchased from Applied Biotechnology Company. SCoT primers were designed based on the conserved ATG start codon region, providing gene-targeted polymorphic markers [4], while ISSR primers targeted inter-microsatellite regions, known for their high reproducibility and discriminatory power [17], [18].

Table 1: Name, sequence and expected bands range size of Scot primers used in the current study

Primer name	Sequences	Range Size bp
SCoT 1	ACG ACA TGG CGA CCA CGC	200-930
SCoT 4	ACC ATG GCT ACC ACC GCA	200-900
SCoT 5	CAA TGG CTA CCA CTA GCG	220-340
SCoT 7	ACA ATG GCT ACC ACT GAC	230-360
SCoT 8	ACA ATG GCT ACC ACT GAG	260-325
SCoT 9	ACA ATG GCT ACC ACT GCC	330-410

Table 2: Name, sequence and expected bands range size of ISSR primers used in the current study

Primer name	Sequences	Range Size bp
ISSR3	5` ACA CAC ACA CAC ACA CT 3`	280-455
ISSR11	5` ACA CAC ACA CAC ACA CC 3`	285-460
ISSR15	5` CTC TCT CTC TCT CTC TG 3`	260-925
UBC820	5´ GTG TGT GTG TGT GTG TC 3`	230-1340
UBC850	5` ACA CAC ACA CAC ACA CA 3`	380-915

PCR Conditions:

SCoT primers were obtained from Operon Technologies, Alameda, USA. These primers were designed based on the consensus sequences reported in previous studies by Collard and Mackill [1] and Mohamed et al. [14], and synthesized by Bio Basic Inc. All SCoT primers were 18-mer oligonucleotides derived from Dataset I, targeting highly expressed genes as described by Sawant et al. [66]. The core design of the SCoT primers included a conserved start codon (ATG) at positions +1 to +3, followed by a fixed nucleotide sequence: ‘G’ at +4, ‘C’ at +5, and ‘A’, ‘C’, ‘C’, and ‘A’ at positions +7 to +10, respectively (5′–ATGGCTACCA–3′).

PCR amplification for both ISSR and SCoT markers was conducted according to the protocols outlined by Fathi et al. [31] and Xiong et al. [66], respectively. Reactions were performed in a Techni TC-512 Thermal Cycler under the following conditions:

- Initial denaturation at 94°C for 4 minutes
- 40 cycles of:
  - Denaturation at 94°C for 1 minute
  - Annealing at 47–50°C depending on the primer
  - Extension at 72°C for 2 minutes
- Final extension at 72°C for 10 minutes
- Holding at 4°C for storage

Gel Documentation and Data Analysis:

The resultant DNA banding patterns were observed and photographed using the Bio-1D Gel Documentation System. The banding profiles were examined with Gel Analyzer 3 software. Clear and distinct bands were scored manually as either present (1) or absent (0) for each primer, yielding a binary data matrix.

This matrix was used to create DNA profiles for both ISSR and SCoT markers, following the methods of Adhikari et al. [9]. Based on the binary data, molecular dissimilarity (genetic distances) was determined using the Dice coefficient [33]. Cluster analysis was then performed using agglomerative hierarchical clustering (AHC), utilizing the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) via PAST software.

3. Results

SCoT-PCR Analysis

The results obtained from the SCoT-PCR analysis demonstrated a moderate level of genetic diversity among the five studied *Solanum tuberosum* cultivars (Figure 1). The amplification of 18 bands across six primers, with molecular weights ranging between 200 and 930 bp, is consistent with the expected band size range previously reported in potato and other Solanaceae species [4].

The overall polymorphism percentage of 50% indicates a significant level of genetic variation within the tested material, although it also suggests that some cultivars may share a large portion of their genome (Table 3). The variation in polymorphism percentages among primers, which range from 33.33% (SCoT7) to 80% (SCoT1), highlights the differing ability of individual primers to target polymorphic loci. The high polymorphism identified with SCoT1 suggests that this primer may target genomic regions that are rich in genetic diversity, making it especially useful for diversity studies. In contrast, the lower polymorphism observed with SCoT7 implies that this primer amplifies more conserved regions among the studied cultivars.

The finding that primer SCoT5 produced the highest number of bands (five) further confirms its broad genomic coverage and efficiency. Conversely, primers SCoT8 and SCoT9, which generated only two bands each, indicate either limited binding sites or higher specificity for conserved genomic sequences. This variation in amplification efficiency among primers is a common observation in SCoT marker studies [21], [23].

The detection of nine monomorphic bands points to genomic regions that are conserved across the five cultivars, which may be associated with essential functions such as tuber development, disease resistance, or stress tolerance. In contrast, the identification of eight unique bands is particularly noteworthy, as they represent cultivar-specific genetic markers. These unique bands can serve as valuable tools for cultivar identification, protection of plant breeder rights, and the selection of parental lines in breeding programs.

The relatively moderate level of polymorphism detected aligns with the clonally propagated nature of potatoes, where limited genetic recombination occurs. However, it also suggests that despite clonal propagation, somatic mutations and historical hybridizations have introduced a reasonable amount of variability.

Comparing these findings with previous studies in potato genetic diversity using molecular markers [10], [8], it is evident that SCoT markers provide a reliable and efficient alternative to traditional SSR or RAPD markers. The ability of SCoT markers to reveal both polymorphic and monomorphic bands makes them highly informative for comprehensive genetic diversity assessments.

Overall, the current SCoT analysis emphasizes the importance of molecular approaches in elucidating the genetic structure of potato cultivars grown in Egypt. These insights are critical for developing effective breeding strategies aimed at improving yield, quality traits, and adaptability to environmental stresses, especially in the context of climate change and increasing disease pressure.

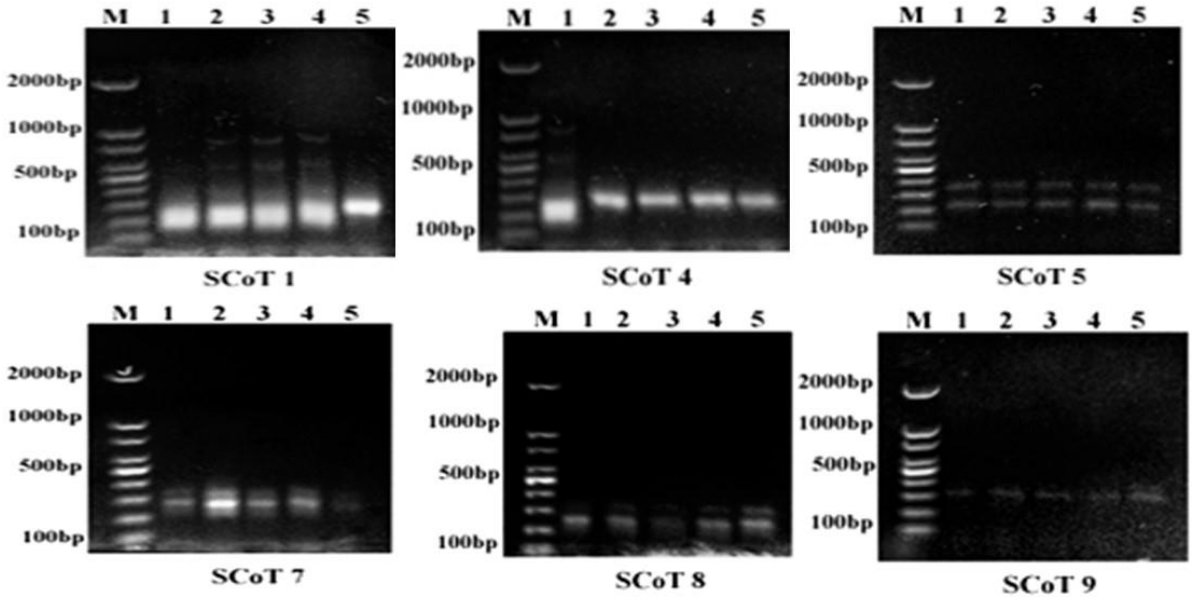

Fig. (1) Banding patterns of SCoT-PCR products for five potatoes Solanum tuberosum cultivars: 1, Caruso; 2, Diamant; 3, Lady Rositta; 4, Santana 5, Spunta generated with six primers. M refers to ABT 100bp DNA ladder (Applied Biotechnology).

Table 3: Banding patterns data as estimated for five Potato *Solanum Tuberosum* cultivars using SCoT technique.

Primer Name	Sequences	Range Size bp	Total bands	Monomorphic Bands	Polymorphic bands	Unique bands	Polymorphic %
SCoT 1	ACG ACA TGG CGA CCA CGC	200-930	5	1	4	3	80%
SCoT 4	ACC ATG GCT ACC ACC GCA	200-900	4	1	3	3	75%
SCoT 5	CAA TGG CTA CCA CTA GCG	220-340	2	2	-	-	-
SCoT 7	ACA ATG GCT ACC ACT GAC	230-360	3	2	1	1	33.33%
SCoT 8	ACA ATG GCT ACC ACT GAG	260-325	2	2	-	-	-
SCoT 9	ACA ATG GCT ACC ACT GCC	330-410	2	1	1	1	50%
Total			**18**	**9**	**9**	**8**	**50%**

ISSR-PCR Analysis

In this study, molecular genetic diversity among the five selected *Solanum tuberosum* cultivars was assessed using ISSR-PCR markers. Five ISSR primers were used to amplify a total of 22 bands, with fragment sizes ranging from 230 to 1340 bp (Figure 2 and Table 4). This size range is consistent with those reported in previous studies that employed ISSR markers for genetic diversity analysis in potato and other plant species. These results highlight the effectiveness of ISSR technology in detecting genetic diversity in plant species, including potato, through simple sequence repeat-based amplification.

Overall, 10 of the bands were polymorphic, resulting in a polymorphism percentage of 45.45%. This indicates a moderate level of genetic variability among the cultivars studied, which aligns with previous research showing that although potato is a clonally propagated species, substantial genetic diversity can still be detected using molecular markers [41]. The observed level of genetic variability reflects the natural genetic variation that arises from somatic mutations, as well as from selective breeding practices aimed at improving specific traits.

There was notable variation in polymorphism rates across the primers used. The highest polymorphic rate (75%) was observed with primer ISSR3, indicating that this primer targets highly variable regions of the potato genome. Conversely, primer ISSR15 showed the lowest polymorphism rate (20%), suggesting it likely amplifies more conserved DNA sequences among the cultivars.

This primer-specific variation in polymorphism levels has also been seen in earlier studies on potato genetic diversity [8], emphasizing the importance of choosing primers based on the target genomic regions.

Regarding amplification efficiency, primer UBC820 generated the highest number of bands (9), demonstrating its broad genome coverage and ability to bind efficiently to diverse regions of the potato genome. In contrast, primers ISSR3 and UBC850 produced the fewest bands (2 each), suggesting that these primers may target more specific and conserved genomic regions, which may contribute to their lower band production. Such primer-dependent variation in amplification efficiency has been reported in other plant species as well [23].

An important outcome of this analysis was the identification of 12 monomorphic bands, which are highly conserved across all cultivars, and 6 unique bands, which are specific to certain cultivars. These unique bands are valuable for cultivar identification, as they serve as molecular fingerprints that can be used for distinguishing between different cultivars. The presence of these unique bands is consistent with findings from other studies, where cultivar-specific markers have been identified as essential tools for molecular fingerprinting and variety protection [59].

When comparing the ISSR results with the findings from the SCoT analysis conducted in this study, it is evident that the SCoT markers exhibited a higher level of polymorphism. This suggests that the use of multiple molecular marker systems, such as ISSR and SCoT, provides a more comprehensive assessment of genetic diversity. This approach, combining both techniques, improves the reliability of genetic relationship analyses among the studied cultivars [41].

In conclusion, the ISSR-PCR analysis confirmed that ISSR markers are a highly efficient, reproducible, and cost-effective tool for assessing genetic diversity in potato cultivars. These results contribute to the growing body of knowledge on the genetic structure of commercial potato cultivars and provide essential information for breeding programs aimed at improving the resilience, productivity, and adaptability of potato crops under varying environmental conditions.

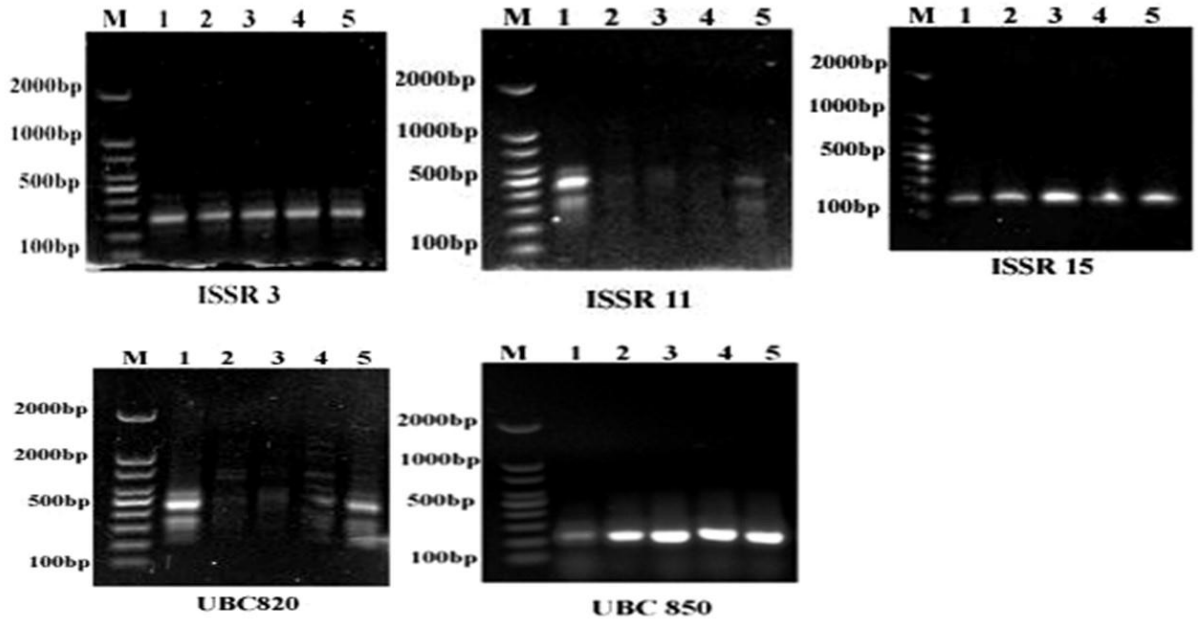

Fig. 2: Banding patterns of ISSR-PCR results for five potato *Solanum tuberosum* cultivars: cultivars 1, Caruso 2, Diamant 3, Lady Rositta 4, Santana 5, Spunta produced using five primers. M refers to ABT100bp DNA ladder (Applied Biotechnology).

Table 4: Molecular banding patterns data estimated for five Potato *Solanum Tuberosum* cultivars using ISSR technique.

Primer Name	Sequences	Range Size bp	Total bands	Monomorphic bands	Polymorphic bands	Unique bands	Polymorphic %
ISSR3	**5` ACA CAC ACA CAC ACA CT 3`**	**280-455**	2	2	-	-	-
ISSR11	**5` ACA CAC ACA CAC ACA CC 3`**	**285-460**	4	1	3	1	75%
ISSR15	**5` CTC TCT CTC TCT CTC TG 3`**	**260-925**	5	4	1	1	20%
UBC820	**5´ GTG TGT GTG TGT GTG TC 3`**	**230-1340**	9	3	6	4	44.44%
UBC850	**5` ACA CAC ACA CAC ACA CA 3`**	**380-915**	2	2	-	-	-
	Total		**22**	**12**	**10**	**6**	**45.45%**

Comprehensive Analysis of Genetic Diversity in Solanum tuberosum Cultivars Using SCoT and ISSR Markers

The combined molecular analysis of genetic diversity among five Solanum tuberosum cultivars, utilizing both SCoT (Start Codon Targeted) and ISSR (Inter Simple Sequence Repeat) primers, offers significant insights into the genetic makeup of these potato varieties (Table 5). The analysis yielded a total of 40 bands across all primers employed, consisting of 19 polymorphic bands and 21 monomorphic bands, resulting in an overall polymorphism percentage of 47.5%. Moreover, 14 unique markers were identified, further underscoring the genetic distinctiveness among the potato cultivars under study.

Table 5: Polymorphic, Monomorphic, Specific Markers and Polymorphic percentage generated by the (SCoT and ISSR) analysis for five Potato Solanum Tuberosum cultivars.

Primer Name	Total Band	Monomorphic Band	Polymorphic band	Unique Band	Polymorphic %
SCoT	**18**	**9**	**9**	**8**	**50%**
ISSR	**22**	**12**	**10**	**6**	**45.45%**
Total	40	21	19	14	47.5%

Genetic Diversity and Polymorphism:

The observed level of polymorphism suggests that the potato cultivars exhibit notable genetic variation, which is a key factor for their adaptability and breeding potential. The polymorphism percentage of 47.5% indicates a moderate level of genetic diversity, which is essential for plant breeding, as it enables the selection of traits that enhance crop yield, disease resistance, and environmental adaptability. These findings align with those reported by Zietkiewicz et al. [60], who utilized SSR markers to assess genetic variation in various plant species, and Martínez et al. [41], who highlighted the role of molecular markers in the characterization of potato cultivars.

The identification of 14 unique markers among the cultivars is particularly noteworthy. These unique markers are specific to certain cultivars, providing a molecular fingerprint that is useful for cultivar identification and the management of plant genetic resources. The ability to distinguish between cultivars using molecular markers is crucial for seed certification, variety protection, and maintaining genetic purity, as emphasized by Bornet and Branchard [17]. These unique markers can also serve as powerful tools for tracking genetic variation in breeding programs, ensuring that new cultivars are selected based on both genetic diversity and desirable traits.

Marker System Comparison: SCoT vs. ISSR:

The use of both SCoT and ISSR markers in this study provides a comprehensive understanding of the genetic diversity within the potato cultivars. SCoT markers, derived from functional regions of the genome, are particularly effective in detecting polymorphisms associated with gene expression. These markers allow for a deeper understanding of the functional genetic variation that may directly influence agronomically important traits, such as disease resistance or stress tolerance. The utility of SCoT markers in plant breeding is well documented, with studies by Mohamed et al. [35]

on apricot and Abd El-Aziz et al. [13] on potato rootstocks demonstrating their effectiveness in evaluating genetic diversity and identifying functional genomic regions associated with specific traits.

ISSR markers, on the other hand, primarily target non-coding regions of the genome, but they have proven to be valuable in detecting broader genetic variation across different plant species, including potato. While the ISSR markers in this study generated a higher number of monomorphic bands, they still provided important insights into conserved genetic regions of the cultivars, as noted by Esselman et al. [53], who explored genetic diversity using ISSR markers in various plant species. The combination of SCoT and ISSR markers allows for a more holistic view of genetic diversity, with SCoT focusing on functional genomic regions and ISSR providing broader genomic coverage.

Genetic Divergence and Breeding Implications:

The substantial level of genetic divergence observed in this study suggests that the potato cultivars are genetically distinct from one another. Such genetic divergence is critical for breeding programs, as it opens the possibility of selecting genetically diverse parental lines that can be crossed to produce offspring with superior traits. Genetic diversity is a fundamental resource for improving traits such as disease resistance, tuber quality, and environmental adaptability—traits that are essential for enhancing potato production under varying climatic conditions.

Additionally, the complementary nature of both SCoT and ISSR markers highlights their collective power in molecular breeding. By combining both marker systems, researchers and breeders can gain a more detailed understanding of the genetic variation present in the cultivars, enabling the selection of parental lines with both functional and broad genetic diversity. This integrated approach has been increasingly adopted in various crops, as seen in the work of Dora et al. [20] and Luo et al. [21], who successfully applied SCoT markers in barley and mango, respectively, to assess genetic diversity and support breeding efforts.

Genetic Similarity Analysis Based on SCoT, ISSR, and Combined Data:

The genetic diversity of the five *Solanum tuberosum* cultivars, evaluated through the application of SCoT and ISSR molecular markers, reveals important insights into their genetic relationships. This combined molecular analysis not only emphasizes the complementary nature of both marker systems but also enhances the understanding of the genetic diversity present within the potato cultivars. The Dice similarity coefficient was used to calculate genetic similarity indices, and the resulting data were analyzed to determine molecular distances between the cultivars.

SCoT-Based Genetic Similarity

SCoT markers, derived from functional regions of the genome, offer a distinct advantage in revealing genetic polymorphisms associated with gene expression, making them particularly valuable for understanding the molecular basis of traits related to productivity and stress tolerance. In this study, SCoT-based genetic distances ranged from 0.72 (between Caruso and Spunta) to 1.0 (between Lady Rositta and Santana). The minimum genetic distance of 0.72 indicates substantial genetic divergence between Caruso and Spunta, which may reflect different genetic backgrounds or breeding histories. Conversely, the maximum genetic distance of 1.0 between Lady Rositta and Santana suggests that these two cultivars have nearly identical genetic profiles according to the markers tested, which could indicate shared ancestry or similar selection pressures during breeding (Table 6).

These findings are consistent with those reported by Mohamed et al. [35], who applied SCoT markers to assess genetic diversity in apricot cultivars and demonstrated the ability of this marker system to identify both closely related and genetically distant varieties. Similarly, Abd El-Aziz et al. [15] reported that SCoT markers effectively distinguished between tomato cultivars with high genetic resolution, reinforcing the robustness of this technique for characterizing genetic variation in a wide range of crops.

Table 6. **Molecular distances (MD) between five** Potato Solanum Tuberosum **cultivars based on Dice-dissimilarity index for SCoT data.**

	Caruso	**Diamant**	**Lady Rositta**	**Santana**	**Spunta**
Caruso	1				
Diamant	0.827	1			
Lady Rositta	0.857	0.962	1		
Santana	0.857	0.962	1	1	
Spunta	0.72	0.75	0.782	0.782	1

ISSR-Based Genetic Similarity

The genetic distances calculated based on ISSR markers revealed variability in the genetic relationships among the potato cultivars. Genetic similarity indices ranged from 0.787 (between Diamant and Spunta) to 0.914 (between Santana and Spunta). The ISSR results further emphasize the genetic closeness between Santana and Spunta

(0.914), suggesting these cultivars may share a recent common genetic origin or have been bred using similar parental lines. On the other hand, the genetic distance of 0.787 between Diamant and Spunta points to more significant genetic divergence, possibly due to differences in their breeding history or adaptation to distinct environments (Table 7).

Table 7. **Molecular distances (MD) between five Potato Solanum Tuberosum cultivars based on Dice-dissimilarity index for ISSR data.**

	Caruso	Diamant	Lady Rositta	Santana	Spunta
Caruso	1				
Diamant	0.827	1			
Lady Rositta	0.812	0.848	1		
Santana	0.903	0.812	0.857	1	1
Spunta	0.875	0.787	0.888	0.914	

Combined SCoT and ISSR Data

ISSR markers have been widely used in potato breeding and other crops for their ability to detect genetic variation without requiring prior sequence information [53]. Their application in potato cultivar characterization has significantly contributed to elucidating genetic relationships and understanding the ancestry of different varieties. For instance, Martínez et al. [41] utilized ISSR markers to evaluate the genetic diversity of potato cultivars and reported notable genetic differentiation among them, highlighting the effectiveness of ISSR markers even in crops subjected to intensive breeding and selection pressures.

To achieve a more comprehensive and robust assessment of genetic diversity, this study integrated the data derived from both SCoT and ISSR markers. The combined molecular analysis enabled the estimation of genetic similarity indices among the five *Solanum tuberosum* cultivars, providing a nuanced understanding of their genetic relationships. The similarity values ranged from 0.784 (between Diamant and Spunta) to 0.909 (between Lady Rositta and Santana), as shown in Table 8.

The high genetic similarity recorded between Lady Rositta and Santana (0.909) confirms the close genetic relationship between these two cultivars, possibly reflecting shared breeding lineages or parallel selection for similar agronomic traits. This close similarity is consistent with the SCoT-only data and is further reinforced by the combined marker analysis, underscoring the reliability of these molecular approaches in revealing true genetic proximity.

Conversely, the lower genetic similarity between Diamant and Spunta (0.784) emphasizes their distinct genetic makeup, which can be exploited in breeding programs to increase heterozygosity and broaden the genetic base of future hybrid combinations. Such information is critical for parental selection strategies in potato improvement programs, as crossing genetically distant cultivars often leads to progeny with superior agronomic performance due to heterosis.

Overall, the integration of SCoT and ISSR data enhances the resolution of genetic relationship analyses, offering a balanced representation of both functional (gene-based) and structural (repeat-based) genomic variation. This dual-marker strategy not only improves the accuracy of cultivar differentiation but also provides breeders with essential tools for optimizing germplasm selection and designing effective breeding schemes, as also supported by previous studies in other crops [20], [21].

Table 8. **Molecular distances (MD) between five Potato Solanum Tuberosum cultivars based on Dice-dissimilarity index for SCoT and ISSR combined data.**

	Caruso	Diamant	Lady Rositta	Santana	Spunta
Caruso	1				
Diamant	0.84	1			
Lady Rositta	0.830	0.905	1		
Santana	0.884	0.884	0.909	1	
Spunta	0.823	0.784	0.851	0.867	1

Advantages of Combining SCoT and ISSR Markers

The advantage of combining SCoT and ISSR markers lies in their complementary roles in assessing plant genetic diversity. While SCoT markers target gene-rich regions and provide insights into functional polymorphisms associated with key agronomic traits, ISSR markers amplify non-coding regions, offering a broader and more neutral perspective of genomic variation [20], [21]. This dual-marker strategy thus ensures a comprehensive overview of both functional and neutral genetic diversity—an approach that is particularly valuable in crops like *Solanum tuberosum*, where maintaining variability is crucial for adaptation, resilience, and productivity.

The higher resolution achieved by combining both marker systems improves the accuracy of genetic similarity studies. This is especially important for molecular breeding programs, where identifying genetically diverse parent lines helps maximize heterosis and enhances trait heritability across generations. Dora et al. [20] and Luo et al. [21] have previously highlighted the effectiveness of this integrated approach, confirming its superiority over single-marker systems.

4. Discussion

Implications for Potato Breeding and Genetic Improvement

The results of this study hold practical implications for the future of potato breeding. For instance, the pronounced genetic divergence between Caruso and Spunta indicates their potential as complementary parental lines for crossing, offering the possibility of enhancing genetic variability in breeding populations. In contrast, the genetic closeness between Lady Rositta and Santana suggests that these cultivars could serve as sources of uniform traits such as disease resistance or tuber yield, which could be selectively maintained or improved through backcrossing or marker-assisted selection.

Moreover, the identification of 14 unique molecular markers across cultivars presents opportunities for cultivar authentication and protection of breeder rights. These markers can act as molecular fingerprints, helping to preserve cultivar identity in seed certification schemes and germplasm conservation strategies [53], [41]. Maintaining such genetic records is essential for traceability, intellectual property management, and the long-term sustainability of breeding programs.

The use of molecular markers also facilitates the pyramiding of desirable traits and shortens the breeding cycle, offering breeders a powerful toolkit to respond more rapidly to biotic and abiotic stresses. In the context of climate change, such precision breeding approaches are indispensable for developing cultivars with improved drought tolerance, heat resistance, and resistance to emerging pathogens.

Genetic Similarity and Cluster Analyses Based on Molecular Markers

The genetic similarity matrix derived from both SCoT and ISSR data provided an insightful assessment of the relationships among the five potato cultivars. Based solely on SCoT markers, the genetic similarity ranged from 0.72 to 1.0, indicating significant variation. The closest genetic relationship was observed between Lady Rositta and Santana, while the most distinct pairing was Caruso and Spunta (Figure 3; Table 6). These findings support previous studies in other crops where SCoT markers have effectively captured diversity among closely related genotypes [2], [20].

ISSR markers, by contrast, indicated a similarity range of 0.787 to 0.914, with Diamant and Spunta being the most divergent, and Santana and Spunta the most similar (Figure 4; Table 7). The broader genome coverage of ISSR markers makes them well-suited for detecting variation in non-coding regions, thus offering valuable insights into background genetic structure [53], [41].

When data from both marker systems were combined, a refined picture of cultivar relationships emerged. The combined similarity indices ranged from 0.784 to 0.909 (Figure 5; Table 8), with Lady Rositta and Santana clustering tightly together across all dendrograms, reinforcing their close genetic affiliation. Meanwhile, Spunta remained consistently isolated, reflecting its distinct genetic background—possibly due to differing origin, selection history, or mutation accumulation over time. Similar patterns were reported by Arya et al.

[18] and Rakha et al. [24], supporting the validity of the clustering outcomes in this study.

UPGMA dendrograms based on SCoT, ISSR, and combined datasets grouped the cultivars into consistent clusters, providing a robust framework for cultivar differentiation. The reliable classification of genotypes using combined markers confirms earlier conclusions by Xanthopoulou et al. [47] and Joshi et al. [45], who highlighted the importance of integrating different marker systems to improve genetic resolution and clustering accuracy.

5. Conclusion

The present study demonstrated the utility of combining SCoT and ISSR molecular markers in the assessment of genetic diversity among Egyptian potato (*Solanum tuberosum* L.) cultivars. The complementary strengths of these marker systems allowed for the detection of both functional and non-coding genomic variation, providing a comprehensive understanding of the genetic landscape of the tested cultivars.

The results revealed moderate genetic diversity, with valuable genetic distances and unique markers identified for cultivar differentiation and selection. The insights gained from this analysis offer strong support for molecular-assisted breeding, parental line selection, and conservation efforts in potato breeding programs. Moreover, the identification of both genetically similar and divergent cultivar pairs can guide future strategies aimed at maximizing genetic gain, improving trait heritability, and sustaining long-term crop productivity

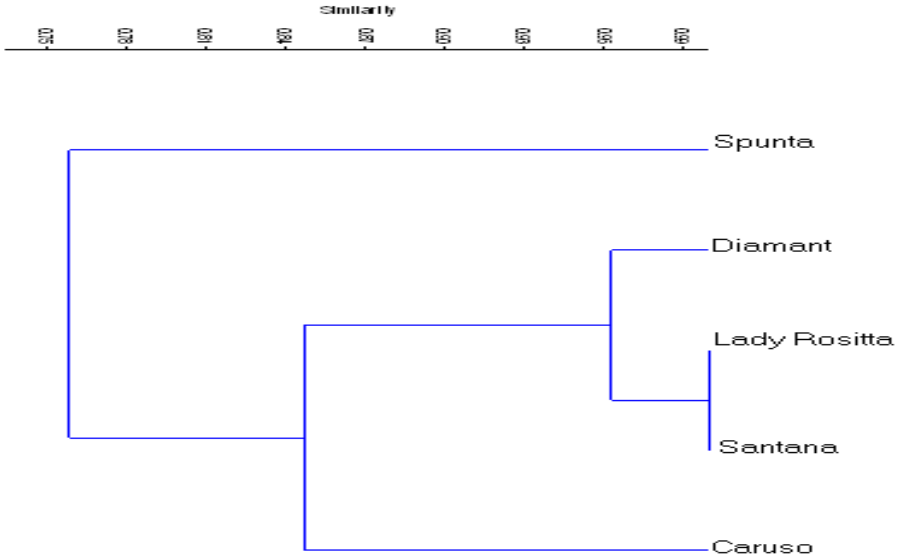

Fig. 3. Dendrogram derived by UPGMA method using Dice-dissimilarity coefficient for combined binary data of SCoT techniques for five Potato Solanum Tuberosum cultivars.

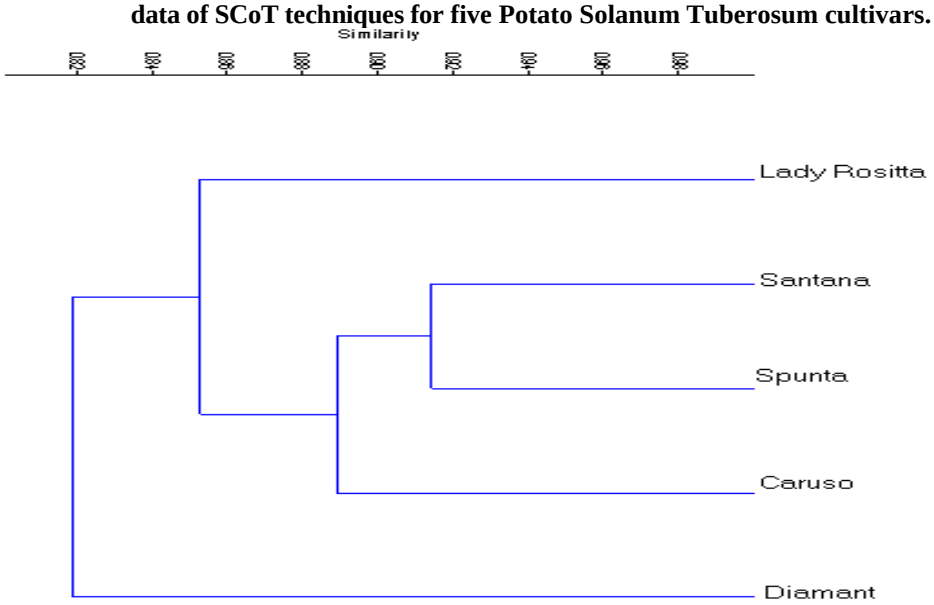

Fig. 4. Dendrogram derived by UPGMA method using Dice-dissimilarity coefficient for combined binary data of ISSR for five Potato Solanum Tuberosum cultivars.

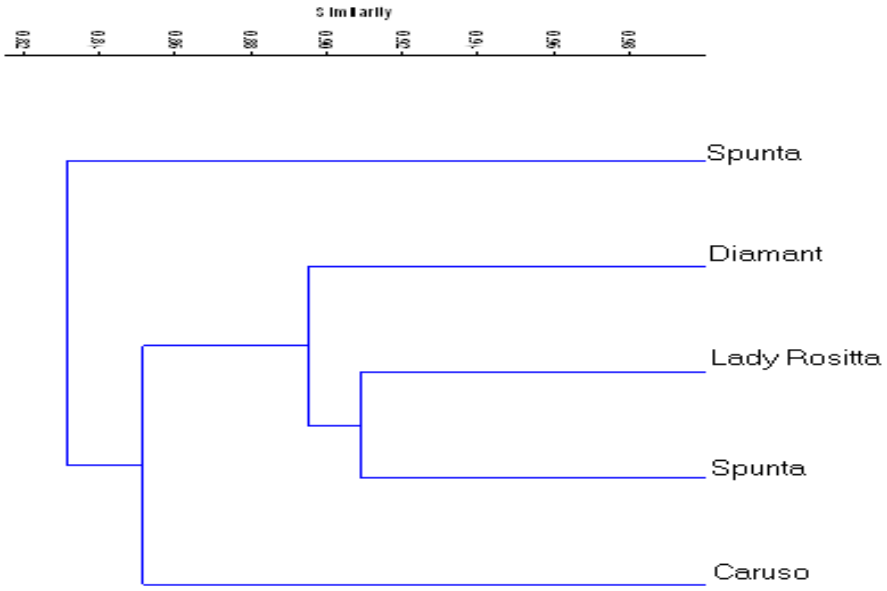

Fig. 5. Dendrogram created by UPGMA method utilizing the Dice-dissimilarity coefficient for concatenated binary data of SCoT and ISSR approaches for five Potato Solanum Tuberosum cultivars.

Cluster Analysis Based on ISSR and Combined SCoT-ISSR Data

Cluster Analysis and Interpretation of Genetic Relationships

The dendrogram generated from ISSR marker analysis (Figure 4) successfully classified the five

Solanum tuberosum cultivars into two main clusters, reflecting substantial genetic diversity. The Diamant cultivar was placed in an independent cluster, indicating a significant degree of genetic divergence from the other cultivars. This observation corroborates the sensitivity of ISSR markers in distinguishing even closely related genotypes, as demonstrated by Bornet and Branchard [17].

The second main cluster was subdivided into two distinct subgroups: one containing only Lady Rositta, and the other comprising Caruso, Spunta, and Santana. This clustering pattern suggests that Lady Rositta shares partial genetic similarity with the latter group but retains a unique genetic identity. Such structuring is in agreement with Rakha et al. [24], who reported the capability of ISSR markers to resolve fine-scale genetic differences among *Solanum* cultivars.

Similarly, the combined dendrogram generated using both SCoT and ISSR markers (Figure 5) offered an even more refined classification. The Spunta cultivar appeared as a distinct lineage in the first main cluster, consistent with its unique genetic profile revealed by individual marker analyses. The second major cluster was further divided into two subgroups: the first containing only Caruso, while the second comprised Diamant, Lady Rositta, and Santana. Interestingly, Diamant and Lady Rositta, which were separated in the ISSR dendrogram, clustered together in the combined analysis, highlighting the advantage of using multiple marker systems to resolve ambiguous relationships, as supported by Joshi et al. [45] and Xanthopoulou et al. [47].

These clustering patterns are critical for guiding breeding decisions. The identification of genetically distant cultivars such as Diamant and Spunta enables breeders to select parents with high genetic divergence, which is essential for maximizing heterosis [47]. Furthermore, understanding genetic structure assists in germplasm conservation, especially in clonally propagated crops like potato, where natural recombination is limited.

Summary of Genetic Diversity and Marker Efficiency

In this study, molecular diversity among five commercial *Solanum tuberosum* cultivars was evaluated using two complementary molecular marker systems—Start Codon Targeted (SCoT) and Inter-Simple Sequence Repeat (ISSR). The application of both marker types provided a comprehensive understanding of the genetic structure and relationships among cultivars, facilitating effective selection and conservation strategies.

The SCoT analysis amplified 18 total bands, of which 9 were polymorphic, yielding a polymorphism rate of 50%. The ISSR analysis amplified 22 bands, with 10 being polymorphic (45.45% polymorphism). Overall, the combined polymorphism percentage across both marker systems was 47.5%, indicating moderate but significant genetic variation among the tested cultivars. These findings align with earlier studies

using SCoT [46] and ISSR [17] in crop genetic diversity analysis.

Moreover, 14 unique markers were identified across the five cultivars, reinforcing the effectiveness of both marker systems in detecting cultivar-specific DNA fragments. Such unique markers are valuable for varietal identification, intellectual property protection, and the development of molecular fingerprinting protocols.

Genetic similarity coefficients ranged from 0.784 (Diamant vs. Spunta) to 0.909 (Lady Rositta vs. Santana), indicating the presence of both closely related and genetically divergent pairs. This range supports the suitability of these cultivars for targeted breeding strategies and underscores the necessity of molecular tools for efficient cultivar discrimination.

Comparative Clustering

Insights and Literature Context

The UPGMA cluster analysis based on SCoT markers grouped Spunta as a separate cluster, reflecting its distinct genetic identity. Caruso formed a separate cluster as well, while Diamant, Lady Rositta, and Santana were grouped together, indicating closer genetic affinity. Conversely, ISSR-based analysis revealed Diamant as an independent group, while Caruso, Spunta, and Santana formed a major cluster. These discrepancies reflect the functional (SCoT) versus neutral (ISSR) nature of the markers and further justify their complementary application.

The combined dendrogram derived from integrated SCoT-ISSR data provided the most consistent clustering, supporting earlier recommendations (e.g., Duran et al. [21]; El-Bolok et al. [25]) that combining marker systems enhances the accuracy of genetic relationship analysis. Similar patterns of polymorphism and clustering have been reported in potato by Moisan-Thiery et al. [48] and Rosa et al. [52], and in other fruit crops like apricot and pomegranate [34], [27].

Breeding and Conservation Implications

The genetic diversity observed among the cultivars in this study has important implications for potato breeding. Genetically distant cultivars such as Diamant and Spunta are ideal candidates for hybridization, which can lead to offspring with increased vigor and improved stress tolerance. The close genetic relationship between Lady Rositta and Santana may reflect shared ancestry or breeding selection for similar agronomic traits [10], and such cultivars could be utilized to fix specific desirable traits.

The identification of unique markers also enables the development of cultivar-specific diagnostic tools and contributes to the conservation of genetic resources. This supports sustainable breeding efforts and ensures the preservation of diverse genetic backgrounds essential for future crop improvement under changing environmental conditions.

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