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Genetic Diversity Analysis of Chinchilla and V-line Rabbit Strains Using RAPD Markers for Breeding Optimization

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Abstract: This study explores the genetic diversity between the Chinchilla and V-line rabbit strains using Random Amplified Polymorphic DNA (RAPD) markers. A total of 45 bands were analyzed for the Chinchilla strain, while 74 bands were assessed for the V-line strain. Polymorphism metrics, including percentage polymorphism (% *P*), Polymorphic Information Content (*PIC*), Marker Index (*MI*), Diversity Index (*DI*), and Resolving Power (*Rp*), were evaluated to determine the extent of genetic variation within and between the strains. The V-line strain exhibited higher genetic diversity with 71.4% polymorphism and an average PIC of 0.57, compared to 66.7% polymorphism and a *PIC* of 0.52 in the Chinchilla strain. The V-line strain also had higher *MI* (5.81) and *DI* (0.71), indicating greater marker discriminatory power and potential for breeding optimization. The OPA02 marker was the most informative, with a PIC of 0.62. Cluster analysis using Unweighted Pair Group Method with Arithmetic Mean Algorithm (UPGMA) revealed clear genetic clustering according to the strains, with intra-strain variation higher in the V-line population. The cophenetic correlation coefficient (0.74) confirmed the reliability of the clustering, reflecting meaningful genetic similarities and differences. These results highlight the importance of genetic diversity in breeding programs and underscore the potential of the V-line strain for trait improvement through selective breeding. Both strains remain valuable for breeding strategies focused on production traits and genetic conservation, with RAPD markers serving as effective tools for genetic characterization.
Keywords: Genetic diversity, Chinchilla rabbit, V-line rabbit, RAPD markers, Polymorphic Information Content (*PIC*).

1. Introduction

Rabbits are becoming increasingly important in Egypt as a source of meat and fur due to their high reproductive rate, efficient feed conversion, and ability to thrive in various environmental conditions. Initially, New Zealand White (NZW) rabbits were introduced, followed by breeds such as Grey Giant, White Giant, and Soviet Chinchilla, which were bred in both the northern and southern regions. These breeds have contributed significantly to local rabbit production systems, with recent efforts focused on enhancing their performance through genetic selection [1].

With the impact of climate change, there is growing interest in selecting heat-tolerant animals that can maintain productivity under heat stress. Breeds exhibit variations in cooling capacities, driven by allelic differences in genes regulating thermoregulation and heat shock resistance [2]. Genetic adaptation for rising global temperatures is essential for optimizing production in challenging environments, making genetic diversity a critical focus for rabbit breeding programs.

RAPD is a powerful molecular marker technology that employs PCR to detect DNA polymorphisms across genomes [3, 4]. RAPD uses short arbitrary primers to amplify random segments of genomic DNA, which are then separated by gel electrophoresis. This technique is simple, fast, cost-effective, and requires minimal DNA, making it widely applicable for studying genetic diversity across animal, plant, and microbial populations [5]. RAPD markers have proven valuable for genetic mapping, population structure analysis, gene identification, and the estimation of genetic distances between individuals and populations [6].

Although RAPD markers offer advantages for genetic analysis, issues with experimental reproducibility have led some researchers to explore more stable alternatives such as microsatellite markers. Nevertheless, RAPD remains a popular tool in population genetics due to its ability to differentiate genetically distinct individuals and detect associations with economic traits [7]. For example, RAPD markers have been used to study body fat traits in broiler chickens [7] and body size traits in Qinglong goats [8]. Studies on meat ducks have identified correlations between RAPD markers and abdominal fat content [9], while RAPD analysis has also been employed to investigate the genetic relationships among rabbit breeds [10].

Recent research emphasizes the growing importance of molecular markers like RAPD in evaluating genetic diversity in rabbits, though the focus has shifted towards more robust markers such as SNPs and microsatellites in some studies.

Nonetheless, RAPD remains valuable for rapid screening of genetic variation and population structure due to its simplicity and cost-effectiveness. For example, RAPD markers have been utilized to assess relationships among different rabbit genotypes in Egypt and guide breeding strategies [11]. Also, RAPD markers were used to explore molecular variations among Alexandria, V-line, and New Zealand White rabbits, revealing distinct genetic clusters based on locality [12].

Recent studies also reveal that molecular markers, including RAPD, play a vital role in identifying distinct genetic clusters and monitoring selection signatures related to traits like reproductive performance and disease resistance [13]. These studies demonstrate the relevance of RAPD in identifying valuable genetic traits and supporting conservation efforts, reinforcing its importance in breeding programs aimed at enhancing rabbit productivity and environmental adaptability.

This study investigates the genetic diversity of Chinchilla and V-line rabbit strains using RAPD markers to identify polymorphisms that could contribute to breeding optimization. The Chinchilla and V-line strains are particularly relevant for rabbit breeding programs due to their distinct origins and performance traits. Chinchilla rabbits are valued for their fur quality, while V-line rabbits are known for their rapid growth and high reproductive performance. Understanding the genetic structure of these strains is essential for developing effective breeding strategies that enhance desirable traits such as growth rate, disease resistance, and heat tolerance.

By applying RAPD technology, this study aims to contribute to the conservation of genetic diversity within rabbit populations and provide insights that will inform breeding practices for sustainable production.

2. Material and Methods

2.1. Animals

This study involved the 6th generation of two rabbit breeds, Chinchilla and V-line. A total of 32 mature rabbits (16 males and 16 females), aged between 18 to 24 months, were included. The animals were initially obtained from the Faculty of Veterinary Medicine, Alexandria University. They were subsequently bred and maintained at the Department of Animal Behavior and Husbandry, Faculty of Veterinary Medicine, Sohag University, until the sixth generation. Both breeds were housed under isolated conditions with standardized management protocols, including feeding, watering, disinfection, and vaccination.

Ethical Considerations: All procedures followed the ethical guidelines approved by the Veterinary Medical Research Ethics Committee, Faculty of Veterinary Medicine, Sohag University, Sohag, Egypt (Approval No. Sohag Uni.Vet. /00074R).

2.2. Housing and Management

The experiment was conducted in a 7 × 7 × 3-meter room, oriented northwest. The room was equipped with 3 air suction fans (25 × 25 cm), 2 shaded windows (100 × 40 cm) with cooling cellulose sheets, and an air-cooling system for temperature regulation. The rabbits were housed in galvanized iron batteries spaced 1 meter apart to facilitate ventilation and waste disposal. Individual cages (60 × 40 cm) provided ample space for each rabbit. The two breeds were kept in separate batteries, each equipped with nipple drinking systems and isolated feeders (15 × 15 cm) [14]. The cages were designed to be easy to clean, offering a safe environment for the rabbits to live, and raise [15]. Rabbits were fed a commercial pellet diet ad libitum. Crude protein levels varied by physiological stage. Maintenance: 12%, Growth: 16%, Pregnancy: 15%, and Lactation: 18%. Ventilation was maintained using exhaust fans, an air conditioner, and shaded windows. Nest boxes were provided before parturition to allow does to prepare bedding and protect kits from extreme weather.

2.3. Characterization

- **Genotype**

Blood samples were collected from the marginal ear vein of each rabbit into 2 mL vacutainer tubes containing EDTA as an anticoagulant and stored at -20 °C until further use. A total of six RAPD primers were used for genotyping [16].

2.4. DNA Extraction and RAPD Technique

- **DNA Extraction**

DNA was extracted using the Gene JET Whole Blood Genomic DNA Purification Mini Kit following the manufacturer's protocol [17]. 20 µl of proteinase K was added to 200 µl of whole blood, 400 µl of lysis solution was added, and the mixture was incubated at 56°C for 10 minutes, 200 µl of ethanol (96–100%) was added, and the mixture was transferred to a spin column and centrifuged, and DNA was eluted with 200 µl of elution buffer and stored at -20 °C. DNA concentration and purity were measured using a Nano-drop spectrophotometer (Q5000, Thermo Fisher Scientific, USA) and confirmed by electrophoresis on an agarose gel.

- **Random Amplified Polymorphic DNA (RAPD)**

RAPD markers are decamer DNA fragments amplified using arbitrary primers. Seventeen primers were used following the protocols of previous studies [16].

Table 1: Decamer Sequences Used in RAPD-PCR (Operon Technologies Inc., USA).

No.	Nomenclature	Sequence (5'3').	GC (%)
1	OPA02	5'-TGCCGAGCTG-3'	70
2	OPA03	5'-AGTCAGCCAC-3'	60
3	OPA08	5'-GTGACGTAGG -3'	60
4	OPA13	5'-CAGCACCCAC-3'	70
5	OPA15	5'-TTCCGAACCC-3'	60

- **PCR Components**

As shown in Table (2) PCR reactions were carried out in a total volume of 25 µl containing 2 µl of genomic DNA, 10 p moles of random primer (1.5 µl each), 2 mM dNTP mix (dATP, dCTP, dTTP, and dGTP) (ABgene, Surrey, UK), 5X

PCR buffer, 25 mM MgCl2, and 1 unit of Taq DNA polymerase. A master mix was prepared, and 12.7 µl was dispensed into each PCR tube, containing 2 µl of template DNA. DNA-free water was added to complete the final volume to 25 µl.

- **PCR Amplification Cycle**

PCR was performed in a Biometra T Gradient thermal cycler (Yumpu, Switzerland) using the following conditions: 35 cycles after an initial denaturation at 95ºC for 5 minutes. Each cycle included denaturation at 95ºC for 60 seconds, annealing at 37ºC for 60 seconds, and elongation at 72ºC for 120 seconds. A final extension was performed at 72ºC for 5 minutes, followed by soaking at 4ºC until removal from the PCR machine as described by Bowditch et al. [18].

Table 2: Components of RAPD-PCR reaction

Master Mix	Amount (1X)
5X PCR buffer	5 µl
$MgCl_2$ (25mM)	2 µl
dNTP's mix (2mM)	2.5 µl
Primer F (10 *p* moles/µl)	1.5 µl
Primer R (10 *p* moles/µl)	1.5 µl
Taq (5 U/ µl)	0.2 µl
DNA (10 ng/ µl)	2 µl
Deionized H_2O	10.3 µl
Reaction	**25 µl**

- **Electrophoresis and Visualization of RAPD Products**

RAPD-PCR products were separated by 2.5% agarose gel electrophoresis in 1x TBE buffer using a Bio-Rad electrophoresis unit. Ten µl of each PCR product was mixed with 2 µl of loading buffer and loaded into the gel. The gel was run at 90 volts for 40 minutes. Bands were visualized using a Gel Documentation System (Bio-Rad, USA). The DNA fragment bands were analyzed using the GelAnalyzer23.1.1 software and compiled for each primer in Tables 3& 4.

- **Statistical Analysis of Genetic Diversity**

Genetic distances and similarities were analyzed using NTSYSpc software ver. 2.20s following Jaccard's coefficient [19]. Dendrograms were generated using theUPGMA *via* the SHAN module in NTSYSpc.The reliability of dendrograms was evaluated using bootstrapping with 1,000 replicates in the Free Tree program.

- **Marker Informativeness and Polymorphism Metrics**

The following marker features were calculated to assess marker performance:

- **Percentage of Polymorphism (*P*%):**

$$P\% = \frac{(\text{Number of polymorphism})}{\text{Total Band}} \times 100$$

- **Polymorphic Information Content (*PIC*)**:

$$PIC = 1 - \sum P_i^2$$

Where pi is the frequency of the i^{th} allele (presence/absence).

- **Resolving Power (Rp):**

$$Rp = \sum I^b \text{where} I^b = 1 - |2 \times (0.5 - p|$$

Here, p is the proportion of individuals showing the band.

- **Marker Index (*MI*):**

$$MI = PIC \times Number\ of\ Polymorphic\ Bands$$

- **Diversity Index (*DI*):**

$$DI = \frac{Total\ Polymorphic\ Bands}{Total\ Bands}$$

These metrics were calculated by Mandal et al. [20] to evaluate the informativeness, discriminatory power, and diversity of the RAPD markers used.

3. Results

The study analyzed 45 bands for the Chinchilla strain and 74 bands for the V-line strain, providing insights into the genetic similarity, variability, and relationships between these two breeds (**Figs. 1& 2; Tables 3 & 4**).

3.1. Polymorphism Metrics Analysis

3.1.1. Comparison of % Polymorphism

The V-line strain exhibited a 71.4% polymorphism, which is slightly higher than the 66.7% polymorphism observed in the Chinchilla strain (Tables 3& 4).

3.1.2. Polymorphic Information Content (PIC)

PIC measures how informative a marker is for identifying genetic differences. The V-line strain showed a higher average ***PIC*** value of 0.57, compared to 0.52 for the Chinchilla strain. The OPA02 marker in the V-line strain had the highest ***PIC*** value (0.62), making it the most informative marker for detecting genetic variation (Tables 3& 4).

3.1.3. Marker Index (MI) and Diversity Index (DI)

The *MI* and *DI* provide insight into the discriminatory power of the RAPD markers and the extent of genetic variability within the Chinchilla and V-line rabbit strains. These metrics not only reflect the markers' efficiency in differentiating individuals but also help gauge the genetic richness of the populations.

For the Chinchilla strain, the calculated *MI* of 4.16 reflects a moderate ability of the markers to capture genetic diversity within the population. The *DI* of 0.67 (66.7%) confirms that a substantial portion of the bands analyzed were polymorphic, though slightly less diverse than the V-line population.

In contrast, the V-line strain exhibited an *MI* of 5.81 and a *DI* of 0.71 (71.4%), indicating higher genetic diversity and greater discriminatory power of the RAPD markers.

Table 3: Polymorphism Data for Chinchilla Rabbit Strain

Marker	Total bands	Mono-bands	Poly-bands	*P* (%)	*PIC* value	*Rp*
OPA02	5	2	3	60%	0.48	4.0
OPA03	4	1	3	75%	0.56	2.0
OPA15	3	1	2	66.7%	0.52	1.9
Total	12	4	8	66.7%	0.52	7.9

Table 4: Polymorphism Data for V-line Rabbit Strain

Marker	Total bands	Mono-bands	Poly-bands	*P (%)*	*PIC* value	*Rp*
OPA02	5	1	4	80%	0.62	2.0
OPA03	5	2	3	60%	0.53	4.0
OPA15	4	1	3	75%	0.58	2.0
Total	14	4	10	71.4%	0.57	8.0

3.1.4. Resolving Power (Rp)

Rp reinforces the distinctions between the two strains by evaluating the markers' ability to distinguish individual genetic profiles. For the Chinchilla strain, the *Rp* was calculated to be 7.9, indicating the markers were sufficiently capable of identifying genetic differences among individuals, though with some limitations. In comparison, the V-line strain displayed a slightly higher *Rp* of 8.0, suggesting that the markers were marginally more effective at capturing finer genetic distinctions within the population.

3.2. Cluster Data Analysis

3.2.1. Clusters

The dendrogram generated using UPGMA clustering (Fig. 3) revealed clear grouping patterns for the samples, with most individuals clustering according to their respective rabbit strains. These clusters demonstrate the distinctiveness of each strain, as well as some internal similarities among individuals belonging to the same group.

3.2.2. Inter-strain Similarity

At certain intermediate clustering levels, branches from both the Chinchilla and V-line strains merged, suggesting some genetic overlap between the two strains. However, as the linkage distances increased, the clusters separated, indicating that despite some shared genetic features, the two strains also maintain distinct genetic identities (Fig. 3). This suggests that the strains may share a common ancestral background or have experienced gene flow at some point but have since diverged into distinct genetic lineages.

3.2.3. Intra-strain Diversity

The Chinchilla strain exhibited tighter clustering among its samples, indicating lower intra-strain polymorphism in comparison with the V-line strain. In contrast, the V-line strain showed more variation in band patterns across its individuals, suggesting higher intra-strain diversity.

3.2.4. Cophenetic Correlation Coefficient

The cophenetic correlation coefficient was calculated as 0.74, indicating a strong correlation between the original Jaccard distance matrix and the UPGMA dendrogram. This value suggests that the dendrogram accurately represents the underlying genetic relationships between and within the rabbit strains (Fig. 3).

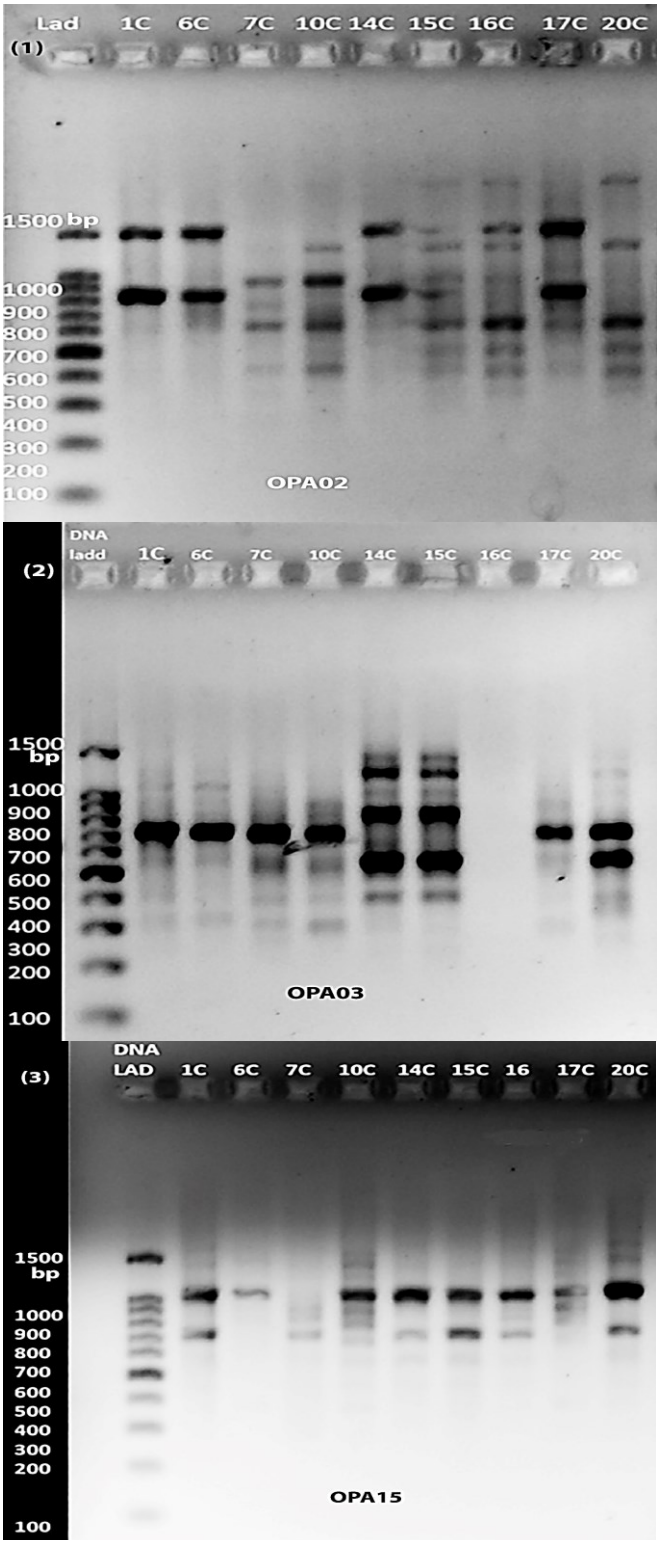

Figure 1: A typical RAPD banding pattern amplified with primer OPA02, OPA03, and OPA15 (Operon Tech. Inc.) that resolved in 1.5% agarose gel stained with ethidium bromide. Template DNA was from the Chinchilla strain (1-3). DNA-lad: A 100 bp ladder.

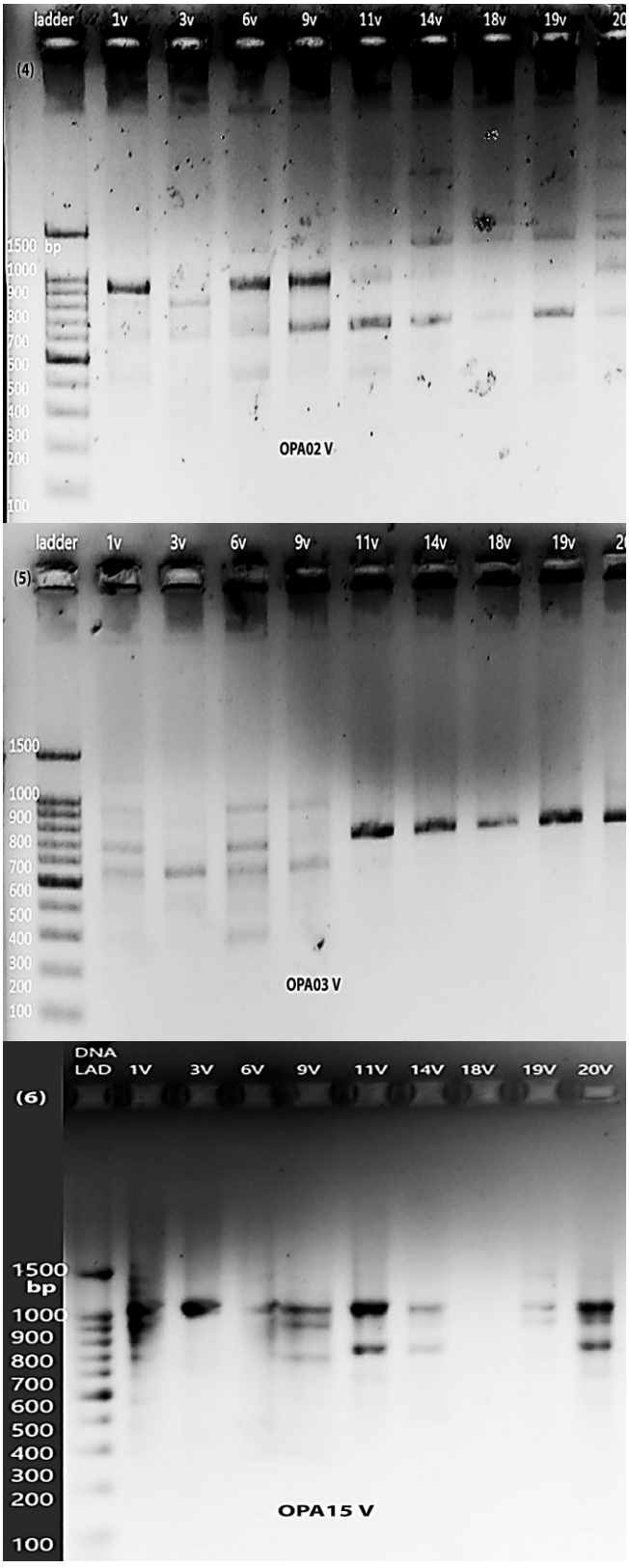

Figure 2: A representative RAPD banding pattern generated using primers OPA02, OPA03, and OPA15. The template DNA samples were obtained from the V-line strain (4 – 6). DNA-Lad: A 100 bp DNA ladder was used as a molecular size marker.

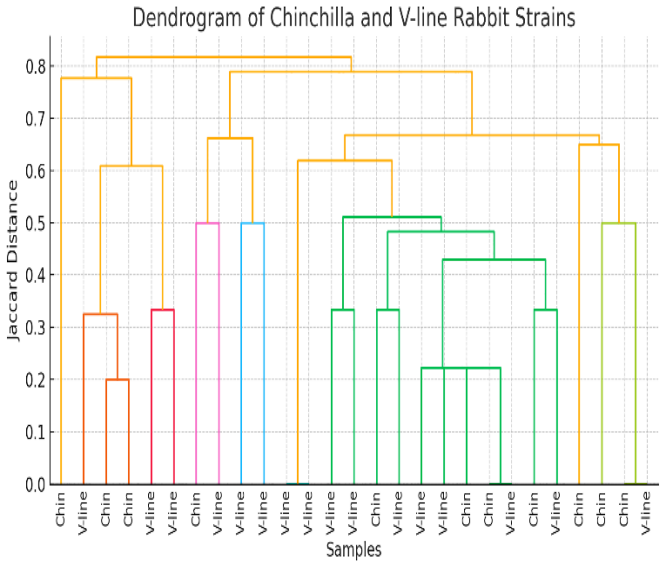

Figure 3: The dendrogram shows the hierarchical clustering of the 45 RAPD bands of the Chinchilla rabbit strain and the 74 bands of the V-line rabbit strain using Jaccard's coefficient and the UPGMA clustering method. The cophenetic correlation coefficient for this clustering is 0.74, indicating a good correlation between the original Jaccard distances and the clustering results.

4. Discussion

This study provides a comprehensive analysis of the genetic diversity between Chinchilla and V-line rabbit strains using RAPD markers, highlighting important insights into their genetic variability, polymorphism, and breeding potential. The findings demonstrate subtle but meaningful differences in genetic diversity metrics, which have implications for breeding programs focused on optimizing production traits.

Polymorphism and Genetic Diversity

The higher polymorphism observed in the V-line strain (71.4%) compared to the Chinchilla strain (66.7%) indicates greater genetic variability within the V-line population. This enhanced diversity suggests that the V-line strain may have a broader genetic pool, providing more opportunities for selective breeding to improve traits such as growth performance and disease resistance [13, 21]. In contrast, while exhibiting lower polymorphism, the Chinchilla strain maintains significant genetic variability, making it suitable for selective breeding programs targeting fur quality and other specific traits [11, 22].

The *PIC* further reinforces these findings, with the V-line strain showing a higher average *PIC* value (0.57) than the Chinchilla strain (0.52). The OPA02 marker in the V-line strain displayed the highest *PIC* (0.62), identifying it as the most effective marker for capturing genetic differences. These *PIC* values reflect the markers' ability to detect meaningful genetic variability, confirming the potential of RAPD markers for population genetics studies in rabbits [23].

Marker Index (MI), Diversity Index (DI), Resolving Power (Rp)

MI and *DI*, which assess both the discriminatory power of

the markers and the extent of genetic variation, further highlight the superior genetic diversity of the V-line strain. With an *MI* of 5.81 and a *DI* of 0.71 (71.4%), the V-line strain demonstrates a richer genetic composition than the Chinchilla strain, which showed an *MI* of 4.16 and a *DI* of 0.67 (66.7%). These metrics suggest that the V-line strain offers greater potential for trait optimization and genetic improvement through breeding strategies targeting growth and reproductive traits [20].

Rp, which measures the effectiveness of the markers in distinguishing individuals within each population, also supports these conclusions. The V-line strain's Rp of 8.0 was marginally higher than the Chinchilla strain's Rp of 7.9, indicating that the RAPD markers were slightly more effective in identifying genetic differences within the V-line population. This slight edge in resolving power aligns with the V-line strain's higher MI and DI values, suggesting it holds more potential for adaptive breeding programs.

Cluster Analysis and Genetic Relationships

The dendrogram generated using UPGMA clustering revealed distinct clusters for both strains, with individuals generally grouping according to their respective populations. This confirms the distinct genetic identities of the Chinchilla and V-line strains, which may reflect independent breeding histories or selection pressures. The separation of clusters at higher linkage distances further indicates that, despite some genetic overlap, the two strains maintain distinct genetic lineages [24].

At intermediate clustering levels, however, some branches from both strains merged, suggesting genetic overlap between the two populations. This overlap may indicate shared ancestry or the possibility of gene flow between the strains at some point in their breeding history. Such findings are consistent with studies suggesting that genetic exchange between populations can occur in domestic animals through controlled breeding or environmental proximity [25].

Intra-strain Diversity

The tighter clustering observed within the Chinchilla strain indicates lower intra-strain polymorphism, reflecting a more uniform genetic background. This may result from selective breeding efforts aimed at maintaining consistency in traits such as fur quality [22, 26]. In contrast, the V-line strain exhibited more variation in band patterns across individuals, suggesting higher intra-strain diversity. This greater diversity may enhance the V-line strain's adaptability to environmental conditions and improve its potential for trait optimization in breeding programs.

Cophenetic Correlation Coefficient

The cophenetic correlation coefficient of 0.74 indicates a strong relationship between the original Jaccard distance matrix and the UPGMA dendrogram. This high correlation confirms that the dendrogram accurately represents the underlying genetic relationships between and within the two rabbit strains. These results support the reliability of the clustering approach in capturing the genetic structure of both populations, which is essential for designing effective breeding programs and conservation strategies [27, 28].

Limitations and Future Directions

While RAPD markers provide a useful method for assessing genetic variation, they are dominant markers, meaning that heterozygosity cannot be directly measured. Future studies could incorporate more advanced molecular markers, such as microsatellites or SNPs, to provide a more comprehensive view of genetic diversity [11, 29]. Additionally, environmental and management factors influencing genetic variation should be further explored to better understand the full scope of genetic divergence between the Chinchilla and V-Line strains.

5. Conclusion

The results of this study underscore the importance of maintaining genetic diversity within rabbit breeding programs. The V-line strain demonstrated higher genetic variability and greater marker informativeness, making it a valuable resource for breeding efforts targeting growth performance and disease resistance. Meanwhile, the Chinchilla strain, with its more uniform genetic structure, remains a suitable candidate for specialized breeding programs focused on fur quality or other economic traits.

These findings emphasize the need for sustainable breeding practices that preserve genetic diversity and optimize trait selection in both strains. The RAPD markers used in this study proved effectiveness in capturing meaningful genetic differences, supporting their continued use in genetic studies and breeding optimization.

Future research could explore the potential of molecular markers such as SNPs or microsatellites to complement RAPD analysis and provide deeper insights into the genetic architecture of rabbit populations.

Credit authorship contribution statement:

The authors confirm their contribution to the paper as follows: study conception and design: M.M. & T.H; data collection: G.S.; results analysis and interpretation: M.M. & T.H; draft manuscript preparation: G.S. and F.M; All authors reviewed the results and approved the final version of the manuscript.

Data availability statement

The data used to support the findings of this study are available from the corresponding author upon request.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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