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# Evaluating the Phenotypic and Genotypic Diversity of *Plantago ciliata* in the Ha'il Region, Saudi Arabia

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**ABSTRACT**

This study aimed to assess the genotypic and phenotypic diversity of *Plantago ciliata* samples collected from two distinct locations in the Ha'il Region of Saudi Arabia. The two species of *P. ciliata* were collected from local rangelands in the spring of 2016. Phenotypic variation was assessed by measuring various traits including the primary root length, number of stems (spikes), longest stem length, number of leaves, longest leaf length, and the fresh weight of the entire plant. The RAPD (Random Amplified Polymorphic DNA) method was used to assess genotypic variation. Phenotypic data were analyzed using analysis of variance (ANOVA), while cluster analysis and similarity matrices were applied to the data, utilizing the unweighted pair group method with arithmetic mean (UPGMA) to construct a dendrogram. The results indicated significant differences ($P \leq 0.05$) in the phenotypic characteristics of *P. ciliata* between and within the two studied populations. The data showed that the plant population from location I showed the highest number of measurements, while the population from location II showed the lowest. Regarding the RAPD markers, two primers successfully amplified both populations, while 3 primers failed to produce any amplification. Out of the five primers used, two primers exhibited polymorphism and showed variation in band patterns. In summary, these findings are valuable for plant breeding programs and biodiversity conservation efforts in the Ha'il Region of Saudi Arabia.

**Keywords:** Morphological, Markers, Diversity, Ha'il, Molecular

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## Introduction

The genus *Plantago*, part of the Plantaginaceae family, is widespread globally, encompassing approximately 200-256 species. It plays a significant role as forage for grazing animals and in pharmaceutical applications [1, 2]. Native to Central Asia, it now grows extensively across Europe, temperate regions of Asia, Australia, and North America, thriving in temperate grasslands worldwide. While it is found naturally in areas with poor soils lacking essential elements like sodium and potassium, its growth is positively impacted by nitrogen, which promotes an increase in biomass, stem, and leaf development, though its effect on root growth is limited [3, 4].

Many *Plantago* species are perennial, long-lived plants with small roots and multiple vertical or arching stems that are roughly the same length as the leaves, often lacking gaps. The leaves are typically oval, with prominent veins and serrated edges, long petioles, and are relatively broad. Some species, however, are annuals, growing 5-20 cm tall with fewer stems and a more or less hairy surface. Their leaves are narrow-lanceolate to linear-lanceolate. The scape is thick and either vertical or ascending, usually longer or shorter than the leaves. The spikes are rigid, condensed, and barely cylindrical, ranging from 3-10 cm. The fruit is a capsule, containing 3-5 seeds [5]. *Plantago* is a crucial medicinal plant, known for its rich array of secondary metabolites [6]. It has been used to treat conditions like high blood sugar and elevated cholesterol levels [7]. The World Health Organization has

endorsed its use as a laxative agent [3, 8]. However, research on *Plantago*, particularly related to its genomic resources, remains relatively scarce [9-15].

Despite the significant medicinal and economic value of *Plantago*, the yield is heavily influenced by environmental factors, leading to a substantial decline in both seed and husk quality. Additionally, efforts to enhance genetic diversity have largely been unsuccessful due to limited genetic resources [16]. As *Plantago* has been introduced in several countries across South and West Asia, the genetic variation within the available gene pool remains quite narrow [17, 18]. Due to this limited diversity, various breeding methods, including selection, hybridization, induced mutations, polyploidy, and tissue culture, have been utilized to improve the genetic characteristics of *Plantago* [19-22]. Morphological differences between species within the genus are minimal, and the classification of species remains uncertain. The entire family has three recognized genera, and *Plantago* is divided into 6 sections [5]. However, genetic variation is primarily assessed using molecular markers, which are key in evaluating the status and development of *Plantago* populations [23, 24]. The wild relatives of *Plantago* species are vast and of medicinal importance, serving as a source of valuable genes for *Plantago* cultivation [25]. Some studies have employed Randomly Amplified Polymorphic DNA (RAPD) for genetic analysis, and a combination of RAPD and ISSR markers has been used to assess genetic diversity in *Plantago* [26-29]. Several researchers emphasize the importance of characterizing cultivated *Plantago* genotypes, as understanding their genetic diversity and utilizing molecular markers can help identify polymorphisms among different genotypes [1]. The phylogenetic analysis of *Plantago ovata* for breeding programs and crop variety identification has been conducted by Rohila *et al*. [1], who highlighted the value of RAPD analysis in determining genetic relationships and estimating genetic diversity among *P. ovata* genotypes. Given the economic significance of the *Plantago* genus, the present study aimed to evaluate the phenotypic and genotypic variations in *Plantago ciliata* collected from 2 distinct locations in Ha'il Region, Saudi Arabia.

## Materials and Methods

*Study area*

The *Plantago* species for this study were gathered from the Ha'il region, located in the central-northern part of Saudi Arabia. This region spans between 25°29′ N and 38°42′ E and covers an area of 118,322 km². Ha'il experiences a temperature range from 10.8 °C in winter to 34.1 °C in summer, with an average annual rainfall of approximately 104.4 mm, most of which occurs in the winter months [30]. As such, Ha'il is classified as an arid zone with a long dry period and a brief, scattered rainy season that lasts for the majority of the year.

*Sample collection*

Two species of *P. ciliata* were collected during the spring season of 2016 from the natural rangelands of Ha'il **(Table 1)**. Fresh grass samples were extracted by digging up the plants and placed into polyethylene bags for transport. These specimens were then transferred to the laboratories at the Department of Biology, Faculty of Science, University of Ha'il for further identification and analysis. The collected samples were dried in a vacuum oven at 105 °C for 24 hours. Following this, 50 grams of each dried sample were packed into paper sacks and stored for additional examination. The phenotypic traits measured included main root length, number of leaves, length of the longest stem, number of stems, and length of the longest leaf, in addition to the fresh weight of the entire plant, as well as dry weights of the shoot and root.

**Table 1.** Plant species collected from the natural rangelands of Ha'il, Kingdom of Saudi Arabia, during the spring season of 2016.

| No. | Name | Location | Coordinates |
|---|---|---|---|
| 1 | *P. ciliata* (Population I) | Al-Qaed district; Ha'il (Location I) | **27°44'25" N 41°36'23" E** |
| 2 | *P. ciliata* (Population II) | Al-Qaed district; Ha'il (Location II) | **27°51'8" N 41°43'32" E** |

*RAPD analysis*

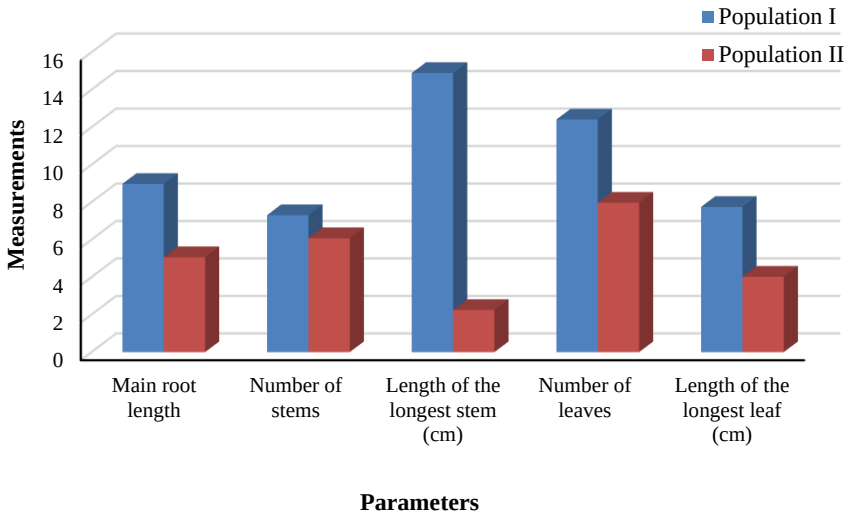
Random amplified polymorphic DNA (RAPD) analysis was used to evaluate genetic diversity for the conservation of wild populations. This method relies on PCR amplification of genomic DNA. DNA extraction was performed on young leaf tissue using the protocol described by Wolff [31]. Standard polymerase chain reaction (PCR-RAPD)

conditions were employed for amplifying the genomic DNA. The PCR reactions were carried out in a 25 µL volume containing 12 ng of genomic DNA, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.01 µM of primer, and 1 U of Taq polymerase. DNA quality was checked using 0.8% agarose gel electrophoresis and quantified with a Nanodrop spectrophotometer (Thermo Scientific, Wilmington, DE, USA).
The PCR products were analyzed by electrophoresis on a 2.5% agarose gel at 85 V for 45 minutes and visualized under UV light. The size of the alleles was compared to a 100 bp DNA ladder.

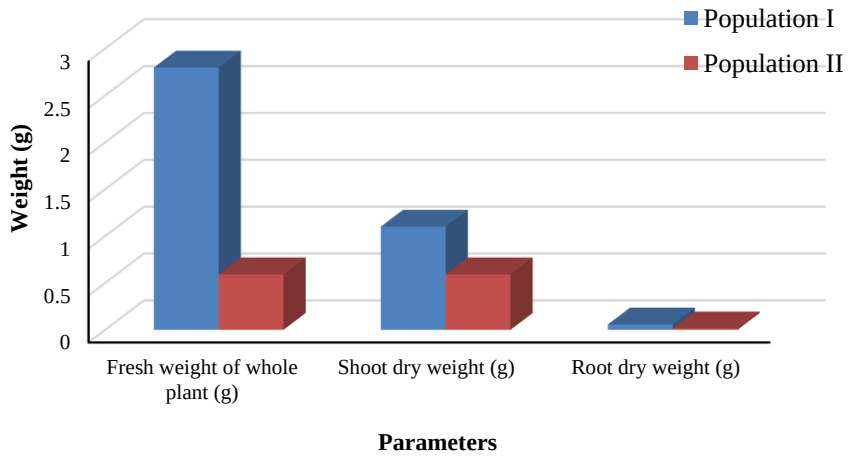
*Statistical analysis*
Phenotypic data were subjected to analysis of variance (ANOVA), and significant differences were identified using Duncan's Multiple Range Test (DMRT) at $P \leq 0.05$. Statistical analyses were conducted using SPSS software (SPSS-17, Inc., Chicago, IL, USA). The number of polymorphic bands and polymorphic percentage were calculated. Cluster analysis of phenotypic data was performed based on similarity matrices, using the Unweighted Pair Group Method with Arithmetic Mean.

## Results and Discussion

*Phenotypic variations*
The findings revealed considerable variations ($P \leq 0.05$) in the phenotypic characteristics of *P. ciliata*, with notable differences observed both within and between the 2 populations examined **(Figures 1 and 2)**.



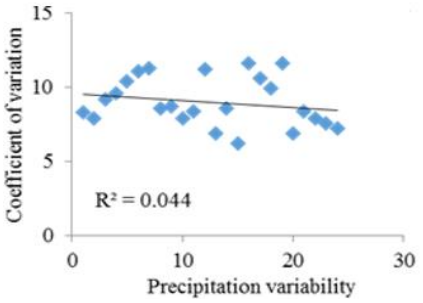

a)




b)

**Figure 1.** Variations in phenotypic traits of *P. ciliata* gathered from two different sites during the 2016 spring season from the natural rangelands of Ha'il region, Saudi Arabia.

The traits assessed at the individual level included the number of leaves, main root length, number of stems (spikes), length of the longest leaf, length of the longest stem as well as the fresh weight of the entire plant, shoot, and root dry weight. The overall trend in the variation between the 2 populations displayed that the plant population from location I exhibited the highest measurements, while the population from location II had the lowest measurements **(Figures 1a and 1b)**. Within each population, significant differences were observed. These variations were analyzed by comparing the relationship between morphological differences in life traits within *P. ciliata*, calculated as the coefficient of variation across individuals and linked to precipitation variability **(Figures 2a-2n)**.

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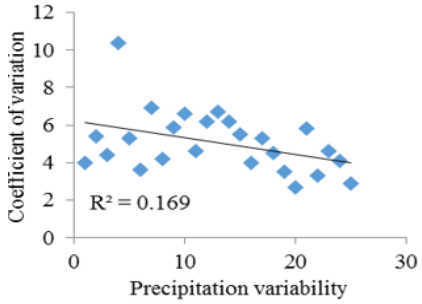


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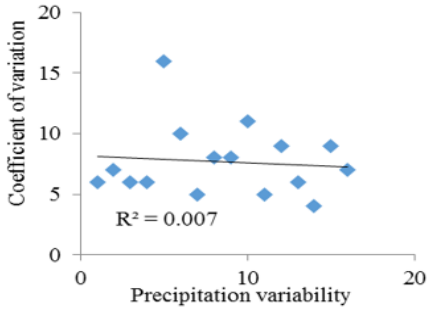


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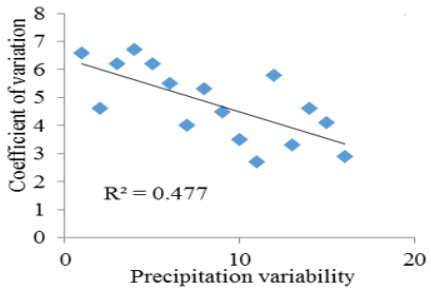
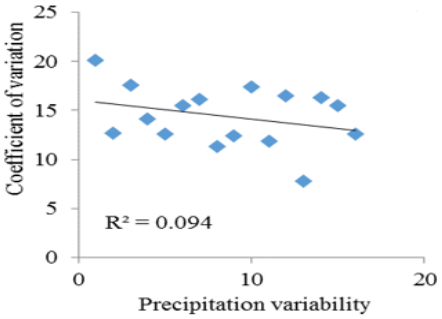
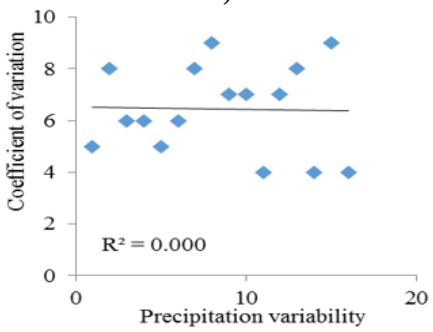



d)




e)

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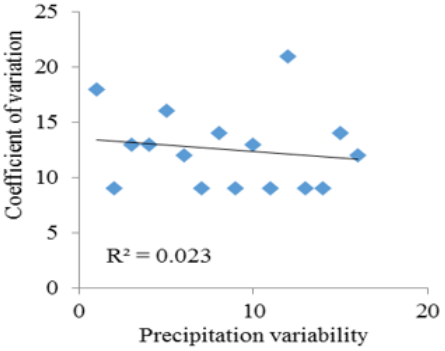


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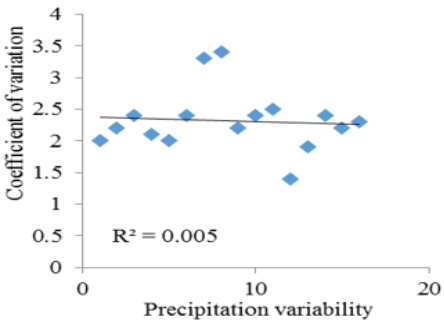


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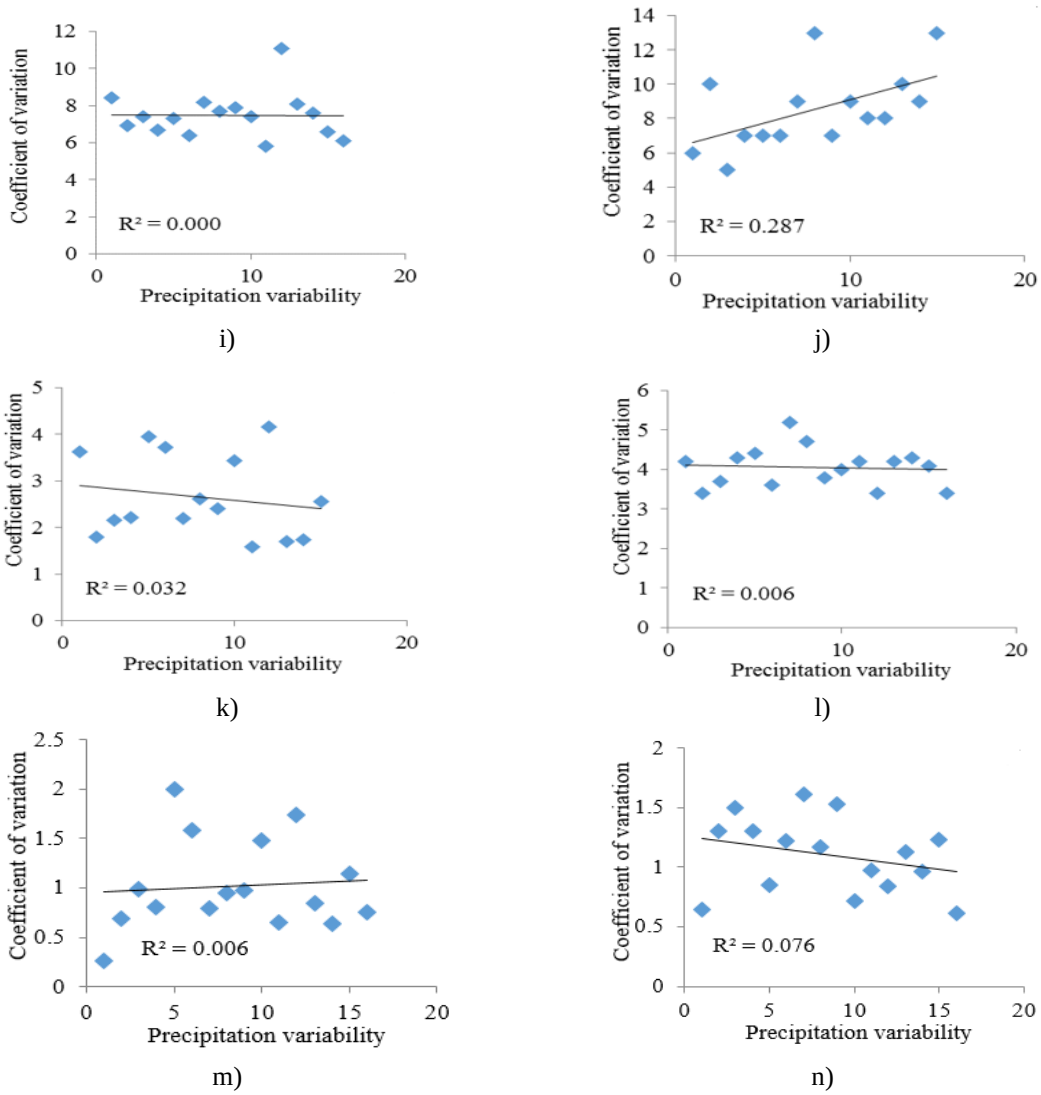


**Figure 2.** The correlation between phenotypic variation in life traits within *P. ciliata*, measured as the coefficient of variation (CV) among individuals, and precipitation variability. The traits for population I and population II are represented in a) and b) for main root length, c) and d) for the number of stems (spikes), e) and f) for the length of the longest stem, g) and h) for the number of leaves, i) and j) for the length of the longest leaf, k) and l) for the fresh weight of the entire plant, and m) and n) for shoot dry weight. The $R^2$ value for each regression analysis is provided.

Additionally, the unweighted pair group method with arithmetic mean phenogram illustrated the relationships within *P. ciliata* based on eight chosen morphological traits **(Figure 3)**.

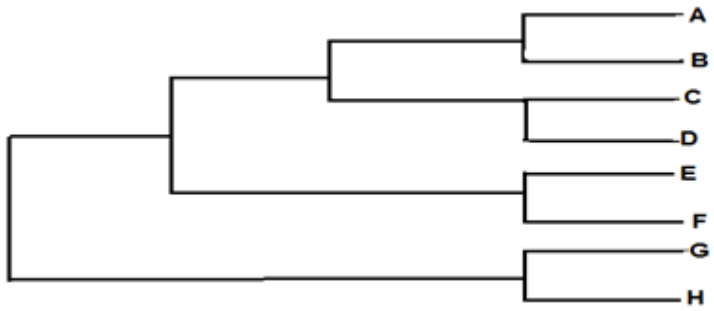


**Figure 3.** Phenogram derived from the unweighted pair group method with arithmetic mean (UPGMA) depicting the relationships within *Plantago* based on eight selected morphological traits.

Various studies have utilized both molecular and morphological variations to investigate the phylogeny and diversity of *Plantago* species. Evaluating and classifying landraces of *Plantago* is a crucial part of collective efforts, given their significant genetic diversity, which has been shaped by multiple generations of selection by farmers and breeders. These landraces also serve as valuable sources of unique genes that provide resistance to environmental stress [32]. The findings of this study align with those of Vahabi *et al*. [33], who explored genetic variations among 22 populations of *P. ovata* using molecular techniques. Their research revealed substantial diversity across all morphological traits among the populations.

*Genotypic variations*

A total of 34 DNA fragments were produced by the primers, 6 of which were polymorphic and 28 were monomorphic. The observed polymorphisms between the two *P. ciliata* populations were highlighted through the photometric bands recorded in this study **(Figure 4; Table 2)**. 5 random APOM primers (APOM1, APOM3, APOM4, APOM5, and APOM6) were selected for the RAPD analysis to detect polymorphism within the populations. Among these, only APOM5 and APOM6 amplified bands in both populations, whereas APOM1, APOM3, and APOM4 failed to produce any bands. This lack of amplification might be due to the absence of complementary binding sites in the genomic DNA of these two *P. ciliata* populations, or it could indicate that the primers need specific conditions to perform effectively [34, 35].

In a similar study, Vahabi *et al*. [33] identified one hundred forty-two polymorphic PCR products (with an average of 4.05 bands per primer) using 35 RAPD primers in *P. ovata*. Singh *et al*. [36] also used 20 random primers to detect 102 bands in 36 genotypes of *P. ovata*, of which 89 (87.25%) were polymorphic, 5 (4.9%) were monomorphic, and 8 (7.8%) were unique, as shown in **Figure 3**. In addition, RAPD markers were employed to analyze the genomic relationships among 22 *P. ovata* populations. The clustering based on RAPD data revealed that populations from similar geographic areas tended to group. The resulting phylogenetic tree displayed a clear differentiation between two major groups [37], emphasizing both molecular and phytochemical variations among the 5 populations of *Plantago* major. Similarly, Singh *et al*. [36] conducted RAPD profiling on eighty accessions of *Plantago* spp., grouping them into seven clusters based on their genetic differences.

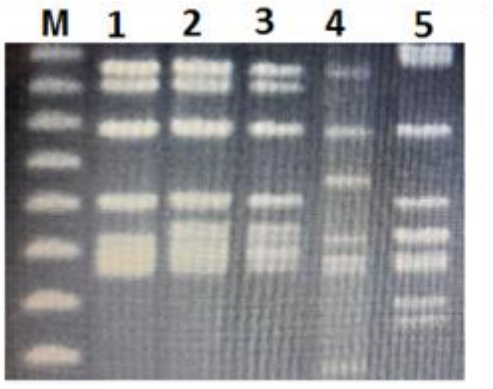


**Figure 4.** Electrophoretic analysis of *P. ciliata* populations using 5 random primers: APOM1, APOM3, APOM4, APOM5, and APOM6. The PCR products from the RAPD analysis were separated on a 2% agarose gel stained with ethidium bromide.

**Table 2.** Random primers demonstrating polymorphism in the two populations of *P. ciliata* collected from distinct sites in the Ha'il Region, Saudi Arabia

| Sr. No | Primer code | Primer sequence (F/R) | No. of genotypes | Total amplified bands | Polymorphic bands | Monomorphic bands | Percentage of polymorphism |
|---|---|---|---|---|---|---|---|
| 1 | APOM 1 | TGGCACTTGGGCAAATCT ACTTGG TTGGTATCCACGGATGAA CAGCCT | 2 | 7 | 0 | 7 | 0.00 |
| 2 | APOM3 | GTTTACCTTGCTCAAGTG CTTGCT | 2 | 7 | 0 | 7 | 0.00 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | AACTCCTTCACCCTTCGC CTAACA | | | | | |
| 3 | APOM4 | TGTCACACACACACACAC ACACAC AGGGAAACTGCCATGACT CCTCTT | 2 | 7 | 0 | 7 | 0.00 |
| 4 | APOM 5 | ATGGAAGGAGGGTGGTG GAAGTTT AGCTTTATCACAGCGACG GAGCTT | 2 | 6 | 3 | 3 | 50.00 |
| 5 | APOM 6 | AATTGAAGACTGTGCACT TGGGCG AAAGGAGAGAGAGAGAG AAGCACG | 2 | 7 | 3 | 4 | 42.86 |

The findings of the present study align with those of Vahabi *et al*. [33], who carried out a field study to evaluate the morphological and molecular variations among 22 populations of *P. ovata* using molecular markers. To assess the relationship between accessions, clustering analysis, and principal component analysis were applied based on the mean values of 8 morphological traits. A total of 142 polymorphic bands were identified using 35 RAPD primers, with an average of 4.05 bands per primer. The RAPD-based clustering, performed using the Unweighted Pair Group Method, revealed a significant correlation between the morphological and RAPD dendrograms, although no such relationship was observed between morphological variations and ISJ-GS with RAPD. In addition, populations from geographically close regions tended to group in the RAPD clustering. The ISJ marker framework generated 95 DNA fragments, with an average of 2.55 polymorphic bands per semi-random primer. However, the ISJ-based dendrogram did not align with the morphological, geographic, or RAPD variations. The study highlighted the potential of RAPD and ISJ markers in assessing genetic diversity, managing genetic resources, and identifying duplicate accessions in *P. ovata*.

## Conclusion

The study observed significant genotypic and phenotypic differences in *P. ciliata* populations collected from two distinct sites in the Ha'il Region, Saudi Arabia. These results are valuable for enhancing breeding programs and ensuring the conservation of biodiversity.

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**Conflict of Interest:** None

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**Ethics Statement:** None

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