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Assessing the Phenotypic and Genotypic Variations of *Plantago ciliata* in Ha'il Region, Saudi Arabia

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ABSTRACT

The present work aimed to assess the phenotypic and genotypic variations of *Plantago ciliata* collected from two different locations in Ha'il Region, Saudi Arabia. Two *P. ciliata* species were collected in 2016 spring season from local natural rangelands. The phenotypic variations were assessed by measuring the main root length, number of stems (spikes), length of the longest stem, number of leaves, and length of the longest leaf as well as the fresh weight of the whole plant. Moreover, the genotypic variations were assessed by using the RAPD technique. Data on phenotypic traits were subjected to analysis of variance (ANOVA) procedure. Cluster analysis supported similarity matrices was additionally deployed on phenotypic data using the Un-Weighted Pair Group Methodology with Arithmetic mean (UPGMA) to get a dendrogram. The results showed significant variations ($P \leq 0.05$) in the *P. ciliata* phenotypic traits within and between the two studied populations. The general pattern of the variation between the two populations showed that the highest measurements were recorded by plant population collected from location I, while the lowest measurements were recorded by population collected from location II. Out of the five APOM, random primers applied, two primers showed amplification in the two populations, while three primers did not show any amplification in any of the populations. Out of these five primers, two primers showed polymorphism and two primers gave the variation in bands. In conclusion, the findings of this study are important for breeding programs and biodiversity conservation in Ha'il Region, Saudi Arabia.

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

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INTRODUCTION

The genus *Plantago* which belongs to the Plantaginaceae family dominates landscapes across the world and includes about 200 -256 species, which plays an important role as s feed for grazing animals and pharmaceutical purposes [1, 2]. This genus grows in a very wide area of Europe and temperate regions of Asia, Australian state in addition to North America. It has plants with wide geographic distribution in temperate grasslands of the world. It grows naturally in the geographic region in Central Asia however currently it's nearly found all over the world [2, 3]. This genus is sometimes found in poor soils that are deficient in elements such as sodium and potassium. When nitrogen is present, the number of total biomass increases, stem growth, and leaves. The impact of nitrogen, however, is restricted on root growth [4].

Most of the *Plantago* plants are perennial forage plants, which last for many years, seemingly glabrous, have a small root and several vertical or bending stems that are equal to or slightly longer than the leaves and lack spaces. Its leaves are oval (the main type) with large veins and serrated ends, comprehensive, and relatively long petioles. Some of the *Plantago* plants are yearly, 5-20 cm high, less stemmed, more or less appressed-hairy. Leaves resolute, closely oblong-lanceolate to linear-lanceolate. Scape thick, vertical or ascending, lengthier or smaller than leaves. Spikes inflexible, very condensed, hardly cylindrical, 3-10 cm. Fruitlet capsule, 3-5 seeded [5]. *Plantago* is a vital medicinal plant that has numerous combinations of secondary metabo-

lites [6]. It has been used for the treatment of some conditions such as high blood glucose and elevated cholesterol levels [7]. Moreover, the WHO has approved its use as a laxative agent [3, 8]. Nevertheless, relatively few studies have been conducted on the plant, especially studies related to genomic resources of the plant [9-15]. Despite its enormous medical and economic value, the yield of *Plantago* is subject to the restrictions of environmental factors, which causes a massive reduction in the quality and yield of seed/husk. Moreover, the efforts concerning genetic diversity generation were relatively unsuccessful because of inadequate genetic resources [16]. Moreover, given that it is a crop introduced to many countries in west and south Asia, the variations in value significant characters in the accessible gene pool are very shallow [17,18]. Based on this low diversity, there are different breeding approaches, like selection, induced mutations, hybridization, tissue culture, and polyploidy, which have been used for the genetic enhancement of *Plantago* [19-22]. The morphological differences among species in the genus *Plantago* are low and how the species are grouped is unclear. Three genera in the whole family were recognized and the genus *Plantago* has been subdivided into six sections [5]. Nevertheless, the genetic variation depends on molecular markers which have been used to evaluate the situation and development magnitude of populations [23,24]. The wild allies in Plantago species are huge and have medical significance. These wild types are a source of genes that are important for *Plantago* production [25]. The use of the randomly amplified polymorphic DNA (RAPD) has been shown by few reports [26,27] and included a combination of RAPD and (ISSR) markers for assessing genetic diversity in *Plantago* [28,29]. Several authors necessitate the urgency for characterizing the genus *Plantago* cultivated genotype, as their genetic diversity and proposed molecular markers could be useful for detecting polymorphism among the genotypes [1]. The phylogenetic analysis of the Plantago ovata (Forsk.) crop for its use in future breeding programs to identify crop varieties was reported by Rohillaet al. [1] who indicated the usefulness of RAPD analysis in genetic relationships determination and genetic diversity estimation among the genotypes of *P. ovata* [1]. Considering the economic importance of the genus *Plantago* The present work was aimed to assess the phenotypic and genotypic variations in *Plantago ciliata* collected from two different locations in Ha'il Region, Saudi Arabia.

MATERIALS AND METHODS

Study area

The *Plantago* species in the current study were collected from the Ha'il region, which lies in the middle-north of the Kingdom of Saudi Arabia between 25° 29′N and 38° 42′E and it extends over an area of 118,322 km^2. The mean temperature in Ha'il ranges from 10.8°C in winter to 34.1°C in summer and the annual rainfall is about 104.4 mm which falls mostly in winter [30]. Therefore, rangeland is classified among the arid zones with a prolonged dry period and the short scattered rainy season which lasts most of the year in Ha'il.

Sample collection

Two *Plantago* species (*Plantago ciliata*) were collected in the 2016 spring season from local natural rangelands of the Ha'il region, Saudi Arabia **(Table 1)**. Fresh grass specimens were uprooted by digging the soil and preserved in polyethylene bags. The samples were then transferred to the laboratories of the Department of Biology, Faculty of Science, the University of Ha'il for identification and further analysis. Samples were dried in a vacuum oven at a temperature of 105°C for 24 hours, and 50 grams of each dried sample were then packed in paper sacks and stored for further analysis. The parameters measured (phenotypic traits) included main root length, number of stems (spikes), length of the longest stem, number of leaves, and length of the longest leaf as well as the fresh weight of the whole plant, shoot and root dry weight.

Table 1: Plant species from natural rangeland of Ha'il, Kingdom of Saudi Arabia, collected in 2016 spring season.

No.	Name	Location	Coordinates
1	*Plantago ciliata* (Population I)	Al-Qaed district; Ha'il (Location I)	**$27^0 44^{-} 25^{=}$N $41^0 36^{-} 23^{=}$ E**
2	*Plantago ciliata* (Population II)	Al-Qaed district; Ha'il (Location II)	**$27^0 51^{-} 8^{=}$N $41^0 43^{-} 32^{=}$ E**

RAPD Analysis:

To assess genetic variation for conservation wild populations, a random amplified polymorphic DNA (RAPDs) is a useful approach. It is based on genomic DNA PCR amplification. DNA was extracted from young leaf tissue following the method Wolff [31]. The standard polymerase chain reaction (PCR-RAPD) protocol was used for the Amplification of genomic DNA. Reactions were set in 25ul volume consisting of 12ng of genomic DNA 1.5 mM MgCL2, 0.2 mM dNTPs, 0.01 Mm of primer, and 1U Taq polymerase checked for quality on 0.8% agarose gel, and quantified using spectrophotometer Nanodrop (Thermo Scientific Wilmington, DE, USA).

PCR amplification products were electrophoresed at 85 V for 45min on 2.5 %agarose gel and viewed under UV light. The size of alleles was determined concerning a 100 pb DNA size standard.

Statistical analysis:

Data on phenotypic traits subjected to analysis of variance (ANOVA) procedure. Using Duncan's Multiple Range Test (DMRT), the means were separated for significance ($P \leq 0.05$). Statistical analysis was done using SPSS software (SPSS-17. Inc., Chicago, ll, USA). Product loci (band) numbers of polymorphic bands and polymorphic percentage %. Based on similarity matrices, cluster analysis was also deployed on phenotypic data using Unweighted Pair Group Method with Arithmetic Mean (UPGMA).

RESULTS AND DISCUSSIONS

Phenotypic variations

The results indicated significant differences ($P \leq 0.05$) in the *Plantago ciliata* phenotypic traits, *i.e.* parameters measured within and between the two studied populations **(Figure 1 and 2)**.

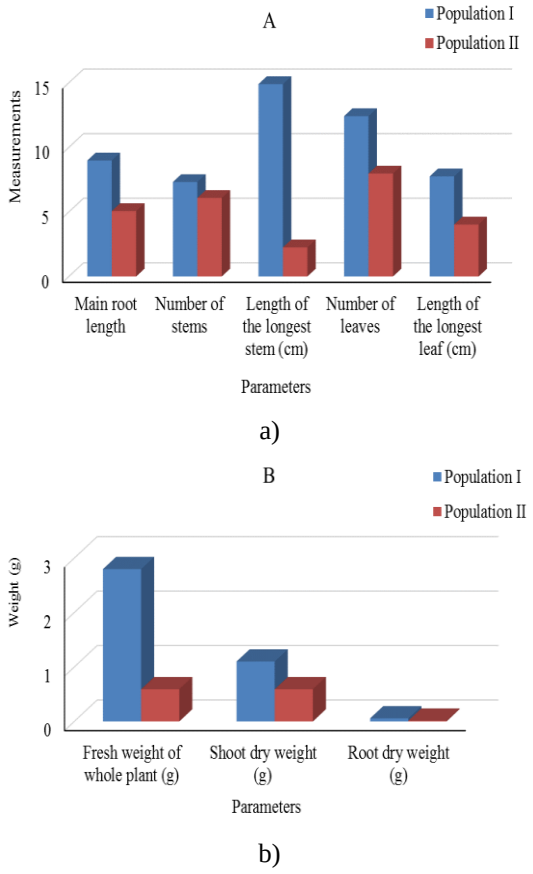
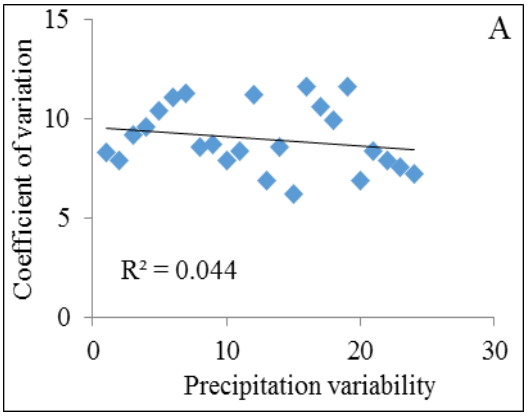


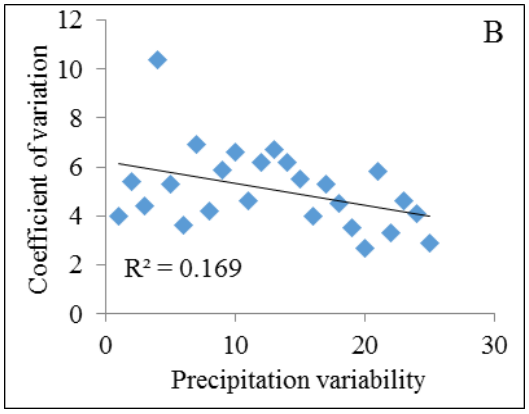
Figure 1. Phenotypic variations in *Plantago ciliata* collected from two locations in the 2016 spring season from local natural rangelands of Ha'il region, Saudi Arabia.

The traits originally measured at the individual level were the main root length, number of stems (spikes), length of the longest stem, number of leaves, length of the longest leaf as well as fresh weight of the whole plant, shoot, and root dry weight. The general pattern of the variation between the two populations showed that the highest measurements of *P. ciliata* were recorded by plant population collected from location I, while the lowest measurements were recorded by plant population collected from location II **(Figure 1 a and b)**. The general pattern of the variation within the populations showed significant variations within the populations. The variations were presented as the relationship between morphology variance in life traits within *P. ciliata* and measured as the coefficient of vari-

ation (CV) among individuals and precipitation variability **(Figure 2 A - N)**.



a)



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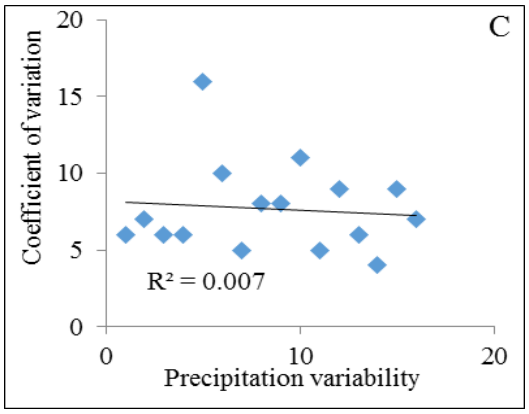

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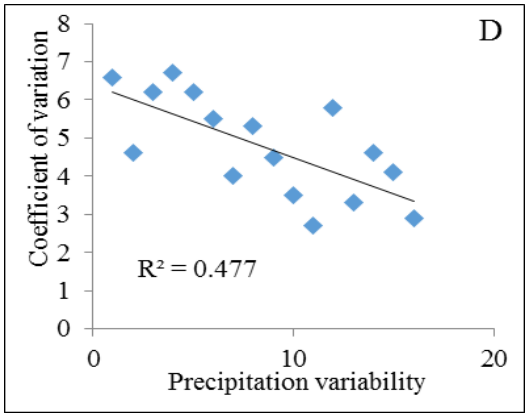

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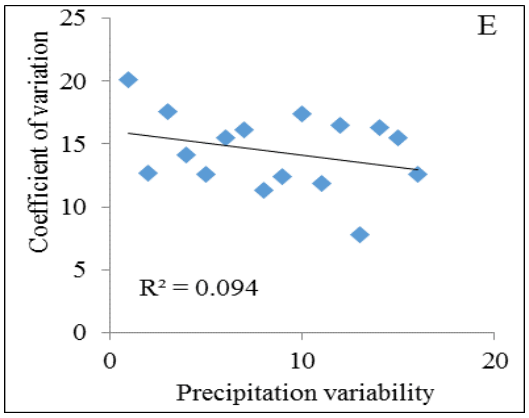
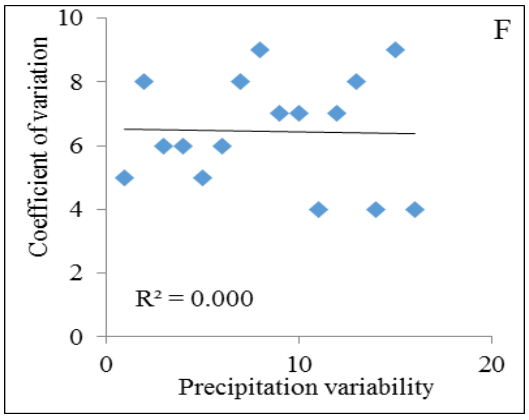
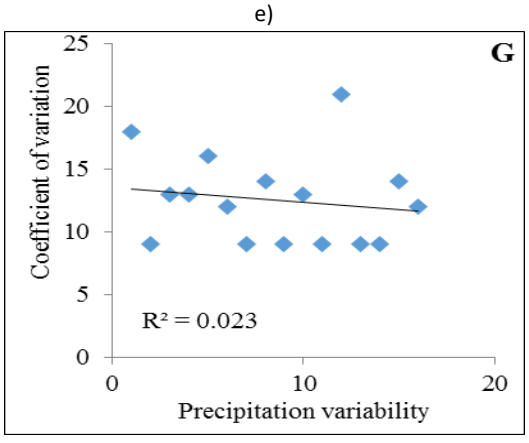


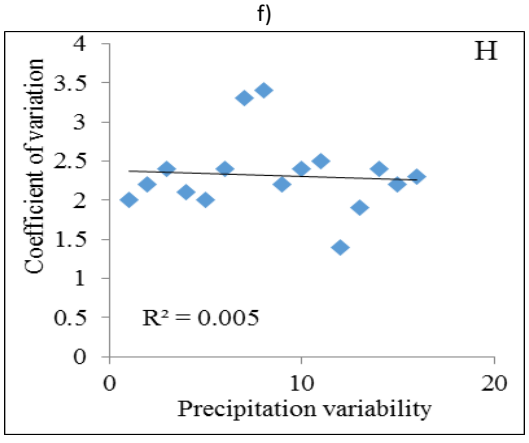
e)



f)



g)



h)

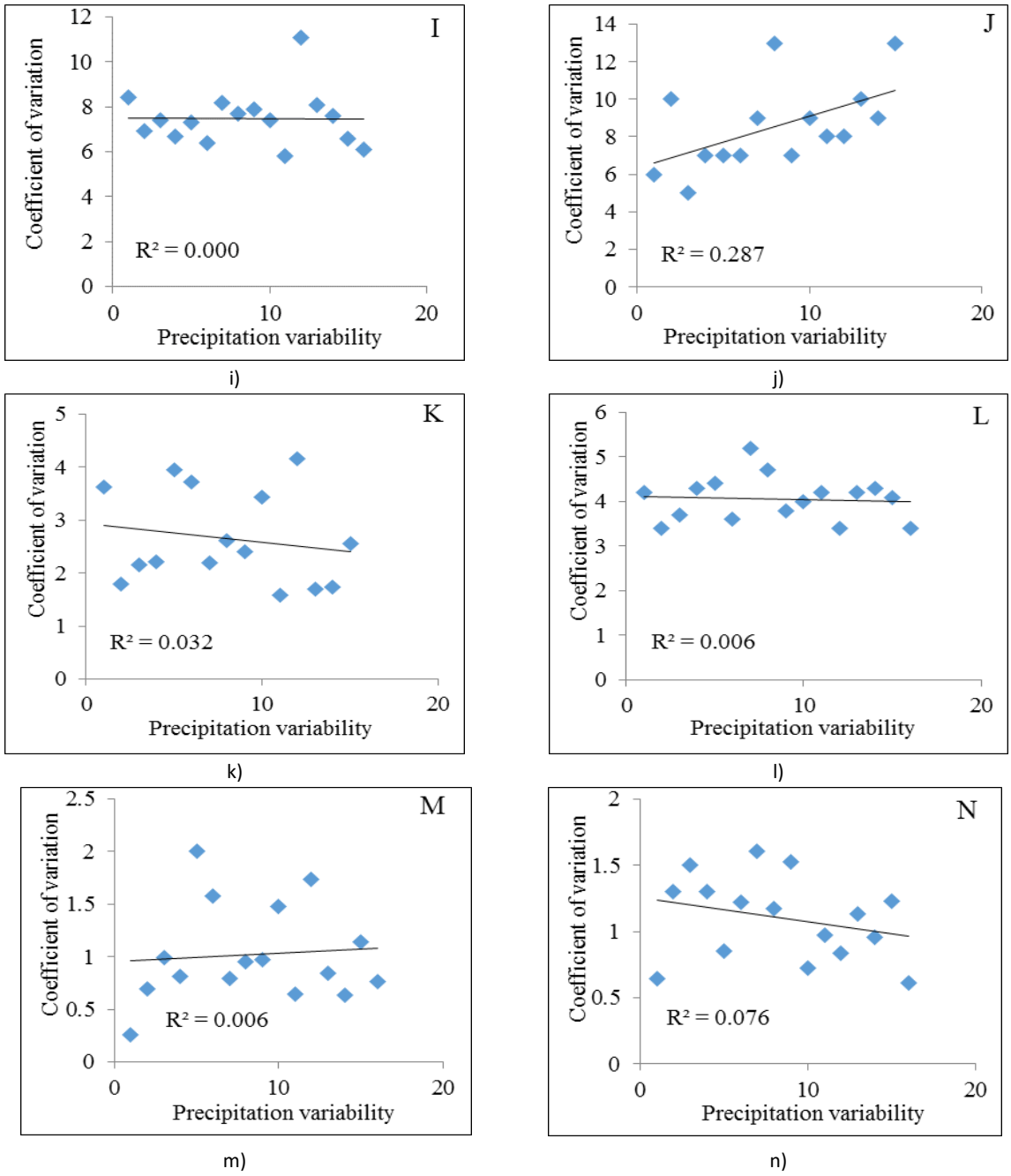


Figure 2. Relationship between phenotypic variation in life traits within *Plantago ciliata* measures coefficient of variation (CV) among individuals and perception variability. Traits for population I and population II are (a) and (b) main root length, (c) and (d) the number of stems (spikers), (e) and (f) length of the longest stem, (g) and (H) number of leaves,(i) and (j) length of longest leaf, (k) and (l) fresh weight of whole plants, (m) and (n) shoot dry weight respectively .R^2 value is given for each regressions analysis.

Moreover, Unweighted Pair Group Method with Arithmetic Mean (UPGMA) phenogram showed the relationships within the *P. ciliate* based on the selected eight morphological characters **(Figure 3)**.

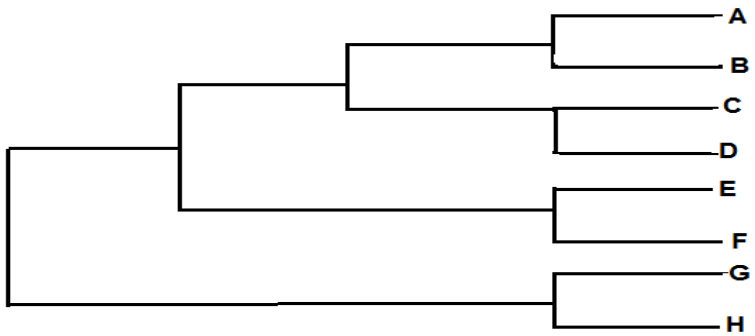


Figure 3. Unweighted Pair Group Method with Arithmetic Mean (UPGMA) phenogram showing the relationships within the *Plantago* based on the 8 selected morphological characters.

Based on morphological and molecular variations, several studies are used to examine phylogeny and the diversity of the *Plantago* species. Assessment and classification of the landrace *Plantago* are key components of group efforts due to its massive in-built genetic diversity because of several generations of rising and selection by farmers and breeders. Landraces also form a good reservoir of distinctive genes conferring resistance to environmental stress [32]. The results of the current study were consistent with those of Vahabi *et al*. [33], who conducted a field experiment to assess genetic variations among 22 populations of *Plantago ovata* using the molecular technique [33] in which he revealed very great diversity among the 22 populations for all morphological traits.

Genotypic variations

Primers produced 34 DNA fragments, 6 were polymorphic bands and 28 were not polymorphic. Polymorphism between the two plants in bands revealed in photometric characters that were recorded in this study **(Figure 4 and Table 2)**. Five random APOM primers (APOM1, APOM3, APOM4, APOM5, and APOM6) were used for RAPD analysis of the two *Plantago ciliate* populations for detecting polymorphism. Out of the five random primers used, two of them (APOM5 and APOM6) showed amplification in the two populations, while three primers (APOM1, APOM3, and APOM4) did not show any amplification in any of the populations. These primers may have found no complementary binding sequences in the genomic DNA of these two *P.ciliata* populations. Furthermore, these primers might work properly in certain conditions [34,35].

Vahabi*et al*. [36] observed 142 polymorphic PCR products (average of 4.05 bands per primer) by using 35 RAPD primers in *P. ovata*. Likewise, by using 20 random primers, 102 bands were detected among 36 genotypes of *P. ovata*. Out of 102 bands, 89 (87.25%) were polymorphic, while 5 (4.9%) were monomorphic and 8 (7.8%) were unique as studied by Singh *et al*. [37] **(Figure 3)**.

Furthermore, using RAPD markers [36] the genomic relations between 22 populations of *P. ovata* were studied. All populations representing the nearest zone formed neighboring groups in the RAPD based clustering. By performance of the RAPD study, the phylogenetic tree showed a noticeable difference between the two main groups [38] and highlighted the phytochemical and molecular variations between and within the five populations of *Plantago major*. Likewise, Singh *et. al*. [37] evaluated germplasm diversity between 80 accessions of *Plantago spp*. through RAPD profiling. The 80 accessions were categorized into seven clusters given the degree of difference.

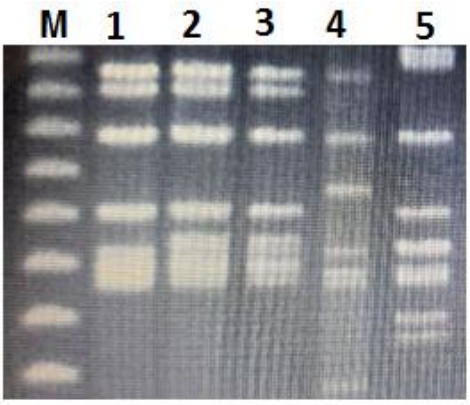


Figure 4. Electrophoretic pattern of Plantago ciliata populations with five random primers; APOM1, APOM3, APOM4, APOM5, and APOM6 (ethidium bromide-stained 2% agarose gel electropherogram of PCR products obtained from the RAPD analysis)

Table 2. Random primers showing polymorphism among the two *Plantago ciliata* populations collected from two different locations in 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	TGGCACTTGGGCAAATCTACTTGG TTGGTATCCACGGATGAACAGCCT	2	7	0	7	0.00
2	APOM3	GTTTACCTTGCTCAAGTGCTTGCT AACTCCTTCACCCTTCGCCTAACA	2	7	0	7	0.00
3	APOM4	TGTCACACACACACACACACACAC AGGGAAACTGCCATGACTCCTCTT	2	7	0	7	0.00
4	APOM 5	ATGGAAGGAGGGTGGTGGAAGTTT AGCTTTATCACAGCGACGGAGCTT	2	6	3	3	50.00
5	APOM 6	AATTGAAGACTGTGCACTTGGGCG AAAGGAGAGAGAGAGAGAAGCACG	2	7	3	4	42.86

The results of the current study were consistent with those of Vahabiet al. [36], who conducted a field experiment to assess morphological and molecular differences among 22 populations of Plantago ovata using molecular markers. Based on the distance between the averages of 8 morphological properties, clustering investigation and central component were utilized to find the relationship between the accessions. Thirty-five RAPD preliminaries gave 142 polymorphic bonds, with a mean of 4.05 groups per primer. Utilizing the Unweighted Match Gather Strategy, the clustering examination method based on RAPD showed that a strong correlation exists among morphological and RAPD dendrograms, but there was no correlation between the morphological variations and ISJ-GS with RAPD. Additionally, populations representing the near zones form close groups in RAPD-based clustering. The ISJ framework marker delivered a 95 DNA part, which has a 2.55 polymorphic band for each semi-random primer. Based on ISJ, the dendrogram marker was not consistent with morphological, topographical, and RAPD variations.

The possibility of the use of RAPD and ISJ markers to estimate genetic diversity, management of genetic resources, and determination of repetitive accessions in Plantago ovata has been demonstrated by the results of this work.

CONCLUSION

The general pattern of the phenotypic and genotypic variations in *Plantago ciliata* collected from two different locations in Ha'il Region, Saudi Arabia showed significant variations between the two populations. These findings are important for breeding programs and biodiversity conservation.

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CONFLICT OF INTEREST: None

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ETHICS STATEMENT: None

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