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Contributions to the knowledge of seven *Plantago* L. accessions (*Plantaginaceae*) from Tunisia

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Abstract:

Background*: The affinities of the Plantaginaceae are not clear and Plantago is a problematic genus to study; most of its species are so similar that identifying stable aspects of variation among them is difficult. In the present study, we attempt to determine the relationships among seven Plantago species combining morphological and ecological data.*

Materials and Methods*: Morphological variations and morphometry of leaves, inflorescence, flowers and seeds were studied in seven Plantago L. accessions, namely P. ciliata Desf., P. albicans L., P. albicans var. nana Boiss., P. weldenii Rchb., P. coronopus L., P. albicans subsp. lanuginosa Chevall., P. serraria L. collected from different locations in Tunisia mainly from arid regions. For edaphic study, soil samples were collected at each collection site near the base of each plant. We conducted a principal components analysis using a multivariate technique to observe the relationships between the edaphic data, the altitude and the studied accessions of Plantago.*

Results*: Ward tree of morphological data were established based on UPGMA-clustering shows that the studied accessions belong to two subgenus Coronopus and Albicans. Main results of the PCA show that the edaphic data and the altitude of the selected sites explain 91.55% of the repartition of the studied taxa. According to our results, it turned out that accessions of subgenus Coronopus could thrive soils with high pH and electrical conductivity. Nonetheless, accessions from the subgenus Albicans prefer the soil with high sand percentage and high altitude.*

Conclusion: *Morphological analysis revealed that the studied accessions should be classified into two subgenera and supports Rahn's classification system, P. weldenii Rchb., P. coronopus L. and P. serraria L. should belong to the subgenus Coronopus, Section Coronopus and P. albicans L., P. albicans var. nana Boiss. and P. albicans subsp. lanuginosa Chevall should belong to the subgenus Albicans, Sections Albicans, Series Albicantes and P. ciliata Desf. to Ciliatae.*

Key Word*: Morphological analysis, edaphic data, Plantago genus, Tunisia*

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I. Introduction

The genus *Plantago* L. (*Plantaginaceae*) has over 200 species with complex nomenclature and taxonomy[1]. The species are usually anemophilous herbs or rarely subshrubs, perennial or annual, and concentrated in temperate and high-elevation tropical regions[2]. The taxonomy of the genus *Plantago* is contentious at the section and subgenus levels.

Barneoud[3] published the first systematic classification of the family, recognizing three genera and subdividing the genus *Plantago* into six sections. Afterward, Decaisne[4] divided the genus into 17 sections. *Plantago* was divided into 12 sections by Engler & Prantl[5], which were divided into two subgenera, one of which contained a single section. Within *Plantago*, Pilger[6] recognized two subgenera: *Euplantago*, which has 247 species divided into 18 sections, and *Psyllium*, which has 13 species in a single section. Rahn[7] published a revision of the genus in which he divided it into 3 subgenera, i.e. *Plantago, Coronopus* and *Psyllium* (Juss.) Harms & Reiche. According to Rahn[8] the genus is divided into 6 subgenera: *Plantago*, *Coronopus*, *Albicans*, *Psyllium*, *Littorella* and *Bougueria.*

In this study, morphological and morphometric data are used to: characterize seven *Plantago* accessions; compare their phenetic relationships using statistical methods and investigate the impact of the ecology of their habitats on their distribution.

II. Material and methods

Plant material

For the initial phylogenetic analysis, we studied Tunisian accessions of *Plantago* L. mainly collected between 2019 and 2021. Seven *Plantago* genotypes were collected from their natural habitats in Tunisia (**Table no1**).

Table no1. Geographical repartition and sampling information of selected sites

N°	Accessions	Latitude	Longitude	Altitude
1	*P. ciliata* Desf.	33°23'00.29" N	10°22'30.34''E	136 m
2	*P. albicans* L.	33°46'48.27" N	10°53'21.10" E	21 m
3	*P. albicans* var. *nana* Boiss.	33°24'51.74" N	10°55'07.19" E	2 m
4	*P. weldenii* Rchb.	35°58'52.95" N	10°31'.38.53" E	2 m
5	*P. coronopus* L.	33°37'41.02" N	10°56'45.31" E	0 m
6	*P. albicans* subsp. *lanuginosa* Chevall.	33°28'25.80" N	10°07'44.59" E	562 m
7	*P. serraria* L.	35°57'47.89" N	9°15'22.16" E	868 m

In order to identify the collected accessions, we checked some specimens from other countries and herbaria. At each studied site, sampling was carried out by randomly collecting twenty-five plants, each plant representing one replication. A list of the accessions and collection data of the specimens representing them is provided in **Table no2**. Identification of *Plantago* accessions was done by using different relevant references[8, 9].

Table no2. Infragenic classification of the studied *Plantago* taxa

N°	Taxa	Subgenera and Sections (Pilger, 1937)	Subgenera and Sections (Rahn, 1978)	Subgenera (Rahn, 1996)	Voucher N°
1	*P. ciliata* Desf.	*Plantago* sect. *Leucopsyilium*	*Psyllium* sect. *Albicans*	*Albicans*	G00148792
2	*P. albicans* L.	*Plantago* sect. *Leucopsyilium*	Psyllium sect. *Albicans*	*Albicans*	P04076052
3	*P. albicans* var. *nana* Boiss.	*Plantago* sect. *Leucopsyilium*	*Psyllium* sect. *Albicans*	*Albicans*	K000779590
4	*P. weldenii* Rchb.	*Plantago* sect. *coronopus*	*Plantago* sect. *coronopus*	*Coronopus*	B101085492
5	*P. coronopus* L.	*Plantago* sect. *coronopus*	*Plantago* sect. *coronopus*	*Coronopus*	C10002786
6	*P. albicans* subp. *lanuginosa* Chevall.	*Plantago* sect. *Leucopsyilium*	*Psyllium* sect. *Albicans*	*Albicans*	MPU005316
7	*P. serraria* L.	*Plantago* sect. *coronopus*	*Plantago* sect. *coronopus*	*Coronopus*	MPU009400

Soil samples collection

Soil samples were collected at 0-20 cm depth, at five distinct points to be analysed for physical and chemical characteristics [pH, electrical conductivity (EC), % Sand, % Clay and % Silt]. Soil samples were then smashed, homogenized, dried at room temperature (20-25 °C and 60-70% relative humidity to a constant weight), and sifted through a 2-mm sieve in preparation for further chemical and physical analyses.

Morphological characterization

The morphological analysis was conducted using 90 characteristics of seven *Plantago* accessions. Multistate characters were transformed into two-state characters in coding and their presence or absence was coded 1 and 0, respectively, when preparing the raw data matrix (**Table no3**). PAST software, version 4.03[12] was used for the data analysis. Clustering was performed using Unweighted Pair-Group Method with Arithmetic average (UPGMA) and represented in phenogram (tree).

Table no3. Basic data matrix used in the numerical classification of *Plantago* genotypes

		1	2	3	4	5	6	7
Altitude	1. Present at sea level 0 m	0	0	0	1	1	0	0
	2. Present between 0 m and 100 m	0	1	0	0	0	0	0
	3. Present ≥ 100 m	1	0	1	0	0	1	1
Plant	4. Perennial	0	1	1	1	1	1	1
	5. Annual	1	0	0	1	1	0	0
	6. Stemless	0	1	1	1	1	1	1
	7. Developed stem	1	0	0	0	0	0	0

	8. Glabrous	0	0	0	0	1	0	1
	9. Hairy	0	1	1	1	0	1	0
	10. Densely hairy	1	0	0	0	0	0	0
Leaves	11. Oval	0	0	0	0	0	1	0
	12. acute lanceolate	0	0	0	0	0	0	0
	13. Lanceolate	0	0	1	0	0	0	1
	14. Linear-Lanceolate	1	1	0	0	0	1	0
	15. Tightly linear	0	0	0	0	0	0	0
	16. Oblong	0	0	0	0	0	1	0
	17. Pennatifid/Spatulate	1	0	0	1	1	0	0
	18. Saw tooth	0	0	0	0	0	0	1
	19. Glabrous	0	0	0	0	0	0	0
	20. Hairy	1	1	1	1	1	1	1
Leaves diameter	21. Length ≤ 3 cm	1	0	0	0	0	0	0
	22. Length ≥ 5 cm	0	1	1	1	1	1	1
	23. Width ≤ 0,8 cm	1	1	1	1	0	1	0
	24. Width ≥ 1,2 cm	0	0	0	0	1	0	1
Scape	25. Beyond the leaves	0	1	1	0	1	1	1
	26. Equal to leaves	1	1	0	1	0	0	0
	27. Shorter than leaves	0	0	0	0	0	0	0
Bracts	28. Oval	0	0	0	0	0	0	0
	29. Scarious	0	0	0	1	1	0	0
	30. Hairy	1	0	0	0	0	0	0
	31. Glabrous	0	0	0	1	1	0	0
	32. Lanceolate	0	0	0	0	0	0	0
	33. Orbicular	0	0	0	0	0	0	0
	34. Sub-orbicular	0	1	1	0	0	1	0
	35. Acute	0	0	0	0	0	0	0
	36. Oval-rounded	0	0	0	0	0	0	0
	37. Oval-acute	0	0	0	0	0	0	0
	38. Faired	0	0	0	0	0	0	1
	39. Brownish	0	0	0	0	0	0	0
	40. Lanceolate-obtuse	0	0	0	0	0	0	0
Inflorescence	41. Linear-elongated	0	0	0	1	1	0	1
	42. Oval-globular	0	0	0	0	0	0	0
	43. Ovoid	0	0	0	0	0	0	0
	44. Oblong	0	0	0	0	0	0	0
	45. Cylindrical	0	1	1	0	0	1	0
	46. Hairy	0	0	0	0	0	0	0
	47. Short	1	0	0	0	0	0	0
	48. Globular	0	0	0	0	0	0	0
Sepals	49. Rounded	0	0	0	0	0	0	0
	50. Oval	0	1	1	0	0	1	0
	51. Faired	0	0	0	1	1	0	0

	52. Ciliate	1	1	0	0	0	0	0
	53. Glabrous	0	0	0	1	1	0	0
	54. Lanceolate	0	0	0	0	0	0	0
	55. Obtuse	0	0	0	0	0	0	1
	56. Ciliated keel	0	0	0	0	0	0	1
	57. Winged keel	0	0	0	0	0	0	0
Corolla	58. Oval	0	0	0	1	1	0	0
	59. Obtuse	0	0	0	0	0	0	0
	60. Acute	0	0	0	1	1	0	1
	61. Acuminate	0	0	0	0	0	0	0
	62. Lanceolate	1	0	0	0	0	0	0
	63. Scarious	0	0	0	0	0	0	0
	64. Largely oval	0	1	1	0	0	1	0
	65. Largely lanceolate	0	0	0	0	0	0	0
	66. Ovoid	0	0	0	0	0	0	1
Seed	67. Angular	0	0	0	0	0	0	0
	68. Large and shiny	0	0	0	0	0	0	0
	69. Smooth	0	0	0	1	1	0	1
	70. Oblong	0	1	1	0	0	1	0
	71. Oval	1	0	0	1	1	0	1
	72. Ellipsoid	0	0	0	0	0	0	0
	73. Shining	0	0	0	0	0	0	0
Seed colour	74. Brown	1	1	1	0	0	1	0
	75. Black	0	0	0	1	1	0	1
Mucilage pore position	76. Median	1	1	1	0	0	1	0
	77. Sub-median	0	0	0	1	1	0	1
Seed diameter	78. Length ≤ 2 mm	1	0	0	1	1	0	1
	79. Length ≥ 3 mm	0	1	1	0	0	1	0
	80. Width ≤ 0,7 mm	0	0	1	1	1	0	1
	81. Width ≥ 1 mm	1	1	0	0	0	1	0
Capsule Shape	82. Ovoid	0	0	0	0	0	0	0
	83. Oval	1	1	1	1	1	1	0
	84. Bilocular	0	0	0	0	0	0	1
	85. Obtuse	0	0	0	0	0	0	0
Inflorescence	86. Width ≤ 0,5 cm	1	1	1	1	0	1	0
	87. Width ≥ 0,7 cm	0	0	0	0	1	0	1
	89. Length ≤ 2 cm	0	0	0	0	0	0	0
	90. Length ≥ 2,5 cm	1	1	1	1	1	1	1

Data analysis

A two-way analysis of variance (ANOVA) was realized for soil analyses and quantitative morphological traits. Results were statistically significant at $p < 0.05$ for Tukey test using the statistical software SPSS statistics version 22.0[11].

Un-weighted pair group method using arithmetic average UPGMA dendrogram were drawn using the SAHN clustering using PAST software, version 4.03[12].

Morphological, ecological and edaphic traits were assessed by a Principal Component Analysis (PCA) in order to determine the relationship between the analysed soil samples, the altitude of collected sites and the studied accessions of *Plantago* genus taxa using PAST software, version 4.03[12]. The PCA was carried out to the data matrix (the altitude of studied sites, 5 edaphic traits and 7 accessions).

III. Results and Discussion

The Ward tree (**Figure no1**), of the selected studied genotypes based on 90 macro-morphological features, had separated *P. ciliata* Desf. from *P. serraria* L. which are split-off in two separate lines sharing 0.2 and 0.42 of similarity respectively. The five other genotypes are divided equally in two main clusters **A** and **B** at 0.7 and 0.74 respectively. Cluster **A** includes *P. albicans* L., *P. albicans* var. *nana* Boiss. and *P. albicans* subsp. *lanuginosa* Chevall. Cluster **B** comprises *P. weldenii* Rchb. *P. coronopus* L. and *P. serraria* L.

These populations differed in leaves (shape and diameter), scape length, flowers (shape, corolla, calyx) and seeds (shape, colour and mucilage pore position). The genotypes in cluster **A** belong the subgenus *Albicans*, adapted to dry habitats and is found in Asia, Europe, Africa, North and South America (absent from New Guinea, Australia and New Zealand). According to Rahn[8], in this sub-genus, leaves are often linear and spike usually short in relation to scape, Flowers spirally arranged, crowded or scattered in a spike. Corolla lobes usually acuminate or apiculate, never cordate at base. The concave side of the seeds partly covered by a ragged white membrane, except for two areas to the left and right of the centre, anterior sepals very asymmetric and with hairs very asymmetrically placed. Anthers 1.8-3.1 mm long. Few ovules develop to mature seeds. Corolla lobes inconspicuously hairy on the back for *P. albicans* L., *P. albicans* var. *nana* Boiss., and *P. albicans* subsp. *lanuginosa* Chevall. (Sub-genus *Albicans*, Section *Albicans*, Series *Albicantes*). However, *P. ciliata* Desf. has corolla lobes densely hairy on the lower surface, lobes usually patent, acuminate. Sepals usually with hairs placed asymmetrically (Sub-genus *Albicans*, Section *Albicans*, and Series *Ciliatae*).

For the cluster B taxa, the accessions belong to subgenus *Coronopus*. This latter is present both in the Mediterranean region and in South Africa.

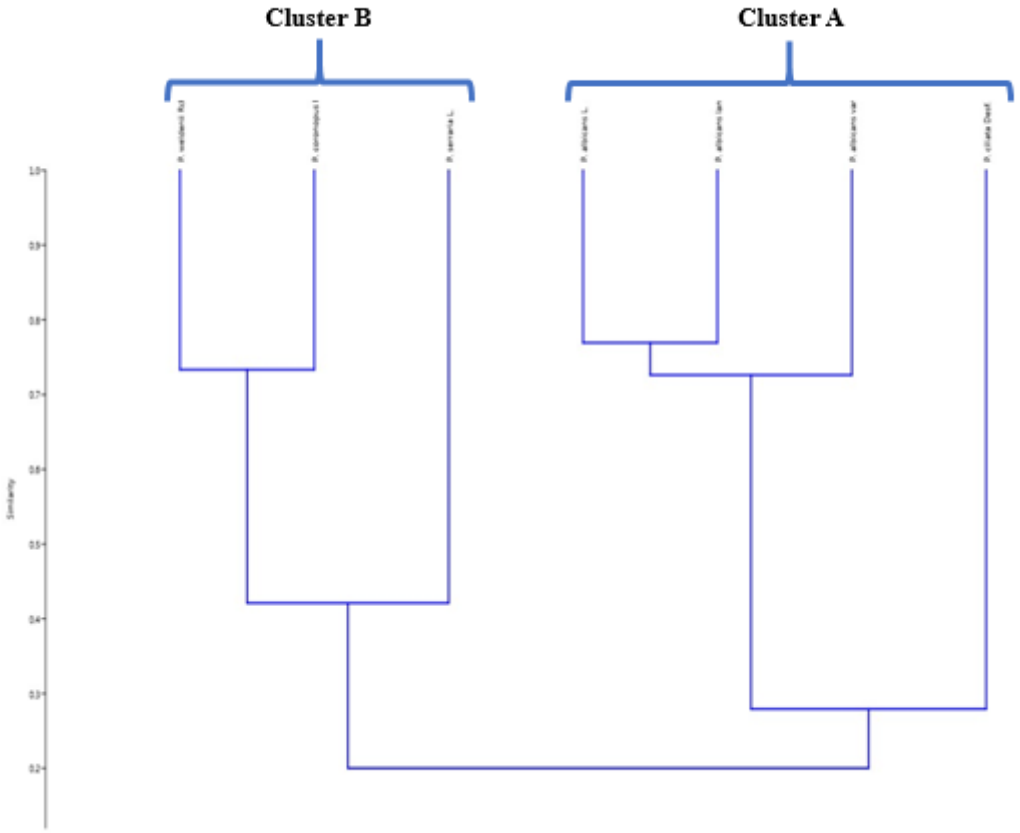

Figure no1. Ward tree of morphological data of the studied *Plantago* genotypes

According to our results of the analysis of soils provided from the sampled sites, the sites 4 and 5 have the highest electrical conductivity (8.69 and 8.49 g/L respectively). Whereas, the other sites present soils with moderate and low salinity. All the collected sites have acidic to neutral soils with a pH ranging from 4.12 in site (**Table no4**).

Table no4. Chemical and physical soil properties of the superficial soil layer (0-20 cm depth) sampled from collected sites

N°	Taxa	pH	EC (g/L)	Sand (%)	Silt (%)	Clay (%)
1	*P. ciliata* Desf.	4.71±0.06	1.3±0.36	75.35±29.3	9.25±2.69	15.4±3.41
2	*P. albicans* L.	6.35±0.07	0.98±0.16	74.51±32.06	8.63±1.23	16.86±4.24
3	*P. albicans* var. *nana* Boiss.	4.17±0.23	2.25±0.35	70.04±31.44	13.8±3.69	16.16±4.36
4	*P. weldenii* Rchb.	7.74±0.13	8.69±2.05	56.9±15.61	20.18±6.63	22.92±7.65
5	*P. coronopus* L.	6.35±0.08	8.49±1.66	71.68±24.62	11.64±3.1	16.68±3.66
6	*P. albicans* subsp. *lanuginosa* Chevall.	5.71±0.03	1.86±0.52	70.35±36.32	9.38±1.75	20.27±7.64
7	*P. serraria* L.	7.2±0.04	0.7±0.26	26.4±10.36	37.8±11.45	37.8±12.06

Eigenvalues by covariance matrix were estimated for soil characteristics, the altitude of the collected sites and the studied *Plantago* genotypes, which show 94.29% of total variability in relationships between the studied taxa and the ecology of their habitats. These results can be explained by the first two principal components (**Figure no2**). The first component explaining 65.83% of the total variance was positively related with pH, % Silt, % Clay and the altitude. The second component explaining an additional 25.72% of the total variability was positively related to the EC.

The scatterplot of the projected variables allowed a good separation of the studied genotypes, the most tolerant to salinity, namely *P. coronopus* L. and *P. weldnii* Rchb. appeared in the upper half of the graph (related to pH and EC of the soil). *P. ciliata* Desf., *P. albicans* L., *P. albicans* var. *nana* Boiss., and *P. albicans* subsp. *langinosa* Chevall are represented on the lower left side of the graph (related to altitude and % of sand in the soil). Whereas, *P. serraria* L. is separating along the first component axis and related to % of clay and silt and the altitude of the collected site.

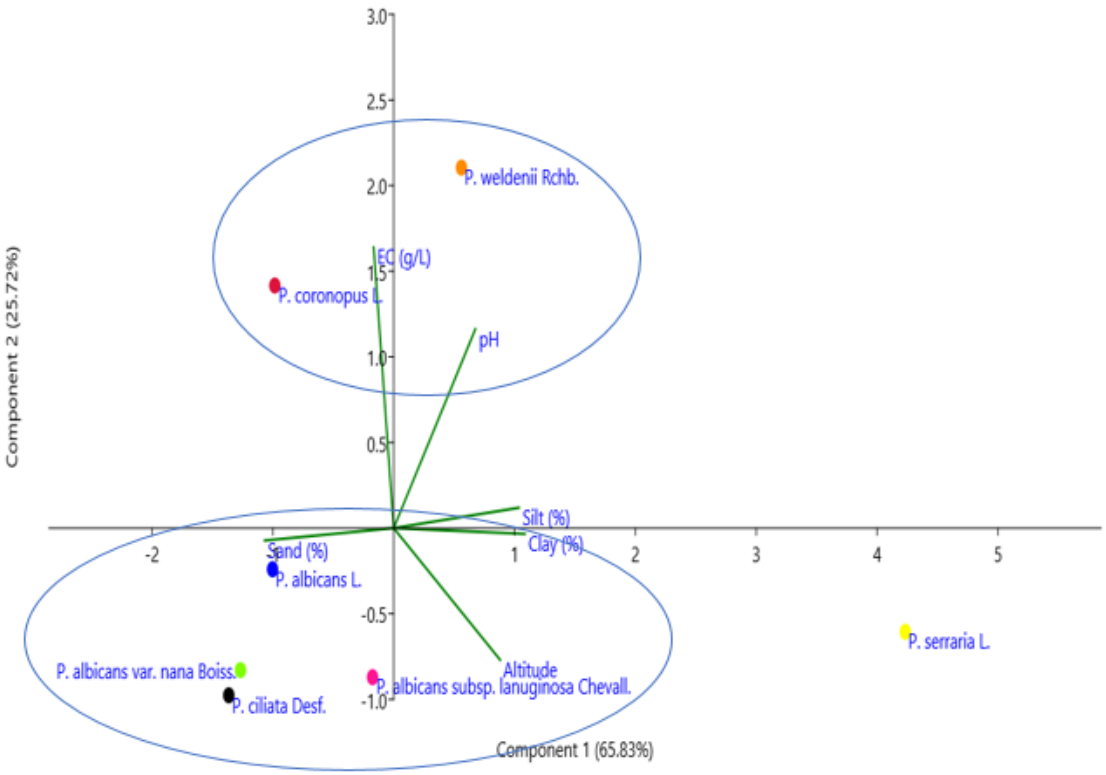

Figure no2. Loading plot and scatterplot of the principal component analysis (PCA), including all the studied *Plantago* genotypes *P. ciliata, P. albicans, P. albicans* var. *nana, P. albicans* subsp. *langinosa, P. coronopus, P. weldenii* and *P. serraria*, soil characteristics (pH, electrical conductivity, %Silt, %Sand, %Clay) and the altitude of collected sites. The first and second principal components account for 65.83% and 25.72% of the total variation, respectively.

IV. Conclusion

The existence and the distribution of the studied taxa are exploratory of diversity in climatic and edaphic factors. The analysis demonstrates the potential of the species sampled to grow under different edapho-climatic conditions. The most discriminant variables that influenced the variability of the studied genotypes were leaves, inflorescence, flowers, seeds and the ecology of their habitats. To construct a more satisfactory classification, a comprehensive study covering all *Plantago* species is required, and it would be much better if future studies focused on the infrageneric classification of *Plantago* using other parameters.

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