# Cytological and electrophoretic relationships Of some *Plantago* L. species

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The relationship among 15 samples representing 9 species and 8 sections of *Plantago*, based on electrophoretic seed protein profile , as well as karyotype data is discussed in the light of their current sectional and sub sectional delimitation. The delimitation of *P. lanceolata* as a separate identity from *P. afra* is congruent with the taxonomic differences between these two species,while its separation from *P. amplexicaulis* and *P. cylindrica* matches the cytological differences between them. The separation of *P. alpina* and *P. coronopus* based on the electrophoretic pattern of their seed protein is congruent with the chromosomal differences between them, *P. alpina* is a tetraploid with 2n=24 and a base number of *X*=6, whereas *P. coronopus* is diploid with 2n=10 and *X*=5. The delimitation of *P. afra* supports its taxonomic separation from the other studied species. The pattern of protein electrophoresis under reducing conditions distinguished *P. major* from the remaining species. This separation is supported by the delimitation of this species in a separate section i.e. *Plantago*. The separation of *P. media* from *P. amplexicaulis* and *P. cylindrica* by the analysis of protein pattern, under reducing condition, confirms their separation based on morphological and chromosomal differences between them. Under non-reducing condition *P. amplexicaulis* and *P. cylindrica* are separated together as one group distinct from *P. major* and *P. media* which are delimited as another group.
Key words: Cytology, Electrophoresis, *Plantago*, Proteins.

## *Introduction*

Several species of the genus *Plantago* L. are cultivated for their various medicinal and other economic uses. *P. lanceolata* and *P. major* have been used as external antirheumatic herbal remedies in the traditional medicine (Caniato, 1982). *P. lanceolata* has been used as an antifertility agent (Herrera *et al.,* 1984). The seeds of *P. ovata*, *P. psyllium* and *P. major* are used as a laxatives (Wasicky, 1961). Biologically active polysaccharides have been isolated from *P. major* (Samuelsen *et al*., 1995). Plantamajoside, a caffeic acid sugar ester from *P. major*, possesses antibacterial activity (Ravn and Brimer, 1988). Antitumor activity of phenolic compounds of *P. asiatica* has been demonstrated by Ravn *et al.* (1990). It is therefore considered of importance to study the species of *Plantago*.

The genus *Plantago* L. comprises 250 - 275 species distributed in diverse habitats throughout the World. Pilger (1937) divided the genus into two subgenera *Euplantago* L. and *Psyllium* L. Rahn (1978) published a revision of the genus in which he divided it into the three subgenera *Plantago* L., *Coronopus* (Lam. & D.C.) Rahn and *Psyllium* (Juss). Harms and Reiche.

Cytological investigations of different species of *Plantago* L. (McCullagh, 1934; Rahn, 1957; Cartier, 1971; Briggs, 1973; Badr, 1980; Badr and El-Kholy, 1987; Badr *et al.,* 1987; Wolff, 1987; Srimanta and Sarmistha, 1997) revealed that this genus has three different basic chromosome numbers of $X$ = 4, 5 and 6. The basic number of $X$ = 6 is the original number from which $X$ = 5 & $X$ = 4 have been derived (McCullagh, 1934; Badr and El-kholy, 1987). This number is present in the majority of species, $X$= 5 is found in

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Determination of nuclear DNA in root tips of *Plantago* species. (Badr *et al.*, 1987; Hamoud *et al.,* 1992; Hooglander *et al.,* 1993; Srimanta and Sarmistha, 1997) revealed that DNA amount per basic genome corresponds to the total length of chromosome. It also corresponds with the ploidy level, from diploid to tetraploid to hexaploid, for corresponding basic number (Badr and El-Kholy, 1987; Sarmistha *et. al.*, 1990; Srimanta and Sarmistha, 1997). These results appear to suggest that evolution in *Plantago*. has probably involved reduction in the nuclear DNA content with the decrease in the number of chromosomes, from $X$=6 to $X$=5 to $X$=4.

Electrophoretic patterns of total seed proteins, as revealed by polyacrylamide gel electrophoresis (PAGE) provide valid evidence for addressing taxonomic and evolutionary problems in plants (Ladizinsky and Hymowitz, 1979; Crawford, 1990). Recently the correlation between SDS-PAGE and chromosomal features and their impact on the interrelationships of some *Trifolium* (Badr, 1995) and *Lathyrus* (El-Shanshoury, 1997) taxa showed that the grouping of species in different sections, depending on protein patterns agrees with the previous taxonomy of these species based on morphological and chromosomal criteria.

The present study is undertaken to evaluate the relationships among selected species of *Plantago* using both cytological and electrophoretic criteria.

## *Material and Methods:*

This study was carried out on 15 samples representing eight species taxonomically delimited in eight sections under the three subgenera of *Plantago*. The source and origin of the examined material are given in Table 1. Most species are from Europe, some are from Canada and one is from Egypt. For karyotype analysis, roots taken from pot-grown plants, were pretreated with saturated solution of 1-4 dichlorobenzene for 2-3 h., fixed overnight in 3:1 ethanol glacial acetic acid, and stored in 70% ethanol at 4°C. Cytological preparations were made using the Feulgen squash method, and were made permanent by mounting in Euparal. C-metaphase chromosomes were counted and their features, such as somatic number, ploidy level, mean chromosome length (MCL) and mean arm ratio (r-value) were calculated from photographic prints enlarged to magnification of 4000 x (Hamoud *et al.*1992).

To extract total seed proteins, 0.1gm mature seeds were mixed with equal weight of pure, clean, sterile, fine sand and powdered using a mortar and pestle. The powder was homogenized with 1 ml of tris-glycine buffer containing 2% NaCl at pH 8.2 for 2 h., by gentle motion. The homogenate was then centrifuged at 12000 rpm for 20 min. at $20^{O}$C. The supernatant (protein extract) was either immediately used for electrophoresis or

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stored at 20$^{O}$C for later use. From this extract 40 µl were mixed with equal volume of digestion buffer (tris-borate buffer, pH = 8), and the mixture was heated for 5 minutes in a boiling water bath. The digestion buffer was used with or without the addition of dithiothreitol (DTE) in order to carry out electrophoresis of seed protein extract under reducing and non-reducing conditions respectively. Three µl bromophenol blue were added as tracking dye and 15 µl of the mixture were then loaded. In the outside lanes of the gel, 6 µl of a marker protein mixture containing 6 different protein subunits with known molecular weight were loaded. Electrophoresis was run in 7.5 - 20 gradient SDS polyacrylamide gel , pH = 8 at 4$^{O}$C and 100 v using small gel slabs (Hoefer) for 2 h. Gels were then stained in 2% solution of Coomassie blue R 250 (Serva) for 30 minutes and destained in a 2 : 1 (v/v) mixture of methanol and acetic acid for 2 - 3 days.

The banding profile of the 15 examined samples was photographed using Agfa pan film and prints were made using Kodak photographic paper. The number of bands was scored by critical observation of gel records and diagramatic representation of the bands were drawn. The best observations were achieved by placing the gel against white background.

The bands produced by each sample, under reducing and non-reducing conditions, were counted and their relative mobilities compared with those of the standard marker protein. The presence or absence of each band was treated as a binary character in a data matrix (coded 1 and 0 respectively) for computation using the program NTSYS pc (numerical taxonomy and multivariate analysis system; Rohlf, 1988). Quantitative variation expressed as difference in intensity and thickness of bands, was also observed, but since this type of variation is often associated with the genotype, it was not taken into consideration when coding for the numerical analysis. The method applied is based on cluster analysis and expresses the relationships of the studied taxa as percent similarity in dendrogram. The bands scored from electropherograms produced under reducing and non-reducing conditions were used as two different sets of data for computer analysis, to produce two separate classifications. A combination of the two sets of data was used for a third classification based on the total number of bands. For the numerical analysis, the samples were numbered as in Table 1.

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### *Results*

Electropherograms of the examined 15 samples revealed a number of bands ranging from 2 to 7 in *P. media* (1) and *P. coronpus* (1) respectively, under reducing conditions (Fig. 1. a) and ranging from 4 bands in *P. afra* to 11 bands in *P. lancoelata* and *P. amplexicaulis* under non-reducing conditions( Fig . 1. b).

The relationships among samples based on the variation in the banding profile under non-reducing condition using the NTSYS program are shown by the dendrogram given in( Fig. 2.a).The studied samples have an average taxonomic distance of about 1.9. At this level the two samples of *P. lanceolata* (12 & 13) are separated from the rest of species. At a taxonomic distance level of 1.5 the two species of *P. coronopus* (8 & 9) are also delimited from the remaining species. The remaining species are divided into two groups at a taxonomic distance of 1.3. One of these groups includes *P. amplexicaulis* (14) and *P. cylindrica* (15) and the other one includes the rest of the species. The four samples of *P. media* (4, 5, 6 & 7) are separated from the other species at taxonomic distance of 1.2. Within the *P. media*, the samples collected from the same country clustered together. *P. alpina* (10) and *P. afra* (11), which are included under two separate subgenera of the genus, were delimited at low taxonomic distance between them. *P. major* represented by 3 samples (1 , 2 & 3), have a low degree of taxonomic distance at 0.6, sample 3 was separated from samples 1 and 2, which showed identical banding profile.

Dendrogram patterns under reducing conditions (Fig. 2.b) revealed that the isolation of most species was similar to that produced under non-reducing conditions. However, some species revealed a change in dendrogram pattern under reducing condition. The two samples of *P. coronopus* (8 & 9) are separated from each other at taxonomic distance of 0.8, despite their grouping under reducing condition at 100% similarities. *P. media* is represented by 4 samples (4, 5, 6 & 7) collected from different countries. Reducing pattern of *P. media* seed protein separated sample 6 from the other samples at a taxonomic distance 0.5. This sample was clustered with sample 5 under non-reducing conditions. The dendrogram based on combining the two sets of data i.e. reducing and non-reducing condition revealed the same patterns as produced under reducing conditions for all samples (Fig. 2.b).

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**Fig**. 1(A): Examples of electropherograms produced by *SDS-PAGE* analysis of seed proteins of *Plantago* L. samples numbered as in Table 1.
A= under reducing conditions M= Marker protein standard
B= undre non-reducing conditions

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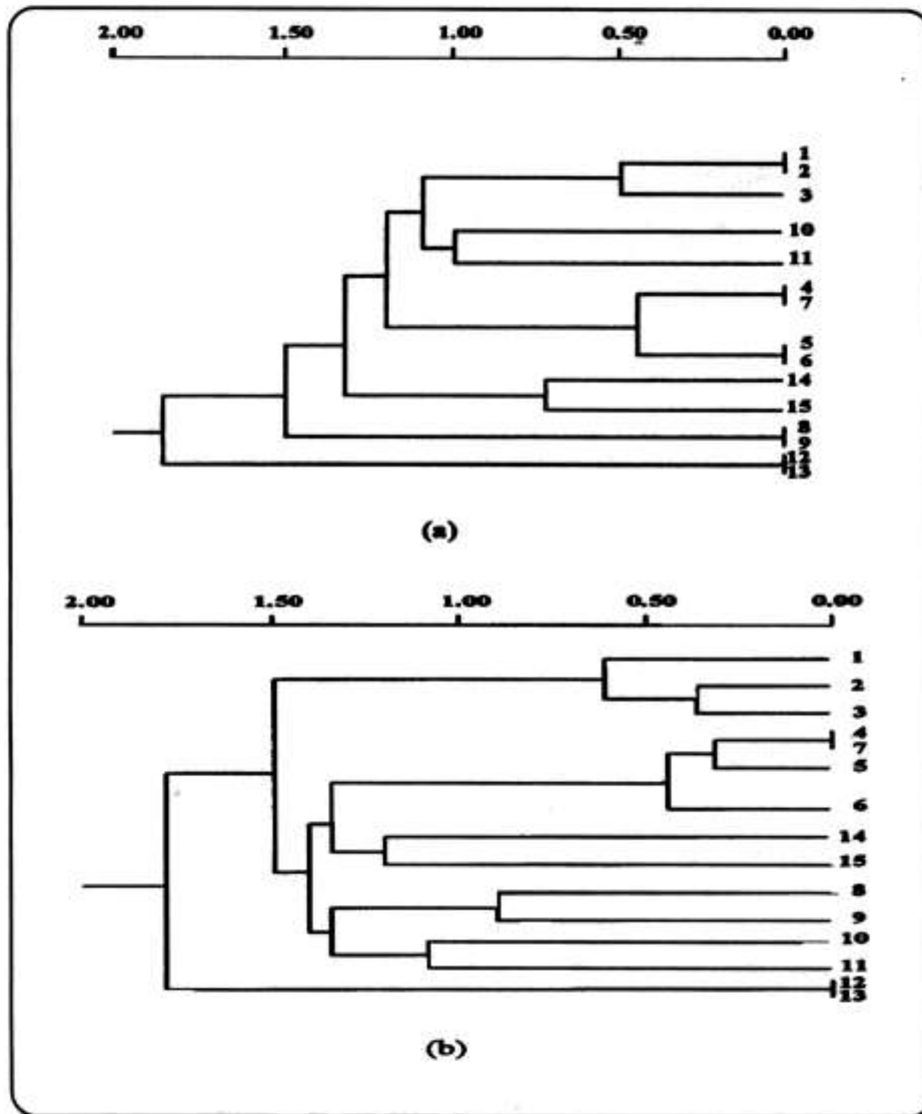


**Fig**. 2 (a, b): Dendograms illustrating the relationship among the 15 studied samples of *Plantago* L. produced by analysis of variation in *SDS-PAGE* of seed proteins samples are numbered as in Table 1.
a= Under reducing conditions
b= Undre reducing conditions and combination of reducing and non-reducing conditions.

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***Discussion***:

The chromosomal features of the examined samples are summarized in Table 1. The diploid chromosome number of 2 n = 12 (*X* = 6) is recorded in three species, *P. major* (1, 2 & 3), *P. afra* (11) and *P. lanceolata* (12 & 13). A tetraploid chromosome number of 2 n = 24 (*x* = 6) was observed in the four samples of *P. media* (4, 5, 6 & 7) and in *P. alpina* (10). A diploid chromosome number of 2n = 10 (*X* = 5) is recorded in the two samples of *P. coronopus* (8 & 9), in *P. amplexicaulis* (14) and *P. cylindrica* Forssk (15).

The phenetic relationships between the studied samples as expressed by the numerical analysis of their seed protein profiles, under reducing and non-reducing conditions, clearly demonstrated the delimitation of *P. lanceolata* (12 & 13) from the rest of the studied samples. This species has been placed in sect. *Arnoglossum* (Decne) in subg. *Euplantago* by Pilger (1937) and in sect. *Lanceifolia* of subg. *Psyllium* by Rahn (1978). Among the studied species in subg. *Psyllium sensu* Rahn only *P. lanceolata* and *P. afra* have *X* = 6, whereas the species, *P. amplexicaulis* and *P. cylindrica* have *X* = 5. Subg. *Psyllium sensu* Pilger only comprises *P. afra*. The delimitation of *P. lanceolata* as a separate identity in this study from *P. afra* is congruent with the taxonomic differences between these two species, while, its separation from *P. amplexicaulis* and *P. cylindrica* matches the cytological differences between them. In these analyses *P. alpina* (10) and the two samples of *P. coronpus* (8 & 9) are separated from each other contradicting the close relationship between these two species as proposed by both Pilger ( 1937) who grouped them in sect. coronopus of subg. Euplantago and Rahn (1978) who delimited them in subg. Coronopus. However, the separation of *P. alpina* and *P. coronopus* based on the electrophoretic pattern of their seed protein is congruent with the chromosomal differences between them, *P. alpina* is a tetraploid with 2 n = 24 and a base number of *X* = 6, whereas *P. coronopus* is diploid with 2 n = 10 and *X* = 5 (Badr, 1992).

*P. afra* is distinguished by its protein pattern under both reducing and non-reducing conditions. The delimitation of this species in the present study supports its taxonomic separation from the other studied species by Pilger (1937). Meanwhile, Rahn (1978) placed *P. afra* in subg. *Psyllium* with other three of the studied taxa, but in a separate section i.e. *Psyllium*. The pattern of protein electrophoresis under reducing condition clearly distinguished the samples of *P. major* (1, 2 & 3) from the remaining species. This separation is supported by the delimitation of this species in separate section i.e. *Plantago* of subg. *Plantago* by Rahn (1978). The variation in chromosome length and the high difference in DNA amounts recorded by Badr (1992) confirm the distant situation of *P. major*. The separation of samples of *P. media* (4, 5, 6 & 7) in a separate group from *P. amplexicaulis* (14) and *P. cylindrica* (15) by the analysis of protein pattern under reducing condition confirms their separation based on morphological characters as proposed by both Pilger (1937) and Rahn (1978), and is matched by chromosomal differences between them (Badr and El-Kholy, 1987; Badr, 1992). Under non-reducing condition *P. amplexicaulis* (14) and *P. cylindrica* (15) are separated together as one group distinct from *P. major* (1,2 & 3) and *P. media* (4, 5, 6 & 7), which are delimited as another group. These two species are placed together in subg. *Plantago* by Rahn (1973) and in two separate sections in subg. *Euplantago* by Pilger (1937).

Further investigation involving more taxa, by protein electrophoresis and other molecular approches, may enable us to reach more satisfactory sectional delimitation and species assignment of species in the genus *Plantago*.

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| No. | *Plantago* species | Sources or origin | 2n | x | Ploidy level | MCL | r-values |
|---|---|---|---|---|---|---|---|
| I | Subgenus : *Plantago L.* | | | | | | |
| | Section : *Plantago L.* | | | | | | |
| | *P. major L.* ssp. *major* (1) | | | | | | |
| 1 | *P. major L.* ssp. *major* (2) | Sheffield. United Kingdom | 12 | 6 | 2x | 1.68+0.03 | 1.3+0.07 |
| 2 | *P. major L.* ssp. *intermedia* (3) | Alberta, Canada | 12 | 6 | 2x | 1.51+0.02 | 1.32+0.11 |
| 3 | Section : *Lamprosntha* | Wageningen, Netherland | 12 | 6 | 2x | 2.32+0.04 | 1.97+0.12 |
| | (*Decne*) | | | | | | |
| | *P. major* (1) | | | | | | |
| | *P. major* (2) | | | | | | |
| 4 | *P. major* (3) | Debyshire, United Kingdom | 24 | 6 | 4x | 3.24+0.05 | 1.57+0.06 |
| 5 | *P. major* (4) | Gottingen, University, Germany | 24 | 6 | 4x | 2.86+0.07 | 1.52+0.08 |
| 6 | | Leipzing, Germany | 24 | 6 | 4x | 2.85+0.08 | 1.63+0.07 |
| 7 | | Dorset, United Kingdom | 24 | 6 | 4x | 2.90+0.08 | 1.59+0.08 |
| II | Subgenus : *Coronopus* (Lam & D.C.) | | | | | | |
| | Section: *Coronopus* (Lam & D.C.) | | | | | | |
| | *P. coronopus* L. (1) | | | | | | |
| 8 | *P. coronopus* L. (2) | Gottingen University, Germany | 10+0-B | 5 | 2x | 2.75+0.20 | 1.23+0.08 |
| 9 | Section: *Maritima* (Rahn Sect. Nov.) | Dorset, United Kingdom | 10 | 5 | 2x | 2.04+0.15 | 1.43+0.11 |
| | *P. alpina* L. | | | | | | |
| 10 | | Gottingen University, Germany | 24+B | 6 | 4x | 2.22+0.08 | 1.93 |
| III | Subgenus : *Psyllium* (Juss, Harms & Heiche) | | | | | | |
| | Section : *Psyllium* (Juss) (Lam & D.C) | | | | | | |
| | *P. afrea* L. | | | | | | |
| 11 | Section : *Lanceifolia* (Bern) | Gottingen University, Germany | 12 | 6 | 2x | 2.92+0.06 | 1.41+0.07 |
| | *P. lancealata L.* (1) | | | | | | |
| 12 | *P. lancealata L.* (2) | Sheffield, United Kingdom | 12 | 6 | 2x | 2.65+0.1 | 3.22+0.92 |
| 13 | Section : *Bauphula* (Decne) | Ontario, Canada | 12 | 6 | 2x | 2.55+0.09 | 2.35+0.56 |
| | *P. amplexicaulis* Cav. | | | | | | |
| | Section : *Albicans* (Bar.) | | | | | | |
| 14 | Series : Albicantes (Rhn. Ser. Nov.) | Gottingen University, Germany | 10 | 5 | 2x | 3.39+0.06 | 2.2+1.96 |
| | *P. cylindrica* Forssk. | | | | | | |
| 15 | | South Sinai, Egypt | 10 | 5 | 2x | 2.38+0.02 | 1.55+0.14 |

Table 1: A survey of the *Plantago* samples studied and of their chromosomal features. MCL = mean chromosome length (m); r-value = mean chromosome arm ratio.