Protein Bands Homologies and the Evolution of Ploidy in Some Species of Genus *Panicum* L. Poaceae

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Abstract: Total of 15 protein bands were identified in 23 accessions belongs to 11 species of genus *Panicum* investigated belonging three ploidy levels. The bands vary in number and positions between accessions even within the same species. Diploids have at least number of bands up to 4. Similarity matrix and dendrograme analysis observe isolation of South African and European diploid accessions indicate the difference in their evolutionary pathway. Tetraploid accessions have the highest mean number of bands up to 6 reflect their alloploid origin. The mean number of bands of tetraploids is 1.5 times of diploids and low coefficient of variation insure the hypothesis suggest the alloploid genome structure of tetraploids which can be represented as AABB rather than AAAA. On the other hand hexaploids have mean number of bands up to 4, less than tetraploids. Low coefficient of variation might suggest the autoallohexaploid origin (AAAABB or AABBBB). This suggests that AABB tetraploids have backcrossed to diploid parental genome to give the above proposed genome, and the hexaploids have this sourt of origin rather than alloploid AABBCC or autoploid AAAAAAA. Most accessions of the same species lies in the same group of similarity with few exceptions, which might be due to ecological factors. [Journal of American Science 2010;6(9):216-224]. (ISSN: 1545-1003).

Keywords*:* *Panicum*, protein electrophoresis, evolution, poaceae, polyploidy

1. Introduction

Many attempts were carried out to use electrophoretic analysis for seed proteins as evidence to provide additional information concerning taxonomic and evolutionary pathways in addition to that obtained by morphological, taxonomic and cytological evidences (Rahman 1988, Bals *et al* 2007, Sivakumar 2006, Kalinova and Moudry 2006, Fan 2006).

Seed protein profiles are often species specific and highly stable (ladizinsky and Hymowitz 1979). Seed proteins also are immediate accessible supply and do not need extensive purification (Mc Daniel 1970). Robinson and Megarrity (1975) proved that mature seeds of different ages will have the same profile. Also environmental conditions and seasonal fluctuations seems to have little effects on composition of seed proteins (Dinhill and Fodven 1965, Lee and Ronald 1967, Adriaanse, *et al* 1969 Gray *et al* 1973).

The use of seed protein electrophoresis for taxonomic and evolutionary purposes has greatly increased and used witin genera and families (Esen and Hilu 1989, Henry and Taylor 1989, Mc Daniel 1970, ladizinsky and Hymowitz 1979). Johnson and Hall (1965) and Johnson (1972a) have stated the role of seed protein electrophoresis in supporting genomic relationships and tracing the origin and evolutionary pathways of many grasses. It also correct the misunderstand of B genome origin in wheat (Johnson 1972b).

To our knowledge, little works carried out with this genus and found mostly quantitative. This work aims to study the electrophoretic seed proteins profile of some species of genus *Panicum* with different ploidy levels and observe its role concerning taxonomic and evolutionary pathways.

This work is a part of programe study cytogenetics and evolution of grasses.

2. Material and Methods

Seeds of 23 accessions belongs to nine species were investigated using polyacrylamide gel electrophoresis. Seeds were collected from different botanic gardens and seed banks of many countries around the world (Table 1). 200 mg of clean seeds were used for protein extraction, ground in 4 ml aqueous 0.05 M Tris-Hcl, pH 6.7 using a caselle mill, left overnight for extraction at 4ºC. The preparation were then centrifuged at 17000 g (8000 R.P.M) for 30 min using high speed centrifuge at 4º C. The supernatant was recentrifuged at 100,000 g for 1 hr. the supernatant was removed with carful and stored at 4º C if required within the same day or kept at 15º C until required.

The polyacrylamide gels were prepared as in Davis (1964) and Ornstein (1964) and modified by Booth and Richards (1978) and Haroun (1991). Vertical gels

Table 1: code name, sources, chromosome number, ploidy level and number of bands for various accessions of *Panicum*.

Code	species	Native area	2n ploidy level		No. of bands
An	*antidotale*	South and Africa	18	2X	4
c3	*capillare*	South Africa	18	2X	3
c1	*capillare*	Belgium	18	2X	2
c2	*capillare*	Belgium	18	2X	2
Lv	*laevifolium*	Belgium	18	2X	2
co2	*coloratum*	South Africa	18	2X	2
Com	*coloratum* var. makarikarinse	South Africa	36	4X	4
Cof	*coloratum* var. olifantuselei	South Africa	36	4X	3
co1	*coloratum*	Egypt	36	4X	3
Mv	miliaceum var. violaceum	Belgium	36	4X	4
Ml	*miliaceum* var. luteum	France	36	4X	5
Mn	*miliaceum* var. nigrum	France	36	4X	5
m1	*miliaceum*	Egypt	36	4X	4
m3	*miliaceum*	Switzerland	36	4X	4
m4	*miliaceum*	Belgium	36	4X	4
m2	*miliaceum*	South Africa	36	4X	4
Mr	*miliare* lam	France	36	4X	6
mx1	*maximum*	Central africa	32	4X	4
mx2	*maximum*	South Africa	32	4X	3
Dc	*dichotomiflorum*	France	54	6X	3
Es	*esculentum*	France	54	6X	3
b1	*bulbosum*	Switzerland	54	6X	3
b	*bulbosum*	France	54	6X	3

were used for electrophoretic run, where three gels were run for each species.

The number and position of bands for each accession were recorded by visual observation against light background and represented diagrammatically in Figure (1) for three ploidy levels investigated.

Comparisons between bands of all species were made using simple cofficient of similarity C, where

$$C = \frac{2a}{X + Y} \times 100$$

Where

a = number of common bands

x = number of bands in 1st sample

y = number of bands in 2nd sample

Coefficient of similarity was ordered on a kulcinski square Richards (1972) and also on computer dendrogram analysis, which was drawn output from the clustan 1A (Wistard 1969) computer program.

3. Results

Altogether, 15 bands were identified in various accessions investigated belonging to three ploidy levels. The differences in number and position of bands were used in comparison between accessions have different chromosome number using similarity matrix method (Figure 2) and computer dendrograme analysis (Figure 3).

The number of bands vary between diploids to hexaploids. Diploids have at least number of bands up to 4, tetraploids have the highest number of bands up to 6, where hexaploids have number up to 4. The three ploidy level accessions were found have certain number of bands in common based on position of band (Rm) which calculated and represented in Figure (1).

Similarity matrix analysis (Figure 2) shows that high similarity area was found at the top of matrix between accession lies in group a represented by *P. capillare* L1 and L2 with *P. miliaceum* L2. *P. capillare* L1 was also in high similarity with *P. capillare* L3, *P. bulbosum* L2 and *P. esculentum.* Another high similarity area was found in the right edge of the matrix include most accessions lies in group b in Table (3). Small group was also found at the bottom of the matrix blonging to group c, showing high similarity between each others include *P. coloratum* olifantsu, *P. miliare*, *P. miliaceum* L1, *P. miliaceum* luteum and *P.miliceum* nigrum. The last two species show high degree of similarity with most accessions of group a and b.

Regarding dissimilarity between accessions many area were scattered in the middle of the matrix and near the top represented that of *P. antidotale* and *P.miliaecum* luteum with the accessions of all three groups, and *P. capillare* L2 with group b and c and between *P. maximum* L1 with group a and *P.miliaceum* L3 with most accessions of groups a and b.

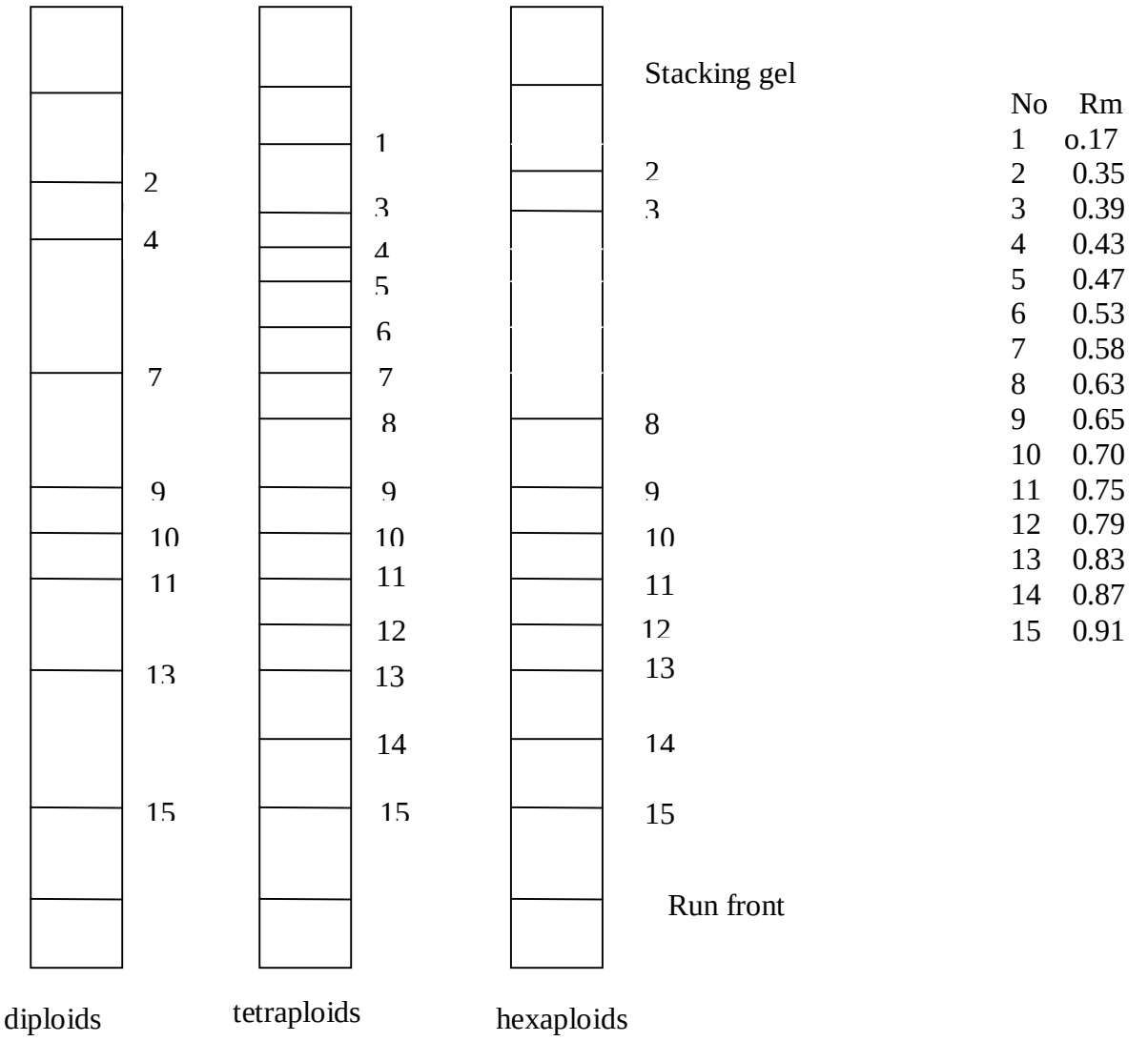
The dendrogame analysis using the average likage method (Figure 3) display more clear the indications given by similarity matrix analysis. The 23 accessions investigated have a total similarity of 36%. At this level the accessions were split off into three main groups (Figure 3). Group a has 6 accessions and is divided into two subgroups at similarity level of 45%.

The first group includes *P. capillare* L1 & L2 with similarity of 80% to each other and with *P. miliaceum* L2 the second group include the two accession of *P. bulbosum* with the highest degree of similarity recorded (86%) and *P. laevifolium.* The second group (b) in the middle includes 9 accessions (Figure 3) the highest degree of similarity within this group was recorded between *P. dichotomiflorum* and *P. coloratum* makarikarensis (75%). Five species of this group were split in similarity of 66.6 % and the rest at 55% similarity level recorded by *P. maximum.*

The third group (c) includes 8 accessions divided into two subgroups at similarity level of 39%. The small one has two accessions *P. miliaceum* L4 and *P. miliaceum villosum* at level of 54% between each others. The second group shows level of 45% for *P. antidotale*, 58% for *P.miliare* and 66.6% for the rest of accessions especially between *P. miliaceum* accessions and *P. coloratum olifantsu* as subgroup.

4. Discussion

Analysis the results of seed protein electrophoresis showed that each accessions is more or less distinguishable from the other at least at one or two bands. These bands are found varied in number and position (Rm) leading to species differentiation. The 23 accessions are found of three ploidy level chromosome number, diploid species with chromosome number of 2n = 18, tetraploids with 2n = 4x =36 and hexaploids of 2n = 6x = 54 based on basic number x = 9. One species recorded 32 chromosomes based on x = 8 which is also recorded for some species of this genus (Haroun *et al* 1992). The chromosome number, basic number, ploidy level and area of collection were listed in Table (1) three of diploid accessions investigated were found in group a (*P.capillare* L1 and L2 and *P. laevifolium*). In *P. capillare* accessions it was found that South African accessions (*P. capillare* L3) is clearly distinguishable from the other two European accessions (lies in group b). This might indicate that this accession has different evolutionary pathway from the other two accessions. This suggestion was previously recommended by cytological work (Haroun *et al.* 1992, Haroun 2002) for the same species and other grass genera. The two European accessions show high resemblance in bands patterns to each other which seems to be further prove to their evolutionary similarity. These results were also confirmed by similarity matrix and dendrogam analysis (Figures 2, 3). In the same manner, Ladizisky and Hymowitz (1979) reported variation in number and position of bands in the accessions of the same species. They reported also differences in the darkness and thickness of various bands in the accessions of the same species, suggesting that bands characters are under control of quantitative gene systems.



No	Rm
1	o.17
2	0.35
3	0.39
4	0.43
5	0.47
6	0.53
7	0.58
8	0.63
9	0.65
10	0.70
11	0.75
12	0.79
13	0.83
14	0.87
15	0.91

Figure1: Diagrammatic representation of common protein bands of various ploidy levels accessions of *Panicum* showing maximum number of bands for each level and the Rm values calculated.

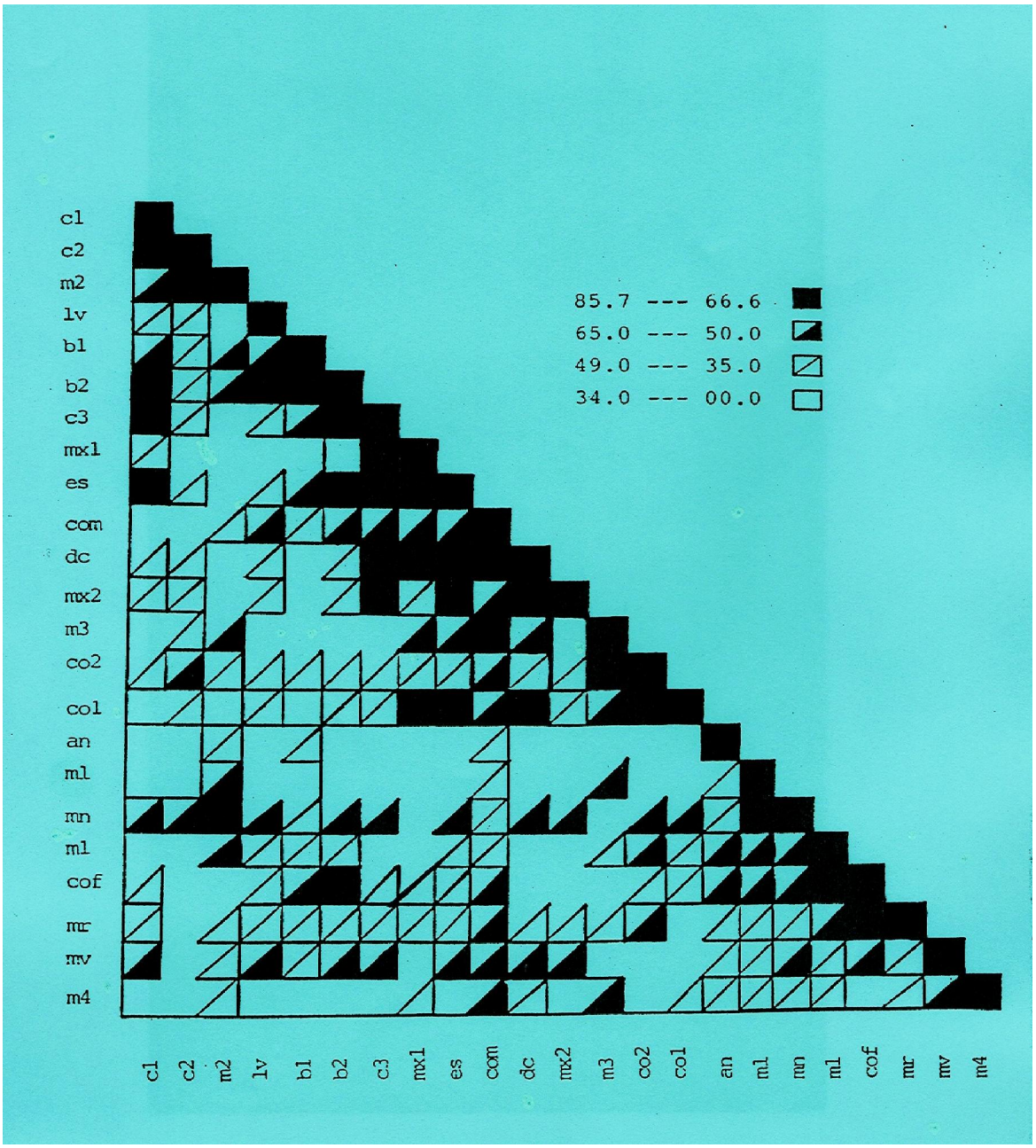


Figure 2: Kulciniski matrix of coefficients of similarity based on protein bands measurements for various accessions of *Panicum*

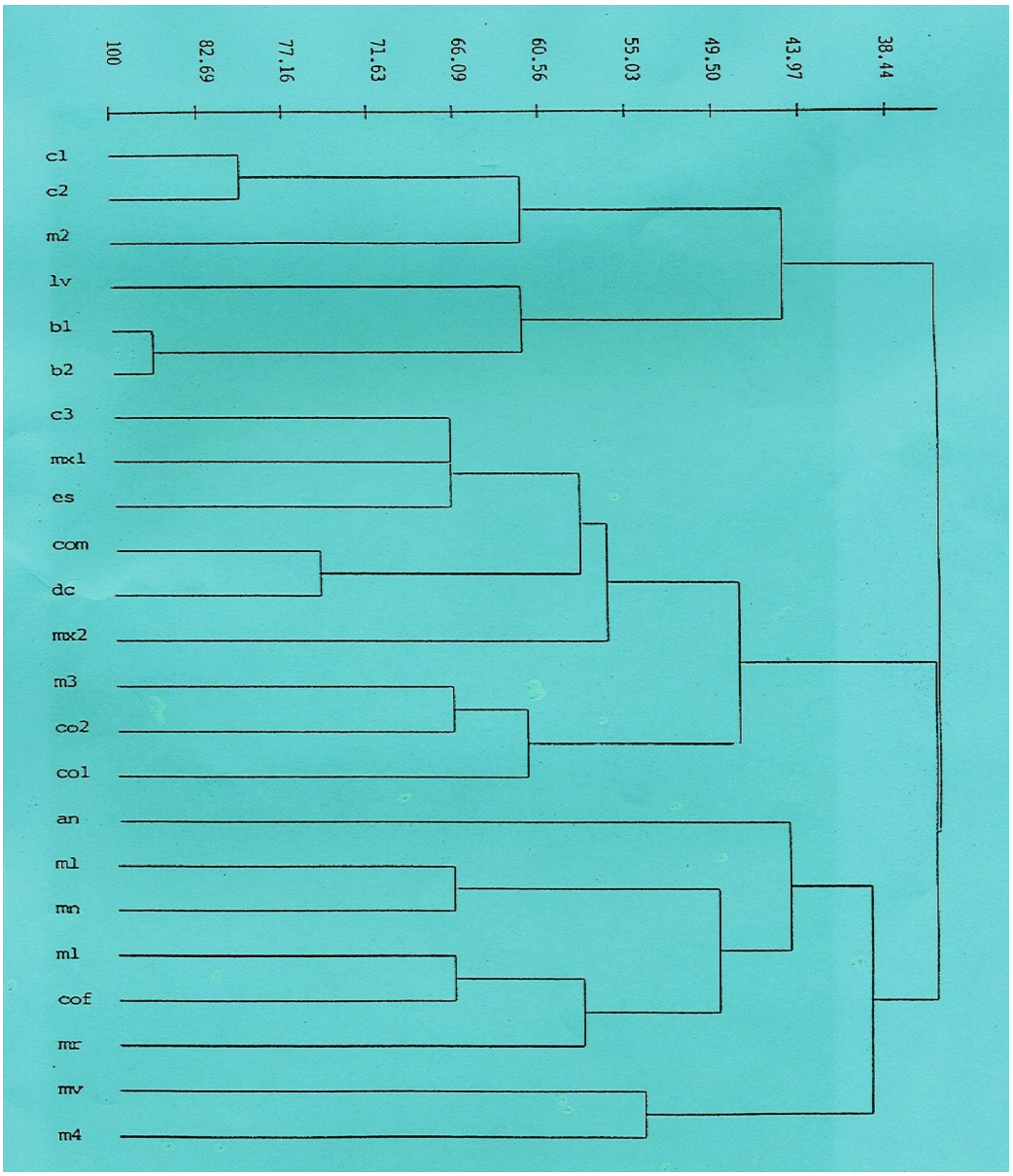


Figure 3: A dendrograme of various accessions of *Panicum* according to protein profile similarity.

In the same group a high similarity was found between two accessions of *P. bulbosum*, showing more or less the same bands patterns in the protein profile indicate the uniformity of their origin and evolution of this species. Clear evidence in this group from similarity matrix and dendrogram analysis show that European accessions of both *P. capillare* and *P. bulbosum* in group a have high similarity within species reflecting their primitive origin.

In the second group b, it was found that high resemblance in bands patterns occurred between *P. capillare* L3 and *P. maximum* L1(both have the same seed source), indicate that ecological factors might have some effects on the evolutionary line in these accessions. In the same manner high resemblance was also found between accessions of tetraploid *P. coloratum* L2 and *P. miliaceum* L4. The two accessions of *P. maximum* L2 and *P. miliaceum* L1 were found quite isolated showing few bands in common with other accessions in this group.

Regarding the third group c it was found that diploid *P. antidotale* (South Africa) is clearly isolated from the rest of species in this group recording low degree of similarity in both similarity matrix and dendrogram analysis and with few bands in common with any other (Figure 1). This degree of similarity was also found between the Belgian accessions of *P. miliaceum* violaceum and *P. miliaceum* L4 and the other accessions of this group. The highest degree of similarity rcorded in this group (66.6%) was found between the two accessions of *P. miliaceum nigrum* and *P. miliaceum luteum* (france), which might have undergone similar pressures from ecological factors with respect to their origin and evolution. At the same level of similarity (66.6%) the two African accessions of *P. miliaceum* L1(Egypt) and *P. coloratum olifantsulei* (South Africa) were also distinguish from each other.

Regarding the relationships between the major groups (Table 3) and taxon distributions it was found that most accessions of the same species were found to be fall more or less in the same group. This is also clear in the dendrograme analysis, i.e. *P. miliaceum* has 5/7 accessions in group c, *P. coloratum* has 3/4 accessions in group b and *P. capillare* has 2/3 accessions in group a. the two accessions of *P. maximum* and *P. bulbosum* were found in group b and a respectively. Few exceptions to this hypothesis might be due to ecological factors or to the effect of quantitative gene system between diploids and polyploids (Ladizinsky and Homowitz 1979, Fan 2006, and Larsen 1967). Another sugestsion by Shepherd (1968) and Kalinova (2006) stated that each band is governed by several genes located at different chromosomes. This might suggest the variation in bands patterns between *P. miliaceum* and *P. coloratum* accessions.

In the same manner Larsen (1967) and Larsen and Caldwell (1968) stated that varieties of many species had the same number of bands but differ in their position. This variation probably due to two co-dominant alleles of the same locus which have subsequently been designated as sp1 and sp2 (Orf and Hymowitz 1976). In *P. coloratum*, where 3/4 accessions were found lies in group b, the diploid accession L2 showed number of bands in common to tetraploid accession L1. This might reflect the evolutionary pathway of the tetraploids in this species. This suggestion agree with that pointed by Johnson (1972a and 1975) and Ladizinisky and Johnson (1972). They found that protein profiles of polyploids are much more uniform in comparison with their diploid progenitors. The mean number of bands between diploids and tetraploids in this species was found to have ratio of 2: 3.7, which more or less follow the ratio of chromosome number.

Calculating the mean number of bands in general for all accessions in the present study show that diploids have mean value of 2.5, tetraploids have 4.08 and hexaploids 3.0 (Table 2).

Table 2: Mean number of protein band and coefficient of variation for three ploidy levels species of genus *Panicum*.

Ploidy level	Mean no. of bands	CV
diploids	2.5	0.26
tetraploids	4.08	0.27
hexaploids	3.0	0.15

The mean number of tetraploid bands is found to be 1.5 times of that in diploids suggest the alloploid origin of most of tetraploid accessions. This in addition to the high number of bands with low coefficient of variation recorded in this ploidy level. Thus, the expected genome for tetraploids can be represented as AABB rather than AAAA.

As the means number of bands in hexaploids less than that of tetraplods this might suggest that all these accessions have an autoallohexaploid origin (AAAABB or AABBBB). This suggests that AABB tetraploids have backcrossed to a diploid parental genome to give the above proposed genome. Also the low coefficient of variation of bands number suggests that hexaploid accessions have the same sort of origin rather than alloploid origin AABBCC or autoploid origin AAAAAA. The number of bands in common (Figure 1) shared between three ploidy levels and their distribution characters might explain the evolutionary relationships between these levels.

Table 3 : Grouping of accessions according to similarity matrix analysis

Group a	Group b	Group c
P. capillare L1	*P. capillare L3*	*P. antidotale*
P. capillare L2	*P. maximum L1*	*P. miliaceum luteum*
P.miliaceum L2	*P. esculentum*	*P. miliaceum nigrum*
P. laevifolum	*P. coloratum maker.*	*P. milaceum L1*
P. bulbosum L1	*P.dichotomiflorum*	*P. coloratum olifant.*
P. bulbosum L2	*P. maximum L2*	*P. miliare*
	P. miliaceum L3	*P. milaceum violac.*
	P. coloratum L2	*P. miliaceum L4*
	P. coloratum L1	

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