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Trends of pollen grain size variation in C3 and C4 Poaceae species using pollen morphology for future assessment of grassland ecosystem dynamics

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Abstract

This pilot study aims to find whether C3 and C4 Poaceae species can be separated by their pollen grain size. One hundred and sixty Poaceae species were prepared for pollen grain size analyses using standard methods. The whole dataset included 70 species from northern Pakistan, 35 species from Tanzania, 25 from South America and 30 from Germany. We measured four pollen grain parameters, i.e. pollen grain length, pollen grain width, pore diameter and annulus width. Results of the statistical tests and multivariate analyses performed on our specific dataset confirmed that C3 polyploid and C4 polyploid species show trends of larger grain size than the respective diploid species. Results of our fresh Poaceae grains should be applied on fossil pollen embedded in environmental archives for reconstructing past climate, for detecting shifts between their C3/C4 Poaceae compositions and assessing the dynamics of grassland ecosystems.

Keywords: pollen grain size, C3 and C4 Poaceae species, ploidy, Pakistan, Europe, Africa, South America

The Poaceae family has about 700 genera and 11 000 species of grasses worldwide (Chen et al. 2006). There are 158 genera with 492 species of Poaceae in Pakistan (Cope 1982; Perveen 2006). Poaceae species are cosmopolitan covering 20% of the Earth's land surface (Shantz 1954; Kellogg 2001). Grassland ecosystems cover different landscapes forming ecosystems such as savannas, páramos, pampas and steppes on different continents. For understanding modern grassland ecosystems, their transformations due to human impact and their response to global change, the history of grassland ecosystems needs to be studied. Since long-term dynamics of grasslands are rather difficult to access using modern Poaceae pollen due to their uniform monoporate pollen type, we investigate to what extent differences in the pollen grain size of C3 and C4 Poaceae species exist and how we can interpret those differences. Of particular interest is the composition of C3 and C4 Poaceae species in different grasslands because they can be interpreted as

climate proxies for past temperature, atmospheric CO₂ concentration and precipitation variation (Schüler & Behling 2011b). C4 grasses are mostly dominant in regions with warm seasonal precipitation, while C3 grasses prefer cool seasonal precipitation (Epstein et al. 1997; Sage 2004). C3 Poaceae species grow under cool moist conditions, preferring high-elevated habitats. The percentage of C3 grasses increases steadily above 2000 m above sea level (a.s.l.) in Ecuador replacing the C4 grass species, which are dominant at lower elevations (Moinuddin et al. 1994). There is a competitive advantage of the C3 metabolism in wetter and cooler areas in contrast to the one of C4 species, which shows higher water use efficiency in more arid and warmer habitats (Boom et al. 2001).

Despite numerous studies on the complex taxonomy and ecology of Poaceae, to our knowledge, there is no published work that compares the pollen morphology of C3 and C4 species, especially on a

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continental scale. Uniform morphology of Poaceae grains poses problems to palaeo-ecologists in the reconstruction of past grassland ecosystems (Andersen 1979). Our aim is to explore whether C3 and C4 Poaceae species from different regions can be separated by their pollen grain size. Spatial variations of tropical and subtropical grassland ecosystems, as well as changes in vegetation composition, locations and boundary shifts play an important role in past climate reconstruction (Prieto 1996; Haberle & Maslin 1999; Behling & Hooghiemstra 2001; Behling 2002; Behling et al. 2004; Behling & Pillar 2007; Brunschön & Behling 2009).

Schüler and Behling (2011a) used Poaceae pollen grain size as a tool to distinguish past grasslands in South America demonstrating that a distinction between grassland ecosystems is possible based on measurable Poaceae pollen grain parameters. Schüler and Behling (2011b) used characteristics of Poaceae pollen grains as a tool to assess palaeo-ecological grassland dynamics in South America concluding that with the help of Poaceae grain characteristics, it is possible to get additional information on the specific grassland characteristics, their development on a temporal and spatial scale and their response to climate change as well as human impact.

Other studies have attempted to use morphology to distinguish different types of Poaceae pollen. Perveen and Kaiser (2012) described the pollen grain morphology of 54 species belonging to 30 genera of the family Poaceae from Pakistan, using light and scanning electron microscopy (SEM). The authors recognised five distinct pollen grain types, based on the exine ornamentation, stating that grain morphology does not correspond with tribe and genus classification, but is significantly helpful at the species level. Ahmad et al. (2011) described the pollen grain morphology of five Poaceae species belonging to three genera of the tribe Chlorideae, stating that grain characters are important in delimiting the Poaceae family at species, genus and tribe levels. The pore diameter as well as the annulus shape and size are the principle characteristics and can be used for distinction between Poaceae species (Beug 1961; Skvarla et al. 2003). The author suggested an intraspecific wide range of grain sizes, but did not report the exact annulus size of Poaceae species.

Dickson (1988) distinguished cereal pollen grains from wild grass pollen grains and reported that *Secale* pollen grains are easily identified, but there are some problems with *Hordeum*, *Avena* and *Triticum* as the pollen grains are swollen with acetolysis and glycerol mounting. *Hordeum* grains can be distinguished by their scabrate sculpturing from that of *Avena* and *Triticum*, which have verrucate grains. Poaceae taxa

seem to display a rather variable grain size range at species level (Salgado-Labouriau & Rinaldi 1990a; 1990b). These authors confirmed that the variation in the grain size of cultivated grasses is higher than the wild grass species. Köhler and Lange (1979) used SEM to distinguish cereal pollen and wild grass pollen. They used the fine sculpture of the exine for this distinction. Mander et al. (2013) used SEM images of 240 grains from 12 species within Poaceae and classified these species by developing algorithmic features that quantify the size and density of sculptural elements on the pollen surface.

Ploidy appears to have a significant effect on grain size. Pollen grain size increases with ploidy level for example in *Avena* (Katsiotis & Forsberg 1995) and *Rumex* (Den Nijs et al. 1980). The authors found that in *Avena sativa* L. the pollen grain size increases with increasing ploidy levels. Our morphometric approach of studying the pollen grains of C3 and C4 species is an attempt to distinguish the uniform monoporate pollen grains within Poaceae family.

Material and methods

Collection of Poaceae pollen

Twelve field visits were made to the central and northern districts of the Khyber Pakhtunkhwa province of Pakistan during March through April 2010 and August through September 2012, and fresh Poaceae species were collected along with flowers.

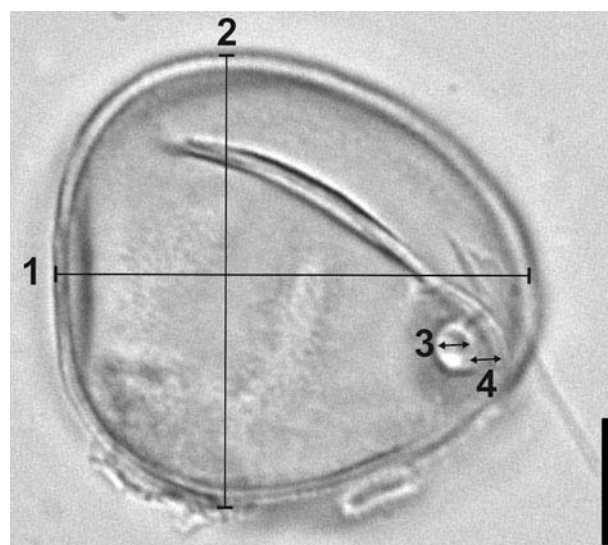


Figure 1. The four parameters of a Poaceae pollen grain considered in the study. 1, Pollen grain length (PGL) is the longest axis of the grain. 2, Pollen grain width (PGW) is the axis of the grain perpendicular to PGL. 3, Pore diameter (PD) excluding the annulus width. 4, Annulus width (AW) is the distance between the pore and the outline of the annulus.

We selected a 200 km long elevational transect from Charsadda to Malam Jabba Swat in Khyber Pakhtunkhwa, investigating grasslands for Poaceae species in the plains of the Peshawar Valley, Charsadda, Nowshera and Mardan (280–350 m a.s.l.). We collected Poaceae species from mid-elevated grasslands at the Malakand Pass, in the Dargai, Batkhela, Thana areas (350–750 m a.s.l.) reaching up to the high altitudes areas of Kabal, Fizagut and Malam Jabba Hill tops (750–2600 m a.s.l.) collecting a total of 70 Poaceae species. The plants were dried and preserved using standard herbarium techniques (Bridson & Forman 1992). Identification was done using reproductive characteristics of flowers and with the help of available literature (Köhler & Lange 1979; Cope 1982; Liu et al. 2004; Andersen & Bertelsen 1972; Ullah et al. 2011; Perveen & Qaiser 2012), with the help of very experienced taxonomists (D. Hojsgaard, Z. Ullah, M. Schwerdtfeger, N. Akhtar) and online research (e.g. www.efloras.org). In order to look for patterns of difference in the pollen grain size within Poaceae, we used additional species representing diverse geographical habitats including 35 species from Tanzania (Africa), 25 from South America including three species each from Brazil and Venezuela and 19 species from Ecuador. We further collected 30 species from the Botanical Garden of the University Göttingen, Germany (European species), represented by ten species each from the genera *Poa*, *Hordeum* and *Bromus*. Hence, the whole data set comprised 160 species including 98 C4 and 62 C3 species (see Appendix Table AI).

Sample preparation for measurement

For analyses of pollen grain size, ten species were processed per day with identical treatment according to the standard method described by Fægri and Iversen (1989). To avoid any swelling of the pollen grains on prepared slides, they were measured on the same day when pollen grains were mounted in glycerine (Cushing 1961; Hamilton 1972; Moore & Webb 1978; Mäkelä 1996; Schüller & Behling 2011a). Pollen residues were safely kept in distilled water after processing for future use. Identical chemical treatment avoids alteration of pollen grain size allowing the comparison of pollen grain size variation. All measurements were made using a Leica Photo Microscope and the accompanying image analyses software Leica QWin. We measured to the nearest 0.01 µm under a magnification of 400× considering the following four parameters (Figure 1):

1. Pollen grain length (PGL) is the longest axis of the grain.

2. Pollen grain width (PGW) is the axis of the grain perpendicular to PGL.
3. Pore diameter (PD) excluding the annulus.
4. Annulus width (AW) is the distance between pore and outline of the annulus.

Measurement setting

Prior to performing the measurements, leading to a differentiation between grass grain size distributions, we determined how many grains had to be measured to get a representative result for the average grain length per species. Hence, we observed the stability of the average grain length within one species with an increasing number of measured pollen grains. This was done for all datasets, which were later pooled for further analyses. Forty-five pollen grains were measured per species. As not all grain size distributions showed a Gaussian distribution, the average grain length is given by the median. The following equations were used to calculate the development of the median grain length with increasing numbers of measured pollen grains:

$$X_{\text{med}} = \sum_1^n X_{n/2+1} / n \quad \text{for all odd } n$$

$$X_{\text{med}} = \sum_1^n 1/2 (X_{n/2} + X_{n/2+1}) / n \quad \text{for all even with}$$

$n = \text{number of measured grains}$

Statistical analysis

The R software package (The R Foundation for Statistical Computing 2013) was used for the statistical analyses. We obtained median values for all four pollen parameters and applied the Shapiro test to both C3 (diploid, polyploid) and C4 (diploid, polyploid) datasets to estimate if the average measures of the four pollen grain parameters were normally distributed. In case our data set was normally distributed, we applied an *F*-test to test the variances and subsequently applied a Student *t*-test to compare the mean of pollen grain measurements. If the data set did not show a normal distribution, we applied the WILCOXON *U*-test for differences in mean pollen grain measurements.

Pearson correlation analyses

Pearson correlation coefficient values were calculated as a measure of linear correlation for determining the strength of relationship among the four pollen grain parameters.

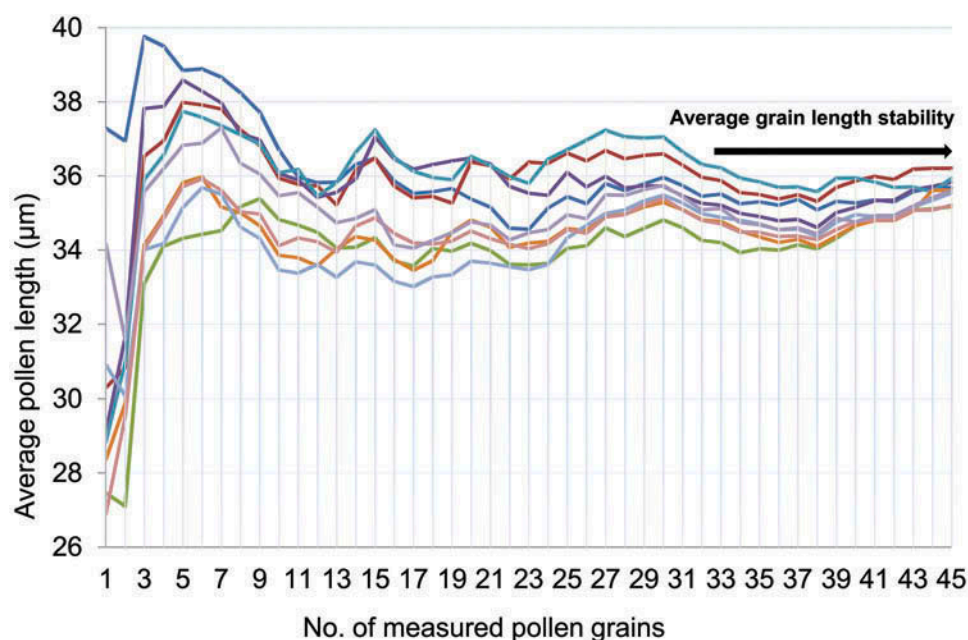


Figure 2. Stability graph showing pollen grain length (PGL) as the representative pollen grain parameter for determining the number of pollen grains, required to be counted to reach stable averages. Stability in PGL is achieved, once the number of counted pollen grains reaches to 30. The X-axis is showing the number of measured pollen grains and the Y-axis is showing the mean value for PGL in micrometres. The same procedure was followed for all the four pollen grain parameters.

Linear discriminant analysis (LDA)

Linear discriminant analysis (LDA) was performed to discriminate a priori defined C3/C4 groups and diploid/polyploid groups as dependent variables, and the four pollen grain parameters as independent variables. Assuming that the groups are linearly separable, the aim was to look for a linear combination of variables that best explains the difference between the respective groups. The prior probability percentages of C3/C4 or diploid/polyploid, out of the total dataset of 160 species, were simply determined based on the number of samples in each group. The mean values and coefficients of linear discriminants for the pollen grain parameters were determined. The percentage of overlaps in C3/C4 and diploid/polyploid scores was estimated by class prediction.

Non-metric multidimensional scaling (NMDS)

Based on the scores of the four pollen grain parameters, we performed separate non-metric multidimensional scalings (NMDSs) both for the C3/C4 group and diploid/polyploid groups [using the meta-multidimensional scaling (MDS) function available in the vegan package of R; Oksanen et al. 2013]. The NMDS scores of the four pollen grain parameters were plotted for C3/C4 species and diploid/polyploid species separately.

Ploidy levels of Poaceae species

For determining the ploidy levels of the species under study, we relied on already published data

(e.g. Cope 1982; Morrone et al. 2006; Hojsgaard et al. 2009; Peichoto et al. 2011; www.efloras.org). The whole dataset of 160 species was divided into small datasets of C3 diploid, C3 polyploid species and C4 diploid, C4 polyploid species.

Results

Measurement setting

The average grain length (given as median) still varies a lot within the first 30–35 measured pollen grains (Figure 2). However, it stabilises after about 40 pollen grains. Above this number, the median remains stable with a variation between 0.1 and 0.8 µm.

Measurement of the four pollen grain parameters

Frequency distribution histograms of the measurements for the four pollen grain parameters along with their measurement range, maximum frequency, average values and their differences (in micrometres) are given in Table I. Values of average pollen grain measurements are larger in C4 than in C3 species and larger in polyploid than in diploid species. The pollen grain length of C3 diploid species (maximum frequency) are in the range 26–28 µm, while those of C3 polyploid species are 32–34 µm, showing that, on average, C3 polyploids species have larger pollen grain length than C3 diploids (Figure 3A, B). The pollen grain length of C4 diploid species (maximum frequency) are in the range 30–38 µm, while those of C4

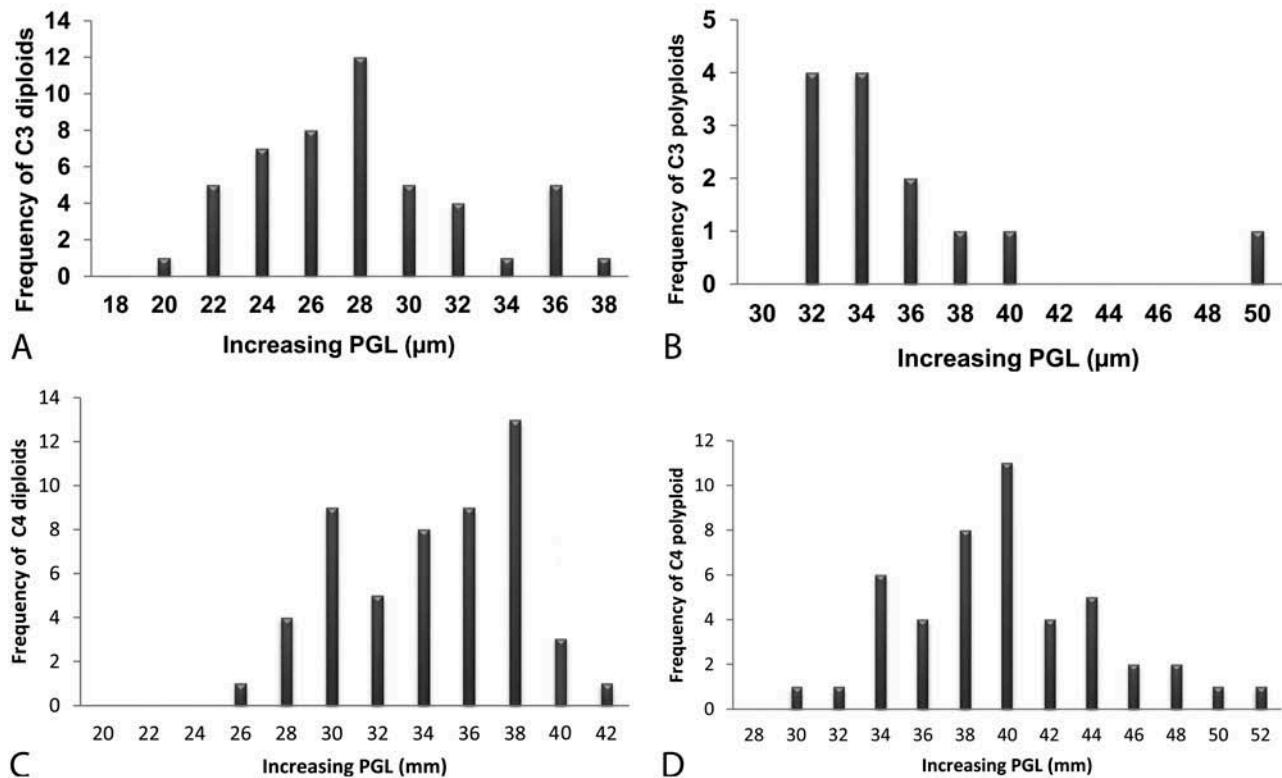


Figure 3. Frequency distribution histograms of the average measures of 62 C3 and 98 C4 Poaceae species. In all the cases, the X-axis is showing the measures of the pollen grain length (μm) while the Y-axis is showing the number of C3 or C4 species, whose respective pollen grain length fall in a certain measurement range. **A, B.** Pollen grain length of C3 diploid and polyploids, respectively. **C, D.** Pollen grain length of C4 diploids and polyploids, respectively.

polyploid species are 38–40 μm , showing that, on average, C4 polyploids have a larger pollen grain length than C4 diploid species (Figure 3C, D).

Statistical analysis

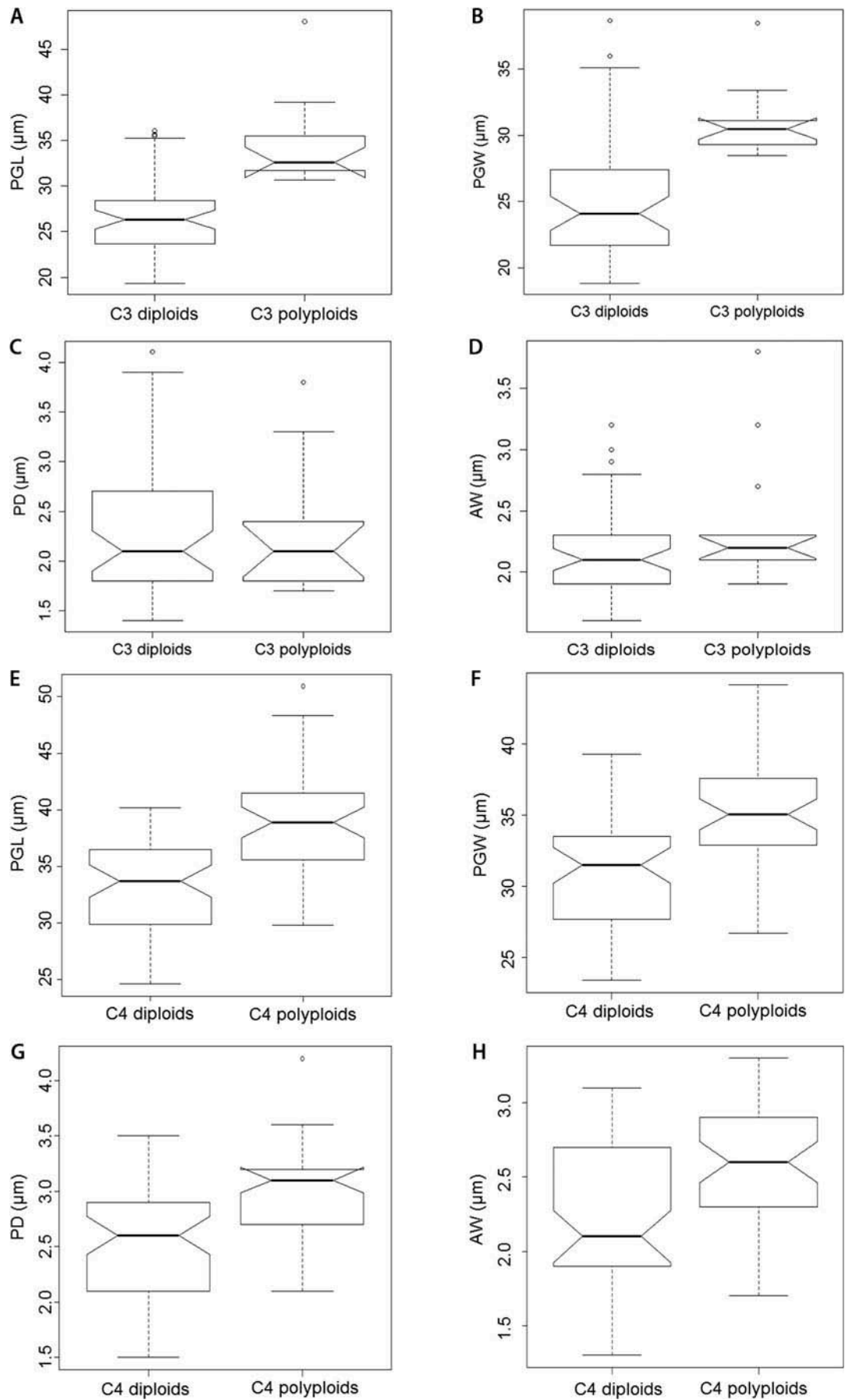
Boxplots for both C3 and C4 datasets (diploid, polyploid), based on the four pollen grain parameters,

show that the median values are higher in polyploid species than in diploid species (Figure 4A–H) except for the pore diameter and annulus width of C3 dataset (Figure 4C, D). The significance of difference in all the four pollen grain parameters between C3, C4 (diploid, polyploid) datasets is evident from the p -values as revealed by the Shapiro–Wilk normality test, F -test, t -test and Wilcoxon U -tests (Table II).

Table I. Measurements of the four pollen grain parameters of C3 and C4 Poaceae species (in micrometres).

S. No	Anatomy	Ploidy	Parameter	Measurement range	Maximum frequency	Average	Difference
1	C3	diploids	PGL	19.3–38.7 μm	26–28 μm	26.9	
2	C3	diploids	PGW	18.8–36.1 μm	22–26 μm	25.2	
3	C3	diploids	PD	1.4–4.1 μm	1.8–2.8 μm	2.3	
4	C3	diploids	AW	1.6–3.2 μm	2.0–2.2 μm	2.1	
5	C3	polyploids	PGL	30.7–48.1 μm	32–34 μm	34.7	C3 poly 7.8 μm > C3 dip
6	C3	polyploids	PGW	28.5–38.5 μm	31–32 μm	31.0	C3 poly 5.8 μm > C3 dip
7	C3	polyploids	PD	1.7–3.8 μm	1.9–2.1 μm	2.3	No difference
8	C3	polyploids	AW	1.9–3.8 μm	2.0–2.2 μm	2.4	C3 poly 0.3 μm > C3 dip
9	C4	diploids	PGL	24.6–40.2 μm	30–38 μm	33.4	
10	C4	diploids	PGW	23.4–39.3 μm	26–34 μm	30.8	
11	C4	diploids	PD	1.5–3.5 μm	1.9–2.2 μm	2.5	
12	C4	diploids	AW	1.3–3.1 μm	2.0–2.8 μm	2.2	
13	C4	polyploids	PGL	29.8–50.9 μm	38–40 μm	38.9	C4 poly 5.5 μm > C3 dip
14	C4	polyploids	PGW	26.7–44.1 μm	33–37 μm	35.4	C4 poly 4.6 μm > C3 dip
15	C4	polyploids	PD	2.1–4.2 μm	2.8–3.2 μm	2.9	C4 poly 0.4 μm > C3 dip
16	C4	polyploids	AW	1.7–3.3 μm	2.7–2.9 μm	2.6	C4 poly 0.1 μm > C3 dip

Note: Maximum range of measurement results given by the frequency of values most often given by the pollen grain parameters. PGL, pollen grain length; PGW, pollen grain width; PD, pore diameter; AW, annulus width.



Pearson correlation analyses

The Pearson correlation coefficient values reveal the strongest relationship of pollen grain length with pollen grain width ($r=0.85$), followed by pollen grain width with pore diameter ($r=0.60$) and pore diameter with annulus width ($r=0.39$; Figure 5).

Results of the LDA

LDA results for dependent variables, i.e. C3/C4 groups (Figure 6A) and diploid/polyploid groups (Figure 6B) are shown as frequency distribution histograms on the Y-axis; the independent variables, i.e. the four pollen grain parameters describing these groups, are shown on the X-axis. In the case of C3/C4 groups, the LDA range on the X-axis varies from -2 to 4 with a threshold value of 0 in between. All scores < 0 on the X-axis represent C3 species, while all scores > 0 represent C4 species (Figure 6A). For the C3 group, the frequency distribution is concentrated in the negative range of the threshold value 0 ($41/62 = 66\%$) having an overlap with C4 ($21/62 = 33\%$). The prior probabilities of the groups revealed C3 = 38.75% and C4 = 61.25% with an area under the curve = 77% . For the C4 group, the frequency distribution is concentrated on the right of the threshold value 0 ($86/98 = 88\%$) having an overlap with C3 ($12/98 = 12\%$).

The same is the case for the diploid/polyploid group (Figure 6B). The prior probabilities of the groups revealed diploid = 63.75% and polyploid = 36.25% with an area under the curve = 68.55% . The coefficient of linear discriminants, values of the mean pollen grain parameters and the percentage overlap estimated by class prediction for both C3/C4 and diploid/polyploid groups are given in Table III.

Results of the NMDS

Results of the NMDS of the four pollen grain parameters based on Euclidean distance, with the dataset square root transformed, centred scaling, PC rotation and standardisation, revealed a stress equal to 0.041 , procrustes equal to $8.601e-05$ and maximum residuals value equal to 0.0005 for the C3/C4 group. The observed dissimilarity is shown on a scale of -0.15 to $+0.15$ along NMDS axis 1 and the ordination distance on the scale of -0.04 to $+0.04$ along NMDS axis 2. The

NMDS based on the four pollen grain parameters does not show strong differences in the scores of C3 and C4 species along NMDS axis 1, as revealed from the t -test ($p = 0.3$; Figure 7A). Similarly NMDS for the diploid/polyploid group revealed a stress = 0.042 , procrustes = $4.134e-05$ and maximum residuals value = 0.0003 . The NMDS based on the four pollen grain parameters does not show strong differences in the scores of diploid/polyploid species along NMDS axis 1, as revealed from the t -test ($p = 0.5$; Figure 7B).

Diploid and polyploid species in the dataset

Out of the total 98 C4 species, 52 species (53%) are diploid and 46 species (47%) are polyploid. Out of the total 62 C3 species, 49 species (79%) are diploid, while 13 species (21%) are polyploid. Hence, in our specific data set of 160 species, polyploidy is more prevalent in C4 species, while diploidy is more prevalent in C3 species.

Discussion

This pilot study dealing with a small dataset of 160 species is certainly limited and the results are specific to our dataset. Hence, the number of Poaceae species needs to be substantially increased for replicating the study in future. Pollen grain studies of paired C3/C4 taxa would allow a more robust comparison. The main results revealed by different analyses are discussed here.

Identical chemical treatment allows us to compare the minute variations of Poaceae grains representing grasslands of diverse habitats. Due to the swelling effect of glycerine on the grain size, we recommend to prepare only a limited number of samples that can be measured on the same day or shortly after sample preparation. As chemical treatment has a clear effect on the size of pollen grains, we recommend standardising the sample preparation to avoid minute alterations in the size of grains due to different chemical treatments (Schüler & Behling 2011a).

The evaluation of changes in median with an increasing number of measured pollen grains revealed that the median grain length per species remains stable after about 40 measured pollen grains. This becomes very important when trying to compare data sets from different habitats and different periods. The median is certainly a rough

←
Figure 4. Boxplots showing the four pollen grain parameters of Poaceae species on the Y-axis and their photosynthetic pathway (A–D) and ploidy levels (E–H) on the X-axis. **A.** Pollen grain length (PGL) in relation to anatomy. **B.** Pollen grain width (PGW) in relation to anatomy. **C.** Pore diameter (PD) in relation to anatomy. **D.** Annulus width (AW) in relation to anatomy. **E.** PGL in relation to ploidy levels. **F.** PGW in relation to ploidy. **G.** PD in relation to ploidy. **H.** AW in relation to ploidy.

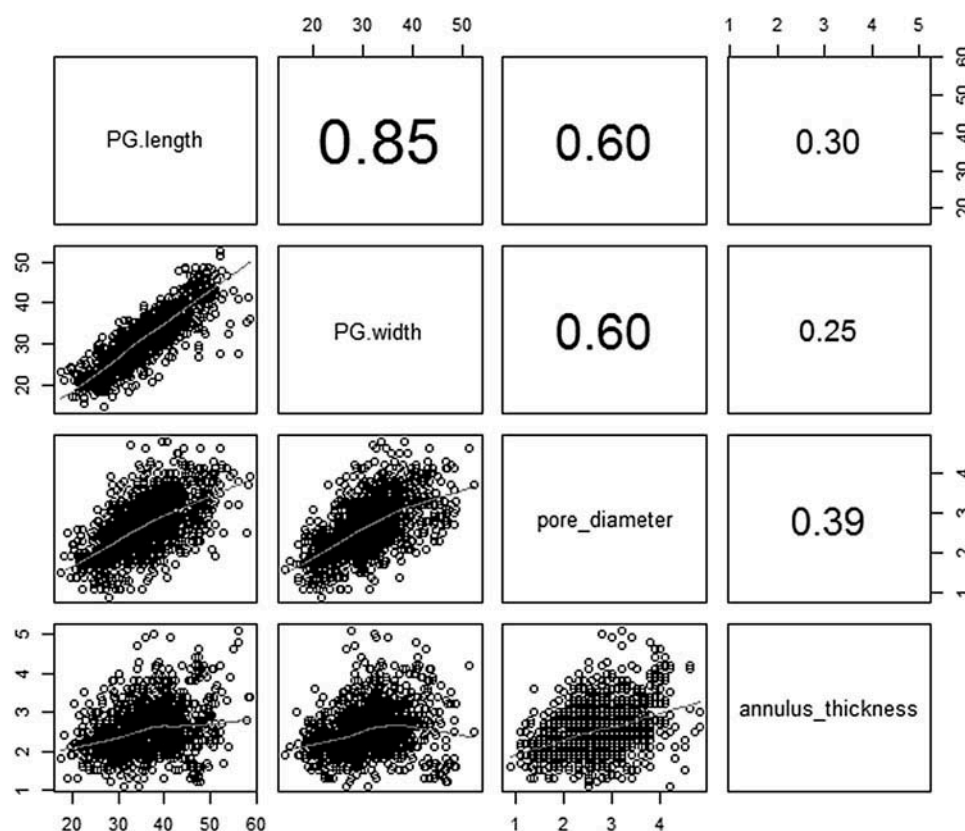


Figure 5. Pearson correlation coefficient values showing the strength of relationship among the four pollen grain parameters in descending order from top to bottom.

measure for an analysis of differences in pollen grain length and their composition. However, it serves well as a first approach to get an impression on the data-set of our species. Despite the stabilisation of the median value above 40 measured pollen grains, we recommend measuring at least 60 pollen grains per species to be completely sure about the representation of the median for the data set.

Schüler and Behling (2011a) described a strong correlation between pollen grain length, pollen grain width and pore diameter, while distinguishing South American past grasslands. Their assertion is based on the studies by Katsiotis and Forsberg (1995), who described a correlation between pollen grain length and pollen grain width in *Avena sativa*. Results of this study are in agreement with the previously obtained results. The correlation analysis revealed a strong relationship between the four pollen grain parameters.

Our results demonstrate that the average measures of pollen grain length, pollen grain width and annulus width are clearly larger in C3 polyploids as compared to C3 diploid species. However, no considerable difference was found in their pore diameter. All measured pollen grain parameters are larger in C4 polyploids than C4 diploid species. Although we used four pollen grain parameters for

distinguishing C3 and C4 Poaceae species, it appears sufficient to use the two most prominent parameters 'pollen grain length' and 'pollen grain width' for determining the grain size. Differences in the measures of pore diameter and annulus width can be neglected due to minor differences.

Due to overlaps, the results of our LDA and NMDS suggest that neither the scores of C3/C4 Poaceae species nor those of diploid/polyploid species are clearly separable using these four pollen grain measurements. However there are clear trends showing that the pollen grain sizes are larger in C4 as compared to C3 species and are larger in polyploids as compared to diploid species. Despite these overlaps, the average difference in the pollen grain sizes counted from a set of samples along a core may indicate shifts between C3 and C4 Poaceae composition of grasslands during the past. Unlike the clear and distinctly identified Poaceae species used in our dataset, in which the stabilisation of the median value for a single species is achieved after about 40 measured pollen grains, a fossil assemblage will always be a mix of unidentified taxa having more variability. To cope with this problem when working with palaeo-samples, the count number of grains needed to reach a stable median value would need to be assessed heuristically each time from sample to

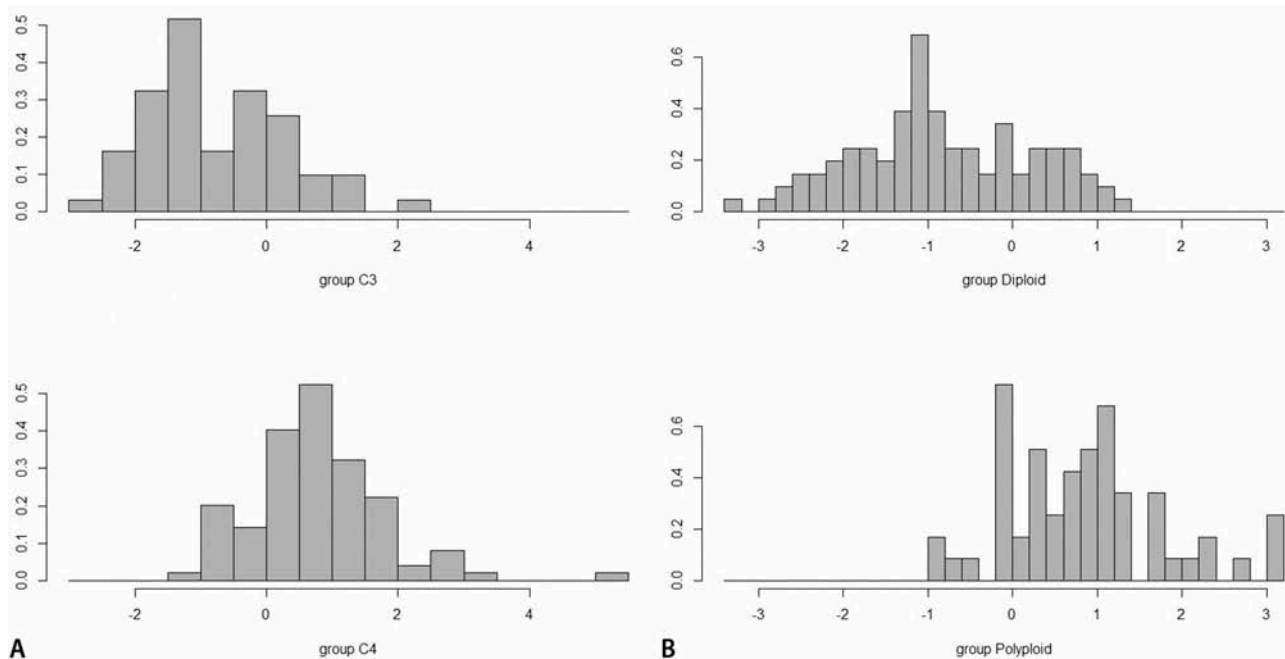


Figure 6. Results of the linear discriminant analysis (LDA). **A.** Comparison of the C3/C4 group. **B.** Comparison of the diploid/polyloid group.

sample. Regarding the relative abundance of C3 and C4 Poaceae species, our developed method provides a useful tool allowing new insights into the dynamics of past grassland ecosystems. Changes may also be tested by the carbon isotopic analysis of Poaceae grains or deposited phytoliths.

Beside the photosynthetic pathway, several factors might contribute to the variation in grain size such as ploidy levels, specific taxa compositions, phylogenetic relatedness and environmental stress. In plants, increases in pollen grain and guard cell sizes are used as surrogate evidence for polyploidy (Tate & Simpson 2004). The percentage of polyploidy in our 98 C4 species (47%) is greater than in 62 C3 species (21%). Similarly, we observed that out of the 70 Pakistani species, polyploidy is higher in C4 species (65.4%)

than in C3 species (34.6%). The larger pollen grain size coupled with higher percentage of polyploidy in C4 species, in comparison to the smaller grain size coupled with higher percentage of diploidy in C3 species, indicate a link between ploidy and grain size. It is difficult to assign a specific range size to a particular Poaceae taxon as the pollen grains of Poaceae show variation in size (Joly et al. 2007; Schüller & Behling 2011a). In our dataset, the general pattern of larger grain size of C4 vs C3 species is contradicted by some specific taxa compositions worth mentioning, of which are the four Ecuadorian C3 *Chusquea* species (Bambusoideae), i.e. *C. dombeyana* Kunth., *C. falcata* L.G.Clark, *C. leonardiorum* L.G.Clark and *C. neurophylla* L.G.Clark, which had much larger grains than many of the C4 species. Some studies

Table II. Results of the statistical tests.

Dataset	PGL	PGW	PD	AW
Shapiro-Wilk normality results for C3 and C4 datasets				
C3 diploids	P = 0.01	P = 0.0002	P = 0.0001	P = 0.005
C3 polyploids	P = 0.001	P = 0.004	P = 0.01	P = 0.001
C4 diploids	P = 0.11	P = 0.21	P = 0.08	P = 0.007
C4 polyploids	P = 0.39	P = 0.43	P = 0.27	P = 0.09
F-test for variances between diploids and polyploids				
In C3 dataset	P = 0.31	P = 0.01	P = 0.37	P = 0.03
In C4 dataset	P = 0.09	P = 0.39	P = 0.08	P = 0.04
U-test for differences in means of C3 diploids and C3 polyploids				
In C3 dataset	P = 1.259e-05	P = 0.0001	P = 0.94	P = 0.21
Student t-test to compare the means of C4 diploids and C4 polyploids				
In C4 dataset	p = 5.92e-09	p = 1.66e-07	p = 6.32e-06	p = 0.0002

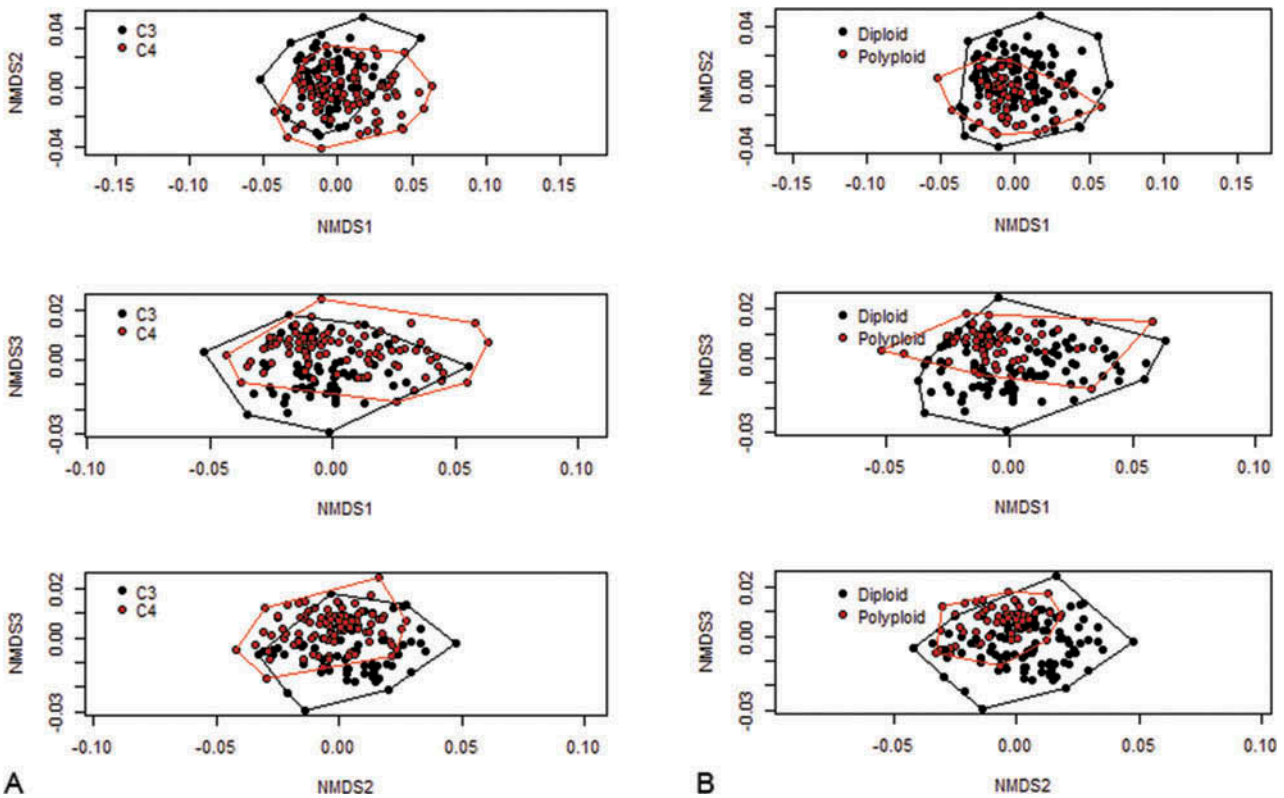


Figure 7. **A.** Non-metric multidimensional scaling (NMDS) results of the first two axes based on the separation of C3 and C4 species scores despite overlaps. C4 scores (red) and C3 scores (black). **B.** NMDS scores of polyploid species (red) and diploid species (black).

have found that in arid climates under higher desiccation stress, pollen grains become larger and more spherical (Ejsmond et al. 2011). Consequently, phenotypic plasticity with in Poaceae may pose a problem when applied to fossil samples, e.g. some C3 species of Pooideae are found in very dry habitats indicating the correlation of grain size variation more to their

characteristic ecological envelope than to the photosynthetic pathway of species in the community. In such cases, the composition and dynamics of existing grasslands based on the grain size analysis of fresh Poaceae species can be studied but interpretation of the palaeosamples for detecting the composition of past grasslands becomes complicated. Hence, the interpretation

Table III. Results of the Linear discriminant analysis (LDA).

Group	PGL	PGW	PD	AW
C3	28.6	26.43	2.30	2.23
C4	35.9	32.91	2.75	2.37
Diploid	30.3	28.14	2.42	2.20
Polyploid	38.0	34.38	2.84	2.52
Coefficient of LD for C3/C4	0.10	0.098	-2.327	2.270
Coefficient of LD for diploid/polyploid	0.21	0.008	2.492	-2.788
Class prediction	C3	C4	Overlap %	
C3	41	21	33	
C4	12	86	12	
Class prediction	diploid	polyploid	Overlap %	
diploid	85	17	16	
polyploid	18	40	31	

Note: LD, linear discriminants.

of signals from fossil samples for detecting the composition of past grasslands shall be managed with caution. It also cannot be ruled out that pollen size is simply a product of phylogenetic relatedness (Little et al. 2010). Members of the monophyletic PACMAD clade (Panicoideae, Arundinoideae, Chloridoideae, Micrairoideae, Aristidoideae and Danthonioideae; Duvall et al. 2007; Strömberg & McInerney 2011), where C4 photosynthesis evolved 22–24 times from their C3 ancestors, might have larger pollen grains than poides, can potentially provide taxonomic information about past grasslands, i.e. which clades were present, but not about the photosynthetic pathway as all PACMADs are not C4. However, phytolith assemblages can indicate the proportion of C3 and C4 grasses in the flora, if supported by evidence of isotopic studies (Wang et al. 1994).

C4 grasses are mostly dominant in regions with warm season precipitation, while C3 grasses prefer cool season precipitation (Epstein et al. 1997; Sage 2004; Urban et al. 2010). C4 Poaceae species with their specialised Kranz anatomy perform faster photosynthesis under high temperature, sufficient light preferring low elevated arid plain habitats. In this regard, our findings fit well with C4 grasses having larger grain size as some C4 species are associated with the drier climates of the low elevated Peshawar, Charsadda, Mardan areas at 275–300 m a.s.l. in northern Pakistan, while some C3 species having smaller grains are associated to the moist climate of the highly elevated Swat region (2200–2600 m a.s.l.) in northern Pakistan.

Conclusions

We demonstrate that in our specific dataset of 160 Poaceae species, C3 and C4 (diploid and polyploid species) are not clearly separable based on the measurement results of their pollen grain length, pollen grain width, pore diameter and annulus width. However, the measurement results and statistical analysis performed on these specific Poaceae species reveal trends showing that C3 polyploids have larger grain sizes than C3 diploid species, and C4 polyploids have larger grain size than C4 diploid species. Our study, even though it is based on a limited dataset (1.45% of all Poaceae species), shows a pattern in the trend of pollen grain size variation between modern C3 and C4 Poaceae species. C4 species have larger grain size than C3 species in our specific dataset. Grasslands dominated by C3 and C4 Poaceae species from different regions and habitats can be separated by studying the pattern of trends in their pollen grain sizes. In our dataset, polyploidy is more common in C4 than in C3 species. This method can be applied on Poaceae pollen grains deposited in environmental archives to

reconstruct past climate and assess the dynamics of past grassland ecosystems. This approach will not only help the ongoing palaeo-ecological studies in unravelling aspects such as changes in vegetation composition and shifts in biomes of past grasslands ecosystems but also provide useful insights for future predictions. The average difference in the pollen grain sizes counted from samples along a core in future studies may well indicate shifts between C3 and C4 Poaceae composition of grasslands during the past.

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References

- Ahmad F, Khan MA, Ahmad K, Zafar M, Iqbal Z. 2011. Palynological studies in tribe Chloridoideae (Poaceae) from salt range of Pakistan. *African Journal of Biotechnology* 10: 8840–8846.
- Andersen ST. 1979. Identification of wild grass and cereal pollen. *Danmarks Geologiske Undersøgelse Aarbog* 1978: 69–92.
- Andersen TS, Bertelsen F. 1972. Scanning electron microscope studies of pollen of cereals and other grasses. *Grana* 12: 79–86. doi:10.1080/00173137209428830.
- Behling H. 2002. South and southeast Brazilian grasslands during late quaternary times: A synthesis. *Palaeogeography, Palaeoclimatology, Palaeoecology* 177: 19–27. doi:10.1016/S0031-0182(01)00349-2.
- Behling H, Hooghiemstra H. 2001. Neotropical savanna environments in space and time: Late Quaternary inter-hemispheric comparisons [Chapter 18]. In Markgraf V, ed. *Inter-hemispheric climate linkages*, 307–323. San Diego, CA: Academic Press.
- Behling H, Pillar VD. 2007. Late Quaternary vegetation, biodiversity and fire dynamics on the southern Brazilian highland and their implication for conservation and management of modern

- Araucaria* forest and grassland ecosystems. Philosophical Transactions of the Royal Society B 362: 243–251.
- Behling H, Pillar VD, Orlóci L, Bauermann SG. 2004. Late Quaternary *Araucaria* forest, grassland (Campos), fire and climate dynamics, studied by high-resolution pollen, charcoal and multivariate analysis of the Camará do Sul core in southern Brazil. Palaeogeography, Palaeoclimatology, Palaeoecology 203: 277–297. doi:10.1016/S0031-0182(03)00687-4.
- Beug HJ. 1961. Leitfaden der Pollenbestimmung. Stuttgart: Fischer.
- Boom A, Mora G, Cleef AM, Hooghiemstra H. 2001. High altitude C4 grasslands in the northern Andes: Relicts from glacial conditions? Review of Palaeobotany and Palynology 115: 147–160. doi:10.1016/S0034-6667(01)00056-2.
- Bridson D, Forman L. 1992. The herbarium handbook. Kew: Royal Botanic Gardens.
- Brunschön C, Behling H. 2009. Late Quaternary vegetation, fire and climate history reconstructed from two cores at Cerro Toledo, *Podocarpus* National Park, south-eastern Ecuadorian Andes. Quaternary Research 72: 388–399. doi:10.1016/j.yqres.2009.07.001.
- Chen SL, Li DZ, Zhu GH, + 20 co-authors. 2006. Flora of China: 22. Poaceae. Beijing: Science Press, and St Louis, MO: Missouri Botanical Garden Press.
- Cope TA. 1982. Flora of Pakistan – Poaceae, 143. Kew: Royal Botanic Gardens.
- Cushing EJ. 1961. Size increase in pollen grains mounted in thin slides. Pollen et Spores 3: 265–274.
- Den Nijs JCM, Hooghiemstra H, Schalk PH. 1980. Biosystematic studies of the *Rumex acetocella* complex (Polygonaceae). IV. Pollen morphology and the possibilities of identification of cytotypes in pollen analysis. Phytom 20: 307–323.
- Dickson C. 1988. Distinguishing cereal from wild grass pollen – some limitations. Circaea 5: 67–71.
- Duvall M, Davis JJ, Clark LG, Noll J, Goldman D, Sánchez-Ken G. 2007. Phylogeny of the grasses (Poaceae) revisited. Aliso 23: 237–247. doi:10.5642/aliso.20072301.18.
- Ejmond MJ, Wronska-Pilarek D, Ejmond A, Dragosz-Kluska D, Karpińska-Kolaczek M, Kolaczek P, Kozłowski J. 2011. Does climate affect pollen morphology? Optimal size and shape of pollen grains under various desiccation intensity. Ecosphere 2: art117. doi:10.1890/ES11-00147.1.
- Epstein HE, Lauenroth WK, Burke IC, Coffin DP. 1997. Productivity patterns of C3 and C4 functional types in the U. S. Great Plains. Ecology 78: 722–731.
- Fægri K, Iversen J. 1989. Textbook of pollen analysis. Chichester, New York, Brisbane, Toronto, Singapore: J. Wiley & Sons.
- Haberle SG, Maslin MA. 1999. Late Quaternary vegetation and climate change in the Amazon basin based on a 50 000-year pollen record from the Amazon fan, ODP site 932. Quaternary Research 51: 27–38. doi:10.1006/qres.1998.2020.
- Hamilton AC. 1972. The interpretation of pollen diagram from highland Uganda. Palaeoecology of Africa 7: 45–149.
- Hojsgaard D, Honfi AI, Rua G, Daviña AJ. 2009. Chromosome numbers and ploidy levels of *Paspalum* species from subtropical South America (Poaceae). Genetic Resources and Crop Evolution 56: 533–545. doi:10.1007/s10722-008-9384-0.
- Joly C, Barillé L, Barreaud M, Mancheron A, Vissela L. 2007. Grain and annulus diameter as criteria for distinguishing pollen grains of cereals from wild grasses. Review of Palaeobotany and Palynology 146: 221–233. doi:10.1016/j.revpalbo.2007.04.003.
- Katsiotis A, Forsberg RA. 1995. Pollen grain size in four ploidy levels of genus *Avena*. Euphytica 83: 103–108. doi:10.1007/BF01678036.
- Kellogg EA. 2001. Evolutionary history of the grasses. Plant Physiology 125: 1198–1205. doi:10.1104/pp.125.3.1198.
- Köhler E, Lange E. 1979. A contribution to distinguishing cereal from wild grass pollen grains by LM and SEM. Grana 18: 133–140. doi:10.1080/00173137909424973.
- Little SA, Kembel SW, Wilf P. 2010. Paleotemperature proxies from leaf fossils reinterpreted in light of evolutionary history. PLoS ONE 5: e15161. doi:10.1371/journal.pone.0015161.
- Liu Q, Zhao N-X, Hao G. 2004. Pollen morphology of the Chloridoideae (Gramineae). Grana 43: 238–248. doi:10.1080/00173130410000776.
- Mäkelä EM. 1996. Size distinctions between *Betula* pollen types – A review. Grana 35: 248–256. doi:10.1080/00173139609430011.
- Mander L, Li M, Mio W, Fowlkes CC, Punyasena SW. 2013. Classification of grass pollen through the quantitative analysis of surface ornamentation and texture. Proceedings of the Royal Society of Biological Sciences 280: 20131905.
- Moinuddin M, Vahidy A, Ali SI. 1994. Chromosome counts in Arundinoideae, Chloridoideae, and Pooideae (Poaceae) from Pakistan. Annals of the Missouri Botanical Garden 81: 784–791. doi:10.2307/2399923.
- Moore PD, Webb JA. 1978. An illustrated guide to pollen analysis. New York: Halsted Press.
- Morrone O, Escobar A, Zuloaga FO. 2006. Chromosome studies in American Panicoideae (Poaceae). Annals of the Missouri Botanical Garden 93: 647–657. doi:10.3417/0026-6493(2006)93[647:CSIAPP]2.0.CO;2.
- Oksanen J, Blanchet FG, Kindt R, Legendre P, Minchin PR, O'Hara RB, Simpson GL, Solymos P, Stevens MHH, Wagner H. 2013. vegan: Community Ecology Package for R, version 2.0–10. <http://CRAN.R-project.org/package=vegan>.
- Peichoto MC, Galdeano F, Arbo MM, Neffa VGS. 2011. Chromosome number and ploidy level of some South American species of *Schizachyrium* (Poaceae, Andropogoneae). Plant Systematics and Evolution 292: 63–71. doi:10.1007/s00606-010-0403-1.
- Perveen A. 2006. A contribution to the pollen morphology of Gramineae. World Applied Sciences Journal 1: 60–65.
- Perveen A, Qaiser M. 2012. Pollen flora of Pakistan – LXIX. Poaceae. Pakistan Journal of Botany 44: 747–756.
- Prieto AR. 1996. Late Quaternary vegetational and climatic changes in the Pampa grassland of Argentina. Quaternary Research 45: 73–88. doi:10.1006/qres.1996.0007.
- Sage RF. 2004. The evolution of C4 photosynthesis. New Phytologist 161: 341–370. doi:10.1111/j.1469-8137.2004.00974.x.
- Salgado-Labouriau MLS, Rinaldi M. 1990a. Palynology of Gramineae of the Venezuelan mountains. Grana 29: 119–128. doi:10.1080/00173139009427742.
- Salgado-Labouriau ML, Rinaldi M. 1990b. Measurements of Gramineae pollen of the Venezuelan mountains. Revista Brasileira de Biologia 50: 115–122.
- Schüler L, Behling H. 2011a. Poaceae pollen grain size as a tool to distinguish past grasslands in South America: A new methodological approach. Vegetation History and Archaeobotany 20: 83–96. doi:10.1007/s00334-010-0265-z.
- Schüler L, Behling H. 2011b. Characteristics of Poaceae pollen grains as a tool to assess palaeoecological grassland dynamics in South America. Vegetation History and Archaeobotany 20: 97–108. doi:10.1007/s00334-010-0264-0.
- Shantz HL. 1954. The place of grasslands in the Earth's cover of vegetation. Ecology 35: 143–145.
- Skvarla JJ, Rowley JR, Hollowell VC, Chissoe WF. 2003. Annulus-pore relationship in Gramineae (Poaceae) pollen: The pore margin of *Pariana*. American Journal of Botany 90: 924–930. doi:10.3732/ajb.90.6.924.

- Strömberg CAE, McInerney FA. 2011. The Neogene transition from C3 to C4 grasslands in North America: Assemblage analysis of fossil phytoliths. *Paleobiology* 37: 50–71. doi:[10.1666/09067.1](https://doi.org/10.1666/09067.1).
- Tate JA, Simpson BB. 2004. Breeding system evolution in TARASA (Malvaceae) and selection for reduced pollen grain size in the polyploid species. *American Journal of Botany* 91: 207–213. doi:[10.3732/ajb.91.2.207](https://doi.org/10.3732/ajb.91.2.207).
- The R Foundation for Statistical Computing. 2013. The R Project for Statistical Computing. Vienna: Institute for Statistics and Mathematics, Wirtschaftsuniversität Wien.
- Ullah Z, Khan MA, Ahmad M, Zafar M, Ullah K. 2011. Systematic implications of foliar epidermis in Andropogoneae (Poaceae) from Hindukush, Himalayas, Pakistan. *Journal of Medicinal Plants Research* 5: 949–957.
- Urban MA, Nelson DM, Jiménez-Moreno G, Châteauneuf J-J, Pearson A, Hu FS. 2010. Isotopic evidence of C4 grasses in southwestern Europe during the Early Oligocene-Middle Miocene. *Geology* 38: 1091–1094. doi:[10.1130/G31117.1](https://doi.org/10.1130/G31117.1).
- Wang Y, Cerling TE, MacFadden BJ. 1994. Fossil horses and carbon isotopes: New evidence for Cenozoic dietary, habitat, and ecosystem changes in North America. *Palaeogeography, Palaeoclimatology, Palaeoecology* 107: 269–279. doi:[10.1016/0031-0182\(94\)90099-X](https://doi.org/10.1016/0031-0182(94)90099-X).

Appendix

Table A1. Measurements of the four pollen grain parameters of the 160 species dataset showing their anatomy, collection countries and ploidy levels.

	Species	Anatomy	PGL	PGW	PD	AW	Ploidy	Country
1	<i>Avena fatua</i> L.	C3	26.8	22.2	2.1	2.3	2	Pakistan
2	<i>Bromus catharticus</i> Vahl.	C3	28.1	24.2	2.8	2.6	2	Pakistan
3	<i>Bromus pectinatus</i> Thunb.	C3	30.2	25.7	2.7	2.1	2	Pakistan
4	<i>Bromus unioloides</i> Kunth.	C3	28.7	27.4	2.8	2.9	2	Pakistan
5	<i>Dactylis glomerata</i> L.	C3	25.3	22.1	3.1	2.2	2	Pakistan
6	<i>Koeleria macrantha</i> (Ledeb.) Schult.	C3	27.1	21.2	1.4	2.2	2	Pakistan
7	<i>Phalaris paradoxa</i> L.	C3	27.2	21.4	2.4	2.6	2	Pakistan
8	<i>Phleum paniculatum</i> Huds.	C3	26.3	22.4	1.9	2.1	2	Pakistan
9	<i>Piptatherum munroi</i> (Stapf) Mez.	C3	28.4	25.3	2.2	2.0	2	Pakistan
10	<i>Poa annua</i> L.	C3	25.2	20.2	1.8	2.2	2	Pakistan
11	<i>Polypogon fugax</i> Nees ex Steud.	C3	27.8	22.0	2.2	2.3	2	Pakistan
12	<i>Polypogon monspeliensis</i> (L.) Desf.	C3	30.7	27.7	2.6	2.8	2	Pakistan
13	<i>Stipa brandisii</i> Mez.	C3	25.7	24.9	2.8	2.6	2	Pakistan
14	<i>Stipa capensis</i> Moraldo	C3	27.4	25.2	2.8	1.6	2	Pakistan
15	<i>Agropyron pilosula</i> Schur.	C3	26.2	21.2	2.2	2.1	2	Pakistan
16	<i>Agrostis gigantea</i> Roth.	C3	20.9	26.7	2.5	2.4	2	Pakistan
17	<i>Ampelesmos mauritanicus</i> (Poir.) T. Durand et Schinz.	C3	21.3	20.6	2.2	2.6	2	Venezuela
18	<i>Stipa carpensis</i> L.	C3	35.3	35.1	2.7	2.0	2	Venezuela
19	<i>Isachne</i> species	C3	26.1	24.9	1.9	1.6	2	Ecuador
20	<i>Holcus lanatus</i> L.	C3	23.7	23.7	2.0	1.7	2	Ecuador
22	<i>Chusquea dombeiana</i> Kunth.	C3	33.2	36.0	3.3	3.0	2	Ecuador
23	<i>Chusquea falcata</i> L.G.Clark	C3	35.5	33.5	3.8	2.9	2	Ecuador
24	<i>Chusquea leonardionum</i> L.G.Clark	C3	35.2	33.0	3.9	2.8	2	Ecuador
25	<i>Chusquea neurophylla</i> L.G.Clark	C3	35.5	38.7	3.1	2.3	2	Ecuador
26	<i>Neurolepis aristata</i> (Munro) Hitchc.	C3	35.7	33.6	4.1	3.2	2	Ecuador
27	<i>Neurolepis elata</i> (Kunth) Pilg.	C3	22.8	28.8	2.7	2.2	2	Ecuador
28	<i>Chusquea</i> species	C3	27.2	25.9	1.7	1.9	2	Ecuador
29	<i>Bromus squarrosus</i> L.	C3	30.2	29.6	2.1	2.1	2	Germany
30	<i>Bromus japonicus</i> Thunb.	C3	27.3	26.2	1.9	2.1	2	Germany
31	<i>Bromus rubens</i> L.	C3	28.4	25.3	2.2	1.8	2	Germany
32	<i>Bromus carinatus</i> Hook. et Arn.	C3	27.9	23.4	1.8	2.3	2	Germany
33	<i>Bromus willdenowii</i> Kunth	C3	28.1	25.1	2.0	1.9	2	Germany
34	<i>Bromus macrostachys</i> Desf.	C3	22.8	21.2	1.9	2.0	2	Germany
35	<i>Hordeum spontaneum</i> K.Koch	C3	25.4	22.7	2.4	2.1	2	Germany
36	<i>Hordeum distichon</i> L.	C3	22.5	21.7	1.8	1.9	2	Germany
37	<i>Hordeum caespitosum</i> Scribn.	C3	21.8	29.6	1.7	1.9	2	Germany
38	<i>Hordeum compressum</i> Griseb.	C3	22.6	20.2	1.8	1.9	2	Germany
39	<i>Hordeum glaucum</i> (Steud.) Tzvelev	C3	30.8	28.7	1.8	2.1	2	Germany
40	<i>Poa supina</i> Schrad.	C3	22.6	20.8	1.6	1.8	2	Germany
41	<i>Poa trivialis</i> L.	C3	19.3	18.8	1.7	2.1	2	Germany
42	<i>Poa pratensis</i> L.	C3	21.2	20.4	1.8	1.9	2	Germany
43	<i>Poa angustifolia</i> L.	C3	20.8	20.1	1.7	1.7	2	Germany
44	<i>Poa annua</i> L.	C3	25.1	22.3	1.8	1.9	2	Germany
45	<i>Poa infirma</i> Kunth.	C3	23.5	20.6	1.9	2.1	2	Germany

46	<i>Poa chaixii</i> Vill.	C3	24.1	22.3	1.8	2.2	2	Germany
47	<i>Poa compressa</i> L.	C3	24.6	21.9	2.1	2.2	2	Germany
48	<i>Poa palustris</i> L.	C3	26.2	24.1	1.8	2.1	2	Germany
49	<i>Poa bulbosa</i> L.	C3	25.1	23.3	1.7	1.9	2	Germany
50	<i>Hordeum murinum</i> L.	C3	37.9	33.4	3.2	2.7	3	Pakistan
51	<i>Leersia oryzoides</i> Hedw. ex Batsch	C3	35.5	28.5	2.4	2.2	3	Pakistan
52	<i>Phalaris minor</i> Retz.	C3	39.2	32.5	3.3	3.8	3	Pakistan
53	<i>Aulonemia patula</i> (Pilg.) McClure	C3	48.1	38.5	3.8	3.2	3	Ecuador
54	<i>Bromus erectus</i> Hudson Smith ex Morris	C3	31.7	30.5	2.1	2.3	3	Germany
55	<i>Bromus rigidus</i> Roth.	C3	31.3	28.6	1.7	1.9	3	Germany
56	<i>Bromus diandrus</i> Roth.	C3	32.6	29.8	2.1	2.2	3	Germany
57	<i>Bromus arvensis</i> Lam.	C3	33.4	30.6	1.8	2.2	3	Germany
58	<i>Hordeum marinum</i> Huds.	C3	32.2	30.5	1.7	2.1	3	Germany
59	<i>Hordeum vulgare</i> L.	C3	30.7	29.3	2.4	2.2	3	Germany
60	<i>Hordeum murinum</i> L.	C3	34.2	29.3	1.9	2.1	3	Germany
61	<i>Hordeum bulbosum</i> L.	C3	32.4	31.1	2.1	2.2	3	Germany
62	<i>Hordeum jubatum</i> L.	C3	31.7	30.6	1.8	1.9	3	Germany
63	<i>Acrachne racemosa</i> Wight et Arn. ex Chiov.	C4	33.7	31.4	1.9	2.5	2	Pakistan
64	<i>Apluda nutica</i> L.	C4	34.8	32.2	2.7	2.7	2	Pakistan
65	<i>Chrysopogon gryllus</i> (L.) Trin.	C4	32.7	29.9	2.4	2.4	2	Pakistan
66	<i>Dactyloctenium aegyptium</i> (L.) Willd.	C4	31.7	27.4	2.0	2.7	2	Pakistan
67	<i>Helictotrichon virescens</i> Nees ex Steud.	C4	37.9	35.4	3.5	2.7	2	Pakistan
68	<i>Imperata cylindrica</i> (L.) P.Beauv.	C4	32.5	28.9	2.8	2.2	2	Pakistan
69	<i>Microstegium nudum</i> (Trin.) A.Camus	C4	38.1	36.0	2.9	2.4	2	Pakistan
70	<i>Setaria viridis</i> (L.) P.Beauv.	C4	37.2	31.6	3.2	3.1	2	Pakistan
71	<i>Themeda anathera</i> (Nees ex Steud.) Hack.	C4	34.6	32.0	3.3	3.1	2	Pakistan
72	<i>Bracharia repans</i> (L.) C.A.Gardner et C.E.Hubb.	C4	36.8	34.9	2.9	2.7	2	Pakistan
73	<i>Chrysopogon aucheri</i> (Boiss.) Stapf	C4	29.8	28.5	2.9	2.6	2	Pakistan
74	<i>Desmostachya bipinnata</i> (L.) Stapf	C4	35.4	33.6	2.4	2.9	2	Pakistan
75	<i>Imperata Cylindrica</i> (L.) P.Beauv.	C4	31.9	29.1	2.8	2.1	2	Pakistan
76	<i>Panicum antidotale</i> Freckmann et Lelong	C4	39.5	38.7	2.9	2.7	2	Pakistan
77	<i>Paspalum paspalodes</i> (Michx.) Scribn.	C4	36.5	35.8	3.2	3.1	2	Pakistan
78	<i>Pennisetum flaccidum</i> Griseb. ex Roshev.	C4	38.9	36.8	3.2	3.1	2	Pakistan
79	<i>Pennisetum glaucum</i> (L.) R.Br.	C4	36.5	35.5	2.9	2.8	2	Pakistan
80	<i>Saccharum griffithii</i> Munro ex Aitch.	C4	33.3	32.5	2.8	2.6	2	Pakistan
81	<i>Saccharum rufiplum</i> Steud.	C4	37.2	32.1	2.8	2.6	2	Pakistan
82	<i>Stipa capensis</i> Moraldo	C4	36.9	35.7	2.8	1.5	2	Pakistan
83	<i>Aristida adscensionis</i> L.	C4	35.6	33.2	3.0	1.9	2	Tanzania
84	<i>Bothriochloa insculpta</i> (Hochst. ex A.Rich.)	C4	36.9	33.5	2.0	1.5	2	Tanzania
85	<i>Bracharia brizantha</i> (Hochst. ex A.Rich.) Stapf	C4	34.7	32.7	2.7	1.9	2	Tanzania
86	<i>Chloris roxburghiana</i> Schult.	C4	31.4	33.7	2.5	2.0	2	Tanzania
87	<i>Cymbopogon caesiatus</i> (Nees ex Hook. et Arn.) Stapf	C4	27.1	28.6	2.1	2.2	2	Tanzania
88	<i>Dactyloctenium aegyptium</i> (L.) Willd.	C4	32.9	25.7	2.1	1.6	2	Tanzania
89	<i>Digitaria dragonalis</i> L.	C4	32.1	29.8	2.2	1.6	2	Tanzania
90	<i>Digitaria abyssinica</i> (Hochst. ex A.Rich.) Stapf	C4	35.6	29.5	2.0	1.5	2	Tanzania

(Continued)

Table AI. (Continued).

	Species	Anatomy	PGL	PGW	PD	AW	Ploidy	Country
91	<i>Diheteropogon Amplectens</i> (Nees) Clayton	C4	37.5	33.5	3.0	1.9	2	Tanzania
92	<i>Elionurus muticus</i> (Spreng.) Kuntze	C4	29.9	25.0	2.7	2.1	2	Tanzania
93	<i>Emicpogon cenchroides</i> (Licht. ex Roem. et Schult.)	C4	29.2	25.1	2.2	2.1	2	Tanzania
94	<i>Enteropogon macrostachyus</i> K.Schum. ex Engl.	C4	34.4	30.9	2.8	1.8	2	Tanzania
95	<i>Eragrostis schweinfurthii</i> Chiov.	C4	31.7	30.8	2.0	1.7	2	Tanzania
96	<i>Eragrostis cilianensis</i> (All.) Link ex Vignolo	C4	26.2	24.3	2.4	1.6	2	Tanzania
97	<i>Eragrostis kinensis</i> Wolf	C4	36.3	24.5	1.7	1.6	2	Tanzania
98	<i>Eragrostis superba</i> Peyr	C4	33.2	23.3	3.3	2.7	2	Tanzania
99	<i>Eustachys paspaloides</i> (Vahl) Lanza et Mattei	C4	28.8	30.5	3.3	2.7	2	Tanzania
100	<i>Leptothrium senegalense</i> (Kunth) Clayton	C4	31.2	27.6	3.1	2.1	2	Tanzania
101	<i>Oropetium minimum</i> (Hochst.) Pilg.	C4	29.2	26.2	1.9	1.8	2	Tanzania
102	<i>Panicum coloratum</i> L.	C4	28.8	27.9	1.5	1.3	2	Tanzania
103	<i>Setaria nervosum</i> (Rottler ex Roem. et Schult.) Stapf	C4	40.2	39.3	2.5	1.9	2	Tanzania
104	<i>Sporobolus juncea</i> (P.Beauv.) Kunth	C4	28.7	24.4	2.1	1.9	2	Tanzania
105	<i>Sporobolus pellucidus</i> Hochst.	C4	27.4	24.4	2.1	1.9	2	Tanzania
106	<i>Sporobolus tenuissimus</i> (Mart. ex Schrank) Kuntze	C4	28.9	24.7	2.6	1.9	2	Tanzania
107	<i>Tetrapogon cenchroides</i> (A.Rich.) Clayton	C4	37.7	26.3	2.3	1.5	2	Tanzania
108	<i>Tetrapogon tenellus</i> (J.Koenig ex Roxb.) Chiov.	C4	36.4	31.5	1.7	2.1	2	Tanzania
109	<i>Tragus berteronianus</i> Schult.	C4	27.9	37.4	3.1	2.6	2	Tanzania
110	<i>Trachypogon spicatus</i> (L.f.) Kuntze	C4	35.7	35.0	2.0	2.7	2	Venezuela
111	<i>Paspalum urvillei</i> Steud.	C4	28.6	27.7	1.9	1.8	2	Brazil
112	<i>Sporobolus indicus</i> (L.) R.Br.	C4	24.6	23.4	2.3	2.0	2	Brazil
113	<i>Andropogon species</i>	C4	35.2	32.5	2.1	1.9	2	Ecuador
114	<i>Melinis minutiflora</i> P.Beauv.	C4	32.8	32.2	2.3	1.9	2	Ecuador
115	<i>Rhytidocladum species</i>	C4	36.5	32.3	3.1	3.1	2	Ecuador
116	<i>Arthraxon prinoides</i> P.Beauv.	C4	44.9	41.6	2.9	3.0	3	Pakistan
117	<i>Cenchrus ciliaris</i> L.	C4	41.1	35.5	3.1	2.3	3	Tanzania
118	<i>Cymbopogon javarancosa</i> Spreng.	C4	39.8	34.4	2.7	2.2	3	Pakistan
119	<i>Cynodon dactylon</i> (L.) Pers.	C4	37.7	33.5	2.3	2.3	3	Pakistan
120	<i>Dichanthium annulatum</i> (Forssk.) Stapf	C4	33.8	31.3	2.7	2.4	3	Pakistan
121	<i>Digitaria ciliaris</i> (Retz.) Koeler	C4	42.3	35.8	3.2	2.5	3	Pakistan
122	<i>Echinochloa crus gallii</i> (L.) P.Beauv.	C4	39.7	33.9	3.1	2.7	3	Pakistan
123	<i>Eleusine indica</i> (L.) Gaertn.	C4	33.3	33.3	3.5	3.1	3	Pakistan
124	<i>Eragrostis cilianensis</i> (All.) Link ex Vignolo	C4	29.8	27.7	2.7	2.8	3	Pakistan
125	<i>Heteropogon contortus</i> (L.) P.Beauv. ex Roem. et Schult.	C4	39.1	32.2	3.1	2.7	3	Pakistan
126	<i>Paspalum flavidum</i> (Retz.) A. Camus	C4	44.2	37.2	3.2	2.8	3	Pakistan
127	<i>Pennisetum orientale</i> Rich.	C4	36.8	35.4	3.4	2.9	3	Pakistan
128	<i>Pennisetum typhoides</i> Rich.	C4	41.1	37.5	3.5	2.9	3	Pakistan
129	<i>Saccharum rufipilum</i> Steud.	C4	37.4	31.8	3.1	2.7	3	Pakistan
130	<i>Cenchrus pennisetiformis</i> Hochst. et Steud	C4	37.5	36.4	3.2	2.8	3	Pakistan
131	<i>Bothriochloa ischaenum</i> (L.) Keng	C4	38.7	34.1	2.9	2.6	3	Pakistan
132	<i>Brachiaria deflexa</i> (Schumach.) C.E.Hubb.ex Robyns	C4	37.5	35.6	3.1	2.8	3	Pakistan
133	<i>Brachiaria eruciformis</i> (Sm.) Griseb.	C4	34.7	33.6	2.9	2.7	3	Pakistan
134	<i>Brachiaria ramosa</i> Stapf	C4	33.6	32.9	2.8	2.6	3	Pakistan

135	<i>Chrysopogon gryllus</i> (L.) Trin.	C4	32.4	28.8	2.4	2.4	3	Pakistan
136	<i>Isachne himalaica</i> Hook.f.	C4	35.2	33.8	2.9	2.6	3	Pakistan
137	<i>Panicum maximum</i> L.	C4	36.8	35.4	3.1	2.9	3	Pakistan
138	<i>Panicum repens</i> L.	C4	37.5	35.3	2.8	2.5	3	Pakistan
139	<i>Panicum turgidum</i> Nees	C4	33.1	31.5	2.6	2.5	3	Pakistan
140	<i>Paspalum dilatatum</i> Poir.	C4	35.6	34.6	3.1	3.0	3	Pakistan
141	<i>Pennisetum flavidum</i> Griseb. ex Roshev.	C4	47.8	42.9	3.4	3.0	3	Pakistan
142	<i>Saccharum spontaneum</i> L.	C4	37.6	31.4	3.4	2.7	3	Pakistan
143	<i>Setaria intermedia</i> Roem. et Schult.	C4	41.5	40.3	3.1	3.1	3	Pakistan
144	<i>Setaria verticillata</i> (L.) P.Beauv.	C4	42.5	40.6	3.2	2.9	3	Pakistan
145	<i>Setaria pumila</i> (Poir.) Roem. et Schult.	C4	48.3	41.9	3.2	2.8	3	Pakistan
146	<i>Sorghum bicolor</i> (L.) Moench	C4	39.8	37.6	3.1	2.9	3	Pakistan
147	<i>Sorghum halepense</i> (L.) Pers.	C4	39.1	34.5	3.0	2.5	3	Pakistan
148	<i>Urochloa panicoides</i> P.Beauv.	C4	40.5	34.7	2.7	3.3	3	Pakistan
149	<i>Brachiaria deflexa</i> (Schumacher) C.E.Hubb. ex Robyns	C4	31.2	32.8	3.2	2.6	3	Tanzania
150	<i>Digitaria milanjiana</i> Henrard	C4	42.5	38.7	4.2	3.1	3	Tanzania
151	<i>Heteropogon contortus</i> (L.) P.Beauv. ex Roem. et Schult	C4	50.9	44.1	3.4	1.7	3	Tanzania
152	<i>Panicum maximum</i> Jacq.	C4	38.6	26.7	2.4	1.8	3	Tanzania
153	<i>Paspalum scrobiculatum</i> L.	C4	39.2	34.8	2.3	1.8	3	Tanzania
154	<i>Rhynchosyrrum repens</i> Nees	C4	42.2	36.4	2.8	2.3	3	Tanzania
155	<i>Themeda triandra</i> Forssk.	C4	42.5	40.6	3.5	2.1	3	Tanzania
156	<i>Paspalum notatum</i> Alain ex Flüggé	C4	34.1	31.1	2.3	2.1	3	Brazil
157	<i>Axonopus compressus</i> (Sw.) P.Beauv	C4	47.8	42.7	2.8	2.1	3	Ecuador
158	<i>Eleusine species</i>	C4	38.1	35.4	2.1	1.9	3	Ecuador
159	<i>Panicum species</i>	C4	39.5	38.4	3.6	2.5	3	Ecuador
160	<i>Paspalum paniculatum</i> L.	C4	33.3	32.3	2.6	1.8	3	Ecuador

Note: PGL₃, pollen grain length; PGW, pollen grain width; PD, pore diameter; AW, annulus width. Ploidy level 2 means diploid and ploidy level 3 means polyploid.