human β-globin section on chromosome 11 does reveal only two locations that appear to have the characteristic vertical shape of the γ-globins; the other globins are relatively harder to identify, but can be made possible using sophisticated pattern recognition techniques.

We may note here that when matching the patterns of two homologous sequences we would be looking for a close rather than an exact match since the base distribution along the sequences will be close but not necessarily identical, and this may reduce Hamori and Ruskin's[1] H-curve method's effectiveness. In the Hamori–Ruskin model, the three-dimensional curve obtained is open and stretched according to the length of the DNA and therefore makes pattern recognition and matching that much more difficult. In the current technique since we plot differences between pairs of nucleotides along the two axes, the pattern is compressed while at the same time it can show sharp variations in the slope of the graph as the differences between the bases swing through zero values. On the other hand, minor variations in base composition cause only slight changes to the overall pattern, making the task of its recognition comparatively easier, while major variations in base composition over a significant length of the DNA produce distinctly different patterns as exemplified in the case of the globin genes discussed above.

It is instructive also to compare the plots of gene sequences in the present model with the diagrams obtained in the chaos generator (CGR) model of Jeffrey[5]. CGR diagrams are not very informative for few hundreds or thousands of bases, unlike the current plotting technique which can be used for tens of bases to megabases of any length to provide characteristic features according to the scale of the plot. CGRs of gene families such as the globins show similar depletion zone patterns but there are no noticeable differences between the different types of β-globin genes. However, where there are sharply contrasting regions of nucleotide abundances, both the CGR and the present model can be used to indicate the differences, with the present model having the advantage of visually identifying regions of differing abundances directly.

In summary, we may state that we have presented a novel graphical method for visual display of nucleotide distribution patterns in DNA sequences and have demonstrated the usefulness of the method by taking the globin gene family as an example; study of other gene families is in progress. From the studies presented here we may conclude that this method can be of use in identifying groups of nucleotides that may be related evolutionarily as in the case of the β- and δ-globins. The method can also be used for visual global homology which we have seen exists in the case of highly conserved sequence families such as the globin genes; where such characteristic shapes can be identified this leads to the possibility of rapid homology search on megabase sequences that are fast becoming part of the global sequence databases. While this graphical technique bears some similarity to the H-curves of Hamori and Ruskin[1], it is a two-dimensional graph that is easier to construct and visualize; at the same time, the major advantage accruing out of the present method is that since this plots the differences of pairs of bases along the two axes, it is very sensitive to significant changes in the base composition. However, in representing a 4-independent parameter object like a gene sequence on a two-dimensional plane there will be shortcomings; in this case parallel maps with *C*, *G* and *G*, *T* axes interchanged may lead to more information. In this context we may mention the extensions to the chaos generator diagrams proposed by Burma *et al.*[6] and submit that the current method is simple to implement, extends sequence analyses techniques in different ways and readily complements the various methods in existence for understanding the nature of the distribution of nucleotides along a gene sequence.

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Comparative study of some methods of oxidation of plant materials for elemental analysis

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Four different methods were used to dissolve eleven different plant materials for simultaneous estimation

of several elements. In comparison to wet digestion in nitric acid, dry-ashing at 500°C resulted in lower estimates of S, As, Pb and Cd. The digestion with di-acid caused the loss of P, K, Ca, Mg, Na, Zn, Cu, Mn and B, the loss being statistically significant and much higher when the ratio of the two acids was 3:1 as compared to 10:1. The loss in K values was higher when concentration was greater than 1%. Therefore, among the four tested methods, the digestion with nitric acid is proposed for elemental analysis.

FOR elemental analysis, plant materials are oxidized either by dry-ashing at high temperature in a muffle furnace or by wet digestion in different mixtures of two or three of the acids such as nitric, perchloric and sulphuric. The choice depends upon the elements required to be determined. Sulphuric acid cannot be used when calcium, magnesium and sulphur are among the elements to be estimated because of the formation of sulphates which are sparingly soluble. Perchloric acid is potentially hazardous during digestion of biological materials and can cause the loss of K by precipitation as potassium perchlorate and B by volatilization[1]. Halvin and Soltanpour[2] proposed the use of only nitric acid for dissolution of plant materials. In dry-ashing some of the more volatile elements are likely to be lost through volatilization at high temperature. Munter *et al.*[3] observed incomplete elemental recovery with ashing method and recommended both ashing and digestions with nitric and perchloric acids to determine the elements normally required from plant analysis. A laboratory study was therefore conducted to standardize a method for simultaneous extraction of different elements to be determined with a flame photometer, atomic absorption spectrometer or an inductively-coupled argon plasma emission spectrometer (ICAP-AES).

Eleven oven-dried and powdered leaf samples (40 mesh) of Egyptian clover (*Trifolium alexandrium*), wheat (*Triticum aestivum*), mustard (*Brassica compestris*), sugarcane (*Saccharum officinarum*), kinnow

Table 1. Concentrations of seventeen elements in the plant material under study (means of 4 methods of oxidation)

		Plant material										
		#1	#2	#3	#4	#5	#6	#7	#8	#9	#10	#11
P (mg/kg)	Mean	4758	5581	4180	1349	1405	1737	1164	1104	2648	3570	3941
	C.V.	13 4	6 8	14 6	6 0	4 9	9 4	8 6	8.6	8 0	8 5	15 8
K (mg/kg)	Mean	22916	22544	19518	6315	9874	9213	6026	2812	15077	20023	22385
	C.V.	35.9	38 6	31 6	4 4	8 9	6.7	5 5	6 5	21 6	33 7	34 7
Ca (mg/kg)	Mean	11068	4518	12653	3688	22383	1754	13021	3272	18014	11868	8088
	C.V.	1.2	4 9	5 9	3.3	3 8	4 8	3 6	5 7	4.1	5 9	6 2
Mg (mg/kg)	Mean	3527	2451	4102	1370	3611	2678	2698	1136	5452	8963	4743
	C V	2 5	1 9	6 3	2 3	5 4	5 6	4 6	7 1	5 9	6 7	4 6
S (mg/kg)	Mean	1978	3067	3999	1159	2363	993	1454	771	9204	3014	3260
	C.V	19 4	18 6	15.9	9 1	16 4	13.9	16 3	20 3	6 2	18 9	19 6
Na (mg/kg)	Mean	1923	316	2016	64	269	134	38	22	587	132	1256
	C.V.	5 3	9 2	10.1	12.2	7 1	12 5	16 2	9 8	2 6	10 4	8 3
Zn (mg/kg)	Mean	27.5	46 9	36.4	22 8	43 6	69 6	137 8	52 4	24 0	23 8	37 8
	C.V	7 9	8 0	5 9	7 2	4 0	6 9	4 1	5 0	4.4	4 8	4 4
Cu (mg/kg)	Mean	3 01	7 28	4 63	4.15	22 04	6 65	6.18	2 22	7 14	12 65	9 01
	C.V.	4 0	8 0	9 1	2 4	5 9	4 8	4 5	10.0	3.1	5.8	6 0
Fe (mg/kg)	Mean	173	167	198	139	116	1359	100	124	239	290	419
	C V.	21	36	17	5	11	14	24	7	6	2	12
Mn (mg/kg)	Mean	30 0	38 1	45 6	33 1	36 9	47 8	445.3	484 6	51 4	34 4	33 9
	C V	5 0	3 9	6 5	6 3	6 1	7 5	4 9	6 0	5 9	6 5	7 0
B (mg/kg)	Mean	27.2	6 0	15 1	8 3	128 8	6 6	34 2	14 1	49 9	23 3	16 9
	C V	5 3	4 8	9 8	8 6	3 2	8 8	6 4	4 0	8 3	8 4	9 5
Al (mg/kg)	Mean	61	55	67	82	67	349	905	365	167	223	297
	C.V	30 0	15 7	24 5	11 5	28.7	6 7	4 8	8 0	25 3	15 7	9 4
As (mg/kg)	Mean	4 22	5.22	5 86	4.30	5 65	5.36	6 22	4 01	6 50	8 57	6 29
	C.V.	12 8	29 0	16 6	33 4	27.4	29 6	17 7	36 7	8 3	7 7	34 7
Pb (mg/kg)	Mean	4 4	4 3	4 0	4 5	4 7	7.2	8.7	9 7	5 8	5 7	6 0
	C V.	25 7	24 3	19.7	29 5	27 8	28 8	28 5	29 8	7 9	17 7	22 2
Cd (µg/kg)	Mean	105	86	193	76	102	699	438	176	219	283	123
	C.V	16 7	20 4	20 2	33.4	35 9	11.5	11 6	18 2	33 1	22 5	21 8
Co (µg/kg)	Mean	1214	1094	1431	495	1975	773	1900	652	2064	1870	1163
	C V.	16 5	3 4	24 3	28 1	11 5	24 0	5 9	21 8	4 3	9 1	24 6
Ni (µg/kg)	Mean	983	995	836	749	911	684	348	1712	1144	1451	1373
	C.V.	17 4	19 7	19 3	17 7	16 6	26 4	22 7	15 6	8 4	8 6	18 4

#1 Egyptian clover (Berseem), #2 Wheat, #3 Mustard, #4 Sugarcane, #5 Kinnow orange, #6 Maize, #7 Pecan, #8 Pine, #9 Cotton, #10 Potato, #11 Pea

(*Citrus nobilis* × *Citrus delicosa*), maize (*Zea mays*), pecan (*Carya illinoensis*), pine needles (*Pinus longifolia*), cotton (*Gossypium hirsutum*), potato (*Solanum tuberosum*) and pea (*Pisum sativum*) were oxidized by the following four methods:

Method 1: Dry ashing

Samples (0.50 g) taken in low form silica crucibles were dry-ashed at 500 °C for 4 h in a muffle furnace. The ash was treated with 2 ml of 6 N HCl (glass-distilled), which was evaporated to near dryness at low heat on a hot plate. The residue was then dissolved in 10 ml of 20% aqua regia (HCl + HNO_3 in 3:1 ratio) and transferred to test tubes for settlement of insoluble material. The clear solution was used for analysis without filtration.

Method 2: Wet digestion in HNO_3

Samples (0.5 g) were transferred in 100 ml conical flasks to which 10 ml of glass-distilled conc. HNO_3 was added and kept overnight. Digestion was carried out by low heating of the flasks till about 2-3 ml of the acid was left. Most of the digests were yellow in colour. After cooling, the digests were diluted and made to 10 ml volume with deionized water, and then transferred to test tubes for letting the insoluble material settle down before analysis.

Method 3: Wet digestion in di-acid (HNO_3 + $HClO_4$ in 3:1 ratio)

After adding 10 ml of the acid mixture, wet digestion was carried out as in method 2. The digests obtained were colourless.

Method 4: Wet digestion in di-acid (HNO_3 + $HClO_4$ in 10:1 ratio)

After adding 10 ml of the acid mixture, wet digestion was carried out as in method 2. The digests in this method were also slightly yellow in colour.

The digests prepared by the above methods were analysed for P, K, Ca, Mg, S, Na, Zn, Cu, Fe, Mn, B, Al, As, Pb, Cd, Co and Ni simultaneously on the ICAP-AES of Labtam make (Plasmalab-8440).

The average concentrations of different elements in plant materials and the coefficients of variation for the four methods of oxidation are given in Table 1. These reveal that the variations in concentrations of different elements due to oxidation methods were not consistent for different plant materials. The per cent variations were found to be 4.9–14.6 for P, 4.4–38.6 for K, 1.2–6.2 for Ca, 1.9–7.1 for Mg, 6.2–20.3 for S, 2.6–16.2 for Na, 4.0–8.0 for Zn, 2.4–10.0 for Cu, 2–36 for Fe, 3.9–7.5 for Mn, 3.2–9.8 for B, 4.8–30.0 for Al, 7.7–36.7 for As, 7.9–29.8 for Pb, 11.5–35.9 for Cd, 3.4–28.1 for Co and 8.4–26.4 for Ni. Thus, the elements for which methods of oxidation differed up to 10% of the mean values were Ca, Mg, Zn, Cu, Mn and B. On the other hand, for K, S, Fe and all the trace elements the variations were generally higher than 20%. The differences among the methods for estimation of K were much wider when its concentration in plant materials was greater than 1%.

The concentrations of different elements obtained by the four methods are given in Table 2 for comparison. The data were analysed statistically using randomized block design. It is seen that the differences among methods were significant for all elements except for Co, Fe and Ni. The dry-ashing method resulted in significantly lower values of As, Cd, Pb and S than with wet oxidation methods. The use of 3:1 di-acid (method 3) resulted in significantly lower estimates of B, Ca, Cu, K, Mg, Mn, Na, P and Zn as compared to nitric acid alone (method 2). Although the estimates of some elements (B, Ca, Cu, K and Mg) obtained by using di-acid of 10 : 1 ratio (method 4) were also lower than those obtained by nitric acid alone, the differences were not significant.

The data presented above indicate that oxidation of plant materials by dry-ashing resulted in lower estimates of As, Cd, Pb and S. At high dry-ashing temperature (500°C), sulphur (B.pt. = 445°C) and arsenic (sublimation pt. = 613°C) are expected to be lost through volatilization. Pb and Cd may have formed their oxides which would not dissolve in the acid treatment of ash or that the temperature of ashing in the present study was too low to recover them completely from organic combinations. On the other hand, the use of perchloric acid in the di-acid mixtures has been found to result in lower estimates of a number of elements of which K is the most spectacular. The magnitude of loss depended on the ratio of perchloric acid used in the mixture. It was also observed that the per cent loss during K estimation was much higher in plant materials containing K more than 1%. Zarcinas *et al.*[4] ascribed this loss to the formation of sparingly soluble potassium perchlorate (0.015 g/ml at 25 °C). The loss in estimation of other elements, viz. Ca, Cu, Mg, Mn, Na, P and Zn, may either be due to their adsorption on particles of perchlorates or the formation of their sparingly soluble perchlorates.

The present study clearly indicates that plant materials can be completely oxidized exclusively by nitric acid and, therefore, can be suggested as a suitable medium for estimation of different elements on flame photometer, atomic absorption spectrometer and plasma emission spectrometer since the yellow colour of the

Table 2. Comparison of different methods of oxidation of plant material for elemental analysis (means of 11 plant materials)

Element	Dry-ashing	Nitric acid	Di-acid (3 : 1)	Di-acid (10 : 1)	C.D. (5%)
P (mg/kg)	2807	2922	2679	3024	230
K (mg/kg)	16083	16957	8662	15281	3192
Ca (mg/kg)	10133	10320	9463	10203	308
Mg (mg/kg)	3673	3862	3487	3789	168
S (mg/kg)	2283	3058	2943	3083	218
Na (mg/kg)	631	635	553	638	59
Zn (mg/kg)	46.5	48 4	46 1	49.0	2.4
Cu (mg/kg)	7 65	8 04	7.34	7 87	0.42
Fe (mg/kg)	307	318	286	297	N.S.
Mn (mg/kg)	116	120	108	121	8 8
B (mg/kg)	30 8	31 5	27 4	30 5	2.5
Al (mg/kg)	271	232	228	229	16 2
As (mg/kg)	4 58	6 26	5.62	6 16	1.01
Pb (mg/kg)	3.96	6 57	6.62	6.53	0 99
Cd (μg/kg)	197	217	243	252	36
Co (μg/kg)	1283	1336	1328	1373	N.S.
Nı (μg/kg)	947	1020	1003	1096	N.S.

digest solutions does not interfere in these techniques. The use of nitric acid alone will not only save the expensive perchloric acid but also minimize potential dangers as it is explosive and improve the estimates of different elements. Both dry-ashing and wet digestions with mixtures of nitric and perchloric acids were not suitable for oxidation of plant materials for simultaneous estimation of different elements.

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Introduction of *Puccinia polysora*, Polysora rust of maize in India

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The rust found on certain maize cultivars in Karnataka in 1991 has been determined to be *Puccinia polysora* Underw., Southern or Polysora rust – a rust which caused widespread devastation in many countries of Africa in the fifties. A description of the rust found in India is provided. It is clear that steps should be taken to limit its spread.

WHILE on a visit to Karnataka, there was an opportunity to assess the state of maize cultivation in the Tibetan refugee colonies in Mysore district. Maize was introduced in the southern parts of the state about three decades ago (post-1960 period). What the farmers grow are materials (hybrids/composites) released by the All India Coordinated Maize Improvement Project and no local cultivars are planted. For a long period, the double-cross hybrid, Deccan, dominated the scene. Hybrid Ganga-5, which is a double-top or three-way cross, began its appearance in 1968. However, sorghum downy mildew (*Peronosclerospora sorghi*, maize-sorghum strain) proved very destructive[1] and gave way to newer hybrids such as Deccan 101, Deccan 103 and Deccan 105 and more recently Ganga-11 (which possesses a high level of resistance to downy mildew). It is during the last five years or so that these materials have reached a sizeable proportion of farmers. Meanwhile, under the liberalized seed import policy, hybrids developed in private sector abroad began to be introduced under various tie-ups with indigenous seed companies or independently under the original brand names. Karnataka has a sizeable area under seed production programmes of both public and private sector companies. In this context, disease occurrence and incidence assumes greater significance than is the case where only commercial maize production is prevalent.