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Chromosomal Biotypes of *Andrographis paniculata* in India and Bangladesh

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Use of herbal and natural ancient medicines is gaining a gradual popularity, instead of isolated or synthesized compounds for their side effects and many hazards. But the discoveries of different genotypes, cytotypes, chemical races etc. raised the question of selecting the most suitable ones. *Andrographis paniculata* (Burm. f.) Nees, highly valued throughout the world as a liver toner, has a wide distribution, covering highly varying climatic and edaphic conditions. Naturally the possibility of discovering chromosomally distinguishable gentotypes in different populations and finally graduating such genotypes according to yield of medicinal compounds was worth exploring. Chromosome study offers a quick way of recognizing genotypes, if the chromosomes show detectable structural or numerical difference which may arise by addition, deletion, translocation, transposition, fragmentation, aneuploidy, euploidy etc. (Spurna *et al.* 1981), e.g., *Scilla* (Sato 1942), *Datura fastuosa* (Bhaduri and Sharma 1946), *Lathyrus sativus* (Datta 1955), *Ipomoea quamoclit* (Sharma and Datta 1958), *Lathyrus odoratus* (Sharma and Datta 1959), *Adhatoda vasica* (Datta and Maiti 1968a), *Centella asiatica* (Datta and Maiti 1968b), *Capsicum annuum* (Datta 1968), *Abelmoschus esculentus* (Datta and Naug 1968), *Cajanus cajan* (Datta and Deb 1970), etc.

This investigation on populations of *A. paniculata* revealed 13 such chromosomal biotypes, the analysis of which helped in having a tentative idea of the path of geographical migration of the species in India and Bangladesh.

Materials and methods

Plants were raised from seeds of different populations of *A. paniculata*, collected from different regions of Bangladesh and India (Table 1) in the Experimental-cum-Botanic Garden of the University of Calcutta. The specimens have been cited (Table 1) following Stern and Chamber (1960).

The best configuration of somatic chromosomes was obtained by prefixation treatment of healthy fresh root tips with 0.5% colchicine (Burrell 1939, Sharma and Sharma 1965) at 4°C for four minutes and then at 14–16°C for 2 hours washing in water, fixing in aceto-alcohol (1: 2) for one hour and staining by usual aceto-orcein squash technique. The stain was 2% aceto-orcein : (N)HCl (9: 1) in which the tips were kept for more than 12 hours.

Chromosomes of metaphase plates were drawn using a camera lucida at a constant table magnification (under oil immersion objective and ×15 eye-piece). The drawings were then magnified further by an enlarger, finally to ×4000.

Idiograms were prepared on the basis of the average mean value of five selected cells of five plants of each population. Positions of the primary constrictions was indicated by centromeric index (Kumkum and Sharma 1979), but slightly modified to suit well in the present observation (Table 2). Differentiation of the primary constriction from the secondary was difficult and had to depend upon the microscopic researches of slight bending at the position.

Observation

The somatic chromosome number of all the thirteen population was constant. Analysis of chromosomes of all these biotypes revealed nine chromosome types, represented in the following idiograms (Fig. 1):

Type A: Two constrictions forming three segments, the longest and the shortest being the two terminal ones.

Type B: Two constrictions forming three segments, two adjacent being short and equal.

Type C: Two constrictions forming three segments, two adjacent equal and longer than the third.

Type D: Three almost equal segments, formed by two constrictions.

Type E: Similar to D but the middle segment shorter.

Table 1. Collection number, locality and population types of *Andrographis paniculata*

Population type	Source, collection	Locality
I	CUh; Roy 701, CU	Ballygunge (Calcutta)
II	CUh; Roy 702, CU	Hooghly (W. B.)
III	CUh; Roy 703, CU	Howrah (W. B.)
IV	CUh; Roy 704, CU	Sundarban (W. B.)
V	CUh; Roy 705, CU	Sonarpur (W. B.)
VI	CUh; Roy 706, CU	Trivandrum (Kerala)
VII	CUh; Roy 707, CU	Lucknow (U. P.)
VIII	CUh; Roy 708, CU	Goa (South India)
IX	CUh; Roy 709, CU	Jessore (Bangladesh)
X	CUh; Roy 710, CU	Rajshahi (Bangladesh)
XI	CUh; Roy 711, CU	Chittagong (Bangladesh)
XII	CUh; Roy 712, CU	Ranchi (Bihar)
XIII	CUh; Roy 713, CU	Hyderabad (A. P.)

Table 2. Determination of the centromeric position by the centromeric index or F% (F%=short arm length/total length of a particular chromosome $\times 100$)

F%	50	25–49.9	12.5–24.9
Centromeric position	Median (M)	Submedian (SM)	Subterminal (ST)

Type F: The middle segment of the three (formed by two constrictions) longer.

Type G: A single median constriction forming two equal arms.

Type H: A submedian constriction forming two unequal arms (Table 2).

Type I: A subterminal constriction forming two very unequal arms (Table 2).

The range of chromosome length differed in the different cytotypes (Fig. 2 for total diploid chromosome length). Difference in number of chromosomes having a secondary constriction, remarkable difference of positions of primary and secondary constrictions in chromosome types helped in distinction of cytotypes (Figs. 3a to 15b).

Comparison of details of the individual chromosome of each cytotype (Table 3) were useful in tracing relations of the cytotypes also.

With the object of following the course of evolution of cytotypes, in relation to soil and latitude, we collected the following informations of the habitat of each type (Raghavan and Kachroo 1963, Rashid 1977).

Type I: Ganga alluvial soil, deficient in nitrogen, in Ballygunge (South Calcutta). Lat. 22. 2N.

Type II: Sandy alluvial soil with high K_2O, P_2O_5, low CaO (below 0.6%) and nitrogen (below 0.2%) in Hooghly of West Bengal. Lat. 22.5N.

Type III: Alluvial soil with sticky heavy loam in Howrah of West Bengal. Lat. 22.3N.

Type IV: Saline and greyish black soil in Sundarban area of West Bengal. Lat. 21.5N.

Type V: Ganga alluvial soil, deficient in N_2, in Sonarpur, 24-Parganas of West Bengal. Lat. 22.0N.

Type VI: Rocky coastal soil of Trivandrum in Kerala. Lat. 8.29N.

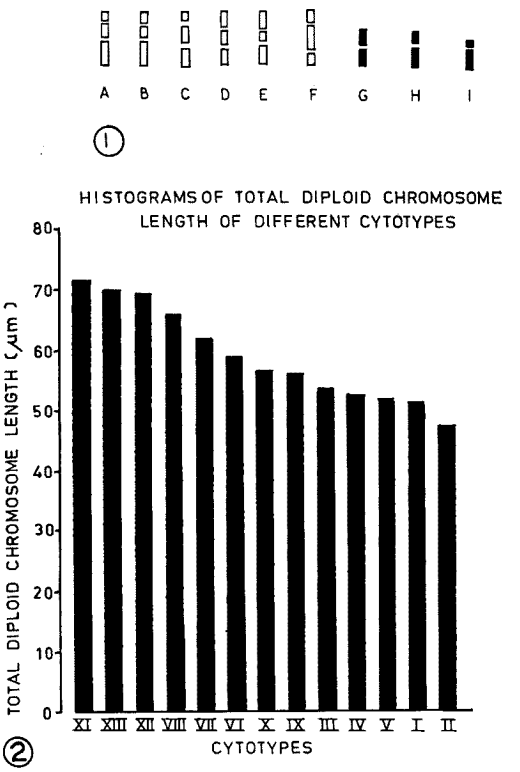
Type VII: Calcareous soil of Lucknow. Lat. 26.5N.

Type VIII: Sandy sea shore of Goa. Lat. 20.5N.

Type IX: Ganga type alluvial soil of Jessore, Bangladesh. Lat. 23.1 N.

Type X: Sandy Barind track of Rajshahi, Bangladesh. Lat. 24.2N.

Type XI: Red soil of hilly Chittagong,

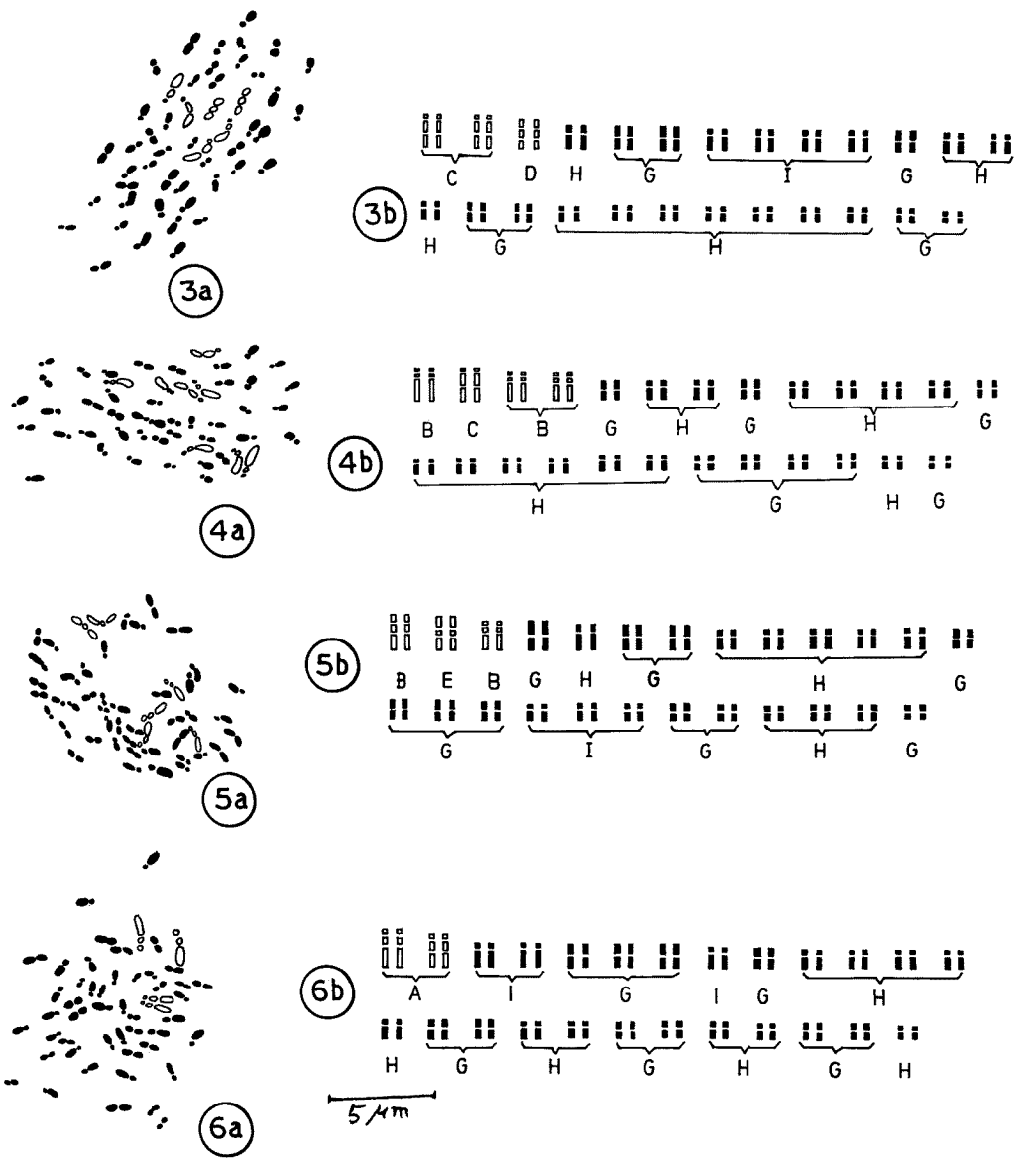


Figs. 1–2. 1(A-I), idiogram representing the types of chromosomes, covering all the cytotypes of *Andrographis paniculata*. 2, histogram showing the total diploid chromosome length of different cytotypes of *Andrographis paniculata*.

Table 3. Karyomorphological detalis of the thirteen population types of *Andrographis paniculata*

Population types studied	Karyotype formula (diploid)	Chromosomes with secondary constrictions		Chromosomes with 40–50% F	Length range (individual chromosome) (μm)	Total diploid chromosome length (μm)
		Number	Types number of complements			
I	4C 2D 14G 22H 8I	SIx	4C 2D	18	0.50–1.75	51.66
II	6B 2C 16G 26H	Eight	6B 2C	18	0.62–1.75	47.46
III	4B 2E 20G 18H 6I	Six	4B 2E	24	0.74–1.88	53.84
IV	4A 20G 20H 6I	Four	4A	24	0.62–2.00	52.62
V	4B 2E 12G 16H 16I	Six	4B 2E	14	0.74–1.75	52.28
VI	2B 2C 12G 30H 4I	Four	2B 2C	26	0.93–2.25	59.12
VII	4A 14G 14H 18I	Four	4A	20	0.73–1.88	61.90
VIII	4A 2C 6G 26H 12I	Six	4A 2C	14	0.81–2.02	65.52
IX	6B 2E 2F 16G 24H	Ten	6B 2E 2F	28	0.74–2.00	56.52
X	2A 2B 2E 2F 10G 32H	Eight	2A 2B 2E 2F	14	0.75–1.88	56.68
XI	4A 18G 24H 4I	Four	4A	32	1.00–2.50	71.32
XII	2A 4C 10G 24H 10I	Six	2A 4C	14	1.00–2.50	69.46
XIII	2A 2E 16G 20H 10I	Four	2A 2E	26	1.00–2.00	70.00

Bangladesh, Lat. 21.0N.
Type XII: Reddish yellow loamy sandy soil of Ranchi, Bihar. Lat. 23.2N.
Type XIII: Red sandy soil, deficient in organic matter, of Hyderabad. Lat. 17.2N.

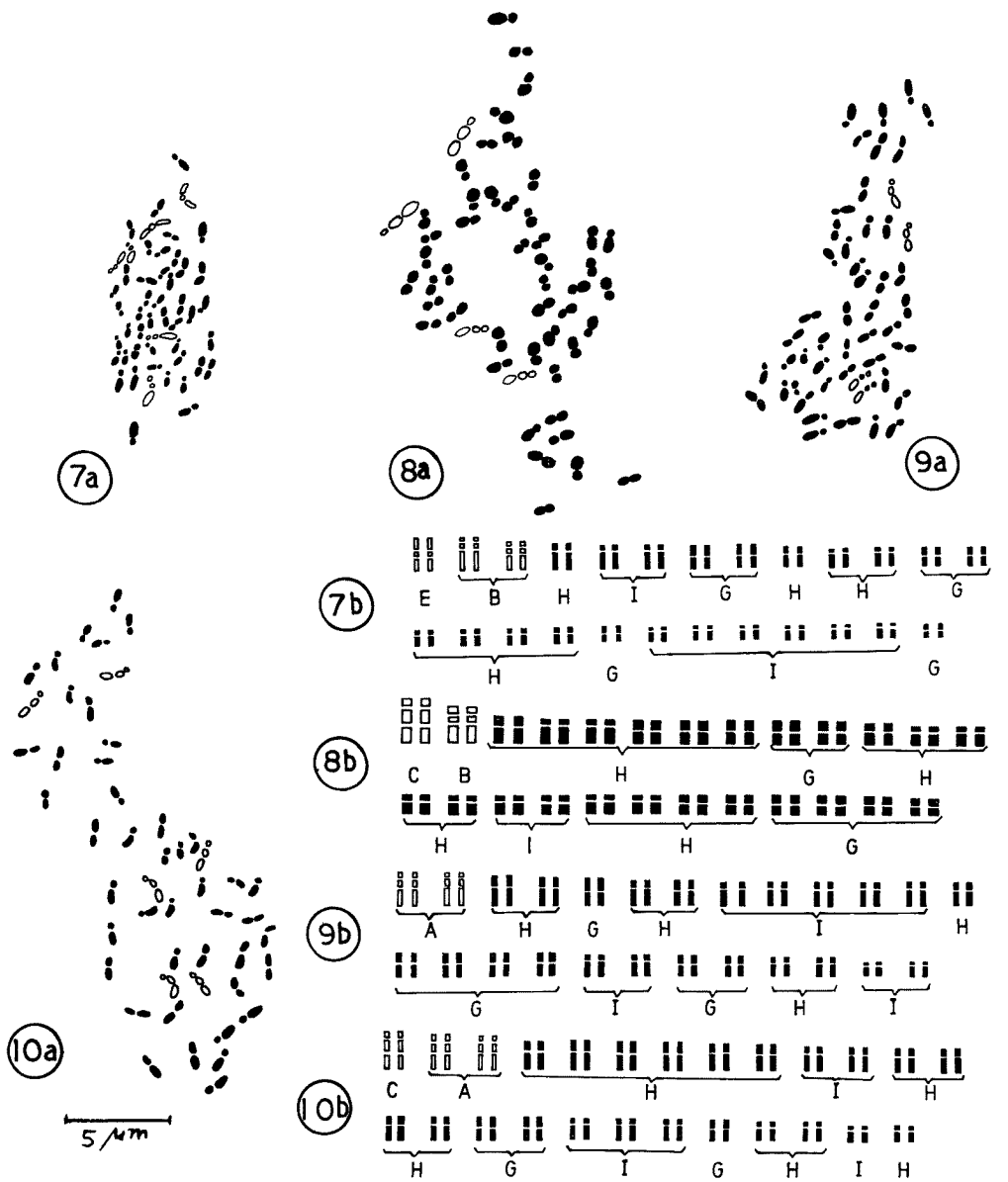


Figs. 3–6. Camera lucida drawings of mitotic metaphase plate with 2n=50 chromosomes (a) and idiograms (b) of cytotypes I, II, III and IV respectively.

Discussion

The chromosome number of *A. paniculata* as found here (2n=50), confirmed the previous reports (Raghavan 1957, Ellis 1962, Mitra and Datta 1967, Fedorov 1969, Datta and Maiti 1970). Inspite of such constancy and homogeneity of chromosome complements in all cytotypes collected from different parts of the Indian subcontinent, from Goa (India) to Chittagong

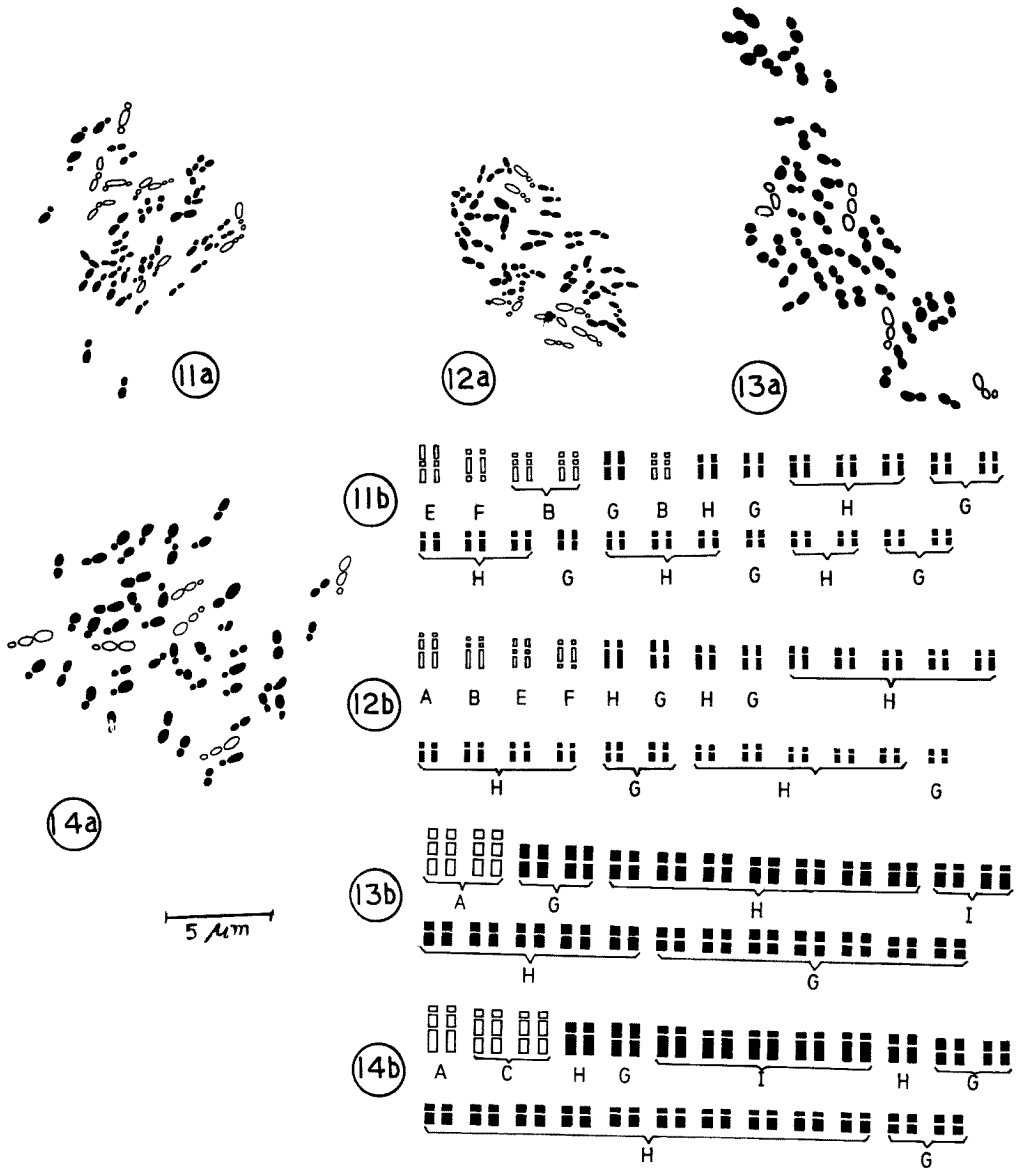
(Bangladesh), structural hybridity, deletion, inversion and many other changes made these types quite distinct. Chromosome morphology was quite useful for selecting the suitable biotypes for further investigation on the pharmacognostic potentialities.



Figs. 7–10. Camera lucida drawings of mitotic metaphase plate with 2n = 50 chromosomes (a) and idiograms (b) of cytotypes V, VI, VII and VIII respectively.

Investigations of populaltions of the same species from different regions of the world often show cytological races (Harada 1956, Larsen 1963). Such collection even within a small area differ in chromosome constituents (Mishra 1966, 1971, Sharma and Chatterjee 1967, Datta 1968, 1971, 1975, Datta and Maiti 1968a, 1968b, Singh and Sharma 1981a, 1981b).

According to the established ideas on evolution the primitive characters of chromosomes are: a) high frequency of median constriction, b) lowest frequency of secondary constriction and c) longest chromosomes.



Figs. 11–14. Camera lucida drawings of mitotic metaphase plate with 2n=50 chromosomes (a) and idiograms (b) of cytotypes IX, X, XI and XII respectively.

Among the types of chromosomes with secondary constrictions, type A appeared as most primitive (as it occurred in all long chromosome cytotypes) which might produce by inversion of the arm having secondary constriction, to type E in one direction. 'A' can also produce 'C' by elimination of one part of the longest arm, 'D' or 'B' by addition of a segment to the shortest terminal one. 'D' can produce 'F' by deletion of the parts at the two end segments (Fig. 16).

On the basis of the structural details of chromosome complements, the cytotypes could be arranged in a tentative plan of evolution (Fig. 17). Type XI with 4 secondary constrictions of chromosomes (4A) and 32 median primary constrictions and more than 70 μm total chromo-

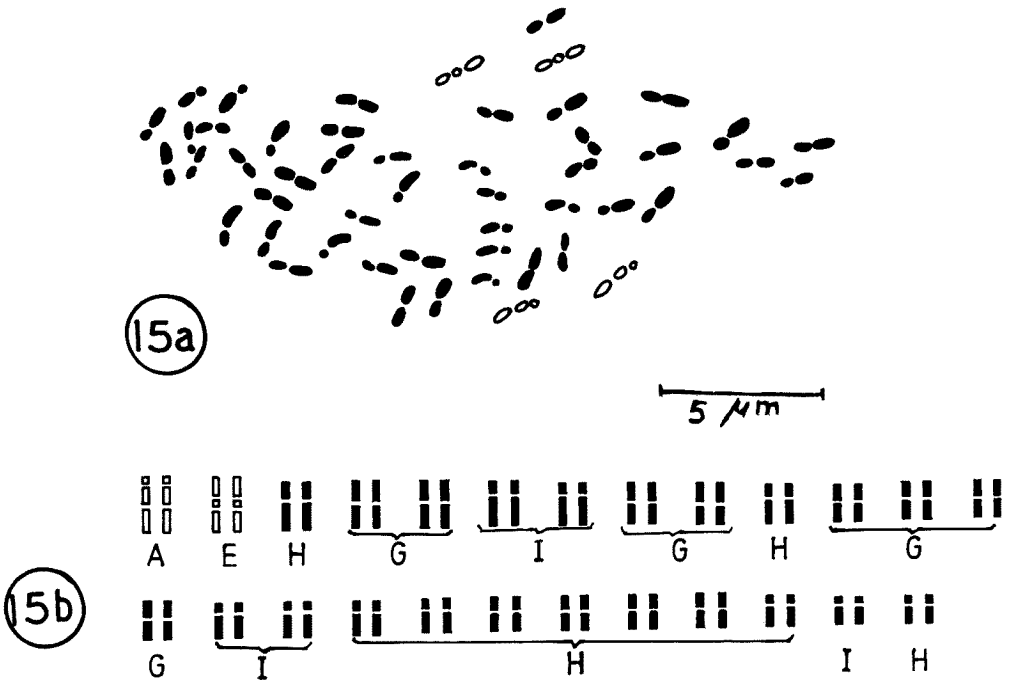


Fig. 15. Camera lucida drawing of mitotic metaphase plate with 2n=50 chromosomes (a) and idiogram (b) of cytotype XIII.

some length appeared as the most primitive type, occurring in the red soil of hilly Chittagong. Type X evolved much in chromosome structure (eight secondary constrictions) but had comparatively long chromosomes, grew well in sandy Barind tract of Rajshahi. Another line produced the type IV, having chromosomes without much structural specialization (over XI), but with much reduction of length, evolved in saline soil of Sundarbans. This probably gave the type IX having highly specialized secondarily constricted chromosomes (10 in number), not found in other cytotypes, growing on the Ganga type of alluvial soil of Jessore. From IV evolved III and V, having much similarity in chromosome morphology, which grew on alluvial soil with heavy loam of Howrah and on Ganga alluvial soil of Sonarpur (deficient in nitrogen) respectively. From III probably evolved the types I and

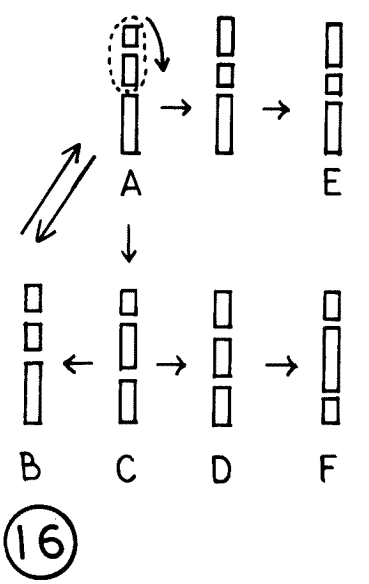


Fig. 16. A tentative plan of evolution of chromosomes bearing secondary constrictions of *Andrographis paniculata*.

II (with further specialized chromosomes), I at South Calcutta (soil similar to Sonarpur) and II in Hooghly (sandy alluvial soil with high K, low P, N and Ca).

Probably another centre (primary or secondary) of distribution was somewhere in western or middle parts of India, from which evolved XIII (with less specialized and long chromosomes)

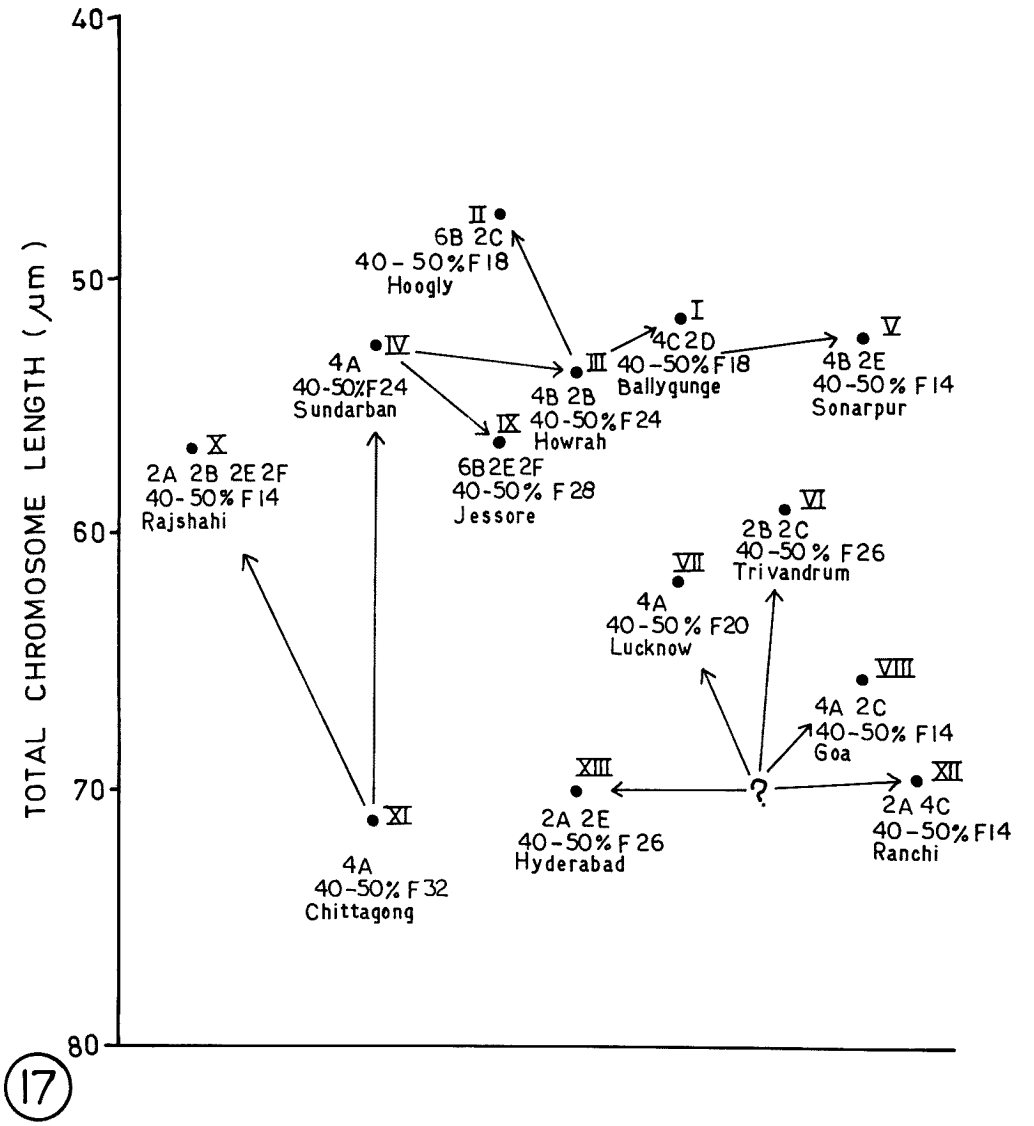


Fig. 17. Evolutionary sequence of different cytotypes of *Andrographis paniculata* on the basis of karyomorphology.

on the red sandy soil (deficient in organic matter) in Hyderabad. VII (whith chromosomes less specialized in structures, but comparatively shorter in length), evolved in calcareous soil of Lucknow. Further specialized were XII, VIII and VI chromosomes including 'C' and 'A' or 'B', evolved in Ranchi (reddish yellow loamy sand), Goa (sandy sea-shore) and Trivandrum (rocky coastal soil), respectively.

Evolution of chromosomes, studied in a species, particularly in a medicinal one was highly significant, because these genetic biomasses, adapting in different edaphic or climatic conditions must have gene controlled variations of enzymatic and hormonal system, distinct synthesis patterns of secondary metabolites. This should have a relation to the types of compounds regarded as active principles, their balancing biochemical network and the efficiencies.

Summary

Andrographis paniculata an well known indigenous drug plant of the whole Indian subcontinent, attracted attention for the diversity of ecological conditions of its abodes. Discovery of genetically distinct types, if chemically distinct, would certainly help in the selection of best strains. The simplest and quickest way of detecting genetical difference is the cytological techniques and study of chromosome morphology. Of course these cytological methods could detect only such genotypes which had structural alterations of chromosomes, but not pure genic changes or point mutations.

The chromosomal biotypes differed only in structure suggesting the occurrence of cytotypes within this species, brought about by translocation, deletion etc. These chromosomal changes probably made the cytotypes best suited to their environments, where they normally grew.

As in reports, many such cytotyes of other taxa, differ in micro- and macromorphological characters and chemical contents, these cytotypes were also suitable for analyticl study of quantitative anatomy, morphology and of the active principle available in this species.

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