# Chromosomal Biotypes of *Adhatoda vasica* Nees. Growing in the Eastern Part of India

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*Received August 12, 1967*

## Introduction

*Adhatoda vasica* Nees. (Acanthaceae) is a very well known indigenous drug of India. In Sanskrit literature, it occurs in the name of *Vasaka* and has been prescribed in many Ayurvedic Texts (Chopra, Nayar and Chopra 1956, Biswas and Ghose 1950). Leaves and roots are used in cough, chronic bronchitis, asthma and phthisis. Leaves are also used in rheumatism and as an insecticide. Flowers, leaves and roots are used as antispasmodic. The leaves contain the alkaloid *vasicine* (to which the therapeutic properties are attributed) and an essential oil. The plant, wild or cultivated, grows throughout the plains of the Indian subcontinent from the Punjab and Assam to Ceylon (Clarke 1885).

Eastern part of India consists of different climatic zones such as gangetic plain, gangetic delta, temperate zone, tropical submontane zone, eastern coastal region, etc. This part covers alluvial soil, old alluvial or red soil, lateritic soil, saline and deltaic soil, hill soil, calcareous soil, red sandy soil and red loams (vide Ghose *et al.* 1960). It is naturally expected that a particular plant species growing in such different climatic and edaphic conditions must undergo genetical changes through the process of evolution for adapting best in the environments. In this way the different ecotypes differing in genetical constitution generally evolved in nature. These genetically diverging plant types though belong to the same species must be provided with some distinct structural or biochemical characters. If these genetical changes are only genic, they are unquestionably beyond the resolving power of microscopes. But if the changes involve structural change of chromosomes, they can be recognised easily by the modern techniques evolved (Sharma 1956). Naturally, therefore, different cytotypes are expected in eastern India and they may be linked with certain economically important characters such as the quantity and nature of alkaloids.

Such a survey of cytotypes may also reveal the place of origin and the pathway of the distribution of the species concerned and the process of evolution by which they have adapted after migration, in their new abodes.

With these objects in view, samples of plants of the species *Adhatoda vasica* have been collected from different parts of eastern India and the karyotype has been analysed. This preliminary survey reveals six chromosomal biotypes.

## Materials and methods

*Adhatoda vasica*, collected from different regions of eastern India, growing under different ecological conditions were grown in the University College garden and maintained for cytological investigation.

Somatic chromosomes were studied from root-tips mainly. Several techniques were tried in order to obtain best structural configurations of chromosomes. The techniques mainly involved a pre-fixation of freshly cut root tips at low temp. 12-16°C in 1) Aesculin (Sharma and Sarkar 1955), 2) Paradichlorobenzene-saturated solution (Sharma and Mookherjea 1955), 3) Colchicine—0.5% solution (Sharma and Sarkar 1963), and 4) Coumarin (Sharma and Bal 1953), for varying periods of times: 1, 1½, 2, 2½ and 3 hours. The roots after these

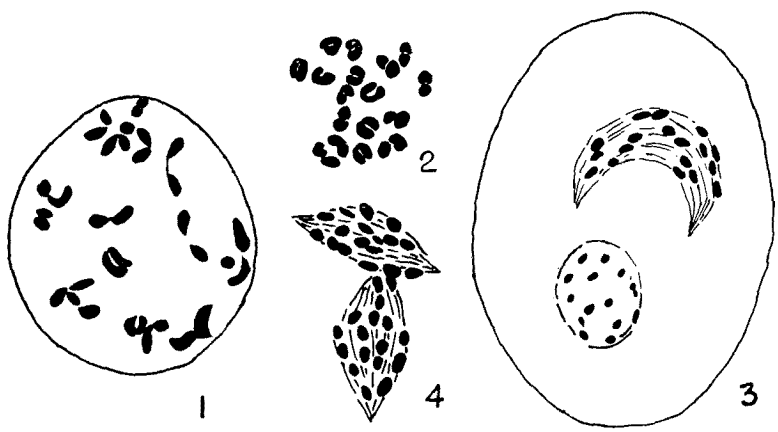
pre-treatment were kept in a mixture of acetic acid and alcohol (1:2) for 30 to 45 min for clearance and then were kept for 10 minutes in 45% acetic acid. This is followed by hydrolysing and staining the root tips by heating for 6 seconds in a mixture of 2% aceto-orcein solution and normal hydrochloric acid in the proportion of 9:1 and then smearing in 1% aceto-orcein (Tjio and Levan 1950, Sharma and Ghosh 1950, Sharma and Bal 1953, Sharma and Mukherjea 1955). The best configuration was obtained by pre-fixation with colchicine for three hours and then following the procedure mentioned above.

Meiotic behavior of chromosomes was studied by smearing with propiono-carmine (1% carmine in 45% propionic acid).

Observations were made under oil immersion with 100/1.3 o.e. apochromatic objective and 12.5 eye-piece. Figures were drawn with camera lucida on a drawing board at a magnification of 2500 approximately.

## Observations

*Meiosis.* Meiotic behaviour of the type (Type IV, Calcutta) has been carefully studied. Both, diakinesis and metaphase plates with normal 34 bivalents (Figs. 1 and 2) have been generally noticed. A few cases of disharmony in movement at anaphase II (Figs. 3 and 4) have also been encountered.




Figs. 1-4. *A. vasica*, Type IV: meiotic stages. ×2000.

*Mitosis.* In the present investigation the normal chromosome number in different collections was found to vary from 34 to 50. Of these, the most frequently occurring number is 2n=34. The chromosome number of the square variety is 2n=50 but variation plates with 46 and 34 chromosomes have also been encountered in this cytotype.

*Adhatoda vasica* collected from different localities did not show any variation in chromosome number except the plant specimen collected from the saline area of Diamond Harbour, West Bengal and a square-stem type. The normal chromosome numbers (2n) of different collections for each variety are tabulated (Table 1).

The chromosomes of different cytotypes though represent gross similarity have got minute structural differences. The total amount of chromatin matter is slightly different in different varieties (Fig. 5).

Size differences of chromosomes (ranging from 0.825-4.68 $\mu$) are not well marked but lengths of chromosomes are more or less in a graded series in

Table 1.

| Types | 2n | Types | 2n |
|---|---|---|---|
| Type I (Orissa) | 34 | Type IV (Calcutta) | 34 |
| Type II (Diamond Harbour) | 40 | Type V (Tripura) | 34 |
| Type III (Lucknow) | 34 | Type VI (Square, Calcutta) | 50, 46, 34 |

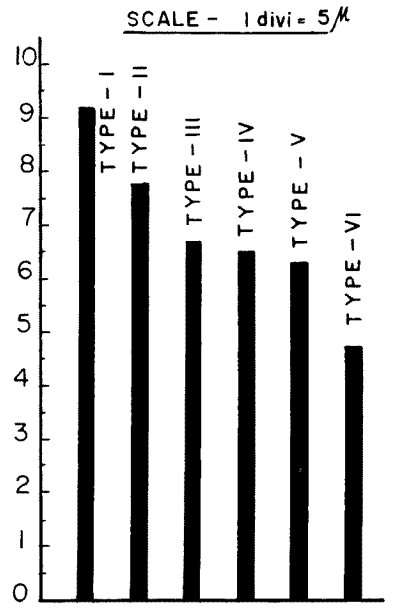



Fig. 5. Histogram showing the total haploid chromatin length of the different cytotypes of *A. vasica*.

each variety. Lengths of chromosomes are not much distinguishable from one another. So types are not well distinguishable by lengths but distinguishable in structural configuration of chromosomes. The primary constrictions are median, submedian and subterminal. The number of chromosomes with secondary constrictions, some of which bear a satellite, generally vary from 2 to 6 in different cytotypes. The Table 2 will indicate these features.

The chromosomes of different cytotypes of *A. vasica* can be classified into the following types (Fig. 6):

Type A (1.98–4.68$\mu$ approx.): A medium sized chromosome type with three constrictions, one primary and two secondary, forming three small more or less equal and one large segments.

Type B (1.93–4.40$\mu$ approx.): A medium sized chromosome type with two constrictions (one primary and one secondary), one submedian and the other nearly subterminal.

Type C (2.20–3.54$\mu$ approx.): A medium sized chromosome with two constrictions (one primary and one secondary). one nearly median and the other nearly subterminal forming one large and two nearly equal small segments.

Type D (2.75–3.30 $\mu$ approx.): A medium sized chromosome type with two constrictions, one primary and one secondary, producing two equal large adjacent segments and a small terminal one.

Type E (3.30$\mu$ approx.): A medium sized chromosome type with two constrictions, one primary and one secondary, dividing the chromosome into three equal segments.

Type F (1.65–4.78$\mu$ appox.): A medium sized chromosome type with two constrictions one primary and one secondary, forming one large middle segment and two smaller equal terminal segments.

Type G (1.18–5.64$\mu$ approx.): A medium sized chromosome type with

a nearly median primary constriction.

Type H (1.65–2.34$\mu$ approx.) A medium sized chromosome type with a submedian primary constriction.

Type I (1.825–1.52$\mu$ approx.): A short chromosome type having a nearly median primary constriction.

Type J (0.825–1.10$\mu$ approx.): A short chromosome type with a submedian primary constriction.

Table 2.

| Cytotypes | Total chromatic length (haploid) in $\mu$ | Primary constrictions | Number of secondary cons. in 2n complement |
|---|---|---|---|
| Type I. (collected from Orissa) | 45.75 | Median, submedian and subterminal | Twelve |
| Type II. (collected from Diamond Harbour) | 38.99 | Median and submedian | Eight |
| Type III. (collected from Lucknow) | 33.69 | Median and submedian | Four |
| Type IV. (collected from Calcutta) | 32.55 | Median and submedian | Six |
| Type V. (collected from Tripura) | 31.45 | Median, submedian and subterminal | Four |
| Type VI. (square stem, collected from Calcutta) | 23.34 | Median and submedian | Six |

On the basis of the above description of chromosome types, the karyotypes of the different species may be represented in the following way:

1. Type I (collected from Orissa) (2n=34)

Size difference among the chromosomes ranges from 1.67–4.68$\mu$. The karyotypes of this cytotype may be represented as 2A, 4B, 2C, 2D, 2E, 10G, 8H, 4J. Of these, twelve chromosomes bear secondary constrictions, two of which bear satellites (Figs. 7 and 8). Details of karyotype analysis reveal the following chromosome types.

i) Fifteen pairs of medium sized chromosomes, one of A, two of B, one of C, one of D, one of E, five of G and four of H types.

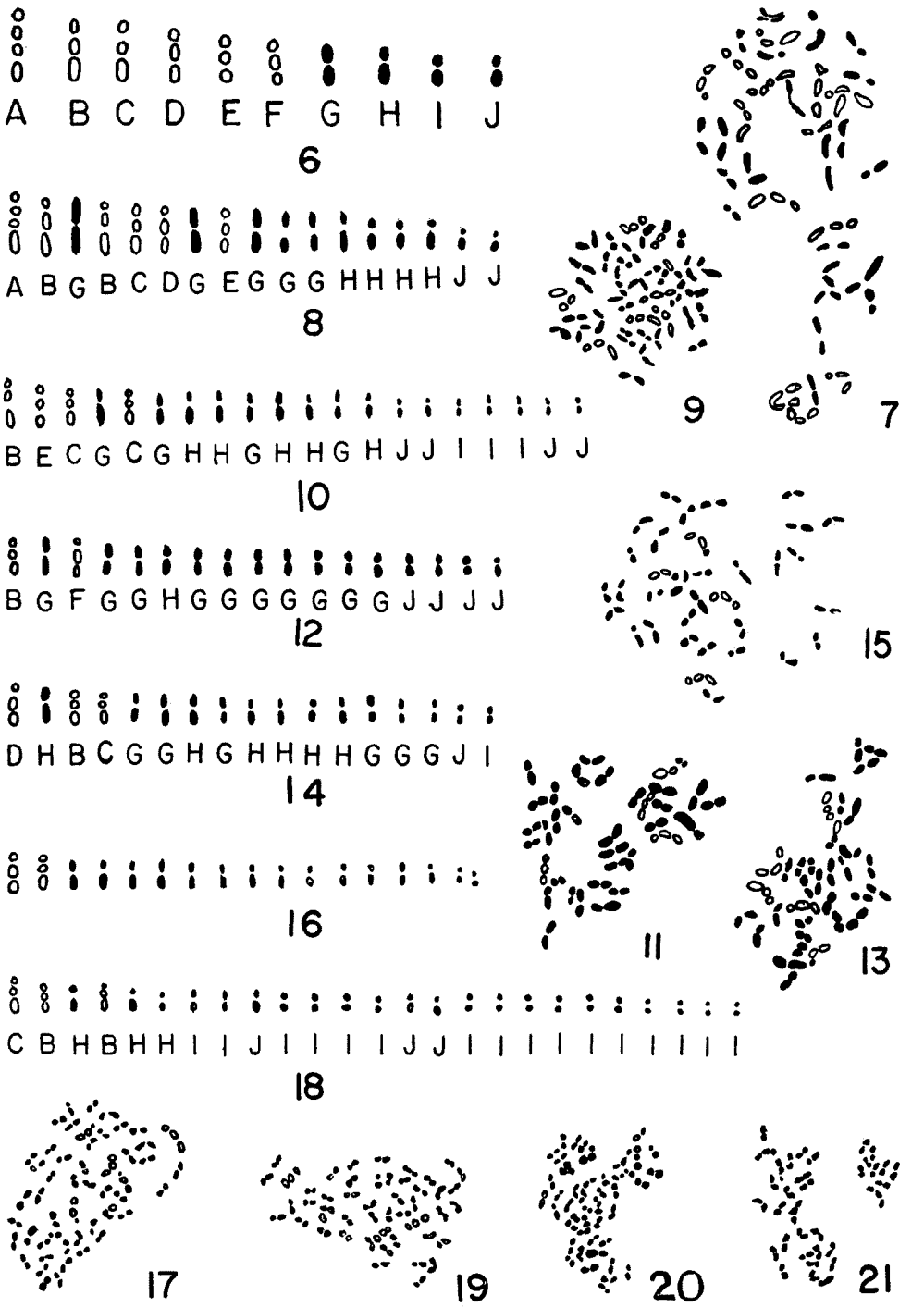
ii) Two pairs of short sized chromosomes of J type.

2. Type II (collected from Diamond Harbour, West Bengal) (2n=40)

The size ranges from 0.98$\mu$–9.58$\mu$. The karyotype may be represented as 2B, 4C, 2E, 8G, 10H, 6I, 8J types. Of these chromosomes, eight chromosomes are seen to bear secondary constrictions (Figs. 9 and 10). Detailed

analysis of karyotype reveals the following types of chromosomes.

i) Thirteen pairs of medium sized chromosomes, one of B, two of C,




Figs. 6-21. 6, diagram showing the different types of chromosomes found in the present study. 7-18, somatic plates and idiograms given alternately, 7 and 8, Type I; 9 and 10, Type II; 11 and 12, Type III; 13 and 14, Type IV; 15 and 16, Type V; 17 and 18, Type VI. 19-21, variation plates of Type VI; showing 46, 48 and 34 chromosome respectively.

one of E, four of G and five of H types.

ii) Seven pairs of short chromosomes, three of I and four of J types.

3. Type III (collected from Lucknow, U.P) (2n=34)

Size difference ranges from 1.10-2.61$\mu$. The karyotype may be represented as 2B, 2F, 20G, 2H, 6I, 2J. Of these four chromosomes are seen to bear secondary constrictions (Figs. 11 and 12). Karyotype analysis of the cytotype may be represented as:

i) Thirteen pairs of medium sized chromosomes, one of B, one of F, ten of G and one of H types.

ii) Four pairs of short chromosomes, three of I and one of J types.

4. Type IV (collected from Calcutta) (2n=34)

Size of chromosomes ranges from 1.10-3.16$\mu$. The karyotype may be represented as 2B, 2C, 2D, 12G, 12H, 4J. Of these six chromosomes are found to bear secondary constrictions (Figs. 13 and 14). The karyotype analysis reveal the following types:

i) Fifteen pairs of medium sized chromosomes, one of B, one of C, one of D, six of G and six of H types.

ii) Two of short chromosomes of J type.

5. Type V (collected from Tripura) (2n=34)

The size ranges from 1.10-2.85$\mu$. The karyotype may be represented as 2B, 2D, 4G, 12H, 4I, 10J type. Of these four chromosome are seen to possess secondary constrictions (Figs. 15 and 16). A detailed study of the karyotype shows the presence of the following types of chromosomes:

i) Ten pairs of medium sized chromosomes, one of B, one of D, two of G and six of H types.

ii) Seven pairs of short chromosomes, two of I and five of J types.

6. Type VI (Square stem type—collected from a village near Calcutta (2n=50)

Size difference is not pronounced, which range from 0.82-2.58$\mu$. The karyotype may be represented as 4B, 2C, 6H, 32I, 6J. Of these six chromosomes are found to bear secondary constrictions (Figs. 17 and 18). Detailed study reveals the following types of chromosomes:

i) Six pairs of medium chromosomes, two of B, one of C and three of H types.

ii) Nineteen pairs of short chromosomes, sixteen of I and three of J types.

In addition to the normal complement (2n=50) described above, somatic nuclei with varying number of chromosomes such as forty-six, forty-eight and thirty-four have been recorded (Figs. 19, 20 and 21).

## Discussion

1. Role of structural change of chromosomes in adaptation to different ecological conditions

Six cytotypes have been come across in the present cytological investigation of *Adhatoda vasica* collected from different regions of the eastern part of India. The type I (Figs. 7 and 8) was found in plants growing near Berhampore of Orissa where the soil is a mixture of sandy and red or old alluvial type. Climatically it belongs to eastern coastal region of India. Type II (Figs. 9 and 10) has been found in the deltoid zone of West Bengal where the soil is saline. Type III (Figs. 11 and 12) has been encountered in plants growing in the calcareous soil of Uttarpradesh (near Lucknow). Type IV (Figs. 13 and 14) occurs in the plants adapted in Calcutta and its neighbourhood which belongs to the gangetic plain having alluvial soil in general. Type V (Figs. 15 and 16) has been discovered in this survey of cytotypes in the State of Tripura having the forest and hilly soil with comparatively humid condition and high proportion of iron in soil. Type VI (Figs. 17 and 18) seems to be a different variety of *A. vasica* which is morphologically distinct and cultivated as a garden plant in Calcutta and its suburbs.

These types show homogeneity in cytological characters. All the types have got median to submedian primary constrictions, 2-6 pairs of secondarily constricted chromosomes and graded variation of chromosome length (within 0.825 and 4.68$\mu$ in most of the types).

In spite of this similarity among all the types, each type can easily be distinguished by position and number of primary and secondary constrictions of chromosomes. The following classification will distinguish the cytotypes:

I. 6 pairs of secondary constriction-bearing chromosomes with one pair of supernumerary constriction.....Type I.
II. 4 pairs of chromosomes bearing secondary constrictions with no supernumerary constriction.....Type II.
III. 3 pairs secondary constriction-bearing chromosomes.
  A. Nucleolar chromosomes-2B, 2C and 2D.....Type IV.
  B. Nucleolar chromosomes-4B and 2C.....Type VI.
IV. 2 pairs of secondary constriction-bearing chromosomes.
  A. Nucleolar chromosomes B and F.....Type III.
  B. Nucleolar chromosomes-B and D.....Type V.

Structural change of chromosomes has no doubt played an important role in evolution, which is indicated by the presence of differences in minute karyotypic details in different cytotypes.

On the basis of the total chromatin length it seems that the type I is the most primitive amongst the cytotypes studied here. Type II seems to be highly evolved because there is an increase in number of chromosomes. There is a decrease in total chromatin length but the reduction in number of secondary constrictions might be brought about by deletion. Fragmentation of

chromosomes involving centromeric regions and subsequent translocation may also increase the number of chromosomes and decrease the number of secondary constrictions. Types III, IV and V, more or less of the same chromatin length and of the same chromosome number, show some grades of evolution. Types III and V possess two chromosome pairs with secondary constrictions. Type IV bearing three pairs of secondary constriction seems to be more highly evolved if the increase in number of secondary constrictions is regarded as a criterion of advancement (Sharma 1964). The type VI is no doubt a highly advanced type where the chromosome number has been increased upto fifty in the somatic cell and the chromosomes have the most reduced length. The total chromatin length is also less than any other type examined. Number of nucleolar chromosomes is only three. This decrease in length of chromosomes and number of secondary constrictions and increase in number of chromosomes might easily be brought about by fragmentation and deletion, the possibility of which has been hinted at by Sharma and Datta (1959).

2. The role of numerical change of chromosomes

In addition to the details of chromosomes morphology, they also differ in chromosome number. Types I, III, IV and V have got thirty-four chromosomes in their somatic complements but the type II, though morphologically not much distinguishable from Type I, III, IV and V excepting by the larger leaf size, has got forty chromosomes in the diploid set. The type VI is found to possess fifty chromosomes in most of the clear somatic plates. Of course a few variation plates with thirty-four, forty-six and forty-eight chromosomes were observed.

Addition and multiplication of chromosomes in the complement not only make a proportional change in genes carried by them but also causes proportionate increase in cell size and change in growth and differentiation (Jorgensen 1928, Sinnott, Houghtaling and Blakeslee 1934, Schkwarnikov 1934, Pandey 1956, Brandley 1954, Kupila 1958, and so on). In the present investigation, *A. vasica* having slight morphological modification is found to be accompanied by numerical change in chromosomes.

Though in most of the collections, the number is thirty-four which corroborates the report of Mukherji (1952), the types II and VI are characterised by larger number of chromosomes. Grant (1955, as referred in Darlington and Wylie 1955) reports the occurrence of 56 chromosomes in this species. From these observations it is evident that numerical change of chromosomes has certainly played some role in the evolution within the species. Critical survey may reveal many polyploid and aneuploid types evolved in different environmental conditions.

3. Role of somatic variation in the evolution of species

The role of somatic variation in the speciation has been emphasised by various authors (Witsch and Fleugel 1952, Babcock 1942, Babcock *et al.* 1937,

Duncan 1945, Vaarama 1949, Sachs 1952, Snoad 1954, Sharma and Bal 1954, Sharma and Das 1954, Sharma and Bhattacharyya 1956, Mookherjea 1956, Sharma and Datta 1960). Somatic change in chromosome number has been encountered in the type VI of *A. vasica*. This suggests the possibility that numerical change in chromosomes might be brought about by this process in different plants.

4. Economic implication of the occurrence of different cytotypes

The morphological difference of chromosomes is no doubt an indication of genetical difference amongst the cytotypes studied. The formation of alkaloids and different active principles are also related to gene-controlled mechanisms. Though the ecological condition has an influence on the formation of alkaloids (Wakhloo 1963), variation in composition of different groups of alkaloids and resins in the roots of *Rauvolfia serpentina* collected from different parts of India (Bal and Gupta 1955) is not expected to be purely an environmental effect. The different chromosomal biotypes of *Adhatoda vasica* are, therefore, expected to differ in the quantity and composition of chemical constituents. In addition, variation is also expected in the details of structures such as quantitative anatomical and pharmacognostic characters.

## Summary

*Adhatoda vasica* Nees, an well known indigenous drug plant of India, was collected from different regions of the eastern part of India. These places of collection differ as regards edaphic and climatic conditions. Six chromosomal biotypes have been encountered when studying these collections.

The chromosomal biotypes differ in structure and sometimes in number of chromosomes, which suggests that there has been an evolution of cytotypes within this species, brought about by translocation, fragmentation, deletion etc. as well as by polyploidy or aneuploidy. These chromosomal changes have probably made the cytotypes best suited to their natural environments, where they normally grow.

It is expected that these chromosomal differences are also related to the quantitative anatomical characters and to the quantity and quality of chemical substances available in this species.

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