Short communication

Karyotype analysis in *Saraca asoca* (Roxb.) de Wilde

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Abstract

A study on karyomorphological analysis in *Saraca asoca* was carried out in College of Forestry, Thrissur, Kerala. The study revealed the presence of 34 chromosomes in the somatic cells of *Saraca asoca* for the first time, in contrast to the earlier report of 2n = 24. Pre-treating with 8-hydroxyquinoline and fixing it in Carnoys II and using the stain acetocarmine gave the best cytological preparation.

Key words : *Saraca asoca*, Chromosome number, Karyomorphology, Ideogram

Saraca asoca (Roxb.) de Wilde, commonly known as ashoka is an indigenous small evergreen tree of Fabaceae family, attaining a height of 6-9 m. Ashoka, is popularly known for its use in treating gynecological disorder, biliousness, dyspepsia, dysentery, colic and piles (Warrier et al., 1996). Bark, flower, leaves, roots and seeds of ashoka are used as medicine. The indiscriminate use and unscientific extraction of ashoka bark has lead to acute scarcity of the genuine raw drug and this in turn has lead to cost escalation and widespread adulteration or substitution of the drug. This tree is classified as vulnerable and is fast vanishing from the wild. Knowledge on the cytology of ashoka will help in establishing suitable breeding programmes for improvement of the existing population of ashoka. The present study tried to investigate the karyomorphology of *Saraca asoca*, to confirm its chromosome number.

Seeds of ashoka were collected from the trees growing in Kerala Agricultural University main campus, Vellanikkara, Thrissur (10° 32' latitude and 76°16' longitude) and were sown in a bed of absorbant cotton and moistened daily. In order to do the karyotype analysis of *Saraca asoca,* mitotic studies were carried out using root tip squash method. Prior to the slide preparation pre treatment was tried with 8-hydroxy quinoline and colchicine. Roots of about 2 cm, collected from freshly germinated seeds and thoroughly washed were used for the treatment. Roots were collected and pre treated at thirty minutes interval from 8 AM to 11 AM. Saturated solution of 8 – hydroxyl quinoline was prepared and the root tips were immersed in the solution for 4 hours under refrigeration. In case of colchicine, root tips were immersed in 0.5% solution for two hours at room temperature.

Two fixatives, viz, Carnoys I and Carnoys II were tried for the study. The pre-treated roots were washed and put into the fixatives for 24 hours. The fixed roots were taken out and treated with acetocarmine and fuelgen stains for identification of ideal staining procedure.

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Photographs of well spread metaphase stages were used for the preparation of karyotype. Chromosome count of ashoka was made from a well spread mitotic metaphase stage. Length of each chromosome was measured using the image analyser (LABOMED iVu 3000 model). Two parameters, namely, total chromosome length (TCL) and relative chromosome length (RCL) were calculated from the measurements. While TCL was estimated as the sum total of the haploid complement, RCL was estimated as the per cent of TCL. RCL was calculated for each of the chromosomes as shown below.

$$RCL = \frac{\text{Length of individual chromosome}}{\text{Total haploid chromosome length}} \times 100$$

Pre-treatment is done to arrest cell division at the mitotic metaphase stage by acting on spindle fibers. As per the present investigation, the excised, roots subjected to 8-hydroxy-quinoline for four hours under refrigeration, was found to be the most ideal pre-treatment for ashoka. Treatment of 8 - hydroxyquinoline for four to five hours at 6°C in case of *Betula papyrifera* and *Populous tremula* was reported earlier (Hommo and Sakilahn 1986). Chromosomes were distinctly visible when pre-treated with 8-hydroxyquinoline (Fig. 1,2). Thus it can be assumed that condensation and chromosome separation was better in the case of 8-hydroxyquinoline than colchicines (Fig. 3). Apart from this, root tips pre-treated at 10.30 AM gave the maximum number of dividing cells in *Saraca asoca.*

After pre-treatment, the excised root tips were transferred to suitable fixatives for killing and fixing of the cells. Among the two fixatives used, Carnoys II was found to be the effective one. Chromosomes usually appeared to be darker in Carnoys II reagent (Fig. 2,3). In case of Carnoys I, cytoplasm staining was frequently observed in the slides when viewed through the microscope (Fig. 4,5). Clusters of shrunk cells were generally observed in root tips fixed in Carnoys I solution. Chloroform in the Carnoys II might have helped for rapid penetration and differential staining of chromosomes. Chloroform might have also helped in clearing the cytoplasm of ingredients, thereby preventing cytoplasmic staining in the cytological preparations.

Among the two stains used in the present study, acetocarmine (Fig. 2,3,4) was found to be better than the fuelgen stain (Fig. 5,6,7). In case of acetocarmine staining, the root tips were gently heated for 10 - 15 minutes, by occasionally adding acetocarmine stain on the root tip kept over the slide. This was continued till the tissue was softened. The

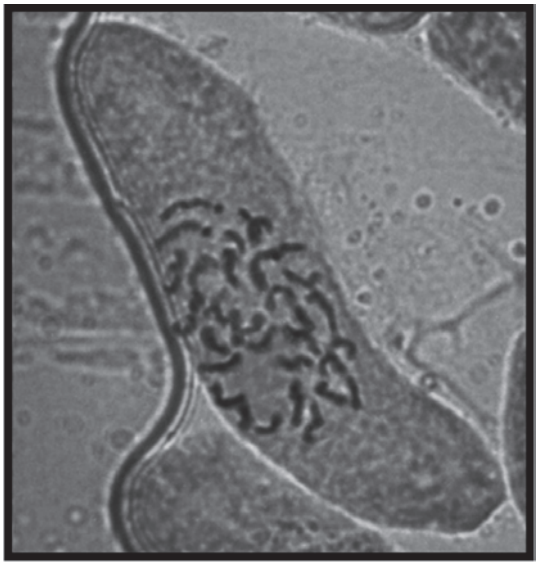

Figure 1. Photograph of *Saraca asoca* cell with chromosomes

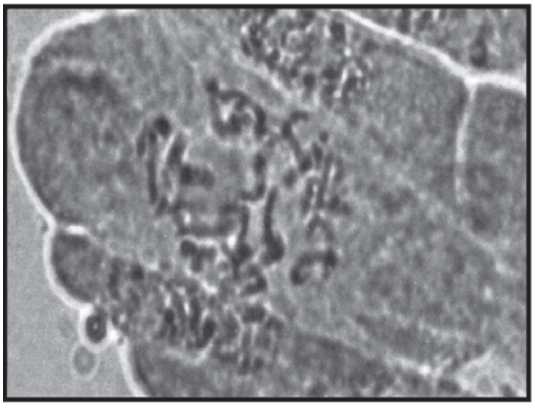

Figure 2. View of cell treated in 8-hydroxyquinoline, Carnoys II and acetocarmine (X 10000)

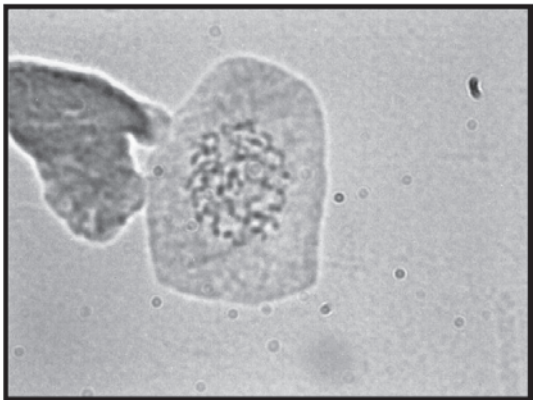

Figure 3. View of cell treated in colchicine, Carnoys II and acetocarmine (X 10000)

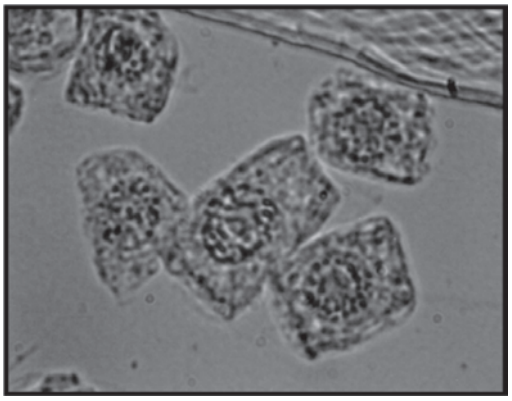

Figure 4. View of cell treated in 8-hydroxyquinoline, Carnoys I and acetocarmine (X 10000)

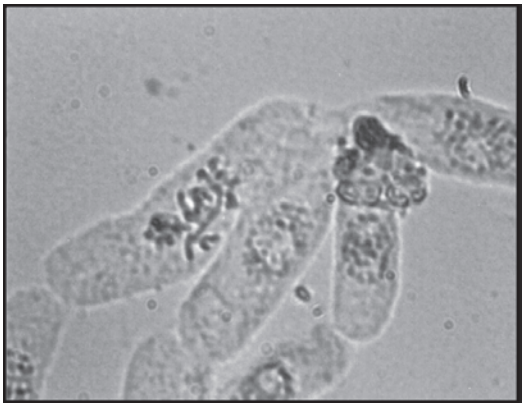

Figure 5. View of cell treated in colchicine, Carnoys I and fuelgen stain (X10000)

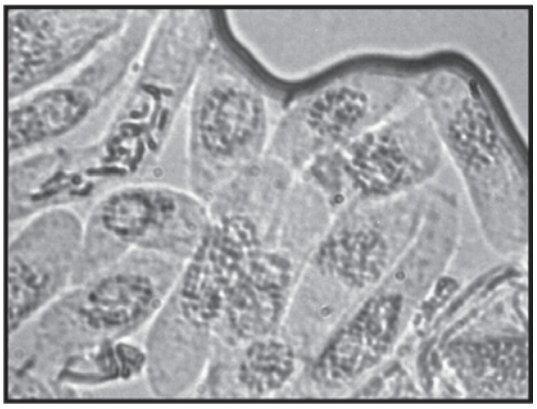

Figure 6. View of cell treated in 8-hydroxyquinoline, Carnoys II and fuelgen stain (X6000)

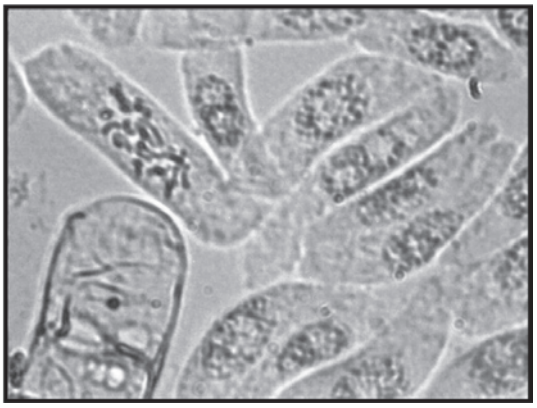

Figure 7. View of cell treated in 8-hydroxyquinoline, Carnoys I and Fuelgen stain (X6000)

softened root tips were easily squashed over a glass slide after covering it with a cover slip. It was observed that while squashing, the cell separation was very good in case of acetocarmine staining. Tapping the cover slip using a rubber edged pencil and pressing over it using the thumb helped in proper spreading of the cells in a single layer.

Careful observation indicated the presence of 34 chromosomes in the somatic cell of the ashoka (Fig. 1). This is in contrary to earlier report (Singhal, et al; 1990. Singh. 2002), that the basic chromosome number of this species was 12. Karyotype of ashoka was prepared from a well spread cell in the metaphase stage (Fig. 8). Chromosomes were classified into 17 sets of homologous pair and arranged according to decreasing length. The pairs were numbered from one to seventeen in the prepared karyotype. This shows that *Saraca asoca*

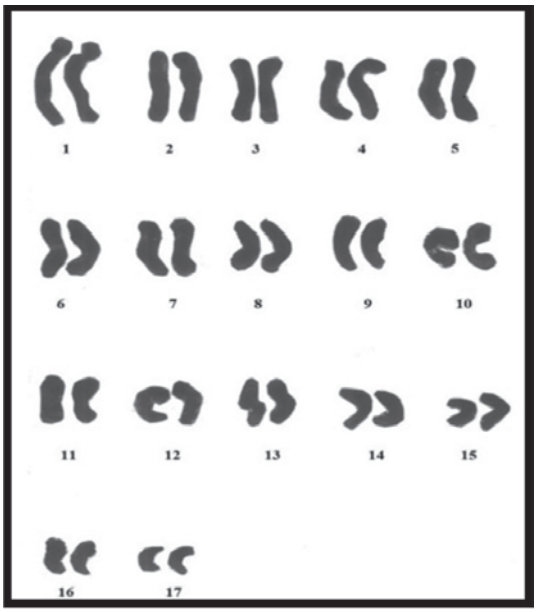

Figure 8. Karyotype of *Saraca asoca* showing chromosomes 2n=34

is a diploid with the basic chromosome number x =17. The presence of two satellite chromosomes also indicated the diploid nature of the plant.

It was not possible to detect exact centromere location and hence only the individual chromosome length was measured with the help of image analyser (LABOMED iVu 3000 model) and the data are presented in Table 1. Idiogram based on relative chromosome length (RCL) and absolute chromosome length were constructed (Fig. 9 and Fig. 10). Based on the measurements it was found that the length of chromosome in ashoka ranged from 12.1 µm to 5.3 µm. Total chromosome length (TCL) was obtained as 127.2 µm. Relative chromosome length in ashoka ranged from 9.5 µm to 4.2 µm. The individual chromosome of ashoka was found to be comparatively longer in comparison with other species of the family Fabaceae namely *Acacia auriculiformis* (1.61µm to 0.75 µm), *A. mangium* (2.6 µm to 0.67 µm), *A. nilotica* (1.25 µm to 0.51 µm) and *A. ferruginea* (1.44 µm to 0.56 µm) (Abideen, 1998 .)

The present study could standardize the procedures

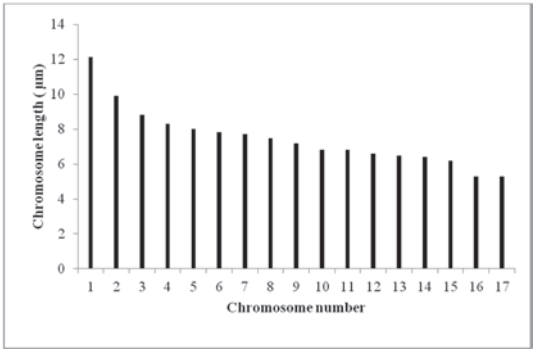

Figure 9. Idiogram based on absolute chromosome length

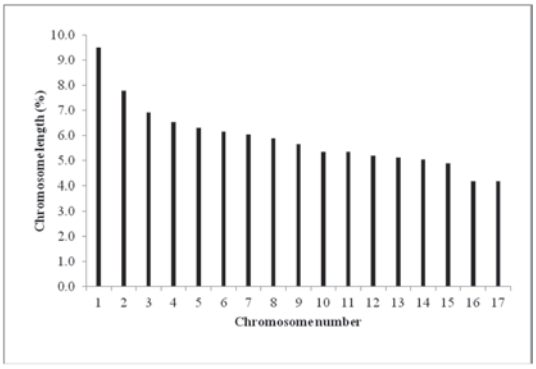

Figure 10. Idiogram based on relative chromosome length (RCL)

Table 1. Total chromosome length (TCL) and relative chromosome length (RCL) measured in *Saraca asoca*

Chromosome number	Total chromosome length (µm)	Relative chromosome length (%)
1	12.1	9.5
2	9.9	7.8
3	8.8	6.9
4	8.3	6.5
5	8.0	6.3
6	7.8	6.1
7	7.7	6.1
8	7.5	5.9
9	7.2	5.7
10	6.8	5.3
11	6.8	5.3
12	6.6	5.2
13	6.5	5.1
14	6.4	5.0
15	6.2	4.9
16	5.3	4.2
17	5.3	4.2

for good mitotic slide preparation of ashoka. A number of cells with metaphase stages was viewed when the root tips are pre-treated at about 10.30 AM with 8-hydroxyquinoline and stained with acetocarmine. The somatic chromosome number was found to be 2n=34 in contrast to the earlier report of 2n= 24.

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