Inconstancy in Chromosome Number in Some Species of *Cassia* L. Found in Nepal

B.P. Chaulagain\(^1\) and S.R. Sakya\(^2\)

\(^1\)Himalayan College of Agricultural Sciences and Technology (HICAST), Bhaktapur
\(^2\)Central Department of Botany, Tribhuvan University, Kirtipur
e-mail: bidurchaulagain@hotmail.com

Received August 2001; accepted January 2002

Abstract

Chromosome count of five species of the genus *Cassia* L. (sensu lato, s.l.) viz. *C. nairobensis* Hort. and taxa of sensu stricto (s.s.) like *C. mimosoides* L. (Chamaecrista mimosoides (L.) E. Greene, s.s.), *C. floribunda* Cav. (*Senna septemtrionalis* H. Irwin & Barneby, s.s.), *C. occidentalis* L. (*Senna occidentalis* (L.) Link., s.s.) and *C. tora* L. (*Senna tora* (L.) Roxb., s.s.) was carried out. Inconstancy in somatic chromosome number of the species was recorded. The dominant frequency of diploid chromosome number of *C. floribunda* was \(2n = 14\), the variation in chromosome number ranging from \(2n = 14\) to \(26\). Similarly, \(2n = 18\) for *C. mimosoides* (varying from \(14\) to \(30\)), \(2n = 20\) for *C. nairobensis* (varying from \(16\) to \(28\)), \(2n = 26\) and \(28\) for *C. occidentalis* (varying from \(16\) to \(38\)) and \(2n = 22\) for *C. tora* (variations, \(2n = 16\) to \(38\)) were observed. The chromosome number of *C. nairobensis* Hort. is reported here for the first time. The inconstancy of chromosome number with the instability of heteromorphic nature of chromosome depicts the chromosomal numerical polymorphism in the taxa. The numerical polymorphism of the chromosomes seems to be the factor responsible for the evolution of Linnaean genus *Cassia* L. s.l. and its complex (*Cassia*, s.s. *Chamaecrista* & *Senna*) formation.

Keywords: *Chamaecrista*, Heteromorphic nature, Numerical polymorphism, *Senna*

Introduction

The Linnaean genus *Cassia* belongs to the pea family Leguminosae Juss. (Fabaceae Rendl.). In Nepal, the species of genus *Cassia* are mostly tropical and subtropical in distribution and comprises about nine species. At present, the floristic and taxonomic study of Nepal Himalayas is in considerable progress. Since the legumes are one of the major floras of the country their biosystematics investigations are equally important to know their interrelationship and general evolutionary tendencies. The present investigating genus *Cassia* L. is generally in the life form of herb, erect shrub to under tree. Their propagation means is mainly due to its seeds. The taxon of the genus *Cassia* L. has been split into three segregated genera such as *Cassia sensu stricto* (s.s.), *Chamaecrista* and *Senna*. The newly formed genera share a characteristic appearance, however, they are distinguishable by morphological characters, floral ontogeny, and other genetic marker (Irwin & Barneby 1981, 1982, Wiersema et. al 1990, Tucker 1996, Whitty et. al 1994). Of the world flora, out of 650 genera of legumes, the cytological information's of 370 genera (57%) is known (Polhill & Raven 1981). The cytotgenetical data of more than the half of the genera of the sub-family Caesalpinioideae including more than three-fourth of the species of *Cassia* too are lacking. The present study has been carried out to analyze chromosome numbers during mitosis in five species of *Cassia*.

Materials and Methodology

The materials for the study were collected from different parts of Nepal. The phenological period of the taxon is noted. The identification of the specimens was based on the taxonomic treatments of Malla (1986). They were latter tallied with the herbarium specimens deposited in the Central Department of Botany, Tribhuvan University (TU), National Herbarium and Plant Laboratory, Royal Botanical Garden, Godawari, Nepal. Voucher specimen of the herbaria was deposited in the Central Department of Botany (TU).

Germination of seeds: The fresh ripened seeds were
directly collected from the plant except the two-year-old seed of *Cassia mimosoides* collected from herbarium specimens. The seeds were subjected to germination in petri dish provided with wet wool cotton in room temperature. The species of *C. floribunda*, *C. occidentalis* and *C. tora* were germinated well (above 50%) in control condition. But the rest of the species *C. nairobiensis* and *C. mimosoides* germinated in very low percentage and subjected to concentrated sulfuric acid treatment for 5 minutes to obtain higher frequency of germinating seeds as suggested by Porter (1949) and Koler and Cohen (1959).

**Root tip Excision:** The seeds subjected for germination were allowed to grow the root tip up to 2-3 cm long. As the time of root tip collection has an effect on the frequency of mitotic cells (Sakai, 1941), all collections were made between 10:00 a.m. and 11:00 a.m. Then the root tips were washed thoroughly in running water after exposing them to sunlight for about 10 minutes to fasten the successive cell divisions as well as to ensure turgidity of the cells before fixation (Wagley 1984).

**Pretreatment and fixation:** After exposing to sunlight the root tip was treated in 0.5% colchicine for 20 to 30 minutes at room temperature. Then the tips were washed thoroughly and subjected to fixation. In present work, glacial acetic-alcohol (Carnoy’s fluid) was applied and some modifications were made for the effectiveness. The species of *Cassia* have been found very poor in taking fixative and took longer times. Malakar (1978) also noticed such experience in the species *floribunda*. The blackening or browning of the tissue was observed during the fixation. The blackening of tissue is due to the reaction of tannin present in the meristem with the metal dissolved in the acetic acid (Li 1954, 1957).

To get well fixed tissue some modification was made in the fixative. The preparation of the fixative was modified by replacing the acetic acid by chloroform and formaldehyde in ratio of 90 ml ethyl alcohol (70%): 5 ml chloroform: 5 ml formaldehyde. The root tips were then fixed in above prepared fixative and then stored in room temperature. Root tips under microscopic observations were immersed in acetic acid 45% for 15 minutes to soften, then hydrolyzed in 1N-HCl at 60°C for 10-15 minutes and washed in running water for 20 minutes as suggested by Naruhashi and Iwatsuho (1993).

**Staining and slide preparation:** After hydrolysis the select root tips were transferred to mixture of 2% acetic-orcein and 1N HCl solution in a proportion of 9:1 in a test tube and gently heated over a spirit flame near to boil for 5-10 minutes interval. Then the root tips were allowed for at least one day to take the stain. The much densely stained apex portion of the root meristems were cut out and washed in 45% acetic acid to remove the debris and impurities. The tip was macerated in 45% acetic acid solution in a clear glass slide. The permanent slide was made using Celartari’s acetic acid -T-butyl alcohol schedule and mounted in euparal.

**Results and Discussion**

Different workers have reported chromosome number of *Cassia* species from different regions of the globe. For the species *C. floribunda*, Irwin and Turner (1960), Mehta (1976) and Tandon & Bhat (1970) have reported 2n=28. At present observation, the counting of chromosomes shows that there has been an inconstancy in somatic chromosome number from one cell to other cell even in the same individual plant. The dominant chromosome number of *C. floribunda* (Coll. No. 1 & 2) was 2n=14 (Table 1A & 1B, Fig.1). The other chromosome numbers were recorded from 2n=16 to 24. The observed number 2n=14 is shown its frequency in two collections as 20.94 and 23.30 percent respectively. The present result agrees with that of Irwin (1960), Irwin and Turner (1960) and Malakar (1978) for 2n = 14 for the taxa.

Similarly, the dominant frequency of chromosome counts was 2n=18 in *C. mimosoides* with its other variable numbers as 2n = 14, 16, 20, 22, 24, 26, 28 and 30 (Table 1C). Such variations were observed within an individual root cells as well as from root meristem of different seeds. The heteromorphic nature of some somatic chromosomes was observed in some cells (Fig. 1.2-3). Earlier reports show that the chromosome number of species *C. mimosoides* is 2n=16 (D’Amato-Avanz 1956, Miege 1960, Irwin 1964, Bogman 1964, Kodama 1967, 1970, & Bir & Kumari 1970), and 2n = 32 (Irwin & Turner 1960). The haploid number n=8 is reported by George & Bhavanandan (1994). The variable numbers of somatic chromosome of the above taxon has been reported as 2n=16, 28, 32, 42, 48, and 56 by Kowakami (1930) and Randel (1970).

Present work also shows the inconstancy in chromosome numbers in *C. occidentalis* where collection of species from three different populations have shown their chromosome variations as 2n=16, 18, 19, 20, 20,22, 23, 24, 25, 26, 28, 30, 32, 34, 36, and 38 within individual or in different collections (Table 1E,1F & 1G). The highly established frequency was 2n = 26, which accounts 12.3 percent of total cells observed. The heteromorphic nature of chromosomes was also observed in this species (Fig. 1.7-10). The
### Table 1. Chromosome counts in five species of *Cassia* L. s.l. collected from different parts of Nepal

<table>
<thead>
<tr>
<th>Species</th>
<th>Collection</th>
<th>Total no. of cells observed</th>
<th>Chromosome number (2n)</th>
<th>Number of cells observed</th>
<th>Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A. C. floribunda</strong></td>
<td>Coll. No. 1</td>
<td>Kirtipur</td>
<td>14, 16, 18, 20, 22, 24</td>
<td>40, 36, 31, 29, 34, 21</td>
<td>20.94, 18.85, 16.23, 15.18, 17.80, 10.99</td>
</tr>
<tr>
<td><strong>B. C. floribunda</strong></td>
<td>Coll. No. 2</td>
<td>Bhaktapur</td>
<td>14, 16, 18, 20, 22, 24</td>
<td>58, 47, 39, 23, 24, 18</td>
<td>20.94, 18.1, 15.9, 8.9, 6.9</td>
</tr>
<tr>
<td><strong>C. C. mimosoides</strong></td>
<td>Coll. No. 3</td>
<td>Taplejung</td>
<td>14, 16, 18, 20, 22, 24</td>
<td>12, 34, 55, 49, 42, 36</td>
<td>4.65, 13.18, 21.31, 19.0, 16.28, 13.95, 19.43, 5.43</td>
</tr>
<tr>
<td><strong>D. C. nairobensis</strong></td>
<td>Coll. No. 4</td>
<td>Kirtipur</td>
<td>14, 16, 18, 20, 22, 24</td>
<td>18, 36, 38, 42, 36, 14</td>
<td>20.94, 18.85, 16.23, 15.18, 17.80, 10.99</td>
</tr>
<tr>
<td><strong>E. C. occidentalis</strong></td>
<td>Coll. No. 5</td>
<td>Swoyambhu</td>
<td>14, 16, 18, 20, 22, 24</td>
<td>58, 47, 39, 23, 24, 18</td>
<td>20.94, 18.1, 15.9, 8.9, 6.9</td>
</tr>
<tr>
<td><strong>F. C. occidentalis</strong></td>
<td>Coll. No. 6</td>
<td>Swoyambhu</td>
<td>14, 16, 18, 20, 22, 24</td>
<td>58, 47, 39, 23, 24, 18</td>
<td>20.94, 18.1, 15.9, 8.9, 6.9</td>
</tr>
<tr>
<td><strong>G. C. occidentalis</strong></td>
<td>Coll. No. 7</td>
<td>Santapur</td>
<td>14, 16, 18, 20, 22, 24</td>
<td>58, 47, 39, 23, 24, 18</td>
<td>20.94, 18.1, 15.9, 8.9, 6.9</td>
</tr>
<tr>
<td><strong>H. C. tora</strong></td>
<td>Coll. No. 9</td>
<td>Swoyambhu</td>
<td>14, 16, 18, 20, 22, 24</td>
<td>58, 47, 39, 23, 24, 18</td>
<td>20.94, 18.1, 15.9, 8.9, 6.9</td>
</tr>
<tr>
<td><strong>I. C. tomentosa</strong></td>
<td>Coll. No. 8</td>
<td>Santapur</td>
<td>14, 16, 18, 20, 22, 24</td>
<td>58, 47, 39, 23, 24, 18</td>
<td>20.94, 18.1, 15.9, 8.9, 6.9</td>
</tr>
</tbody>
</table>

Previous chromosome reports have shown the somatic number as 2n=28 (Senn 1938, Pantulu 1940, Turner 1956, HSU 1968, Tandon & Bhat 1970, Larsen 1971, Gupta & Gupta 1971, Bir & Kumari 1977, Gill 1978a) and 2n=26 (Bir & Sidhu 1966) and 2n=26 to 28 (Mehra 1972).

Present investigation in the species *Cassia tora* indicates 2n=22 for its dominant diploid number The variation was 2n=16, 18, 19, 20, 21, 23, 24, 25, 26, 28, 30, 32, 34, 35, 36, and 38 (Table 1D). The heteromorphic nature of chromosomes was noticed which were variable. Some chromosomes were found sticky in nature causing disparity in chromosomes during polar movement (Fig. 1.11-15). Previous investigation in this species were reported as 2n=26 (Datta 1933, Senn 1938, Bir & Sidhu 1966, Larsen 1971), 2n=28 (Jacob 1940, Tandon & Bhat 1970, Sharma 1970), and 2n=26 and 32 (Katayama 1953, Miege 1960). Similarly, 2n=20 has been recorded in the species.
Fig. 1.1. *C. floribunda* (Coll. No. 2), metaphase. Fig. 1.2-6. *C. mimosoides* (Coll. No. 3) metaphase chromosomes with variable number and heteromorphic nature. Fig. 1.4-6. *C. nairobensis*, metaphase with sticky chromosomes. Fig. 1.9-10. *C. occidentalis* (Coll. No. 7), metaphase. Fig. 1.11-12. *C. tora* (Coll. No. 9), metaphase chromosomes with heteromorphic nature. Fig. 1.13-14. *C. tora* (Coll. No. 10), metaphase. Fig. 1.15. *C. tora* (Coll. No. 10), sticky metaphase.

*C. nairobensis* with variable chromosome number as 2n=16, 18, 22, 24, 26 and 28 (Table 1C). The frequency of predominant number was 23.45 percent. The chromosome morphology found differing from cell to cell and some were sticky in nature (Fig. 1.4-6).

At present, the instability in somatic chromosome number within individual taxa should not be subjected to artificial treatments. The results, however, should be interpreted upon the basis of naturally occurring phenomena. Sharma and Sharma (1956), Hegwood and Hough (1958), Sarvella (1958), Nielson and Nath (1961), Thompson (1962), Lewis (1962), Wagley (1984), Sakya and Joshi (1990) had also observed such cases in different plant species. Wagley (1984) argued such phenomenon in *Codium variegatum* as cytomoxis while Love (1938) believed such somatic variation of chromosome numbers in hybrid wheat is due to hybridity. Darlington and Thomas (1937) has reported anomalous numerical polymorphism of chromosomes in *Festuca-Lolium* hybrid and argued for the cause due to spindle deformities.

The occurrence of variations of somatic
chromosome numbers has been confined greatly in the plant species of those families that have highest species diversity viz, Asteraceae (1250-1300 genera, 20,000-25,000 species), Orchidaceae (750 genera, 20,000-25,000 species) and Poaceae (900 genera, 11000 species). The Cassias belongs to such family with one of the largest species diversity with 650 genera and 18,000 species (Takhtajan 1981, Cronquist 1988). The occurrence of somatic chromosome number variation in Cassia could be depicted to the causes that have been found in the species of families like Asteraceae, Poacene, and Orchidaceae.

Gildenhuys and Brix (1958a, 1958b) observed somatic instability, abnormal meiosis, and apomixis in Pennisetum dubium and marked it as gene control mechanism. Snoad (1955a, 1955b) proposed that aberrant chromosome numbers are heritable through incompatible gene combinations rather than to the germinal line and that even chromosomally unbalanced gametes can be functional. Pantulu and Rao (1977) observed intra-plant variation in chromosome in a cross between Pennisetum purpureum and P. typhoides and their backcross progenies and interpreted it as attribution to spindle abnormalities as the result of ‘duality’ of the nucleus (hybrids) in foreign cytoplasm with resultant unbalanced enzyme and amino acid. The nature of the abnormal achromatic figures of chromatin and chromosome number variations can be attributed, perhaps, to unbalanced nucleo-protein systems that resulted from the combination of distantly related gametes in the formation of the inter- or intra-generic hybrids as suspected by Neilsen and Nath (1961). However such breeding experiments in the present taxa are not known except fertile natural hybrids of X floribunda (S. septentrionalis X S. multiglandulosa) (Wiersema et al 1990). Besides the above-mentioned facts, cytomixis as well as accessory chromosomes may responsible to display such somatic instability in the presently studied taxa.

Acknowledgments

We are thankful to Central Department of Botany, Tribhuvan University for providing laboratory facilities during work. We are also thankful to National Herbarium and Plant Laboratory, Department of Plant Resource, Godawari, for herbaria facilities and to Dr K.K. Shrestha for providing seeds of Cassia mimosaed. A part of the work was presented at the III National Conference on Science and Technology-1999 organized by RONAST in Kathmandu.

References


