CHROMOSOME NUMBERS OF SOME INDIGENOUS TREE SPECIES OF ETHIOPIA

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ABSTRACT: Chromosome data are essential information for any organism and many chromosome investigations have been performed, providing important characters for plant systematic and evolutionary analysis as well as for germplasm improvement. Trees are important to the wellbeing of people in every country, as they have essential ecological, economic and cultural values. In Ethiopia, there are over 300 tree species. However, for most of the country's tree species, chromosome data are not available in the literature. The present investigation was aimed at carrying out mitotic chromosome studies on some important indigenous tree species of Ethiopia. Somatic chromosome counts were carried out on somatic cells from root tips. In this study, chromosome numbers for the following twelve tree species from ten genera of seven families are reported: Cordia africana 2n=48, Acacia abyssinica 2n=104, Acacia senegal 2n=26, Erythrina brucei 2n=42, Acacia albida 2n=26, Millettia ferruginea 2n=22, Ficus sur 2n=26, Ficus sycomorus 2n=26, Olea europaea subsp. cuspidata 2n=46, Podocarpus falcatus 2n=24, Ziziphus spina-christi 2n=24, and Allophylus abyssinicus 2n=28. Counts on all the species are reported for the first time for Ethiopian materials and six of the counts are initial species reports. For A. abyssinica and Z. spina-christi, new chromosome numbers are reported. Metaphase chromosome microphotographs are presented for all the species studied.

Key words/phrases: Chromosome number, Ethiopia, Indigenous trees.

INTRODUCTION

Chromosome data are essential information for any organism and many chromosome investigations have been performed, providing important characters for plant systematic and evolutionary analysis (Stace, 2000). Chromosome information is also important for germplasm improvement through breeding as well as for selecting model genetic organism. For example, *Arabidopsis thaliana* (2n=2x=10) became a model genetic plant partly because of its very small genome size (Leutwiler *et al.*, 1984). But, if any angiosperm with only one chromosome is discovered, almost certainly it would become an interesting model system for genome studies (Bennett, 1998). However, no chromosome count exists for more than 70% of angiosperm species (Bennett, 1998).

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The flora of Ethiopia is estimated to contain between 6,500 and 7,000 species, of which 12% is considered to be endemic (Institute of Biodiversity Conservation, 2007). There are over 300 tree species in the country (Institute of Biodiversity Conservation, 2005). Trees are important to the wellbeing of people in every country, as they have essential ecological, economic and cultural values. To maximize the services that trees provide, studying their biology including chromosome data is essential. However, for most of the Ethiopian tree species, chromosome data are not available in the literature.

The present investigation was aimed at carrying out mitotic chromosome counts as a contribution toward reduction of deficiencies of cytological information on Ethiopian tree species. Chromosome counts are reported for twelve important indigenous tree species of Ethiopia belonging to ten genera and seven families. Of these, six are initial species reports and two new records.

MATERIALS AND METHODS

The following twelve species were studied for chromosome number: Cordia africana, Acacia abyssinica, Acacia senegal, Erythrina brucei, Acacia albida, Millettia ferruginea, Ficus sur, Ficus sycomorus, Olea europaea subsp. cuspidata, Podocarpus falcatus, Ziziphus spina-christi, and Allophylus abyssinicus. Their sources, taxonomic family to which they belong as well as the number of plants studied for each species are presented in Table 1.

Somatic chromosome counts were made from tips of the radicle grown from the seeds placed between moist filter paper in petridishes, and root tips obtained from seedlings that were potted in the greenhouse.

Techniques for somatic chromosome preparation were as follows. Root tips about 5-15 mm long were excised, immersed in 0.002M 8-hydroxyquinoline for 4 hours at room temperature in the dark, fixed in a 3:1 mixture of ethanol and acetic acid (v/v) for at least 16 hours at 4°C. Then, enzyme maceration, air drying and staining were performed by the method of Kifle Dagne and Heneen (1992) as follows. Fixed root tips were rinsed in distilled water several times for 5 minutes in each change and macerated in an enzyme solution containing 4% cellulase and 4% pectinase for 1-4 hours at 37°C in a water bath. After rinsing in distilled water twice for 5 minutes, the meristematic root tips were placed on a clean glass slide. A drop of the fixative was added to the root tip and meshed with flat end of mounted needle. Two more drops of the fixative were added, and the slide was then

air dried at room temperature. Air-dried slides were stained in 2% Giemsa for 3-4 hours or more until the chromosomes got stained satisfactorily, the slides were rinsed twice in distilled water, air dried and mounted in Depex.

Photomicrographs of good metaphase chromosome spreads were taken under oil immersion at x1000 total magnification using an Olympus compound microscope fitted with MEM1300 Digital Eyepiece camera. Chromosomes were counted on a minimum of five cells per plant from photomicrographs and/or directly under the microscope.

Literature concerning chromosome numbers for publications from 1979 to 2004 was checked using the *Index to plant chromosome numbers* database (Goldblatt and Johnson, 1979-2004) which is available at http://mobot.mobot.org/W3T/Search/ipcn.html and other sources were consulted for publications before 1979 and after 2004.

RESULTS AND DISCUSSION

In the present study, only chromosome numbers are reported; no karyotype analyses were done as chromosomes were too small to allow observation of morphological variations. Chromosome counts are summarized in Table 1 and the photomicrographs of mitotic metaphases are presented in Figures 1-12. To the best of our knowledge, there are no previous reports on chromosome count for all those species for Ethiopian materials. But chromosome counts are available in the literature for six of the species from non-Ethiopian materials. However, for two of the six species, *A. abyssinica* (2n=104) and *Z. spina-christi* (2n=24), new chromosome numbers were recorded in the present study. Reports of different chromosome numbers for a species mainly from different geographical regions are not unusual in the literature. For example, Oballa and Olng'otie (1993) reported two different chromosome counts for *Acacia tortilis*, 2n=52 from Niger, Senegal and Israel and 2n=104 from Kenya.

1. Podocarpus falcatus (Thunb.) R. Br. ex Mirb. (PODOCARPACEAE)

The previous chromosome report for this species was 2n=24 (Mehra and Khoshoo, 1956), which is in agreement with the present count (Fig. 7). This count is the first for the flora of Ethiopia.

2. Cordia africana Lam. (BORAGINACEAE)

Somatic chromosome count for *C. africana* showed 2n=48 (Fig. 1). To our knowledge, this is the first chromosome count for the species. This count is in agreement with the previous chromosome count of *Cordia myxa* L.

(2n=48) (Mehra, 1976). Chromosome counts previously reported for other species of this genus include 2n=16, 18, 28, 30, 32, 36, 48, 50, 70, 104 and n=9, 16 and 18 (Goldblatt and Johnson, 1979-2004).

3. Acacia abyssinica Hochst. ex Benth. (FABACEAE)

The present somatic chromosome count for *A. abyssinica* was 2n=104 (Fig. 2). However, this number was previously reported for *A. tortilis* (Forssk.) Hayne (Oballa and Olng'Otie, 1993). For *A. abyssinica*, a different chromosome number (2n=52) was reported (Goldblatt and Johnson, 1979-2004). According to our data, the present chromosome count is a new record for the species and the first count for the Ethiopian flora. Though the commonest chromosome count for *Acacia* species is 2n=26, there are also other chromosome counts including 2n=40, 52, 56, 78, 104, 208 and n=12, 13, and 26 (Goldblatt and Johnson, 1979-2004).

4. Acacia senegal (L.) Willd. (FABACEAE)

Somatic chromosome count of 2n=26 was previously reported for *A. senegal* from Pakistan by Bukhari (1997), which is in agreement with our count (Fig. 3). However, as far as our knowledge goes, this is the first count for the Ethiopian flora.

5. Erythrina brucei Schweinf. (FABACEAE)

Somatic chromosome count for *E. brucei* showed 2n=42 (Fig. 4). This chromosome number is the commonest report for the genus *Erythrina* (Goldblatt and Johnson, 1979-2004). Previous chromosome counts on other species in this genus include 2n=42 and n=12, 21, 42, and 84 (Goldblatt and Johnson, 1979-2004). To the best of our knowledge, this is the first chromosome count for *E. brucei*.

6. Acacia albida Del. (syn. Faidherbia albida (Del.) A. Chev.) (FABACEAE)

Somatic chromosome count of 2n=26 was previously reported for *Acacia albida* from Pakistan by Bukhari (1997), which is in agreement with our count (Fig. 5). According to our data, this count is the first for the Ethiopian flora.

7. Millettia ferruginea (Hochst.) Bak. (FABACEAE)

Somatic chromosome count for *M. ferruginea* showed 2n=22 (Fig. 6). This number is reported for several other species in the genus (Goldblatt and Johnson, 1979-2004). However, according to our knowledge, this is the first

chromosome count for *M. ferruginea*. Previous chromosome counts for other species of the genus *Millettia* include 2n=20, 22, 32, 48, and n=8, 10, 11, 12, and 18 (Goldblatt and Johnson, 1979-2004).

8. Ficus sur Forssk. (MORACEAE)

Somatic chromosome count for F. sur was 2n=26 (Fig. 10). This number is the commonest chromosome count for the genus Ficus though other numbers including 2n=26, 39, n=12, 13, and 14 were also reported (Goldblatt and Johnson, 1979-2004). To our knowledge, this is the first chromosome count for the species.

9. Ficus sycomorus L. (MORACEAE)

Somatic chromosome count for F. sycomorus showed 2n=26 (Fig. 8). Though this chromosome number is the commonest for the genus (Goldblatt and Johnson, 1979-2004), this is the first chromosome count for F. sycomorus according to our knowledge.

10. Olea europaea L. subsp. cuspidata (Wall. ex G. Don) Cif. (OLEACEAE)

Somatic chromosome count of 2n=46 was previously reported by Breviglieri and Battaglia (1954) as cited in Minelli *et al.* (2000), which is in agreement with our count (Fig. 9). This count is the first for the Ethiopian flora.

11. Ziziphus spina-christi (L.) Desf. (RHAMNACEAE)

Somatic chromosome count in our sample for *Z. spina-christi* showed 2n=24 (Fig. 11). According to our knowledge, this is a new chromosome count for this taxon. Previous reports for *Z. spina-christi* are n=36 from Pakistan by Khatoon and Ali (1993) cited in Ghaffari (2008), and 2n=96 from Iran by Ghaffari (2008). This and other chromosome counts on *Ziziphus* species indicate that the genus has various chromosome numbers of 2n=20, 24, 26, 40, 48, 60, 72, and 96 (Goldblatt and Johnson, 1979-2004).

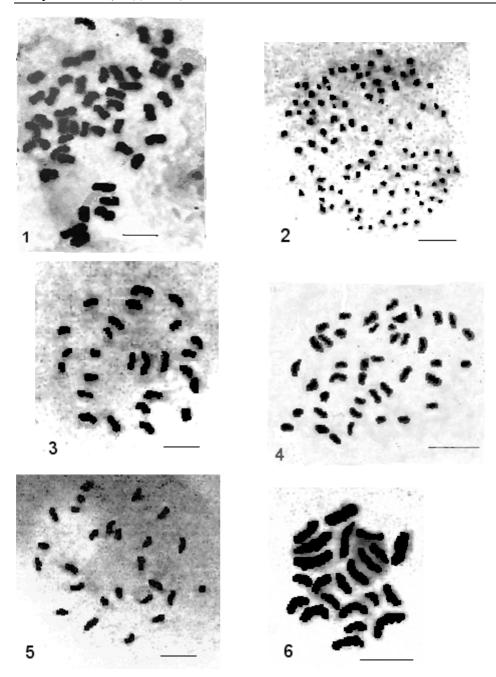
12. Allophylus abyssinicus (Hochst.) Radlk. (SAPINDACEAE)

Somatic chromosome count for *A. abyssinicus* was 2n=28 (Fig. 12). Previous chromosome counts on other species in the genus *Allophylus* are 2n=28, 56, and n=14 (Goldblatt and Johnson, 1979-2004). Chromosome number 2n=28 was reported for *A. edulis* (A. St. -Hil., A. Juss. & Cambess.) Hieron. ex Niederl. and *A. pauciflorus* Radlk. (Goldblatt and Johnson, 1979-2004). According to our knowledge, the present chromosome count is the

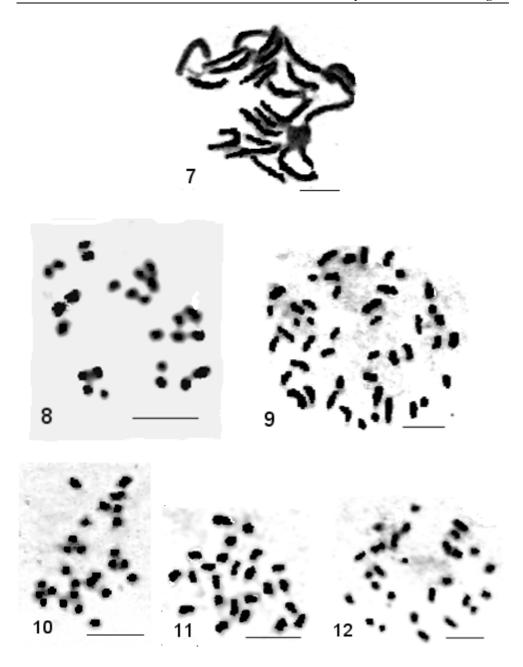
first for A. abyssinicus.

Table 1. Chromosome counts, material sources and number of individuals investigated for some indigenous tree species of Ethiopia.

Taxon	Chromosome number (2n)	No. of plants investigated	Source of plant material
Family Podocarpaceae			
Podocarpus falcatus	24	3	Debre Zeit
Family Boraginaceae			
Cordia africana	48	4	Arjo
Family Fabaceae			
Acacia abyssinica	104	5	Langano
Acacia senegal	26	4	Bulbula
Erythrina brucei	42	1	AAU, College of Natural Sciences Campus
Acacia albida	26	5	Langano
Millettia ferruginea	22	4	Bahir Dar
Family Moraceae			
Ficus sur	26	4	AAU, College of Natural Sciences Campus
Ficus sycomorus	26	5	AAU, College of Natural Sciences Campus
Family Oleaceae			
Olea europaea subsp. cuspidata	46	1	Chilimo Forest
Family Rhamnaceae			
Ziziphus spina-christi	24	2	Metehara
Family Sapindaceae			
Allophylus abyssinicus	28	4	AAU, College of Natural Sciences Campus



Figs. 1-6. Photomicrographs of mitotic metaphases: 1. Cordia africana 2n=48; 2. Acacia abyssinica 2n=104; 3. Acacia senegal 2n=26; 4. Erythrina brucei 2n=42; 5. Acacia albida 2n=26; and 6. Millettia ferruginea 2n=22. Initial magnification x1000. Bars= 5μ m.



Figs. 7-12. Photomicrographs of mitotic metaphases: 7. *Podocarpus falcatus* 2n=24; 8. *Ficus sycomorus* 2n=26; 9. *Olea europaea* subsp. *cuspidata* 2n=46; 10. *Ficus sur* 2n=26; 11. *Ziziphus spina-christi* 2n=24; and 12. *Allophylus abyssinicus* 2n=28. Initial magnification x1000. Bars=5µm.

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REFERENCES

- Bennett, M. D. (1998). Plant genome values: How much do we know? *P. Natl. Acad. Sci. USA* **95**: 2011-2016.
- Bukhari, Y.M. (1997). Cytoevolution of taxa in *Acacia* and *Prosopis* (Mimosaceae). *Aust. J. Bot.* **45**(5): 879-891.
- Ghaffari, S.M. (2008). Chromosome records for some plant species from Iran. *Iran. J. Bot.* **14**(1): 39-46.
- Goldblatt, P. and Johnson, D.E. (1979-2004). Index to plant chromosome numbers. http://mobot.mobot.org/W3T/Search/ipcn.html. Missouri Botanical Garden, St. Louis.
- Institute of Biodiversity Conservation (2005). Ethiopia: Third national report to CBD.
- Institute of Biodiversity Conservation (2007). Ethiopia: Country report on the state of PGRFA to FAO.
- Kifle Dagne and Heneen, W.K. (1992). The karyotype and nucleoli of *Guizotia abyssinica* (Compositae). *Hereditas* **117**: 73-83.
- Leutwiler, L.S., Hough-Evans, B.R. and Meyerowitz, E.M. (1984). The DNA of *Arabidopsis thaliana. Mol. Genet. Genomics* **194**: 15-23.
- Mehra, P.N. (1976). Cytology of Himalayan Hardwoods. Sree Saraswaty Press, Calcutta.
- Mehra, P.N. and Khoshoo, T.N. (1956). Cytology of conifers. II. J. Genet. 54: 181-185.
- Minelli, S., Maggini, F., Gelati, M.T., Angiolillo, A. and Cionini, P.G. (2000). The chromosome complement of *Olea europaea* L.: Characterization by differential staining of the chromatin and *in-situ* hybridization of highly repeated DNA sequences. *Chromosome Res.* **8**: 615-619.
- Oballa, P.O. and Olng'Otie, P.A.S. (1993). Chromosome numbers in two African *Acacia* species. *Kew Bull.* **49**: 107-113.
- Stace, C.A. (2000). Cytology and cytogenetics as a fundamental taxonomic resource for the 20th and 21st centuries. *Taxon* **49**: 451-477.