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Meiotic Studies in Some Medicinal Angiosperms from Doaba Region of Punjab, India

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Abstract

The chromosome number and their behavior in 15 medicinal plant species collected from Doaba region of Punjab, India have been studied. The species have shown chromosome numbers like *Basella rubra* L. (n=22), *Coccinia cordifolia* Cogn. (n=12), *Delphinium ajacis* L. (n=8), *Lens culinaris* Medic. (n=7), *Melilotus alba* Desr. (n= 8), *Nicotiana plumbaginifolia* Viv. (n=10), *Nymphoides cristata* (Roxb.) Kuntze (n=9), *Papaver rhoeas* L. (n=7), *Raphanus sativus* L. (n=9), *Sesamum indicum* L (n=13), *Tinospora cordifolia* (Wild.) Hook.f.et Thoms. (n=13), *Triticum aestivum* L. (n=21), *Vitex negundo* L. (n=17), *Xanthium strumarium* L. (n=18) and *Zea mays* L. (n=10). Most of the species are diploid with normal course of meiosis. But meiotic abnormalities in the form of univalents, stickiness and presence of supernumerary B-chromosomes have also been observed in some species. All the species have some medicinal value. The chromosome counts in these species are in conformity with the earlier reports, but behavioral changes have been observed in some species. This shows the genetic stability and resistance of these species to various biotic and abiotic stresses.

Keywords: Chromosomes, Meiosis, Medicinal plants, Doaba region, Punjab, India

Introduction

Angiosperms are representing the major group of plants with nearly 2, 60,000 species belongs to 453 families [1]. Angiosperms are essential for human beings as majority of the world's crops are Angiosperms. They are of great importance in day to day life, especially in herbal medicines for human and animal diseases. The medicinal properties of these plants vary not only with different seasons and environmental conditions, but also with the ploidy level of the plant. Thus the knowledge of the chromosome number of medicinal plants has its own significance.

Meiosis is an important component in angiosperms fertilization. Meiosis reduces the chromosome number and results in the formation of gametes. Meiosis brought variation which is important for evolution. Variations occur due to recombination between homologous chromosomes. Therefore the chromosomal study is an important tool to understand the evolution and diversification of the species. The chromosomal study has also been proved useful in plant taxonomy. After morphological characterizations, chromosomal study is the next step to authenticate the identity of a species. The correct identification of medicinal plants is very important to make herbal remedies for a particular disease.

To best of our knowledge, very little work has been done on the chromosome study of angiosperms of this particular region of Punjab. Keeping this in view, chromosomal investigation has been carried out to confirm the morphological characterization of species recorded from the study area. Chromosomal study of some of the traditionally used medicinal plant species of this area has already been carried out [2]. Present study is focused on chromosome number and their meiotic behavior in 15 angiosperm species.

Materials and Methods

Chromosomal study was carried out using floral buds collected from the plants of study area. Buds of appropriate size were fixed in a freshly prepared 3:1 (alcohol: glacial acetic acid) solution for 24 hours at room temperature. The fixed material was then transferred to 70% ethanol and stored in refrigerator. One or two immature anthers were excised from the bud and squashed on a clean slide in a drop of 2% aceto-carmine stain. Then the cover slip was placed over the squashed material, avoiding the air bubbles. The slide was heated gently and pressed by thumb under the two fold of filter paper to spread the pollen mother cells uniformly. The chromosome number and behavior in different species were observed and photographed at different stages of meiosis. The photomicrographs of pollen mother cells showing different stages of meiosis have been taken, using Eclipse 200 Nikon digital imaging system.

Results and Discussion

Chromosome analysis of fifteen wild and cultivated species belonging to 15 genera and 13 families has been carried out. The chromosome number varies from n=7 to n=22. All the species are medicinally important and most of them were used in traditional medicines.

Basella rubra L.

Basella rubra is known as Indian spinach, belongs to family Basellaceae. It is a wild climber having white-pink tiny flower and reddish-purple stem. It is also used as green vegetable and believed to have more iron than spinach. The meiotic preparations have revealed the presence of 22 bivalents at late diplotene stage in pollen mother cells (Figure.1). Normal segregation of chromosome at anaphase stage has also been observed. This shows regular meiosis in this species. Peter [3] recorded the same chromosome number in this species which tallies with the present chromosome count. The chromosomes are long and sticky in nature, hence not well separated which makes their counting difficult.

Coccinia cordifolia Cogn

Coccinia cordifolia is a wild climbing herb of family Cucurbitaceae. The flowers are white and unisexual in nature. Young fruits are used as vegetable and have antidiabetic property. The chromosomal investigation in this species is little difficult as the chromosomes are very small in size. During present investigation, 12 bivalents have been observed at diakinesis stage (Figure. 2). However some pollen mother cells possess univalents that indicate the meiotic abnormality. Bhowmick et al. [4] also studied the chromosome number of this species, which is in conformity with the present findings.

Delphinium ajacis L.

It is an annual herb of family Ranunculaceae and commonly known as Larkspur. It bears deep purple flowers on the long spike. Spur, a modification of petal is the most attractive part of flower. Arial parts are used as pediculicide *i.e.* to remove hair lice [5]. Meiotic study has shown the presence of n=8 chromosomes. Similar chromosome count has earlier been recorded by Singh [6]. The chromosomes are large enough and are easily countable at metaphase stage (Figure. 3). In all the studied pollen mother cells, two pairs of chromosomes are very large and two are usually smaller than the others. The meiosis was found to be normal with no chromosomal irregularities.

Lens culinaris Medic.

The herbaceous plant is a member of family Fabaceae. It is cultivated as pulse crop. This leguminous crop improves the fertility of soil by biological nitrogen fixation**.** Cooked seeds are given to cattle as body tonic and for reproductive problems. Meiotic preparations possess n=7 chromosomes. All the chromosomes are aligned at their respective positions at metaphase-II (Figure. 4). Most of the pollen mother cells have normal segregation of chromosomes at anaphase stage. Some PMCs have witnessed the formation of chromatin bridges and unequal segregation of chromosomes. Rehman and Altaf [7] studied the n=7 chromosomes in this species from Pakistan which is in agreement with the present study.

Melilotus alba Desr.

White sweet clover is a member of family Fabaceae with white flowers. The plant is used to cure chicken pox, joint pain and as thermoregulator. The chromosomal study depicts eight bivalents at diakinesis stage (Figure. 5). Both ring and rod shaped bivalents have been observed in many pollen mother cells. Formation of chiasma in some pollen mother cells is clearly visible. Pavlova and Tosheva [8] studied the karyology of *Melilotus alba* in Bulgarian species and Hijazy [9] in Egyptian species which tallies with the results of present investigation.

Nicotiana plumbaginifolia Viv.

The plant is commonly known as “Jangli tambakoo” is a member of family Solanaceae. It is a wild herb growing in moist and shady waste lands. Traditionally the plant is used to cure many skin problems [10]. The meiotic study has represented ten bivalents in pollen mother cells. This number is in accordance with the previous reports for this species [11; 12]. The chromosomes are larger and clearly visible at metaphase stage. The chiasma formation has been observed in many pollen mother cells (Figure.6). In few PMCs, two to three bivalents overlap with each other which show the sticky nature of the chromosomes. In some PMCs supernumerary B-chromosomes have also been observed which are smaller than A-chromosomes and they do not pair with the A-chromosomes during meiosis (Figure. 7-8). El-Nahas et al. [13] while doing the karyology of Solanaceous species, found the presence of B-chromosomes in *Nicotiana alata.* This shows that B-chromosomes are present in genus *Nicotiana.*

Nymphoides cristata (Roxb.) Kuntze

It is an aquatic plant of family Menyanthaceae. The plant bears small white colored flowers. A cluster of tuberous roots are attached underside the floating leaves. These roots are used to cure Jaundice [14]. The chromosome study depicts n=9 chromosomes at metaphase stage of meiosis (Figure. 9). Li et al. [15] studied the cytology of five species of *Nymphoide*s and reported that *N. hydrophylla* (Synonyms *N. cristata*), and *N. indica* were diploids with 2n=18, *N. lungtanensis* is triploid with 2n= 27 and *N. coreana* is tetraploid with 2n= 36 chromosomes. This has corroborated with the presently studied chromosome number for this species. It has also been confirmed that the presently studied species is diploid in nature.

Papaver rhoeas L.

Papaver rhoeas is a member of family Papaveraceae. It grows wild in agriculture fields, along road sides and at waste lands. But it is also grown as an ornamental plant because of its red colored beautiful flowers. The flowers and leaves are used as tonic, anti anemic and aphthae for children [16]. Meiotic observations made on large number of pollen mother cells showed that the species is diploid with n=7 chromosomes at metaphase stage (Figure. 10-11). This observation is in accordance with the findings of Asghari et al. [17]. Majority of PMCs have rod shaped bivalents except one which is ring shaped (6 rods + 1 ring).

Raphanus sativus L.

Raphanus sativus is a vegetable of Brassicaceae family**.** It is a small herb bearing rosette of leaves. It is good for gastrointestinal problems, jaundice, kidney stone and piles. Chromosomal study has shown nine bivalents at metaphase-I stage (Figure. 12). Equal distribution of chromosomes at each pole during anaphase-I has been observed but in anaphase-II, non- segregation and unequal segregation of chromosomes were quite frequent. Zhang et al. [18] also recorded 9 bivalents at diakinesis stage in this species which strengthened our results. They also found some irregularities in spindle formation and as laggards.

Sesamum indicum L.

It is a member of family Pedaliaceae and have large bell shaped white flowers. It is cultivated as oilseed crop. Seed oil is used for the treatment of joint pains and in veterinary problems. The chromosomal study has indicated 13 bivalents arranged at the equatorial plate during metaphase-I (Figure. 13-14). The chromosomes are well separated and distinct which makes the study easy. Anaphase-I stage has witnessed the normal disjunction of chromosomes. Ashri [19] had also reported 2n=26 chromosomes in this species which confirms the present investigation.

Tinospora cordifolia (Willd.) Miers ex Hook.f.et & Thoms.

Tinospora cordifolia belongs to family Menispermaceae and is well known for its medicinal importance. Traditionally, the plant extract is used to cure, typhoid, malaria, joint pain, diarrhoea and cardiovascular problems. The meiotic study has presented 13 bivalents at diakinesis stage (Figure. 15). Meiotic abnormalities in the form of univalents have also been observed in many pollen mother cells. Mathew [20] while studying the members of family Menispermaceae, observed the same chromosome number in this species. This shows the genetic stability of the species since last five decades.

Triticum aestivum L.

Triticum aestivum is a cultivated wheat species of family Poaceae. It is one of the major staple food crops. Wheat flour is used in traditional remedies to cure burns, headache, cold and also given as body tonic to human and cattle. The meiotic study has revealed the presence of 21 bivalents at diakinesis (Figure.16) and metaphase stages of meiosis. Normal segregation of chromosomes during anaphase-I has been observed in some pollen mother cells (Figure.17). Regular course of meiosis at metaphase and anaphase stages is an indicator of fully fertile plant which ultimately enhances the crop yield. The basic chromosome number in genus *Triticum* is x=7. Hence the presently studied species is hexaploid in nature with 2n=6x=42 chromosomes. The same chromosome number has also been studied previously [21; 22].

Vitex negundo L.

It is a shrub or small tree of family Verbenaceae. It is commonly known as five-leaved casted tree**.** The leaves are used to cure gastrointestinal problems in cattle, feet swelling, wound and also act as an antidote for snake bite**.** During meiotic study, 17 bivalents have been found in many pollen mother cells. In some pollen mother cells, during diakinesis stage, all chromosomes are well separated and clearly seen except three chromosomes which are not well separated (Figure.18-19). Sandhu and Mann [23] reported the haploid chromosome number n=16 in this species. Later on Kathoon and Ali [24] recorded 17 bivalents. This shows that both n=16 and n=17 are prevalent in *Vitex negundo*. The presently studied population is in agreement with the one of the earlier report.

Xanthium strumarium L.

It is a weed growing on waste places, agricultural fields and along road sides. Unlike other members of family Asteraceae, the plant bears male flowers at the terminal position and female flowers in the axils of branches. The roots, fruits and seeds are used to cure stomach problems, small pox and also act as demulcent [25]. The presently studied population of this species is diploid with n=18 chromosome at diakinesis stage (Figure. 20). Equal segregation of chromosomes has been observed at anaphase stage. These findings are in conformity with Singhal et al. [26] who also recorded the same chromosome number in this species from Mandi district of Himachal Pradesh.

Zea mays L.

The maize is an annual grass of family Poaceae and is one of the major cereal crops in the world. The plant is monoecious unisexual with terminal raceme of male flowers and axillary female inflorescence. Maize flour is used for skin problems, diarrhoea and jaundice by the natives of study area. The diakinesis stage of meiosis has well separated and clear 10 bivalents (Figure. 21-22). Most of the bivalents are of ring shaped. Fisk [27] reported the same number of chromosomes for this species which is in accordance to the present study.

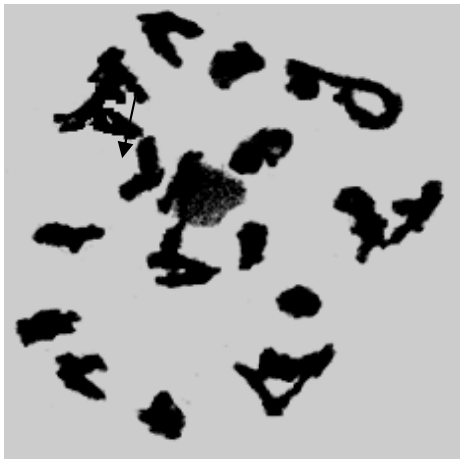


Figure. 1

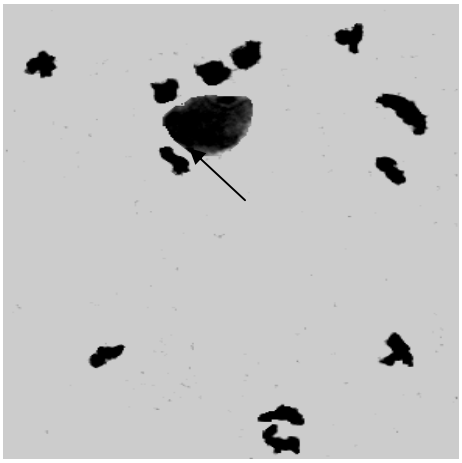
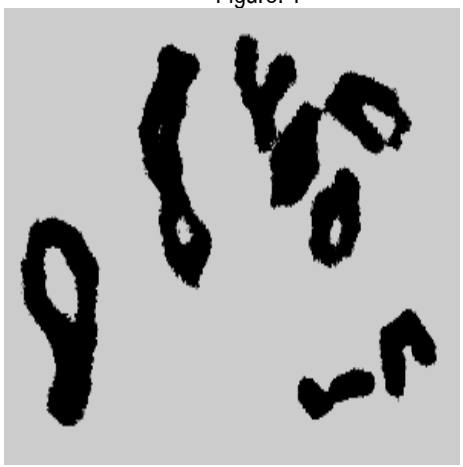


Figure. 2



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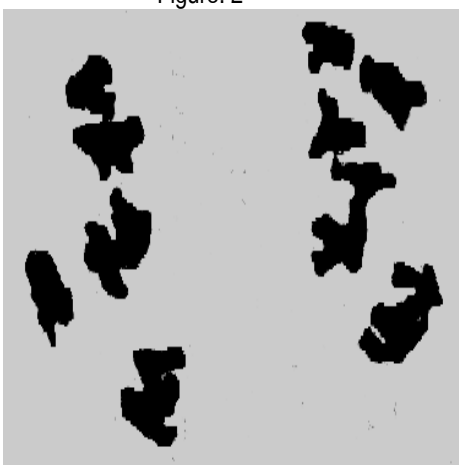
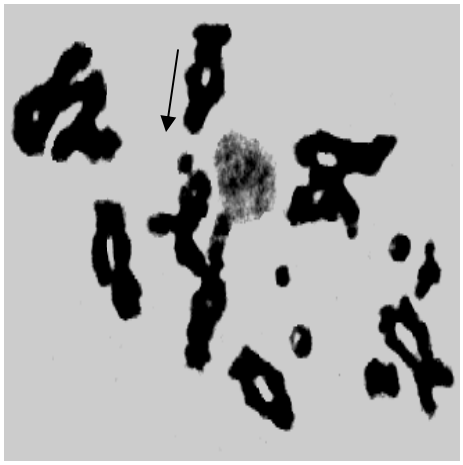


Figure. 4

Figure. 5

Figure. 6



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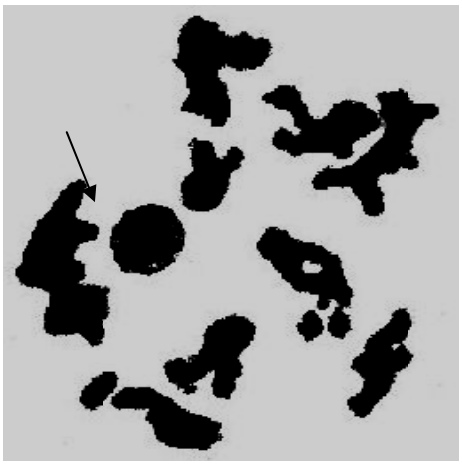

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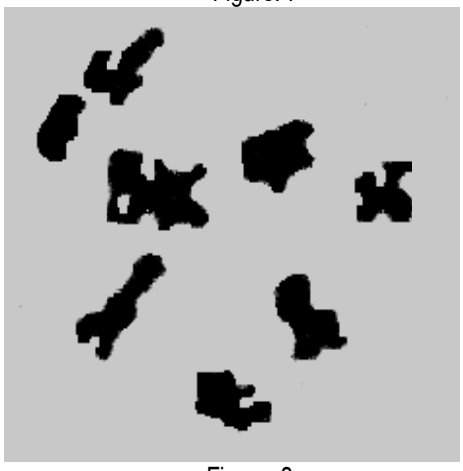


Figure. 9

Figure. 10

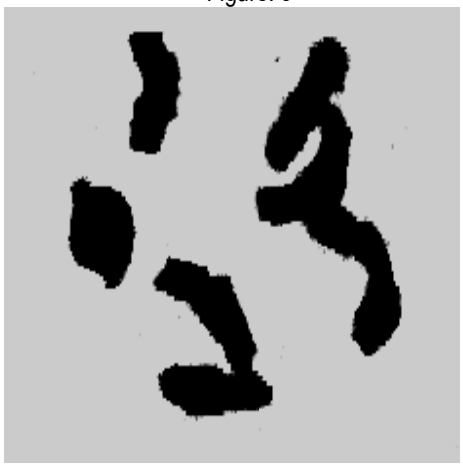
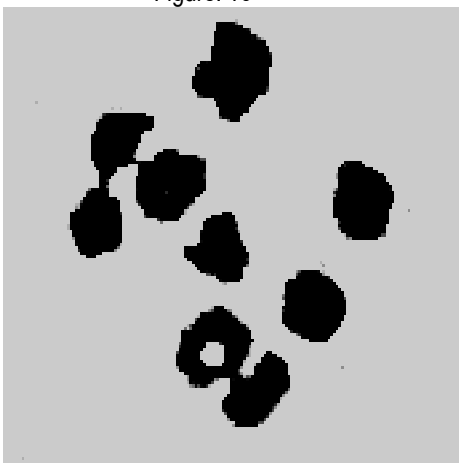


Figure. 11



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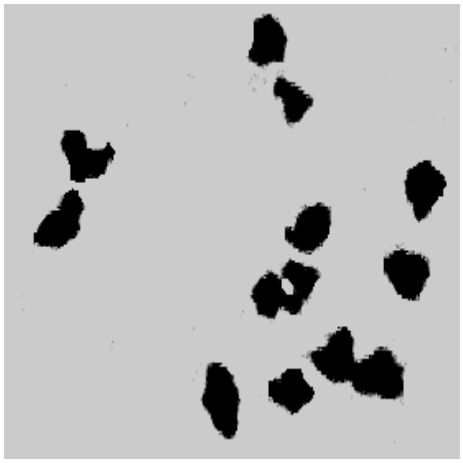

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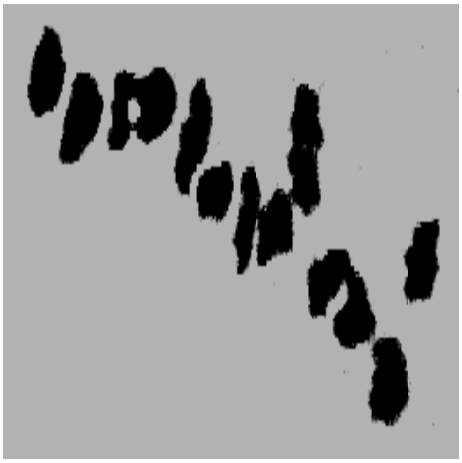

Figure. 14

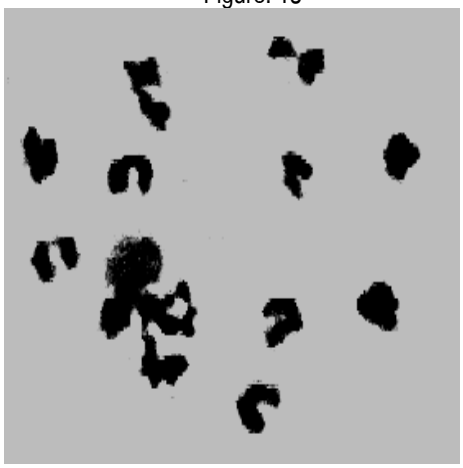

Figure. 15

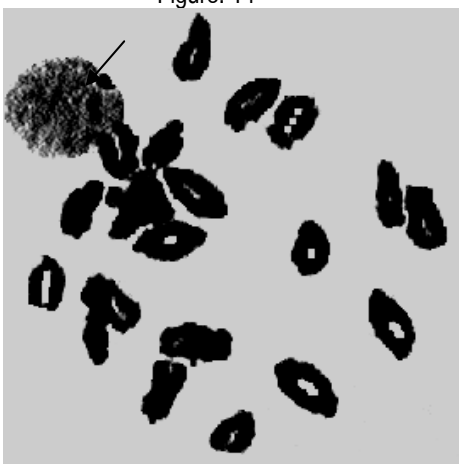

Figure. 16

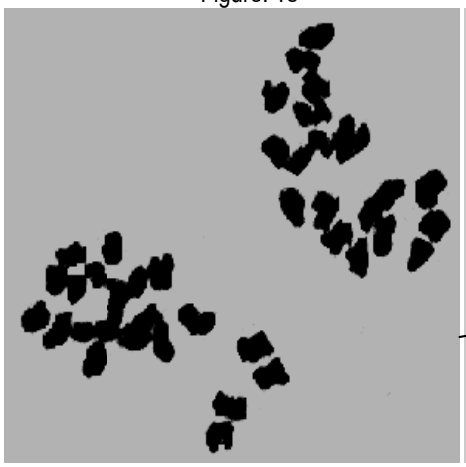

Figure. 17

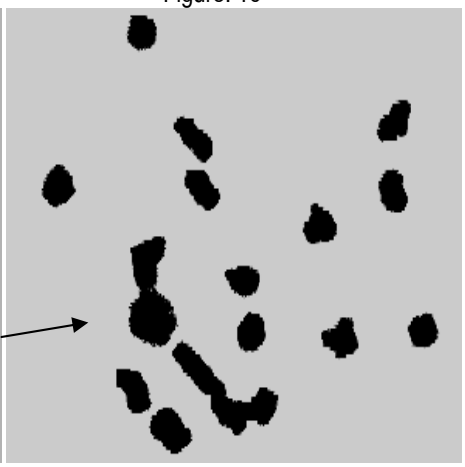

Figure. 18

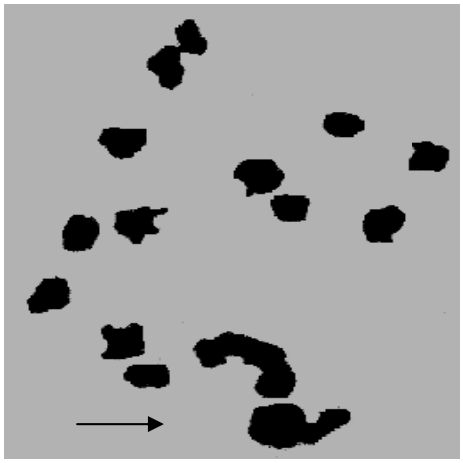

Figure. 19

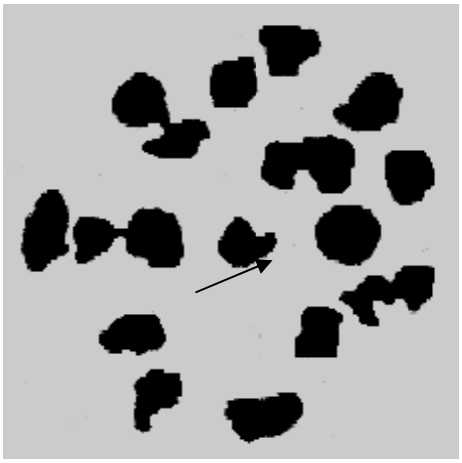

Figure. 20

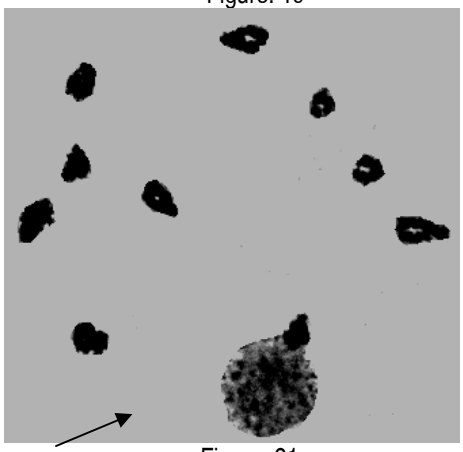

Figure. 21

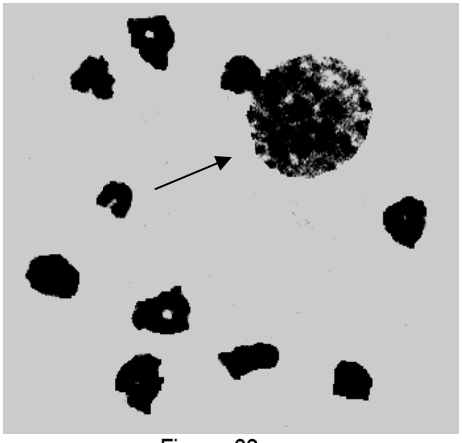

Figure. 22

Figs. 1-22: (1) *Basella rubra* n=22^{II} at Late diplotene. (2) *Coccinia cordifolia* n =12^{II} at diakinesis. (3) *Delphinium ajacis* n=8^{II} at metaphase-I. (4) *Lens culinaris* n= 7^{II} at metaphase-II. (5) *Melilotus alba* n=8^{II} at diakinesis. (6) *Nicotiana plumbaginifolia* n=10^{II} at early diplotene and (7-8) showing supernumerary B chromosomes. (9) *Nymphoides cristata* n= 9^{II} at metaphase-I. (10-11) *Papaver rhoeas* n=7^{II} at metaphase-I. (12) *Raphanus sativus* n=9^{II} at metaphase-I. (13) *Sesamum indicum* n=13^{II} at metaphase-I and (14) arranged on equatorial plate. (15) *Tinospora cordifolia* n=13^{II} at diakinesis. (16) *Triticum aestivum* n= 21^{II} at diakinesis and (17) at anaphase-I. (18-19) *Vitex negundo* n= 17^{II} at diakinesis. (20) *Xanthium strumarium* n= 18^{II} at diakinesis. (21-22) *Zea mays* n= 10^{II} at diakinesis.

Conclusion

Meiotic studies have been carried out in 15 plant species belonging to 13 families for their chromosome numbers and behavior. The course of meiosis is regular in all the species except few cases where some meiotic irregularities have been reported in the form of univalents and B-chromosomes. The chromosome number of these species varies from n=7 to n=22. The chromosomes were very small in *Coccinia grandis* whereas, *Triticum aestivum* chromosomes are comparatively large in size. Out of 15, one species is hexaploid (*Triticum aestivum*) and another one is tetraploid (*Basella rubra*). The remaining 13 species are diplolid in nature. The present findings related to chromosome number in these species tallies with the earlier reports. However behavioral changes have been observed in some cases. This constancy of chromosome number in these species has confirmed the genomic stability.

Authors' Contributions

Kuljinder Kaur carried out the meiotic study and wrote the manuscript. Dr. M. C. Sidhu has made interpretation of data and drafted the manuscript. All authors have read and approved the final manuscript.

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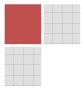

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