Journal of Pharmacognosy and Phytochemistry
Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
www.phytojournal.com
JPP 2020; 9(5): 549-553
Received: 18-06-2020
Accepted: 20-07-2020

Anu Bala
Ph.D. Research Scholar, Department of Floriculture and Landscaping, College of Horticulture and Forestry, PAU, Ludhiana, Punjab, India

Madhu Bala
Assistant Floriculturist, Department of Floriculture and Landscaping, College of Horticulture and Forestry, PAU, Ludhiana, Punjab, India

Vikrant Khare
Ph.D. Research Scholar, Department of Plant Breeding and Genetics, College of Agriculture, JNKVV, Jabalpur, Madhya Pradesh, India

Corresponding Author:
Anu Bala
Ph.D. Research Scholar, Department of Floriculture and Landscaping, College of Horticulture and Forestry, PAU, Ludhiana, Punjab, India

A review on cytological study in *Chrysanthemum* species

Anu Bala, Madhu Bala and Vikrant Khare

Abstract
Chrysanthemum produce showy flowers with different colors that make it highly suitable for pot culture, bedding purpose and for production of loose flowers that can be used for making garland, worship and for decoration purposes and thus it occupies a place of pride both as commercial crop and popular exhibition flower. As per ploidy level consideration, correlations exist between and the mode of reproduction of any organism. Cytological changes lead to change in flowering and reproductive behavior. Determination of the ploidy level of plants is important for identification of species, for the selection of parental lines, for finding the relationships between species and for determining evolutionary patterns.

Keywords: Chrysanthemum, chromosomes, cytological, varieties

Introduction
Chrysanthemum (*Chrysanthemum morifolium* Ramat.) is one among the important flower crops belonging to family Asteraceae commonly known as ‘Autumn Queen’ or ‘Queen of East’. The word ‘chrysos’ means ‘the gold’ and ‘anthemum’ means ‘flower’. There is hardly any other flower crop which has such diverse and beautiful range of flower colors, shape, form, size and height as present in chrysanthemum. In south-east Asia and European countries, its abundance of diversity in flower type and colour and plant architecture makes it to occupy a considerable proportion in flower industry. It is native to Northern hemisphere, chiefly Europe and Asia [1]. It is generally believed that the specie is a hybrid complex derived from the chance hybridization that naturally occurred between species as follows *Chrysanthemum vestitum*, *Chrysanthemum indicum*, *Chrysanthemum lavandulifolium* and *Chrysanthemum zawadskii* [4, 5, 30]. Major chrysanthemum cultivating countries are Columbia, China, Japan, USA, UK, Holland, Norway, Eucador and Canada. It ranks 3rd after rose and carnation in international cut flower trade. In India chrysanthemum is among the most commercial used flowers and it falls in category of growing traditional flowers and major chrysanthemum producing states of India are Tamil Nadu, Karnataka, Andhra Pradesh, Madhya Pradesh, Himachal Pradesh, West Bengal, Maharashtra, Assam, Telangana, Chhattisgarh, Haryana, Meghalaya and Jammu & Kashmir. The area under chrysanthemum cultivation in India in 2016-17 was 20.55(000 hectare) with loose flower and cut flower production 188.81(000MT) and 15.38 (lacs no.) respectively.

As per the ploidy level of chrysanthemum many statements and reports are given by different authors. Cultivated chrysanthemums are allohexaploid (2n=6x=54) with basic chromosome x=9 and somatic chromosome numbers range from 2n=47-63 and 2n=36 to 45, 47, 51-57 [8, 22]. There is another report that chrysanthemum constitutes a polyaneuploid complex having arisen from a complex hexaploid Chinese species (chiefly two *C. indicum* L. and *C. sinense* Sabine) through repeated cycles of hybridization and selection extending for over a period of 2,500 years [6]. There is a wide range of ploidy variation among chrysanthemum germplasm, including diploids, tetraploids, hexaploids, octoploids, aneuploids and even pentaploids [25, 7, 8, 20, 21, 33, 9, 18].

Chromosomal study is important aspect as we all know chromosomes are the carriers of genetic material and changes in the number of chromosomes affect the morphology, anatomy, physiology, and biochemistry of an organism and lead to many changes in genetic characteristics. Chromosomes of chrysanthemum species often have different degrees of differentiation, both between and within species. Therefore, elucidation of ploidy of species provides foundation for understanding its genetic background, which further will be helpful for germplasm conservation and other applications [2]. The perennial species of chrysanthemum have a number of polyploid cytotypes, whereas annuals are mainly diploid in nature. Many of these diploids are self-incompatible and heterozygous for reciprocal translocations [24, 10].

Polyploids play an important role in cross hybrids as they are closely related to each other and it further plays an important role in chromosome evolution and would perform introgressive nature of hybridization [18].

Results and Discussion

Ploidy level determination by different methods

Different methods are used to check the ploidy level of crops. It can be determined directly by direct chromosome counting or through indirect means. Macroscopic to microscopic different ploidy assessment methods are available. Determination of ploidy level by various means as stomata size, pollen grains, pollen mother cells, root tips, chloroplast number in guard cells and through morphological observations. In chrysanthemum flower different methods has been used by different researchers. In this review paper, we have tried to compile all work done on ploidy determination till date in chrysanthemum.

Root tip and pollen mother cells

Chromosome counting done directly by counting the number of chromosomes in mitotic and meiotic cells. Mitosis occurs in somatic cells and chromosomes present in somatic cells are termed as somatic chromosomes and somatic chromosome number is represented by 2n. On the other hand, all sexually reproducing organisms undergo meiosis at the time of spore formation and as a result cells formed having haploid (n) chromosome number. Following cytological studies are made by different authors through root tip and pollen mother cells (PMCs) which are compiled as:

Cytological studies were made from both root tip and bud materials from the clones of *Chrysanthemum yoshinaganthum* collected from three different locations [26]. In this study plants used were grown and maintained in pots and a total of 96 clones collected were studied cytologically. Meiosis study of all clones was done and all clones showed similar meiotic behaviour in PMCs and found to be 2n=36 but variation in karyotypes found in material collected from three locations and 2n=36 chromosomes were further classified into 18 pairs, five pairs of which were satellite chromosomes. Author stated that among the different species *Ch. boreale* (2n=18), *Ch. Makinoi* (2n=18), *Ch. nipponicum* (2n=18), *Ch. lineare* (2n=18), *Ch. rupestre* (2n=18), *Ch. vulgare* (2n=18), *Ch. indicum* (2n=36) and *Ch. wakasaense* (2n=36), *Ch. Yoshinaganthum* was found to closely resemble *Ch. Makinoi* in characteristics of karyotypes and external morphology. Therefore *Ch. yoshinaganthum* is presumed to be autotetraploid which originated from *Ch. Makinoi*.

Natural hybrids *Ch. yoshinaganthum* x *Ch. indicum* were also made and a total of 31 clones of the natural hybrids were studied for meiotic behaviour. It was also observed that leaves of the hybrids varied in form ranging from ovate-cuneate to ovate. Most clones of the natural hybrids were morphologically intermediate between *Ch. yoshinaganthum* and *Ch. indicum*, and some clones either resembled *Ch. Yoshinaganthum* or *Ch. indicum*. Eighteen clones of the hybrids were studied cytologically. Sixteen clones were found to be 2n=36 and two were found to be 2n=34. Karyotypes were studied in 16 clones and variation in karyotypes was also recorded.

Study on physiological characteristics associated with chromosomal structure in *Chrysanthemum carinatum* carried out as it was found that there was need to find out, to what extent differential viability or fertility of the homozygous and the heterozygous individuals can account [23]. He used flower buds as a meterial for meiotic studies which were fixed in Carnoy's fixative mordanted with ferric chloride and anthers were squashed in acetocarmine to study the number of chromosomes. Cytological observations on several populations of *Chrysanthemum carinatum* revealed the presence of two types of plants: those regularly forming nine bivalents (homozygotes or the rearranged karyotype) and other showing a multiple of four chromosomes along with seven bivalents (interchange heterozygotes). These two chromosomal type plants were compared with regard to their reproductive potential and it was observed that the interchange heterozygotes produce more flowers and seed per plant than the homozygotes. It was concluded by authors that, the predominace of interchange heterozygotes in populations is determined primarily by their superior reproductive fitness.

An attempt was made to investigate the chromosomal complement and behaviour of certain compositae available locally at Gorakhpur, which is situated in Eastern U. P. In this study a total number of 21 taxa belonging to 16 genera and 17 species studied cytologically. Among 17 species *Chrysanthemum coronarium* Linn. Was also studied which found as a weed in the spring flowering beds. The chromosomes of *Chrysanthemum coronarium* found were much bigger in size than in other taxa studied, showing an aberrant cell with large number of chromosomes. Anaphase I showing 9 chromosomes at each pole and at anaphase II, distribution of chromosome was regular and no lagging chromosomes or bridges were observed. In *Chrysanthemum coronarium* Linn multivalents, particularly quadrivalents and hexavalents were commonly found and pollen fertility was observed normal suggesting uniform alternate disjunction [12].

Studies on the control of diploid-like meiosis carried out in polyploid taxa of chrysanthemum hexaploid *Ch. japonense* Nakai and in meiosis study of *Ch. japonense* (Saka strain) 2n=6x=54 chromosome number found [31]. Crosses were made between hexaploid *Ch. japonense* (Saka strain) and diploid *Ch. Boreale* and eight F1-hybrids were obtained with ploidy level 2n=4x=36 and one with 2n=4x+1=37 and seven F2-hybrids with 2n=4x=36 ploidy level. F1-hybrids were partially fertile 0%-1.1% in interbreeding and 0.3%-59% in intrabreeding. Triploid B1-hybrids were obtained to diploid *Ch. boreale*, and on backcrossing to tetraploid F2-hybrids obtained by intrabreeding. Out of nine in eight B1-hybrids 2n=3x=27 ploidy level found. These B1-hybrids with 2n=3x=27 were partially fertile 0%-0.2% in interbreeding and 0%-3.7% in intrabreeding and gave rise to B2-hybrids with 2n=24, 26, 27 and 36 on backcrossing to the diploid *Ch. boreale* and progenies with 2n=30, 32, 33, 34, 35 and 36 chromosome number were found by intrabreeding.

Nineteen out of twenty F3-hybrids obtained with chromosome number 2n=4x=36 and the twentieth with 2n=4x+1=37 and eleven F3-hybrids with 2n=4x=36. In *Ch. japonense* (Nakamura strain) with 2n=6x=54. Author reported that in hexaploid *Chrysanthemum japonense* Nakai genetic stabilization of diploid-like meiosis occurs and in Fl-hybrids between diploids and hexaploids homoeologous chromosome pairing from different base sets derived from the parental polyploidy also observed. Homoeologous pairing was observed in the B1-hybrids. In tetraploid-hybrids most of chromosomes formed either bivalents or quadrivalents, and the frequency of trivalents and univalents was extremely low. In most of triploid, however, approximately two-thirds of the chromosomes form trivalents. A gradual increase of quadrivalent formation from F1 to F3 was observed. The average frequencies of quadrivalents was extremely low in spite of the fact that all sets of corresponding chromosomes were capable of associating as quadrivalents in the tetraploid hybrids.

Chromosome number of 183 cultivars of chrysanthemum studied with ploidy ranging from 2n= 36, 45, 53-60, 62-65, 67, 68 and 72 [22]. They reported that there is no correlation between the grade of ploidy and capitulum size, and the taxon as a whole is outbred, supplemented by the sporophytic incompatibility. Detailed analysis of the chromosome complement also reveals that there was decrease in size of chromosome with an increase in grade of ploidy. Pollen stainability degree was high might be due to high polyploidy nature of taxa.

Autotriploid of *Chrysanthemum coronarium*, a winter ornamental with considerable structural heterozygosity, was obtained from the seeds of open pollination of induced autotetraploid [13]. The autotriploids found with luxuriant vegetative growth with lesser number of large sized yellow radiate capitula in comparison to diploids and auto-tetraploids. Leaves and stomata in the autotriploid show appreciable increase, on the other hand there was decrease in stomatal frequency. Pollen size found increased with the polyploidy which was slightly larger in triploid in comparison to tetraploids and in triploids there was no viable seed formation. The chromosomal associations observed were highly varied which are represented from heptavalent to univalent, in none of the diakinesis and M-I and nine trivalents were observed. Anaphase I of autotriploid was quite abnormal due to the presence of laggards and irregular distribution of chromosomes. They compare autotriploid with diploids and revealed that in spite of large chromosome size and high chiasma frequency in the diploid, only 55% chromosomes were involved in multivalents and of the trivalents, ‘chain’ type chromosomes were the most common. The triploids in spite of 56% pollen fertility, completely lacks achene formation. Microsporogenesis found quite abnormal due to the partial or complete failure of cytokinesis in 33.3% PMCs observed in study. They suggested that maintenance of triploid cannot be possible for the species which lacks vegetative multiplication.

Annual chrysanthemum (*Chrysanthemum carinatum*) chromosome number of 2n=18 studied to find out the chromosomal aberrations [29]. Material used by them was root tips of annual chrysanthemum and this material was easily available, easy to grow and handle, has very high frequency of dividing cells per root tip, large and intensely stained chromosomes and high frequency of chromosomal aberrations. In this study procedure followed was as, seeds were harvested and germinated on filter paper. After two days from germinated seeds about 1cm long primary root tips, pretreated with 0.02% aqueous solution of colchicine for 3hr at room temperature and were fixed in a freshly prepared fixative (1:3 acetic acid: absolute alcohol). Next day root tips were hydrolyzed in N HCl at 60 ºC for 10min, stained in leucobasic fuchsin and squashed in 1% acetocarmine. The main object of the present study was to find out suitable material, other than the traditionally used ones, for the study of radiation induced chromosomal aberrations and the most significant being the recovery of unusually large number of clear dividing cells and scorable chromosomal aberrations, facilitating authentic determination of dose-response and qualitative analysis of various categories of aberrations. This study provided a new cytogenetic system in which various theories of induced chromosomal aberrations can be tested.

Chinese 30 large flowered pot chrysanthemum cultivars studied karyotypically, differing with respect to flower type, petal type, and flower colour [3]. They found that interphase nuclei and prophase chromosomes of all the cultivars respectively were, of complex chromocentre and interstitial type. Somatic chromosome number of 30 varieties varies from 49 to 62 and most were lying in the range 51-56 or 58. Most of the cultivars showed chromosomal mosaics, with three showing 2n = 6x = 54, and two 2n +1= 6x + 1 = 55. At mitotic metaphase, most of the chromosomes are of the metacentric or submetacentric type, with a small number of acrocentrics and telocentrics.

Artificial crosses between Japanese, octoploid *Chrysanthemum shiwogiku* Kitamura and *Ch. vestium* chinese, hexaploidy were made and through hybridization 48 hybrids were produced and 181plants germinated grown upto mature stage. The hybrids in morphological character showed more or less intermediate type plants between the two parents and found more close to *Ch. vestium* as white color ligule flowers inherited from this spp. Chromosome number of 48 hybrids were studied and out of 48 in 19 hybrids ploidy level varied from 2n=63-81with various aneuploids and in this study some individuals showed very small chromosomes. From this study it was suggested that hybrids can produce back cross progenies after hybridization with *C. vestium* as parents and can hybridize with sister plants of the F_1 hybrids. Based on chromosome number hybrid plants categorized into five groups: One individual having 2n=63, one individual with 2n=67, eight individuals with 2n= 72, one individual with 2n= 78 and two individuals found with 2n=81. Based on the present study it was revealed that, polyploidy nature of *Chrysanthemum sensu* stricto and its closely related genera *Chrysanthemum sensu* lato are more complex as self-incompatibility and sexual reproduction present. From the results it was concluded that polyploid hybrids might involve several mutations as deletion, translocation or inversion of genes in their genetic composition. Mutations might be very helpful for hybrid survival. By producing high polyploidation level or by additional chromosomes in aneuploid progenies may compensate the existence of such kind of mutations. In other words, mutations are helpful for the survival of hybrid population [28].

The chromosome number of 40 cultivars of chrysanthemum studied and it was reported that chromosome number of cultivars varied from 44 to 72. Authors mentioned that 27 (67.5%) cultivars were hexaploid (2n = 6x = 54), based aneuploidy (51–59, 55 on average), 9 (22.5%) cultivars were heptaploid (2n =7x = 63)-based aneuploid (60–67, 63 on average), and one cultivar was octoploid (2n=8x=72): *Chrysanthemum morifolium* “Feitian”. The remaining three cultivars (7.5%) were pentaploid (2n = 5x =45) on this basis aneuploid (44–50, 48 on average): *C morifolium* “Lijin” (44), *C. morifolium* “Jinhongmaoci” (46) and *C. morifolium* “Ziyanfanfei (50). They also studied coefficient of karyotypes and found that there was no obvious difference in the asymmetry coefficient of karyotypes, but the discrepancy in the variance of karyotype asymmetry index and relative length of chromosomes was quite distinct. In terms of karyotype parameters, the petal types of chrysanthemums were classified to five groups as flat, tubular, spoon, abnormal, and anemone. They did not observe any obvious orderliness among flower head types, while they considered the relationship between karyotype parameters and phenotypic characters, variation of long-/short-arm ratio and asymmetry coefficient of karyotypes had the greatest relevance toward most phenotypic characters. From this study it was indicated that karyotype parameters possess great values for cultivar identification, classification, and genetic analysis in chrysanthemums [32].

Detailed cytological studies on seven ornamental species of chrysanthemum were conducted. The species studied by them include namely, *C. carinatum* (2n=18), *C. cinerarifolium* (2n=18), *C. coronarium* (2n=18), *C. leucanthemum* (2n=36), *C. morifolium* (2n=36), *C. paludosum* (2n=18) and *C. segetum* (2n=36) [15]. They firstly reported the chromosomal study for *C. paludosum* species. Diploid species involving variable number of chromosomes in reciprocal translocations are mostly heterozygous for chromosomal alternations. In the present study, attempt was made to study detailed meiotic behaviour and chiasmata frequency in *Chrysanthemum* species. Authors found that large-sized, ring-shaped bivalents accommodate two chiasmata each, and multiple associations are also mostly ring-shaped with distal localization of chiasmata. The researchers stated that presence of multivalents leads to unbalanced chromosomal distribution with formation of laggards and abnormal microsporogenesis, which results in low pollen fertility. In the *C. morifolium* meiotical analysis, it was found that specie had 2*n*=36 chromosome number with a lot of structural heterozygosity in PMCs along with the formation of quadrivalents (0.75), trivalents (0.08) and univalents (0.41) besides bivalents (16.01) in each PMC. From this study, it has been made clear that the annual cultivated species of *Chrysanthemum* with distal localization of chiasmata and large size of the chromosomes were well adapted to structural changes, particularly reciprocal translocations. From the study, it has been suggested that for the improvement of these ornamental species, induced chromosomal aberrations and mutations should be considered rather than auto polyploidys and due to self-incompatibility, the interspecific hybrids among these *Chrysanthemum* species to be tried for improvement of their floriculture value.

Studies on meiotic behaviour, chromosome pairing and chiasma frequency were performed on 12 species of Chrysanthemum belongs to family Asteraceae [16]. All the species studied by them based on x=9, the commonest base number of the genus. In this study the record of 2n=36 for *C. maximum* is a new tetraploid cytotype for the species and *C. koreanum* (2n=54) and *C. paludosum* (2n=18) were the two species studied cytologically for the first time. Chromosomal counts on Indian accessions of species *viz*. *C. coccineum*, *C. indicum*, *C. multicaule*, *C. pyrethrum* and *C. segetum* were made for the first time. Chiasma frequency ranges from 1.80-1.99 per bivalent in diploids, 2.47-3.01 per bivalent in tetraploids and 1.37 in hexaploidy and multivalents were noticed in both diploids and tetraploids.

Genome differentiation, hybridity and relationship was investigated in an interspecific hybrid produced by crossing between *Chrysanthemum latifolium* (2n= 36) and *C. grandiflorum* Ramat. Cv. ‘Red Betty’ (2n=54) by [27]. Ploidy level of F_1 hybrid was checked through GISH and FISH method and it was found with ploidy level 2n=45. It was revealed from this study that 27 chromosomes of hybrid were inherited from the *Chrysanthemum latifolium* and 18 could be the characteristics of *C. grandiflorum* Ramat. Cv. ‘Red Betty’ nine out of 27 chromosomes were homologous chromosomes of the two genomes *Chrysanthemum latifolium* and *C. grandiflorum* Ramat. Cv. ‘Red Betty. From this study it was suggested that interspecific hybridization between chrysanthemum cultivars and wild species of chrysanthemum is an effective way for the development of new strain.

Study on cytomorphological diversity in some members of family Asteraceae carried and cytomorphological survey was done, including meiotic studies and chromosome counts in 42 species under 93 accessions collected from the various localities of Solang Valley in Kullu district, Himachal Pradesh. The material collected from different altitudes ranging 2,400 to 3,100 m. They studied *Chrysanthemum leucanthemum* chromosome number. Based on *x*=9, the individuals studied by them exist at 4*x* level and these individuals showed the presence of multivalents, laggards, chromatin bridges and abnormal microsporogenesis with the formation of dyads, triads, polyads and sporads with micronuclei and consequently reduced pollen fertility i.e. 68% [17].

Cytology of polyploidy induced plants

Cytological and morphological screening was made on the different cultivated material of *Chrysanthemum coronarium* Linn [14]. As the species is highly heterozygous and has good prospectives in floriculture, it was considered by authors to induce autopolyploidy. The purpose was also to produce trisomics and to study their cytogenetics. As a result of colchicine treatment followed by studies on the generation, autotetraploids (2n=36), autotriploid (2n=27), hypertriploids (2n=28 and 30), trisomics (2n=19 and 20), and mixoploid (2n=16, 18) individuals were produced. In current study autotetraploids were studied further for various characters and following points were presented. Autotetraploids formed found inferior and do not show any increase in size of its parts. Meiosis of autotetraploids characterised by multiple associations ranging from octavalent to univalents. The results revealed that there was moderate frequency of multivalent formation, but there was tendency for normal distribution at A-I and high pollen fertility, viable achenes were not observed.

Ploidy determination through flow cytometry

Flow cytometry is a simple, rapid, and highly accurate method for identifying ploidy levels in chrysanthemum species. Flow cytometry method was used to determine the ploidy level of 405 Chinese large-flower chrysanthemum (*Chrysanthemum morifolium* Ramat.) cultivars [11]. In this finding sixty-three cultivars found triploid, 175 cultivars tetraploid, 32 cultivars pentaploid, 46 cultivars hexaploid and 1 cultivar found heptaploid. Forty-eight cultivars were then randomly selected by authors for confirmation by chromosome-counting. Most of the cultivars were found aneuploid. High % age of tetraploid and triploid in this research represents the first evidence of low ploidy in large-flower chrysanthemum, which showed that a wider range of ploidy variation lies in this population.

Flow cytometry method was also used to analyze the ploidy levels of nine species of *Chrysanthemum* L. [17]. They reported three diploids corresponded to *Chrysanthemum lavandulifolium* and two species of *C. nankingense,* on the other hand three tetraploids related to *C. indicum* and two species of *C. chanetii*. Two hexaploids corresponding to *C. vestitum* and *C. zawadskii*. In this study no significant differences among the same species of Chrysanthemum collected from different locations with respect to ploidy level were found which indicates that ploidy level is not associated with geographic regions.

Conclusion

From above reviews following conclusions has been made. Ploidy level affects many factors like pollen viability, size of chromosomes, pollen size, viability of achenes and polyploidy of population affected by the self-incompatibility. Mutations at chromosomal level helpful for the survival of population. Presence of multivalent lead to unbalanced chromosomal distribution with formation of laggards and abnormal micro-sporogenesis resulting in low pollen fertility.

References

1. Anderson RL. Reclassification of genus Chrysanthemum, Hort Sci. 1987; 22:313.
2. Bennett MD, Leitch IJ. Plant genome size research: A field in focus, Ann Bot. 2005; 95:1-6.
3. Chang LI, Chen S, Chen F, Zhen L, Fang W. Karyomorphological studies on Chinese pot chrysanthemum cultivars with; large inflorescences, Agricultural sciences in China. 2009; 8(7):793-802.
4. Chen FD, Chen PD, Fang WM, Li HJ. Cytogenetics of F1-hybrids between two small-headed cultivars of *Dendranthema* x *grandiflorum* and two wild *Dendranthema* species, Acta Hortic Sin. 1998; 25:308-309.
5. Dai SL, Chen JY, Li WB. Application of RAPD analysis in the study on the origin of Chinese cultivated chrysanthemum, Acta Bot Sin. 1998; 40:1053-1059.
6. Darlington CD. Chromosome botany and origins of cultivated plants, George Allen and Unwin Ltd, London, 1973, 161.
7. Dowrick GJ. The chromosome of Chrysanthemum (I). Heredity. 1952; 6:365-75.
8. Dowrick GJ. The chromosomes of chrysanthemum (II). Garden varieties. Heredity. 1953; 7:59-72.
9. Fukai S, Zhang W, Goi M. Some *Dendranthema* species native to Japan. Acta Hort. 1998; 454:85-90.
10. Gill BS, Gupta RC. Structural hybridity in *Chrysanthemum coronarium* L. In: Manna GK, and Sinha, U. (eds.), Cytology and Genetics. 1981; 3:523-530.
11. Guo X, Luo C, Wu Z, Zhang X, Cheng X, Huang C. Polyploidy levels of chinese large-flower chrysanthemum determined by flow cytometry, African Journal of Biotechnology. 2012; 11(31):7789-7794.
12. Gupta PK. Cytological investigations in some Indian compositae, *Cytologia*. 1969; 34:429-438.
13. Gupta RC, Gill BS. Cytomorphology of Induced Autotriploid (2n-27) in *Chrysanthemum coronarium* Linn. *Cytologia*. 1984; 49:629-633.
14. Gupta RC, Gill BS. Induced autotetraploidy in *Chrysanthemum coronarium* Linn. Cytologia. 1985; 50:117-123.
15. Gupta RC, Bala S, Sharma S, Kapoor M. Department of Botany, Punjabi University, Patiala-147002, Punjab, India, Chromosome Botany. 2013; 8:69-74.
16. Gupta RC, Bala Santosh, Suruchi. Cytogenetical Studies in Seven Ornamental Species of Chrysanthemum (*Asteraceae*), Cytologia. 2013; 78(4):439-48.
17. Kaur M, Singhal VK. Cytomorphological diversity in some members of family Asteraceae from the ecologically disturbed habitats of Solang valley, Kullu District, Himachal Pradesh Cytologia. 2015; 80(2):203-222.
18. Kondo K, Abd El-Twab MH, Idesawa R, Kimura S. Genome phylogenetics in Chrysanthemum *Sensu lato*. In: Plant Genome Biodiversity and Evolution, Science Publishers. 2003; 1:117-200.
19. Ma YP, Wei J, Yu Z, Qin B, Dai S. Characterization of ploidy levels in *Chrysanthemum* L. by flow cytometry J For Res. 2015; 26(3):771-75.
20. Nakata M, Hong DY, Qui JZ, Uchiyama H, Tanaka R, Chen SC. Cytogenic studies on wild chrysanthemum sensu lato *in* china, a natural hybrid between *Dendranthema indicum* (2n=54) from Hubei Province, J Jap Bot. 1992; 62:92-100.
21. Nakata M. Species of wild Chrysanthemum in Japan: cytological and cytogenetical view on its entity, Acta Phytotaxon Geo bot. 1987; 38:241-59.
22. Nazeer MA. Khoshoo, Cytogenitical evolution of garden chrysanthemum, Curr Sci. 1982; 51(12):583-85.
23. Rana RS. Monosomic interchange heterozygote of diploid Chrysanthemum, Nature. 1965; 206:532-533.
24. Rana RS. Physiological Characteristics Associated with Chromosomal Structure in *Chrysanthemum carinatum*. 1966; 31:160-165.
25. Tahara M. Cytological studies in Chrysanthemum (A preliminary note), Bot Mag Tokyo. 1915; 29:48-50.
26. Tanaka R. On the speciation and karyotypes in diploid and tetraploid species of *Chrysanthemum yoshinaganthum* (2n=36), *Cytologia*. 1960; 25:43-58.
27. Twab Kondo Chrysanthemum latifolium x C. *grandiflorum* cv. Red Betty crossed to new cultivars: Hybrid genome characterization and species relationship analyzed by FISH and GISH Chromosome Botany. 2014; 9:7-11.
28. Twab MHAE, Kondo K. Hybridity and relationship between *Chrysanthemum shiwogiku* Kitam. And *C. vestium* (Hemsl.) Stapf, *Chromosome Botany*. 2009; 4:65-70.
29. Verma RC, Chandel S. Annual Chrysanthemum: A new and novel material for the study of radiation induced chromosomal aberrations at mitosis, *Cytologia*. 1994; 59:339-343.
30. Wang BQ, Porter AH. An AFLP-based interspecific linkage map of sympatric hybridizing colias butterflies, Genetics. 2004; 168:215-225.
31. Watanabe K. Studies on the control of diploid-like meiosis in polyploid taxa of chrysanthemum hexaploid *Ch. japonense* Nakai, *Cytologia*. 1981; 46:459-498.
32. Zhang Y, Zhu ML, Dai SL. Analysis of karyotype diversity of 40 Chinese chrysanthemum cultivars, Journal of Systematics and Evolution. 2013; 51(3):335-352.
33. Zhou SJ, Wang JW. The cytological study on ten species of *Dendranthema* (in Chinese), J Wuhan Bot Res. 1997; 15:289-92.