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Ploidy study of chrysanthemum (*Chrysanthemum morifolium*) through PMC's method

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Chrysanthemum (*Chrysanthemum morifolium* Ramat.) is an important flower crop belonging to the family Asteraceae. It is commonly known as 'Autumn Queen' or 'Queen of East'. This flower is a leading flower cultivated for its versatile uses as cut flower, loose flower, pot culture and bedding throughout the world (Kaushal and Bala 2019). Chrysanthemum flowers have high market value because of different shapes, sizes, forms flower colour. In India, it is one of the commercial flower crop grown as traditional crop in Tamil Nadu, Karnataka, Andhra Pradesh, Madhya Pradesh, Himachal Pradesh, West Bengal, Maharashtra, Assam, Haryana and Jammu & Kashmir.

It is generally believed that the chrysanthemum species is a complex hybrid derived from the chance hybridization that naturally occurred between *Chrysanthemum viaticum*, *C. indicum*, *C. lavandulifolium* and *C. zawadskii* (Dai *et al*. 1998, Wang *et al.* 2004). Cultivated chrysanthemums are allohexaploid (2n=6x=54) with basic chromosome x=9; somatic chromosome numbers range from 2n=47 to 63 and 2n=36 to 45, 47, 51–57 (Dowrick 1953, Nazeer and Khoshoo 1982). Changes in chromosome number and size affect the morphology, anatomy, physiology, and biochemistry of an organism which leads to many changes in genetic characteristics (Bala *et al*. 2020) Chrysanthemum constitutes a poly aneuploid complex having arisen from a complex hexaploid Chinese species (chiefly two *C. indicum* L. and *C. sinense*) through repeated cycles of hybridization and selection over a period of 2,500 years (Darlington 1973). There is a wide range of ploidy variation including diploids, tetraploids, hexaploids, octoploids, aneuploids, and even pentaploids (Dowrick 1953, Kondo *et al.* 2003). Chromosomes often have different degrees of differentiation even between and within species (Bennett and Leitch 2005). In present study, thirty chrysanthemum genotypes were selected from germplasm maintained at Floriculture Research Farm, PAU, Ludhiana and pollen study was carried out at Department of Plant Breeding and Genetics, PAU, Ludhiana during 2019. Procedure adopted to study the ploidy level of selected chrysanthemum genotypes was through meiotic study of pollen mother cells. For chromosomal study, young flower buds of selected germplasm were collected from the plants in the morning hours from 7:00 a.m. to 8:00 a.m. Carnoy's solution-II with ethanol; chloroform; glacial acetic acid in the ratio of 6:3:1 was used as fixative (Sharma and Sharma 1965). Buds were kept in Carnoy's fixative for 48–72 h and afterwards buds were transferred in 70% ethanol. Buds in fixative medium were stored at room temperature; flower bud samples were shifted to a temperature of 20°C till they were not used for slide preparation. Anthers were crushed by gentle tapping to bring out the dividing cells in the acetocarmine solution and all debris containing somatic cells was removed with the help of needle from the acetocarmine smear. The prepared glass slides were observed under the compound microscope and fine and coarse adjustments were made. The whole slide was used to find out the right stage of development of dividing cells and number of chromosomes were also observed and counted.

A range of ploidy level was encountered in current study of selected chrysanthemum germplasm and found to be between 2n= 52-116 as shown in Table 1 and Fig 1. The results reveled that out of 30 genotypes, in four genotypes, viz. White Bouquet, Mother Teresa, Baggi and Bravo the level of ploidy lies between 52–56; in five genotypes, Reagan Emperor, Yellow Charm, White Staphour, Shanti and Punjab Mohini chromosome number lies between 60–62; in five genotypes, Gul-e-Sahir, Rage, Anmol, Yellow Delight and Garden Beauty chromosome number was between 64–66, in three genotypes, Punjab Gold, Flirt and Royal Purple it lies between 68–70; four genotypes, Winter Queen, Ratlam Selection, Ajay and Kelvin Mandarin exhibited ploidy level between 80–82; Basanti, Kelvin Tattoo, Punjab Shyamli and Punjab Anuradha showed ploidy between 104–106; in five genotypes, Reagan White, Autumn Joy, Jaya, Punjab Shingar and NBRI Suneel chromosome number lies between 112–116.

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The variation in ploidy level of chrysanthemum germplasm found in present study is in accordance with previous studies that also found disparity in ploidy level. Majority of Japanese genotypes appeared to be euhexaploid, but many English and American ones were aneuploid reported by (Dowrick 1953, Endo 1969a, Endo 1969b). The earlier cytological research of cultivated chrysanthemum varieties showed that there is a broad hexaploidy based aneuploid aberrant phenomenon in cultivated chrysanthemum varieties (Tanaka 1960).

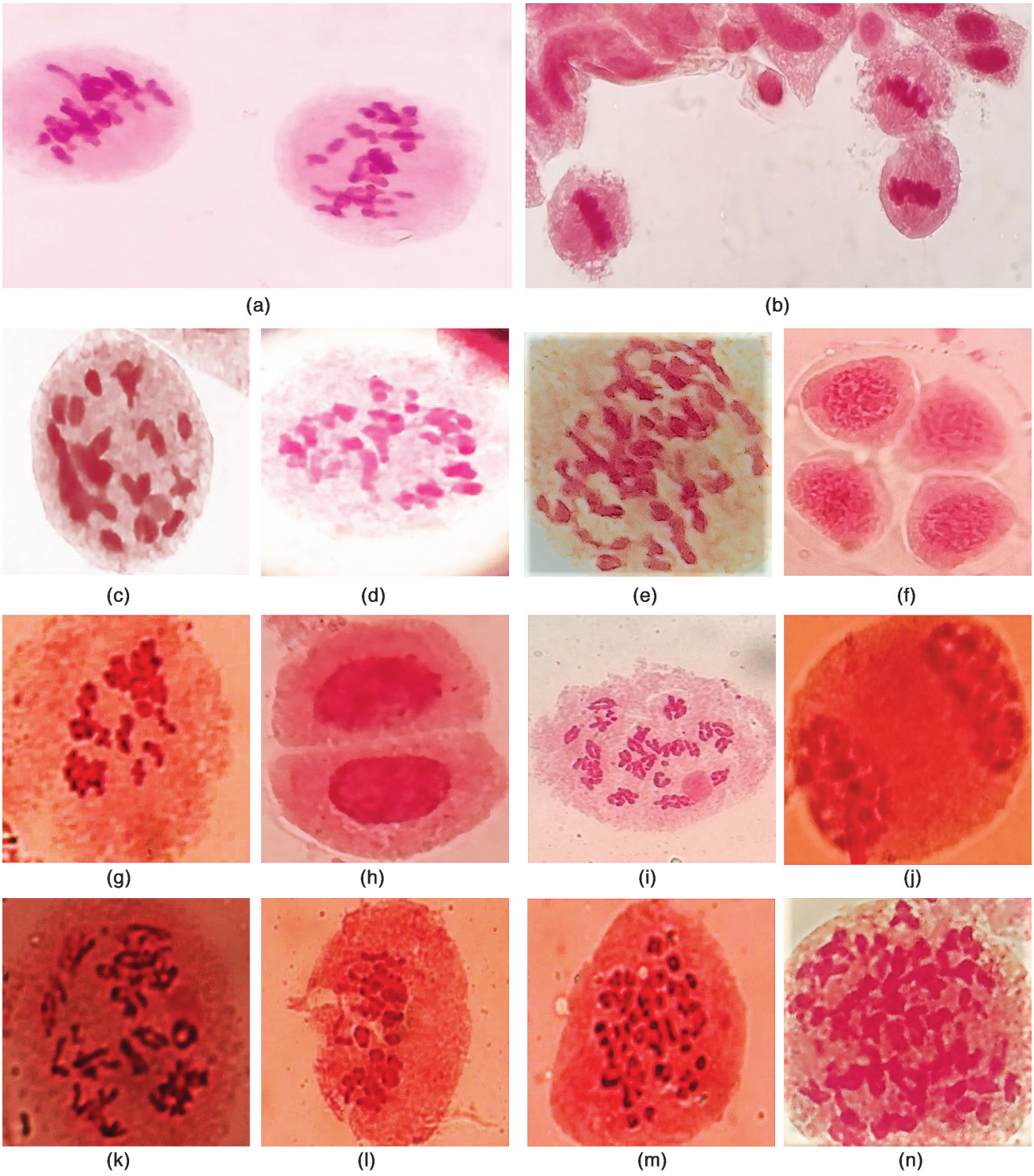

Fig 1 Pollen mother cells with different meiotic stages: (a-b) PMC at metaphase stage showing chromosome stickiness (c) PMC with ploidy level 2n=52 (d) PMC with ploidy level 2n=64 (e) Pollen mother cells with ploidy 2n=80 (f) Tetrad cell (g) PMC with ploidy 2n=64 (h) Dyad stage (i) PMC with ploidy 2n=56 (j) PMC at Anaphase-I (k) PMC with chromosome number 2n=60 (l) PMC with ploidy 2n=68 (m) PMC with chromosome number 2n=104 (n) PMC with ploidy 2n=116.

Table 1 Chromosome numbers in meiotic cells of 30 genotypes of chrysanthemum

	Pollen mother cells with (2n) number of chromosomes															
Cultivar	52	54	56	60	62	64	66	68	70	80	82	104	106	112	116	Total no. of PMC's observed
White Bouquet	8	3	1													12
Punjab Gold								7	2							9
Gul-e-Sahir						6	2									8
Reagan Emperor				9	2											11
Flirt								8	2							10
Winter Queen										5	2					7
Mother Teresa	7	2	1													10
Rage						7	1									8
Yellow Charm				7	2											9
White Staphour				8	1											9
Ratlam Selection										8	2					10
Baggi	8	1	2													11
Basanti												7	2			9
Anmol						8	2									10
Royal Purple								7	3							10
Ajay										7	2					9
Kelvin Mandarin										6	2					8
Kelvin Tattoo												6	2			8
Reagan White														3	9	12
Yellow Delight						7	3									10
Punjab Shyamli												8	2			10
Autumn Joy														2	6	8
Garden Beauty						6	2									8
Bravo	6	1	1													8
Jaya														1	5	6
Punjab Shingar														2	7	9
Shanti				7	3											10
Punjab Mohini				8	2											10
NBRI Suneel														2	8	10
Punjab Anuradha												9	3			12

Variation in chromosome numbers of large flowered Chinese chrysanthemum genotypes also varied from 46 to 73 and proportions of pentaploid and hexaploids found was 5.3% and 84.10%, respectively (Zhu *et al.* 2011). Chromosome number ranges from 44–72 and the results revealed that most of the genotypes were hexaploid and proportion of pentaploid was 7.5% (Zhang *et al*. 2013). Fifteen Chinese genotypes also proved to be having variations with respect to chromosome number that ranges from 52 to 71 (Li *et al*. 1983). Somatic chromosome number of 30 varieties varies from 49 to 62 and most were lying in the range 51–56 as reported by Chang *et al*. (2009).

It is very clear from the Table 1 that polyploidy variation is present in 30 varieties of chrysanthemum. Polyploids have multiple set of chromosomes in excess of the diploid number (Acquaah 2007, Chen 2010, Comai 2005). Polyploidy is common in nature and provides a major mechanism for adaptation and speciation (Chen *et al.*

2007). Polyploids may be classified into either euploids or aneuploids on the basis of their chromosomal composition. Euploids are polyploids with multiples of the complete set of chromosomes specific to a species. Aneuploids are polyploids that contain either an addition or subtraction of one or more specific chromosome(s) to the total number of chromosomes that usually make up the ploidy of a species (Acquaah 2007).

No correlation between the grade of ploidy and capitulum size was reported by Nazeer and Khoshoo (1982) and our results are in agreement with their study. In present study, when flower diameter of selected chrysanthemum varieties was compared with ploidy level, it was observed that there was no relationship between ploidy level and diameter of flowers for example in both Gul-e-Sahir and Yellow Delight genotypes chromosome number lies between 64–66 and flower diameter of both genotypes is 4.70 cm and 5.20 cm, respectively.

Flower colour analysis revealed that there were five different colour patterns that included white, yellow, pink, red and purple. Among these 30 genotypes, the ploidy variation was found within the genotypes having same flower colour for example in cv. White Bouquet ploidy ranges from 2n=52–56 and in cv. White Staphour 2n= 60–62. Our results are in accordance with the study of Zhang *et al.* (2013) where more than one ploidy level existed in all colours i.e. in white, yellow, orange, pink, red, purple and brown except in yellow green. Thus, it is concluded that ploidy level and colour of flowers have no correlation.

Ploidy determination helps find the genetic variation which can be helpful for further breeding programs. Ploidy study is important aspect due to increase in fertility, viability and heterosis as the process of hybrid formation for polyploids should not be without setbacks and vice-versa. Many interspecific hybrids have low fertility and viability due to hybrid incompatibilities (Chen 2010). To increase the heterosis, fertility and viability of interspecific hybrids, several factors should be considered and one of them is that parents used should be of diverse genetic background and preferably heterozygous (Acquaah 2007, Chen 2010). Ploidy study could be found beneficial in identifying the genotypes with high pollen stainability that may be further helpful in breeding programs as Nazeer and Khoshoo (1982) also found that pollen stainability degrees were high which might be due to high polyploidy nature of taxa.

Chromosomal study of chrysanthemum could be helpful for the development of new genotypes. In artificial crosses and their cytological study done to estimate polyploidy (Twab and Kondo 2009) it was reported that mutations can be very helpful for hybrid survival and production of high polyploidation level, or by additional chromosomes in aneuploid, progenies may compensate the existence of such kind of mutations. Mutations are very much helpful for the survival of hybrid population. Thus, it can be concluded that by estimating the ploidy level, new genotypes could be developed by addition or deletion of chromosomes in genotypes with known ploidy level. It was also detected that polyploid hybrids might involve several mutations such as deletion, translocation or inversion of genes in their genetic composition.

SUMMARY

Determination of the ploidy level of plants is utmost important for identification of species, selection of suitable parental lines and also to identify the relationships between species to determine the evolutionary patterns. In chrysanthemum species, chromosomes often have different degrees of differentiation, both between and within species. Total 30 genotypes of Chrysanthemum (*Chrysanthemum morifolium* Ramat.) were used to study the cytology in the Department of Floriculture and Landscaping and Department of Plant Breeding and Genetics, PAU, Ludhiana during 2019 with the objective to find out the genetic variation among the diverse genotypes of chrysanthemum. The ploidy level of 30 chrysanthemum genotypes was studied through meiotic study of pollen mother cells and for this study of meiotic chromosomes, stage and timings of bud collection is very much important. A range of ploidy level was encountered in chromosomal study of selected chrysanthemum germplasm and ploidy level was 2n= 52–116. This study indicated that polyploidy variation was found in all varieties of chrysanthemum selected for study. The elucidation of ploidy level of various crops and species provides foundation for understanding their genetic background, therefore, the results of present study will be further helpful for germplasm conservation of chrysanthemum flower crop.

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