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MEIOTIC STUDIES OF TWO CYTOTYPES OF *CHENOPODIUM ALBUM* L. FROM NORTHERN INDIA

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ABSTRACT

The analysis of 10 populations of *Chenopodium album* L. showed all to be tetraploid (2n=36) and hexaploid (2n=54). The analyzed populations displayed two cytotypes. Detailed meiotic course and pollen fertility studies have been carried out. Meiosis was normal with equal segregation of chromosomes at anaphase-I. Six species showed the presence of Laggards, bridges and vagrants chromosomes in good amount of PMCs. Among the analyzed populations 3.22% -24.07% PMCs showed the presence of cytoplasmic channels among themselves involving 2-3 to 19-21 PMC at a time. These abnormalities in chromosome segregation leads to formation of diads, triads, polyads and tetrad with micronuclei. Pollen size ranges from 20.23X20.19 to 17.33X16.51 and pollen fertility ranges from 74.32%—100%. The small sized micro pollens were mainly sterile in nature and contributed to overall pollen fertility.

KEYWORDS: *Chenopodium album* L., meiosis, pollen fertility.

INTRODUCTION

Chenopodium album is fast growing weedy plant in family Chenopodiaceae and is vernacularly known as *Bathua sag*, *Chandan betu*, *Parupukkirai*, *Pappukuru*, *Katu ayamoddakam*. The standard British name is Fat-hen and is also known as lamb's quarters. The plant has a very complex taxonomy and has been divided in numerous subspecies and varieties, but it is difficult to differentiate between them. *Chenopodium album*, a worldwide weed frequently occurring in tropics, is adaptable to climate with average temperature ranging from 5-30˚C. Habitat includes cropland, old fields etc. The major bioactivities shown by *Chenopodium album* include antifungal, antipruritic, antinociceptive, anthelmintic, antiphlogistic, antirheumatic hyposensitive, odontalgic, and sperm immobilizing agent. It is

used in various pharmacological activities like appetite enhancement, memory enhancement, clearing worms, laxative, antipyretic, curing of anorexia, cough, dysentery, oedema etc. The cytological work on *Chenopodium* was initiated in the earlier parts of last century. First ever, chromosome count for *C. album* L. appeared in 1917 when Winge worked out 2n=18 chromosome number of this species.[1] was first to observe the existence of diploid (2n=18) and tetraploid (2n=36) cytotypes in the species.[2] reported the hexaploid cytotype (2n=54) for the species.[3] reported all the three cytotypes 2x, 4x and 6x from different populations of *C. album* in India. Chromosomes of *Chenopodium album* L. are very small and regular, differing in overall size by less than a micron.[4]

MATERIALS AND METHODS

Natural populations of *Chenopodium album* L., growing in different locations in various districts of Punjab were collected for investigation. The information regarding the altitude, latitude and longitude of various localities from where *Chenopodium album* L. populations were collected is given in Table. 1. The parameters selected for present study were Cytological analysis.

Table 1: Sampled populations of *Chenopodium album* L. with geographical location.

Sr. No.	Population	Localities	Latitude	Longitude	Altitude
1.	Amritsar	Wheat fields	31˚20'N	76˚24'E	252mt
2.	Barnala	Sewage irrigated fields	30˚23'N	75˚31' E	226mt
3.	Bathinda	NFL sewage disposal	30˚11'N	75˚ 00'E	210mt
4.	Hoshiarpur	Wheat fields	31˚32N	75˚57E	295mt
5.	Jalandhar	Leather complex disposal	31˚19'N	35˚18'E	221mt
6.	Ludhiana	Wheat fields	30˚55'N	75˚54'E	262mt
7.	Mohali	Wheat fields	30˚46'N	76˚41'E	220mt
8.	Mukatsar	Sewage disposal	30˚30'N	74˚43'E	184mt
9.	Patiala	Punjabi University campus	30˚20'N	76˚24'E	252mt
10.	Sangrur	Sewage disposal (Bhasaur)	30˚12'N	75˚53'E	231mt

CYTOLOGICAL ANALYSIS

For meiotic analysis, unopened and young floral buds from the healthy plants were collected and were fixed in Carnoy' fixative (Absolute alcohol: Chloroform: Glacial acetic acid:: 6:3:1) for 24 hours. The materials were subsequently transferred to 90% Alcohol and kept at 4˚C in refrigerator till use.

The developing anthers were squashed in 1-2 drops of 2% Acetocarmine, prepared by dissolving 2gm of standard strain Carmine (BDH) in 100ml of 45% Acetic acid. A number of

freshly prepared slides were carefully examined for meiotic analysis and chromosome counts. The incidence of various types of meiotic abnormalities like late disjunction, laggards, bridges at Anaphase-1 or Telophase-1 etc., were scored from various slides at random.

Detailed observations were made on PMCs at tetrad stage to study the microsporogenesis. Pollen fertility in each species were estimated by squashing the mature anthers from different flowers in 1:1 Glycero-acetocarmine at 60˚C for 5 minutes and examined after 24 hours. Well filled pollen grains with stained nuclei were scored as fertile, while shriveled and unstained cytoplasm were counted as sterile. Photomicrographs of chromosome counts, sporads and Pollen grains were made from freshly prepared/temporary slides using Nikon 80i eclipse microscope.

RESULTS

Cytological investigation has been carried out on ten populations of *C. album* growing at different locations. The data regarding the meiotic course, pollen fertility and pollen size are provided in Tables 3, 4, 5. The details are as under.

Population-I

The plants were collected from wheat fields at the outskirts of Amritsar city. Meiotic studies on Amritsar population revealed the presence of 27 bivalents at diakinesis and metaphase-I (Figs. 1, 2). Further course of meiosis was normal in majority of the PMCs. The phenomenon of cytomixis i.e. migration of chromatin material or chromosome between meiocytes through cytoplasmic connection channels, however, was observed in 3.82% of the observed PMCs. The PMCs at diakinesis, metaphase-I, and telophase-II were observed to be involved in cytomixis (Figs. 4-6). The number of PMC involved at a time varies from 2-3. The other abnormality observed was the presence of vagrant chromosome at metaphase- I (Fig. 3). Besides the presence of tetrad with micronuclei (Fig. 7) and polyads (Fig. 8) were also observed .The pollen fertility was 96.14%.

Population-II

The plants were collected from the population of species inhabiting waste lands along the roadside at Barnala town in Punjab. Several PMC at diakinesis (Fig. 10) in Barnala population revealed 2n=36. Few PMCs of this population showed the presence of bridges at anaphase-I (Fig. 11). Investigations on microsporogenesis revealed that besides normal tetrads, aberrant PMCs like tetrads with micronuclei (Fig. 12) and triads (Fig. 13) were

observed in 7.8% of the PMCs observed at this stage. The abnormal microsporogenesis resulted in the formation of different sized pollen grains (Fig. 14). The pollen fertility was quite high i.e. 98.76%.

Population-III

Plants of this population were collected from National Fertilizer Ltd sewage disposal at Bathinda. Meiotic observations made on Bathinda population revealed the haploid chromosome number n=18 at metaphase-I (Fig. 15). Some of the PMCs (3.92%) show presence of laggards (Figs. 16, 17) at anaphase-I and telophase-II. Some PMC at anaphase-I show the presence of bridge (Fig. 18). About 3.30% of the PMCs observed were involved in cytomixis at tetrad and triad stages (Figs. 19, 20). Besides tetrads, few tetrads with micronucleus (Fig. 21), triad (Fig. 22) and polyad (Fig. 23) were also observed. The pollen fertility was 96.98%.

Population-IV

Plants were collected from wheat fields of Hoshiarpur. Meiotic analysis showed haploid chromosome number n=27 at diakinesis (Fig. 25). Further meiotic course was slightly abnormal with formation of cytomictic channels at the early stages of meiosis (Fig. 26) and at tetrad stage (Fig. 27). Laggards were also observed at telophase-II (Fig. 28). Besides normal tetrads, tetrads with micronuclei (Fig. 29) were also observed. Heterogeneous sized pollen grains were observed (Fig. 30) and pollen fertility was 74.32%.

Population-V

The plants were collected from population of the species growing at effluent laden canal in Leather complex area at Jalandhar. The plants collected from Jalandhar population were tetraploid with 2n=36 at metaphase-I (Fig. 31). As many as 24.07% of the observed PMCs showed the presence of cytomixis with clear cytoplasmic channels among PMCs at different stages i.e. diakinesis, metaphase-I, anaphase-I, telophase-II (Figs. 32-35) involving 19-21 PMCs at a time showing multidimensional transfer of genetic material that may leads to the formation of hypoploids and hyperploids (Fig. 32, 33). Besides tetrads the presence of diad (Figs. 37, 38), triads (Fig. 39) and polyads (Fig. 41) was also observed. Diad and triads with two micronuclei were also found may be due to cytomixis (Fig. 38, 39). The extra genetic material gained by PMCs may remain separate in the cytoplasm resulting in the formation of micronuclei in tetrads. The pollen fertility was estimated to be is 92.82% (Figs. 42, 43).

Population-VI

The plants were collected from weed components of wheat fields at Ludhiana. Meiotic analysis conducted on Ludhiana population showed the haploid chromosome number n=27 confirmed from PMCs at diakinesis and metaphase-I (Figs. 44 and 45). Further course of meiosis and microsporogenesis was normal with high pollen fertility of 99.22%.

Population-VII

The plants were collected from heat fields. Meiotic study in plants of Mohali population showed several PMCs at metaphase-II showing on 2n=54 (Fig. 46). Nearly 4.65% of the observed PMCs showed the presence of cytomixis with cytoplasmic channels among PMCs at telophase-II (Fig. 48). In addition to cytomixis PMCs with meiotic abnormality at anaphase- II showing laggards (Fig. 47) have been observed. Microsporogenesis showed the tetrads with micronuclei (Fig. 49). Pollen fertility was 98.21% (Fig. 50).

Table 3: Data on cytomixis and meiotic course in the different populations of *Chenopodium album*

Sr. No.	Population	Chr. No. (n)	Cytomixis			Meiotic abnormalities (%)				
			% of PMC	PMC associated	Meiotic stages	Total PMC observed	Lag	Brid	Vag	Abberant PMC
1.	Amritsar	27	3.82	2-3	Dia, M-I,T-II.	109	—	—	2.75	2.75
2.	Barnala	18	—	—	—	114	—	1.75	—	1.75
3.	Bathinda	18	3.30	2-3 units	Triad, tetrad	98	3.06	1.02	—	4.25
4.	Hoshiarpur	27	3.49	3-4 units	Tetrad	132	2.72	—	—	2.72
5.	Jalandhar	18	24.07	19-21	Dia, M-I,A-I,T-II.	—	—	—	—	—
6.	Ludhiana	27	—	—	—	—	—	—	—	—
7.	Mohali	27	4.65	2-3	T-II	78	1.28	—	—	1.28
8.	Mukatsar	18	—	—	—	—	—	—	—	—
9.	Patiala	27	3.44	2-3	T-II	87	—	—	3.44	3.44
10.	Sangrur	18	3.22	2-3	T-II	75	1.33	—	—	1.33

Lag=Lagard, Vag=Vagarnt, Brid=Bridge

Population-VIII

The plants were collected from wild plants in the roadside vegetation at Mukatsar. Meiotic study on the plants belonging to Mukatsar population showed the presence of 2n=36. The chromosome number was confirmed from several PMCs (Figs. 51, 52). These plants showed perfectly normal meiosis and microsporogenesis leading to apparently fertile pollen grains.

Population-IX

The plants were collected from the weed population of wheat fields at Punjabi University Campus, Patiala. Plants collected from Patiala population were hexaploid with 2n=54.

Several PMCs at diakinesis and metaphase-I (Figs. 53, 54) showed the 27 bivalents. Some PMC showed vagrant chromosome at metaphase-I (Fig. 55). Cytomixis has been observed in 3.44% PMCs involving 2-3 PMCs at a time. PMCs at telophase-II (Fig. 56) were involved in the transfer of genetic material. Analysis of microsporogenesis revealed the presence 2.88% of tetrads with micronuclei and 1.92% triads (Figs. 57, 58). Pollen fertility was 96.78%.

Population-X

Several PMCs of plants collected from Sangrur population showed 2n=36 at Metaphase-I and anaphase-I (Figs. 60,61). More than 3% of the PMC were observed to be involved in cytomixis showing transfer of genetic material and 2-3 PMCs at a time (Fig. 63). At anaphase-I abnormal meiotic behavior in the form of laggards was observed (Fig. 62). Analysis of microsporogenesis revealed the presence of triads in 4.6% (1.84% triads with micronuclei) of the observed PMCs (Fig.64,65) besides tetrads with micronuclei (Fig.66). Pollen fertility observed was 98.31% (Fig 67).

Triad	
N	Mn

Table 4: Data on microsporogenesis in different populations of *Chenopodium album* L.

Sr. No.	Population	PMC observed (%)								
		Total	Diad				Tetrad		Polyad	Abnormal PMC
			N	Mn			N	Mn		
1.	Amritsar	182	—	—	—	—	90.10	7.69	2.19	9.89
2.	Barnala	192	—	—	3.12	—	92.18	4.68	—	7.81
3.	Bathinda	213	—	—	1.40	—	94.83	2.81	0.93	5.16
4.	Hoshiarpur	131	—	—	1.52	0.76	87.78	8.39	—	12.21
5.	Jalandhar	226	4.86	1.76	3.53	0.88	84.51	2.65	1.76	15.48
6.	Ludhiana	132	—	—	—	—	100	—	—	NIL
7.	Mohali	114	—	—	—	—	93.85	6.14	—	6.14
8.	Mukatsar	167	—	—	—	—	100	—	—	NIL
9.	Patiala	208	—	—	1.92	—	95.19	2.88	—	4.80
10.	Sangrur	217	—	—	2.76	1.84	91.70	3.84	—	8.29

N = Normal, Mn = Micronuclei

Table. 5: - Data on chromosome number, ploidy level, pollen fertility and pollen size of *Chenopodium album* L.

Sr. no.	Population	n	Ploidy level	Pollen fertility (%)	Pollen size (µm)
1.	Amritsar	27	6X	96.14	17.58X17.10
2.	Barnala	18	4X	98.76	17.70X17.10
3.	Bathinda	18	4X	96.98	19.25X19.21
4.	Hoshiarpur	27	6X	74.32	23.82X22.82
5.	Jalandhar	18	4X	92.82	20.23X20.19
6.	Ludhiana	27	6X	99.22	16.81X16.44
7.	Mohali	27	6X	98.21	17.33X16.51
8.	Mukatsar	18	4X	100	17.88X17.32
9.	Patiala	27	6X	96.78	17.64X17.50
10.	Sangrur	18	4X	98.31	17.91X17.74

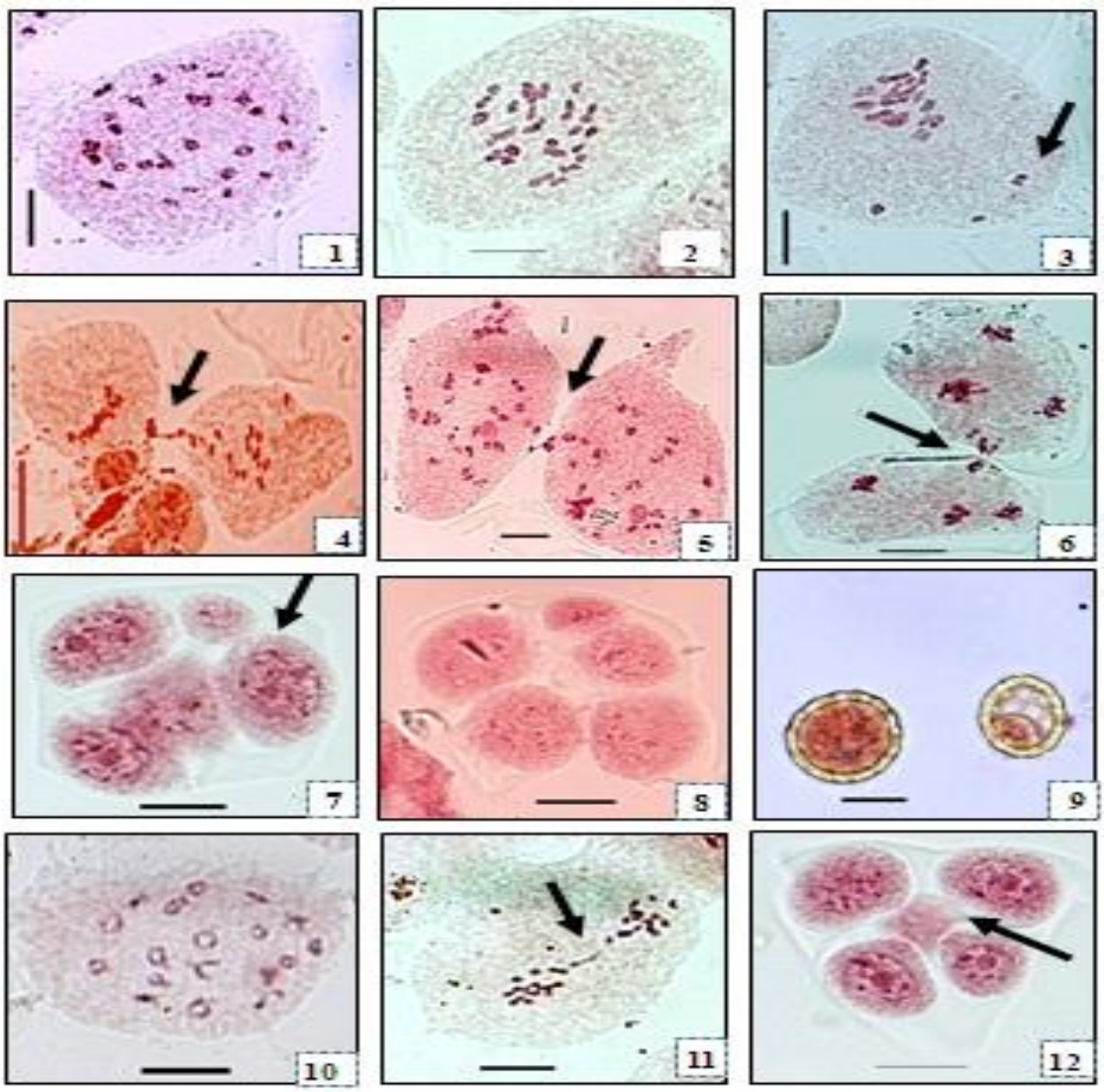

FIG. 1.

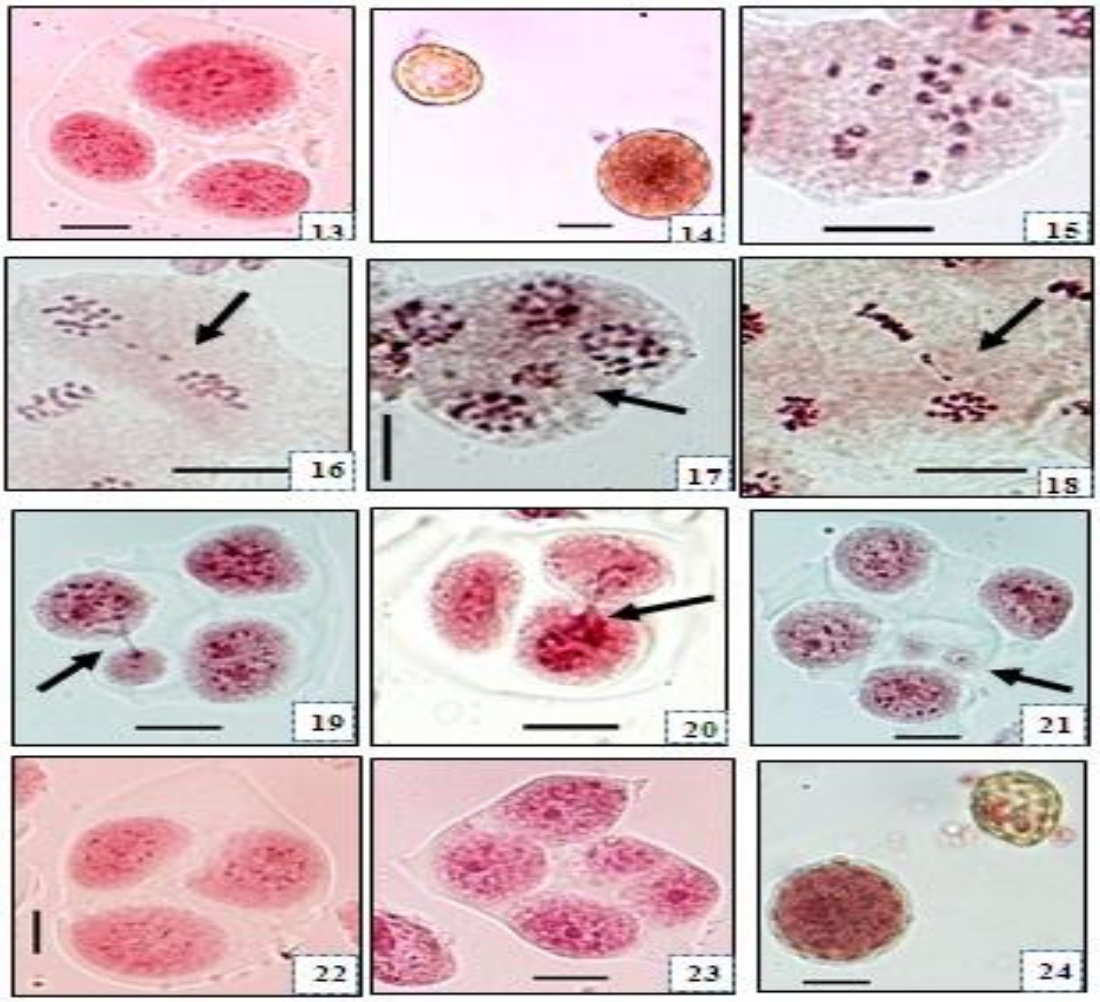

FIG. 2.

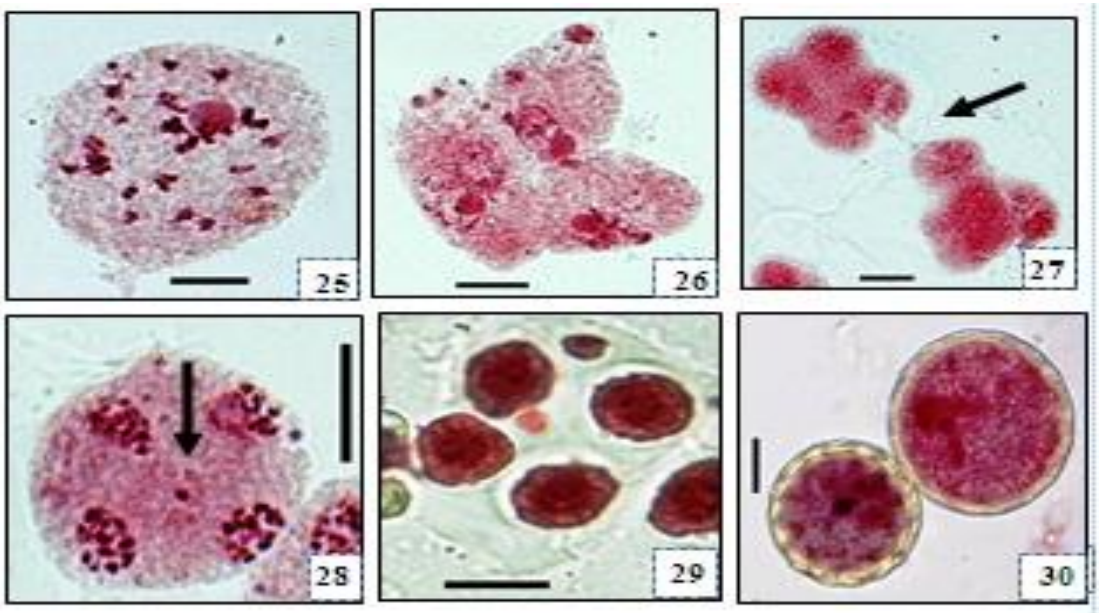

FIG. 3

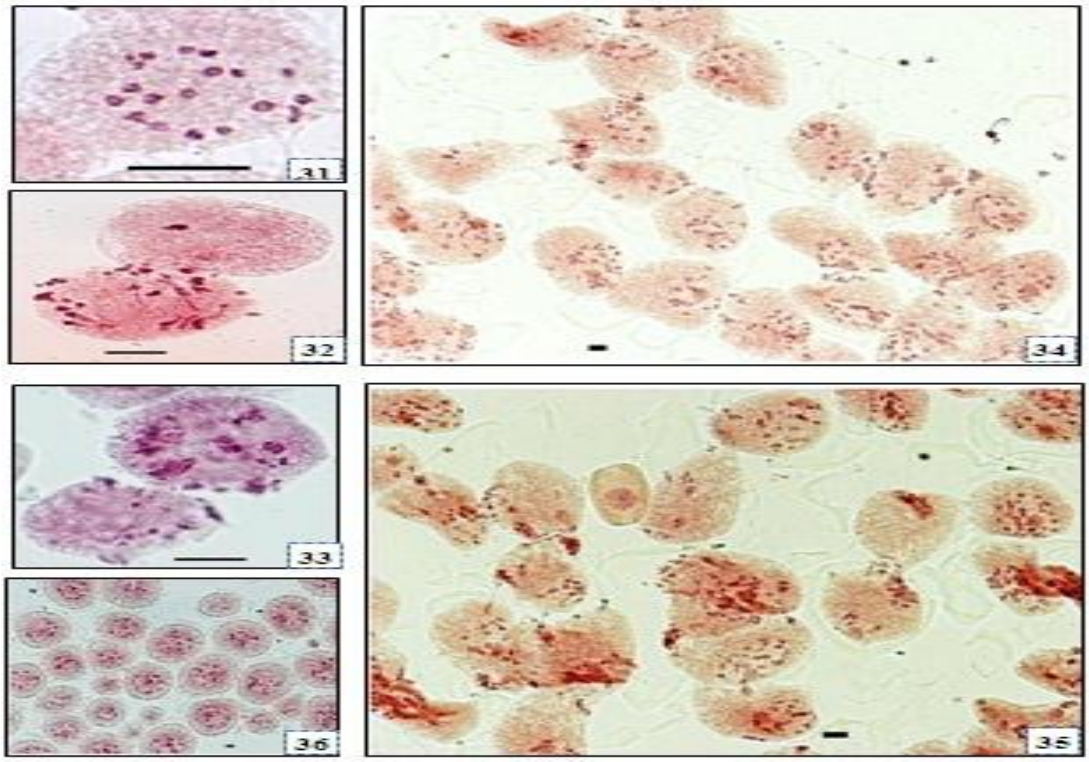

FIG. 4.

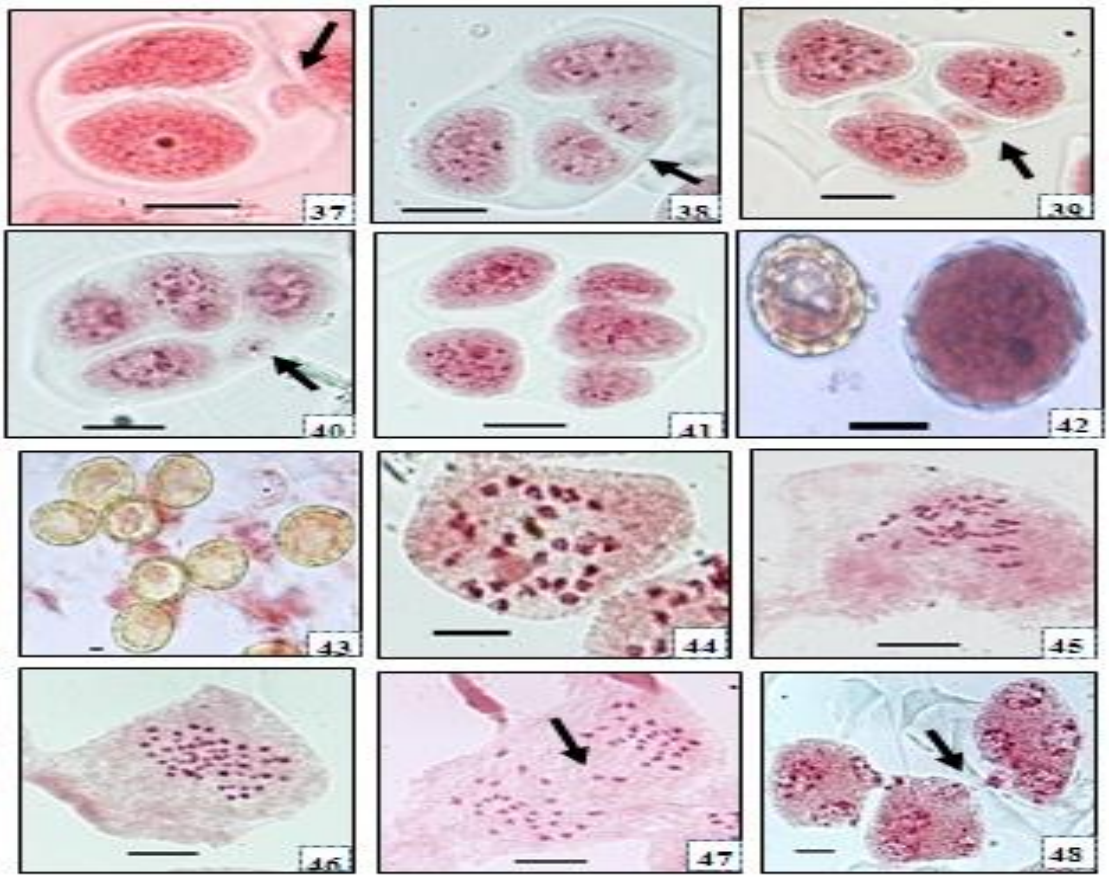

FIG. 5.

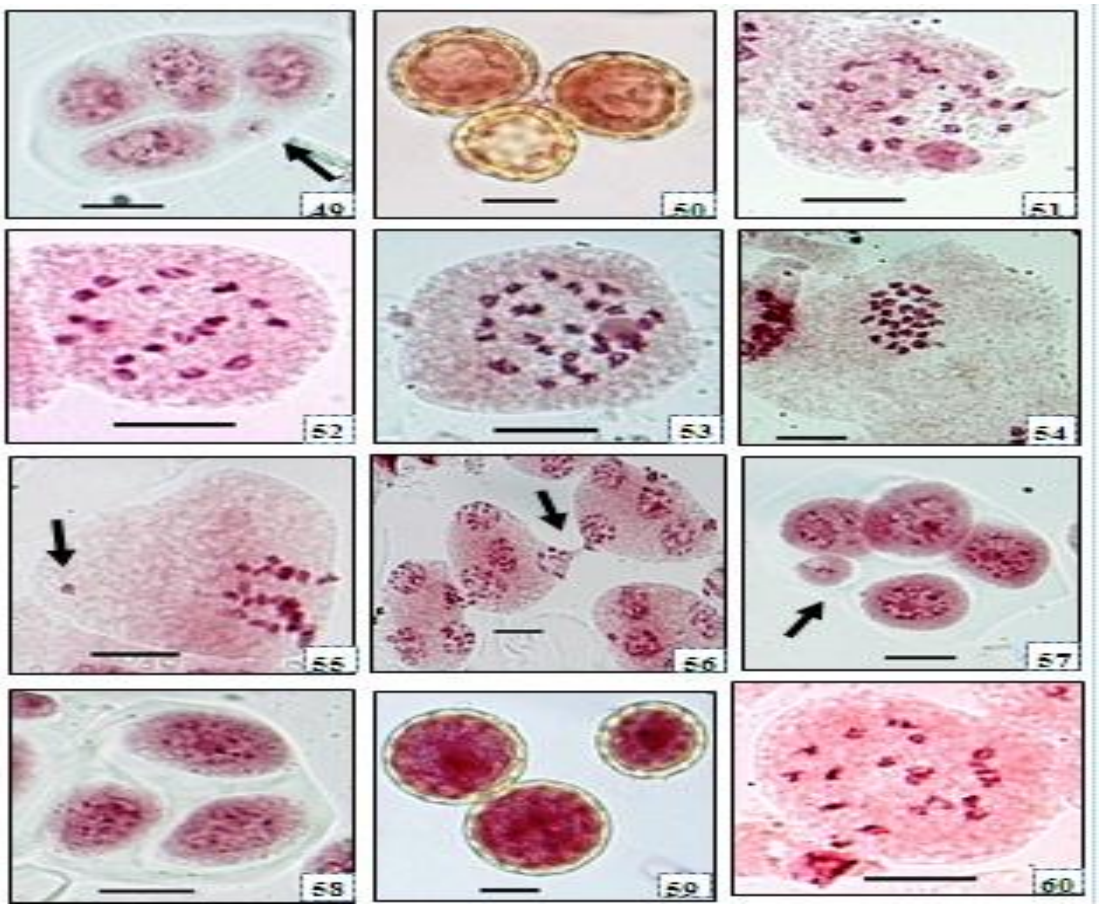

FIG. 6.

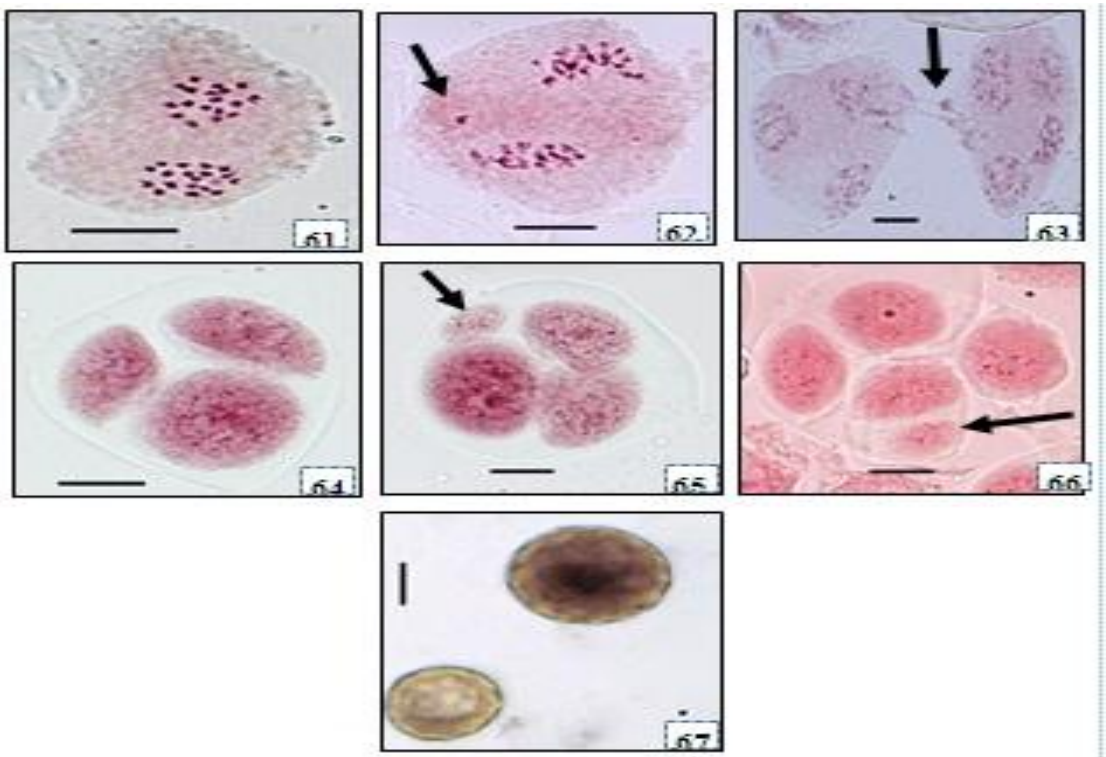

FIG. 7.

DISCUSSION

Presently the species is based on x=9.The present count of chromosome number of 2n=36 and 2n=54 is in line with earlier records for the species. The presently studied plants are at tetraploid (2n=36=4x) and hexaploid level (2n=54=6x). The present count of chromosome number of 2n=36 and 2n=54 is in line with earlier records for the species by[3][5] etc. Presently, the meiotic analysis in the plants of different populations of *Chenopodium* revealed that the majority of PMCs showed normal meiosis. However, few abnormal PMCs Showing cytomixis, bridges, laggards, etc. were often encountered in these plants. Cytomixis is the formations of cytoplasmic channels between neighboring cells and it represent an important mean of cell to cell communication. The formation of cytoplasmic channels through which chromatin material can migrate from one cell to another was first documented as Cytomixis long ago.[6] It has been observed at higher frequency in the pollen mother cells (PMCs) in many species of flowering plants.[7][8] Its origin is not clear. Among the factors considered responsible to cause cytomixis are the influence of genes[9], abnormal cell wall formation during the premeiotic divisions[10], changes in the biochemical process that involves microsporogenesis modifying the microenvironment of affected anthers etc. Presently, the phenomenon of cytomixis has been observed in the PMCs of as many as six populations of *Chenopodium album* L. of the ten populations of the species where meiotic analysis was carried out. The process involved minimum of 2-3 and maximum 19-21 PMCs interconnected through cytoplasmic channels. The amount of chromatin transferred from one PMC to another varied from a small part of chromatin to sometimes complete chromatin involving the entire complement of the PMC making the donor cell completely empty (Fig. 32). In the plants of Jalandhar population the chromatin transfer during cytomixis was observed simultaneously in 19-21 PMCs at a time, involving 24.07% of the total PMCs. The present study revealed that cytomixis was directly responsible for abnormal meiotic behavior, pollen grains of different sizes and pollen sterility in *C. album*. Similar findings regarding the effects of cytomixis on meiotic course have been reported in *Coix*[11] etc. The present observation of cytomixis at tetrad and triad stage in PMCs of Ludhiana population of *C. album* L. is in line with earlier observations of[12] in *Vigna glaberscens.* Microsporogenesis was abnormal to the extent that 4.80% to 15.48% of PMCs showed formation of micronuclei at tetrad as well as diad and triad stages. Polyads were also observed in some populations (Table. 4). The pollen size showed heterogeneous nature in al the populations of *C. album* L. Large sized pollen grains varied from 21-23 µm in diameter and whereas small sized were below 16 µm in size. Small and some of the medium pollens were mainly sterile in nature and

contributed to overall pollen sterility. The medium sized pollen were maximum in number in all the cases. Pollen fertility ranges from 74.32% in the Hoshiarpur population to 100% in the Mukatsar population (Fig. A). The present record of different sized pollens in plants of *C. album* may be contributed due to cytomixis (chromatin transfer) and associated meiotic abnormalities in the species. The formation of micronuclei is entirely due to laggards as suggested by.[13] The abnormalities in the meiotic behavior were quite clearly reflected in pollen.

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