



## Cytomorphological diversity of *Ageratum* Species from North-West India.

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Received: February 29, 2016; Revised: March 05, 2016; Accepted: March 18, 2016.

**Abstract:** *Ageratum* belongs to tribe Eupatorieae of family Asteraceae. In the present study floristic forays revealed the prevalence of two species of the genus in North India namely, *A. conyzoides* and *A. houstonianum*. Two morphovariants were observed in both the species. The plants were found to differ on the basis of leaf shape and flower colour. Further, analysis of male meiosis revealed the presence of three cytotypes of *A. conyzoides* and two cytotypes of *A. houstonianum*. The specimens of *A. conyzoides* with purple flowers were found to have chromosome count of  $n=10$  (diploid cytotype,  $2x$ ) and rarely plants with chromosome count of  $2n=30$  (triploid cytotype,  $3x$ ) were also observed. This is the first report of  $3x$  plants of *A. conyzoides* from wild. The white flowered plants were found to be tetraploid with  $n=20$ . In the specimens of *A. houstonianum*, the diploid number  $n=10$  was found in white flowered plants while tetraploid number  $n=20$  was found in purple flowered plants. The meiotic course varies from normal to abnormal in some populations of both the species.

**Key words:** *A. conyzoides*; *A. houstonianum*; Cytotype ( $2x$ ,  $3x$  and  $4x$ ); Morphotype.

### Introduction

*Ageratum* is one of the plants of family asteraceae that shows wide distribution throughout the World and in India two species, i.e. *Ageratum conyzoides* and *A. houstonianum* are known to exist (Singh *et al.*, 2011). *Ageratum* is derived from the greek word 'ageras' which means 'non-aging' and refers to the long lasting nature of flowers. The genus *Ageratum* includes approximately 30 species of annual and perennial herbs and shrubs that are all native to Central and South America (Okunade, 2002). Belonging to tribe Eupatorieae, the genus is characterised by the absence of yellow corolla and ray florets, presence of tubular corolla with varying shades of blue, lavender or white.

*Ageratum conyzoides* is also known as *Ageratum album* Steud., *A. caeruleum* Hort. ex Poir., *A. coeruleum* Desf., *A. cordifolium* Roxb., *A. hirtum* Lam., *A. humile* Salisb., *A. latifolium* Cav., *A. maritimum* H.B. & K., *A. mexicanum* Sims., *A. obtusifolium* Lam., *A. odoratum* Vilm. The plants are malodorous and have peculiar odour like that of goat, hence it is commonly known as Goat weed, Billy goat weed and Chick weed. *A. houstonianum* is also known as *A. mexicanum* Sims. and commonly known as Blue billy goat weed, Blue mink, Floss flower, Garden *Ageratum* and Mexican *Ageratum*. The species are postulated to have originated in Central America from where they spread as an ornamental to both the Americas and was first introduced in gardens in Europe prior to 1697 from where they became pantropical (Johnson, 1971).

A wide range of bioactive chemical compounds including terpenoids, flavonoids, alkaloids, steroids, chromenes have been recognised from *Ageratum* species (Kamboj and Saluja, 2008; Adebayo *et al.*, 2011; Singh *et al.*, 2013; Kumar, 2014; Kaur and Dogra, 2014). *Ageratum* is

considered an important medicinal plant in folk medicine. It is used for wound dressing, skin diseases, ophthalmia, colic, ulcers, in mouthwash for toothache, killing lice and as anti-itch, antitusive, vermifuge, tonic (Girthers, 1948; Burkill, 1985; Kapur, 1993). The decoction of the plant is used for stomach ailments such as diarrhoea, dysentery, intestinal colic, rheumatism, fever and gynaecological diseases (Sharma and Sharma, 1995; Chopra *et al.*, 2002).

*A. conyzoides* is distributed throughout India and *A. houstonianum* is known to occur in Northern parts of the country (Sahu, 1982). These plants have naturalised in India. So, the present study has been undertaken to investigate cytomorphological diversity of *Ageratum* species from North West India.

### Materials and Methods

**(a) Area surveyed:** Material for male meiotic study was collected on population basis from the different districts of Punjab, Himachal Pradesh, Haryana, Uttarakhand and Jammu & Kashmir (Table-1).

**(b) Morphological studies:** The morphology of specimens was studied *in situ* as well as *in vivo*. The plants showing variability of apparent morphological features like plant size, leaf shape, size and flower colour were grown *in vivo* to rule out any differences due to environmental variability between the populations. The comparative study chart was prepared on the basis of *in vivo* studies. The morphological parameters like plant height, leaf shape, size, flower colour and floral projections were studied for each morphotype to have proper insight of morphological variations.

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**(c) Cytological studies:** Young flower buds from plants were fixed in carnoy's fixative (Ethanol: Chloroform: Glacial Acetic Acid:: 6:3:1) for 24 hours and then transferred to 70% alcohol and kept at 4°C in the refrigerator until analysis. For male meiotic studies young anthers were smeared in acetocarmine (2% carmine in 45% Acetic acid) and number of temporary slides were prepared for each population. Chromosome number was confirmed from several Pollen Mother Cells (PMCs) and microphotographs were taken using Nikon Eclipse 80i microscope fitted with Digital Imaging System.

Apparent pollen fertility will be determined as given by Marks (1954) using glyceracetocarmine for which the mature anthers from different flowers were squashed in glyceracetocarmine (Glycerol: acetocarmine :: 1:1) mixture. Well-filled pollen grains with stained nuclei and cytoplasm were scored as fertile while pollen grains that were shrivelled and poorly stained were scored as sterile.

The scanning electron microscopy (SEM) of pollen grains was done in IPLS, DBT Laboratory Punjabi University, Patiala to know the variation in pollen grains at inter and intraspecific levels.

## Results

Cytomorphological studies were conducted on fifty-five populations of *Ageratum* species from various regions of North West India. The studies revealed prevalence of two species, i.e. *A. conyzoides* and *A. houstonianum*. The populations of *Ageratum* showed variation of morphological characters *in situ*. These were collected and grown *in vivo* at plant conservatory Punjabi University, Patiala and studied further to substantiate *in situ* observations. The species were observed to be represented by two morphotypes each (Table-2). Morphotypes of *A. conyzoides* were found to be different on the basis of colour of corolla and size of the plant, while those of *A. houstonianum* showed variability of corolla colour as well as shape of the leaves (Fig. 1a-t).

**Table 1:** Information about locality and altitude, accession number, latitude longitude of different types of *Ageratum* species from different regions of North-West India.

S.No.	Species	Locality & Altitude	Latitude Longitude	Accession No. (PUP)
1.		Plant Conservatory, Patiala (PB) (251m)	30.36°N 76.45°E	59721
2.		Plant Conservatory, Patiala (PB) (251m)	30.36°N 76.45°E	59859
3.		Karheri, Patiala (PB) (251m)	30.32°N 76.40°E	59825
4.		Ganda Kheri, Patiala (PB) (251m)	30.36°N 76.45°E	59826
5.		Sunam, Sangrur (PB) (231m)	30.13°N 75.80°E	59827
6.		Sunam Nursery, Sangrur (PB) (231m)	30.13°N 75.80°E	59856
7.		Ahmedgarh, Sangrur (PB) (231m)	30.67°N 75.82°E	59857
8.		Raikot, Ludhiana (PB) (254m)	30.65°N 75.60°E	59828
9.		Ludhiana (PB) (254m)	30.88°N 75.85°E	59855
10.		Barnala (PB) (227m)	30.38°N 75.55°E	59833
11.		Moga (PB) (252m)	30.81°N 75.17°E	59832
12.		Joga, Mansa (PB) (217m)	30.14°N 75.42°E	59843
13.		Hoshiarpur (PB) (293m)	31.58°N 75.98°E	59831
14.		Faridkot (PB) (196m)	30.40°N 75.45°E	59830
15.		Kotakpura, Faridkot (PB) (196m)	30.40°N 75.45°E	59844
16.		Sirhind, Fatehgarh Sahib (PB) (246m)	30.36°N 76.23°E	59852
17.	<i>A. conyzoides</i> (Purple, diploid)	Ropar (PB) (260m)	31.23°N 76.50°E	59829
18.		Anandpur Sahib, Ropar (PB) (260m)	31.23°N 76.50°E	59862
19.		Leisure Valley, Chandigarh (321m)	30.75°N 76.79°E	59835
20.		Mohali (PB) (316m)	30.78°N 76.69°E	59860
21.		Pathankot (PB) (335m)	32.27°N 75.65°E	59854
22.		Ambala (Haryana) (264)	30.38°N 76.78°E	59853
23.		Panchkula (Haryana) (365m)	30.74°N 76.80°E	59838
24.		Una (H.P.) (369m)	31.63°N 76.34°E	59846
25.		Bilaspur (H.P.) (673m)	31.33°N 76.75°E	59847
26.		Barwana, Bilaspur (H.P.) (673m)	31.33°N 76.75°E	59848
27.		Hamirpur (H.P.) (785m)	31.68°N 76.52°E	59845
28.		Sundernagar, Mandi (H.P.) (1020m)	31.53°N 76.90°E	59836
29.		Rewalsar, Mandi (H.P.) (1360m)	31.63°N 76.83°E	59849
30.		Dharmasala, Kangra (H.P.) (1457m)	32.21°N 76.32°E	59837
31.		Palampur, Kangra (H.P.) (1472m)	32.11°N 76.53°E	59861
32.		Shimla (H.P.) (2205m)	31.10°N 77.17°E	59858
33.		Mussorie (Uttarakhand) (2000m)	30.27°N 78.06°E	59834
34.		Mussorie (Uttarakhand) (1826m)	30.45°N 78.06°E	59850
35.		Kathua, Jammu (J&K) (307m)	32.27°N 76.45°E	59839
36.		Kathua, Jammu (J&K) (307m)	30.36°N 75.52°E	59851
37.	<i>A. conyzoides</i> (Purple, triploid)	Hamirpur (H.P.) (785m)	31.68°N 76.52°E	59863
38.		Rewalsar, Mandi (H.P.) (1360m)	31.63°N 76.83°E	59864
39.		Rewalsar, Mandi (H.P.) (1360m)	31.63°N 76.83°E	59865
40.		Plant Conservatory, Patiala (PB) (251m)	30.36°N 76.45°E	59722

41.		Plant Conservatory, Patiala (PB)	30.36°N 76.45°E	59723
42.		Plant Conservatory, Patiala (PB)	30.36°N 76.45°E	59866
43.		Plant Conservatory, Patiala (PB)	30.36°N 76.45°E	59867
44.		Barsar, Hamirpur (H.P.) (785m)	31.68°N 76.52°E	59773
45.	<i>A. conyzoides</i>	Barsar, Hamirpur (H.P.) (785m)	31.68°N 76.52°E	59782
46.	(White, tetraploid)	Bilaspur (H.P.) (673m)	31.33°N 76.75°E	59774
47.		Bilaspur (H.P.) (673m)	31.33°N 76.75°E	59775
48.		Galma, Mandi (H.P.) (1189m)	31.62°N 76.87°E	59776
49.		Galma, Mandi (H.P.) (1189m)	31.62°N 76.87°E	59777
50.		Mandli, Una (H.P.) (369m)	31.63°N 76.34°E	59778
51.		Mandli, Una (H.P.) (369m)	31.63°N 76.34°E	59779
52.		Rewalsar, Mandi (H.P.) (1360m)	31.63°N 76.83°E	59780
53.		Rewalsar, Mandi (H.P.) (1360m)	31.63°N 76.83°E	59781
54.	<i>A. houstonianum</i>	Plant Conservatory, Patiala (PB)	30.36°N 76.45°E	59724
	(Purple, tetraploid)	(251m)		
55.	<i>A. houstonianum</i>	Plant Conservatory, Patiala (PB)	30.36°N 76.45°E	59725
	(White, diploid)	(251m)		

**Table 2:** Comparison of cytomorphotypes of *Ageratum* species from North-West India.

S. No.	Characters	<i>Ageratum conyzoides</i>			<i>Ageratum houstonianum</i>	
		Purple	Purple	White	Purple	White
1.	Ploidy level	2x	3x	4x	4x	2x
2.	Habit	Medium sized herb	Medium sized herb	Small sized herb	Medium sized herb	Small sized herb
3.	Habitat	Ranging from plains to high altitude mountains, along roads, water bodies and wheat fields.	Produced <i>in vivo</i> from diploid and tetraploid plants.	Along with purple plants in hilly regions only.	Escaped ornamental on low altitude mountains and plains usually under trees.	Escaped ornamental on low altitude mountains and plains usually under trees.
4.	Shape of leaves	Leaves are 6.7x5.9cm ovate with obtuse base never cordate.	Leaves are 5.0x3.1cm ovate with acute apex.	Leaves are 6.7x3.7cm elliptic-oblong with acute apex and base.	Leaves are 7.2x6.5cm orbiculate with cordate base.	Leaves are 6.3x5.5cm ovate with acute apex and cordate base.
5.	Colour of inflorescence	Purple	Purple	White	Purple	White
6.	Size of inflorescence per capitula	6-7mm	5-6mm	5-6mm	6-8mm	5-6mm
7.	Involucral bracts	hairy	hairy	less hairy	hairy	less hairy
8.	Size of floral projections	Large	Large	Small	Large	Small

**Table 3:** Association of chromosomes of triploid plant produced *in vivo*.

No. of PMCs observed	Association of Chromosomes		
	I	II	III
4	9	6	3
5	7	10	1
3	8	11	-
1	8	8	2
2	4	13	-
3	4	10	2

I: univalent, II: bivalent, III: trivalent.

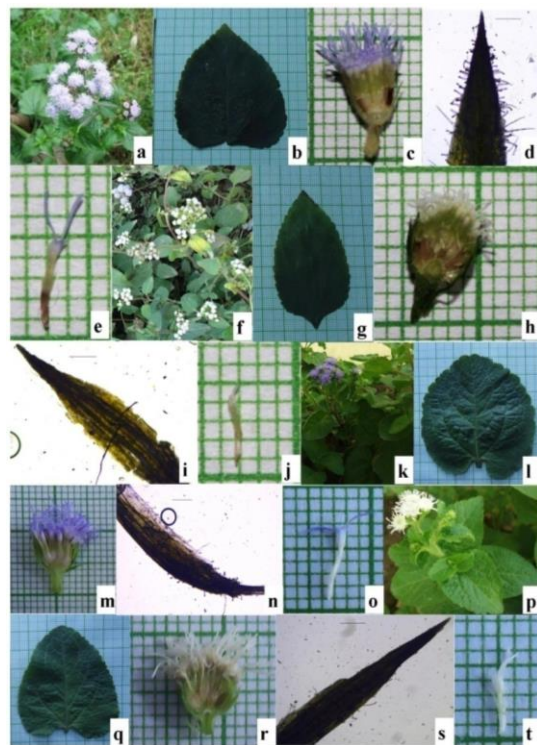
**Table 4:** Details of abnormal populations of *Ageratum* collected from different regions of North-West India.

S. No.	Species	Accession Number PUN	Ploidy level	Abnormalities						
				i	ii	iii	iv	v	vi	vii
1.		59825	2x	-	-	-	-	2.8%	-	-
2.		59826	2x	-	-	1.9%	-	20%	4.7%	-
3.		59827	2x	-	-	2.5%	-	11.5%	-	-
4.		59856	2x							
5.		59828	2x	2.5%	-	-	-	23.3%	-	-
6.	<i>A. conyzoides</i>	59843	2x	2.2%	-	-	-	8.8%	-	-
7.	(purple)	59847	2x	-	-	-	6.6%	16.6%	-	-
8.		59848	2x	-	-	-	-	8.1%	-	-
9.		59845	2x	-	6.2%	-	-	-	-	11.5%
10.		59849	2x	-	-	-	-	-	-	6.8%
11.		59837	2x	-	-	-	-	-	8.6%	-
12.		59834	2x	-	-	-	-	7.9%	-	-
13.	<i>A. conyzoides</i>	59863	3x	2.1%	2.3%	3.1%	-	-	-	-

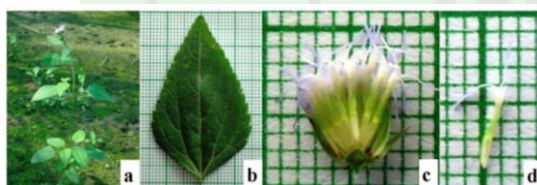


14.	(purple)	59864	3x	1.9%	2.9%	1.05%	-	-	-	-
15.	<i>A. conyzoides</i> (white)	59778	4x	-	-	-	-	-	1.7%	2.5%
16.	<i>A. houstonianum</i> (purple)	59724	4x	2.0%	3.4%	1.3%	-	15.5%	3.3%	-

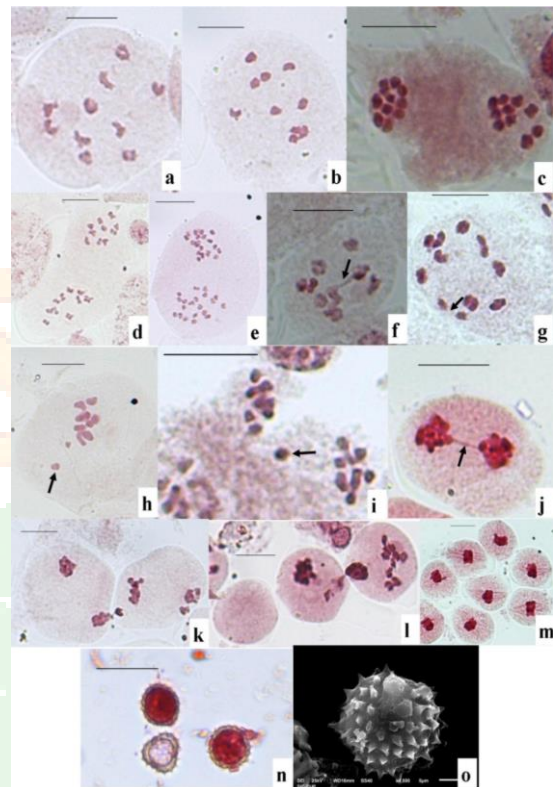
i: Vagrant, ii: Laggards, iii: Bridge, iv: Cytomixis, v: Chromosome stickiness, vi: Inter bivalent connection, vii: Early disjunction of bivalent.



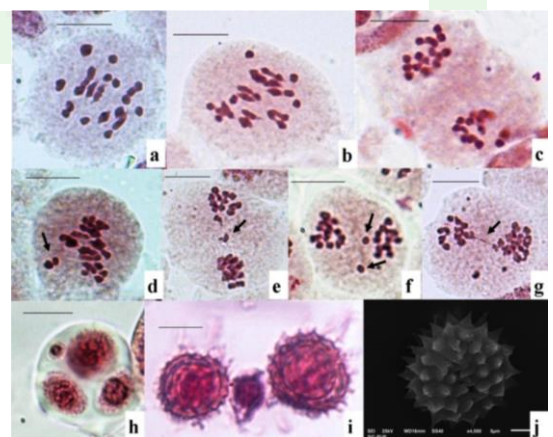
**Fig. 1:** a) Field photograph of *A. conyzoides* (purple, 2x) b) leaf c) L.S. of capitulum d) involucre bract e) floral projection f) Field photograph of *A. conyzoides* (white, 4x) g) leaf h) L.S. of capitulum i) involucre bract j) floral projection k) Field photograph of *A. houstonianum* (purple, 4x) l) leaf m) L.S. of capitulum n) involucre bract o) floral projection p) Field photograph of *A. houstonianum* (white, 2x) q) leaf r) L.S. of capitulum s) involucre bract t) floral projection.



**Fig. 2:** a) Field photograph of *A. conyzoides* (purple, 3x) b) leaf c) L.S. of capitulum d) floral projection.

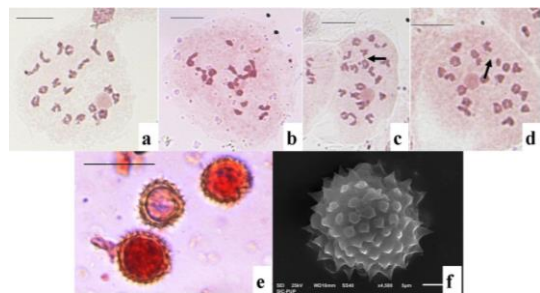


**Fig. 3:** *A. conyzoides*  $n=10$  a) PMC showing 10 II at Diakinesis b) PMC showing 10:10 chromosomes at A-I c) PMC showing 10:10 chromosomes at M-II d) PMC at mixed A-II e) PMC showing interbivalent connections f) PMC showing early disjunction of bivalents g) PMC showing vagrants h) PMC showing laggards i) PMC showing bridge formation j) PMCs showing cytomixis k) Hypo-ploid and Hyper-ploid cells l) PMCs showing chromosome stickiness m) Fertile and sterile pollen grains n) Pollen morphology.

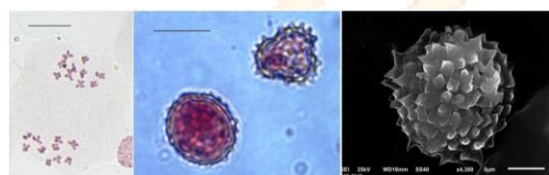


**Fig. 4:** *A. conyzoides*  $2n=30$  a) PMC showing 3 III, 6II and 9 I at M-I b) PMC showing 1 III, 10 II and 9 I at M-I c) PMC showing 1 III, 10 II and 9 I at M-I d) PMC showing 1 III, 10 II and 9 I at M-I e) PMC showing 1 III, 10 II and 9 I at M-I f) PMC showing 1 III, 10 II and 9 I at M-I g) PMC showing 1 III, 10 II and 9 I at M-I h) PMC showing 1 III, 10 II and 9 I at M-I i) PMC showing 1 III, 10 II and 9 I at M-I j) PMC showing 1 III, 10 II and 9 I at M-I

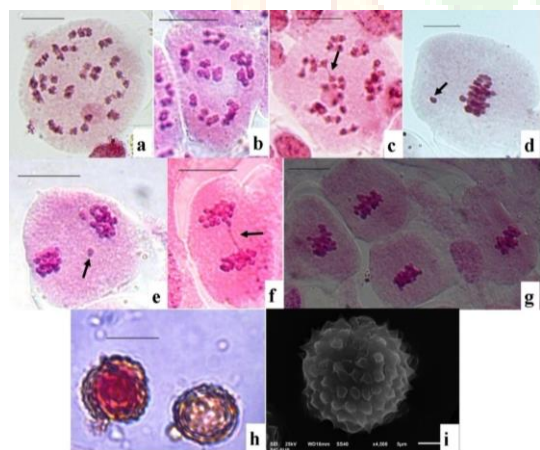
7 I at M-I **c)** PMC showing unequal distribution i.e. 16:14 chromosomes at M-II **d)** PMC showing vagrants **e), f)** PMC showing laggards **g)** PMC showing bridge formation **h)** Tetral with micronuclei **i)** Heterogenous sized fertile pollen grains **j)** Pollen morphology.



**Fig. 5:** *A. conyzoides* n=20 **a)** PMC showing 20 II at Diakinesis **b)** PMC showing 20 II at M-I **c)** PMC showing interbivalent connections **d)** PMC showing early disjunction of bivalents **e)** Fertile and sterile pollen grains **f)** Pollen morphology.



**Fig. 6:** *A. houstonianum* n=10 **a)** PMC showing 10:10 chromosome distribution at M-II **b)** Fertile and sterile pollen grains **c)** Pollen morphology.



**Fig. 7:** *A. houstonianum* n=20 **a)** PMC showing 20 II at Diakinesis **b)** PMC showing 20 II at M-I **c)** PMC showing interbivalent connections **d)** PMC showing vagrants **e)** PMC showing laggards **f)** PMC showing bridge formation **g)** PMCs showing chromosome stickiness **h)** Fertile and sterile pollen grains **i)** Pollen morphology.

A study of male meiosis showed that the white flowered *A. conyzoides* were tetraploid with n=20 (Fig. 5a-b) while purple flowered *A. conyzoides* were diploid with n=10 (Fig. 3a-e) as well as triploid with 2n=30 (Fig. 4a-c). Out of fifty-three populations of *A. conyzoides*, thirty-six were diploid, four were triploid and thirteen were tetraploid. In

the populations of *A. houstonianum*, one was found to be diploid with n=10 (Fig. 6a) and one was tetraploid with n=20 (Fig. 7a-b).

The seeds of cytomorphotypes grown *in vivo* were collected and grown in the subsequent season. The plants that grew from seeds of diploid plants were all diploid while those from tetraploid were also tetraploid in both species. However, some plants growing at the border of 2n and 4n populations of *A. conyzoides* were found to be 3n. This is a first report of *in vivo* production of triploid *A. conyzoides* plants due to intraspecific hybridization of 2n and 4n populations. The 3n plants resembled 2n parents morphologically (Fig. 2a-d).

The meiotic course was observed to be abnormal in some populations of *Ageratum* species. In *A. conyzoides* with n=10 the meiotic abnormalities like interbivalent connections (Fig. 3f), early disjunction of bivalents (Fig. 3g), vagrants (Fig. 3h), laggards (Fig. 3i), bridge formation (Fig. 3j), cytomicis (Fig. 3k), hyper-ploid and hypoploid cells (Fig. 3l), chromosome stickiness (Fig. 3m) were observed in the populations collected from Karheri, Ganda kheri, Sunam, Sunam nursery, Raikot, Mansa, Bilaspur, Barwana, Hamirpur, Rewalsar, Dharmasala and Mussorie (Table-4). These spindle abnormalities resulted into heterogenous sized fertile and sterile pollen grains (Fig. 3n). A detailed study of meiotic course revealed that triploids showed secondary association of chromosomes (Table-3) and meiotic irregularities like secondary associations vagrants (Fig. 4d), laggards (Fig. 4e-f), bridges (Fig. 4g) and abnormal microsporogenesis (Fig. 4h) were also observed. The white plants of *A. conyzoides* collected from Una district showed some meiotic abnormalities like interbivalent connections (Fig. 5c) and early disjunction of bivalents (Fig. 5d). The meiotic course was observed to be abnormal in purple plants of *A. houstonianum* with some meiotic irregularities like interbivalent connections (Fig. 7c), vagrant (Fig. 7d), laggard (Fig. 7e), bridge formation (Fig. 7f) and chromosome stickiness (Fig. 7g). Meiotic abnormalities in the species may lead to some pollen sterility and heterogenous sized pollen grains (Fig. 7h).

The scanning electron microscopic studies of pollens of *Ageratum* revealed that pollen grains are rounded in shape, tritreme and trichotomocolpate. Exine was observed to be echinate with similar morphology and size in all the morphotypes (Fig. 3n-o, 4i-j, 5e-f, 6b-c, 7h-i).

## Discussion

In *A. conyzoides* the meiotic chromosome number of 2n=20 (Fig. 3a-e) was in line with the previous records by large number of workers including Ishikawa (1916), Mehra and Sidhu (1960), Kaul



(1967), Bir and Sidhu (1979), Sidhu and Pelia (1987), Gupta and Gill (1989), Gaonkar and Torne (1991). Earlier Nazeer *et al.*, (1981) have reported the triploid cytotype as interspecific hybrid of *A. conyzoides* and *A. houstonianum*. The chromosome number of  $2n=40$  agrees with the earlier records of Love and Love (1961), Turner and King (1964), Kaul (1971), Subramanyam and Kamble (1971), Mehra and Remanandan (1975), Keil *et al.*, (1988), Nirmala and Rao (1989) and Xie and Zheng (2003). Earlier Gill and Gupta (1971) reported the presence of 0 – 3B chromosomes in PMCs of *Ageratum conyzoides*. Dey (1979) reported the diploidy and tetraploidy in *A. conyzoides*. The species is also known to have chromosome counts of  $2n = 36$  (Husaini and Iwo, 1990),  $2n = 38$  (Sharma and Verma, 1960; Chen *et al.*, 2003). Singhal *et al.*, (2013) recorded the early disjunction of bivalents in diploid cytotype from Mandi district of Himachal Pradesh. Meiotic abnormalities in *Ageratum conyzoides* from Rajasthan was reported by Ramanpreet and Gupta (2015).

In the plants of *A. houstonianum*, the diploid cytotype with  $2n=20$  in white flowered plants was in line with the previous records by Copper and Mahony (1935), Hsu (1967, 1970), Sharma and Dhakre (1981). The chromosome counts of  $2n=40$  was reported previously by Shukur *et al.*, (1977).

The cytological studies in both the species of *Ageratum* was done by Mehra *et al.*, (1965), Miyagi (1971), Mathew and Mathew (1983, 1988), George *et al.*, (1989), Morton (1993) and Razaq *et al.*, (1994).

## Conclusion

The present study showed prevalence of two species of *Ageratum* in the study area of which *A. conyzoides* was found growing luxuriously in wild however *A. houstonianum* was collected only from cultivation. There are two cytomorphotypes of *A. houstonianum* and three of *A. conyzoides* prevalent in the area. The present investigations put forward a report of 3x intraspecific hybrid ( $2x \times 4x$ ) of *A. conyzoides* for first time.

## Acknowledgement

The authors acknowledge the financial and material facilities provided by University Grants Commission, New Delhi under DRS and BSR programmes. Thanks are due to the Head, Department of Botany and DBT IPLS project, Punjabi University, Patiala for providing necessary infrastructural facilities.

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#### Cite this article as:

Arneet Gill and Puja Garg. Cytomorphological diversity of *Ageratum* Species from North-West India. *Annals of Plant Sciences* 5.3 (2016): 1291-1295.

Source of support: Nil

Conflict of interest: None Declared