

CYTOMORPHOLOGY OF SOME MEDICINAL GRASSES FROM HANGRANG VALLEY OF DISTRICT KINNAUR, HIMACHAL PRADESH

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ABSTRACT

Objective: The present study is focused on the chromosome number, their meiotic behavior, pollen fertility and medicinal importance of 6 species of grasses from the remote high altitude region of Kinnaur, Himachal Pradesh.

Methods: The young spikes of suitable sizes were fixed in carnoy's fixative (6 ethanol: 3 chloroform: 1 acetic acid v/v) for 24 h and preserved in 70% alcohol at 4 °C until use. For meiotic studies, anthers were squashed in 2% acetocarmine. Pollen fertility was examined using the glycerol-acetocarmine method.

Results: The species studied have shown chromosome numbers like *Arundo donax* (n=12), *Avena fatua* (n=21), *Cynodon dactylon* (n=18), *Cymbopogon distans* (n=20), *Eleusine indica* (n=9), *Sorghum halepense* (n=20). The medicinal uses, other traditional uses and the cytological analysis of these grasses belonging to family Poaceae are studied for the first time from the study area.

Conclusion: The present exploration gives useful information regarding the chromosome number and various meiotic irregularities including their medicinal importance.

Keywords: Grasses, Medicinal value, Meiotic behavior, Cytomixis, Kinnaur, Himachal Pradesh

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INTRODUCTION

The family Poaceae is the true grasses and includes most plants grown as grains for pasture and for lawns, numbering approximately 11000 species belonging to about 700 genera in the World [1, 2]. The vegetation of Kinnaur region has been classified in to 29 types that belong to Himalayan moist and dry temperate forests, dry and moist alpine scrub and meadows [3]. The lower valleys of Kinnaur receive monsoon rains, and upper valleys are very dry which remain under snow cover for 4-6 mo and are remarkable for the variety of beautifully colored flowers forming a rich storehouse of medicinal and aromatic plants [4]. The local inhabitants of Kinnaur practice the Tibetan Amchi system of medicine. Amchi practitioners (Buddhist) are highly respected both socially and spiritually.

The local Amchi prescribed the use of the medicinal plant in the form of paste, powder, decoction and oil. During June 2012 to September 2015, various localities were visited, and ethno botanical information was collected through interviews with elderly people, women, shepherds and local amchi, to highlight the medicinal and traditional use of the grasses by the native people. Some of the medicinal grasses used by the tribals of Kinnaur are shown in table I. Besides their fodder and medicinal value, some grasses have various other significant uses also, like *Cynodon Dactylon* is considered as sacred and are used for worshipping and offered in the Gompas especially during special ceremonies, *Cymbopogon distans* are used for flavoring food products, Some other important grasses like *Eleusine indica* and *Arundo donax* are used in weaving mats, baskets, and thatching of roofs.

Earlier very little work has been done on ethnobotanical studies of medicinal and aromatic plants of District Kinnaur, but no serious attempts has been made on chromosome study of grasses from this particular area of Kinnaur. The present study is focused on the medicinal value, chromosome count and meiotic behavior in six species of grasses from the study area. The species studied, their accession number, a locality with altitude, chromosome number, ploidy level, previous chromosome reports and pollen fertility are given in table 2.

MATERIALS AND METHODS

Study area

The present study was undertaken in Kinnaur (30 °22'40"N to 33 °12' 40" N Latitude and 75 °47'55" E to 79 °04'20" E Longitude), which spans over an area of 6,400 km in the Western Himalayas. Systematic surveys were conducted to cover different altitudinal zones and the area surveyed is shown in fig. A.

Methodology

For meiotic studies, young wild spikes of appropriate size were collected from different localities of Kinnaur, Himachal Pradesh. The plant specimens were identified with the help of regional floras [5, 6, 7] and at the Botanical Survey of India (BSI) herbarium, Dehradun. The young unopened spikes were fixed in carnoy's fixative for 24 h and then stored in 70% alcohol at 4 °C until use. For meiotic studies, anthers were squashed in 2% acetocarmine. Pollen fertility was estimated using the glycerol-acetocarmine technique. Photomicrographs were made from freshly prepared slides using a Nikon 80i Eclipse Microscope.

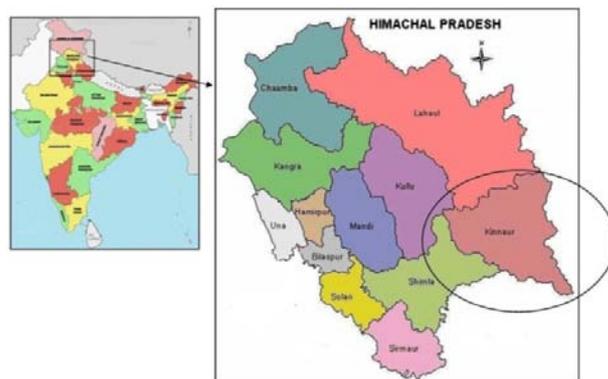


Fig. A: A map of the study area showing different localities surveyed

RESULTS AND DISCUSSION

Tribe: arundineae

Arundo donex L.

Arundo donex, commonly called "Giant reed," is a tall, perennial grass growing in damp and saline soils and is used to make fishing rods, baskets, and garden fences. Leaves are used as stuffing for pillows or mattresses, culms for walking sticks [8]. Medicinally the rhizome is used against diuretic and in the treatment of cancer. Local people of the study area use this plant for soil stabilization to control landslides. The plant was collected from Moorang (3591m). The meiotic study reveals diploid chromosome count of $n=12$, which is in conformity with the previous report [9] from India. Meiotic course is abnormal with the presence of Laggards (6.7%), unoriented bivalents (7.5%) and cytomixis (6%) which leads to reduced pollen fertility (75%) (fig. 1-5).

Tribe: andropogoneae

Cymbopogon distans (Nees ex Steud.) wats

Cymbopogon distans, commonly called "lemongrass," is an aromatic perennial grass widely growing in xerophytic grasslands of the upper Himalayan region. The species is used for the production of essential oil which is used in soaps, cosmetics and perfumery industries. Tetraploid chromosome count of $n=20$ is confirmed on the basis of plant collected from Spillow (3000 m) which is in conformity with the previous report [10]. Further the species shows abnormal meiosis with the presence of cytomixis (3.6%) which leads to reduced pollen fertility (60%) (fig. 15-17).

Sorghum halepense (Linn.) pers

Sorghum halepense commonly called "Johnson grass," is a good pasture grass. Plant parts are used for the treatment of stomach ache, epilepsy and decoction of seeds is used against diarrhea. Local people of Kinnaur use boiled seeds to cure dysentery. Meiotically, the species showed tetraploid chromosome count of $n=20$, which was collected from Kanam (2900m). The present report confirms the previous reports from Canada [11] and India [12]. Further, meiotic course is abnormal with the formation of quadrivalent and presence of laggards (9.8%) at anaphase leading to reduced pollen fertility (70%) (fig. 21-28).

Tribe: aveneae

Avena fatua L.

Avena fatua, commonly called "wild oat," is edible and the roasted seed is a coffee substitute. Further, the seeds are diuretic, emollient and refrigerant. Straw has a wide range of uses such as in papermaking, thatching and as forage. Boiled seeds are used by the local people for curing stomach disorder. Chromosomally the species is found to be hexaploid with chromosome number, $n=21$ at M-I. Hexaploid chromosome count ($n=21$) was determined on the basis of plant collected from Hango (3080m).

The meiotic behavior is observed to be abnormal with the presence of late disjunction (21.4%), laggards (11.3%) and bridges (13.33%) which result in reduced pollen fertility (65%) (fig. 6-10). The present chromosome count is in line with the previous report [13] from India and outside India [14].

Table 1: Data showing Ethnobotanical and Ethnomedicinal uses of some species of grasses from District Kinnaur, H. P (information collected from elderly people, women, shepherds and local amchi of the region)

S. No.	Botanical name	Local name	Part used	Ethnobotanical and Ethnomedicinal uses
1	Tribe: Arundineae <i>Arundo donax</i> L.	(Rajal)	Roots/stems	Stems used for walking sticks and as support for climbing trees. A decoction of roots is used by local amchi for curing cancer.
2	Tribe: Aveneae <i>Avena fatua</i> L.	(Yukpa)	Seeds	The plant is used as forage for cattle and yaks. Roasted seeds are eaten for curing stomach disorder and in the case of fever seeds are mixed with milk and boiled milk are taken.
3	Tribe: Cynodonteae <i>Cynodon dactylon</i> (L.) Pers.	(Changma cha)	Leaves	This grass is considered as sacred and used for the worship of deity by the local people. Leaves are crushed and applied on the cut area to stop bleeding.
4	Tribe: Andropogoneae <i>Cymbopogon distans</i> (Steud.) Wats.	(Kurcha)	Leaves	Plants parts are used as forage. Aromatic oils are extracted from the leaves and used in preparing amchis medicine. Oil is applied in inflammation and joint pain.
5	<i>Sorghum halepense</i> (L.) Pers.	(Pakche)	Stem/seeds	Seeds are boiled and mixed with salt and given to the patient to cure dysentery. Stem are used in weaving baskets, mats and in making brooms for cleaning roadsides.
6	Tribe: Eragrostideae <i>Eleusine indica</i> (L.) Gaertn.	(Kangli)	Whole plant	A decoction of the fresh plant is used against dysentery and constipation. The whole plant is used for making ropes, brooms, and mats.

Table 2: Data showing Taxa, Locality with altitude, Ploidy level, Chromosome number reports and pollen fertility

S. No.	Taxa/Accession number(PUN)	Locality with altitude (m)	Ploidy level	Chromosome number reports		Pollen fertility
				Present (n)	Previous	
1	<i>Arundo donax</i> L. (58707)	Nichar (2700m)	Diploid	$n=12$	$2n=24, 36, 64, 72, 100, 110, 112$	75%
2	<i>Avena fatua</i> L. (59162)	Hango (3080m)	Hexaploid	$n=21$	$2n=14, 42, 44$	65%
3	<i>Cynodon dactylon</i> (L.) Pers. (58510)	Moorang (3591m)	Tetraploid	$n=18$	$2n=18, 36, 54, 27, 40$	78%
4	<i>Cymbopogon distans</i> (Nees ex Steud.) Wats. (59166)	Reckong Peo (2670 m)	Tetraploid	$n=20$	$2n=20, 40, 60$	60%
5	<i>Eleusine indica</i> (Linn.) Gaertn. (58710)	Bhaba Nagar (2700m)	Diploid	$n=9$	$2n=18, 36$	84%
6	<i>Sorghum halepense</i> (Linn.) Pers. (58703)	Bhaba Nagar (2700m)	Octaploid	$n=20$	$2n=10, 18, 20, 30, 40$	70%

Tribe: cynodonteae

Cynodon dactylon (L.) pers

Cynodon dactylon, commonly called "Dhoob grass" is a hardy perennial grass and is one of the most commonly occurring weed in

India. Although it is a potential weed, dhoob grass is a valuable herbal medicinal and used as first aid for minor injuries [15].

This plant is bitter, sharp, hot taste, good odor, laxative, brain and heart tonic and useful against pains, inflammations and toothache [16]. Local people of Kinnaur use this grass for worshipping of deity

in their religious place. The species has been studied chromosomally on the basis of plants collected from Moorang (3591m) and tetraploid chromosome count of $n=18$ (based on $x=9$) is confirmed, which is in conformity with the previous report [17] from Pakistan and from India [18]. Meiotic course is abnormal with the presence of Laggards (5.7%) which leads to reduced pollen fertility (78%) (fig. 11-14).

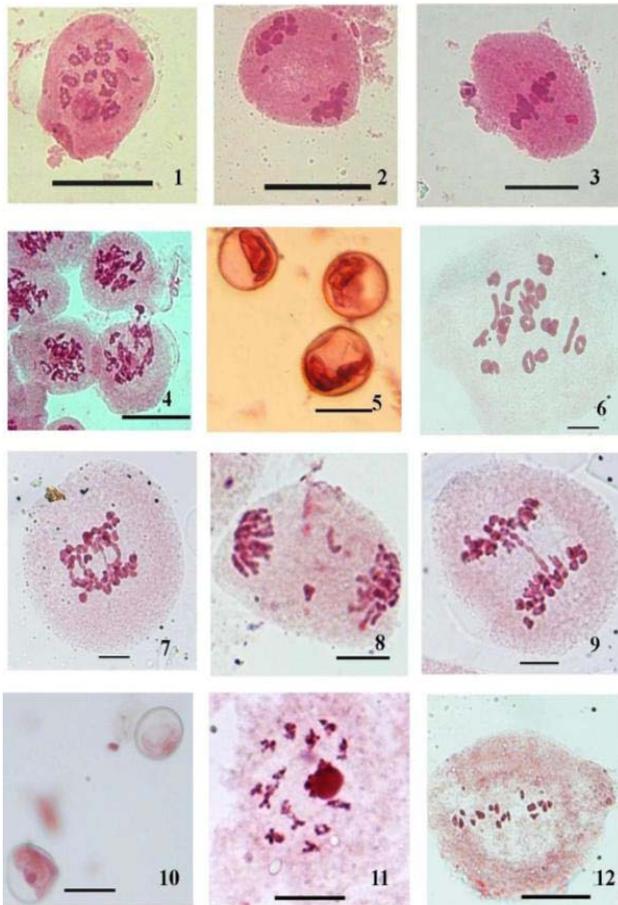


Fig. 1-12: *Arundo donex*. 1: A PMC showing 12 bivalents at Diakinesis; 2: A PMC showing laggards at anaphase I; 3: A PMC showing unoriented bivalents at Metaphase; 4: A PMC showing cytomixis at Metaphase; 5: Partially sterile and fertile pollen grains; 6: *Avena fatua*. A PMC with 21 bivalents at metaphase I; 7: A PMC with late disjunction; 8: A PMC with laggards at anaphase I; 9: A PMC with Bridges at anaphase I; 10: A sterile and fertile pollen grain; 11: *Cynodon dactylon*. A PMC showing 18 bivalents at Diakinesis; 12: A PMC showing 18 bivalents at metaphase I

Tribe: eragrostideae

Eleusine Indica (Linn.) gaertn

Eleusine Indica, Commonly called "Indian Goosegrass," is a small annual grass distributed throughout the warmer areas. The whole plant especially the root is used for the treatment of hypertension and influenza. The seed is sometimes used as a famine food and also used in the treatment of liver complaints.

For the meiotic study, the plant was collected from Chango (2830m) which shows diploid chromosome count $n=9$ at metaphase-I. The present report confirms the previous report from China [19] and from India [20]. The meiotic course is abnormal with the presence of laggards (7.6%) at anaphase-I leading to reduced pollen fertility (84%) (fig. 18-20).

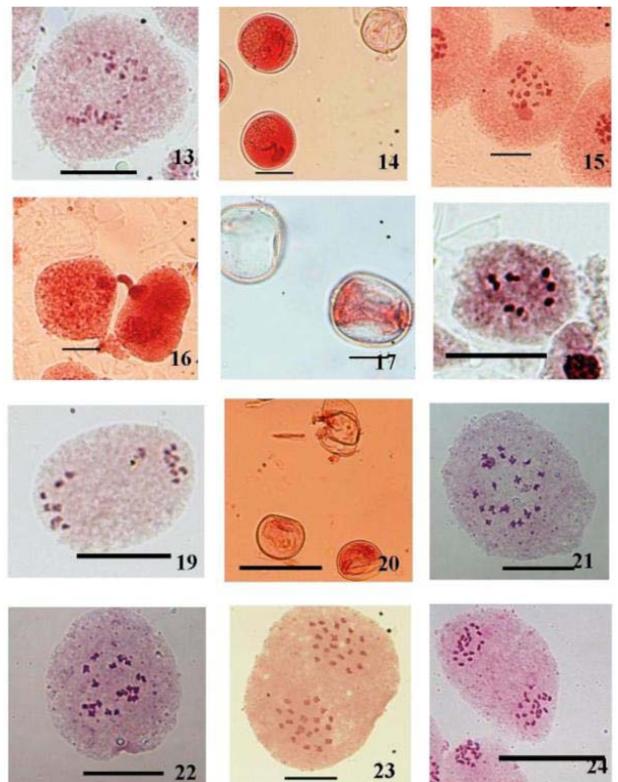


Fig. 13-24: 13: A PMC showing laggards at anaphase I; 14: Sterile and fertile pollen grains; 15: *Cymbopogon distans*. A PMC showing 20 bivalents at diakinesis; 16: A PMC showing cytomixis at Diakinesis; 17: Sterile and fertile pollen grains; 18: *Eleusine Indica*. A PMC showing 9 bivalents at metaphase I; 19: A PMC showing laggards at anaphase I; 20: Sterile and fertile pollen grains; 21: *Sorghum halepense*. A PMC showing 20 bivalents at Diakinesis; 22: A PMC showing 20 bivalents at metaphase I; 23: A PMC showing 20 chromosomes at metaphase II; 24: A PMC showing 20:20 distribution of chromosomes at anaphase I

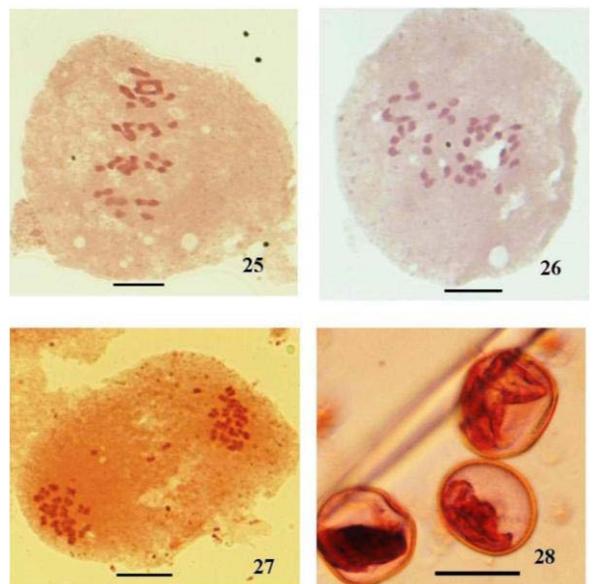


Fig. 25-28: 25: A PMC showing 18 bivalents and one quadrivalent; 26: A PMC showing 36 univalents and one quadrivalent in chain form; 27: A PMC showing laggards at anaphase I; 28: Sterile and fertile pollen grains. Scale bar=10 μ m

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CONCLUSION

In the present study, we are here of the view that the climatic conditions, particularly high altitude and the low temperature during the flowering period may be a possible cause for various meiotic abnormalities. It is thus concluded that due to various meiotic abnormalities during meiosis the pollen fertility was reduced (60% to 84%) in all the medicinally important plant species collected from the study area.

CONFLICT OF INTERESTS

Declared none

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