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Abnormal Meiosis in Tetraploid (4x) *Cannabis sativa* (L.) from Lahaul-Spiti (Cold Desert Higher Altitude Himalayas)-A Neglected But Important Herb

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ABSTRACT: Present paper deals with the chromosome numbers, meiotic course and pollen viability of *Cannabis sativa* L., which was assessed cytologically in 12 populations growing in different localities of Lahaul-Spiti of Himachal Pradesh (H.P.) viz. Trilokinath (3,020m), Kaza (3,820m), Udaipur (2,743m), Losar (4,079m), Keylong (3,350m), Manali (2,050m), Gramphoo (3,060m), Chamba (920m), Kullu (1,362m), Khoksar (3,140m), Batal (3,960m) and Darcha (3,360m). Except, the population growing in Darcha (2n=4x=40), all the other populations were reported with diploid chromosome count of 2n=2x=20. The tetraploid (4x) meiotic chromosome count of 2n=40 in *Cannabis sativa* L. from cold desert area of Lahul-Spiti is reported here for the first time. Microsporogenesis was normal in most of the populations. It was present with Cytomixis (33.36%), laggards (19.52%), bridges (15.60%), chromosome stickiness (27.17%), diads (07.50%), triads (06.33%), polyads (09.50%), micronuclei and unequal daughter cells in plants of Darcha population (2n=4x=40). The consequences of these meiotic abnormalities were in the form of heterogenous sized pollen grains and low (67.33%) pollen viability.

Keywords: Chromosome number; Meiotic course; Pollen viability; Population; Tetraploid (4x) and *Cannabis sativa* L.

INTRODUCTION: *Cannabis sativa* (L.) known well as Marijuana is one of the important, annual and herbaceous member of family Cannabaceae. Species is taxonomically variable in terms of morphology and adaptation (Hill 1983). Currently, *cannabis* is the most widely consumed illicit and illegal drug in the world. It is most distributed and domesticated plant in Russia, China, India, Pakistan, Iran and cultivated elsewhere in world. It is used for its psychoactive effect of cannabinoid delta-9-tetrahydrocannabinol (THC), durable fiber, oil and protein rich nutritious seed. Locally in India it is known by the name of Bhang, Ganja, Chillum, etc. and cultivated for its valuable fibers for making ropes, strings, etc. Species also possess important place in religious ceremonies and festivals. *C. sativa* L. is very adaptable in Northern regions from plains of Punjab to high altitude areas (3,500 m) in Lahaul-Spiti of Himachal Pradesh and grows abundantly on roadside especially. Cytologically the species is studies from the plain areas by Kaur and Singhal (2010) and from Shimla hills by Suman Kaushal (2012). As the species is widely distributed in cold regions of India and no cytological data are available for the species from the study areas, an attempt has been made to study the species for cytomorphological diversity from these study regions.

MATERIAL AND METHODS: Materials for meiotic study were collected during the months of June–August. For the meiotic preparations appropriate sized of young floral buds growing in their natural habitats were fixed in Carnoy`s fixative (6:3:1=Absolute ethanol: chloroform: glacial acetic acid, v/v) for 24 h and preserved in 70% ethanol at 4^{o}C. Anthers were squashed in 1% acetocarmine. More than 2000 pollen mother cells (PMCs), including microsporocyte in meiosis I/II, and meiotic products were analyzed in each population. Pollen stainability in glycerol–acetocarmine (1:1) was used to calculate pollen viability. The diameter measurement of viable pollen grains was done using ocular micrometer and for photog-

raphy a Nikon microscope Eclipse 80i digital system was used.

The accessions of *Cannabis sativa* (L.) were collected from various study areas of higher altitude Himalayas. Plants specimens were identified by the Botanical Survey of India (B.S.I., Northern Circle), Dehradoon, Punjabi University Patiala, Punjab, India and Department of Biodiversity, IHBT (CSIR), Palampur, Himachal Pradesh. Specimens were also identified by consulting different floras of the western Himalayan region (Hooker 1885; Aswal and Mehrotra 1994) and finally deposited in the Herbarium, with accession number (V. Sharma-20125-20136).

RESULTS AND DISCUSSION: Presently detailed meiotic studies were made on 12 taxa belonging to the species of *Cannabis sativa* L. The taxa were collected from the different populations in the high altitude localities of Lahaul-Spiti. The data regarding localities herbarium accession number (PUN), meiotic chromosome number (2n), previous reports and pollen viability of worked out taxa are provided in Table 1. Following the cytological study on the collected taxa from 11 different populations revealed almost similar diploid chromosome count of 2n=2x=20 (Figure 1a-b). Meiotic course in these populations were normal except taxa of few populations (Table 2) where less than 5% proximate pollen mother cells (PMC's) were observed with the phenomenon of chromosome stickiness and cytomixis. Pollen fertility was high in these populations and observed to be above 90% (see Table 1). Only the result of the taxa with present cytological interest is described here.

Meiotic observations in the floral buds, collected from the plant population growing in Darcha area (2,850m) of Keylong district were reported with tetraploid chromosome count of 2n=4x=40 (Figure 1c-g). Meiotic course in this population was differing from that of diploid populations collected from the study area (Table 2). At M-I nearly 27.17% of PMC's were observed with chromosome stickiness (Figure 1h-i). Multiple bridges, laggards and poles were present at anaphase-I (A-I) in nearly 17.52% PMC's (Figure 1j-n). Approximately 23.33% of PMCs were present with inter PMC's chromatin transfer i.e. cytomixis (Figure 1o). Microsporogenesis was observed with diads, triads, micronuclei and unequal daughter cells (Figure 1p-t). The consequences of these meiotic abnormalities were observed in the form of heterogenous sized pollen grains and low (67.33%) pollen viability (Figure 1u-w). Heterogenous sized pollen grains are studied in three categories namely, small size (P; falls in averaged size ranged of x ; $x \leq 27.82$ x 25.83 µm), normal medium size (Q; falls in averaged size ranged of y ; 27.82 x 25.83 µm < y ≤ 34.75 x 32.89 µm) and large size (R; falls in averaged size ranged of z ; > 34.75 x 32.89 µm). Pollen grains falling in range of y (category Q) and z (category R) were observed in all the collected populations, only tetraploid population of Darcha area was observed with pollens falling in all the ranges of x, y and z i.e. categories P, Q and R. The relative frequency % (Rf-value) of medium (category Q) sized (T; Table 2) fertile pollen grain was high in almost all the populations while it was lowest (Rf %=55.98) in tetraploid population (P12) of Darcha area and highest (Rf %=96.84) in diploid population (P8) of Chamba (Table 2).

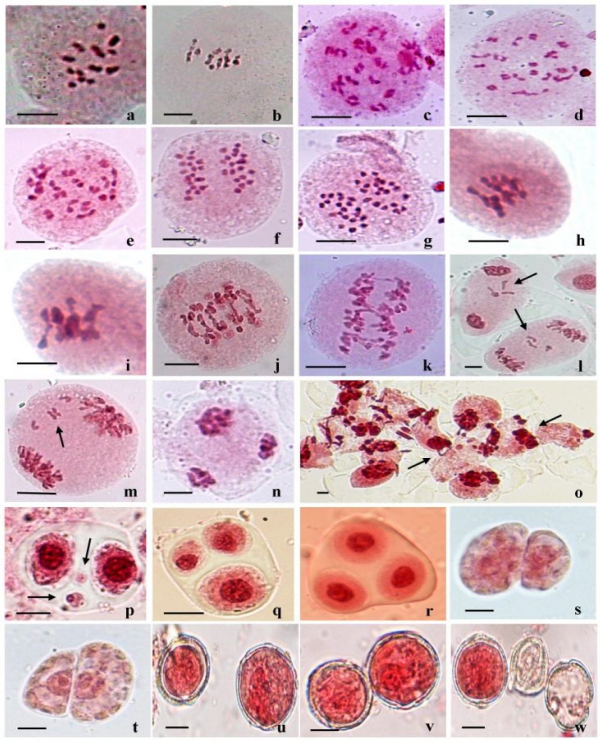

Figure 1: Meiotic observations in *Cannabis sativa* (L.)

[*a-b: PMCs showing 10_{II} at M-I; c-d: A PMC with 20_{II} at diakinesis; e: PMCs having 20_{II} at pre metaphase-I; f-g: PMCs showing 20:20 chromosome distribution at A-I; h-i: PMCs showing chromosome stickiness at M-I; j-k: PMCs with multiple bridges at A-I; l-m:. PMCs showing laggards at A-I; n: A PMC with three meiotic poles having unequal chromatin content; o: PMCs involving in cytomixis; p: A diad with two microneuclei; q-r: Triads; s-t: Cytokinesis with unequal sized daughter cells; u-v: Heterogenous sized pollen grains; w: Sterile pollen grains. (Scale=10µm)*]

Table 1: Data showing locality with voucher specimen numbers, 2n chromosome counts/ ploidy and previous reports with remarks of *Cannabis sativa* (L).

Populations (P)	Accession Number	Locality (Altitude)	2n (Ploidy)	Pollen viability %	(2n) Previous reports/ Remarks
1	20125	Trilokinath (3,020m)	20 (2x)	088.76	2n=2x=20: Krasnikov and Schaulo 1990; Stepanov and Muratova 1995 Murín and Svobodová 1992; Kaur and Singhal 2010; Kiehn et al 2000; Kaushla 2012.
2	20126	Kaza (3,820m)	20 (2x)	097.55	
3	20127	Udaipur (2,743m)	20 (2x)	100.00	
4	20128	Losar (4,079m)	20 (2x)	100.00	
5	20129	Keylong (3,350m)	20 (2x)	096.81	
6	20130	Manali (2,050m)	20 (2x)	095.83	
7	20131	Gramphoo (3,060m)	20 (2x)	097.66	
8	20132	Chamba (920m)	20 (2x)	100.00	
9	20133	Kullu (1,362m)	20 (2x)	094.59	
10	20134	Khoksar (3,140m)	20 (2x)	091.33	
11	20135	Batal (3,960m)	20 (2x)	099.91	
12	20136	Darcha (3,360m)	40 (4x)	067.33	2n=4x=40: First Report*

**Tetraploid= First report of 2n=4x=40.*

Table 2: Data showing analysis of cytomixis, meiotic course, microsporogenesis and pollen character in *Cannabis sativa* (L).

P	Cytomixis			Meiotic course (PMC's)			Microsporogenesis			Pollen		
	PMC's involved (%)	No. of PMC's involved	Stages	Laggards at A/T (%)	Bridges at A/T (%)	CS (%)	Diad %	Triad %	Polyad %	Fertility %	Size (Range in µm)*	Rf % S, T, U
1	00.00	00.00	00.00	00.00	00.00	00.00	00.00	00.00	00.00	100	-, Q, R	00.00, 86.66, 13.34
2	00.00	00.00	00.00	00.30	00.00	03.09	00.00	00.00	00.00	97.81	-, Q, R	00.00, 91.21, 08.79
3	00.00	00.00	00.00	00.00	00.00	00.00	00.00	00.00	00.00	99.01	-, Q, R	00.00, 89.91, 10.09
4	2.18	2-3	M-I	00.00	00.00	05.33	00.00	00.00	00.00	93.73	-, Q, R	00.00, 83.33, 16.67
5	00.00	00.00	00.00	00.00	00.01	00.00	00.00	00.00	01.00	92.55	-, Q, R	00.00, 95.56, 04.44
6	00.00	00.00	00.00	00.00	00.00	00.25	00.00	00.00	00.00	100	-, Q, R	00.00, 87.20, 12.80
7	00.01	2-3	M-I	02.00	00.00	04.50	00.01	00.10	00.00	100	-, Q, R	00.00, 85.10, 14.90
8	00.00	00.00	00.00	00.00	00.00	00.00	00.00	00.00	00.00	91.11	-, Q, R	00.00, 96.84,

												03.16
9	00.00	00.00	00.00	01.50	00.00	03.80	00.00	00.00	00.00	93.86	-, Q, R	00.00, 91.22, 08.78
10	00.00	00.00	00.00	00.00	00.00	00.00	00.00	00.00	00.00	95.50	-, Q, R	00.00, 89.96, 10.04
11	00.00	00.00	00.00	00.00	00.00	00.00	00.00	00.00	00.00	98.20	-, Q, R	00.00, 96.13, 03.87
12	33.36	2-3	EP-I/M-I	19.52	15.60	27.17	07.50	06.33	09.50	67.80	P, Q, R	10.27, 55.98, 33.74

Where, P= Populations (as referred in table 1); A/T (%) = At Anaphase-I/II or Telophase-I/II; CS= Chromosome stickiness; P= Small sized pollens; Q= Normal medium sized pollens; R= Large sized pollens; RF%= Relative frequency for small (S), medium (T) and large (U) sized pollen grains [Observed number of (small, medium, large) pollen grains / total number of fertile pollen grains].

The report of 2n=2x=20 in the present species is in agreement with the previous reports of 2n=20 by Kaur and Singhal (2010), and Kaushla (2012) from India and by several authors from outside (see Table 1). Apart from the present species, chromosome number of the other species viz. *C. ruderalis* Janisch (2n=20, Krasnikov and Schaulo 1990; Stepanov and Muratova 1995) and *Cannabis sativa* subsp. *spontanea* (Czer.) Serebr. (2n=20, Murín and Svobodová. 1992; Kiehn *et al.* 2000) have also similar counts. The tetraploid (4x) meiotic chromosome count of 2n=40 in *Cannabis sativa* L. from cold desert area of Lahul-Spiti is reported for the first time. Cytologically all the diploid species have normal meiotic behavior with high (>90%) pollen viability. Presently the irregularities and disturbances in meiosis contributed to the degenerative processes of pollen development in tetraploid (4x) population and resulted in low (<70%) viability. The low pollen viability in (4x) population reflects the role of meiotic abnormalities especially laggards, bridges and stickiness of chromosomes as suggested in other species by Saggoo and Srivastava (2009); Srivastava (2012) and Kumar and Dwivedi (2013). Presence of heterogenous size pollen grains and high frequency of large sized pollen grains in (4x) population may be partially due to meiotic irregularities and partially due to cytomixis which are responsible for unequal chromatin distribution in these pollens. Similar observation and concept of cytomixis was made in other flowering plants by different workers (Saggoo and Srivastava 2009; Kalinka and Achrem 2010; Saggoo et al. 2011; Srivastava 2012; Kravets 2013). Now it is well known that cytomixis is a natural and normally genetically controlled phenomenon. But the influence of environmental factors (Jones and Newell 1948; Kurtz and Liverman 1958), nutrition (Bell 1959), ploidy (Gould 1957, Kapadia and Gould 1964) and geographical variation (Cain and Cain 1944) on pollen size and fertility in flowering plants could not be underestimated.

CONCLUSION: As per the results, it is clear that tetraploid (4x) meiotic chromosome count of 2n=40 in *Cannabis sativa* L. from cold desert area of Lahaul-Spiti is reported for the first time. Further, due to the absence of any significant morphological differences in between (2x) and (4x) populations, evolution towards 4x from 2x population under stress can be predictable, but in the absence of molecular and biochemical evidences it can only be hypothesized.

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