

MALE MEIOSIS AND MORPHOMETRIC ANALYSIS OF ETHNOBOTANICALLY IMPORTANT *ALLIUM CAROLINIANUM* DC. FROM KINNAUR DISTRICT OF HIMACHAL PRADESH, INDIA

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ABSTRACT

Objective: This study is focused on the brief review on the meiosis, ethnobotany and cultivation practices of *Allium carolinianum* (Family: Alliaceae) from District Kinnaur, Himachal Pradesh.

Methods: The local inhabitants were interviewed for concerning utilization of this medicinal plant. For meiotic studies the young unopened buds were fixed in Carnoy's fixative, anthers were squashed in 2% acetocarmine. A number of freshly prepared slides were examined for chromosome counts and meiotic abnormalities. Pollen fertility was examined using glyceracetocarmine (1:1). Photomicrographs were made using Nikon 80i Eclipse Microscope.

Results: The present tetraploid chromosome count was in line with the previous report, but the behavioral changes and the medicinal importance of the plant were reported for the first time from the study area.

Conclusion: At present, the biodiversity of Kinnaur is threatened by the continuous removal of plant species for various purposes. Hence, the efforts should be made through cultivation practices for the conservation of the medicinal plants.

Keywords: Morphometric analysis, Meiosis, Ethnobotany, Cultivation, *Allium carolinianum*, District Kinnaur.

INTRODUCTION

Temperate and alpine regions of the Himalayas in India are the home of about 40 species of wild onions [1-3]. Each species differing in taste, forms and color but known to have close biochemical, phytochemical, and nutraceutical properties [4]. *Allium carolinianum* is one such wild *Allium*. This ethnobotanically important annual or perennial plant produces egg-shaped nearly 2.5 cm wide solitary bulbs covered with leathery scales.

The species is found in the alpine zone between 3000 and 4000 m in Kinnaur area of Himachal Pradesh. The plants are widely distributed in the high altitude regions of Himalayas in India, Pakistan, Afghanistan, mountains in Soviet central Asia and China. In India, it has been reported from alpine regions of Himachal Pradesh and Uttaranchal. Karyomorphological studies carried out in this plant from Qinghai-Tibetan plateau [5] and Uttarakhand in India [6] revealed diploid and tetraploid cytotype, respectively. Both the previous studies were restricted to karyotype analysis from root tip cells. The present study is the first report on the analysis of male meiosis and meiotic abnormalities in the wild accessions of the species of the cold desert region of Himachal Pradesh. This study is part of our attempts to compile cytological and Ethnobotanical knowledge about monocotyledonous plant wealth of Kinnaur valley and adjoining high altitude regions of Himachal Pradesh, India.

METHODS**Study area**

Plant specimens were collected during snow-free period of April 2013 to September 2015 from Hango village (3600m) of Kinnaur, Himachal Pradesh. The district lies between 31° 05' 50" to 32° 05' 15" N latitude and 77° 45' to 79° 00' 35" E longitude. Plants were identified with the help of regional floras as well as by comparing with dried specimens at the Herbarium of Botanical Survey of India (BSI), Dehradun. The voucher specimens (accession number PUN 59604) were deposited in the Herbarium, Department of Botany, Punjabi University, Patiala.

Ethnobotany

The local amchi, shepherd, old men and women whose empirical evidence was respected by the local inhabitants were interviewed randomly concerning utilization of this medicinal plant, mode of preparation, occurrence in the area, etc. in different regions of Kinnaur.

Meiotic studies

The young unopened buds of suitable sizes were fixed in Carnoy's fixative for 24 hrs and then stored in 70% alcohol at 4°C until use. For meiotic studies anthers were squashed in 2% acetocarmine. A number of freshly prepared slides were examined for chromosome counts and meiotic abnormalities. Pollen fertility was examined using glyceracetocarmine (1:1) method [7]. Well filled pollen grains with stained nuclei were considered fertile and unstained or partially stained shriveled pollen grains were counted as sterile. Photomicrographs were made from the temporary mounts using Nikon 80i Eclipse Microscope.

RESULTS AND DISCUSSION**Morphology and ecology**

A. carolinianum commonly called "Demokh" is an annual or perennial plant, having large broadly linear glaucous, flat leaves. The scape is cylindrical up to 60 cm tall and bears many pink purplish flowers in a round umbel. The bulbs remain dormant inside the soil during winter and during April-May new shoots come up from the soil. Flowering takes place during the month of July-August and by the end of October-November seeds start dispersing (Fig. 1). In Kinnaur area, the plant grows in small isolated patches distributed along stream sides, open cliffs, along roadsides, open green slopes between 2800 and 4000 m. It has also been observed to grow at unapproachable slopes and cliffs in the regions.

Meiosis

Cytological analysis of *A. carolinianum* revealed the tetraploid chromosome count of $n=16$ (based on $x=8$) at diakinesis and metaphase-I (Fig. 2a and b) which is in line with the previous report [8-10]. Further the plant showed some meiotic irregularities



Fig. 1: The wild plant *Allium carolinianum* growing in its natural habitat

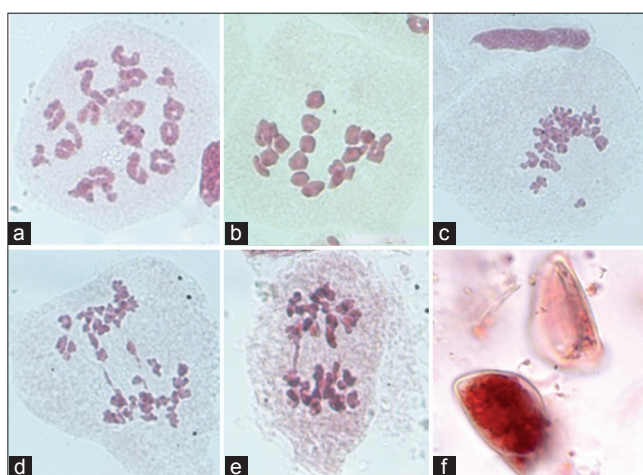


Fig. 2: *Allium carolinianum*. (a) A pollen mother cell (PMC) showing 16 bivalents at Diakinesis; (b) a PMC with 16 bivalents at metaphase I; (c) a PMC showing unoriented bivalents at metaphase; (d) a PMC with laggards at anaphase I; (e) a PMC with bridges at anaphase I; (f) a sterile and fertile pollen grain. Scale bar=10 μ m

such as unoriented bivalents at metaphase-I (Fig. 2c) and chromosomal bridges and laggards at anaphase-I (Fig. 2d and e), due to which the pollen fertility was reduced (77.46%) (Fig. 2f). Spindle apparatus plays important role in chromosome alignment during metaphase and any disturbance may lead to random unorientation of chromosomes in pollen mother cells [11]. The causes laggards and bridges may be due to interlocking of bivalents [12] or paracentric inversion [13].

Ethnobotany

There are reports of use of this plant by tribal and locals of other areas in Himalayas. In Spiti valley, vegetable prepared from leaves and rhizome is consumed to cure stomach disorders [14]. In Lahaul valley, Leaf and bulbs are used as stimulant, diuretic, and given with milk after delivery; soup is used for stomach infections [15]. In Lahaul and Spiti valley, paste of fresh parts is taken orally with water to cure stomach pain [16]. In Ladakh, leaves are collected by local peoples to prepare condiments, vegetable, and for medicinal use [17,18].

Whole plant is also considered to be highly nutritious and used by women after post-parturition as a substitute of meat in the Manang district of central Nepal [19]. In Northern Pakistan regions, whole plant is used as vegetable and medicinally used for swellings, dysentery and

joints pain, as a tonic [20], against flu and cough [21]. When added to diet on regular basis it helps to reduce blood cholesterol levels, act diet tonic to digestive system. Plant juice is used as moth repellent. The plant repels insects and moths. The predominating tribes living in high mountain areas of Kinnaur called Bhotia, depend on traditional medicine for treating different ailments. Fresh leaves of *A. carolinianum* are cooked as vegetable and local people dry the leaves and stored for winter as vegetable when no fresh vegetable is available. Local amchi use the dried seeds and leaves in powdered form to cure headache, stomach infections, urinary disorder, and restlessness.

The medicinal potentials of this plant have been equally supported by the bio constituents in the species. The plant contains essential oil with organic sulfur compounds, allin, allicin, scordinina, scordine, anthocynine, glycosides of kempferol. Vitamins such as riboflavin and niacin have also found [22]. The extracts from leaves and bulbs of *A. carolinianum* are rich in cysteine sulfoxide compounds such as alliin, isoalliin, methiin, and propiin. The species contain non sulfur compounds mainly carbohydrates, flavonoids, and saponins. This plant also shows antimicrobial, antifungal, and anti-algal activity [23].

Cultivation

The Bhotiya of Uttarakhand have been known to cultivate *A. carolinianum* on their agricultural fields and these plants are traded throughout the Himalayan region for centuries [24]. These plants are propagated through bulbs and their leaves are harvested twice a year. The Bhotiya sell these herbs or exchange them for grains and other spices at lower altitudes in the foothills of Himalayas [25]. The cultivation of medicinal plants helped to conserve indigenous knowledge about medicinal herbs among the Bhotiya [26-29].

CONCLUSION

Despite of a biodiversity rich region, no attempt has been made on chromosomal and Ethnobotanical study of traditionally used medicinal plant species from this particular region of Kinnaur. The biodiversity of Kinnaur is threaten by continuous removal of plant species for various uses, overgrazing by migratory livestock, construction of roads, development of hydro power projects, and climatic alterations that has resulted in desertification and loss of biodiversity. If the naturally occurring plant resources are not timely conserved then they may soon become extinct. Hence, the efforts should be made through cultivation practices by local inhabitants for the conservation of the medicinal plants.

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