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Male meiotic investigation in *Zanthoxylum armatum* Roxb.

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SUMMARY

One of the genus of *Zanthoxylum i.e. Z. armatum* Roxb. is a plant highly used for medicinal as well as religious purposes by the Hindus in Northern India. The other species found are *Z. planispinum, Z. alatum subtrifoliolatum e*tc. *Zanthoxylum* is also known as winged prickly ash, tejbal, timroo and Nepali dhania.The plant is found in the hot valleys of Himalaya from 600 to 1800 m. In the present work the genera was explored for detailed male meiotic analysis. The male flowering material was collected from the Kotbanglow, Uttarkashi. The material was fixed in Carnoy's fluid II (6 absolute alcohol: 3 Chloroform: 1 Glacial acetic acid). The smearing and squashing was done in 1% aceto-orcein. The genus was explored cytogenetically using parameters like chromosome configuration at metaphase I/diakinesis, chiasmata/chromosome frequency, number of spores/tetrad, pollen size and pollen sterility. The number of bivalents were observed to be 33 (2n = 66). High amount of asynchrony (stages from metaphase I to telophase II) was observed within the same anther. The chiasmata/cell and chiasmata/chromosome were observed to be 61.2 % and 0.93 %, respectively. Meiotic anomaly like retarded movement of chromosomes and chromatids were also observed in about 10 % PMCs (Pollen mother cells). The spore arrangement was found to be tetrahedral (four spores per PMCs), monads (single spore per PMCs), dyads (two spores per PMCs), heptads (seven spores per PMCs) and polyads (more then seven spores per PMCs). The mean number of pollen per anther was found to be varied from 1200-3200. The mean pollen diameter was found in a range of 10 -16 µm and the pollen sterility was found to be 13 %.

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Key words : Asynchrony, 33 bivalents, Metaphase I/diakinesis, Retarded movement, Sterile pollen grains

The genus *Zanthoxylum* is distributed worldwide from tropical to temperate zones. There are over 200 species from small shrubs to large trees. It has some other synonyms as *Z. planispinum, Z. alatum subtrifoliolatum* (French.), etc. It is known as winged prickly ash, tejbal, tejphal, timroo timber or Nepali dhaniya. It is widely distributed throughout the warmer region of the world, extending into temperate region of Europe, Asia and Australia. About 50 species among 20 genera are reported from India. Out of which 9 species are classed as commercial timbers (Pearson and Brown, 1932). The range of the plant is from Eastern Asia –China to the Himalayas. *Zanthoxylum* is recognized as having medicinal qualities for curing stomachache, toothache, intestinal worms, rheumatism, scabies, snakebites, fever, cholera and used as a flavouring agent or spice for preparation of certain traditional dishes. During winter, a soup made from the dried fruit (locally known as hag) is consumed by the entire family to keep warm in winter. A chutney (like a sauce), locally known as dunkcha, is also a popular food item (Kala *et al.,* 2004). The seed is ground into a powder and used as a condiment (Facciola, 1990). The fruit is rather small but is produced in clusters which make harvesting easy. Each fruit contains a single seed and young leaves are used as condiments (Gupta, 1945; Tanaka, 1976; Facciola, 1990). The fruit contains 1.5% essential oil (Chopra *et al.*, 1986). The oil obtained from plant is known as *Zanthoxylum* oil or Nepali pepper oil. The essential oil is obtained by stem distillation of the dried fruits. The oil being rich in linalool, and also containing limonene, methyl cinnamate and cineole. It is used as anti infectious, sedative, and for curing diseases like arthritis, cholera and toothache. It is also used as a spice, and as pepper substitute (Gupta, 1945; Tanaka, 1976). All the plant parts like seeds, bark, fruits, branches,

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thorns are used in different ailments (Gupta, 1945; Uphof, 1959; Usher, 1974; Chopra *et al.*, 1986).

Hooker (1875) has described eleven species of genus *Zanthoxylum* in India, of which 6 occur in the Himalayan region. The Uttarakhand Himalaya harbours 4 species of *Zanthoxylum,* namely *Z. armatum* DC. *Z. acanthopodium* DC. *Z. oxyphyllum* Edgew and Z. *budrunga* (CSIR 1989). The genus is represented by *Z. limonella* in the plains but the other species are restricted to montane and sub-montane regions. All the 8 species, namely *Z. ovalifolium*, n=18, 34; 2n=ca. 136; *Z. acanthopodium,* n=32; *Z. armatum*, n=33; *Z. nitidum*, n=34; *Z. scandens*, n=34; *Z. limonella*, n=34; *Z. oxyphyllum*, n=36; and Z. *tomentella* n=36 are cytologically investigated (Mehra and Khosla, 1973). The family Rutaceae embraces 1,800 species in 150 genera (Brizicky, 1964).

In the present work, the genera was exposed to detailed male meiotic analysis. The aim of the work has been to determine their chromosomal constitution, process of meiosis, pollen fertility and sterility. Till date the most of work reported on *Zanthoxylum* was through vegetative and micro propagation method. Less work has been reported on cytogenetic aspects of *Zanthoxylum* (Singhal *et al.,* 1983) studied many plants of family rutaceae cytopalynologically. The different plant parts have a number of alkaloids like dictamine, γ-fagarine, etc.

MATERIALS AND METHODS

In the Garhwal Himalayas the plant is mostly dioecious. Material for meiotic studies was collected from the wild forest of Uttarkashi (Kotbanglow, Uttarkashi 1150 m.asl) at the morning time (Fig. 1). Flower buds were fixed in Carnoy's fluid II (6:3:1; absolute alcohol: chloroform: glacial acetic acid) for 24 hours and after that transferred to 70% ethanol and stored in a refrigerator.

Anthers were smeared and squashed in 1% aceto-orcein for studying meiosis (Fig. 2). Slides were made permanent in Euparal. Using electric binocular microscope, *Z. armatum* male flowering shoot, premature anther, monad were studied at a uniform magnification of (× 45), while diakinesis, metaphase I, metaphase II, spore tetrads, pollen/anther, pollen size, pollen sterility at a magnification of (×100). All the observation and photomicrographs were taken from unsquashed and squashed temporary preparation.

Fig. 1 : Male flowering shoot

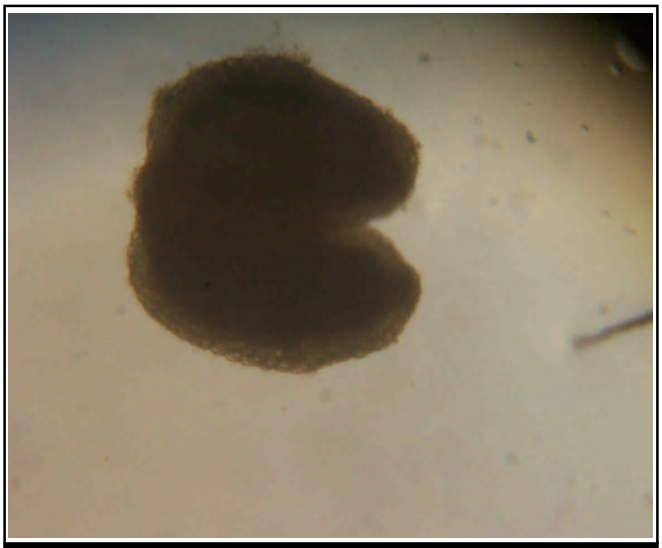

Fig. 2 : Premature anther

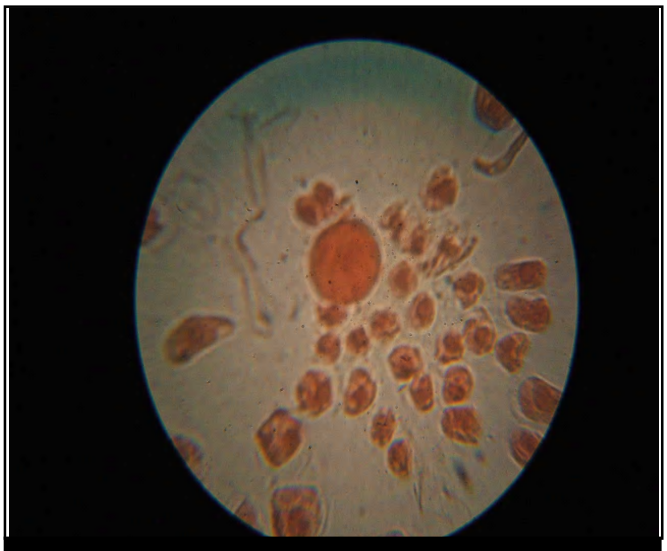

Fig. 3 : Monad

For the study of pollen size variation, anther of desirable age was kept in a slide and crushed with the helps of a needle, and then these pollen grains were stained with 1% aceto-orcein.

Various cytological parameters (Fig. 1-12) that were analyzed in the present study are:

Pollen size, Number of spores per tetrad, Total no. of pollen grains in an anther, Pollen sterility, Asynchrony for meiotic divisions within an anther, Chromosome configuration at metaphase I/diakinesis, including the chiasmata frequency, Types and frequency of various meiotic anomalies.

For the study of pollen sterility and fertility pollen grains were stained with 1% aceto-carmine for 24 hours.

RESULTS AND DISCUSSION

The chromosome count was confirmed by metaphase II analysis (Fig.4). During diakinesis/ metaphase I investigation 33 bivalents were observed. The meiosis within an anther was asynchronous, stages from prophase I to telophase II was noticed in the same anther. All the observations produced only bivalents. The mean number of chiasmata/cell and chiasmata/ chromosome were observed to be 61.2 and 0.93, respectively. The chiasmata frequency per chromosome

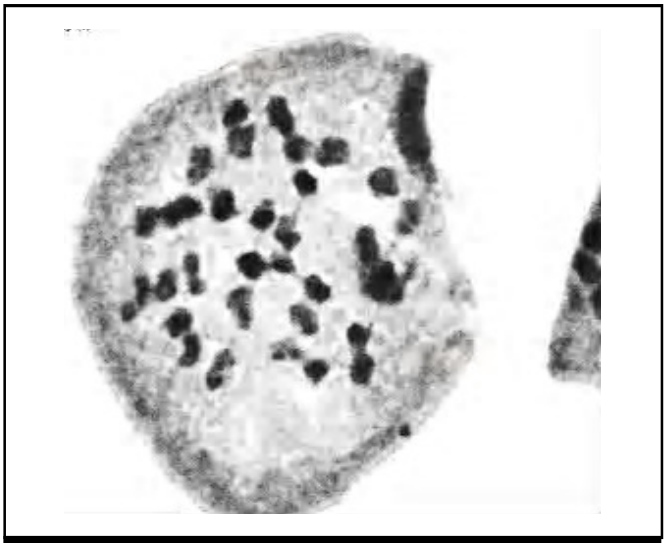

Fig. 4 : Diakinesis showing

indicating that both arm's of a homologous chromosome pair is mostly involved in synapsis and that the two gametophytic sets share a significantly higher degree of homology.

On examining these pollen grains in binocular microscope two types of pollen grains were found *i.e.* stained and stainless. The result revealed that stainless pollen grains were sterile, while stained pollen grains were fertile.

The frequency of ring bivalents was found to be higher than rod bivalents. During the present asynchrony analysis telophase II were observed in highest frequency (32%) though metaphase I (29.33%), metaphase II (28.00%), and telophase I (10.66%) were also observed. The meiotic course was normal in majority of all the observations but different amounts of irregularities in meiotic course were also observed as retarded movement of bivalents for metaphase I alignment (Fig.5) and chromosomes for metaphase II (Fig.6). Asynchrony in meiotic stages between sister dyad cells were also noticed.

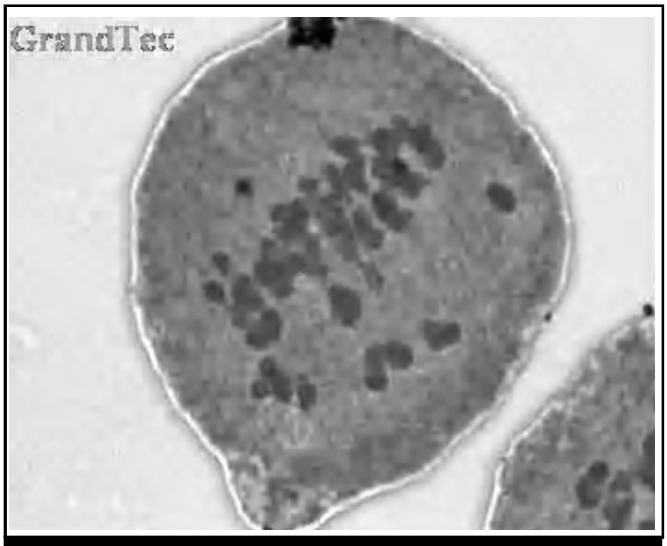

Fig. 5 : Retarded movement of chromosomes at metaphase I

Fig. 6 : Retarded movement of chromatids at metaphase II

During the spore tetrad analysis tetrahedral tetrads were observed 42.41% (Fig.7) in highest frequency though Monads (12.00%), dyads (15.65%), heptads (9.00%) and Polyads (22.00%) were also observed.

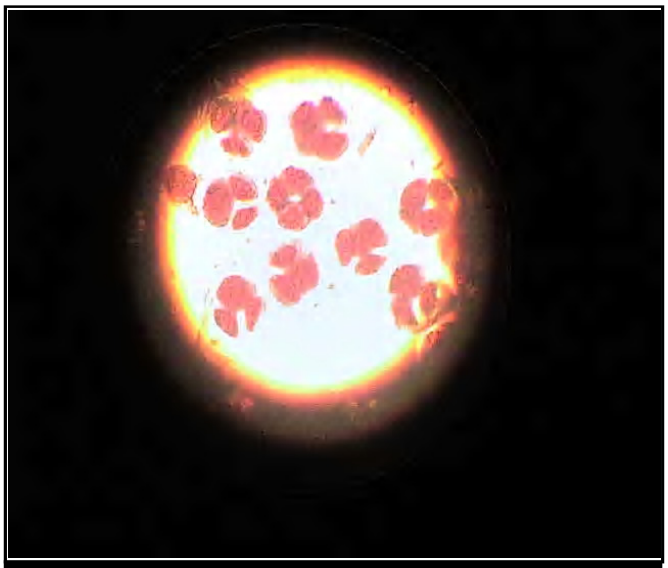

Fig. 7 : Spore tetrads

Mean data related to pollen sterility, number of pollen grains per anther (Fig. 8) and pollen diameter were noticed. The pollen sterility was found to have 13%. The mean number of pollen per anther was found to be varied from 1200-3200. The mean pollen diameter was found in a range of 10 -16 µm and the pollen sterility was found to be 13 % (Fig. 9).

Frequency distribution of PMCs within an anther at different stages of meiosis showing asynchrony, present within an anther at different stages of spores like monads, dyads, and polyads, chromosome analysis at metaphase I, No. of pollen /anther, pollen diameter and pollen sterility within an anther were tabulated in Tables 1, 2, 3 and 4, respectively.

The plant life cycle alternates between a diploid saprophyte and a haploid gametophyte. Meiosis in plants represents the transition from the saprophytes to the gametophyte generation. In higher plants, meiosis takes place in specialized cells, the sporocytes, which are formed in the anthers and ovules. Many of the genes encoding basic structural components of the meiotic machinery that is common to all eukaryotes, such as that required for chromosome organization, segregation and conservation.

Fig. 9 : Size variation of pollen grains

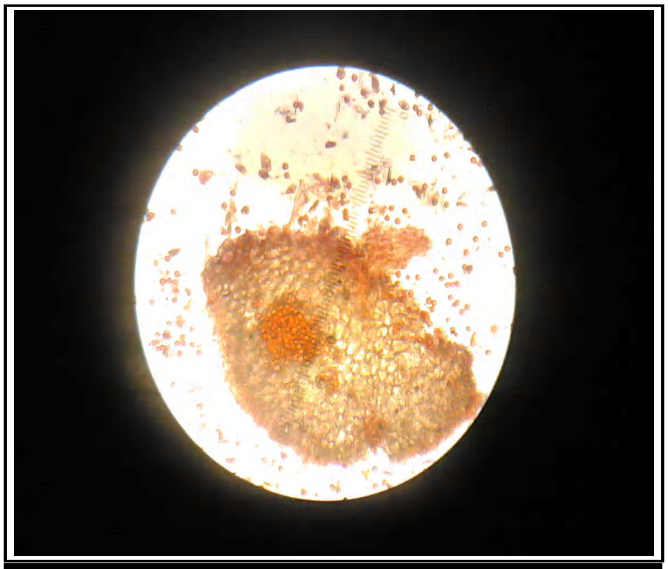

Fig. 8 : No. of pollen /anther

Fig. 10 : Fertile and sterile pollen grains

Table 1 : Freq. (%) distribution of PMCs within an anther at different stages of meiosis showing asynchrony in *Z. armatum*

Stages	% Asynchrony
Metaphase I	29.33
Metaphase II	28.00
Telophase I	10.66
Telophase II	32.00

Table 2 : Data showing per cent within an anther at different stages of spores like monads, dyads and polyads in *Z. armatum*

Type	%
Monad	12.00
Dyad	15.65
Tetrad	42.41
Heptad	9.00
>7	22.00

Table 3 : Chromosome analysis at metaphase I in *Z. armatum*

	Mean	SE	Range		
Ring	28.21	± 0.97	21.00	-	33.00
Rod	4.79	± 0.97	0.00	-	12.00
x-ta/cell	61.21	± 0.97	54.00	-	66.00
X-ta/chromo.	0.93	± 0.01	0.82	-	1.00

Table 4 : Data showing number of pollen /anther, pollen diameter and pollen sterility within an anther in *Z. armatum*

	Mean	SE	Range
No. of pollen /anther	2112.75	± 147.253	1180-35.30
Pollen diameter µm	18.0155	± 0.58188	13.0-21.9
Pollen sterility	15.7	± 1.32407	9.00-29.00

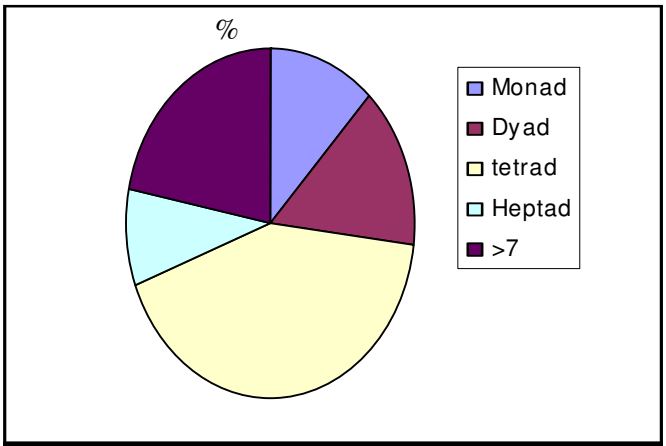

Fig. 11 : Pie diagram showing per cent of different spores like monads, dyads, tetrads, and polyads within an anther in *Z. armatum*

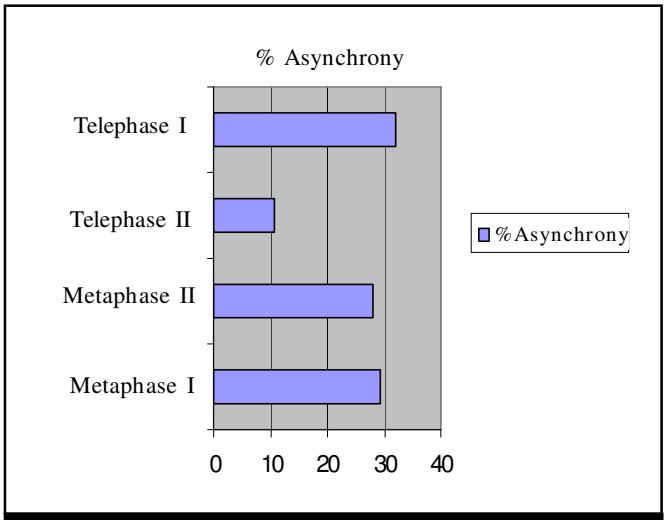

Fig. 12 : Bar diagram showing per cent asynchrony within an anther at different stages of male meiosis in *Z. armatum*

Meiosis is a "form of mitosis" in which the nucleus divides twice while the chromosomes divide only once, so that each of the meiotic products receives one representative of each chromosome type. The temporal pattern of meiotic stages is definitely ordered in both time and space and is genetically controlled. The chromosome count in the earlier works had been found to be 66 (Singhal *et al.,* 1983). The present work confirms the earlier count *i.e.* 2n=66.

Retarded movement of bivalents and chromosome for metaphase I and alignment, respectively may be due to malfunctioning of microtubules responsible for the displacement of chromosomes. Asynchrony in division between sister dyad cells could be due to differential speed of second meiotic division in these cells.

Dyads and polyads were observed. Dyads were formed due to arrest of meiosis in both sister dyad cells after first meiotic division, the polyads were formed due to cytoplasmic partitioning of additional nuclei at the end of meiosis.

Synchrony within an anther can be theoretically defined as the presence of only one meiotic stage in all the PMCs of the anther. For quantifying the degree of asynchrony, the highest present frequency of PMCs with the same meiotic stage was calculated against the total number of PMcs present in an anther.

The scientific work in progress is inadequate to save the biodiversity of this plant in mountain regions. Many improvements must be made. This group of plant should be studied well to understand their distribution, quantity and quality of plants available, selection of superior plants and to establish proper methods of conservation. Great

challenges lie ahead to select and save germplasm of good quality. More research input is necessary and many of the modern methods have to be adopted for mass propagation of good quality plants to increase production and economic value. With such inputs, changes are good that higher altitude plant will be saved and be used on a sustainable basis.

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