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Cytological studies and pollen viability of *Euphorbia wallichii* Hook.f.an important medicinal plant of Kashmir Himalaya

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ABSTRACT

Euphorbia wallichii, an important medicinal plant species of Kashmir Himalaya was examined for pollen mother cell (PMC) meiosis from four different wild populations of Kashmir Himalaya. Buds of suitable size were collected from all the four study sites and fixed in 1:3 acetic alcohol for 24 hours and stored in 70% alcohol under refrigeration. For slide preparation, the anthers were squashed in 2% acetocarmine and2% propiono-carmine. The results revealed the mean meiotic chromosome configurations of the species is x=10 having 2n=2x=20. Pollen viability show positive correlation with altitude and increase with increase in the altitude. Pollen viability found to be more at high altitude population (Sinthantop) and less at low altitude population (Hallan). Pollen viability ranged from 65.05 to 87.76% from low altitude to high altitude population.

Keywords: Euphorbia wallichii, Kashmir Himalaya, PMC meiosis, acetocarmine, pollen viability.

I.INTRODUCTION

Family Euphorbiaceae is of worth prominence due to immense diversity of chromosome numbers and chromosome sizes both between and within so called natural groups. First chromosomal survey in the family was carried out by [1]. There are two fundamental base numbers in the family x=7 and x=13 of which latter is probably derived from the first by doubling and anueploid reduction [2]. The original number was probably x=10 with other numbers originating in descending anuepliod series. The lowest chromosome number in the family is 2n=12 in *Euphorbia cornuta* and the highest is in *Antides mabuninus* 2n=234 [3].

The species of *E.spinidens, E. marschalliana, E. bungei, E.buhsei* and *E.szovitsii* showed 2*n*=2*x*=20, while *E. orientalis* had 2*n*=2*x*=18 and *E. helioscopia* showed 2*n*=4*x*=42. The chromosome numbers obtained for *E. szovitsii* and *E.helioscopia* support the earlier reports, while the chromosome number of *E. spinidens, E. marschalliana, E. bungei* and *E. buhsei* are new to science. *Euphorbia* species differ in their karyotype formulae indicating the occurrence of structural changes in their chromosomes supported by meiotic chromosome pairing as quadrivalents were formed in diploid species due to heterozygote translocations [4]. The base chromosome

number of the family varies from *x*= 6–18 and both polyploidy and aneuploidy have played role in the species diversification [2].

Euphorbiaceae a large and almost cosmopolitan family, exhibit a considerable range of variation in habit morphology, embryology and chromosome number. A comprehensive cytological survey in such a family is desirable from the evolutionary aspect.

II. METHODOLOGY

The study sites for the present study were four wild populations of Kashmir Himalaya located at Hallan (2800 m), Brinal (3080 m), Khillanmarg (3125 m) and Sinthantop (3600 m) asl. For investigation of pollen mother cell (PMC) meiosis, buds of suitable size were collected from all the four study sites in the morning hours between 7 to 9 am and were fixed in Carnoy's fixative 1:3 acetic alcohol for 24hours. After 24 hours the material was preserved in 70% ethanol under refrigeration at 4^0C until use. Then anthers were squashed in 1% acetocarmine to observe phases with countable chromosomes numbers (metaphase and anaphase). Acetocarmine was prepared by dissolving 10 g carmine in 1 L of 45% glacial acetic acid and reflux for 24 h. It was filtered into dark bottles and stored at 4 °C. Chromosomes were counted from good prepared slides at metaphase and anaphase. Chromosome number was determined at diakinensis. Propiono-carmine was also used which was prepared by taking 45ml of 45% propionic acid with 55ml of distilled water to which 2gm of carmine powder was added to prepare 100ml of 2% propiono-carmine.

To test viability, pollen grains from ready to dehisce anthers of randomly selected flowers were stained in 1% acetocarmine and 1% aniline blue –lactophenol [5]. The slides were then observed under light microscope. The darkly stained pollen grains with regular margins were recorded as fertile. Counts of viable and non-viable pollen grains were made from randomly chosen fields of view at 10X. Percentage pollen fertility/viability was calculated as follows:

$$\%\text{age pollen viability} = \frac{\text{No of viable pollen grains}}{\text{Total no of pollen grains observed}} \times 100 \quad (1).$$

III. RESULTS

During meiotic analysis ten rod and ring shaped bivalents were observed at diakinensis confirming the diploid number 2n=20 chromosomes. Based on x=10 having 2n=2x=20. The 20 chromosomes pair into ring and rod bivalents at metaphase I. During meiotic analysis ten rod and ring shaped bivalents were observed at diakinensis confirming the diploid number n=20 chromosomes (Fig 1). The anaphasic segregation is fairly normal which confirms the higher percentage pollen viability. Viability of pollen grains is necessary for the vitality of the plant species and also for the efficient sexual reproduction. The species produces good quantity of pollen grains

and pollen fertility is more as compared to sterility. The percentage of healthy plump and viable pollen grains of the species studied in four different Kashmir Himalayan populations varies between 65.05-87.76%(Fig 2).

Table: Pollen viability of *Euphorbia wallichii* from Kashmir Himalayan populations.

Population	No. of pollen grains scanned	No of viable pollen grains	Percent viability
Hallan(2800m)	114.6±41.86	74.8±29.92	65.05
Brinal(3080m)	59.7±38.81	44.9±32.27	73.16
Khillanmarg(3125m)	74.4±37.58	63.7±32.23	85.38
Sinthontop(3600m)	52.5±13.4102	46.2±12.31	87.76

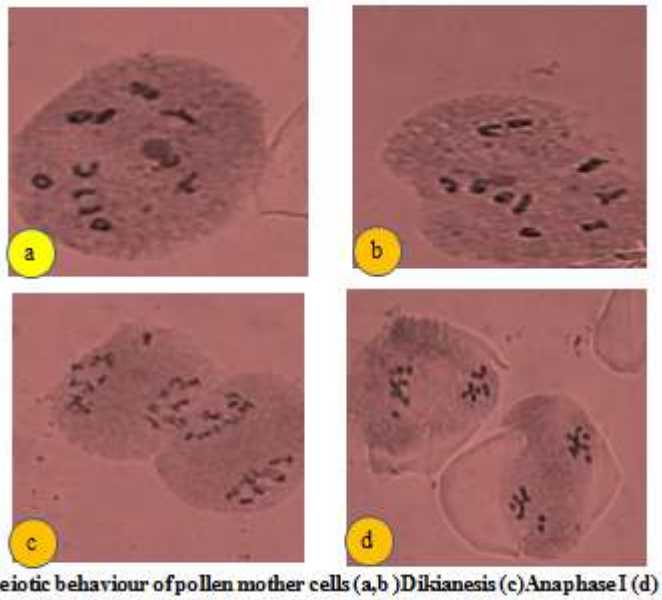

Fig1: Meiotic behaviour of pollen mother cells (a,b)Dikianesis (c)Anaphase I (d) Anaphase II

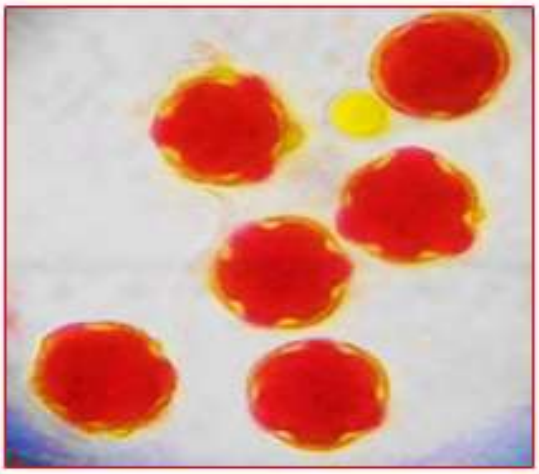

Fig 2: Pollen viability.

IV. DISCUSSION

The base chromosome number of the family varies from *x*= 6–18 and both polyploidy and aneuploidy have played role in the species diversification [2]. Present studies revealed that pollen viability show positive correlation with altitude and increase with increase in the altitude. Pollen viability was found to be more at high altitude population (Sinthantop) and less at low altitude population (Hallan). Pollen viability ranged from 65.05 to 87.76% from low altitude to high altitude population.

Pollen viability is considered to be an important parameter of pollen quality [6]. Research in controlled environments on different types of beans has shown that exposure to high temperature reduced pollen viability and seed set of the beans [7]. Lower pollen viability at high temperatures could be related to degeneration of tapetum layer [8], and/or decreased carbohydrate metabolism all of which could significantly influence nourishment of pollen mother cells which could lead to infertile pollen [9,10,11].

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