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MITOTIC CHROMOSOME OBSERVATION AND KARYOMORPHOLOGY IN *Aloe vera* (L.) Burm. *f.*

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ABSTRACT

Aloe vera (L.) Burm. *f.* commonly known as Ghritakumari which belongs to family Alliaceae or Asphodelaceae widely used in Ayurveda, as well as cosmetic industry and has as well as for its health benefits. In present work the cytological observation was performed. Fresh root tips from *ex vitro* plants were used for cytological study and different dividing stages were observed and photographed. The chromosome number was counted and a typical mitotic metaphase somatic cell contains 14 chromosomes (2n=14). The aim of the present work is to study the cytology of *Aloe vera*.

KEY WORDS: Cytology; *Aloe vera;* Mitosis; ex vitro.

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1. INTRODUCTION

Aloe vera (L.) Burm. *f*. syn. *Aloe barbadensis* Miller, were utilized for centuries earning the name “Plant of Immortality” and the Arabs called *Aloe* the ‘Desert Lily’ for its internal and external uses. The genus Aloe belonging to family Alliaceae or Asphodelaceae is a succulent herb of 80-100 cm in height which matures in 4-6 years and survives for nearly 50 years under favourable conditions. *Aloe vera* (L.) Burm. *f*. is most biologically active among 400 species [Joshi 1997; West & Zhu 2003; Yagi et al., 2003]. Cytology is that branch of life science, which deals with the study of cells in terms of structure, function and chemistry. Levitsky seems to have been the first to define the karyotype as the phenotypic appearance of the somatic chromosomes (Levitsky, 1931). Pre-treating cells in a hypotonic solution, which swells them and spreads the chromosomes, Arresting mitosis in metaphase by a solution of fixative and squash preparation. In recent years with refinement of techniques the study of chromosomes, structure becomes easier and more accurate for the study of physical & genetic nature of chromosomes.

1.1 MORPHOLOGY OF ALOE VERA

Aloe vera (L.) Burm. f. syn. *Aloe barbadensis* Miller, succulent perennial herb has triangular, sessile stem, shallow root system, fleshy, serrated leaves, arranged in rosette having 30 - 50 cm length and 10 cm breadth at the base; colour pea-green. The bright yellow tubular flowers, length 25 - 35 cm, axillary spike and stamens are frequently projected beyond the perianth tube and fruits contain many seeds [Yeh et al., 2003].

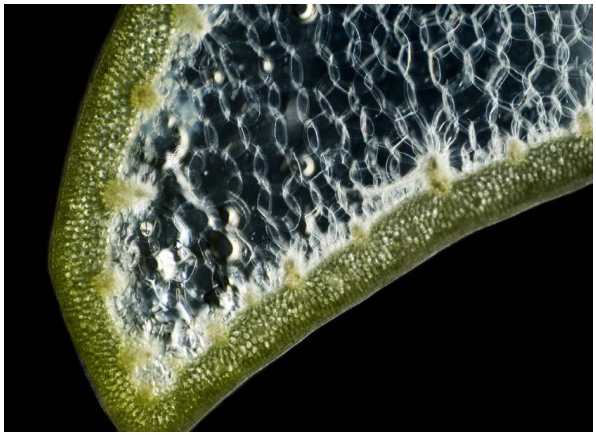

Figure 1
The transverse section of the leaf exhibiting three cells layers, the protective layer, middle layer and colourless inner layer.

Figure 1 represents the leaves have three layers, the outer most layers consist of 15-20 cells thick protective layer synthesizing carbohydrates and proteins [Brown 1980]. The juice that is originated from cells of the pericycle and adjacent leaf parenchyma, flowing spontaneously from the cut leaf get dried with or without the aid of heat and get solidified should not be confused with *Aloe vera* gel which is also the colourless mucilaginous gel that is obtained from the parenchymatous leaf cells [Bruneton 1995]. Aloe vera is predominantly characterized by the 2n=2x= 14 chromosomes with four long and three small chromosomes in its basic haploids set (Vig 1968).

1.2 CHEMICAL CONSTITUENTS

Aloe has complex chemical ingredients, from literature, there are over 100 secondary metabolites in leaf (Xiong, 2002). *Aloe vera* has marvelous medicinal properties due to nutritional ingredients in *Aloe vera*. There seems to be no single magic ingredient. The ten main areas of chemical constituents of *Aloe vera* include: amino acids,

anthraquinones, enzymes, minerals, vitamins, lignins, monosaccharide, polysaccharides, salicylic acid, saponins, and sterols (Barcroft & Alasdair, 1999).

2. MATERIALS & METHODS

The investigation was done during 2011-2012 with the targeted species for the present experiment is *Aloe vera*. The plant species collected from the Green House of Plant Biotechnology under West Bengal State Council of Science & Technology at Banabitan Complex, Salt Lake.

2.1 Pretreatment

Principle: Pretreatment is done for disorganization of spindle apparatus to spread out and flattened the chromosomes in metaphase at the equators. The main principle involved is that pretreatment chemical increase cytoplasmic viscosity level of the spindle and their by spindle losses its identity. The main objectives of pretreatment are as follows: Clearing the cytoplasm, Separation of middle lamella, causing softening of tissue, scattering of chromosomes with clarification of constricted region due to different hydration of chromosome segment, to remove undesirable tissue deposits and facilitated rapid penetration of fixatives and secure high frequency of metaphase stages obtained through spindle inhibition.

Pretreating agents: A number of chemicals are required for pretreatment. The time and treatment varies with the type of chemical used and also on the plant material used. Usually pretreatment is done in low temperature as it insures a slow and shady process of consideration and hydration of chromosomes whereby they become shortened and straightened. In our study pretreatment of root tips was performed in saturated para-dichloro bengene solution with trace amount of aesculine for 4.5 hours at 12-14°C. A chilling shock at 0°C to 4°C for 5 minutes (brief exposure) is applied at the initial stage of pretreatment for better scattering of chromosomes (Table1). Fixation may be defined as a process by which tissues or other components are fixed selectively to a desired extend. The purpose of fixation is to kill the tissue without causing any distortion of the compound to be studied. It should not only increase the visibility of the chromosomes structure but also clarify the details of chromosome structure and the common fixative given in Table 2. In our study, the roof tips after pre-treatment were fixed in glacial acetic acid: ethanol (1:3) mixture for 2-4 hours. 70% ethanol may be used for preservation of roof tip tissues at room temperature for a longer time period.

Table 1
represents the common pre-treating agents

Chemicals	Concentration	Time	Temperature
Para-dichlorobenzene (PDB) (Effective for high chromosome no.)	Saturated solution	3-5 hrs.	12-16°c
Colchicine	0.5-1%	½ hrs.	8-10°c
Aesculin	Saturated and semi-saturated solution	5min-24 hrs.	4-10°c
Coumarin	Saturated	3-6 hrs	Cold (4°c)

Table 2
shows the common fixatives

Non metallic	Concentration	Molecular weight
Acetic Acid	40-100%	60.45
Ethyl Alcohol	50-100%	46
Chloroform	100%	119.53
Formaldehyde	50%	30

Staining

Bio-colouring agents with capability to combine with cellular components by chemical combination. Staining is a combination of physical absorption of chemical reactions. Staining of chromosomes is carried out to make the visible under light microscope. Chromosomal stain may be acidic. e. g. Aniline blue (When the stain has only the protein part and basics. When the stained DNA, e.g. caramel). An Amphoteric stain is that which colour both protein & DNA e. g. Orcein.

Preparation of 2% Aceto-orcein stains stock solution

Requirements: 2 gm Orcein Powder, 100ml of 45% Acetic acid, Measuring cylinder, conical flask, Funnel, Filter Paper, Burner, glass rod, and Electronic balance.

Procedure

2 gm of orcein was taken and 100ml of 45% acetic acid added in beaker & heated to boil. When it reaches the boiling point orcein powder was slowly added to it & continuously stirs by a glass rod. Then heated for 10 min. keep it in a simmering point, it was allowed for 10 min. to cool down. After this filter and stored in a dark bottle. In last step 2% Aceto-orcein was mixed with 1N HCl to 9:1 ratio of 2% Aceto-orcein and 1N HCl. The roof tips preserved in 70% ethanol are transferred to 45% acetic acid (CH_3COOH) at room temperature for 10-15 minutes. The roof tips are subsequently stained in 2% aceto-orcein: HCl (9:1) staining mixture for 2 hours. 1(N) HCl is added in the staining mixture usually help in tissue softening through digestion of middle lamella. Finally the tip portions of 0.5-1.0 mm. were carefully excised from the roofs and squashed in a drop of 45% acetic acid.

Squash Preparation

Study of mitosis is done by squashed preparation. In this process the root tip was pretreated in suitable agents under necessary condition. It is a fix in Acetic acid: Absolute alcohol (1:2) for 1 hrs. Then the fix tissue was kept in 45%in CH_3COOH (Acetic Acid) for 10-15 min. for softening of tissue. After that it was gently treated in 2% Aceto orcein: 1N HCl in 9:1 proportion for few seconds; kept in staining mixture for 30-40 min. The root tip was then transferred in a clean grace free slide in a drop of 45% Acetic Acid. A cover slip was applied, squashed by exerting uniform pressure, sealed with paraffin and observed on the microscope.

Methods of preparation of permanent slide from temporary squash

In 1st step Paraffin seal is carefully removed with help of blade, slide is inverted in a Petridis containing Acetic acid: Ethyl alcohol (1:2) solution and kept their till the cover glass is detached off. In 2nd step slide and cover glass are then transfer to the 2nd Petridis containing ethyl alcohol, mix in a proportion of 1:1 and kept of 3-5 min. Then the slide and cover glass transferred to a 3rd Petridis containing ethanol and butanol mixed in proportion 1:2 and kept their for 3-5 min. 4th step slide and cover glass are then transferred to 4th Petridis containing ethanol & butanol mixed in proportion 1:3 for 3-5 min. Finally, the slide & cover glass transferred to a Petridis containing pure butyl alcohol and kept for 3-5 min. The cover glass mounted on a clean grease free slide in Euperol and the slide is covered with clean grease free cover glass.

RESULTS

Different stages were observed in the mitotic study of *A. vera*. The study shows well dividing stages along with non dividing cells. Different stages was photographed and presented above. From the present study, chromosome number was counted and 2n=14. Figure 2 representing the cell plate during division and different stages of mitosis. Cell plate was observed in microscope under 45x, and Figure b-e representing divisional stages of mitosis i.e. Prophase, Metaphase, Anaphase and Telophase under 100x respectively.

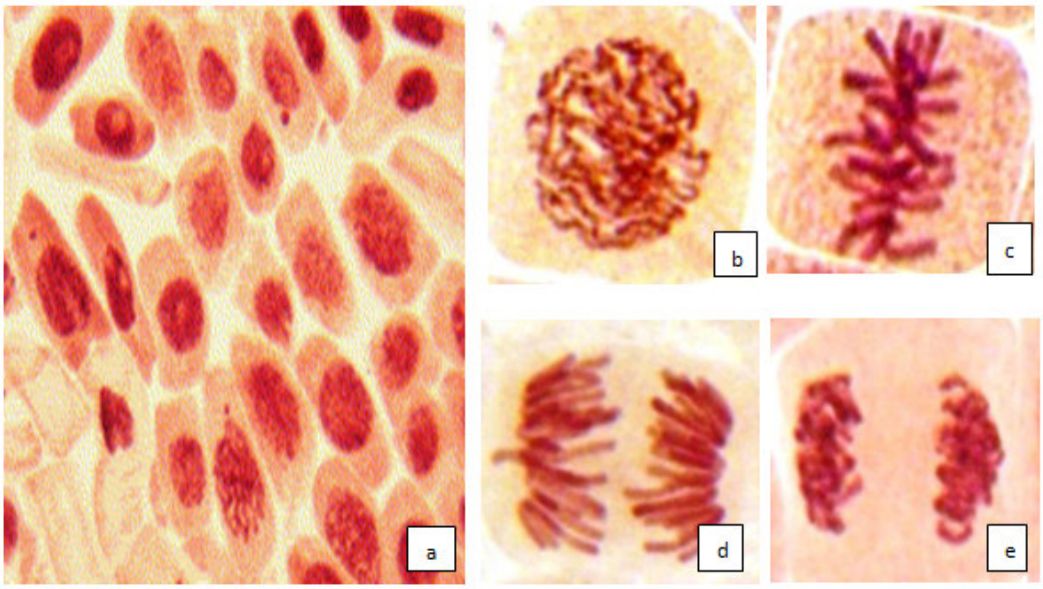

Figure 2(a-e)
shows the cell plate and different stages of mitosis a. Cell plate observed under 45x, b. Prophase (100x), c. Metaphase (100x), d. Anaphase (100x) and e. Telophase (100x)

Table 3
represents the Centromeric index and chromosome type

Centromeric Index (F %)	Name of Constriction	Types of chromosome
49.9- 37.5%	Median	Metacentric
37.4- 25.5%	Nearly median	Nearly metacentric
25.4- 12.5%	Submediam	Sub metacentric
12.4- 8.5%	Subterminal	Acrocntric
8.4-1.0%	Nearly subterminal	Nearly acrocentric
<1%	Terminal	Telocentric

Depending on the centrometric Index and formation of position of constriction can distinguished the type of chromosomes i.e. metacentric, sub metacentric, acrocentric, telocentric, nearly metacentric (Table 3).

DISCUSSION

Many studies showed that the chromosome number for somatic cell of most *Aloe* is 2n = 14, and the haploid set genome consists of three short chromosomes and four long ones (Brandham and Doherty, 1998 ; Ji *et al*., 2002; Alam and Khanam, 2005). Many studies demonstrated that most plants have *Arabidopsis*-type telomeres consisting of many repeat copies of the sequence 5'-TTTAGGG-3' (Adams *et al*., 2000), nevertheless, this kind of telomeres are not found in *Aloe*, similar to *Allium*, *Nothoscordum* and *Tulbaghia*. *Aloe* lacks *Arabidopsis* type telomeric repeats, but it has vertebrate-like telomeric sequences (T_2AG_3) and according to the report by Weiss and Scherthan (2002). According to the classification criterion reported by Stebbins (1971), the karyotypes of *Aloe* belong to '3B', '4B', '3C' or '4C' (Zheng *et al*., 2005). For example, K (2n) = 4sm + 10st of *Aloe ferox* Miller and K (2n) = 4sm + 10st of *Aloe arborescens* were belonged to 4C and 3B, respectively (Liang and Bo, 2001). The investigations based on seven methods revealed the separation of different karyotype asymmetry and parameters of the broad intervals used by Stebbins, explaining only one quantitative parameter (Paszko, 2006). Recently, cytogenetical studies have been used as taxonomic information complementary to biochemical, molecular, morphological and anatomical studies. Earlier studies suggested that *Aloe* has great

potential of chromosomal stability due to the presence of bimodal constant karyotype, n=7 with eight large and six small chromosomes (Vij *et al*. 1980; Takahashi *et al*. 1997; Brandham and Doherty 1998).

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