RESEARCH ARTICLE

CHROMOSOME MORPHOLOGY AND BEHAVIOUR IN *ALOE VERA* L. PLANTS GROWING AT JAMMU, J&K STATE, INDIA

Seema Devi*, Swati Gupta and Namrata Sharma

Department of Botany, University of Jammu, Jammu-180006, India
Email: dseema254@gmail.com

Received-02.07.2019, Revised-21.07.2019

Abstract: *Aloe vera* L., a medicinal plant belonging to family Asphodelaceae, has a long ethnobotanical and medical history. Though a prolific flower producer, seed formation occurs rarely in this species. Propagation occurs mainly through suckers. In order to probe the reasons behind the seedlessness, we investigated the meiotic system of the plants growing as escapes in our area i.e. Jammu, J&K state, India. Cytological characteristics of both sporophytic as well as gametophytic cells of these plants were investigated by studying pollen mitosis, nucellar cell mitosis and pollen mother cell meiosis. The species showed bimodal karyotype with karyotype formula as 6sm+8st in nucelllar cells and 3sm+4st in pollen grains. No significant difference was noted between chromosomes characteristics of haploid and diploid cells. The chromosome number of *Aloe vera* was 2n=14 (in nucellus cells) and n=7 (in pollen). While chromosome pairing was normal at metaphase I where 7II were observed, a large number of meiotic abnormalities was observed (69%) in the form of laggards, bridges and chromosome stickiness etc. during later stages. This reduced the pollen viability. Interestingly reduction in pollen viability had a correlation with environment factors in particular temperature. It showed a range from 2.45% to 79.47%. All the viable pollen were however cytologically stable with an expected haploid chromosome number as n=7 and karyotype formula as 3sm+4st.

Keywords: Karyotype, Gametophytic cell, Sporophytic cell, Bimodal, Meiotic system

INTRODUCTION

Chromosome studies are indispensable for any species. These gives us important information about chromosome number, morphology, ploidy level, homology, behaviour and aberration if any in numerical as well as structural form. Such abnormalities are known to affect the sexual system in terms of gametic mortality and efficiency (2.45%) and thus the reproductive output. In the present study we studied the somatic chromosomes of *A. vera* in both diploid nucellar cells (2n) and haploid (n) pollen, and tried to compare the various characteristics of chromosomes from both. We also studied chromosome behaviour during pollen mother cells meiosis and correlated the same with pollen viability. The studies were undertaken in view of the seedlessness in *A. vera* L. *A. vera* L. is a prolific flower producer but a very poor seed setter. The species relies on vegetative means for its propagation in most of the parts of the world including the present area of study i.e. Jammu, J&K, India. In the study area it shows meagre amount of fruit and seed set. The seeds produced are chaffy and without any embryo. Although some earlier studies have reported *Aloe* to be having stable karyotype with four large and three small chromosomes in the basic haploid complement (Brandham 1971, Das et al.,2010, Sapre 1975); present study was undertaken to report details of the karyotype of both haploid and diploid cells of *A. vera* L. and provide some basic information about its meiotic behaviour and gametic viability. Besides this, the studies tend to explore the role of cytological features in the extreme low sexual reproductive output of the species. A need is also highlighted to study the meiosis in the female tract i.e. in the megaspore mother cells to find out its contribution in the failure of sexual reproduction.

MATERIALS AND METHODS

Plants of *A. vera* growing as escapes in and around Jammu were identified and tagged. Few of these were transplanted in experimental beds (containing garden soil and cow-dung in the ratio of 1:1) in the Botanical Garden, University of Jammu, Jammu.

Nucellar cell mitosis: The floral buds of appropriate sizes were fixed in freshly prepared Carnoy's solution (ethanol: acetic acid, 3:1) and stored in 70% ethanol for further use. The pistils were removed from flowers, washed and their ovaries were hydrolyzed in 1N HCL at 60 °C for 10 minutes. These were subsequently stained in fuelgen for 15-20 minutes. Ovules were then dissected out of these ovaries under a dissection microscope on a glass slide and squashed in 1% propiocarmine. Slides were scanned for somatic chromosome complement in the nucellar cells.

Pollen mitosis and meiotic studies: For meiotic and mitotic studies in male track floral buds were fixed in early morning hours in carnoy's solution (ethanol: acetic acid, 3:1) for 24 hours. After overnight fixation buds were transferred to 70% ethanol at 4 °C until further study. The anthers were then squashed in 1% propiocarmine to work out details of meiosis in pollen mother cells and mitosis in pollen. Staining ability of the fresh pollen sample was determined by 1% acetocarmine. Round and deep red coloured pollen were considered viable whereas the shrivelled and colourless were non-viable. The percentage

*Corresponding Author

viability of the pollen was determined by using the formula,

$$\frac{\text{number of viable pollen}}{\text{number of viable+ non-viable pollen}} \times 100$$

Six well scattered metaphase preparations were selected for studying chromosome details each for gametophytic (pollen) and sporophytic (nucellus) cells. All these slides were examined and photographed by zeiss microscope using axiocamERc5s camera. The karyotype was prepared by following Stebbins chromosome classification. The homologous chromosomes were tentatively paired on the basis of their morphology and size and were arranged in decreasing order of their length.

RESULTS

Plant and floral characteristics: The plants of *A. vera* L. are stemless, spiny and succulent perennials. In the subtropical region of Jammu, the flowering period of *A. vera* L. ranges from mid- October to March. Beautiful orange coloured tubular flowers arranged in dense racemes on a colourless scape appear on these plants during this period. Each raceme bears an average of 62 flowers. Individual flower is hermaphrodite, actinomorphic and pedicellate consisting of six stamens and a single pistil. Each anther on an average produces 2477.5 pollen grains and thus pollen count per flower averages about 15783. Ovule count per flower averages 30.54. Thus the pollen-ovule ratio per flower is 516.79:1. Meagre amount of fruit as well as seed formation occurs in this plant (Gupta and Sharma 2011).

Cytological details: All the cells scanned for metaphase preparations revealed n= 7 for haploid pollen cells and 2n=14 for diploid nucellar cells (Fig.1& 2). The karyotype formula was 3sm+4st and 6sm+8st for gametophytic and sporophytic cells resp. Average chromatin length for haploid pollen cells was 71.13 $\pm$ 3.83µm with the longest and the shortest chromosome measuring 15.63 $\pm$ 1.19µm and 4.36 $\pm$ 0.26µm respectively (table 1). The ratio between the longest and the shortest chromosome was 3.58. The total chromatin length for diploid nucellar cells was 150 $\pm$ 4.03µm; the longest and the shortest chromosome measured 17.19$\pm$0.54µm and 3.80 $\pm$ 0.13µm respectively and the ratio between them was 4.52 (table 2). The karyotype was bimodal with long chromosomes as subtelocentric and the short as submetacentric (Fig.1b & 2b). The species falls into class 3C karyotype asymmetry category (Stebbin 1971) and thus has an asymmetric karyotype. No aberrant cell was recorded in nucellus or pollen as far as chromosome number and morphology is concerned.

Pollen mother cell meiosis revealed the cells carrying 14 chromosomes which pair and form seven bivalents, of which four were large and three small. At metaphase I, the large bivalents were mostly ring and the small bivalents rod shaped. Anomalies like chromosome lagging, chromatin bridges and stickiness of chromosomes were frequent during anaphase I & II. Lagging chromosomes and bridges were observed at anaphase II and stickiness at anaphase I & II both (Fig.3). However, these anomalies were more frequent in the intervening cold period spanning the months of January and February when the temperature dropped to 5.3°C (max.26.8°C during daytime) in the area of study. Average of 69% abnormal pmcs were observed in the month of February whereas about 32% in the month of November. Similar results were also found for pollen viability which also showed seasonal variability. The maximum pollen viability was recorded at the initiation and termination of flowering (average 60%) during the months of November and March (average temp. max. 30°C, min.10.2°C, relative humidity max.100%, min. 24%) It declined to very low to a minimum of 2.45% also, in the month of February when it becomes very cold in the area of study (average temp. max.26.8°C, min.5.3°C & relative humidity max.100%, min.44.4%). (Gupta and Sharma 2011)

DISCUSSION

Sexual reproduction is not only a means of propagation but also the source of generation of genetically variable plants. Breeding and meiotic systems are its integral parts and together constitute the ‘‘genetic system’’ (Darlington 1939). The failure of sexual reproduction is thus a huge set back to any species. *Aloe* is a native of North-Africa and is introduced to India. In the present area of study i.e. Jammu, J&K, India, inspite of profuge flower production, the setting of fruits is extremely rare in the species and whenever the seed is set, it is chaffy and embryoless. The effective means of propagation is through suckers. The failure of sexual reproduction in the species is attributed mainly to high rates of pollen sterility (Sapre 1975); although self incompatibility is also suggested by Berger (1908) for some *Aloe* species. To investigate the role of meiotic system in the same, we probed the chromosome constitution and meiotic behaviour of these plants. We also observed extremely low pollen viability and the seasonal variation of this feature in plants growing at Jammu. All plants studied by the author presently were diploid bearing 14 chromosomes which pair and form 7 bivalents, 4 large and 3 small. The number confirms to count made earlier (Brandham 1971 and Cavallini 1993). First nuclear division of pmc meiosis was normal except for few cells at anaphase-I displaying stickiness. The second division displayed anomalies like chromosome lagging, chromatin bridges and stickiness etc (fig.3d-h). Abnormal segregation at anaphase I and II could be hypothesized to have a genetic base or environment effect. These

abnormalities resulted in differentiation of genetically unbalanced pollen, which perhaps do not tolerate these anomalies and appear non-viable. All the viable pollen produced in plants studied presently were genetically balanced and carried a complete haploid set as n=7. So the species is appearently cytologically stable with gametophytic number n=7, and sporophytic number 2n=14. The karyotype was asymmetric and bimodal as has been described for the entire tribe, Aloinae (Darlington, 1963 and Stebbin, 1971).

Table 1. Chromosome parameters of haploid pollen grains of *Aloe vera*

Chromosome number	Total length (µm)	Long arm(L) (µm)	Short arm(S) (µm)	Arm ratio (L/S)	Centromeric position
1	15.63±1.19	13.07±1.35	2.56±0.49	5.10	st
2	14.74±1.09	12.06±1.011	2.68±.16	4.5	st
3	13.74±0.85	11.28±2.53	2.46±0.13	4.59	st
4	13.29±0.80	10.39±0.82	2.90±0.13	3.58	st
5	5.58±0.26	3.69±0.31	1.89±0.19	1.95	sm
6	4.91±0.26	3.35±0.32	1.56±0.20	2.15	sm
7	4.35±0.26	2.79±0.08	1.56±0.13	1.79	sm

*Mean±SE; sm: submetacentric; st: subtelocentric
Average chromatin length = 71.13±3.83µm

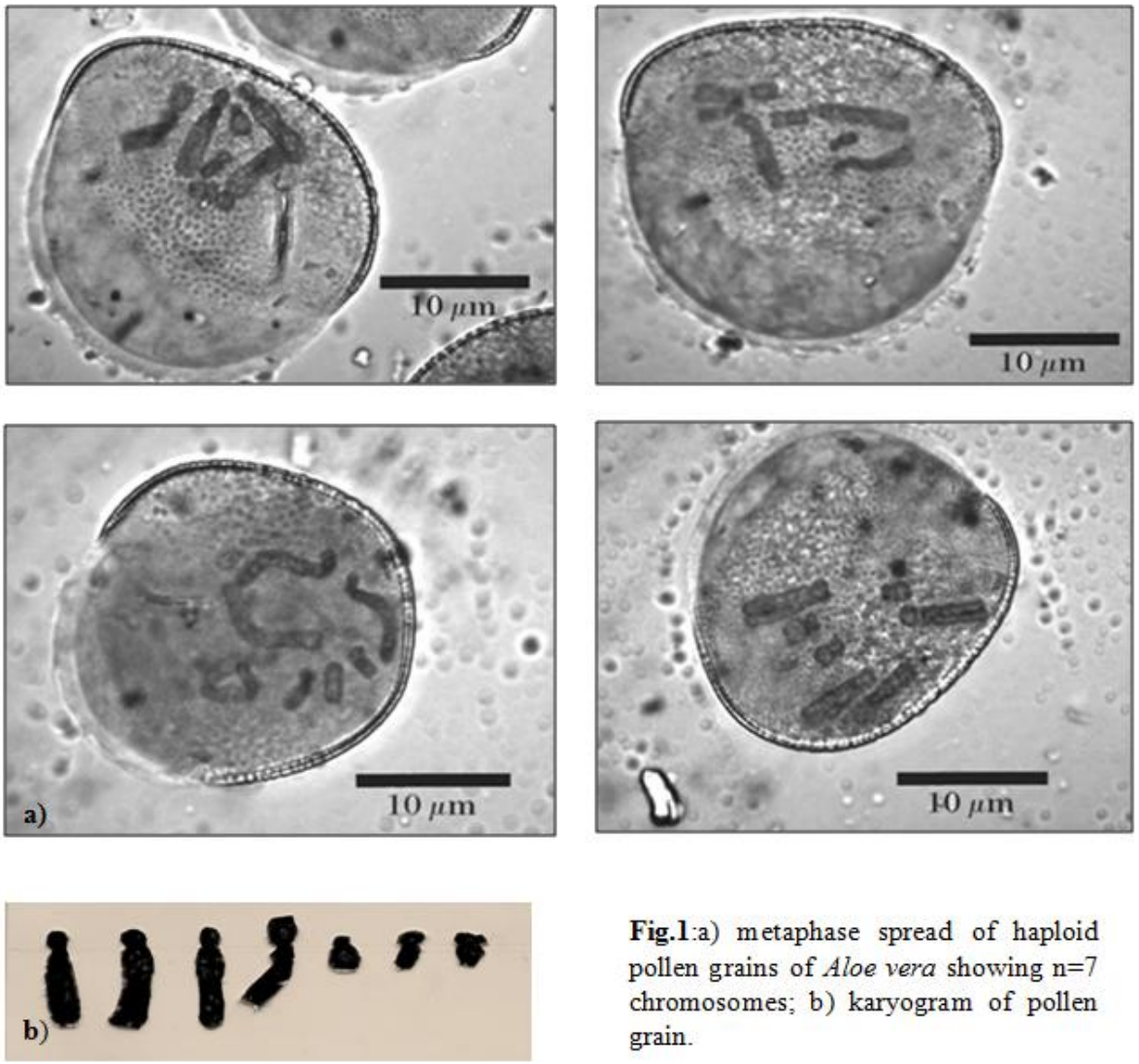

Fig.1:a) metaphase spread of haploid pollen grains of *Aloe vera* showing n=7 chromosomes; b) karyogram of pollen grain.

Table 2. Chromosome parameters of diploid nucellus cells of *Aloe vera*

Chromosome number	Total length (µm)	Long arm(L) (µm)	Short arm(S) (µm)	Arm ratio (L/S)	Centromeric position
1	17.19±0.54	14.29±0.41	2.90±0.30	4.93	st
2	16.64±0.43	13.85±0.58	2.79±0.53	4.96	st
3	16.19±0.53	13.85±0.49	2.34±0.34	5.91	st
4	15.74±0.52	13.51±0.40	2.23±0.30	6.05	st
5	14.96±0.56	12.40±0.54	2.56±0.10	4.84	st
6	14.51±0.56	12.28±0.44	2.23±0.26	5.50	st
7	13.74±0.41	11.73±0.30	2.01±0.22	5.83	st
8	13.07±20	11.06±0.14	2.01±0.16	5.50	st
9	6.14±0.29	4.13±0.29	2.01	2.05	sm
10	5.14±0.20	3.35	1.79±0.20	1.87	sm
11	4.91±0.26	3.24±0.18	1.67±0.14	1.94	sm
12	4.80±0.21	3.13±0.20	1.67±0.14	1.87	sm
13	4.69±0.27	3.02±0.20	1.67±0.14	1.80	sm
14	3.80±0.13	2.46±0.13	1.34	1.83	sm

*Mean±SE; sm: submetacentric; st: subtelocentric
Average chromatin length = 150±4.033µm

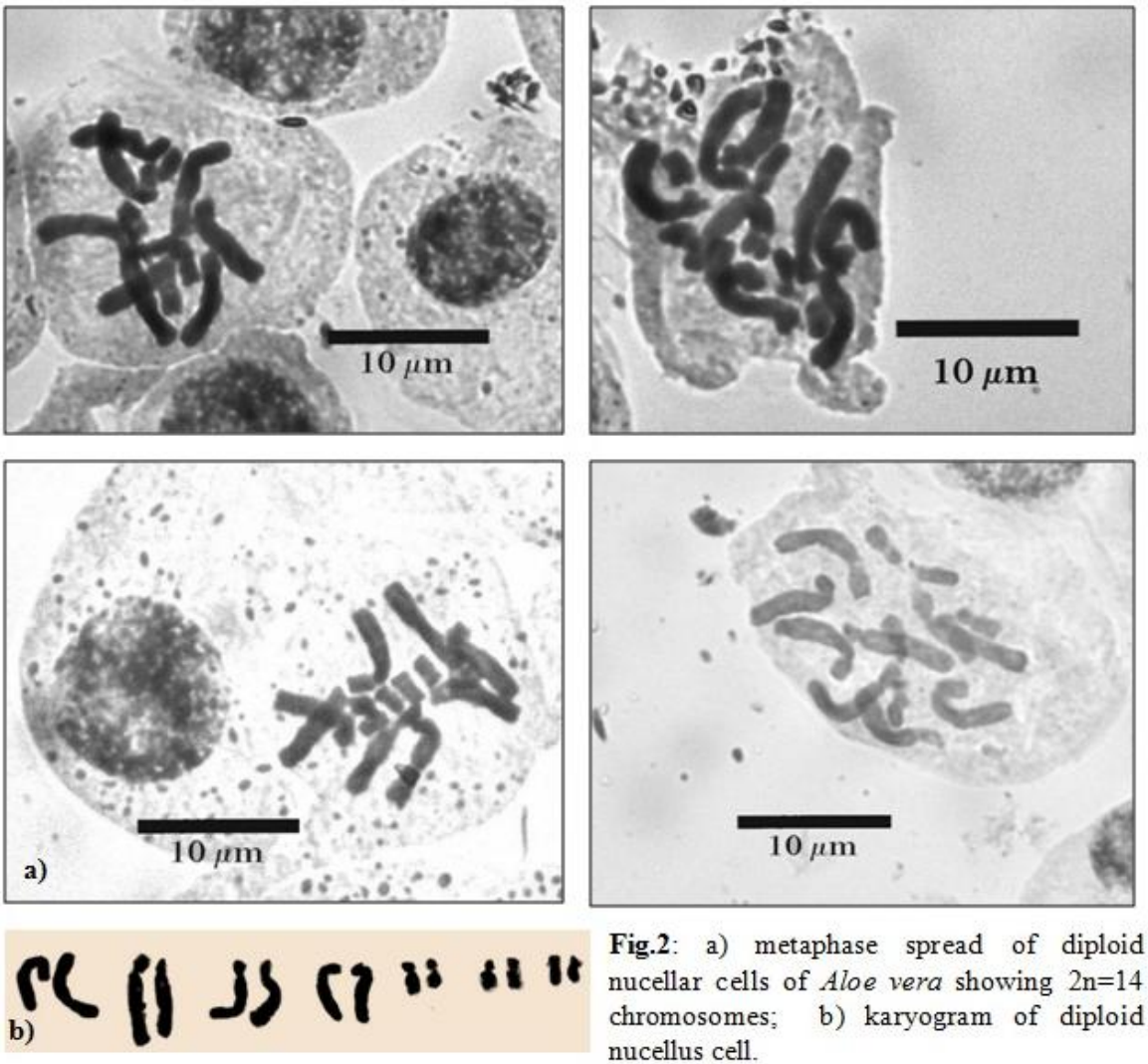

Fig.2: a) metaphase spread of diploid nucellar cells of *Aloe vera* showing 2n=14 chromosomes; b) karyogram of diploid nucellus cell.

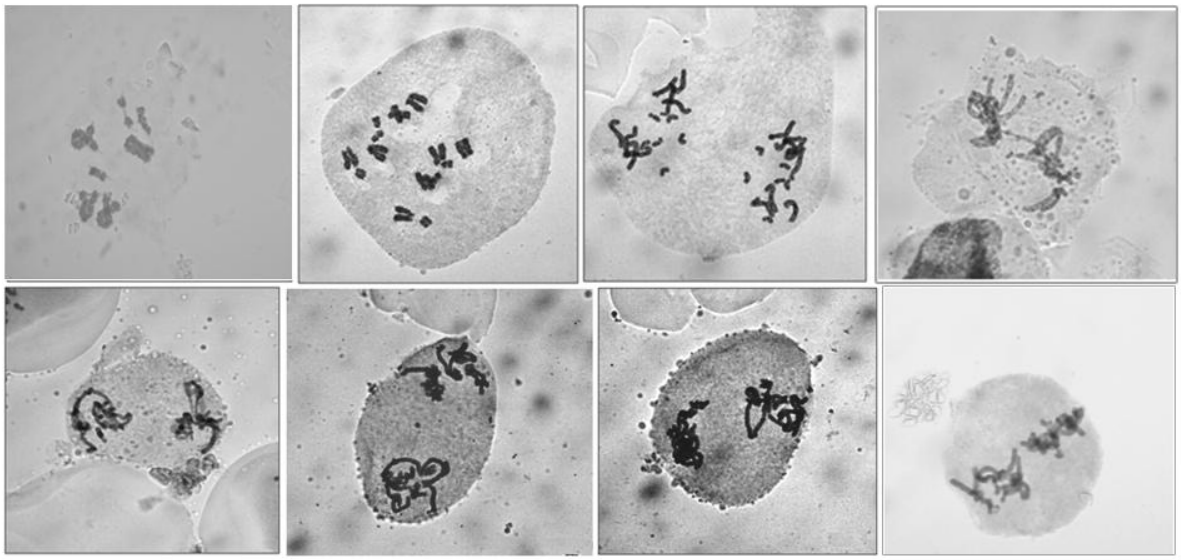

Fig.3: Pmc meiosis (a) bivalents at metaphase I (b) chromosomes moving to opposite poles at anaphase I (c) chromatids at four poles at early anaphase II (d) stickiness and laggard at anaphase II (e) and (f) bridge formation (g) and (h) stickiness at anaphase II.

CONCLUSION

Logically the viable pollen carrying complete haploid set as n=7, should be capable of causing fertilization and producing seed which is however not happening in the plants studied. Total breakdown of sexual reproduction and formation of no or chaffy seeds whatsoever reflects that even these pollen grains were not inducing seed set and that some other factors are also playing a role in suppressing sexual reproduction in the species. A need is there to study meiosis in female track also.

ACKNOWLEDGEMENTS

The author is thankful to the Head, Department of Botany, University of Jammu, for providing all necessary laboratory and library facilities.

REFERENCES

Berger, A. (1908). Liliaceae - Asphodeloideae - Aloinae. In: Englers Pflanzenreich IV: 38, III, II.

Brandham, P. E. (1971). The Chromosomes of the Liliaceae II. Polyploidy and karyotype variation in the Aloineae. Kew Bulletin, 25:381-399.

Cavallini, A. (1993). Cytophotometric and biochemical analysis of DNA variations in the genus *Aloe* L. International Journal of Plant Sciences, 154(1):169-173.

Darlington, C. D. (1939). The Evolution of Genetic Systems. Cambridge University Press, Cambridge.

Darlington, C. D. (1963). Chromosome Botany and The Origin of Cultivated Plants. George Allen and Unvin Ltd., London.

Das, A., Mukherjee, P., Ghorai, A. and Jha, T. B. (2010). Comparative karyomorphological analysis of in vitro and in vivo grown plants of *Aloe vera* L. BURM. f. Nucleus, 53(3):89-94.

Gupta, S. and Sharma, N. (2011). Seedlessness in *Aloe vera* L.- Role of male track in failure to harness benefits of sexual reproduction. The International Journal of Plant Reproductive Biology, 3(2):123-126.

Sapre, A. B. (1975). Meiosis and pollen mitosis in *Aloe barbadensis* Mill. (*A. Perfoliata* var. *vera* L., *A. vera* Auth. non Mill.). Cytologia, 40:525-533.

Stebbin, G. L. (1971). Chromosomal Evolution in Higher Plants. Edward Arnold Ltd., London.