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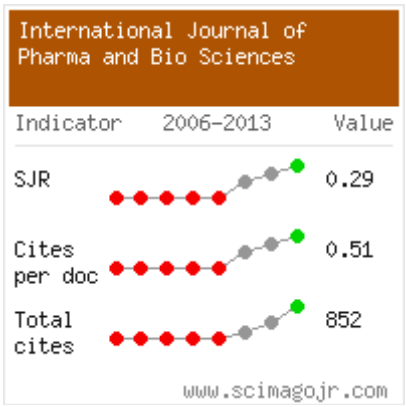

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SEEDLESSNESS IN ALOE VERA L. - ROLE OF ENVIRONMENTAL FACTORS IN FAILURE TO HARNESS BENEFITS OF SEXUAL REPRODUCTION

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ABSTRACT

Aloe vera L., is a perennial, evergreen, freely suckering plant, distributed throughout the world and widely recognized for its medicinal and cosmetic values. Species have naturalized well in tropical to sub tropical regions of India. Plants growing in these regions are prolific flower producer but do not set any seed. The flowering period of the species in the subtropical climates of Jammu J & K state, India (area of present study) spans over a period of 6 -7 months (mid October to April). During the peak flowering period (in the months of January and February), the minimum and maximum temperature recorded was quite low and averaged 2.9°C and 26.4°C respectively. Yellowish orange flowers are hermaphrodite and produce a good amount of pollen and ovules both. These are peculiar in showing seasonal variability in pollen viability. Pollen viability is high at the time of initiation and termination of flowering. As the temperature declines, blooming increases, but the pollen viability get decreased. In nature, no pollen germination was ever recorded on the stigmas, although they carried some pollen load. During the flowering period, when the pollen viability was adequate, both manual - self and cross pollinations were carried out. On manual – self pollination, no pollen grain germinated on self – stigma. On manual cross- pollination some pollen germination was recorded (15.38%). Pollen tubes so produced were however inhibited at various levels in the style, revealing some sort of abnormality in pollen tube growth or a stylar inhibition leading ultimately to loss of sexual reproductive output in this plant species.

KEYWORDS: ***Aloe vera,*** **viability, reproductive output, hermaphrodite, manual pollination**

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INTRODUCTION

Sexual reproduction in plants is not just a means of propagation, but a major source of genetic variation too. The same is generated through breeding and meiotic systems, which are integral parts of the sexual cycle and together constitute the "genetic system"[1]. In such taxa, where this important means of propagation gets suppressed because of one reason or another, the major source of genetic variation gets obliterated [1 2 3]. Suppression of sexuality however does occur in many angiosperms right from the cases where flowers do not develop at all to the ones where seeds abort after fertilization. A number of taxa are on record that flower profusely, but set lesser number of fruits/seeds, e.g. *Lophocereus schottii, Quercus alba, Pyrus malus, Ceiba pentandra* etc [4 5], few taxa are however peculiar in producing a good number of flowers, but no fruit/seed at all e.g. *Allium sativum* [6 7]. *Aloe vera* L., the material of present study belongs to this peculiar group. The species also known as burn plant, lily of the desert and elephant's gall is semi–tropical, medicinal plant, indigenous to Africa. The name *Aloe* is derived from the Arabic word "Alloeh" meaning 'shining bitter substances' and "vera" in latin means 'true" [8]. This perennial, evergreen, monocot crassulacean acid metabolism (CAM) plant, is distributed throughout the world and is widely recognized for its medicinal and cosmetic values. More than 360 species of *Aloe* are on record of which four occur in India [9]. Here Aloes, in particular *Aloe vera* are found both in cultivated form and in wild in Rajasthan, Andhra Pradesh, Gujarat, Maharashtra, Chhattisgarh, Madhya Pradesh and Tamil Nadu [10]. These are also found as wild along the coast of South India [11]. The Aloes growing in India in general are high flower producers but set fruits and seeds very rarely, also the seeds in fruits wherever produced are chaffy, do not germinate and are probably without an embryo[12]. These species are propagated by suckers. Though vegetative propagation by suckers is an efficient means of propagation available to these species, they are left bereft of genetic variation and are mostly clones. Meiotically *Aloe vera* is interesting. Lot of cytological work done on the species in India and the world over has brought to light several anomalies in meiotic system [13-26]. Whether these anomalies are solely responsible for the seedlessness in this economically important species however is not clear. Present work was planned to answer this question for *Aloe vera* growing in the area of study i.e. Jammu and its adjoining areas in J & K state, India. The plants growing at different sites were analysed for

A) Details of floral structure and floral biology,
B) Pollination mechanism and breeding system.

MATERIALS AND METHODS

Plant Material

Plants of *A. vera* were localized at different places in and around Jammu district. While at most of the locations these were under cultivation, in a remote village named Thein (636 msl), few of these (n = 68) were found growing on their own on a slope. This group was taken as wild. A total of more than 500 plants growing on three distinct sites (1 wild and 2 cultivated) were scanned regularly for 5 years (2009 – 2013). Individual plants of *Aloe vera* are succulent, perennial and stemless. These are freely suckering forming dense groups (Fig. 1) and possess a shallow root system. Plant propagates well through these suckers. Inspite of the majority of these producing good amount of flowers, not a single individual was found to produce fruits/ seeds.

METHODS

Morphology

Details on morphological features such as height of the plant, number of leaves/ plant, size of leaf, number of flowers/ spike etc. were collected from the plants growing in the field. Floral structure, including anther size, corolla length, pistil size, position of anther and pistil, stigma length and breadth, style length, papillae length, ovule length and breadth, pollen size,

etc was studied in the laboratory using the standard measuring scale and wherever required ocular and stage micrometer.

Anthesis and Anther Dehiscence

Anthesis as well as anther dehiscence was observed at regular intervals of time in plants growing in the field. Flowers were regularly monitored throughout the day so as to record the time of anthesis and anther dehiscence. Time required by an individual inflorescence to bloom was recorded.

Stigma Receptivity and Pollen tube growth

For checking stigma receptivity, stigmas of different ages were removed from flowers blooming in the field and fixed in 3 parts of absolute alcohol and 1 part of acetic acid (Carnoy's fixative) for 3 h. These were later washed in distilled water and stained with a mixture of 4ml of 1% aqueous acid fuchsin, 2ml of 1% light green, 40ml of lactic acid and 46ml of distilled water. These were then mounted in lactophenol (mixture of lactic acid, distilled water, glycerine and phenol in the ratio of 1:1:1:1). Slides were subsequently examined under a compound microscope. Stigmatic receptivity was also tested by checking Stigmatic Peroxide Activity (SPA) as suggested by Kearns and Inouye (1993). Intact stigmas of emasculated flowers of different ages were placed on a glass slide in a drop of 3% Hydrogen peroxide on which a coverslip was laid. Stigmas that produced the bubbles within 2-3 minutes were considered receptive. Some stigmas were manually pollinated for different time duration to estimate the duration of stigma receptivity and pollen tube growth. For this purpose, flowers were emasculated and cross pollinated by dusting the pollen grain from freshly dehisced anthers on the stigma and then bagging them for different time intervals. Pistils from these flowers were fixed in 3 parts of absolute alcohol and 1 part of acetic acid (Carnoy's fixative) for 24 h. These were then transferred to 70% ethyl alcohol. Before mounting in lactophenol, each carpel was given a quick washing in distilled water and stained in Lewis stain. The number of pollen grains attached to the stigmatic surface and number of pollen grains germinating on the stigmatic surface were recorded from each preparation.For fluorescence microscopy, the carpels were fixed in Carnoy's fixative for 24h. and then transferred to 70% ethanol. After 24h., the carpels were thoroughly washed in distilled water and cleared in 4N NaOH in an oven maintained at 60°C for 4h. The carpels were then washed in distilled water and kept overnight in 0.005% decolourised aniline blue. These carpels were mounted in glycerin and studied under a fluorescence microscope (Nixon Eclipse 80 i).

Pollen : Ovule ratio

Pollen count per flower was estimated by calculating the number of pollen grains per anther just prior to dehiscence by squashing it in a drop of 1% acetocarmine. The actual count was made by scanning the slide from one end to the other on the mechanical stage of Olympus (HB) microscope. Ovules were counted by carefully dissecting out the individual ovaries with the help of a pair of fine needles. Pollen/ Ovule ratio was calculated by dividing the pollen count per flower with the number of ovules per flower. From the data, thus obtained, pollen – ovule ratio was calculated using the following formula

$$\text{P/O ratio} = \frac{\text{Pollen output/ flower}}{\text{No. of ovules/ flower}}$$

Dimensions of both pollen and ovule were measured with the help of calibrated ocular and stage micrometers.

Pollen Stainability and Viability

Pollen stainability and viability were determined by stainability test (1% acetocarmine) and by enzyme assay (TTC test). For the former freshly dehisced anthers were squashed in 1% acetocarmine on a clear glass slide and observed under compound microscope. All well stained pollen grains were considered to be viable while unstained empty pollen grains with irregular outline were taken as sterile (non-viable). Viability was estimated by TTC (2,3,5-triphenyltetrazolium chloride) test. For the same

freshly dehisced anthers were squashed in TTC solution prepared by dissolving 0.5% TTC in 10% sucrose solution. Pollen grains stained red were considered as viable and unstained deformed pollen grains as non-viable. Pollen viability was calculated by applying the formula:

$$\text{Percentage Pollen Viability} = \frac{\text{No. of viable pollen grains}}{\text{No. of viable pollen grains} + \text{No. of non-viable pollen grains}} \times 100$$

Pollination studies

For checking the role of wind in pollination, hanging slide method was employed. For this purpose, glass slides (7.5 x 2.5cm) smeared with Mayer's albumen (mixture consisting of glycerine and egg albumen in the ratio of 1:1) were suspended from T-shaped wooden stands (almost of the same height as the plants) fixed around the plant at a distance of varying between 0.5-2m from the blooming plants. These slides were left exposed for 24 h. and examined microscopically thereafter, for the number of pollen grains trapped. The plants of *A. vera* were also observed in the field during the peak flowering period for the presence and types of visitors, if any. Plants were also checked for the nature of attractants and rewards in their floral and extra-floral parts. The visitors were trapped, anaesthetized and scrutinized for pollen load on their body parts. These were subsequently identified upto the genus level.

Sucrose Concentration in Nectar

To check the sucrose concentration in nectar, a drop of nectar was taken from opened flower and was put on Hand Refractometer.

RESULTS

Plants of *Aloe vera* studied at different locations did not display much variation in most of the features explored, thus the description follows a general pattern, with the differences wherever found highlighted individually.

Plant and Floral Morphology

In the sub tropical climate of Jammu and its adjoining districts, plants of *Aloe vera* remain in vegetative phase for 5-6 months (May to September) and bear flowers for the rest of the year (6-7 months, October to April). Temperature during the flowering period fluctuates between 2.9°C to 31°C while the relative humidity is 24% to 100%. Dense rosette of green, fleshy, waxy leaves which are erect to slightly spreading form the above ground part of *Aloe vera* (Fig. 1a). These attain an average height of 1.35m. At maturity, leaves are alternate and without stipules and petiole. Leaf blade is linear to lanceolate with acuminate apex with deltoid margins. The number of leaves per plant averages 11.2 ± 0.31 (7 – 18), while the average size of single leaf in a mature blooming plant is 45.01cm ± 1.06 (25cm – 62cm) X 4.81cm ± 0.13 (2cm – 6.8cm). Flowers arise in a terminal dense raceme. Inflorescence is long and averages 0.85m ± 0.03 (0.46m – 1.44m) in size. Number of inflorescences per plant averages 1.1 per season (Fig. 1b). Peduncle is simple or sometimes 1 – 2 branched above the middle. Number of flowers per inflorescence varies between 38 – 110 (60.80 ± 2.51). Individual flower is bisexual, regular, actinomorphic, bracteate, pedicillate and averages 3.48cm ± 0.02 (3.1 – 3.9) at maturity. Bract is linear, green and 0.6cm – 1.1cm in length (0.84cm ± 0.02). Perianth is tubular and bright yellowish – orange in colour. Each flower bears six stamens and a single pistil (Fig. 1c). Length of single stamen varies between 2.4cm to 3.7cm (3.06 ± 0.99). Anthers are yellowish – brown in colour, exerted, dorsifixed with a longitudinal line of dehiscence. Size of single anther was invariably same in all the flowers scanned (0.3cm X 0.1cm). Difference in length of stamen being the result of difference in length of filament which ranges between 1.4cm to 3.7cm. The gynoecium consists of single carpel with an average length of 2.82cm ± 0.032 (2.0cm – 3.4cm). The ovary

is superior, approximately 0.5cm X 0.2cm in size and trilocular. The number of ovules per flower is high averaging 30.54 ± 1.15. It however, displayed an extreme range, varying from 8 to 46 per ovary. Ovules are borne on an axile placenta. The style is elongated averaging 2.1cm ± 0.05 (1.2cm – 3.0cm) in length. The style terminates into stigma which is dry, head shaped, papillate and 0.40mm ± 0.01 (0.30mm – 0.61mm) X 0.60mm ± 0.01 (0.45mm – 0.87mm) in size. The papillae are long varying from 0.09mm to 0.22mm (0.15mm ± 0.005). Nectar is secreted from the gynoecium via septal nectaries located in ovary (Fig. 1d). This nectar has an average sucrose concentration of 21.5%.

Flowering phenology

The flowering period of *Aloe vera* in the subtropical climates of Jammu spans over a period of 6 -7 months (mid October to April). Maximum and minimum temperature during the onset of flowering period was recorded as 30°C and 23.7°C respectively, while the relative humidity was recorded between 50% to 75%. During the peak flowering period (in months of January and February), the minimum and maximum temperature recorded was quite low and averaged 2.9°C and 26.4°C respectively; the relative humidity recorded was between 35% – 100 %. Flowers in *Aloe vera* as described before are borne in racemes. The inflorescence axis elongates and brings the flowers above the foliage, where they bloom. The blooming takes place in an acropetal succession. The average time required for an inflorescence to senescence is quite long and averages 3.1 months. The opening of the flowers initiates during morning around 10:00 h; there after the buds keep on opening throughout the day till 1800 hrs. Single bud takes quite a long time to expand its petals completely. The time taken varies with temperature and shows a wide range in different seasons. In the months of November and December flowers takes about 3 – 5 h. to bloom whereas in January they take about 28 hrs. to open out completely. Opening of flowers is marked by expansion of the petals whereby the anthers and stigma are exposed. Anthers generally start dehiscing along with the expansion of the petals; temperature plays an important role in it also as the dehiscence normally occurs during afternoon time. Dehiscence is longitudinal, commencing from apex to base.

Pollen Output and Size

Pollen in *Aloe vera* is shed as single grains and are released at 2 – celled stage. These are mono-aperaturate having smooth and thick exine. Pollen varies in shape from round to slightly elliptical. The average size of viable pollen grain is around 45µm and is constant. Pollen output per flower displayed wide difference in plants growing in wild (Site 1) and in cultivation (Site 2 and 3). A single anther of a flower of a plant in wild produces an average of 6850.33 pollen whereas that of cultivated plants produces an average of 2415.17 pollen. Since a single flower has six anthers, average pollen output per flower comes out to be 41102 in case of wild population and 14581.39 in case of cultivated plants, revealing an almost threefold difference.

Ovule Number and Size

The number of ovules per flower varies both between wild and cultivated plants as well as within each type. In wild plants, the number of ovules per flower ranges from 23 to 52 (30.46 ± 2.07) while in cultivated plants it ranges from 8 to 46 (30.54 ± 1.15). The average value, thus remains same for both types. The size of single ovule averages 0.41mm ± 0.01 (0.33mm – 0.55mm) X 0.31mm ± 0.008 (0.22mm – 0.40mm). The ovules are bitegmic and anatropous.

Pollen – Ovule Ratio

With the number of pollen grains averaging 41102 per flower and number of ovules averaging 30.46, the pollen – ovule ratio in case of wild population comes out to be 1349.37 : 1. In cultivated plants, the number of pollen grains averages 14581.39 per flower while the number of ovules averages 30.54 and thus the pollen – ovule ratio is 477.45 : 1.

Pollen viabilaity

The pollen viability in *Aloe vera* plants growing in Jammu displayed a unique trend. During the onset of flowering in the month of October, the pollen viability as calculated through acetocarmine stainability test was quite high in all the plants scanned. It averaged 79.47%, thereafter it declined and dipped to almost nil in the month of February. With a rise in the temperature during the termination of the flowering period in April, it again rose and averaged 77% (Table 1) (Fig. 3) Pollen viability with enzyme assay was maximum at the end of the flowering season in the month of April and averaged 71.18%. Nil pollen viability was recorded during January by TTC test in all the plants scanned at a time when the plants were in full bloom.

Stigma Receptivity

In all the populations scanned, pollen load on stigma collected from open pollinated plants at the time when the flowers were fully opened varied between 0 to 190. No pollen germination was however recorded on any of these stigmas.Manual pollinations were performed during the different times of flowering (October, November, December, January, February and March). No pollen germination was observed on stigmas dusted with self pollen. Although the pollen load ranged between 5 to 51 (n = 22). A few (n = 26) emasculated and bagged flowers when dusted with pollen of flowers from plants from other sites revealed pollen germination to some extent during the month of November. 15.38% pollen were seen germinating on the stigmas of these flowers (Fig. 2a). Fluorescence microscopy showed that the pollen tubes thus emerging enter the style, grow a little and soon the growth is ceased by a plug – like structure formed at the end of pollen tube. These pollen tubes are thus unavailable for fertilization (Fig. 2b).

Pollination Mechanism

Wind Pollination

To confirm whether the pollen of *A. vera* is carried by wind, glass slides smeared with Mayer's albumen were hanged on the wooden stands fixed around the individual plant in full bloom at a distance of about ½ m. The slides were scanned for pollen load after 24h. Few pollen were observed on these slides with average pollen count per slide averaging 35.

Entomophily

During the blooming period of these plants, few insects like ants (*Camponotus ligniperda)* and dragon fly were observed visiting these flowers. No pollen load was ever observed on the body parts of these visitors confirming that they acted as Casual visitors. No major pollinator was ever seen hovering around these plants.

Reproductive effort

Fresh biomass estimations showed that in the open pollinated plants, investment in the sexual structures is low and averages only 41.80 g. In vegetative structures, which include both root stock and above ground parts, it is quite high and was found to average 1128.16 g (n = 12). The sexual reproductive effort (SRE) as estimated from fresh biomass allocation averaged 3.12%, while the vegetative reproductive effort (VRE), i.e. root stock investment averaged 5.39 %. The vegetative effort (VE) thus comes out to be quite high which is 91.48%.

Table 1
Pollen Viability

S. No.	Method	Percentage viability in months of						
		Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.
1.	Acetocarmine Stainability Test. (n = 58)	79.50%	52.93%	40.11%	16.24%	7.12%	32.54%	76.95%
2.	Enzyme Assay (TTC) (n=52)	29.19%	8.65%	4.12%	0	0.27%	18.69%	71.18%

n = number of plants

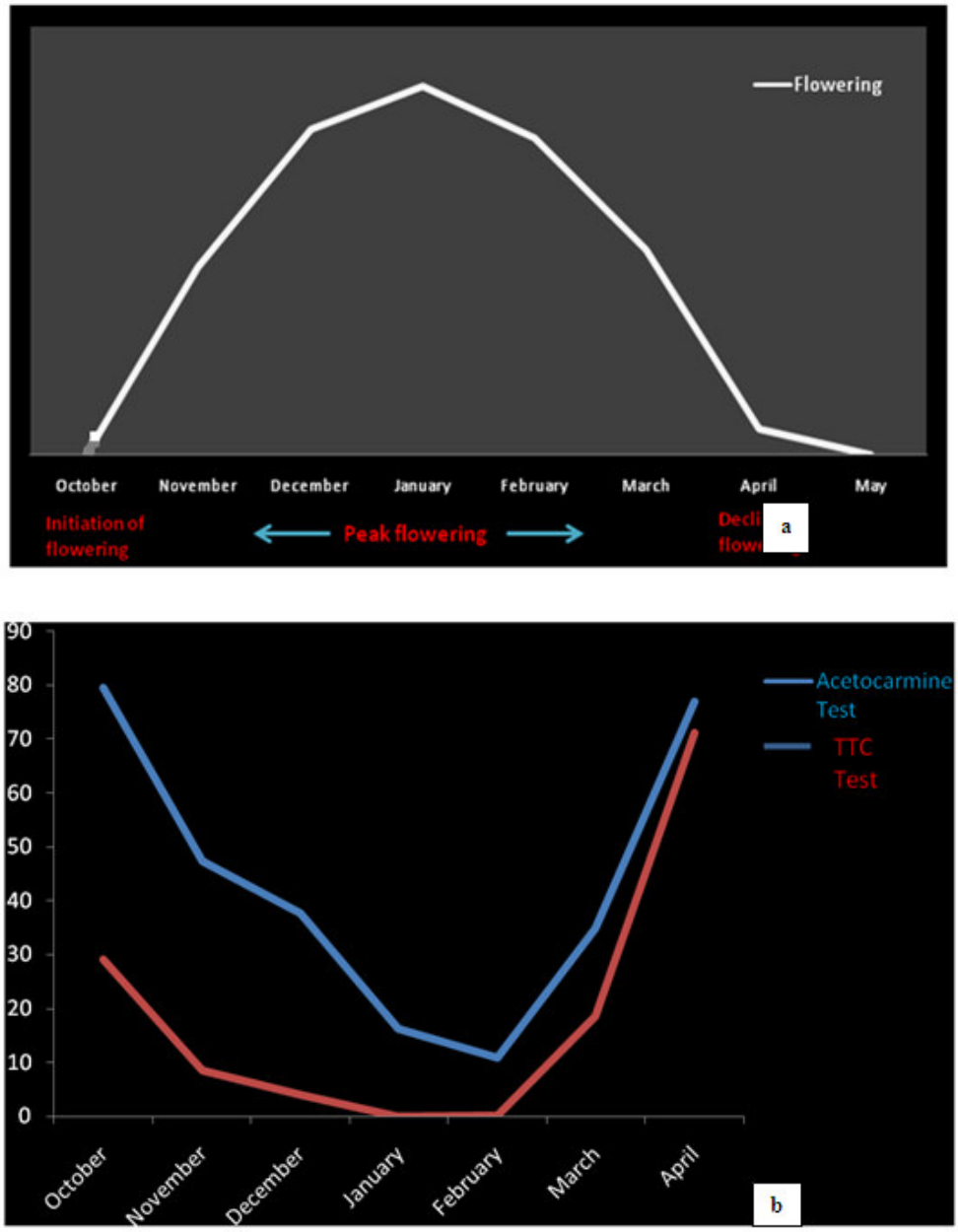

Figure 3
Line graphs showing

a). the frequency of flowering in different months of blooming.
b). pollen viability calculated during different months of flowering.

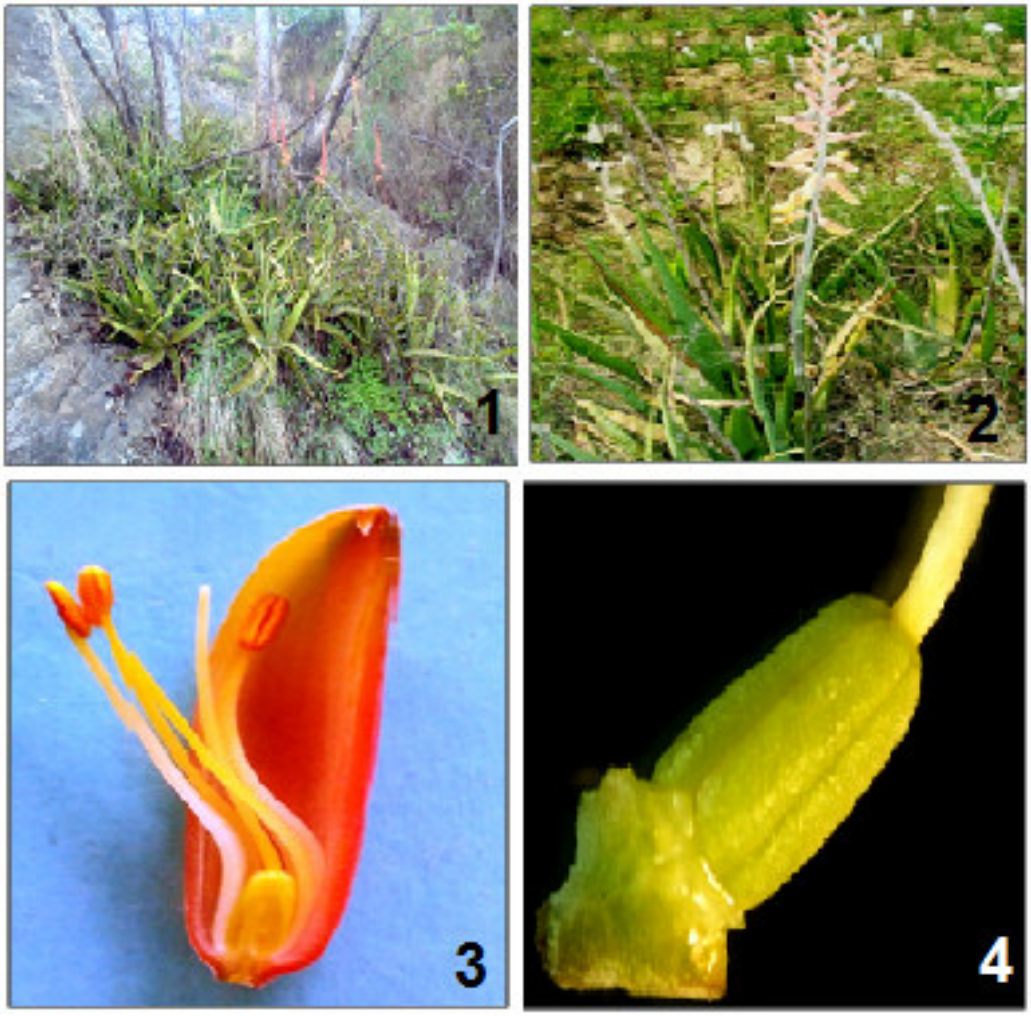

Figure 1- Plants of *Aloe vera* growing wild
Figure 2- A flowering individual of *Aloe vera*
Figure 3- V.S. of flower (x 1.2).
Figure 4- Ovary with nectar protruding out (x 6).

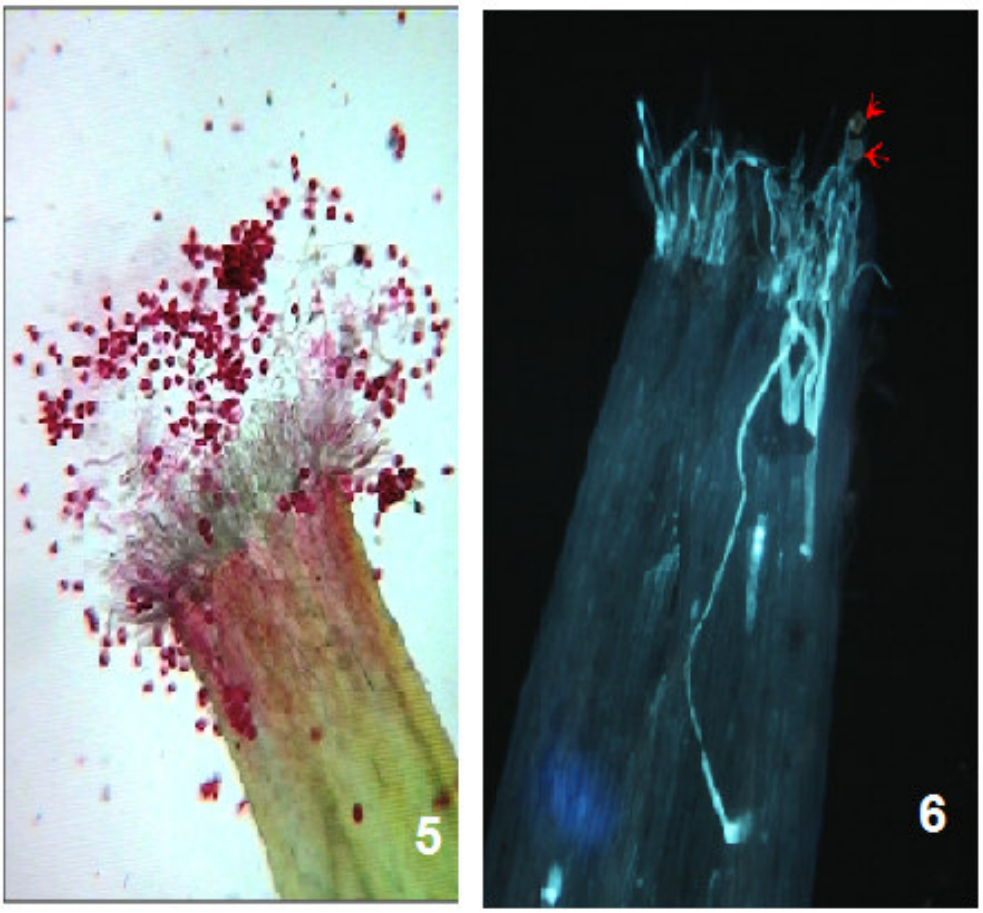

Figure 5- Pollen germination observed on manually cross- pollinated stigmas.
Figure 6- Flourescence micrographs showing pollen tubes entering style and terminating prematurely

DISCUSSION

At Jammu, *Aloe vera* remains in vegetative stage from May – September and subsequently flowering begins from October onwards. Plants in each group are found to be either flowering or non – flowering. Flowering individuals are larger with an average height of 0.87m ± 0.03. Each of these produced 1/ 2 spike in a flowering season, with the number of flowers per inflorescence varying between 38–110. Flowers are actinomorphic, hermaphrodite with bright yellowish – orange tubular perianth. These are equipped with a well defined sexual apparatus in the form of six stamens and a single pistil, both of which are seemingly normal. Flowers also possess a septal nectary. *A. vera* anthers are plump and produce a good amount of pollen. Pollen production per flower, however shows a wide range. Pollen production is higher in wild plants where it ranges between 28542 and 56214. In case of the cultivated plants range is wider, i.e. 5358 – 37,380. Single carpel consists of long style, dry and papillate stigma and superior ovary. The ovule number per ovary ranges from 23- 52 in case of wild plants and 8 – 46 in case of cultivated plants. With the pollen and ovule number per flower high, the pollen – ovule ratio comes out to be 1349.39:1 in case of wild populations and 477.45:1 in case of cultivated plants. This accounts for nearly three – fold difference. Taking pollen : ovule ratio as

indicator of breeding system *A. vera* falls in the category of in-breeders, though the wild population can be taken as slightly outcrossed too [27-29]. Seasonal variability in pollen viability has been observed in plants studied presently. During the onset of flowering in the month of October (Average maximum temperature- 31°C and Average minimum temperature- 23.7°C), the pollen viability averaged 79.50% with acetocarmine stainability and 29.19% with TTC. Thereafter pollen viability kept on declining as the temperature dipped to a minimum of 2.9°C in the month of January and averaged 16.24% with acetocarmine stainability and nil with enzyme essay. During the termination of the flowering period in April, with a rise in temperature (Avg. Max.- 36.8°C and Avg. min.- 16°C) pollen viability again rose and averaged 76.95% with acetocarmine stainability and 71.18% with TTC. As it is evident from the figure, pollen viability is high at the time of initiation and termination of flowering. As the temperature declines, blooming increases but the pollen viability decreases. It again rises a bit for some time but almost at the termination of flowering period (Fig. 3). Although reports on pollen viability in different *Aloe* species are scanty, the few reports available do not show highly reduced viability. Chaudhari and Chaudhary (2012), report that in *A. vera* plants scanned at Botanical Garden, Banaras Hindu University (Varanasi, India) pollen viability as estimated by Acetocarmine stainability test is 87.60%, though the plants there were also seedless [9]. Army et al. (2004) have evaluated the percentage of viable pollen using TTC test and acetocarmine method in *A. saponaria* (Ait.) Haw. as 65.48% and 64.02% respectively. In *A. yuanjiangensis*, these values were 52.28% and 55.46% [30]. Although pollen sterility has been reported as a common cause for low fruit or seed set following chilling temperature at flowering in many plants like rice, sorghum, tomato, strawberry etc. a positive correlation between full bloom and low pollen viability operating in *Aloe vera* studied presently is an interesting feature [31]. In nature, pollen load on stigmas recorded at different timings of the blooming period (October – April), and at the stage when the flowers were fully opened, averaged 31 in plants of *A. vera* studied in different populations. No pollen germination was however ever recorded on these stigmas (n = 55). During the flowering period (October- November and March - April), when the pollen viability was adequate, both manual - self (n =12) and cross (n =26) pollinations were carried out. On manual – self pollination, no pollen grain germinated on self – stigma revealing the species to be self – incompatible. On manual cross- pollination some pollen germination was recorded. An average of 15.38% pollen germination was observed but only in the month of November, when the pollen viability recorded was 52.93% with acetocarmine stainability test and 8.65% with enzyme assay. The average minimum and maximum temperature recorded during this period was 9.9°C and 28.85°C respectively with a relative humidity of 24 % - 100%. Inspite of being able to germinate on stigma, pollen tubes produced by these pollen were unable to reach the ovules and affect fertilization. These were inhibited at various levels. In most of the cases, callose plugs were formed in the pollen tubes just near the stigma, in others a little further away from the stigma or in extreme cases in the middle of the style, revealing some sort of abnormality in pollen tube growth or a stylar inhibition. Stylar inhibition of pollen tube growth is a feature normally associated with Gametophytic Self- Incompatibility. For the same, various explainations have been forwarded [32 33]. However the inhibition of the pollen tubes of cross- pollen in styles of the same species is an interesting feature for self - incompatible *A. vera*, which needs further investigation for its correlation with genotypes of parents involved in crossing, genetic imbalance in pollen grains and/ or the environmental conditions. As per present data, *Aloe vera* plants growing at Jammu can be treated as both self as well as cross incompatible. Lack of seed/ fruit set in these plants is thus because of lack of fertilization. Though *Aloe vera* plants studied presently also exhibit several features associated with ornithophily, including brightly coloured flowers with lack of scent and good quantity of nectar; no account is available for bird pollination in this species. Several other

Aloe species have on the other hand been reported to be effectively pollinated biotically [34 35 36 37 38]. During the 5years (2009-2013) of study on diverse populations of the species, no bird and/ or bee visitation was ever recorded on plants in bloom. Pollen load recorded in open – pollinated stigmas are thus in all probability self – pollen. This may explain the lack of pollen germination in these plants. *Aloe vera* has an efficient vegetative method of propagation through suckers. It slowly offsets to form a clump [9]. Since it is perennial also, it incorporates a chunk of its resources to vegetative reproduction (5.39%). Most of the resources are however diverted to leaves (91.48%) which are the major exploited part. Sexual reproductive effort for the species was calculated to be only 3.12%, which is quite low as compared to several other perennial herbaceous taxa investigated from this angle [39 40] . This little effort also goes a waste in sub – tropical climate of Jammu, as it is never used for future propagation. Only option of propagation that remains is thus vegetative.

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