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GENETIC VARIABILITY FOR POLLEN TRAITS PROVIDES INSIGHTS INTO POLLINATION AND FRUIT SET IN ALOE (*Aloe barbadensis* MILL.)

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ABSTRACT

Aloe (*Aloe barbadensis*) is an important xerophytic medicinal plant used in various traditional medicines. Seedlessness is a major problem in improving the Aloe through recombination breeding. Genetic variability for seedlessness and its related traits help to choose parents for recombination breeding however, no information is available on genetic variability for pollen and fruit set traits in Aloe. Hence, the study deciphers the genetic variability on pollen and fruit set traits. The pollen grains shape varied from subspheriodal to prolate with mean polar and equatorial length was 40.70 ± 3.73 µm and 27.21 ± 2.10 µm, respectively. Pollen surface ornamentation was reticulate. Number of pollens ranged from 23974.07-142370.36 among the accessions. Male fitness and reproductive efficiency ranged from 0.03-0.35 and 40.47-117.91, respectively. Higher (>60%) heritability and genetic advance over mean (>20%) observed for pollen count, pollen stainability, pollen viability, *in vitro* and *in vivo* pollen germination, capsules per inflorescence and seeds per capsule. The Pearson's correlation analysis revealed that a positive significant correlation was observed for pollen viability with pollen size ratio, pollen stainability, *in vitro* pollen germination and *in vivo* pollen germination. The accessions IC112527 and IC112517 exhibited superiority for pollen fertility traits, while, N127, IC112527 and NR72 were found superior for fruit set parameters and the accessions J4, GUJ3 and IC112531 had higher seed set per cent. Superior accessions identified will form base for genetic improvement of Aloe.

Key words : Pollen traits, Pollen germination, Male fitness, Reproductive efficiency, Fruit set, Seedlessness.

Introduction

Aloe (*Aloe barbadensis* Mill. syn. *A. vera* Linn.) is a valuable medicinal plant that belongs to the family Asphodelaceae. The name is derived from the Arabic word "Alloeh'' meaning "bitter" because of the bitter liquid found in the leaves. It is also known as "Desert Lily" and the plant of immortality with qualities to serve as alternative medicine (Arunkumar and Muthuselvam, 2009). *A. barbadensis* is native to Africa and was introduced to India. It is widely cultivated and has been introduced in the dry lands, the Mediterranean, tropical, and subtropical regions of the world. It is mostly cultivated or seen wild in the dry parts of India. It is a xerophytic, hardy succulent plant with triangular fleshy green leaves that grows up to 60-100 cm. Aloe is reported to have anti-inflammatory, anti-cancer, anti-oxidant and anti-diabetic and anti-ulcerogenic properties (Benson *et al*., 2015).

Crop improvement work in Aloe, even though very limited, is largely restricted to clonal selection from germplasm collections. Developing improved cultivars/ varieties with higher leaf yield with enhanced gel and aloin contents is an important breeding objective of Aloe. So far, only few varieties released for cultivation in Aloe *viz*., Gujarat Anand Kunwarpath-1 (GAKP-1) is a new variety form Anand Agricultural University, Anand (https:/

/www.aau.in/variety_released). Recombination breeding through hybridization and selection is limited in Aloe due to the limited knowledge of genotypes having higher pollen fertility and fruit set behaviour with viable seed set. Due to the presence of sterility, which is a barrier to rapid propagation, sexual reproduction by seeds is almost not effective (Natali *et al*., 1990). Seedlessness is reported in *A. barbadensis* (Swati *et al*., 2015). It is a major problem affecting recombination breeding. The cause of seedlessness may be attributed to environmental, genetic, genotype environmental interaction and cytological factors (Rana and Kanwar, 2017; Ahmad and Gupta, 2016; Sharma and Sharma, 2016). Meiotic abnormalities like precocious movement of chromosomes in anaphase, formation of chromosomal bridges and laggards reported in meiosis I and II might be the reason for high percentage of pollen sterility (Sapre, 1975; Lv *et al*., 2005; Chaudhari and Chaudhary, 2012; Sharma and Sharma, 2016). The seed germination per cent is reported to be very low *i.e*., 0-25 per cent in *A. barbadensis* (Das *et al*., 2015). But, when germinated under *in vitro* condition germination per cent reach up to 60-70% which provides a scope for development of cultivars through recombination breeding (Das *et al*., 2015).

The Aloe accessions exhibited genetic variation for leaf fresh weight, gel yield per leaf and plant spread (Ahmad and Gupta, 2016). Genetic diversity among various accessions of Aloe indicated intra and inter population variability (Bhaludra *et al*., 2014; Deore and Samantaray, 2015; Kumar *et al*., 2022). However, no much information is available on genetic variability pollen and fruit/capsule set traits. Hence, it is essential to find the genotypes with better pollen traits and fruit set traits which can serve as a potential source for improvement of Aloe through recombination breeding. In this regard, the present investigation was undertaken to identify Aloe genotypes having high pollen fertility and fruit set. Further, information on genetic variability for pollen traits provides foundation for recombination breeding and seed propagation of Aloe.

Materials and Methods

Study site and plant materials

The study was carried out during the *Rabi* season of the year 2022 at ICAR-Directorate of Medicinal and Aromatic Plants Research (ICAR-DMAPR), Boriavi, Anand, Gujarat, India. The experimental site is situated in the Agro-climatic zone III. It is geographically positioned at 22º35 N latitude, 72º55 E longitude and altitude of 45.1m above sea level. The experimental material for the present study consisted of 25 accessions namely N106, N118, IC112531, N127, IC112532, GUJ4, N156, IC112527, NR72, NR31, IC112517, N119, GUJ3, NMRM2, IC310618, NR30, N109, J4, J1, IGKV1, GAKP1, AB9, IC112521, RAJ1 and GUJ2 maintained at ICAR-DMAPR were used for the study. Pedigree details are provided in Table S1. The accessions evaluated for pollen traits in completely randomized design (CRD) design with three replications.

Traits studied

The observations were recorded for fourteen traits relating to pollen and fruit set in Aloe *viz*., time of flower opening per inflorescence, time of pollen dispersal/anther dehiscence, pollen size, pollen quantification, pollen surface ornamentation, pollen stainability (%), pollen viability (%), *in vitro* pollen germination (%), *in vivo* pollen germination (%), number of ovules per flower, pollen ovule ratio, pollination efficiency, capsules per inflorescence and seed per capsule. (1) Time of flower opening per inflorescence: Number of flower opening per inflorescence per day on per cent basis was noted at various interval of time *i.e.*, 6.00 am to 9.00 am, 9.00 am to 12.00 pm, 12.00 pm to 3.00 pm, and 3.00 pm to 6.00 pm for seven consecutive days. (2) Time of pollen dispersal/anther dehiscence: Time taken for anther dehiscence from anthesis was noted at different interval of time *i.e.*, 6.00 am to 9.00 am, 9.00 am to 12.00 pm, 12.00 pm to 3.00 pm, and 3.00 pm to 6.00 pm for seven consecutive days. (3) Pollen size ratio: Pollens grains were collected from freshly dehisced anthers. They were mounted on the slide in a drop of glycerol. To assess the shape of the pollen, the polar to equatorial length ratio was calculated. The measurement of length and breadth was noted for ten pollens in each slide and repeated thrice with the help of microscope (Olympus) fitted with AxioVision software. (4) Pollen quantification/count: Haemocytometer was used for pollen quantification study as per standard method (Shore and Barrett, 1984; Barrett, 1985; Dudash, 1991). (5) Pollen surface ornamentation: Acetolysis method was used to study the pollen surface ornamentation (Erdtman, 1952). The observations were noted by observing under scanning electron microscope. (6) Pollen stainability: Pollen stainability was assessed by acetocarmine test (Singh, 2016). (7) Pollen viability: Pollen viability was assessed by Fluorochromatic reaction test (FCR) as per the standard method (Heslop Harrison and Hesiop-Harrison, 1970; Heslop-Harrison *et al*., 1984). (8) *In vitro* pollen germination: Pollens were collected from randomly selected freshly dehisced three inflorescences of each accession. Brewbakers and Kwack's medium (BKM) was used to study *in vitro* pollen germination (Brewbaker and Kwack, 1963). (9)

Table S1 : Details of the *Aloe barbadensis* Mill. accessions and their source.

S. no.	Genotypes	Source
1	N 106	Field Gene Bank, ICAR-DMAPR, Boriavi, Anand
2	N 118	Field Gene Bank, ICAR-DMAPR, Boriavi, Anand
3	IC 112531	ICAR-NBPGR, New Delhi
4	N 127	Field Gene Bank, ICAR-DMAPR, Boriavi, Anand
5	IC 112532	ICAR-NBPGR, New Delhi
6	GUJ 4	Godhra, Gujarat
7	N 156	Field Gene Bank, ICAR-DMAPR, Boriavi, Anand
8	IC 112527	ICAR-NBPGR, New Delhi
9	NR 72	Field Gene Bank, ICAR-DMAPR, Boriavi, Anand
10	NR 31	Field Gene Bank, ICAR-DMAPR, Boriavi, Anand
11	IC 112517	ICAR-NBPGR, New Delhi
12	N 119	Field Gene Bank, ICAR-DMAPR, Boriavi, Anand
13	GUJ 3	Field Gene Bank, ICAR-DMAPR, Boriavi, Anand
14	NMRM 2	Field Gene Bank, ICAR-DMAPR, Boriavi, Anand
15	IC 310618	Field Gene Bank, ICAR-DMAPR, Boriavi, Anand
16	NR 30	Field Gene Bank, ICAR-DMAPR, Boriavi, Anand
17	N 109	Field Gene Bank, ICAR-DMAPR, Boriavi, Anand
18	J 4	Field Gene Bank, ICAR-DMAPR, Boriavi, Anand
19	J 1	Field Gene Bank, ICAR-DMAPR, Boriavi, Anand
20	IGKV 1	Field Gene Bank, ICAR-DMAPR, Boriavi, Anand
21	GAKP 1	Field Gene Bank, ICAR-DMAPR, Boriavi, Anand
22	AB 9	Field Gene Bank, ICAR-DMAPR, Boriavi, Anand
23	IC 112521	ICAR-NBPGR, New Delhi
24	RAJ 1	Banswara, Rajasthan
25	GUJ 2	Field Gene Bank, ICAR-DMAPR, Boriavi, Anand

In vivo pollen germination: The aniline blue fluorescence method was used to study *in situ* pollen germination (Martin, 1959; Kho and Baer, 1968) (10) Number of ovules: Number of ovules per flower of each accession was counted manually under stereomicroscope. (11) Pollen ovule ratio: Pollen ovule ratio was calculated by number of pollens produced per flower (by haemocytometer) divided by number of ovules per flower by dissecting the ovary and counting through stereomicroscope (Cruden, 1977). (12) Pollination efficiency: Pollination efficiency can be estimated in terms of male fitness and reproductive efficiency. Number of pollens on the stigma to number of pollens produced per flower is an index of efficiency in terms of male fitness (Cruden *et al*., 1990). Number of ovules per capsule of flower to number of pollens on the stigma is reproductive efficiency (Richards, 1986). Pollination efficiency is calculated and expressed in terms of percentage. (13) Capsules per inflorescence: Capsules per inflorescence was counted by randomly selecting three inflorescences of each accession. Capsule set (%) was determined by counting number of capsules to number of flowers per inflorescence. (14) Seeds per capsule: Seeds per capsule were counted by randomly selecting three capsules of each accession. Seed set (%) was determined by counting number of seeds per capsule to total number of ovules per flower.

Statistical analysis

The data obtained were subjected to ANOVA test (CRD) to determine whether there is significant difference between accessions for various traits. Statistical differences were evaluated using the least significant difference at $P < 0.05$ and $P < 0.01$. The correlation analysed was carried out between the traits pollen size ratio, pollen count, number of ovules per flower, pollen stainability, pollen viability, *in vitro* pollen germination, *in vivo* pollen germination, capsules per inflorescence and seeds per capsule. Statistical analysis was done using the R-software (R package version 4.3.0).

Results

Mean performance of accessions

Significant differences were observed for all the traits studied indicated presence of genetic variability for pollen and fruit set parameters in 25 accessions (Table S2). Staggered flowering opening observed among the accessions. The maximum flower opening was noted between 9.00 am and 12.00 pm (35.62%), which was followed by 6.00 am-9.00 am (27.05%), 12.00 pm-3.00 pm (21.66%) and 3.00 pm-6.00 pm (15.66%) (Table S3). Wide variation (32.14-60.71 min) was observed in time



Fig. 1 : Floral morphology of *Aloe barbadensis* Mill. (a) Inflorescence (b) Stamen (c) Different stages of development of *Aloe barbadensis* Mill. flower (d) Anther dehiscence pattern longitudinal splitting (e) Completely dehisced anther.

Table S2 : Analysis of variance for various characters of *Aloe barbadensis* Mill. accessions.

Characters	Mean sum of squares	
	Genotype	Error
Degree of freedom (df)	24	50
Pollen size ratio	0.09*	0.03
Pollen count	2475146528.74*	85974969.78
Number of ovules	52.92*	5.65
Pollen stainability	645.15*	20.92
Pollen viability	211.05*	3.33
In vitro pollen germination	113.12*	6.35
In vivo pollen germination	135.04*	50.03
Capsule per inflorescence	73.50*	4.41
Seeds per capsule	18.54*	3.36

period between anthesis and anther dehiscence among the accessions. Those flowers which open from 6.00-9.00 am, 9.00-12.00 am, 12.00-3.00 pm and 3.00-6.00 pm completed anther dehiscence in 60.71 min, 45.71 min, 22.14 min and 32.14 min, respectively (Table S4). Floral morphology of *Aloe barbadensis* is depicted in the Fig. 1. Pollen shape varied from subspheriodal (0.75-1.33) to prolate (1.33-2.00) (Table S5). The accession IC112527 (1.81) had highest pollen size ratio, which was at par with AB9 (1.80), IGKV1 (1.75), IC112521 (1.74), IC112517 (1.66), GUJ4 (1.64), N156 (1.62), NR72 (1.61),

Table S3 : Percentage of flower opening per inflorescence of *Aloe barbadensis* Mill. accessions at different intervals of time.

Accessions	Time of flower opening per inflorescence (%)			
	6.00-9.00 am	9.00-12.00 pm	12.00-3.00 pm	3.00-6.00pm
N 106	**35.59(E)**	30.92	17.60	15.88
N 118	28.57	**30.21**	24.69	16.55
IC 112531	14.34	**44.99**	13.00	27.67
N 127	**52.00(E)**	28.00	15.00	5.00
IC 112532	20.95	**34.33**	25.96	18.76
GUJ 4	23.43	**34.85**	23.43	18.28
N 156	24.60	**33.45**	26.32	15.64
IC 112527	14.30	**38.95**	24.95	21.80
NR 72	**43.83(E)**	32.50	11.50	12.16
NR 31	6.67	**40.23**	38.58	14.51
IC 112517	17.71	**44.38**	25.52	12.39
N 119	19.16	**34.17**	29.17	17.50
GUJ 3	24.00	**31.33**	26.67	18.00
NMRM 2	14.00	20.00	30.00	**36.00(L)**
IC 310618	**40.00(E)**	26.67	6.66	26.67
NR 30	**56.27(E)**	26.16	10.57	7.00
N 109	14.00	**52.00**	29.00	5.00
J 4	**52.00(E)**	28.00	15.00	5.00
J 1	6.67	**44.88**	21.78	26.67
IGKV 1	13.33	**50.00**	31.67	5.00
GAKP 1	**42.92(E)**	**42.91**	7.92	6.25
AB 9	**25.83(E)**	**26.50**	24.50	23.17
IC 112521	25.00	**45.00**	23.75	6.25
RAJ 1	**32.74(E)**	28.45	20.06	18.77
GUJ 2	28.33	**41.67**	18.33	11.67
Mean	**27.05**	**35.62**	**21.66**	**15.66**
Range	**6.67 to 56.27**	**20.00 to 52.00**	**6.66 to 38.58**	**5.00 to 36.00**

E: Early anthesis, L: Late anthesis.

RAJ1 (1.60), GUJ2 (1.59), IC112531 (1.57), IC112532 (1.56). The lowest pollen size ratio was observed in accession N118 (1.19). Number pollens produced per flower ranged from 23974.07 - 142370.36 with a mean of 87254.84 pollens. Maximum number of pollens was observed in J4 (142370.36), which was at par with IC112517 (139318.51) in contrast; the accession RAJ1

Table S4 : Time required for anther dehiscence from anthesis in *Aloe barbadensis* Mill. accessions.

Days	Anther dehiscence after anthesis (min)			
	6.00-9.00 am	9.00-12.00 am	12.00-3.00 pm	3.00-6.00 pm
1	80	45	25	35
2	70	40	20	20
3	60	45	25	30
4	45	55	20	40
5	60	50	20	25
6	50	45	20	35
7	60	40	25	40
Mean	**60.71**	**45.71**	**22.14**	**32.14**

(23974.07) produced lowest number of pollens (Table 1). Pollen surface ornamentation studied using scanning electron microscopy revealed the exine surface consists of honey comb like hexagonal unit hence, indicating reticulate pattern (Fig. 2). The surface pattern with ridges is known as muri and empty gaps/depression known as lumina (Fig. 2). Genetic variation for pollen fertility was observed in the accessions studied. Pollen stainability, a measure for pollen fertility was ranged from 34.75-89.33% with a mean 67.73 ± 14.66 indicating wide variation in pollen fertility. The accessions IC112527, N127, IC112517, NMRM2, IC310618 and IC112521 recorded maximum (>80%) pollen stainability, in contrast, GUJ2 (34.75%) recorded lowest pollen stainability. Wide variation in pollen viability was observed among the accessions. Pollen viability ranged from 11.48-48.33% with a mean value 23.49 ± 8.39%. The accession IC112527 (48.33%) recorded highest pollen viability (Fig. 3). While, the NR30 (11.48%) had the lowest pollen viability. The mean *in vitro* pollen germination of Aloe pollen was 19.16% with range from 9.95-31.96% indicating wide variation among the accessions (Table 1). The accession IC112527 (31.96%) and RAJ1 (30.27%) exhibited highest pollen germination, while the accession NR30 (9.95%) recorded the lowest pollen germination indicating genotypic variation. *In vivo* pollen germination (%) was measured using fluorescence microscopy, it ranged from 19.85-43.92 with a mean 30.04%.

The accession IC112527 (43.92%) recorded highest *in vivo* pollen germination (%), which was at par with RAJ1 (39.07%), NR72 (38.95%), AB9 (38.33%), IC112517 (36.18%), IC112532 (35.87%), IC112531 (35.52%), N 119 (35.05%) and IC310618 (32.68%) while, the lowest *in vivo* pollen germination (%) was noticed in NR30 (11.48%) indicating genotypic variation. *In vivo* pollen germination, pollen tube growth inside the style and pollen tube reaching the ovule is depicted in the Figure 4. Number of ovules per flower was 48.13 that ranged from 40.33-54.00. The accession N 119 (54.00) had highest number of ovules which was at par with AB9 (52.67), NR72 (52.67), N127 (52.33), IC112527 (52.33), N109 (52.00), N156 (51.67), NMRM2 (51.67), IC112521 (51.67), NR30 (51.00) and N106 (50.33). Pollen to ovule ratios is a measure of breeding system of the species. The accession J4 (3444.44) had highest pollen ovule ratio followed by IC112517 (3050.77), IC112532 (2526.75) and GAKP1 (2524.31) indicating xenogamy (cross pollination) while, the minimum was recorded in the accession RAJ1 (409.30; facultative xenogamy) indicating variation in the

Table S5 : Pollen size variation in *Aloe barbadensis* Mill. accessions.

Accessions	Polar length (P)	Equatorial length (E)	P/E ratio
N 106	37.37	27.69	1.35 (P)
N 118	35.90	**30.37**	1.19 (S)
IC 112531	40.21	25.81	1.57 (P)
N 127	37.07	27.04	1.38 (P)
IC 112532	38.37	24.43	1.56 (P)
GUJ 4	39.61	24.34	1.64 (P)
N 156	44.54	27.47	1.62 (P)
IC 112527	42.34	23.41	**1.81** (P)
NR 72	42.57	26.52	1.61 (P)
NR 31	41.93	30.34	1.39 (P)
IC 112517	**48.58**	29.29	1.66 (P)
N 119	35.40	26.56	1.34 (P)
GUJ 3	42.69	29.91	1.43 (P)
NMRM 2	42.23	29.78	1.43 (P)
IC 310618	42.51	28.57	1.48 (P)
NR 30	38.12	26.59	1.44 (P)
N 109	35.28	28.03	1.28 (S)
J 4	35.44	26.50	1.34 (P)
J 1	37.74	28.82	1.31 (S)
IGKV 1	41.94	24.14	1.75 (P)
GAKP 1	38.08	26.39	1.45 (P)
AB 9	44.57	25.00	1.80 (P)
IC 112521	43.34	25.07	1.74 (P)
RAJ 1	44.84	28.25	1.60 (P)
GUJ 2	46.86	30.02	1.59 (P)
Mean	**40.70**	**27.21**	**1.51**
Maximum	**48.58**	**30.37**	**1.81**
Minimum	**35.28**	**23.41**	**1.19**
SD	**3.73**	**2.10**	**0.17**
CV (%)	**9.17**	**7.73**	**11.30**

(S): Subspheriodal **(P):** Prolate.

Table 1 : Mean performance of *Aloe barbadensis* Mill. accessions for pollen and other related traits.

Accessions	PSR	PC	NO	PS	PV	ITPG	IVPG	CPI	SPC
N 106	1.35	45111.11	50.33*	59.52	22.35	21.35	22.83	3.67	7.33
N 118	1.19	37629.63	43.33	54.75	17.56	14.32	20.64	4.00	6.00
IC 112531	1.57*	110148.14	49.33	44.75	29.07	23.77	35.28*	6.00	9.00*
N 127	1.38	70703.70	52.33*	88.44*	27.97	23.46	29.50	17.33*	7.33
IC 112532	1.56*	113703.70	45.00	76.53	29.44	24.21	35.87*	5.33	6.33
GUJ 4	1.64*	93259.26	41.33	68.11	25.55	19.58	31.01	6.67	5.33
N 156	1.62*	66888.89	51.67*	53.48	17.48	12.52	27.16	0.33	7.33
IC 112527	1.81*	108511.85	52.33*	89.33*	48.33*	31.96*	43.92*	16.67*	9.33*
NR 72	1.61*	104888.89	52.67*	58.90	15.41	11.51	38.95*	13.67*	8.33*
NR 31	1.39	96592.59	48.33	71.58	28.14	18.71	23.06	10.33	8.67*
IC 112517	1.66*	139318.52*	45.67	85.66*	21.36	14.92	36.18*	9.33	5.33
N 119	1.34	78888.89	54.00*	74.11	27.28	22.26	35.05*	3.33	8.67*
GUJ 3	1.43	52629.63	47.67	56.25	13.19	11.56	20.31	2.33	10.67*
NMRM 2	1.43	89481.48	51.67*	83.40*	29.29	23.44	27.37	4.33	6.33
IC 310618	1.48	96148.14	46.67	83.02*	27.46	24.25	32.68*	1.67	7.67*
NR 30	1.44	97037.03	51.00*	69.09	11.48	9.95	28.13	6.67	8.67*
N 109	1.28	103629.63	52.00*	55.88	14.01	13.99	21.56	5.00	6.67
J 4	1.34	142370.36*	41.33	71.91	26.71	23.52	29.86	2.33	9.67*
J 1	1.31	72666.66	40.33	80.15	24.31	16.92	31.89	3.33	7.67*
IGKV 1	1.75*	102888.89	49.33	80.96	34.02	25.26	31.02	3.33	7.33
GAKP 1	1.45	107703.70	42.67	55.97	15.42	12.63	27.30	0.00	0.00
AB 9	1.80*	76111.11	52.67*	60.29	23.10	22.10	38.33*	0.00	0.00
IC 112521	1.74*	78740.74	51.67*	82.14*	15.36	13.03	19.85	13.33	7.67*
RAJ 1	1.60*	23974.07	45.00	54.41	30.14	30.27*	39.07*	7.33	6.33
GUJ 2	1.59*	72344.44	45.00	34.75	12.76	13.67	24.12	2.00	6.33
Mean	**1.51**	**87254.84**	**48.13**	**67.73**	**23.49**	**19.16**	**30.04**	**5.93**	**6.96**
Maximum	**1.81**	**142370.36**	**54**	**89.33**	**48.33**	**31.96**	**43.92**	**17.33**	**10.67**
Minimum	**1.19**	**23974.07**	**40.33**	**34.75**	**11.48**	**9.95**	**19.85**	**0**	**0**
S. Em. (±)	**0.09**	**5353.35**	**1.37**	**2.64**	**1.05**	**1.45**	**4.08**	**1.21**	**1.05**
CD (5%)	**0.27**	**15251.62**	**3.90**	**7.51**	**2.99**	**4.14**	**11.61**	**3.45**	**3.01**
CV (%)	**10.94**	**10.62**	**4.94**	**6.75**	**7.77**	**13.15**	**23.55**	**35.41**	**26.34**

Note: PSR: Pollen size ratio, PC: Pollen count, NO: Number of ovules, PS: Pollen stainability, PV: Pollen viability, ITPG: *In vitro* pollen germination, IVPG: *In vivo* pollen germination, CPI: Capsules per inflorescence, SPC: Seeds per capsule.

breeding system in Aloe (Table S6).

The male fitness of aloe ranged from 0.03-0.35 with mean 0.12% among the accessions indicating variability. The highest male fitness was noticed in the accessions RAJ1 (0.35%) followed by N156 (0.19%) and N118 (0.17%). While, the accession J4 (0.03%) had the lowest male fitness (Table S6). Reproductive efficiency (ratio of number of ovules to the number of pollens on the stigma) is a potential indicator of seed set. The reproductive efficiency value ranged from 40.47-117.91% with the mean 62.30% among the accessions. The accessions NR72 (117.91%) followed by J4 (102.48%) and N127 (82.82%) recorded the highest reproductive efficiency (Table S6). Number of capsules per inflorescence ranged from none to 17.33 with mean value 5.93 indicating wide variability among the accessions. The accession N127 (17.33) at par with IC112527 (16.67) and NR72 (13.67) had highest number of capsules. In contrast, the accessions GAKP1 and AB9 have no capsules limiting their use in recombination breeding (Table 1). Capsule set (%) per inflorescence ranged from none to 27.08 (Table S7) with a mean 6.97. Highest fruit set (%) was observed in N127 (27.08%) while, no fruit set in GAKP1 and AB9. Maximum seed set was observed in GUJ3 (10.67), which was at par with J4 (9.67), IC112527 (9.33), IC112531 (9.00), NR31 (8.67), N119

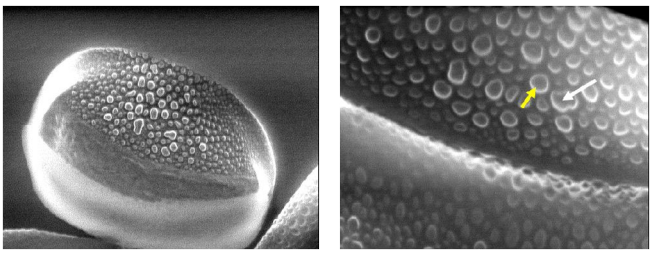


Fig. 2 : Pollen surface pattern of *Aloe barbdensis* (a) Reticulate surface pattern (2500X) (b) Reticulate surface pattern (6500X), yellow arrow indicating muri (ridge) and white arrow indicating lumina (depression) of the pollen surface.

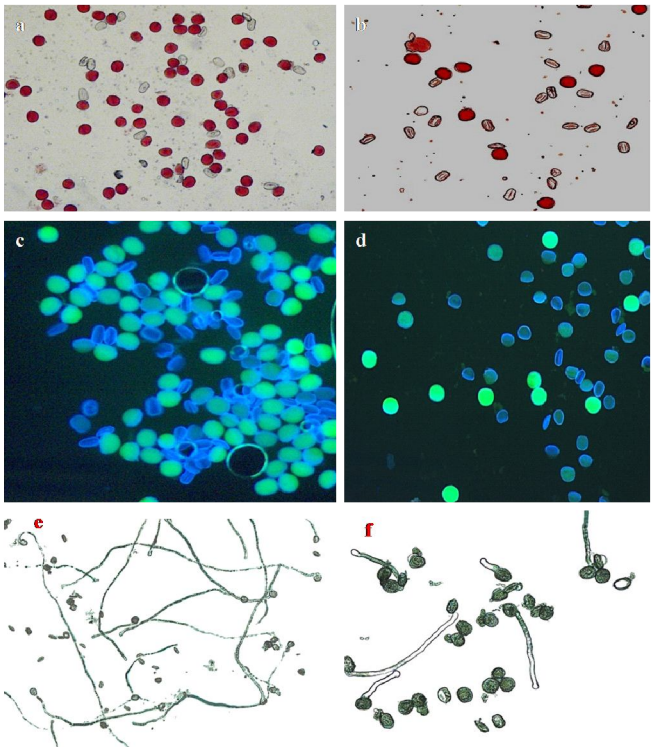


Fig. 3 : Pollen stainability, pollen viability and *in vitro* pollen germination in *Aloe barbadensis* Mill. (a) Acetocarmine staining of pollen grains of IC112527 accession (high pollen stainability) (b) GUJ2 (lower pollen stainability) (c) IC112527 accession (high pollen viability) (d) NR30 accession (lower viability) (e) *In vitro* pollen germination of IC112521 accession (higher germination) (f) NR30 (lower germination).

(8.67), NR30 (8.67), NR72 (8.33), IC310618 (7.67), J1 (7.67) and IC112521 (7.67). In contrast, no seed set in GAKP1 and AB9. Seed set (%; number of seeds per capsule to total number of ovules in ovary) ranged from 0.00-23.39 with the mean 14.9 (Table S7). The maximum seed set per cent was observed in the accession J4 (23.39 %) which was followed by GUJ3 (22.38%), J1 (19.01%) and IC112531 (18.24%) indicating variability for the seed set. In contrast, minimum was noticed in accession GAKP1 (0.00%) and AB9 (0.00%).

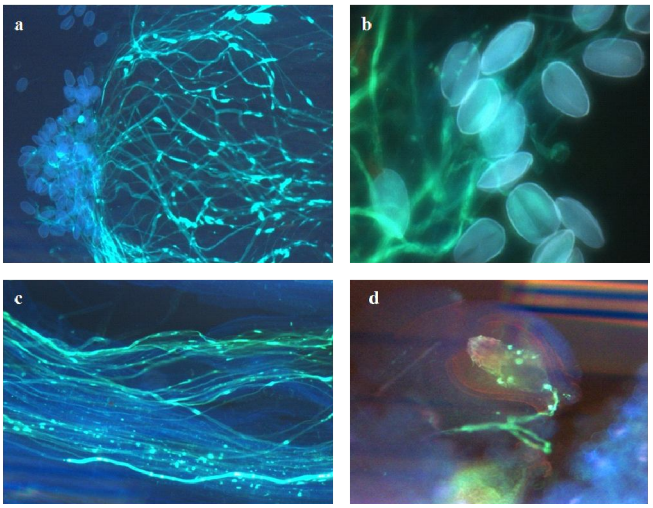


Fig. 4 : *In vivo* pollen germination of *Aloe barbadensis* Mill. (a) Pollen germination on the stigma surface (b) Germinated pollen on stigma 40x (c) Pollen tube growth in the style (d) Pollen tube reaching ovule.

Genetic variability

In general, phenotypic coefficient of variation (PCV) was higher than genotypic coefficient of variation (GCV) for all the nine traits studied indicating existence of sufficient genetic variability among the accessions of Aloe (Table 2). High magnitude of PCV and GCV (>20%) were observed for capsules per inflorescence (88.33% and 80.92%, respectively) followed by pollen viability (36.26% and 35.47%, respectively), seeds per capsule (41.69% and 32.32%, respectively), pollen count (32.34% and 34.04%, respectively), *in vitro* pollen germination (33.78% and 31.12%, respectively) and pollen stainability (22.34% and 21.29%, respectively) indicating greater variability among the accessions.

The difference between GCV and PCV for the traits was minimal (<11%) indicating less influence of environment on the traits studied. The traits are under the control of additive genes thus scope for further improvement of these traits through selection. High heritability coupled with high genetic advance over mean (GAM) was found for pollen viability (95.66% and 36.26%, respectively), pollen stainability (90.86% and 41.81%, respectively) and pollen count (90.25% and 63.29%), *in vitro* pollen germination (84.86% and 59.05%, respectively) capsules per inflorescence (83.93% and 152.73%, respectively) and seeds per capsule (60.09% and 51.60%, respectively) indicating preponderance of additive gene action. Moderate heritability coupled with high GAM was observed for the *in vivo* pollen germination (36.16% and 21.95%, respectively). Moderate heritability coupled with moderate GAM was observed for the trait pollen size ratio (40.00% and

Table 2 : Variance components, heritability and Genetic advance over mean (%) of various characters of *Aloe barbadensis* Mill. accessions.

Characters	Mean	σ^2_p	σ^2_g	PCV (%)	GCV (%)	h^2_b (%)	GAM (%)
Pollen size ratio	1.51	0.05	0.02	14.80	9.36	40.00	12.20
Pollen count	87254.84	882365489.43	796390519.65	34.04	32.34	90.25	63.29
Number of ovules	48.13	21.40	15.76	9.61	8.25	73.64	14.58
Pollen stainability	67.73	228.99	208.07	22.34	21.29	90.86	41.81
Pollen viability	23.49	72.57	69.42	36.26	35.47	95.66	71.46
In vitro pollen germination	19.17	41.94	35.59	33.78	31.12	84.86	59.05
In vivo pollen germination	30.04	78.37	28.34	29.47	17.72	36.16	21.95
Capsules per inflorescence	5.93	27.44	23.03	88.33	80.92	83.93	152.73
Seeds per capsule	6.96	8.42	5.06	41.69	32.32	60.09	51.60

Note: σ^2_g: Genotypic variance, σ^2_p: Phenotypic variance, GCV: Genotypic coefficient of variation, PCV: Phenotypic coefficient of variation, h^2_b (%): Broad sense heritability, GAM (%): Genetic advance over mean.

Table 3 : Pearson's correlation coefficient value for various traits.

Traits	PSR	PC	NO	PS	PV	ITPG	IVPG	CPI	SPC
Pollen size ratio	1.00	0.16	0.22	0.07	0.25*	0.16	0.31**	0.18	-0.20
Pollen count		1.00	-0.02	0.34**	0.17	0.03	0.21	0.12	0.00
Number of ovules			1.00	0.10	0.05	0.04	0.04	0.28*	0.11
Pollen stainability				1.00	0.50***	0.32**	0.20	0.43***	0.14
Pollen viability					1.00	0.85***	0.46***	0.29*	0.15
In vitro pollen germination						1.00	0.42***	0.15	0.08
In vivo pollen germination							1.00	0.18	-0.07
Capsules per inflorescence								1.00	0.25*
Seeds per capsule									1.00

Note: *p<0.05,**p<0.01,***p<0.001

Table S6 : Pollen/ovule ratio, male fitness and reproductive efficiency of *Aloe barbadensis* Mill. accessions.

Accessions	Pollen/ Ovule ratio	Male fitness (%)	Reproductive efficiency (%)
N 106	896.25	0.15	73.30
N 118	868.38	0.17 (III)	69.15
IC 112531	2232.73	0.06	77.49
N 127	1351.03	0.09	79.70
IC 112532	2526.75 (III)	0.05	82.82 (b!)
GUJ 4	2256.27	0.10	45.09
N 156	1294.62	0.19 (II)	40.47
IC 112527	2073.47	0.10	48.01
NR 72	1991.56	0.04	**117.91 (I)**
NR 31	1998.47	0.11	46.33
IC 112517	3050.77 (II)	0.08	42.81
N 119	1460.91	0.14	48.21
GUJ 3	1104.12	0.11	80.79
NMRM 2	1731.90	0.12	48.29
IC 310618	2060.32	0.08	60.61
NR 30	1902.69	0.12	44.61
N 109	1992.88	0.08	59.77
J 4	**3444.44 (I)**	0.03	102.48 (II)
J 1	1801.65	0.11	50.21
IGKV 1	2085.59	0.08	63.52
GAKP 1	2524.31	0.07	52.89
AB 9	1445.15	0.14	50.32
IC 112521	1524.01	0.10	65.96
RAJ 1	532.76	**0.35 (I)**	54.00
GUJ 2	1607.65	0.12	52.73
Mean	**1830.35**	**0.12**	**62.30**
Maximum	**3444.44**	**0.35**	**117.91**
Minimum	**532.76**	**0.03**	**40.47**

Table S6 continued...

Table S6 continued...

P/O: Pollen/Ovule ratio.

12.20%, respectively) indicating trait is controlled by both additive and non-additive gene action.

Correlation among the traits

Seeds per capsule is an important measure of seed set of Aloe. Seeds per capsule had a significant positive correlation with capsules per inflorescence (0.25*) indicating the nature of relationship among the traits. Capsules per inflorescence had a significant positive

Table S7 : Capsule set and seed set per cent of different accessions.

Accessions	Capsule set (%)	Seed set (%)
N 106	3.91	14.57
N 118	4.23	13.85
IC 112531	7.89	18.24
N 127	**27.08**	14.01
IC 112532	6.27	14.07
GUJ 4	6.21	12.90
N 156	0.47	14.19
IC 112527	21.01	17.83
NR 72	16.27	15.82
NR 31	10.95	17.93
IC 112517	8.95	11.68
N 119	3.45	16.05
GUJ 3	2.02	22.38
NMRM 2	4.41	12.26
IC 310618	1.67	16.43
NR 30	7.19	16.99
N 109	5.36	12.82
J 4	2.24	**23.39**
J 1	3.51	19.01
IGKV1	4.35	14.86
GAKP 1	0.00	0.00
AB 9	0.00	0.00
IC 112521	17.02	14.84
RAJ 1	6.83	14.07
GUJ 2	2.96	14.07
Mean	**6.97**	**14.49**
Maximum	**27.08**	**23.39**
Minimum	**0.00**	**0.00**
SD (±)	**6.77**	**5.22**

correlation with number of ovules (0.28*), pollen viability (0.29*) and pollen stainability (0.43***). Pollen viability had significant positive correlation with pollen size ration (0.25*), pollen stainability (0.50***), *in vitro* (0.85**) and *in vivo* pollen germination (0.46**). Pollen size has positive correlation with pollen count (0.34**) indicating nature of relationships among the traits (Table 3).

Discussion

Seedlessness is major problem in propagation and improvement of Aloe. Traditionally, Aloe is propagated through suckers. However, large scale production through sucker is cumbersome and costly. Further, clonal selection has inherent limitation in the genetic improvement of Aloe. Seed production through sexual means occur in Aloe however, a few seeds are produced and show poor seed germination (Mukonyi *et al.*, 2011). Moreover seed propagated plants show long gestation period (Cristiano *et al.*, 2016). Seedlessness may be due environmental, genetic and cytogenetic factors (Rana and Kanwar, 2017; Ahmad and Gupta, 2016; Sharma and Sharma, 2016). Knowledge the factors governing the seedlessness will help improve Aloe through recombination breeding. Further, genetic variability for pollen and fruit set parameters unearths the potential factors hindering the seed formation on Aloe. A number of reasons could be hypothesized for the low fruit set behaviour in flowering plants. These may be genotype-dependent factors related with the floral biology, that influenced fruit set and, consequently, productivity such as flower bud production, flower bud drop, flowering time, ovule development stage at anthesis, pollen production, pollen sterility, pollen germination, architectural difference between the planes of stigma and the anthers, aborted pistils and incompatibility issues, etc. (Guitian *et al.*, 2001; Animasaun *et al.*, 2014; Nair, 2016).

We observed asynchronous flowering among the accessions. The accessions GAKP-1 and AB9 were late flowering compared to rest of the accessions. Being a cross pollinated nature synchronous flowering facilitates more seed set in Aloe. There is positive impact of synchronous flowering on the reproductive output in plants (Michalsk and Durka, 2007). However, slight asynchrony in flowering has been recognized as advantageous for individual fitness when it compels pollinators to visit multiple plants for long time to ensure successful pollination (Rathcke and Lacey, 1985). The accessions GAKP1 and AB9 flowering period did not synchronize with the other accessions this might be one reason for no capsule and seed set in the accessions as Aloe requires cross pollination for seed set and it exhibit self-incompatibility and sterility (Sapre, 1975). We found staggered anthesis among the accessions which may facilitate hybridization and seed set. Anthesis was maximum during 9.00-12.00 pm among the accessions. However, the accession NMRM2 exhibited the peak anthesis between 3.00-6.00 pm indicating genotypic variation for anthesis. In the previous study staggered flower opening was observed in the five species of Aloe (Botes *et al.*, 2008). Earlier, it was reported that flower opening started at 8.00 am and continued till 3.00 pm (Rajasekharan *et al.*, 2023). The flower opening was noticed even up to late evening hours in *A. marlothii* (Symes *et al.*, 2008). Delayed (one-hr after anthesis) anther dehiscence was observed when flower opened early (6.00 am-9.00 am) compared to flower open at 12.00 pm-3.00 pm (20 min after anthesis) which may be due to effect of climatic factors. In the previous study it

was reported in *Holcus* and *Festuca* that the climatic factors like air temperature, relative humidity and light intensity affect the anthesis and pollen dispersal (Liem and Groot, 1973). In date palm that the unfavourable environmental conditions, such as low temperature and high humidity may delay the anther dehiscence and subsequently delay the process of pollination which may cause reduction in fruit set (Al-Obeed and Soliman, 2011). The perianth removal and heating (increase in temperature) can accelerate anther opening whereas high relative humidity around the anther can prevent anthesis in *Gasteria verrucosa* and *Lilium* hybrid (Keijzer, 1987).

The pollen count directly affects the seed set per cent. A higher pollen count ensures increased chance of pollen landing on stigma which in turn increases the rate of fertilization. The number of pollen produced per flower ranged from 23974.07-142370.36. In the previous study the number of pollens produced was reported to be 249487.12 in *Aloe vera* and 331205.22 in *A. Saponaria* (Velasquez-Arenas and Imery-Buiza, 2008).

Pollen fertility was assessed using pollen stainability (acetocarmine), pollen viability (FCR test), *in vitro* and *in vivo* pollen germination. High pollen fertility was observed in pollen stainability (acetocarmine) and pollen viability (FCR test) compared *in vitro* and *in vivo* pollen germination. Wide variation in the pollen fertility was observed among the accessions of Aloe. *In vivo* pollen germination varied from 19.85 to 43.92% among the accessions. The accessions, IC112527, RAJ1 and NR72 had maximum *In vivo* pollen germination indicating their potential role in seed set in Aloe. The pollen to ovule ratio indicates number of pollen grains available for each ovule in a flower. The pollen to ovule ratio indirectly contributes to the reproductive success. The higher pollen to ovule ratio increases the chance of pollination and subsequently fertilization which in turn lead to higher seed set. The mean pollen to ovule of the Aloe accessions was 1830.35. In the previous study pollen to ovule ratio was reported to be 4115.20 and 3247.10 in *A. barbandesis* and *A. saponaria*, respectively (Velasquez-Arenas and Imery-Buiza, 2008). Earlier, Cruden (1977) reported that pollen to ovule ratio in different breeding system varied from 4.70 ± 0.70 (cleistogamy), 27.7 ± 3.1 (obligate autogamy), 168.00 ± 22.12 (facultative autogamy), 796.60 ± 87.70 (facultative xenogamy) and 5859.20 ± 936.50 (Xenogamy). In most of Aloe accessions pollen to ovule ratio was comparatively higher indicating xenogamy breeding system. However, in some of the accession RAJ1 (409.30) showed lowest pollen to ovule ratio indicating facultative xenogamy. Earlier, a Xenogamy (wild populations) mode of breeding systems was observed in Aloe (Swathi *et al*., 2015). Accessions with facultative xenogamy may be having more seed set due more pollens on the stigma. However, this needs further study. Male fitness and reproductive efficiency calculated was 0.12% and 62.30%. Male fitness is influenced by several factors which include pollen production, pollen quality, pollen dispersal and pollen competition. Through investigating the male fitness of the accessions one could explore the ways to enhance the reproductive success. The reproductive efficiency per cent value provides an estimate of potential successful fertilization and seed set. However, it is a simple measure it does not consider the various complex factors that affect reproductive efficiency. In the previous study, male fitness and reproductive efficiency was reported to be 0.06 and 57.30%, respectively in Aloe (Geetha, 2006).

The accessions J4, GUJ3 and IC112531 had maximum seed set per cent and reproductive efficiency hence, could be useful for propagation through seeds. Similarly, the accessions IC112527 and IC112517 exhibited superiority for pollen fertility traits such as pollen size ratio, pollen to ovule ratio, pollen count, pollen stainability and *in vivo* pollen germination. Hence, these accessions could be important candidates utilized as a male parent in hybridization programme to get maximum seed set. The accessions N127 IC112527 and NR72 were superior for fruit setting and related traits such as number of ovules, capsules per inflorescence, seeds per capsule and reproductive efficiency. Hence, the above accessions could be used as female parent in hybridization programme.

In general, PCV was higher than GCV for all the nine traits studied indicating the influence of environment on the traits. Earlier, a similar finding was reported among the traits studied in Aloe accessions (Ahmad and Gupta, 2016). The GCV does not offer the full scope for the estimation of heritable portion of variation. Hence, to differentiate between genetic and environmental factors the estimation of heritability and GAM becomes necessary (Johnson *et al*., 1955). High heritability coupled with GAM was observed for the trait pollen viability, pollen stainability, pollen count, *in vitro* pollen germination, capsules per inflorescence and seeds per capsule indicating heritability are due to additive effect and chance of fixing trait through selection is high and hence, selection is effective. The highest GAM might be due to highest phenotypic standard deviation. Hence, the selection will only be effective for pollen count, pollen stainability, pollen viability and *in vitro* pollen germination.

Among the studied traits, positive and highly

significant correlation was observed between pollen viability, pollen size ratio, pollen stainability, *in vitro* pollen germination, *in vivo* pollen germination and capsules per inflorescence indicating one trait could be used to predict the other. Earlier, a significantly positive correlation between pollen viability and *in vitro* pollen germination (r = 0.92) was observed in date palm (Al-Najm *et al*., 2021). The average size of the pollen is the indication of the metabolic vigour. Increased metabolic vigour would increase fertilization ability and male reproductive fitness. Hence, it might be hypothesized the size may have a potential impact on pollen tube growth rate, fertilization ability, competitive ability and seed set (Cruzan, 1990). The accessions with higher pollen viability could also be used to predict the accessions with higher capsules per inflorescence in turn seeds per capsule.

Conclusion

Identification of the genotypes with better pollen traits and fruit set traits which can serve as a potential source for improvement of Aloe through recombination breeding and also the commercial propagation of through seeds. We found the J4, GUJ3 and IC112531 having higher seed set per cent. The accessions IC112527 and IC112517 exhibited superiority for pollen fertility traits. Hence, these accessions could be important candidates utilized as a male parent in hybridization programme to get maximum seed set. The accessions N127, IC112527 and NR72 were superior for fruit setting and related traits. Hence, the above accessions could be used as female parent in hybridization programme. The results presented here will be useful in selecting the accession as parents for hybridization programmes and propagation of Aloe through seeds.

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Competing interests

The authors declare that they have no competing interests.

Contributions

GKA, SK, AP contributed to design the study. KS carried out the experiments. DJP carried out data analysis. KS. SB and NRR drafted and edited the manuscript. All authors have read and approved the final manuscript.

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