Spontaneous Inversion Heterozygotes in *Aloe barbadensis* Mill.

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Summary A population of cultivated *Aloe barbadensis* Mill. displayed various types of chromosomal configurations at anaphase/telophase-I and II due to inversion heterozygosity. The population was characterized by the presence of bridges and fragments because of various numbers and positions of cross-overs in the inversion loop. The inversion heterozygote had very low pollen fertility.

Key words *Aloe barbadensis* Mill., Inversion, Pollen fertility, Bridge-fragment configuration.

Aloe is a genus of the family Liliaceae, containing about 500 species (Gunjan and Roy 2010). *Aloe barbadensis* Mill. is one of the most important medicinal plant species and widely cultivated throughout the world. It is a xerophytic, succulent, perennial herb with fleshy leaves arising in a rosette from a short stem. Its leaves are used in various skin diseases and diabetes. The chromosome number is $n=7$ and the size of the chromosomes is comparatively large. There have been very few studies on spontaneous inversion heterozygosity in *Aloineae* (Brandham 1975, 1977 a, b). In the present investigation, an inversion heterozygote was discovered, which was subsequently confirmed on the basis of presence of bridges and fragments at anaphase I/II.

Materials and methods

Aloe barbadensis Mill. clones were collected from different locations, such as Govt. Forest. Narsingarh Distt. Rajgarh, Green house nursery Indore, High-tech nursery Ujjain, Malava nursery Ujjain, Botanical garden of the S. S. in Botany Vikram University Ujjain and Central Arid Zone Research Institute Jodhpur. Twenty clones were collected from each location and planted in pots. For meiotic studies, young flower buds of appropriate size were collected and fixed in Conroy's fluid (3 absolute alcohol: 1 acetic acid). Anthers were separated, teased in a drop of 2% iron acetocarmine on a clean slide, and squashed under a cover glass. Pollen mother cells (PMCs) were analyzed for suitable stages of meiosis. Slides with well-spread cells and clear chromosomes were selected for scoring and photomicrographs were taken from temporary slides under oil immersion ($\times 100$) and magnified to $\times 1000$.

Results

Meiosis in normal plants: *Aloe barbadensis* Mill. has the chromosome number $n=7$. Among these, there are four long and three short chromosome pairs. Meiosis in normal plants is regular, with seven bivalents, and the proportion of ring bivalents to rod bivalents is roughly 4:3. Chromosomes are equally distributed (7:7) at each pole at anaphase/telophase-I and II (Figs. 1–3).

Inversion heterozygotes: The inversion heterozygote showed bridge/fragment configuration at anaphase/telophase-I/II of meiosis. At anaphase/telophase-I/II, 616 PMCs analyzed in the plant

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Figs. 1–12. Meiotic stages in control and inversion heterozygotes in *Aloe barbadensis* Mill. 1–3 Control; 1 diakinesis, 2 anaphase I, 3 anaphase II, 4–12 Inversion Heterozygote; 4 bridge at anaphase I, 5 bridge+fragment at anaphase I, 6 bridge+fragment at telophase I, 7 fragment at telophase I, 8 bridge at anaphase II, 9–10 bridge+fragment at anaphase II, 11 fragment at anaphase II, 12 pollen grains, note high sterility.

were found to have various configurations of chromosomes confirming the presence of inversion heterozygosity (Table 1). At anaphase/telophase-I, bridges, fragments and bridges+fragments were observed in 41.47% of PMCs (Figs. 4–7). At anaphase/telophase-II, configurations of bridges, fragments, and bridges and fragments (B/F) were observed in 30.3% of PMCs. Two bridges and two fragments (BB/FF) at different poles were recorded in 2.21% of PMCs. (Table 1 and Figs. 8–11). Pollen fertility was studied in anthers taken from different buds. It was remarkably low in the abnormal plant (25.28%, Fig. 12).

Table 1. Various types of chromosomal configurations at anaphase/telophase-I /II at meiosis of inversion heterozygote of *Aloe barbadensis* Mill

	Abnormalities	Number	(%)
A. Anaphase/Telophase-I	Normal cells	125	59.52
	Bridges+Fragments	34	16.19
	Loops+Fragments	51	24.28
	Total number of abnormal cells	85	41.47
B. Anaphase/Telophase-II	Normal cells	274	67.48
	Bridges	56	13.79
	Fragments	43	10.59
	1 Bridges+1 Fragments	24	5.91
	2 Bridges+2 Fragments	9	2.21
	Total number of abnormal cells	132	32.51
C. Pollen Grains	Fertile	131	25.28
	Sterile	387	74.71
	Total	518	

Discussion

For the development of a new species, the first basic requirement is availability of genetic variability at the gene/chromosome level, which may either be spontaneous or induced. Several researchers have found chromosomal variations in different species of a genus. Spontaneous chromosomal changes were found in *Aloe barbadensis* Mill. (Kaul 1965, Vig 1968), in *Paeonia decomposita* (Wang *et al.* 2008) and induced chromosomal changes as in *Phlox drummondii* (Verma and Raina 1982, Verma and Sharma 2000), in *Crotalaria* (Verma *et al.* 1982), in *Vicia faba* (Sjodin 1971, Verma and Rao 1994, Verma *et al.* 2004). In the present investigation, an inversion heterozygote was found in the cultivated population of *Aloe barbadensis* Mill. and that was confirmed on the basis of chromosomal configurations at meiosis division.

The inversion heterozygote has a bridge-fragment configuration at anaphase-I and II, indicating the paracentric nature of inversion. Various possible types of configurations resulted from different numbers and positions of cross-over inside and outside the inversion loop. The presence of one bridge-fragment at anaphase-I was due to one cross-over inside the inversion loop. In PMCs, where only the fragment was visible, it was presumed that this was due to the overlapping of the loop by other chromosomes at the poles and that the original configuration was loop-fragment.

Newman (1966) suggested that numerous organisms in which the pachytene stage has not been suitable for cytological investigation have been reported to be heterozygous for paracentric inversions on the basis of circumstantial evidence of meiotic anaphase bridge and fragment configurations. That this conclusion was correct is evident from the bridge-fragment configuration, observed at anaphase-II, which could result only from one loop-fragment of anaphase-I. The above configurations were the consequence of one cross-over inside and one outside the inversion loop. On the basis of the meiotic stage, it was difficult to pinpoint the chromosome in which inversion had taken place.

The most obvious genetic effect of inversions is the formation of unbalanced gametes that often causes microspore sterility (Wang *et al.* 2008). As is expected, the loss of chromosomal fragments as a result of paracentric inversion leads to deficient gametes and the high sterility observed in the present case.

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