African Journal of Biotechnology Vol. 6 (22), pp. 2585-2589, 19 November, 2007
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2007 Academic Journals

Full Length Research Paper

Meiotic chromosome behaviour and sexual sterility in two Nigerian species of *Aloe Linn*

B. O. Akinyele

Department of Crop, Soil and Pest Management, Federal University of Technology, P. M. B. 704, Akure, Ondo State, Nigeria. E-mail: yele174@yahoo.co.uk.

Accepted 16 October, 2007

The behaviour of meiotic chromosomes and the subsequent behaviour of the meiotic products were investigated in two Nigerian species of *Aloe*, namely *Aloe keayi* and *Aloe macrocarpa* var *major* with a view to uncovering the cause of their inability to reproduce sexually. The two plant materials used in this study were already under cultivation in the Aloe Research Garden situated in the Crop Type Museum of the Department of Crop, Soil and Pest Management, Federal University of Technology, Akure, Nigeria. All the various meiotic stages from leptotene to pollen development were studied. Number of chromosomes per complement in the two taxa is 2n = 14. The 14 chromosomes were resolved into seven bivalents all of which show incomplete pairing thereby lowering the frequency of chiasma formation. Chromosome aberration involving deletion of a segment from one of the longest chromosomes was detected in *A. macrocarpa* var *major*. Though the percentage of pollen stainability is high in both taxa, the percentage of pollen germinability, both *in vitro* and *in vivo*, is very low in *A. keayi* when compared with what obtains in *A. macrocarpa* var *major*. The morphology and behaviour of chromosomes in the two taxa, the failure of the flowers to form fruits in *A. keayi* and the failure of the fruits to attain maturity in *A. macrocarpa* var *major* are all evidences that sexual sterility in the two taxa is attributed to genetic instability and deficiency in the genotype of the spores as a result of their hybrid origin.

Key words: Sexual sterility, genetic instability and deficiency, *A. keayi, A. macrocarpa* var *major*.

INTRODUCTION

There are over 325 species of aloes in the world, mostly native to Africa (Anselm, 2004). They range in size from little one-inch miniatures to massive plant colonies consisting of hundreds of two-foot diameter plants. They belong to the family Liliaceae and are cultivated for their thick, fleshy leaves from which the dry aloes of commerce are obtained. The sap of the *Aloe* plant is a thick, mucilaginous gel. It is this gel which is used medicinally. Basically, all the various species of *Aloe* are known to have similar chemical constituents but *Aloe vera* is more readily available for use because it propagates itself faster than any other known species of *Aloe* (Anselm, 2004).

Since the life of an individual plant is limited in duration, it has developed certain mechanisms by which it can reproduce itself in order to continue the perpetuation of its kind and also to multiply in number. One of these mechanisms is sexual reproduction. According to Dutta (1979), sexual reproduction consists in the fusion of two sexual reproductive units (gametes) to give rise to a zygote which develops into a new whole plant. In the opinion of Oyewole (1984), successful sexual reproduction is an essential achievement of genetic stability of any organism. While investigating the reproductive abilities of nine accessions of Aloe indigenous to Nigeria, Akinyele et al (2007) observed that two species, namely *Aloe keayi* and *Aloe macrocarpa* var *major* were not able to reproduce sexually even though they produced flowers. This observation raises important questions about the origin and evolution of these two species. According to Oyewole (1984), most of the factors responsible for sexual sterility in plants are linked up with natural hybridization and polyploidy, both of which are known to play important roles in the evolution of plant species.

A. keayi and *A. macrocarpa* var *major* are both known to be diploids, each having a somatic chromosome number of 2n = 14 (Akinyele, 2002). Hence, sexual sterility in the two species cannot be linked up with polyploidization. The cause of the sexual sterility was therefore investigated, and this paper discusses results obtained.

Figure 1. Vegetative habit of *Aloe keayi*.

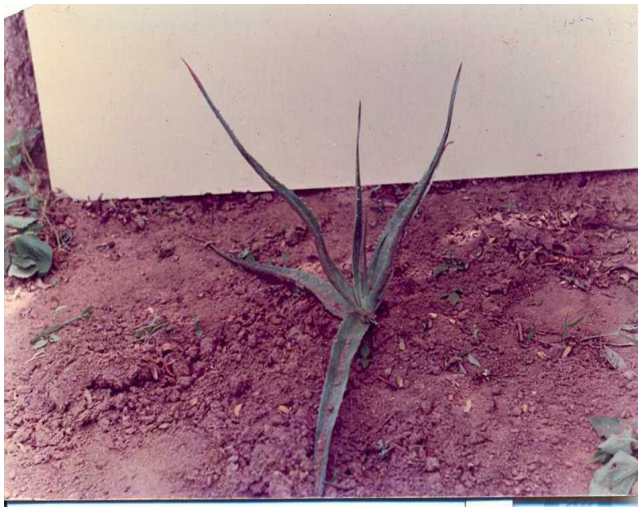

Figure 2. Vegetative habit of *Aloe macrocarpa* var *major*.

MATERIALS AND METHODS

Representatives of the two plant materials, *A. keayi* and *A. macrocarpa* var *major*, are already under cultivation in the Aloe Research Garden situated in the Crop Type Museum of the Department of Crop, Soil and Pest Management, Federal University of Technology, Akure, Nigeria. Figures 1 and 2 show the vegetative habit of the two *Aloe* species. The places from which collections were made are as stated by Akinyele (1999).

Growing tips of young roots, usually not longer than 5 mm, were harvested between 7.00 and 9.00 am (local time). The root tips were washed in distilled water and kept in a dark cupboard at room temperature for 1 h in saturated aqueous solution of Para-dichlorobenzene (p-DCB). They were then fixed in freshly prepared 1:3 glacial acetic alcohol (1 part of glacial acetic acid: 3 parts of absolute ethanol) and stored at 4°C in the refrigerator for at least 24 h. The root tips were later hydrolysed in 5 N HCl at room temperature for 5 min and squashed in 2% solution of acetic orcein. Chromosomes of cells with well spread metaphase plates were studied and photomicro-graphed at X10 eyepiece and X40 objective of the Olympus Research Microscope.

Figure 3. Mitotic metaphase of *Aloe keayi*.

Flower buds, usually 4 – 5 mm long, were harvested between 5.00 and 6.00pm (local time), fixed in a freshly prepared 1 : 3 glacial acetic acid-absolute ethanol and stored at about – 4°C for up to 24 h. The flower buds that were not processed immediately were preserved in 70% alcohol. One anther at a time was extracted from the fixed flower buds, placed on a clean glass slide and squashed in 2% acetocarmine solution. The preparation was covered with a cover-slip already smeared with glycerin albumen and examined under X10 eyepiece and X40 objective of the Olympus Research Microscope. Pollen mother cells (pmc) at the appropriate stages were studied. At zygotene, diakinesis and metaphase I stages, the number of chromosome bodies, the number of bivalents, the frequency of chiasma formation per complement and the extent of chromosome pairing were recorded for each of the *Aloe* species. Photomicrographs were taken as in the root tip cells.

For *A. keayi*, whose flowers did not produce any fruits, its pollen grains from indehisced anthers of mature flowers were used for stainability and germinability investigations. Anthers were opened and squashed in 2% acetocarmine solution on a clean glass slide, covered with a cover-slip and then examined under the microscope for pollen ability to absorb the stain. Such preparation was made from five flowers harvested from five different inflorescences and counts from these preparations were taken randomly. Percentage of pollen stainability was calculated as the proportion of the pollen grains that absorbed the stain out of the total counts. Ability of the pollen grains to germinate was investigated both *in vitro* and *in vivo* according to the methods of Oyewole (1984).

The fruits and the seeds produced by *A. macrocarpa* var *major* were examined under the dissecting microscope with a view to finding out what could be responsible for the inability of the seeds to germinate.

RESULTS

The number of chromosomes per somatic complement (2n = 14) in the two species as revealed by the mitotic metaphase is shown in Figures 3 and 4. The pairing of meiotic chromosomes at diplotene stage is shown in Figures 5 and 6. Cells at the various stages of meiotic division (leptotene to the stage of formation of pollen grains in which gametotype development is in progress) were encountered in each of the anthers squashed. The

Figure 4. Mitotic metaphase of *Aloe macrocarpa var*. major.

Figure 5. Diplotene stage of *Aloe keayi*.

behaviour of meiotic chromosomes, pollen stainability and pollen germinability are shown in Table 1. All the fruits and the seeds of *A. macrocarpa* var *major* examined under the dissecting microscope were found to be wrinkled and to have the appearance of fruits and seeds that are not fully formed or developed.

DISCUSSION

Both *A. keayi* and *A. macrocarpa* var *major* had established themselves vegetatively and were fully adapted to their new habitat. *A. keayi* propagates itself vegetatively by production of suckers but fruits were not formed from its flowers. It was also found out that all the fruits produced by *A. macrocarpa* var *major* aborted before reaching the stage of maturity. This development

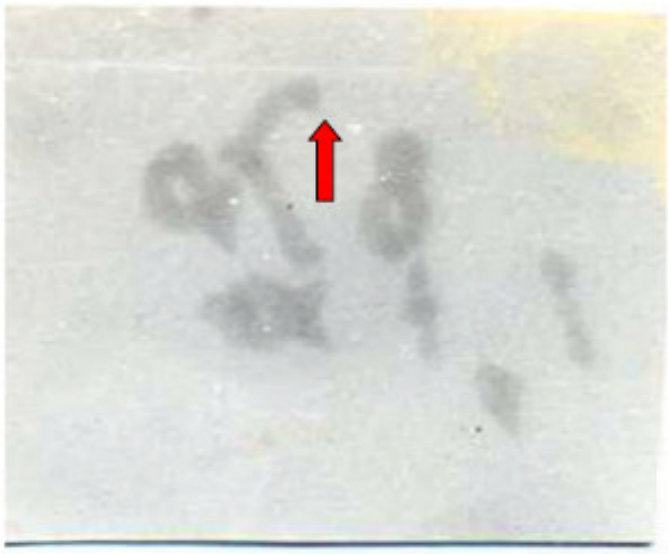

Figure 6. Diplotene stage of *Aloe macrocarpa* var *major* (note the arrow that points to the region at which a chromosome segment was deleted).

implies that the two *Aloe* species are unable to reproduce sexually. In the words of Weaver and Hedrick (1997), "sexual reproduction occurs when individuals produce male and female sex cells, or gametes, that in turn unite to form a zygote, a single cell from which a new individual develops". Though both *A. keayi* and *A. macrocarpa* var *major* produced male and female gametes, new individuals did not develop from the union of these sex cells. According to Abercrombie et al. (1973), a plant is sterile if it is unable to reproduce sexually. In their own explanation, Gardner and Snustad (1981) contend that a plant is sterile if it is not able to produce offsprings. Since neither *A. keayi* nor *A. macrocarpa* var *major* was found to produce offsprings, it is reasonable, therefore, to opine that the two species in question are sexually sterile.

Moore (1976), corroborated by Oyewole (1984), argued that "the chromosomes provide the physical basis by which the genetic stability and continuity of individuals and population is maintained". This infers that chromosome stability, as individuals and as a complement, during the various stages of cell division, greatly influences not only the genetic stability of the organism but also the genetic stability of the population. Hence, the morphological attributes of the organism and the population, as also contended by Oyewole (1984), are a product of the events at the chromosomal level. As further opined by Moore (1976), "these chromosomes are themselves vehicles for generating variation which is essential for evolution to proceed". Lewis (1966) argues that the establishment of any new kind of organism is faced with two unavoidable situations: (i) the stabilization of its genetic system and (ii) the restoration (or acquisition) of sexual fertility. Both situations are an internal battle of chromosomal organization fought at the genetic

Table 1. The behaviour of meiotic chromosomes, pollen stainability and pollen germinability in the two Nigerian *Aloe* species.

Character	No of observations	A. Keayi		A. macrocarpa var *major*	
		No of occurrences	% of occurrences	No of occurrences	% of occurrences
No. of chromosomes per complement	50	14 (all)	100%	14 (all)	100%
No. of bivalents per complement	50	7 (all)	100%	7 (all)	100%
Multivalent	50	0	0.0%	0	0.0%
Normal anaphase movement	50	50	100%	50	100%
Laggards	50	0	0.0%	0	0.0%
Chromosome fragments	50	0	0.0%	0	0.0%
Complete chromosome pairing	50	0	0.0%	4	8.0%
Incomplete chromosome pairing	50	50	100%	46	92.0%
Chromosome aberration	50	0	0.0%	48	96.0%
X no. of chiasma per complement	50	2.80		2.92	
No. of pollen grains that absorbed stain	100	88	88.0%	93	93.0%
No. of pollen grains that germinated *in vitro*	50	6	12.0%	39	78.0%
No. of pollen that germinated *in vivo*	50	0	0.0%	33	66.0%

level. Since the organism would need to establish itself fully in its environment and adapt to the prevailing environmental conditions before attempting to restore sexual fertility, the achievement of these two situations, in most cases, proceeds simultaneously, with stabilization preceding restoration of fertility. The organism, therefore, can afford to be apomictic, reproducing its kinds vegetatively to overcome the stress of its habitat while the process of genetic stabilization and restoration of sexual fertility can come later. This may account for the inability of the two *Aloe* species to produce flowers in the first three years of cultivation as observed by Akinyele et al. (2007).

In the two taxa, the process of meiotic cell division, from leptotene to the stage of spore formation, occurs in the anthers almost normally. There is high success rate in chromosome pairing, chromosome disjunction at anaphase I and II and the distribution of chromatids to the daughter cells which later developed to form the microspores. This means that the centrioles (organelles that bear and control the spindle fibres) are functional and very effective. However, chromosome pairing in the two taxa is incomplete in almost all the seven bivalents in the complement as demonstrated in Figure 2. This lowers the frequency of chiasma formation as shown in Table 1. As contended by Mustapha (1991), the incomplete pairing amongst the bivalents and the lack of sufficient homology (suggested by the precocious separation of bivalent members) observed in the various complements are evidences that the sets of chromosomes in the complements of the two taxa are most likely to be of hybrid origin. The mere fact that all the stages of the meiotic process are encountered in each anther of the two species is an indication that the two genetic systems are not stable.

In *A. keayi*, it is observed that there is high pollen stainability but low germinability. It is also observed that the pollen tube is unable to grow and penetrate the stylar tissue. All these point to the fact that the spore nuclei are genetically deficient; a feature associated with taxa of hybrid origin (White, 1973). In *A. macrocarpa* var *major*, both pollen stainability and germinability are very high. Most of the pollen tubes are able to grow and penetrate the stylar tissue. Fruits are formed but they all abort before reaching the stage of maturity. Two things could account for the fruit abortion. It is either the endosperm, which is supposed to serve as food reserve for the embryo, is not properly formed or the chromosome aber-

ration observed in metaphase II (see the arrow in Figure 6), in which a segment of one of the longest chromosomes is deleted, may have impaired further development of the embryo to attain maturity. This is possible if the lost chromosome segment carries the genes responsible for the development of the embryo. While the inability of the endosperm to form properly is a physiological failure emanating from a genetic deficiency, chromosome aberration in which a chromosome segment is lost is in itself a genetic deficiency. Whichever is responsible for the abortion of fruits is a feature associated with taxa of hybrid origin. It is logical, therefore, to opine that both *A. keayi* and *A. macrocarpa* var *major* are most likely to be of hybrid origin.

Since the two taxa have succeeded in establishing themselves vegetatively, there is no doubt that they have overcome the danger of extinction. What is left now is the acquisition of genetic organization to restore sexual fertility. The process of achieving this will definitely take time and will involve one or more of the strategies highlighted by Sudharshan and Jagadishchandra (1981); Watanabe (1981).

Conclusion

The information obtained from the various investigations carried out in this study suggests that the sexual sterility observed in the two Nigerian species of *Aloe* is caused by genetic instability and deficiency in the genotype of the spores as a result of the hybrid origin of the taxa. As soon as genetic deficiency is ruled out and a stable genetic system is attained in each of the two species, the process of restoring sexual fertility will commence.

REFERENCES

Abercrombie M, Hickman CJ, Johnson ML (1973). The Penguin Dictionary of Biology. 6th ed. Penguin Books Ltd., England. p. 311.

Akinyele BO (1999). Biosystematic Studies in some Nigerian Representatives of *Aloe* Linn. A Ph.D Thesis of the University of Ilorin, Ilorin, Nigeria. p.195.

Akinyele BO (2002). Karyotype studies in four Nigerian species of *Aloe* Linn. App. Trop. Agric. 7: 29-33.

Akinyele BO, Odiyi AC, Taye Temikotan (2007). Reproductive ability as a guide to the selection of a Nigerian *Aloe* Linn. most suitable for cultivation on a commercial scale. Int. J. Plant Breed. Genet. 1(1): 24-28.

Anselm, A (2004). Nature Power. 3rd ed. Fr. Anselm Adodo, OSB Ewu-Esan, Nigeria. p. 288.

Dutta AC (1979). Botany for Degree Students. 5th ed. Oxford University Press, Bombay, India. p. 909.

Gardner EJ, Snustad DP (1981). Principles of Genetics. 6th ed. John Wiley and Sons, Inc. New York, p. 611.

Lewis KR (1966). The evolution of chromosome systems. Ind. J. Genet. 26: 1-11.

Moore DM (1976). Plant Cytogenetics Chapman of Hall. p. 29.

Mustapha OT (1991). Biosystematic Studies in the *Urginea indica* (Roxb). Kunth Complex. A Ph.D Thesis of the University of Ilorin, Ilorin, Nigeria. p. 258.

Oyewole SO (1984). Microporogenesis and sexual sterility in *Drimiopsis barteri.* Cytologia 49: 87-93.

Sudharshan MR, Jagadishchandra KS (1981). Unusual meiotic behavior and the formation of 2n-pollen in tetraploid *Cymbopogon caesius* (Nees) Stapf. (Poaceae). Cytologia 46: 117-123.

Watanabe K (1981). Studies on the control of diploid-like meiosis in polyploidy taxa of *Chrysanthemum* I. Hexaploid *Ch. japonense* Nakai. Cytologia 46: 459-498.

Weaver RF, Hedrick PW (1997). Genetics. 3rd ed. Wm. C. Brown Publishers, USA. p. 638.

White MJD (1973). "Chromosome rearrangement in mammalian populations, Polymorphism and speciation". In Cytotaxonomy and Vertebrate Evolution. eds. Chiarelli AP &E. Capana. Acad. Press. pp. 95-128.