# KARYOMORPHOLOGY OF THREE SUCCULENT SPECIES: *GASTERIA BATESIANA, HAWORTHIA LIMIFOLIA* VAR *LONGIANA* AND *ALOE JUCUNDA* (FAMILY ASPHODELACEAE)

**SAMEER GUNJAN LAKRA* AND KAMINI KUMAR****

*Research Scholar, Laboratory of Cytogenetics, Plant Breeding, Molecular Biology and Biotechnology, University Department of Botany, Ranchi University, Ranchi-834008, Jharkhand, India. **University Professor, Pro-V. C. (On Lien), University Department of Botany, Ranchi University, Ranchi-834008 Jharkhand, India*
*E-mail: gunjansameer5@gmail.com*
*Date of online publication: 30th June 2020*
*DOI:10.5958/2455-7218.2020.00018.2*

In the present investigation, karyomorphological details of *Gasteria batesiana* Rowley, *Haworthia limifolia* var. *longiana* Marloth and *Aloe jucunda* Reynolds has been carried out to establish a affinity among the species. The study revealed that, the species of *Gasteria* and *Aloe* are diploid with 2n=14 chromosomes whereas *Haworthia* with 2n=18 chromosomes. Among the species maximum chromatin length was observed in *Haworthia limifolia* var. *longiana* Marloth with 74µ. Along with this, chromosomes were classified on the basis of its centromeric positions with the help of arm ratios in accordance with Levan *et al*. Karyotype symmetry was carried out with the help of Stebbins (1971) classification. Various other parameters, such as total chromatin length, disparity index, gradient index, symmetry index were used to determine the advanced and primitive nature among the considered species.

**Key words:** *Aloe*, *Gasteria*, Gradient Index, *Haworthia*, Karyotype

*Gasteria batesiana* Rowley, *Haworthia limifolia* var. *longiana* Marloth and *Aloe jucunda* Reynolds are succulent that belongs to the family Asphodelaceae. They are endemic to South Africa (Walkin and Suzanne 2019, Smith and Wyk 1991) but cultivated and found in India too. They are monocots (Russell 1987) with inflorescence in bunches (Gunjan and Roy 2010). These genera come under the category of crassulacean acid metabolism (CAM) which are capable to survive in dry climatic conditions (Anderson and Beardall 1991). *Gasteria*, *Haworthia* and *Aloe* had been used medicinally for the treatment of various diseases and disorders by traditional healers and practitioners in ancient times (Dold and Cocks, 2002) and till date they are used as therapeutically. They were used in the treatment of constipation, wound healing (Jia *et al.* 2008), mental health problems (Stafford *et al.* 2007), AIDS (Wilfred *et al.* 2012) etc. The present investigation deals with the karyomorphological details of the selected species to determine the chromosome number, to establish correlation and to trace out the evolutionary tendencies among them with the help of several other parameters related to the karyotypic studies.

## MATERIALS AND METHODS

The plants were collected from Ahmedabad, Gujarat and were potted in the mixture of vermicompost and sand in the shady condition (Fig. 1 a, b and c). The freshly emerged roots of *G. batesiana* Rowley and *H. limifolia* var. *longiana* Marloth were treated with 8-hydroxyquinoline whereas the roots of *A. jucunda* Reynolds was treated with paradichlorobenzene. The roots were then fixed in 3:1 aceto-alcohol solution followed by preservation in 70% ethanol for the further studies. The preserved roots of *G. batesiana* Rowley and *H. limifolia* var. *longiana* Marloth were stained in 2% aceto-orcein solution and roots of *A. jucunda* Reynolds was stained in 2% aceto-carmine solution. For the preparation of slides, La-Cour (1941) technique and squash technique had been adopted and ten well separated metaphase plates were taken for the measurement of chromosomes. Various parameters used to obtain the karyological data

Received on April 11, 2020 www.indianbotsoc.org Accepted on May 07, 2020

of each genus was calculated to draw a statistical tour to get the final results.

To analyse the karyotype of the considered species following classification has been adopted, based on the length of chromosome. Type A: ≥ 17 µ, Type B: < 17 µ – 13.5 µ, Type C: < 13.5 µ – 11.5 µ, Type D: < 11.5 µ - 8.5 µ, Type E: < 8.5 µ – 6.5 µ, Type F: < 6.5 µ – 5.5 µ, Type G: < 5.5 µ – 4.5 µ, Type H: < 4.5 µ – 3.5 µ, Type I: < 3.5 µ – 2.5 µ, Type J: < 2.5 µ – 1.5 µ.

The karyotype formula was designed based on the centromeric positions and according to the classification of Levan *et al.* (1964) and the karyotype symmetry was determined according to the Stebbins (1971).
The chromosome form or centromeric position was expressed using the d value (difference value) by calculating the arm ratio (r) by the following formula

$$r = \frac{\text{Length of the long arm}}{\text{Length of the short arm}}$$

$$d = \frac{10\ (r-1)}{r+1}$$

Disparity Index (D.I.) was calculated according to the formula adopted by Mohanty *et al.* (1991)

$$\text{D.I.} = \frac{\text{Longest chromosome} - \text{Shortest chromosome}}{\text{Longest chromosome} + \text{Shortest chromosome}} \times 100$$

The total form percent (T.F.%), given by Huziwara (1962), was calculated to know the karyotype symmetry or asymmetry..

$$\text{TF\%} = \frac{\text{Total sum of the short arm length}}{\text{Total sum of the chromosome length}} \times 100$$

Other parameters used to determine the karyotype asymmetry was Gradient Index (G.I.), given by Levitzky (1931) and symmetry index

$$\text{G.I.} = \frac{\text{Length of the shortest chromosome of the complement}}{\text{Length of the longest chromosome of the complement}} \times 100$$

$$\text{S.I.} = \frac{\text{Total length of all short arms}}{\text{Total length of all long arms}} \times 100$$

The idiograms were prepared in decreasing order of the chromosomes, based on the above mentioned calculations

## RESULTS

The karyomorphological data are summarized in Table 2. In the present investigation *G. batesiana* Rowley and *A. jucunda* Reynolds were recorded with 2n=14 chromosomes (Table 2; Fig. 1: d and f) whereas *H. limifolia* var. *longiana* Marloth was recorded with 2n=18 chromosomes (Walker and Suzanne, 2019; Votteiero and Buiza 2015, Ahirwar and Verma 2014, Fentaw *et al.* 2013, Zheng *et al.* 2005, Zooneveld and Jaarsveld 2005, Brandham and Doherty 1998, Sato 1937, Vosa and Bennett 1990) (Table 2; Fig. 1: e). The karyotype symmetry of the species was deduced in accordance with the Stebbins (1971) Table 1 based on the difference between longest and shortest chromosome of the complement which was denoted by symbol B and C along with a numerical prefix. The maximum chromatin length was recorded in *H. limifolia* var. *longiana* Marloth with 74 µ whereas minimum total chromatin length was 47.9 µ recorded in *G. batesiana* Rowley Table 2. In *G. batesiana* Rowley, the chromosomes were classified as 6 submedian (Type D, E, G, H and J) and 1 subterminal (Type E) whereas in *H. limifolia* var. *longiana* Marloth and *A. jucunda* Reynolds chromosomes were 2 median (Type D and G) + 7 submedian (Type A, C, D, I and J) and 1 median (Type D) + 6 submedian (Type C, D, E, G, I and J) respectively Table-2. In *H. limifolia* var. *longiana* Marloth, T.F% was 35.67, S.I. was 55.46 and D.I. was 80.85 which is maximum among all the three considered species while minimum values were observed in *H. limifolia* var. *longiana* Marloth with T.F%=30.06, S.I.=42.98 and D.I=65.22. G.I. was observed maximum in *G. batesiana* Rowley and minimum in *H. limifolia* var. *longiana* Marloth Table 2 and Table 3.There was a variation in the

**Table 1:** The classification of karyotype symmetry based according to Stebbins (1971).

| **Ratio Longest/Smallest** | **Proportion of chromosomes with arm ratio > 2:1** | | | |
|---|---|---|---|---|
| | 1.00 (1) | 0.99-0.51 (2) | 0.50-0.01 (3) | 0.00 (4) |
| **< 2:1 (A)** | 1A | 2A | 3A | 4A |
| **2:1 – 4:1 (B)** | 1B | 2B | 3B | 4B |
| **> 4:1 (C)** | 1C | 2C | 3C | 4C |

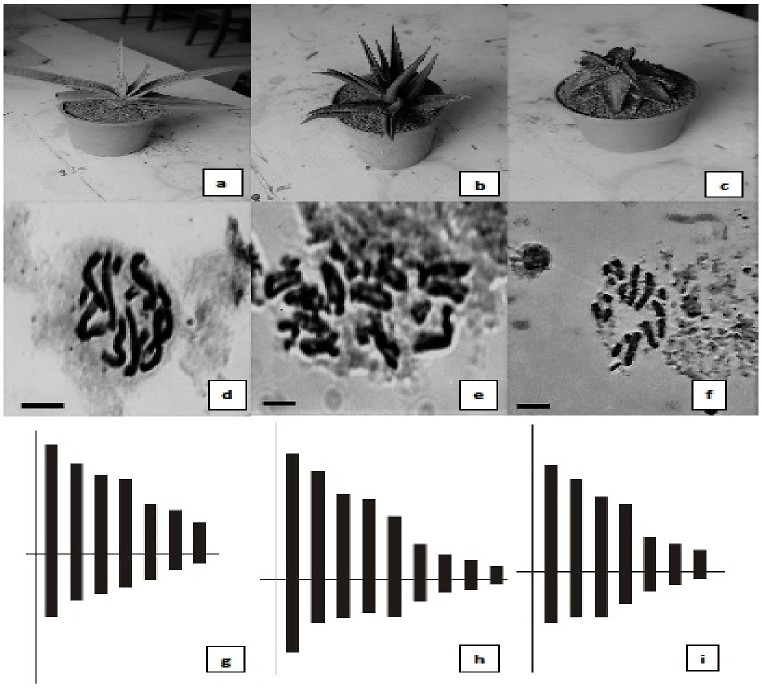


**Figure 1: a**: Photograph of *Gasteria batesiana* Rowley, **b**: Photograph of *Haworthia limifolia* var. *longiana* Marloth, **c**: Photograph of *Aloe jucunda* Reynolds, **d**: Photomicrograph of mitotic metaphase of *Gasteria batesiana* Rowley, **e**: Photomicrograph of mitotic metaphase of *Haworthia limifolia* var. *longiana* Marloth, **f**: Photomicrograph of mitotic metaphase of *Aloe jucunda* Reynolds, **g**: Idiogram of *Gasteria batesiana* Rowley, **h**: Idiogram of *Haworthia limifolia* var. *longiana* Marloth, **i**: Idiogram of *Aloe jucunda* Reynolds.

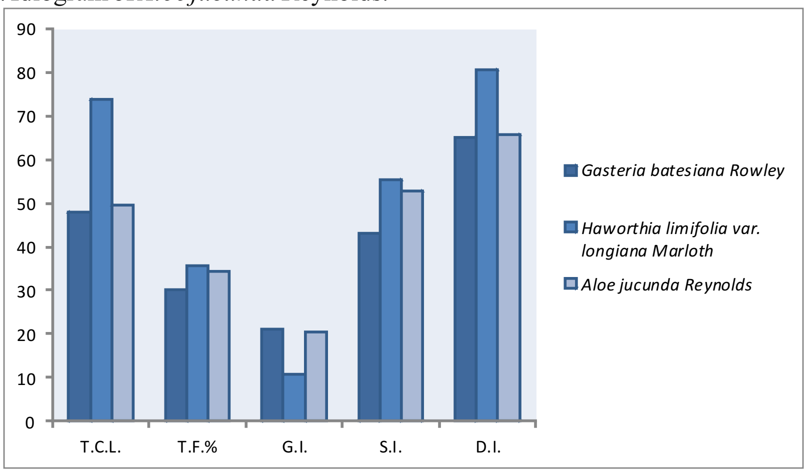


**Figure 2:** Column graph showing comparative total chromatin length (T.C.L.), total form % (T.F.%), gradient index (G.I.), symmetry index (S.I.), disparity index (D.I.) of Gasteria batesiana Rowley, Haworthia limifolia var. longiana Marloth and Aloe jucunda Reynolds.

**Table 2:** Karyological data of *Gasteria batesiana* Rowley, *Haworthia limifolia* var. *longiana* Marloth and *Aloe jucunda* Reynolds.

| Sl No. | Species | 2n no. | Chromosom length Shortest:Longest | Karyotyp symmetr | Disparit Index | T.C.L of haploid set (in µ) | Karyotype formula |
|---|---|---|---|---|---|---|---|
| 1 | *Gasteria batesiana* Rowley | 14 | 1:4.75 | 4C | 65.22 | 47.9 | 2smD+1smE+1stE+1smG+1sm |
| 2 | *Haworthia limifolia* var. *longiana* Marloth | 18 | 1:9.44 | 3C | 80.85 | 74 | 1smA+1smC+2smD+1mD+ 1mG+2smI+1smJ |
| 3 | *Aloe jucunda* Reynolds | 14 | 1:4.85 | 3C | 65.84 | 49.51 | 1smC+1smD+1mD+1smE+ 1smG+1smI+1smJ |

**Note:**
T.C.L = Total chromatin length
Chromosome form based on d value; m = median (0.0 - 2.5), sm = submedian (2.5 - 5.0), and st = subterminal (5.0 - 7.5).

**Table 3:** Data related to karyotype of *Gasteria batesiana* Rowley, *Haworthia limifolia* var. *longiana* Marloth and *Aloe jucunda* Reynolds.

| Species | Chromosome range (µ) | Total Form % | Gradient Index | Symmetry Index |
|---|---|---|---|---|
| ***Gasteria batesiana* Rowley** | 2.4-11.4 | 30.06 | 21.05 | 42.98 |
| ***Haworthia limifolia* var. *longiana* Marloth** | 1.8–17 | 35.67 | 10.59 | 55.46 |
| ***Aloe jucunda* Reynolds** | 2.42–11.75 | 34.48 | 20.59 | 52.91 |

chromosome size of each species. In *Gasteria batesiana* Rowley the size of the chromosome complement ranged from 2.4 µ to 11.4 µ, in *Haworthia limifolia* var. *longiana* Marloth the range was 1.8 µ to 17 µ and in *Aloe jucunda* Reynolds it was 2.42 µ to 11.75 µ. Maximum variation in the chromosome complement variation was observed in *Haworthia limifolia* var. *longiana* Marloth Table 3.

Idiograms were constructed with the help of long arm and short arm value of each chromosome and is depicted in Fig. 1: g, h and i. Comparative data of T.C.L, D.I, G.I and S.I were shown graphically in Fig. 2.

## DISCUSSION AND CONCLUSION

An attempt has been made to find a relative affinity of the considered three taxa based on the karyoevolutionary parameters which includes chromosome number, total chromatin length, karyotype symmetry, karyotype formula, gradient index, symmetry index and disparity index. Based on the above information the karyotype studies revealed that the plants were diploid with 2n=14 in *Gasteria batesiana* Rowley and *Aloe jucunda* Reynolds but variation was recorded in the chromosome count of *H. limifolia* var. *longiana* Marloth with 2n=18 chromosomes Table 2. The results regarding the chromosome number of the considered plant species were earlier confirmed. The maximum total chromatin length, 74 µ, was observed in *H. limifolia* var. *longiana* Marloth whereas minimum total chromatin length was 47.9 µ in *G. batesiana* Rowley. According to Babcock and Cameron (1943) and Sinha and Kumar (1979) plants with least DNA content are responsible for evolutionary process and considered as most advanced. Reduction in the chromosome content may occur due to deletion of chromosome segments during the process of evolution. On the basis of karyotype asymmetry table given by Stebbins (1971) *G. batesiana* Rowley showed the highest asymmetry since it showed the 4C class of karyotype asymmetry. *H. limifolia* var. *longiana* Marloth and *A. jucunda* Reynolds was observed with 3C class of karyotype asymmetry and it may be said that it might have preceded the 4C class of *G. batesiana* Rowley. Stebbins considered the asymmetrical karyotyope as the advanced one. Based on karyotype formula, Stebbins (1950) also showed the asymmetrical nature with the presence of maximum number of submedian chromosomes in the species. *G. batesiana* Rowley was recorded with maximum number of submedian chromosomes along with a subtelocentric chromosome which reflect its asymmetrical and advanced nature whereas *H. limifolia* var. *longiana* Marloth and *A. jucunda* Reynolds was recorded with median and submedian chromosomes which makes it primitive when compared to the other two species. Subtelocentric chromosomes may have arisen due to deletion and deficiency in one arm of the chromosome which results in the displacement of centromeric position. This result was also confirmed by the other karyological parameters which were used and revealed that the maximum values of T.F% and S.I. supports the primitive nature of *H. limifolia* var. *longiana* Marloth, according to Huziwara, 1962. Asymmetrical nature was also confirmed by the value of gradient index. If G.I. value is less than 30 or around 30 then this indicates the asymmetrical nature of chromosomes. The value of D.I. shows the homogeneity in the species when it is low and heterogeneity when the value is quite high (Lavania and Srivastava, 1991). The species were recorded with high value of D.I. ranging from 65 to 80 and maximum was recorded in *H. limifolia* var. *longiana* Marloth this may show the higher level of karyotypic specialization. Based on idiograms [Fig. 1: g, h and i], asymmetrical nature confirms the progressive steps in evolution. This was too confirmed by the shifting of centromeric positions from median to nearly median and nearly submedian, according to Stebbins, 1971. Based on the above information, it may be concluded that there is a relative affinity among the selected species and suggested that *G. batesiana* Rowley shows the tendency towards advancement.

We express our sincere gratitude to Prof. Jyoti Kumar, Dean, Science faculty and Head, University Department of Botany, Ranchi University, Ranchi for providing the laboratory facilities.

## REFERENCES

Ahirwar R and Verma R C 2014 Karyotypic studies in some members of Liliaceae. *J Cytol Genet* **15** 61-74.

Anderson J W and Beardall J 1991 *Molecular activities of plant cells An introduction to plant biochemistry.* Blackwell Scientific Publication Oxford Pp xv +384.

Babcock E B and Cameron D R 1934 Chromosome and phylogeny in *Crepis* II: The relationship of 108 species. *Univ. Calif. Publ. Agr. Sci.* **6 287-324**

Brandham P E and Doherty M J 1998 Genome size variation in the Aloaceae, an angiosperm family displaying karyotypic orthoselection. *Annals of Botany* **82** 67-73

Dold AP and Cocks ML 2002 The trade in medicinal plants in the Eastern Cape Province, South Africa. *South African Journal of Sciences* **98** 598-597.

Fentaw E, Dagne K, Ronsted N, Demissew S and Grace OM 2013 Karyotypes in Ethiopian *Aloe* species (Xanthorrhoeaceae: Asphodelaceae). *Kew Bulletin* **68** 1-9.

Gunjan K and Roy BK 2010 Karyotype studies in dominant species of *Aloe* from eastern India. *Caryologia* **63(1)** 41-49.

Huziwara Y 1962 Karyotype analysis in some genera of Compositae VIII: Further studies on the chromosome of Aster. *American Journal of Botany* **49** 116-119.

Jia Y, Zhao G and Jia J 2008 Preliminary evaluation: The effects of *Aloe ferox* Miller and *Aloe arborescens* Miller on wound healing. *J Ethnopharmacol* **120** 181-189.

Lavania U C and Srivastava S 1992 A simple parameter of dispersion index that serve as an adjunct to karyotype asymmetry. *J. Biosci.* **17(2)** 179-182.

Levan A, Fredga K and Sandberg A A 1964 Nomenclature for centromeric position on chromosomes. *Hereditas* **52** 201-220

Levitzky G A 1931 The karyotype in systematics. *Bulletin of Applied Botany, Genetics and Plant Breeding* **27** 19-174.

Mohanty B D, Ghosh P D and Maity S 1991 Chromosomal analysis in cultured cells of barley (*Hordeum vulgare* L.) : Structural analysis in chromosomes. *Cytologia* **56** 191-197.

Russell G E G 1987 Preliminary floristic analysis of the major biomes in southern Africa. *Bothalia* **17(2)** 213-227.

Sato D 1937 Karyotype alteration and phylogeny I Analysis of karyotypes in Aloinae with special reference to the SAT-chromosome. *Cytologia* **6** 80-95.

Sinha S S N and Kumar P 1979 Mitotic analysis of thirteen varieties of *Cajanus cajan* (L.) Mill. *Cytologia* **44** 571-580.

Smith G F and Wyk B-EV 1991 Generic relationships in the Alooideae (Asphodelaceae). *International Association for Plant Taxonomy* **40(4)** 557-581.

Stafford G I, Pederson P D, Jager A K and Staden J V 2007 Monoamine oxidase inhibition by southern African traditional medicinal plants. *South African Journal of Botany* **73(3)** 384-390

Stebbins G L 1950 *Variation and Evolution in Plants*. Columbia Univ. Press New York.

Stebbins G L 1971 *Chromosomal Evolution in Higher Plants*. Edward Arnold Publishers Ltd. London.

Vosa C G and Bennett S T 1990 Chromosome studies in the Southern African Flora 58-94 Chromosome evolution in the genus *Gasteria* Duval. *Caryologia* **43** 235-247.

Votteiero P V and Buiza J I 2015 Karyotype evolution of seven species of *Aloe* L. (Xanthorrhoeaceae). *Acta Botanica Venezuelica* **38(1)** 1-18.

Walker C C and Mace S 2019 *Aloe erenii, Aloe jucunda* and a new cultivar. *Cactus World* **37(1)** 13-19.

Wilfred M O, Grierson D S and Ndip R N 2012 Phytochemical studies and antioxidant activity of two South African medicinal plants traditionally used for the management of opportunistic fungal infections in HIV/AIDS patients. *BMC Complementary and Alternative Medicines* **12** 43-49.

Zheng M, Sheng Y X, Yong L, Hong W U and Zhang SZ 2005 karyotype analysis of fourteen species and two varieties in *Aloe* L. *Journal of Wuhan Botanical Research* **23(6)** 535-540.

Zonneveld B J M and Jaarsveld E J V 2005 Taxonomic implication of genome size for all species of the genus *Gasteria* Duval (Aloaceae). *Plant Syst. Evol.* **251** 217-227.