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Karyotypic Variation of the *Aloe vera* L. and *Aloe littoralis* Baker in Iran

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Background: We describe karyotypic variations and the in vitro methods for plant propagation and conservation as well as detailed chromosomal analysis of (*Aloe vera* L.) and *Aloe littoralis* from Iran during flowering stage.
Objectives: This karyotypic was discovered because of a difference in the position of the chromosome and due to genomic differentiation in domesticated populations of the Iranian species. We show that highly conserved ortho selected karyotype in (*Aloe vera* L.) both in in vivo and in vitro grown cultivars.
Materials and Methods: Cytological investigation of *Aloe littoralis* in vitro grown plants revealed its tetraploid nature. Chromosome behaviors in miosis cell division were studied in one hundred cells and the populations and the parameters such as number and type of formed chiasmas were recorded.
Results: Despite the large size of the chromosomes, the most portions of the observed chiasmata were one chiasma per pairs of homologes. A population of *Aloe littoralis* showed high level of four chiasmata. The percentage of pollen viability is high in both (*Aloe vera* L.) and *Aloe littoralis,* yet the flowers failed to form fruits.
Conclusions: This finding, in combination with regional differences in the frequency of the karyotype, has important values for future studies using *Aloe* spices.

Keywords: Aloe Littoralis; Chiasma; Karyotypic Variation

1. Background

Aloe vera L. belongs to *Liliaceae* family and the medicinal use of this plants goes back to several thousand years. The applications of this plant have been recorded in ancient cultures of India, Egypt, Greece, Rome and China. *Aloe vera* L. in Egypt and china cultures called as the plant of immortality and elixir of youth, respectively. The *Aloe vera* L. used as burn plant and medicine plant many years ago. The name of *Aloe vera* L. derived from the Arabic term "Alloeh" meaning shining bitter substance. *Aloe Barbadensis mill*, *Aloe chinensis bak*, *Aloe elongate murray*, *Aloe indica royale*, *Aloe officinalis forsk*, *Aloe perfoliata*, *Aloe rubescens dc*, *Aloe vera* L. *var. Littoralis konig ex bak*, *Aloe vera* L. *var. Chinensis berger*, *Aloe Vulgaris lam* are the other names of *Aloe vera* L. in the literatures. Aloe *barbadensis mill* is synonym with *Aloe vera* (L.) *Burm f.* However, *Aloe vera* (L.) *Burm f.* is the legitimate name for this specie in the International Codes of Botanical Nomenclature. According to Adams et al. (1) *Aloe* has also been placed taxonomically in a family called *Aloeaceae*. The *Aloe* is originates from tropical Africa, warm climatic zones of Asia, Europe and America (2). Recently, because of herbal movement initiated by naturopaths, yog gurus, alternative medicine promoters and holistic healers the usage of *Aloe vera* L. is common. Scientific investigations on *Aloe vera* L., believed that this plant is effective in treatment of stomach ailments, gastrointestinal problems, skin diseases, constipation, radiation injury (2, 3), inflammations (3), wound healing (4) and burns, ulcers (2, 3), diabetes (3, 4) and cancers (5). The gel consists of water, amino acids, vitamins, lipids, sterols, tannins, and enzymes (2). The global market for value-added products of *Aloe vera* L. is highly booming (4). Thus commercial application of plant genetic engineering and biotechnology can be of great values. The present study details the collection of *Aloe vera* L.) and *Aloe littoralis* from the arid zone of Iran, micropropagation, its adaptation, conservation and its karyotype analysis. Almost the two morphologically indistinguishable cultivars are phenotypically identified based on the differences in flower color, aloin content ,and degree of bitterness. Previous karyological data have been used to evaluate the numerical changes in chromosomes of *Aloe* species. The concept of symmetry vs. asym-

Implication for health policy / practice / research / medical education:
For plant physiologist.

metry has been proposed on the basis of predominance of metacentric and submetacentric chromosomes which are approximately same sizes.

Whereas, increased asymmetry formation, has been related to the shift of centromeric position from median / submedian to terminal / subterminal or differences in the relative size between the chromosomes of the complement, that makes the karyotype more heterogeneous. Since long, classification of Stebbins (6) has been frequently used as qualitative method for assessing karyotype asymmetry and describing the typical relationships between different taxa, which recently reported by Seijo and Fernandez et al. (7) in Lathyrus and He et al. (8) between Davidia involucrata and Camptotheca acuminata. Recently, cytogenetical studies have been used as taxonomic information besides biochemical, molecular, morphological and anatomical studies. Earlier studies suggested that Aloe has great potential of chromosomal stability due to the presence of bimodal constant karyotype (9-11). Determination of the chromosomal alterations and DNA variation have been proposed, usinf karyotype analysis, (12) and these variants have been used to evaluate the plant species (13). Very few studies of Aloe were conducted on the karyotypic variations.

Aloe has an unusual stable karyotype with four large and three small chromosomes in the basic, haploid karyotype. The highest karyotypic orthoselection level occurs in the *Aloaceae*, which has the same bimodal karyotype of all species, (x = 7); four long and three short acrocentrics (9).

2. Objectives

This investigation was undertaken to prepare a comparative chromosomal database and to evaluate the karyotypic orthoselection level within these two cultivars, as well as in polyploid state.

3. Materials and Methods

3.1. Plant Material

The plant materials selected for the study were collected from the germplasm field of the National Institute of Genetic Engineering and Biotechnology (NIGEB), Tehran, Iran and established in the experimental garden of (NIGEB), Tehran. During the flowering stage both cultivars *Aloe Vera* L. and *Aloe littoralis* L. could be distinguished (Figure 1).

3.2. Micropropagation

For *in vitro* propagation, 35.5 μM BAP and 9.8 μM IBA in combination with 81.4 μM adenine sulphate used to produce the optimum concentration for shoot bud induction in both the cultivars. A single explant (shoot apical meristem) was initially induced 6 shoot buds within 3 – 4 weeks.

The best treatment for highest shoot number and bud proliferation was MS medium containing 8.87 μM BAP and 2.46 μM IBA. Maximum numbers of proliferated shoot buds (22 nos.) from a single explant were obtained in MS medium after 1 – 2 weeks of the first subculture. Both of the cultivars were adventitious root induced in MS medium containing 2.45 μM IBA. In vitro hardening achieved in high humidity and kept under 22 ± 2°C, 1600 lux and photoperiods of 16 / 8 hours for 10 – 12 days and then these plants were moved to green house in larger pots and kept under less humid condition and sunlight intermittently (Figure 1).

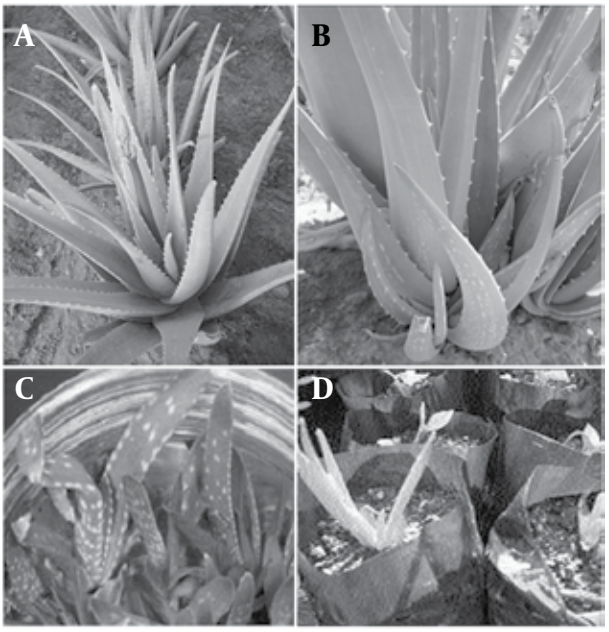

Figure 1. *In vivo* and *in vitro* grown plants of two *Aloe* species. A and B), in vivo, C and D), *in vitro* grown plants of (*Aloe Vera* L.) and *Aloe littoralis* Baker respectively.

3.3. Mitotic Study

Fresh young roots were used from both (*Aloe vera* L.) and *Aloe litoralis* L. and pretreated with 0.002 M 8-hydroxyquinoline for 3 hours at 10 – 12 °C and fixed in acetic-ethanol (3:1) overnight at 10 – 12 °C and then the root tips were treated with 45 % acetic acid for 25 minutes. and hydrolyzed for 5 minutes. in 1N HCl at ambient temperature used by staining with 2 % aceto-orcein (14). Karyotypes were described following the classification and nomenclaturing by Levan et al. (15).

3.4. Meiotic and Pollen Mitotic Study

Plants of both cultivars flowered in late October – November. For meiotic and pollen mitotic studies, 7 – 12 mm long suitable flower buds were collected in the morning from young inflorescences from both cultivars. Buds were fixed in Carnoy's solution at 10 – 12 °C for 24 hours. prepared bud smears for meiotic and pollen mitotic

study was made in 2 % carmine on a clean glass slide (14). Staining ability of fresh pollen samples was determined by acetocarmine technique (16). The deeply stained pollen grains were counted as viable, while weakly stained were recorded as non viable (12). Viable and non-viable pollen grains were examined under light microscope. The percentage of viable, non-viable pollens and number of chiasmata were then determined.

3.5. Detailed Karyomorphological Study

Chromosome identification is indispensable in cytological study. Karyomorphological data in the present study are established using parameters, like chromosome length, co-efficient of variation (17), number of satellites and mean centromeric index value or TF %. Zeiss photomicroscope Progress Capture R C3 software used for observations and photography. Camera lucida, and digital calipers used for karyotype and meiotic analysis. The morphology of chromosomes including the total arm length of each chromosome (long arm length + short arm length) and relationship of the arms (RB = long arm length / short arm length) and arm ratio (AR = short arm length / long arm length) were analyzed. Arm ratio, widely utilized for the classification of chromosome types, (15) has been considered empirically to be a more stable parameter of the chromosomal morphology.

4. Results

4.1. Comparative Diploid Karyotype Analysis of *in vivo and in vitro Grown Plants of (Aloe Vera* L.) *and Aloe littoralis*

According to analysis of numerical data the karyotypes of the (*Aloe vera* L.), although very similar ones, can be distinguished, mainly based on mean chromosome length, type and position of satellites and karyotype symmetry.

4.2. Karyotype of Aloe Vera L.

Mother plant and tissue culture raised plants revealed chromosomal analysis of diploid cells with 2n = 14 and bimodal chromosomes (Figure 2). Parameters of chromosomes are indicated in Table 1. both of in vivo and in vitro plants have the same karyotype formula (6Sm + 8St) (Table 1). Four pairs of the long chromosomes size ranged from 11.8 to 17.3 μm and and three pairs of short chromosomes length were 2.9 to 5.9 μm. Long chromosomes were subtelocentric and short chromosomes were submetacentric. Chromosomes with secondary constrictions could not be found in this cultivar (Figure 2).

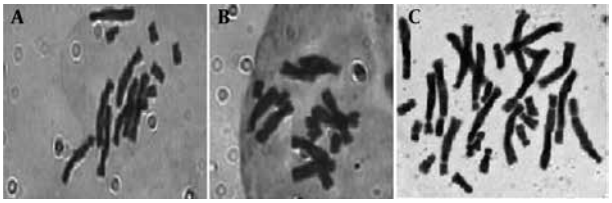

Figure 2. A) Somatic chromosomes (Somatic metaphase of (*Aloe Vera* L.), B) *Aloe littoralis* and C) *Aloe littoralis* tetraploied, respectively.

Table 1. Karyomorphological study of (*Aloe Vera* L.) and *Aloe littoralis Baker*

Name [a]	2n	PL	KF	CP	RLCL	RSCL	TCL	TF	VC
Aloe vera in vivo	14	2x	6Sm + 8St	-	11.8 – 15.1	2.9 – 4.0	61.0	24.83	30.22
Aloe vera in vitro	14	2x	6Sm + 8St	-	13.1 – 17.3	5.0 – 5.9	79.0	20.64	32.49
Aloe littoralis in vivo	14	2x	6sm + 6St + 2St (SAT)	1	19.0 – 21.1	5.0 – 6.4	98.2	19.98	19.13
Aloe littoralis in vitro	14	2x	6sm + 6St + 2St (SAT)	1	13.8 – 18.2	4.1 – 5.0	76.0	20.92	20.87
Aloe littoralis tetraploid	28	4x	12Sm + 16St	2	13.0 – 18.4	4.7 – 5.7	153.5	22.67	44.12

[a] Abbreviations: PL: Ploidy level; KF: Karyotype formula; CP: Chromosome pairs with secondary constriction; RLCL: Range of long chromosome length in μm; RSCL: Range of short chromosome length in μm; TCL: Total chromatin length in μm; VC: Variation coefficient in percentage; TF: Total forma percentage

4.3. Karyotype of Aloe littoralis

The plants of *Aloe littoralis* also showed diploid cells having 2n = 14 chromosomes with bimodal karyotype (Figure 3). Four pairs of long chromosomes are subtelocentric and three pairs of short chromosomes are submetacentric. Table 1 shows the karyomorphological data of chromosomes of this cultivar. It is clear from Table 1 that both plants have a similar karyotype formula 2St (SAT) + 6St + 6Sm. Four pairs of long chromosomes size ranged from 13.8 to 21.1 μm and and three pairs of chromosomes length ranging from 4.1 to 6.4 μm. In this cultivar, one pair of long chromosome with secondary constriction at long arm of the chromosome was found (Figure 3).

This is for the first time that one tetraploid tissue culture raised plant of *Aloe littoralis* is report. Tetraploid level (4X) of the plant was determined by chromosome counting using mitotic examinations (Figure 4). Cytological analysis was carried out on root tip cells showing 4X = 28 chromosomes with bimodal karyotype (Figure 4). Karyotype formula is represented by 12Sm + 16St as shown from

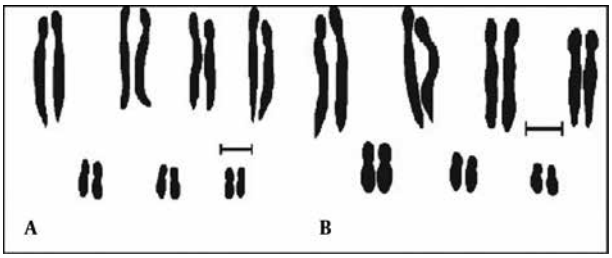

Figure 3. A) Karyotype of *in vitro* grown (*Aloe Vera* L.) and B) *Aloe littoralis*. L Scale Bar = 10 μm

data (Table 1). Four pairs of long chromosomes are acrocentric and their size ranges from 13.0 to 18.4 μm. Three pairs of short chromosomes are submetacentric and their size ranges from 4.7 to 5.7 μm (Figure 4) and (Table 2). A clear difference was found between the gross sizes of the diploid and tetraploid plants.

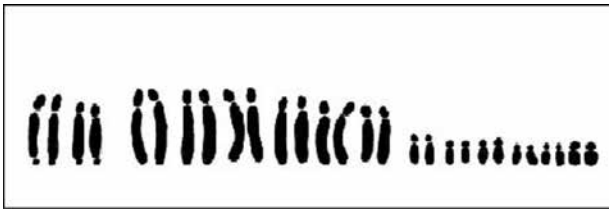

Figure 4. Karyotype of *in vitro* grown tetraploied plant of *Aloe littoralis* (4n = 28). Scale Bar = 10 μm

Table 2. Chromosome Behavior in Meiotic in Aloe Populations

Population	1 chiasma	2 chiasma	3 chiasma	4 chiasma
Aloe vera in vivo	307	257	130	5
Aloe vera in vitro	288	208	194	8
Aloe littoralis in vivo	247	172	213	11
Aloe littoralis in vitro	221	154	187	15
Aloe littoralis tetraploied	331	193	162	66

4.4. Analysis of Meiotic Chromosomes in vivo Plants of (Aloe vera L.) and Aloe littoralis Baker

Both of cultivars for meiotic chromosome analysis had a consistent haploid chromosome number of n = 7 in meiotic metaphase. Different stages of meiosis including metaphase I and II, anaphase I and II and telophase were observed. Predominant bivalent (II) pairing was found in pollen mother cells with basic number x = 7. The pollen viability ranged from 96.5 % and 95.1 % in *Aloe littoralis* and (*Aloe Vera* L.) respectively.

5. Discussion

Due to the uniformity in basic chromosome number and morphology *Aloaceae* is the most stable angiosperm families for karyotypic orthoselection. For example African family *of Aloaceae* had the highest degree of karyotypic orthoselection in the genus *Aloe*. In vivo grown and tissue culture raised plants of (*Aloe Vera* L.) had diploid karyotype data and indicated the chromosomal homogeneity showing consistent bimodal type (11). This is also same in haploid and tetraploid karyotype. The karyotypes of all haploid, diploid and tetraploid plants showed general intra- and inter- chromosome symmetry. The data also reflected the preservation of characteristic gross chromosomal morphology in both cultivars. Evidently, the chromosome number is deep-seated in this vegetatively reproducible species (14). Therefore, the karyotypic orthoselection is reconciled in this study. A marked difference in gross chromatin length has been observed amongst the cultivars of (*Aloe Vera* L.) in this study. One pair of the long chromosome has a secondary constriction on the long arm. The *littoralis* variety has the higher total chromatin length than the (*Aloe Vera* L.). For characterization the cultivars these karyomorphological differences are helpful. The product of complete endomitotic duplications during culturing tetraploid plant, is produce and the autotetraploid nature observed. In *Aloe* genus tetraploidy is quite rare (11, 18). Most species of *Aloe* are diploid (2n = 14) although a very few are tetraploid including *A. Cremnophila* and *A. Inermis* (2n = 28) reported from natural populations of Somalia (9). Occurrence of triploidy in *Aloe* has also been reported. Few specimens of *A. Jucunda* from Somalia showed 2n = 21 bimodal chromosomes. Till today, only one naturally growing autotriploid plant of (*Aloe Vera* L.) (2n = 21) has been reported from regions near Cape Comorin Southern parts of the India (1). This study is the first report of tetraploid *A. littoralis* plant developed under in vitro condition in Iran. Polyploidy is defined with a reduction in chromosome length (6). Karyotype this autotetraploid plant has smaller chromosomes and more symmetry compared with diploid ones. Due to the adaptive corrective mechanism elimination of excessive genetic material represented by the duplicated genes and repetitive DNA sequences polyploidy was occurred (19).

Seed settings were not effective in pollen viability of both (*Aloe vera* L.) and *Aloe littoralis* and reflecting the

meiotic regularity. The pollen viability and coefficient of variation percentage were significantly high in both cultivars (Table 1). To answer the karyomorphological differences amongst the cultivars further detailed study on molecular cytogenetics is recommended.

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Authors' Contribution

The entire manuscript was prepared by the Authors.

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