

## Taxonomic Relationships Between Some Species of *Dracaena* and Their Related Taxa

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In the present study, 350 macro-micro/morphological (222) and macro-micro/molecular (128) attributes were investigated in 16 species belonging to six genera viz. *Dracaena*, *Agave*, *Cordyline*, *Ruscus*, *Sansevieria* and *Yucca*. UPGMA clustering method was established by using the program NTSYS-pc 2.2. The morphological and anatomical characters as well as the produced bands profile by application each of the isozymes and PCR-ISSR techniques were species-specific; useful in delimitation of almost all the studied taxa. A reassessment of the taxonomic relationships was made by comparing the produced classification with the current systems.

**Key words:** *Dracaena* - PCR-ISSR - Isozymes - Cluster analysis - Molecular - UPGMA - Taxonomy.

### Introduction

The genus *Dracaena* L. comprises about 60 species, mainly found in tropical and subtropical Africa (Mabberley, 2008). The center of its diversity is in West Africa, where at least 23 species occur in the Guinea-Congo region (Bos, 1998). *Dracaena* and its related genera especially *Agave* (300 species), *Cordyline* (15 species), *Ruscus* (6 species), *Sansevieria* (60 species) and *Yucca* (40 species) possess common features of

morphology, inflorescence and grow in similar environments (Heywood, 1978). The relationships among the species of *Dracaena* and the related genera have always been a material of much confusion mainly because of the different approaches to species delimitation, resulted in overlapping of many taxa (Dalhgren *et al.*, 1985 & 1989, Sparg *et al.*, 2004 and Takhtajan, 2009). These were all placed in the family Liliaceae *sensu lato* (Bessey, 1915; Hallier, 1923; Pulle, 1938 and Englar & Diels, 1936). Sometimes, they were segregated in six families i.e. Agavaceae, Amaryllidaceae, Dracaenaceae, Laximanniaceae, Liliaceae *sensu stricto* and Rusceae (Dalhgren *et al.*, 1985 & 1989; APG II, 2003 and Takhtajan, 2009). Ambiguously, they are again regrouped but in another family i.e. Asparagaceae *sensu lato* based on the molecular criteria in spite of some clades are not yet completely resolved (APG III, 2009). On the other hand, inconsistency between gross morphology and molecular data at the infrageneric level is reported in *Dracaena* (Buerki *et al.*, 2009).

Many studies were performed on the taxonomic relationships of the genus *Dracaena* and their related taxa by using morphological, anatomical and molecular approaches. However, no combination of more than one line of taxonomic evidence with the molecular criteria was achieved. Thus, the present study consider data from each of the macro (i.e. gross morphology) and micro-morphology; macro-molecular or biochemical markers (i.e. Isozymes) alongside of micro-molecular markers (i.e. Inter-Simple Sequence Repeat; ISSR) in representative species of *Dracaena* and its related genera *viz.* *Agave*, *Cordyline*, *Ruscus*, *Sansevieria* and *Yucca* in order to: (1) test the consistency between their macro & micro-morphology and molecular characters, (2) shed more light on their leaf epidermal characteristics (LM & SEM), (3) examine diversity of their molecular criteria, and (4) reassessment their taxonomic treatments using numerical approaches.

### **Material and Methods**

The present study comprised 16 taxa grown in Egypt, supplied by Egyptian Botanical Gardens, indicated in Table (1). Identification and nomenclature of the wild Egyptian species (*Dracaena ombet*) follows Täckholm (1974) and Boulos (2005), whereas those of the cultivated species follow Bailey (1949) and Bailey & Bailey (1976). Voucher specimens were kept at (CAIA) Herbarium at Botany Department, Faculty of Science, Ain Shams University, Cairo, Egypt.

Table 1. The studied taxa and their sources

No.	Taxa	Source
1	<i>Agave americana</i> L.	BGA
2	<i>A. franzosinii</i> Baker	BGA
3	<i>A. sisalana</i> Perrine	BGA
4	<i>Cordyline fruticosa</i> (L.) A. Chev. Kunth	BGA
5	<i>C. stricta</i> (Sims) Endl.	BGA
6	<i>Dracaena draco</i> (L.) L.	ZBG
7	<i>D. marginata</i> Lam.	BGA
8	<i>D. fragrans</i> (L.) Ker Gawl.	BGA
9	<i>D. ombet</i> Kotschy & Peyr.	OBG
10	<i>D. reflexa</i> Lam.	BGA
11	<i>D. sanderiana</i> hort. Sander ex Mast.	BGA
12	<i>D. surculosa</i> Lindl.	BGA
13	<i>Ruscus aculeatus</i> L.	BGA
14	<i>Sansevieria trifasciata</i> Prain var. <i>laurentii</i> (De Wild.) N. E. Br.	BGA
15	<i>Yucca aloifolia</i> L.	BGA
16	<i>Y. guatemalensis</i> Baker	BGA

BGA: Botanical Garden, Faculty of Science, Ain Shams University, Cairo, Egypt. OBG: Orman Botanical Garden, Ministry of Agriculture, Giza, Egypt. ZBG: Zohria Botanical Garden, Ministry of Agriculture, Gezzera, Cairo, Egypt.

Morphological description of the whole plant was made from the investigated living specimens or compiled from literature. Stem parts were collected and a portion of the middle lamina; including the midrib was cut from the mid. Cuttings were fixed in FAA and stored in 70% ethanol until use. Stems and lamina sections were prepared using hand microtome at 10-20µm; double stained using safranin and light green; mounted in Canda Balsam (Johansen, 1940); inspected by light microscope; photographed using a Reichert Microstar IV microscope. Ab and adaxial epidermal surfaces were carefully separated, stained in safranin, mounted in glycerin on a slide for LM investigation. For SEM sample preparations (abaxial surface), fresh small pieces (7 mm<sup>2</sup>) of the leaf material were fixed on SEM stubs with double-sided tape, coated with gold in SPI-Module sputter coater,

examined and photographed by Joel JSM 5200 scanning microscope at various magnifications.

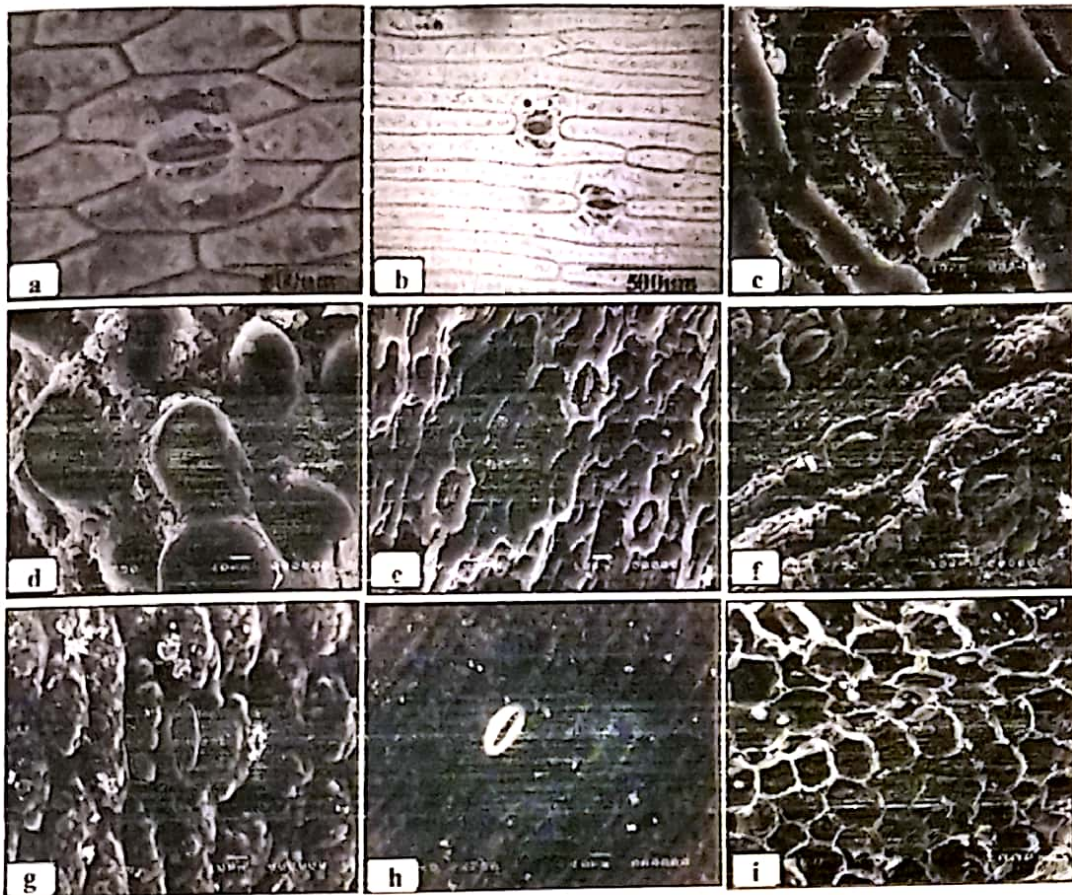
Native-polyacrylamide gel electrophoresis (Native-PAGE) was used to identify isozyme variation among the studied taxa using five isozyme systems; namely  $\alpha$ - and  $\beta$ -esterase ( $\alpha$ - and  $\beta$ - *Est*), acid phosphatase (*Acph*), alcohol dehydrogenase (*Adh*) and aldehyde oxidase (*Alo*) in fresh and young leaf samples as described by Stegemann *et al.* (1985).

Genomic DNA extraction was performed as suggested by DNA extraction kit's manufacturer Jena Biosciences, Plant DNA Preparation Kit, Genomic DNA purification from plant tissue (Martins *et al.*, 2003 and Zahuang *et al.*, 2004).

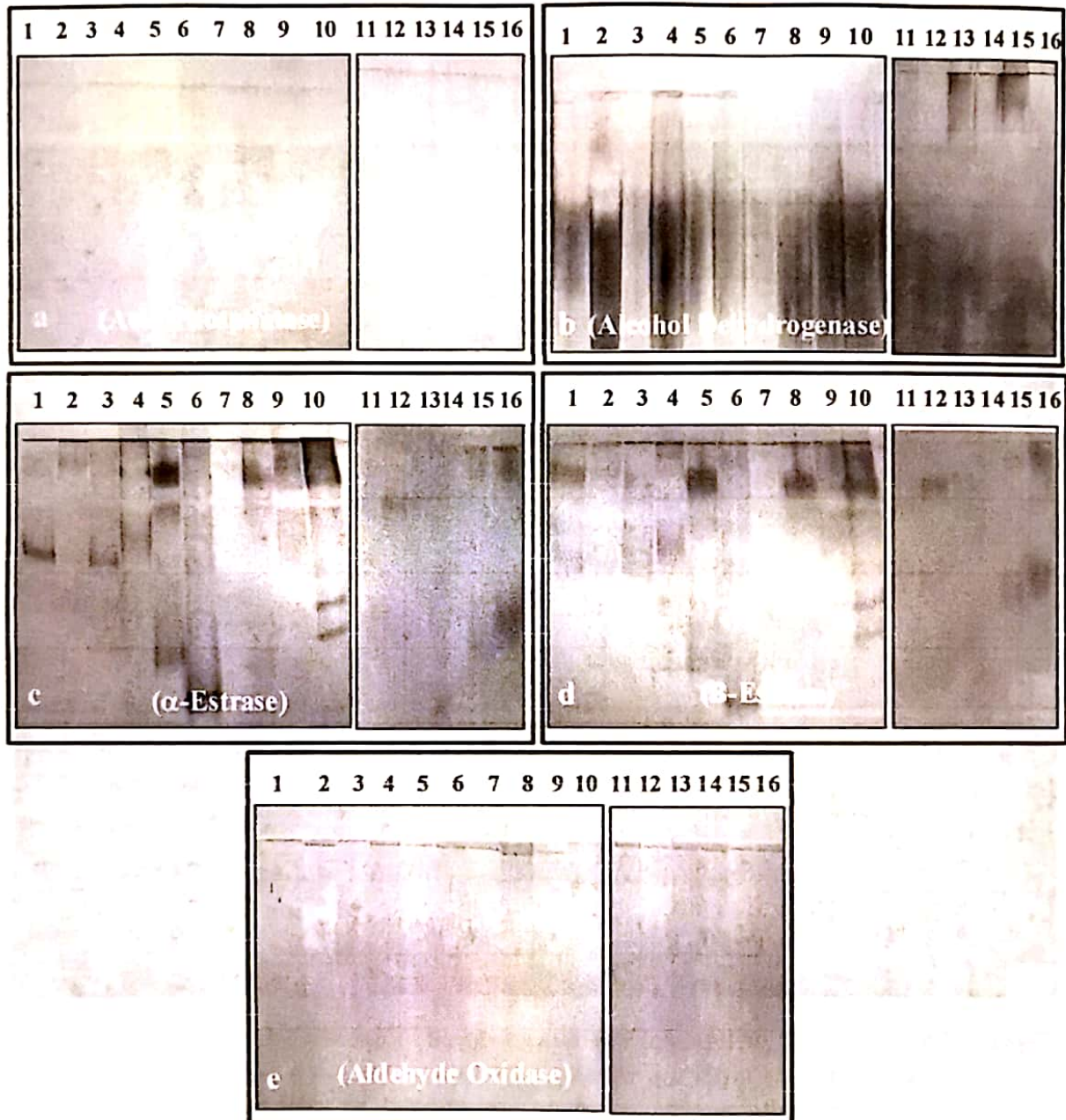
Each of the studied species was considered as Operational Taxonomic Unit (OTU). Multistate characters were transformed into two-state characters in coding i.e. pseudobinary characters (Sneath & Sokal, 1973). The data editor program N'EDIT 2.2 (Rohlf, 2005) was used to prepare two main sets of data matrices based on the macro/ micromorphological and macro/ micromolecular characters. The program NTSYS- pc 2.2 (Rohlf, 2005) was used to perform a cluster analysis for the above mentioned two data sets.

### **Results and Discussion**

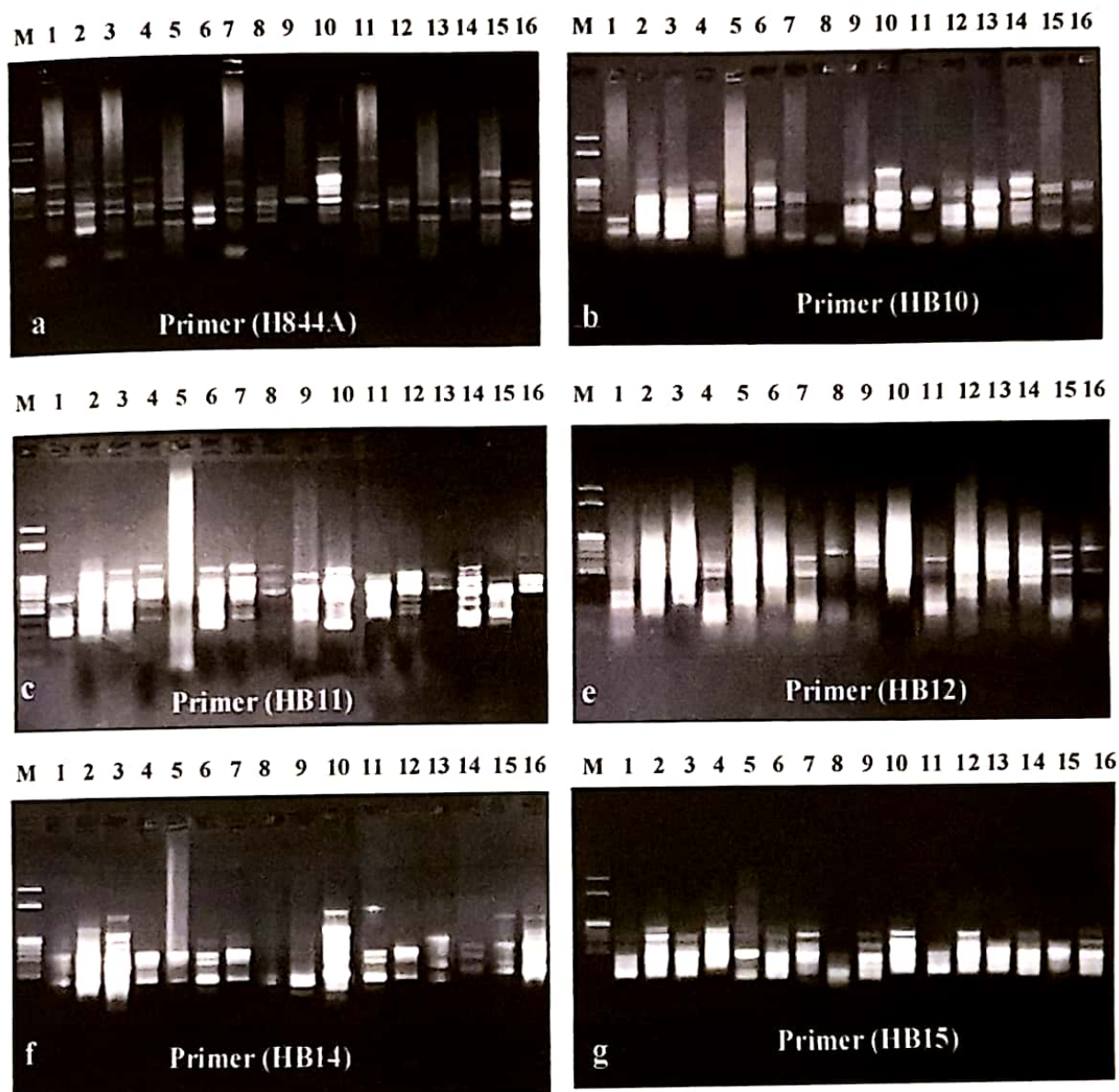
Microphotographs of the observed stomata types as revealed by LM are illustrated in Figures 1a-1b, whereas those of the lamina sculptures as revealed by SEM are illustrated in Figures 1c-1i. Photographs of the produced bands profile by application of the isozymes technique are illustrated in Figures 2a-2e, whereas those of the ISSR technique are illustrated in Figures 3a-3g. The scored 350 macro/micromorphological (141 of gross morphology; 76 of stem & leaf sections and striped off lamina epidermis; 26 SEM of lamina sculpture) and macro/micromolecular (42 isozymes & 86 PCR-ISSR) characters and their data matrix used in the phenetic analysis are given in Appendix 1. The produced UPGMA phenogram showing clustering of the studied taxa based on the used character states is given in Figure 4. A comparison between groups of the studied taxa and their corresponding tribes in the most common systems of classification are given in Table 2.



**Figures 1a-1i.** Two major types of stomata recorded in the studied taxa. **a:** Anomocytic type and **b:** Iris type. **c-i.** Microphotographs of lamina sculpture by SEM. **c:** rugose; **d:** tuberate; **e:** ruminant, **f:** sulcate; **g:** colleculate; **h:** lineate; **i:** reticulate.



**Figures 2a-2e.** Isozyme bands profile of the studied taxa; 1) *D. draco*; 2) *D. fragrans*; 3) *D. marginata*; 4) *D. ombet*; 5) *D.reflexa*; 6) *D. sanderiana*; 7) *Cordyline stricta*; 8) *Dracaena surculosa*; 9) *Cordyline fruticosa*; 10) *Sansevieria trifasciata*; 11) *Agave Americana*; 12) *A. franzosinii*; 13) *A. sisalana* 14) *Ruscus aculeatus*; 15) *Yucca aloifolia*; 16) *Yucca*



**Figures 3a-3g.** ISSR bands profile of the studied taxa; 1) *D. draco*; 2) *D. fragrans*; 3) *D. marginata*; 4) *D. ombet*; 5) *D. reflexa*; 6) *D. sanderiana*; 7) *Cordyline stricta*; 8) *Dracaena surculosa*; 9) *Cordyline fruticosa*; 10) *Sansevieria trifasciata*; 11) *Agave Americana*; 12) *A. franzosinii*; 13) *Yucca aloifolia*; 14) *Agave sisalana*;

The produced UPGMA - phenogram based on the 350 characters states (Figure 4) divided the studied taxa into three clusters. The first cluster (A) comprises three minor groups  $a_1$ ,  $a_2$  &  $a_3$  that are grouped together at the similarity level of 0.29. The first minor group  $a_1$  comprises three species *viz.* *Agave americana* and *A. franzosinii* are grouped together at the level of 0.51, and *A. sisalana* is clustered with them at the level of 0.44. The second  $a_2$  is distinguished as a separate phenetic line including *Sansevieria trifasciata* at the level of 0.32. The third minor  $a_3$  group comprised *Yucca aloifolia* and *Y. guatemalensis* at the level of 0.49, and clustered with *Sansevieria trifasciata* at the level of 0.32.

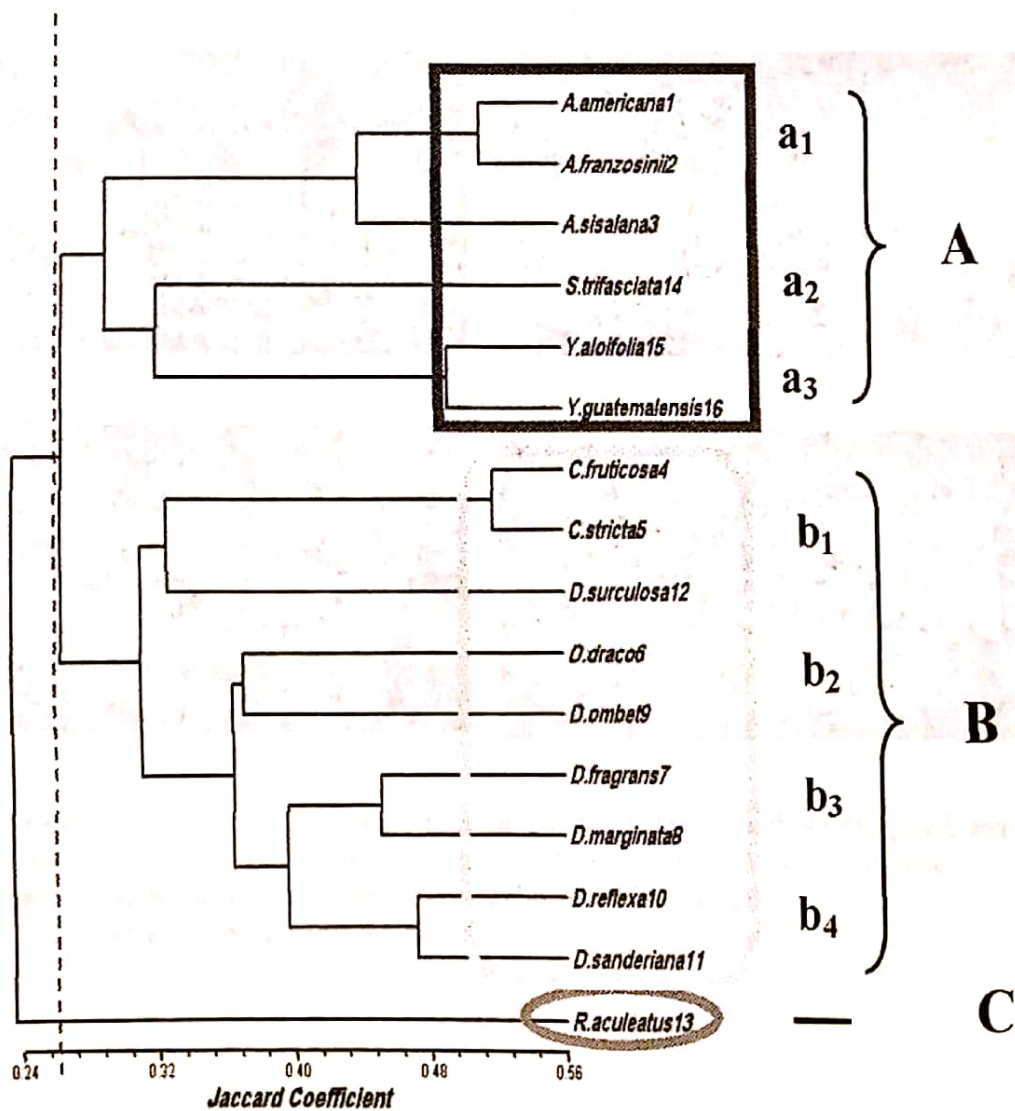


Figure 4. UPGMA - phenogram showing clustering of the studied taxa based on 350 character states.



The second cluster (B) is divided into four minor groups (b<sub>1</sub>, b<sub>2</sub>, b<sub>3</sub> & b<sub>4</sub>). The first minor group b<sub>1</sub> comprised three species *Cordyline fruticosa* and *C. stricta* at the 0.52 level, and *Dracaena surculosa* is clustered with them at the 0.33 level. The second minor group b<sub>2</sub> comprised *Dracaena draco* and *D. ombet* at the level of 0.37. The third group b<sub>3</sub> comprised *Dracaena fragrans* and *D. marginata* at the level of 0.45. The fourth group b<sub>4</sub> comprised *Dracaena reflexa* and *D. sanderiana* at the level of 0.47, and clustered with b<sub>3</sub> at the level 0.39. The Third cluster (C) is distinguished as a separate phenetic line including only *Ruscus aculeatus* at the level of 0.24.

*Sansevieria* was classified in the Agavaceae by Cronquist (1981) alongside *Agave* and *Yucca* with other genera on the basis of their possession of a woody stem produced by a secondary thickening meristem. Rendle (1953) using morphological data divided Agavaceae into two subfamilies: Agavoideae including *Agave* and Dracaenoideae including *Yucca* and *Sansevieria*. Dahlgren *et al.* (1985 & 1989) using a phylogenetic analysis of morphological data moved the genus *Sansevieria* into the family Dracaenaceae which was established earlier by Salisbury (1866) who divided the Agavaceae into two subfamilies Agavoideae and Yuccoideae instead of Dracaenoideae in Rendle's (1953) classification. These data were adopted by APGII (2003) and Takhtajan (2009) except that APG II (2003) placed *Sansevieria* in the family Ruscaceae, whereas Takhtajan (2009) retained it in Dracaenaceae.

In the present study, the representative taxa of *Agave*, *Yucca* and *Sansevieria* are clustered together in the major group A which corresponding to the family Agavaceae. They are morphologically characterized by rosette leaves arrangement, petiole absent, inflorescence panicle, flower bisexual, six lobes of perianth tube and six stamens. Anatomically, they are characterized by the ill-defined epidermis, collenchyma present, leaf vascular bundle embedded in conjunctive tissue and amphistomatic leaf. Molecular data revealed that they are shared band number 12 in the produced ISSR profile by using the primer HBA44 with molecular size (Mr) of 339.97 base pair.

**Table 2.** A comparison between groups of the studied taxa and their corresponding tribes in the most common systems of classification

Proposed classification		Taxa	Bessey (1915), Hallier (1923), Pulle (1938) and Englar & Diels (1936)	Hutchinson (1973), Heywood (1978) and Cronquists (1981)	Dahlgren <i>et al.</i> (1989)	APG II, 2003	Takhtajan (2009)	APG III, 2009
Group	Subgroup							
A	a <sub>1</sub>	<i>Agave americana</i>	Liliaceae	Agavaceae	Agavaceae	Agavaceae	Asparagaceae	
		<i>A. franzosinii</i>						
		<i>A. sisalana</i>						
	a <sub>2</sub>	<i>Sansevieria trifasciata</i>			Ruscaceae			
	a <sub>3</sub>	<i>Yucca aloifolia</i>			Agavaceae			
		<i>Y. guatemalensis</i>						
B	b <sub>1</sub>	<i>Cordyline fruticosa</i>	Liliaceae	Agavaceae	Dracaenaceae	Laxmanniaceae	Asteliaceae	
		<i>C. stricta</i>						
		<i>Dracaena surculosa</i>						
		<i>D. draco</i>						
	b <sub>2</sub>	<i>D. ombet</i>			Ruscaceae	Dracaenaceae		
		<i>D. fragrans</i>						
	b <sub>3</sub>	<i>D. marginata</i>						
		<i>D. reflexa</i>						
b <sub>4</sub>	<i>D. sanderiana</i>							
C	-	<i>Ruscus aculeatus</i>		Ruscaceae				

The first minor group (a<sub>1</sub>) comprised *Agave americana*, *A. franzosinii* and *A. sisalana* were morphologically clustered due to the broad leaf, inflorescence lateral, anther introse, ovary inferior, subsessile setting, three locules, three ovules and style elongated. Anatomically, they are clustered together due to the absence of schlerenchyma fibers, presence of stone cells, absence of extraxylary fibers, stomata anomocytic and sunken, epidermal cells of wide anticlinal walls, raised periclinal walls and smooth wax. Molecular data revealed that they shared bands of numbers 12 in the produced ISSR profile by using the primer HBA44 at Mr of 339.965 bp, 4, 6 & 9 in the produced ISSR profile by using the primer HB11 at Mr of 874.379, 687.034 & 381.661 bp respectively.

The second minor group (a<sub>2</sub>) including *Sansevieria trifasciata* var. *laurentii* which distinguished as a separate phenetic line, molecular data revealed that it shared bands number 1, 3 & 5 in the produced ISSR profile by using the primer HBA44 at Mr of 1853.93, 991.08 & 812.98 bp respectively, band no. 8 in the produced ISSR profile by using the primer HB10 at Mr of 604.94 bp, bands no. 2, 4 & 10 in the produced ISSR profile by using the primer HB14 at Mr of 1518.16, 1175.91 & 372.41 bp respectively; bands no. 1, 2 & 5 in the produced ISSR profile by using the primer HB12 at Mr 923.88, 788.39 & 532.36 bp respectively. Isozymes data revealed that it shared bands number 2 & 3 in Ao enzyme profiles, band number 6 in  $\alpha$ -est enzyme profile.

The third minor group (a<sub>3</sub>) including *Yucca aloifolia* & *Y. guatemalensis* was established morphologically due to: flower sessile, anther introse, three ovules, fruit capsule. Anatomically, they characterized by the rectangular cell of stem epidermal cells, crystals absent, sculpture lineate, anticlinal walls depressed, periclinal walls raised. Molecular data revealed that they share bands number 4, 9 & 10 in HB11 at Mr of 874.38, 381.661 bp respectively, bands number 3 & 10 in HB12 at Mr of 704.75 & 173.457 bp respectively, presence of bands number 4 & 9 in HB14 at Mr of 1363.02, 614.89 bp respectively, band number 9 in HB15 at Mr of 208.28 bp. Thus, the present data confirmed those of Cronquist (1981) and Rendle (1953) regarding the placement of *Agave* and *Yucca* in two different subfamilies of the Agavaceae.

*Dracaena* and *Cordyline* were either placed together in the same family, regardless the family name, or separated in two different families. Bessey (1915), Melchior (1964), Goldberg (1986) and Thorne (1992) placed the two genera in Liliaceae *sensu lato*. Rendle (1953), Hutchinson (1973), Heywood (1978) and Cronquist (1981) placed both genera in Agavaceae. Dahlgren *et al.* (1985 & 1989) placed the two genera in Dracaenaceae. On the other hand, APGII (2003) separated *Dracaena* and *Cordyline* in the Ruscaceae and Laxmanniaceae respectively, whereas Takhtajan (2009) separated them in Dracaenaceae and Asteliaceae respectively.

In present study, *Cordyline* is clustered with the studied species of *Dracaena* in the same major group B; which corresponding to the family Dracaenaceae as defined by Dahlgren *et al.* (1985 & 1989). This group is morphologically characterized by entire leaf, inflorescence terminal, panicle, bisexual, flower actinomorphic, gamotepalous, six stamen, ovary superior, three locules, capitate stigma, fruit small i.e. < 1.5 cm. Anatomically, the group B is characterized by absence stone cells, cuticle thin, stomata level, anticlinal walls depressed.

Molecular data revealed that, group B is characterized by the absence of bands number 1 & 4 in the produced ISSR profile by using the primer HBA44 at Mr of 1853.93 & 891.47 bp respectively; bands number 2 & 7 in the produced ISSR profile by using the primer HB10 at Mr of 1209.64 & 795.34 bp respectively, presence of band number 6 in the produced ISSR profile by using the primer HB11 at Mr. of 687.03 bp, absence of band number 1 in HB12 at Mr of 923.88 bp, absence of bands number 1, 3 & 13 in the produced ISSR profile by using the primer HB14 at Mr of 1685.24, 1363.023 & 362.50 bp respectively, presence of band number 11 in the produced ISSR profile by using the primer HB14 at Mr of 439.173 bp, presence of band number 6 in HB15 at Mr of 429.74 bp.

Isozymes data revealed that group B is characterized by presence of band number 1 in  $\alpha$ -est enzyme profile. These data confirms the placing of *Cordyline* with *Dracaena* in the same family i.e. Dracaenaceae as reported by Rendle (1953), Chupov & Kutiavina (1978 & 1981) and summarized by Dahlgren *et al.* (1985 & 1989).

Klimko & Wiland-Szymańska (2008) reported divergence of both *Dracaena ombet* and *D. surculosa* from the rest of the genus *Dracaena* based on the highest variability in the leaf texture and cuticle features between the adaxial and abaxial surfaces. In the present study, *D. surculosa* is distinguished from the remaining studied species of *Dracaena* and

grouped with those of *Cordyline* due to the following morphological characters: stem branched, leaf lax, tapering/ strap, bright to dark green, inflorescence lateral, perianth greenish to purple, anther extrose, ovary subsessile, one ovule and fruit orange. Anatomically, *Dracaena surculosa* is characterized by stem epidermal cells tangentially arranged, crystals present, square to pentagonal in side view, lineate sculpture, droplet waxes, cuticular flange absent. Molecular data revealed that it is characterized by the presence of bands number 5 & 9 in the produced ISSR profile by using the primer HBA44 at Mr of 991.08 & 529.91 bp respectively, band number 23 in HB10 at Mr of 204.43 bp, band number 3 in HB12 at Mr of 704.75 bp, bands number 12 & 14 in HB14 at Mr of 405.17 & 315.625 bp respectively, band number 9 in HB15 at Mr of 208.28. Isozymes data revealed that it is characterized by the presence of band number 10 in Ad enzyme and band 5 in Ao enzyme. These data are compatible with findings of Klimko & Wiland-Szymańska (2008) regarding the divergence of *Dracaena surculosa* away from other *Dracaena* species. Moreover, such data reveals a potentiality of placing *D. surculosa* with *Cordyline* in a separate subfamily Dracaenaceae, although it needs further studies on larger number of samples.

Klimko & Wiland-Szymańska (2008) reported a close relationship between *Dracaena draco* and *D. ombet* based on SEM criteria of the leaf surface. They pointed out that both species have some characteristics of xerophytes especially the slightly succulent leaves and presence of the cuticular flange around stomata. In the present study, *D. draco* and *D. ombet* are clustered together in the same minor group (b<sub>2</sub>) due to sharing the following morphological characters: leaf slender, entire, sessile, inflorescence panicle, flower actinomorphic, gamotcpalous, six stamens, filament inserted at perianth top, filiform, anther extrose, ovary superior, three locules, stigma capitate, fruit berry.

Anatomically *D. draco* and *D. ombet* shared the following characters: epidermal cells ill-defined, cortical cells suberized, absence of stone cells, absence of crystals, leaf epidermal cells include crystals, cuticle thin, raised anticlinal walls, granular periclinal walls, stomata at the same level, surrounded by a thick cuticular flange, square or rectangular in outline. Molecular data revealed that, *D. draco* and *D. ombet* are characterized by presence of band number 10 in the produced ISSR profile by using the primer HB10 at Mr of 624.74 bp, band number 6 in Hb11 at Mr of 687.034 bp, band 8 in HB12 at Mr 290.39 bp, band 8 in HB14 at Mr 709.92 bp.

Isozymes data revealed that, *Dracaena draco* and *D. ombet* are characterized by the presence of band number 3 in  $\beta$ -est, band 4 in Acph. The present data confirms the close relationships between *D. draco* and *D. ombet* as proposed by White (1983) and Klimko & Wiland-Szymańska (2008) *D. draco* and *D. ombet* could be placed in a separate subgenus (i.e. *Ombet*) in Dracaenaceae.

Bos (1998) and Klimko & Wiland-Szymańska (2008) reported a closer relationship of *D. marginata* to *D. draco* and *D. ombet* than *D. fragrans* based on morphological and anatomical criteria. In the present study *D. fragrans* and *D. marginata* are grouped together in the same minor group (b<sub>3</sub>) due to sharing the following morphological characters: stem single, leaf rosette, inflorescence branched, flower sessile and white, filament inserted at top of perianth, ovary superior, one ovule, style filiform; fruit round. They are anatomically shared the following characters: cork cells well represented, canals absent, vascular bundle absent, lignified conjunctive tissue present, epidermis penta-hexagonal shaped, square/ pentagonal in side view, stomata at the same level, oval shaped, cuticular flanges present.

Molecular data revealed that. *D. fragrans* and *D. marginata* are characterized by presence of bands number 8 & 10 in the produced ISSR profile by using the primer HBA44 at Mr of 604.94 & 468.57 bp respectively: band number 8 in HB10 at Mr of 737.55 bp, bands number 2, 4, 8, 9 & 10 in Hb11 at Mr of 1112.99, 874.38, 504.68, 381.66 & 320.70 bp respectively; bands number 3, 5, 6 & 9 in HB12 at Mr of 704.75, 532.36, 431.67 & 173.46 bp respectively; bands number 6, 8 & 9 in HB14 at Mr of 935.724, 709.919 & 614.894 bp respectively, bands number number 3 and 9 in HB15 at Mr of 708.907 & 208.284 bp respectively. Thus, the present data disagree with those of Bos (1998) and Klimko & Wiland-Szymańska (2008) and reveals a closer relationship between *D. marginata* and *D. fragrans* than to both *D. draco* and *D. ombet*. These two species could be distinguished at least in the same subfamily.

*Dracaena reflexa* and *D. sanderiana* exhibit a close relationship and could be placed in the same subfamily. They are grouped together in the same minor group (b<sub>4</sub>) due to sharing the following morphological characters: leaf acute, entire, petiolate, inflorescence panicle, flowers long, subsessile, filament inserted at the top of perianth, filiform, ovary superior, sessile, with one ovule, and elongated style. They are anatomically shared the following characters: stomata at the same level, oval, lineate, cuticular flanges absent. Molecular data revealed that, they are characterized by presence of band number 12 in the produced ISSR profile by using the primer HBA44 at Mr of

339.97 bp, band number 15 in Hb10 at Mr of 410.75 bp, band number 4 in HB11 at Mr of 874.38 bp. Isozyme data revealed that, both species have the band number 1 in Adh enzyme.

APGIII (2009) placed the genus *Ruscus* and the other studied genera (*Agave*, *Yucca*, *Cordyline*, *Sansevieria* and *Dracaena*) in the family Asparagaceae, whereas Shipunov (2009) and Takhtajan (2009) placed this genus apart of such genera in the family Ruscaceae. In present study, the representative species of the genus *Ruscus* is used as outgroup and is distinguished as separate phenetic line (i.e. group C). This is morphologically due to: leaf elliptic, pale green, flower unisexual, sessile/subsessile, filament green tinged, stigma papillate, fruit brown, oval. Anatomically, it is characterized by thin cell wall stem, cuticle thin, cork absent, conjunctive tissue absent, leaf surface sulcate. Molecular data revealed that it is characterized by presence of bands number 2 & 5 in the produced ISSR profile by using the primer HBA44 at Mr of 1853.93 & 812.975 respectively. Isozyme data revealed that *Ruscus aculeatus* has the band number 2 in  $\alpha$ -est enzyme. These data disagree with those of APGIII (2009) and could support the distinction of the genus *Ruscus* in a separate family (Ruscaceae) as proposed by Shipunov (2009) and Takhtajan (2009).

Carlquist & Schneider (2007) reported the close relationship between *Sansevieria* and *Dracaena* based on some root anatomical characters, the present data disagree with those regarding the close relationship between *Sansevieria* and *Dracaena*. Rather, it reveals the possibility of separation of *Sansevieria* from Dracaenaceae as defined by Dahlgren *et al.* (1989) or from Ruscaceae as defined by APGIII (2003) and retaining it with *Yucca* in the subfamily Yuccoideae.

The distinction of *Dracaena* and *Sansevieria* has previously been reported by Kauff *et al.* (2000) and Rudall *et al.* (2000). They reported that *Dracaena* and *Sansevieria* are very alike, but secondary thickening growth does not occur in roots of *Sansevieria* and the two genera can be distinguished by their exotesta despite its inclusion within *Dracaena* by Bos (1998). Furthermore, Rudall *et al.* (2000) stated that "despite their similarity in overall morphology *Sansevieria* and *Dracaena* should be treated as distinct entities based on palynology, ontogenetic and molecular data" and this gives further support to the present conclusion.

### Concluding remarks

The present data confirmed each of the following: a) Placing of *Agave* and *Yucca* in two different subfamilies of Agavaceae i.e. Agavoideae and Yuccoideae as proposed by Cronquist (1981), Rendle (1953) and Torres-Morán *et al.* (2008). b) Placing of *Cordyline* with *Dracaena* in the same family i.e. Dracaenaceae as reported by Rendle (1953), Chupov & Kutiavina (1978 & 1981) and summarized by Dahlgren *et al.* (1985 & 1989). c) The close relationship between *D. draco* and *D. ombet* that proposed by Klimko & Wiland-Szymańska (2008) and placing them in a separate subgenus (i.e. Ombet) of Dracaenaceae as defined by White (1983).

The present data disagreed each of the following: a- The close relationship between *Sansevieria* and *Dracaena* that proposed by Carlquist & Schneider (2007). b- Rather, it revealed the possibility of separation of *Sansevieria* from Dracaenaceae as defined by Dahlgren *et al.* (1989) or from Rusceae as defined by APG (2003) and retaining it with *Yucca* in the subfamily Yuccoideae of the Agavaceae. c- The closer relationship of *Dracaena marginata* to both *D. draco* and *D. ombet* than to *D. fragrans* as proposed by Bos (1998) and Klimko & Wiland-Szymańska (2008). Rather, it revealed a closer relationship between *D. marginata* and *D. fragrans* than to both *D. draco* and *D. ombet*. d- Merging of the genus *Ruscus* with the other studied taxa in the same family as proposed by APG (2009) and could support the distinction of the genus *Ruscus* in a separate family (Ruscaceae) as proposed by Shipunov (2009) and Takhtajan (2009).

The present data was compatible with those of Klimko & Wiland-Szymańska (2008) regarding the divergence of *Dracaena surculosa* from the rest species of *Dracaena* and revealed a potentiality of placing *D. surculosa* and *Cordyline* in a separate subfamily in the Dracaenaceae, although it needs further studies on larger number samples.



Appendix 1: All the 350 character states and their data matrix used in the phenetic analysis of the present study; 1 = Present; 0 = Absent.

No.	Characters	OTU's	0000000001111111 1234567890123456
<b>I- Macro morphology</b>			
<b>i: Whole Plant</b>			
01	Habit	Shrub	0001000000111000
02		Sub-shrub/ shrub	0010000000000000
03		Shrub/ tree	1100101101000011
04		Rhizome	0000000000000100
05		Tree	0000010010000000
<b>ii: Stem</b>			
06	Branching	Single	1110001100100000
07		Single/ branched	0000110000000011
08		Branched	0000010011011000
09		Reduced	0000000000000100
<b>iii: Leaves</b>			
10	Arrangement	Lax	0000010000111000
11		Rosette	1111101111000111
12	Width	Broad	1110001000000000
13		Slender	0001110111111111
14	Shape	Linear/lanceolate	0110001000000011
15		Lanceolate	0001000110000000
16		Linear	0000000001000000
17		Lanceolate/ elliptic	0000000000100000
18		Tapering/ strap	0000000000010000
19		Elliptic	0000000000001000
20		Strap/ lanceolate	1001100000001000
21	Apex	Acuminate	1001100000001000
22		Acute	0110010110001111
23		Acute-subulate mucro	0000001000000000
24		Acute/ acuminate	0000000001100000
25	Colour	Gray green with yellow	1000000000000000
26		Blue gray	0110000000000000
27		Dark green with purplish	0001000000000000
28		Green tinged purpish	0000100000000000
29		Grayish green	0000010000000000
30		Green broad yellow	0000001000000000
31		Glossy green	0000000100000000
32		Dark green	0000000010000011
33		Olive green	0000000001000000
34		Glossy green with silver	0000000000100000
35		Bright to dark green	0000000000010000
36	Margins	Pale green	0000000000001000
37		Grayish green	0000000000000100
38		Undulate	0110001000000000
39		Entire	0001110111110110
40	Serrate	0000000000000001	
<b>iv: Petiole</b>			

41	Presence	Absent/ present	1110011110000111
<b>v: Inflorescence</b>			
42	Length	Absent	0000000000001000
43		15-40 cm Short	0001100001110000
44		50-80 cm Middle	0010001110000111
45		90-110 cm Long	1100010000000000
46	Position	Lateral	1110000000010000
47		Terminal	0001111111100111
48	Type	Panicle	1111110111111111
49		Raceme	0000001000000000
50	Branching	Branched	111111100110011
51		Branched- unbranched	0000000100000000
52		Unbranched	000000001000100
<b>vi: Flower</b>			
53	Length	0.7-10 cm Small	1011100000001011
54		30-50 cm Medium	0000000100010000
55		60-70 cm Long	0100011011100100
56	Sex	Bisexual	1111111111110111
57		Unisexual	0000000000001000
58	Setting	Subsessile	1111110011110100
59		Sessile	0000001100000011
60		Sessile/ sessile	0000000000001000
61	Symmetry	Dimorphic	1110000000000000
62		Actinomorphic	0001111111111111
63	Colour	Pale yellow	1100000000000000
64		Green to yellowish	0010000000000000
65		Yellowish	0001000000000000
66		Red purple	0000100000000000
67		Creamy white	0000010000000000
68		White	0000001110100000
69		Cream green	0000000001000000
70		Greenish to purple	0000000000010000
71	Green or tinged	0000000000001000	
72	Greenish- white	0000000000000111	
<b>a: Perianth</b>			
73	Cohesion	Polytepalous	1000000000001111
74		Gamotepalous	0111111111110000
75	Tube	0.2-0.8 cm small	0000010011000000
76		0.9-1.2 cm middle	0001101100110000
77		1.8-2.2 cm large	0110000000000000
78	Lobes	6	1110000011011111
79		3	0001111100000000
<b>b: Stamen</b>			
80	Number	6	1111111111110111
81		3	0000000000001000
82	Insertion	At the top of throat	0100011111101000
83		Above the throat	0010000000000000
84		In the throat	0001000000010100
85		On the throat	0000100000000000
86	Filament	Filiform	1101001011111100
87		Linear	0010000000000000
88		Flattened	0000110100000011

89	Anther	Introse	1111000000000011
90		Extrose	0000011111111100
<b>c: Ovary</b>			
91	Position	Inferior	1110000000000000
92		Superior	0001111111111111
93	Length	0.9-2 cm short	0000000000000001
94		2-3.5 cm medium	0011111111111010
95		4-5 cm long	1100000000000100
96	Setting	Subsessile	1110010010010101
97		Sessile	0001101101101010
98	Locules	3	1111111111110101
99		1	0000000000001000
100		6	0000000000000010
101	Ovules	3	1110000000000011
102		2	0001100000001000
103		1	0000011101110000
104		1-2	0000000010000100
<b>d: Style</b>			
105	Shape	Elongated	1110000001101001
106		Slender	0001110000000110
107		Filiform	0000001100010000
<b>e: Stigma</b>			
108	Shape	Capitate	1011111111110000
109		Clavate	0100000000000000
110		Papillate	0000000000001000
111		Simple	0000000000000111
<b>vii: Fruit</b>			
112	Type	Fleshy	1101000100001000
113		Capsule	0010000000000011
114		Berry	0000111011110100
115	Colour	Red	1000000000000000
116		Bright orange	0100001000000100
117		Orange	0010000101010000
118		Yellow red	0001000000000000
119		Purple black	0000100000000000
120		Orange red	0000010000000000
121		Brown	0000000010001000
122		Purple	0000000000000010
123		White yellowish	0000000000100001
124	Shape	Obovoid	1000000000000000
125		linear	0100000000000000
126		Cylindrical	0010000010000000
127		Linear-oblong	0001000000000000
128		Globose	0000000001110100
129		Oval	0000000001110100
130		Round	0000111100000000
131		Oblong -ellipsoid	0000000000000010
132		Oblong-ovoid	0000000000000001
133	Length	5-20 cm small	1101111111111100
134		25-40 cm medium	0010000000000010
135		70-90 cm large	0000000000000001

<b>i. Stem Epidermal Cells</b>			
136		Ruptured /Homogenous	0001101101111000
137		Barrel	0001001000000000
138		Radially elongated	0000100000000000
139		Papillose	0000000101000000
140		Radial	0000000000101000
141		Tangentially	0000000000010000
142	Cell wall	Ill-defined	1110010010000111
143		Thick	0001001001110000
144		Thin	0000000100001000
145	Cuticle	Thick	0001001001110000
146		Thin	0000100000001000
<b>A: Protective tissue</b>			
147	Schlerenchyma	Absent/ Present	1110110110111101
148	Cortical cell	Absent	0001101101011100
149		Non-suberized	1100000000000000
150		Suberized	0010010010000011
151	Stone cells	Absent /Present	0001111111111111
152	Storied cork	Present/Absent	1111111111010111
153	Collenchyma	Absent/Present	1111110111011111
<b>B: Cortex</b>			
154	Canals	Absent /Present	0000111111111111
155	Vascular bundle	Absent /Present	1110100011000111
156	Crystals	Absent /Present	1011110111100010
<b>C: Anomalous 2<sup>nd</sup> growth</b>			
157	Conjunctive tissue	Absent	0000000000001000
158		Thin	1110000010100111
159		Lignified	0001111101010000
160	Width of ring	Very narrow	0000000000000100
161		±Narrow	1110101100010011
162		±Wide	0001010011100000
<b>ii. Lamina Epidermis</b>			
<b>a: Surface view</b>			
163	Shape	Penta-hexagonal	1111111101110100
164		Recta-pentagonal	0000000010000011
165	Thickness	Thin	0000110101000010
166		Thick	1111001010010101
167	Crystals	Absent/ Present	1011011110100111
<b>b: Side view</b>			
168	Shape	Papillose	1000000000000100
169		Barrel	0100010010000011
170		Square	0010000000000000
171		Cupic	0001000000000000
172		±Tangentially elongated	0000100000000000
173		Square - pentagonal	0000001100010000
174		Radially elongated	0000000001100000
175	Cuticle	Thin	0001111111110110
176		Thick	1110000000000001
<b>iii. Leaf</b>			
<b>a- Mesophyll</b>			
177	Composition	Chlo&Par	1110111011100110
178		Parenchyma	0001000000010001

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179		Chlorenchyma	0000000100000000
180	Crystals	Absent/ Present	1100000101100100
181	E. xyellary fibers	Absent/Present	0000100111100010
<b>b- Vasculature</b>			
182	Central portion rows	1-2 rows	1000010100010110
183		2- 3 rows	0001100001111001
184		3 -4 rows	0010000010000000
185		4 - 5 rows	0100000000000000
186		5 -6 rows	0000001000000000
187	Lateral portion	Attach& embedded	0001100000000000
188		Embedded	1110011111101111
<b>c- Stomata</b>			
189	Location	Ab & ad	1110011100110111
190		Ab	0001100011000000
191	Type	Anomocytic	1111101010000111
192		Iris	0000010101110000
193		canna	0100000000000000
194	Guard cells Position	Sunken	1110000000000000
195		Same level	0001111111110111
196	Guard cells Shape	Globose	1110110000000000
197		Oval	0001001111110111
<b>iv. SEM of Leaf Epidermal cells</b>			
<b>A. Sculpture</b>			
198	Shape	Colliculate	0000001000000000
199		Lineate	0000000111110011
200		Sulcate	0000100000001000
201		Reticulate	0000000000000100
202		Rugose	1010010000000000
203		Ruminate	0001000000000000
204		Tuberculate	0100000000000000
<b>B. Anticlinal walls</b>			
205	Range	Narrow	0001111001110111
206		Wide	1110000110001000
207	Level	Level	0100000000000000
208		Raised	0001000000000100
209		Depressed	1010111111111011
210	Texture	Granular	0100000111100001
211		Smooth	1011111000101110
<b>C. Periclinal walls</b>			
212	Level	Depressed	0001000000000100
213		Raised	1110111111111011
214	Texture	Granular	0010111111000001
215		Smooth	1101000000111110
216	Secondary sculpture	Absent	1111010010111111
217		Colliculate	0000101000000000
218		Granulate	0000000101000000
<b>D. Wax</b>			
219	Type	Droplets	0000000010111100
220		Granules	0000110101000000
221		Smooth	1111001000000011
<b>E. Cuticular flange</b>			
222	Occurrence	Absent/Present	1111111110000011

III- Micro molecules			
PCR-ISSR			
	Primer	Band no.	
223	H844A	1	10000000000000100
224		2	0000001010001010
225		3	0001110100000100
226		4	0000000000000001
227		5	0000000000011100
228		6	0100001000010101
229		7	0000010000000000
230		8	1001101101000100
231		9	0110000011011010
232		10	1001111100110101
233		11	0000000000000000
234		12	1110000011111111
235		13	0000001000000000
236		14	0000000000000000
237		15	000000001000010
238		16	0000000000000000
239		17	0011100100000000
240		18	1000010000000000
241	HB10	1	0000000000100000
242		2	0000000000000100
243		3	0000000100000000
244		4	0000001010000100
245		5	0100000000100000
246		6	0000000010001001
247		7	0010000000000010
248		8	0000001100100001
249		9	0111100000000010
250		10	1000001010001100
251		11	0001100101000000
252		12	0010000000100101
253		13	0000001110001010
254		14	1001100000001000
255		15	0100000101100000
256	16	0010011000000100	
257	17	0000001010000000	
258	18	0100000101001100	
259	19	0000001000000000	
260	20	0000010010100010	
261	21	0000000100000001	
262	22	0001100000000000	
263	23	1000000000010000	
264	HB11	1	0000000010000001
265		2	00011011110111101
266		3	0100001000001001
267		4	11111011111111011
268		5	00111010111001001
269		6	11111111111111111
270		7	1100000010000000
271		8	01000011001011111

272		9	110111110101011
273		10	0000011100101110
274		11	0001100100000000
275	HB12	1	0000000000000100
276		2	0011101000000100
277		3	0110001100111011
278		4	1001100001101110
279		5	1010001110000100
280		6	0111101111101111
281		7	1100000000101000
282		8	0100010111000000
283		9	0000011101000000
284		10	1001100010010011
285		11	0000010000000000
286	HB14	1	1000000000000000
287		2	0000000100000100
288		3	0000000000000011
289		4	0000001010000100
290		5	0000000100000001
291		6	0010001101100101
292		7	0000001000001111
293		8	1101111111101101
294		9	1010011100100011
295		10	0000011000001111
296		11	1101111111100000
297		12	0010000000010000
298		13	0000000000000100
299		14	0000000000010000
300	HB15	1	0001101010100000
301		2	0000010000100000
302		3	0101101110101101
303		4	0000000110100101
304		5	0101101000000100
305		6	1111111111111010
306		7	1110011111101101
307		8	0010000101101101
308		9	1110011101111011

## IV- Macro molecules

## Isozymes

	Enzyme	Band no.	
309	Ao.	1	0000010111100000
310		2	0000100000010100
311		3	0001000000000100
312		4	0001010000000000
313	Adh.	1	0010010101110000
314		2	0000100000000010
315		3	0111010000000000
316		4	0000010000000000
317	$\alpha$ -est	1	0001111111111110
318		2	0001000000001010
319		3	0101000000010100
320		4	0000010000000001

321		5	0000000010000010
322		6	0001000000010000
323		7	0000001001000000
324		8	0001000010000000
325		9	0000011001000000
326		10	0001000000100000
327		11	0001100000000000
328		12	0000000010000000
329		13	0000100001000000
330		14	0000000001000000
331		15	0000000001000000
332	$\beta$ -est.	1	0000000001000000
333		2	0000000001000000
334		3	0000011111100111
335		4	0010010000010000
336		5	1100100000000000
337		6	0000000010000001
338		7	0001100000010010
339		8	0000000001000000
340		9	0001100010010000
341		10	0000000100010000
342		11	0000010000000000
343		12	0001000000000000
344		13	0001000000000000
345		14	0000000000100000
346		15	0001000000000000
347	Acph.	1	0000001000000000
348		2	0000010111100000
349		3	0000010001010000
350		4	0001100010000000

01: *Agave americana*, 02: *A. franzosinii*, 03: *A. sisalana*, 04: *Cordyline fruticosa*, 05: *Cordyline stricta*, 06: *Dracaena draco*, 07: *D. fragrans*, 08: *D. marginata*, 09: *D. ombet*, 10: *D. reflexa*, 11: *D. sanderiana*, 12: *D. surculosa*, 13: *Ruscus aculeatus*, 14: *Sansevieria trifasciata* var *laurentii*, 15: *Yucca aloifolia*, 16: *Y. guatemalensis*.

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