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# Epidermal Properties of *Aloe vacillans* Forsskål Leaf and Its Taxonomical Significance in Classifying its Two Forms

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**Abstract:**
About 14 leaf epidermal characters (6 epidermal cell properties & 8 stomatal properties) of two forms (red-flowered & yellow-flowered forms) of *Aloe vacillans* Forsskål (Endemic Species to South West of Arabian Peninsula) were investigated. Only two epidermal cell properties and three stomatal properties show high significance in distinguishing between the two forms of *A. vacillans*. Moreover; 12 epidermal quantitative characters were subjected to numerical analysis (UPGMA), and the resulted dendrogram segregated the two examined forms at a relatively high similarity level (93.53%). This confirms that the two forms (red form and yellow form) of *A. vacillans* are two different infra-specific taxa under *A. vacillans*, which were described as *A. audhalica* Lavranos and Hardy and *A. dhalensis* Lavranos.

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## INTRODUCTION

The genus *Aloe* L. comprises just more than 600 taxa (including subspecies and varieties) (Carter *et al.* 2011; Grace *et al.,* 2011). *Aloe* L. is widespread in eastern and southern Africa, but in Asia is only known from the southwest part of Arabia and Socotra Island (Wood, 1983; Klopper and Smith, 2013). Moreover; few species such as *Aloe vera* which has become naturalized as a garden escapes in tropics and subtropics (including India, Pakistan, and the Americans) (Carter *et al*., 2011).

The general characteristics of the genus *Aloe* is a typical monocotyledonous plant, and its identification is based on morphological characteristics, which include the rosette arrangement of the leaves, the compact racemose inflorescence, and the predominant color of the flowers, as well as the thickened cuticle which has enhanced the capability of the genus to withstand harsh conditions (Coopoosamy and Naidoo, 2011), Those morphological characters exhibit similarities within all species of the genus with the exception of leaf size, growth height and a negligible difference in the floral colors (Cutler, 1969; Cronquist, 1981; Dahlgren *et al.,* 1985). On the other hand Cronquist (1981), Cutler (1985), and Anderson and Beardall (1991) stated that a detailed look at the appearance of the epidermal cells of the leaf provides sufficient characteristics to identify a genus, and sometimes these characteristic identifications can be made at the species level. Moreover in 2011 Coopoosamy and Naidoo, mentioned that the surface morphology, internal anatomical structures, and the nature of the crystals in the *Aloe excelsa* leaves appeared to be an additive diagnostic tool that could be utilized taxonomically at the species level to separate *A. excelsa* from the other *Aloe* species; Furthermore, in 2018 Ibrahim *et al.* discussed the high taxonomical significance value of epidermal properties in the separation between the Arabian endemic *Phragmanthera austroarabica* and Nigerian *Phragmanthera* spp. According to Al Khulaidi (2013), the genus *Aloe* L. in Yemen is represented by 20 species, including 13 endemic species (11 in mainland and 2 in Socotra Island) and 7 near endemic species (Endemic to Arabian Peninsula).

*Aloe vacillans* Forsskål is an endemic species to the southwest of the Arabian Peninsula and it is a common species in the southwestern quarter of the Arabian Peninsula (Wood, 1983; Carter *et al.,* 2011). It was first recorded by Forsskål from Kurma, in the mountains of the southern Yemeni escarpment. Its distribution extends through much of the Yemeni mountains into the Asir Province Mountains in Saudi Arabia, with an altitudinal range 1300-3000m above sea level. The species *A. vacillans* Forsskål is stemless or rarely with a short erect or decumbent stem, usually growing singly, rarely suckering to form small colonies, leaves are rosulate, small, glaucous, ascending or erect with brown marginal teeth. Perianths are red or yellow. *Aloe vacillans* is more variable than most aloes in Yemen. It has two distinct types of flowers; red flowers (red form) and yellow flowers (yellow form), which have been described by Lavranos (1965) as *A. audhalica* and *A. dhalensis* respectively. The distribution of these two morphological forms of *A. vacillans* is of more interest than usual, the yellow form which is predominant in the south part of the country and the red form in the north part of the country, and both forms only occur in about equal frequency in Ibb governorate area and also found together at Sana'a Amran road (Wood, 1983; Al Khulaidi, 2013; Al-Hood, 2020). According to the previous studies, the epidermal cells of the leaf have significant characteristics in identifying and distinguishing between genera, and sometimes these characters can be used at the species level. Moreover, *A. vacillans* in Yemen is represented by two forms, which had been identified as two different species (Wood, 1983). Therefore the current study aims to employ the leaf epidermal characters of *Aloe vacillans* to illustrate the taxonomical relationship among its two morphological forms in Yemen.

## MATERIALS AND METHODS

### Plant samples collection

Fresh samples of the two morphological forms of *Aloe vacillans* Forsskål were collected (Figure 1 and Table 1) during September–October 2019 and implanted in an experimental farm in Sana'a city. Plant samples were identified by utilizing the available taxonomical literature: Wood (1983); Collenette (1999), and Carter *et al.* (2011).

**(A)** **(B)**

**Fig. 1.** General view of *Aloe vacillans*, A: Red form (Red flowers); B: Yellow form (Yellow flowers).

**Table 1.** Locality and Date of Collection of the two of *Aloe vacillans* forms

| Date of collection | Location | Coordinates | | Elevation | *Aloe vacillans* forms |
|---|---|---|---|---|---|
| | | Longitude | Latitude | | |
| October 2019 | Collected from Sana'a – Amran road / Implanted in Sana'a City | 44°11'2 E | 15°21'19 N | 2278 m asl | Red form (Red flowers) |
| September 2019 | Collected from Al- Thary- Ibb Governorate/ Implanted in Sana'a City | 44°34'1E | 14°14'12"N | 2217m asl | Yellow form (Yellow flowers) |

**Epidermal investigation**

At least seven leaves were cut into pieces, each piece with an area of about 10 $cm^2$, then the two layers of the epidermis (adaxial layer and abaxial layer ) were separated using a razor blade, and the two layers were then cleaned with a camel hair brush in water (Ibrahim and Ayodele, 2013; Ibrahim *et al.*, 2016**)**. The two epidermal layers (adaxial & abaxial) were stripped and stained with safranin, Excess stain was rinsed off with clean water, and the samples mounted in glycerol on clean slides were then covered by a cover slide **(**Sreelakshmi *et al*., 2014**)**. The slides were observed by using a Leica (ATC 2000) microscope to determine leaf epidermal (adaxial & abaxial) characters of each species, which were based on the terminology of Dilcher (1974). The characters determined were epidermal cell features (shape, frequency, length, width, size and cell wall thickness) and stomata pore features (frequency, length, width, size and pore wall thickness).

The stomata and epidermis cell frequencies were based on the average obtained from observation of 12 microscope field of view at 400X. The stomatal index (SI) was calculated using the formula SI= [S \ (S+E)] × 100, where S = No. of stomata in a field of view at 400× & E= No. of epidermal cells in a field of view at 400× (Salisbury, 1927; Al-Sanabani, 2020). The stomata ratio (SR) was helpful in defining the type of leaf. It is the ratio of the stomata on

adaxial epidermis to the number of stomata on abaxial epidermis, if SR>1 the leaves are classified as amphistomatic, if 0.1<SR<1 as hypoamphistomatic, and if SR<0.1 as hypostomatic (Szymura and Wolski, 2011). The epidermal cell size (length × width) was based on the average obtained from observation of 40 individuals, while; stomata pore size (length × width) and pore wall thickness, were based on average obtained from observation of 25 individuals, with the help of ocular micrometer calibrated with a stage micrometer (value of 400× 1ocular small division = 2.5 µm) and Image j program.

The taxonomical value of quantitative leaf morphological, epidermal features was determined by T. test using Graph Pad Prism 6.01 program, if P-value P < 0.05 then the quantitative leaf features are significantly different.

Moreover; the taxonomical relationship between the two forms of *A. vacillans* has been illustrated by a dendrogram and similarity matrix based on the quantitative epidermal properties analyzed numerically by Unweighted Pair Group Method Averages (UPGMA) using Primer 5 software version: 5.2.2.

## RESULTS

The results presented in Table 2 and Figure 2 showed the main leaf epidermal characters of the studied leaves of the two forms of *Aloe vacillans* as clarified by a light microscope. Epidermis (adaxial and abaxial) cell shape of the red form and the yellow form of *A. vacillans* varied from round, rectangular to hexagonal with thick cell wall boundaries (Figure 2). The nature of the cell wall composition appeared to be cellulose, hemicellulose, and pectic substances. Also, the cell wall was embedded or characterized with lignin as the epidermal cell wall was stained red with safranin.

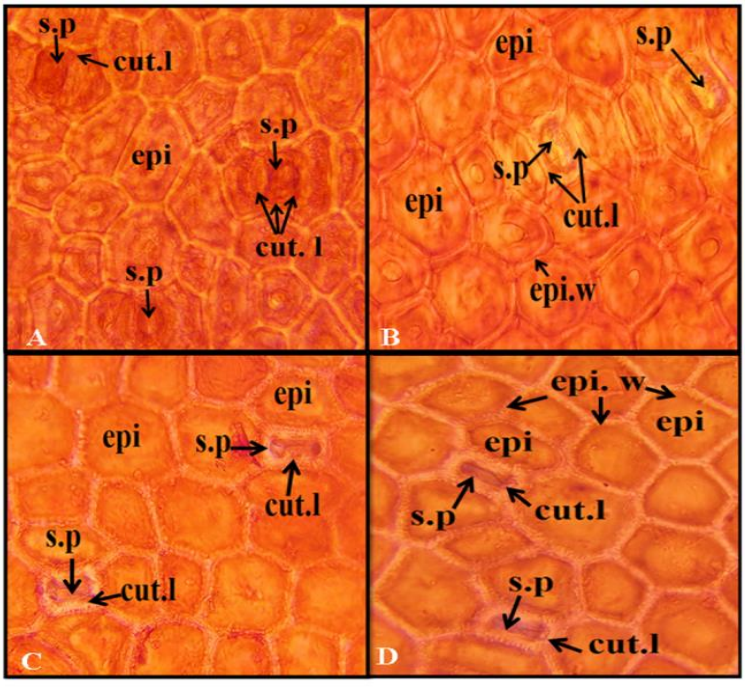


**Fig. 2.** Epidermal Characters of the *Aloe vacillans* leaf, **A** (adaxial surface) - **B** (abaxial surface): red form (red flowers)- **C** (adaxial surface )- **D** (abaxial surface):yellow form (yellow flowers); epi: Epidermal cell, epi.w: epidermal cell wall, S.p: Stomata pore, cut. l: cuticular membrane lobes.

The highest mean of epidermal cells density was found in the adaxial surface of the red form (76 epidermal cell/field of view) followed by 73 epidermal cell/field of view on the abaxial surface of red form; while the lowest mean of epidermal cells density (66 epidermal cell/field of view) was recorded on the abaxial surface of the yellow form. Furthermore, the mean size of epidermal cells is largest in the adaxial surface of the yellow form (2366.3 $\mu m^2$) and the smallest mean of epidermal cell size (1784.7 $\mu m^2$) was recorded in the abaxial surface of the red form (Table 2).

**Table 2.** Quantitative epidermal characters comparison between adaxial and abaxial surface of the red form (red flower) and yellow form (yellow flower) of *Aloe vacillans* leaf.

| Characters | | | *A. vacillans* | | P-Value |
|---|---|---|---|---|---|
| | | | Red form (Red flowers) | Yellow form (Yellow flowers) | |
| Epidermis cells | Number of epidermal cells in 400x view | Ad | 72 (76 ±2.7)79 | 64(68±2.5)73 | < 0.0001**** |
| | | Ab | 71 (73±2)77 | 62(66±2.5)70 | < 0.0001**** |
| | Length of epidermal cells (µm) | Ad | 14.6(48 ± 17.3)78.4 | 17.8 (46±14.11) 84.5 | 0.5307 |
| | | Ab | 28.8 (44.5 ± 10.1)72.1 | 14.5 ( 43.2±13) 75.5 | 0.6127 |
| | Width of epidermal cells (µm) | Ad | 7.85 (38.9 ± 12.9)58.1 | 29.9(49.6±13.6) 84 | 0.0005*** |
| | | Ab | 20.7 (38.9 ±9.0)58.1 | 31 (51.3±14.3)91.2 | < 0.0001**** |
| | Size of epidermal cells ($\mu m^2$) | Ad | 114.7 (2022.3 ±1114.7) 4383.3 | 744.3 (2366.3±1285.4) 7096.7 | 0.2047 |
| | | Ab | 688.7 (1784.7 ±726.1) 3549.6 | 672.9(2260.1±1098.1)5459.7 | 0.0251* |
| | Thickness of cell wall (µm) | Ad | 4 (6 ± 1.5)8.9 | 4.8( 7.5 ±1.2)10.5 | 0.0003*** |
| | | Ab | 5.3 (7.8 ±1.9)12.5 | 4(7.1±1.6)10.7 | 0.1435 |
| Stomata complex | Number of Stomata in 400x view | Ad | 1(4±1.1 )5 | 3(5±1) 6 | 0.0265* |
| | | Ab | 3 (4 ±0.8)5 | 4(5±0.9)7 | 0.0150* |
| | Stomata Ratio | | 1 | 1 | - |
| | Stomatal index | Ad | 1.3(4.6±1.3) 6.5 | 4.1(6.5±1.4) 8.6 | 0.0026** |
| | | Ab | 3.8 ( 5.5± 0.9) 6.5 | 5.6(7.3±1.3)9.9 | 0.0007*** |
| | Length of stomata pore in (µm) | Ad | 19.7 (29.3 ±4.4 )39.8 | 13.9(22.4± 4.6)31.3 | < 0.0001**** |
| | | Ab | 16.6 (21.3± 2.3)25.7 | 18.3(25.1±2.9)32.3 | < 0.0001**** |
| | Width of Stomata pore (µm) | Ad | 8.9 (14.4 ±3.2) 19.9 | 4.1(6.3 ±2)13.5 | < 0.0001**** |
| | | Ab | 4.3 (7.9 ± 2.3)12.5 | 4.9(7±1.1)8.9 | 0.0672 |
| | Size of stomata pore ($\mu m^2$) | Ad | 174.9 (431±148.4) 768.9 | 58.5(145.6±72)423.7 | < 0.0001**** |
| | | Ab | 87.8 (172.1 ±59.6) 304.3 | 89.3(177.6±39) 247.3 | 0.6972 |
| | Thickness of stomata pore wall (µm) | Ad | 4.9 (12.1±2.6)16.3 | 5.3 (7.8±1.4) 12.2 | < 0.0001**** |
| | | Ab | 5.2 (6.8 ±1.1)9.3 | 6.2 (9.1±1.3)11.6 | < 0.0001**** |

On the other hand, the largest mean epidermal cell wall thickens was found to be 7.8 µm in the abaxial surface of the red form, followed by 7.5 µm and 7.1 µm in the adaxial and abaxial surface of the yellow form correspondingly, while the smallest mean of epidermal cell wall thickness was found in the adaxial surface of the red form (6 µm).

The stomata on the adaxial and abaxial surface of the two forms were deeply sunken below the general surfaces of the leaf with a cuticular membrane extending throughout the stomata complex, overarched by four well developed lobes one from each of the four surrounding epidermal cells (Subsidiary cell).

The highest mean of stomata density was observed on the abaxial and adaxial surface of the yellow form (5 stomata/field of view) whose mean of stomatal index was 7.3 and 6 respectively followed by 4 stomata/field of view on the abaxial and adaxial surface of the red form, whose mean of stomatal index is 5.5 and 4.6 correspondingly.

Furthermore; the largest mean size of stomata pore was in the adaxial surface of the red form (431$\mu m^2$) followed by the mean size of stomata pore in abaxial of the yellow form and abaxial of the red form; 177.6 and 172.1 respectively; while the smallest mean of stomata size (145.6 $\mu m^2$) was recorded in the adaxial surface of the yellow form (Table 2). On the other hand; the stomata ratio (SR) of the *A. vacillans* two forms (red form and yellow form) were = 1.

This shows that leaves of the studied *Aloe vacillans* forms are amphistomatic (leaves that have stomata on both surfaces, on the abaxial and adaxial surface). Moreover; the highest mean stomata pore wall thickness was recorded on the adaxial surface of the red form (12.1 µm) followed by the mean stomata pore wall thickness in the abaxial (9.1 µm) and adaxial (7.8 µm) surface of the yellow form, while; the smallest mean stomata pore wall thickness (6.8 µm) was found in the abaxial surface of the red form.

Moreover; based on the 12 quantitative epidermal characteristics (Table 2), numerical analysis was used to perform a similarity matrix to illustrate the relationships among the two studied forms. The resulted dendrogram (Figure 3) clarifies that the *A. vacillans* forms under investigation are divided into two series (SI and SII) at a relative similarity level of 93.53%, series I include *A. vacillans* red form, while series II includes *A. vacillans* yellow form.

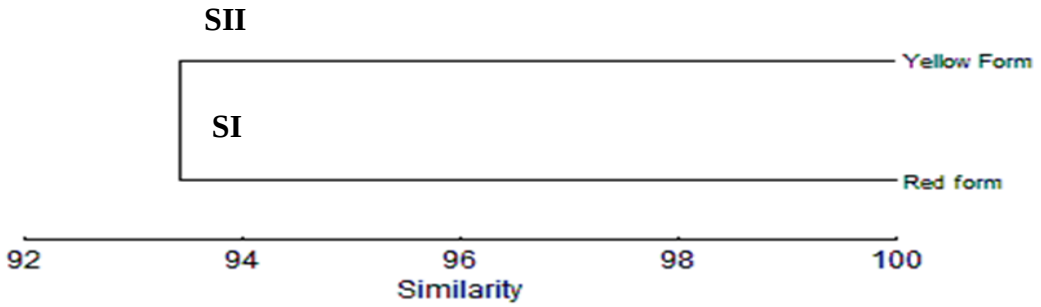


**Fig. 3.** Cluster analysis of the relationship among the two studied forms based on 12 Characters of leaf epidermal layer.

## DISCUSSION

Based on previous results, the two forms of *A. vacillans* possess a thickened (Lignified) epidermal cell wall and deeply sunken stomata, which is agreed with the findings of Coopoosamy and Naidoo (2011) where they mentioned that the epidermal layer of the genus *Aloe* possesses a thickened epidermal cell wall and deeply sunken stomata, typical to plant species inhabiting an arid or xeromorphic environment.

According to the earlier results the frequency of epidermis cells and the stomata pore length, width, size, and stomata pore wall thickness in the adaxial surface of the red form is much larger than in the adaxial surface of the yellow form; while the width of the epidermal cells, thickness of epidermal cells wall, stomata frequency and stomatal index in the adaxial surface of the yellow form is much larger than in the adaxial surface of the red form; Moreover, the frequency of epidermis cells in the abaxial surface of the red form is much larger than in the

abaxial surface of the yellow form; while, the width and size of epidermal cells, number of stomata, stomatal index, length of stomata pore and stomata pore wall thickness is much larger in the abaxial surface of the yellow form than in the adaxial surface of the red form.

Therefore; the frequency and width of the epidermal cells, stomatal index, length of stomata pore, and stomata pore wall thickness in the adaxial and abaxial surface of the red and the yellow forms of *Aloe vacillans* leaf shows a taxonomical significant value in distinguishing between those two forms; In addition; the two forms of *A. vacillans* were described by Lavranos (1965) as *A. audhalica* Lavranos & Hardy (red flowers) and *A. dhalensis* Lavranos (yellow flowers), but according to previous studies (Al-Hemaid 2001; Al Khulaidi, 2013; Al-Hood, 2020) *A. audhalica* Lavranos & Hardy and *A. dhalensis* are synonyms for *A. vacillans*, and this agreed with the numerical analysis since the relative similarity level between the two forms is 93.53%. This illustrates that the two forms are different infra-specific taxa under the species *A. vacillans*.

## CONCLUSION

According to the preceding results, the epidermal quantitative characters of the leaves of the two investigated *A. vacillans* forms show a high significance in taxonomic value for separating them into two different infra-specific taxa under the species *A. vacillans*.

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## CONFLICT OF INTEREST

There is no conflict of interest.

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