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Water relations, transpiration rate, stomatal behaviour and leaf sap pH of *Aloe vera* and *Aloe eru*

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ABSTRACT
Aloe vera L. and *Aloe eru* Berger, were grown in big pots (25 kg soil) under different irrigation regimes of fresh water, irrigation with brackish water, plants supplemented with *Acacia saligna* green manure and under direct and diffused sunlight. *Aloe vera* and *Aloe eru* are succulent CAM plants and exhibited a noticeably economic use of water by virtue of the Crassulacean acid metabolism rhythm, opening stomata by night and closing them by day, thus minimizing water loss. The leaf sap pH is acidic, acidity rose by night and declined by day. Structural adaptive characteristics, in addition to the functional CAM rhythm, are focused upon.

KEYWORDS: *Aloe vera*, *Aloe eru*, CAM plants, Transpiration.

INTRODUCTION

Among the most important species of *Aloe* commonly grown in Egypt are *Aloe vera* and *Aloe eru*, therefore the present work was planned to study the water relations and anatomical adaptive character of *Aloe.* Sundberg (1985) stated that large stomata in succulent CAM plants allow considerable gas exchange to occur at night, but their low density helps to minimize water loss during the day when stomata are usually closed. Woodhouse *et al.* (1983) reported that nocturnal stomatal opening of *Agave deserti* reduced leaf surface temperature by only 1.4°C, with increasing the short wave absorptance maximum leaf surface temperature increase by 8°C. It was stated by Nowak & Martin (1997) that low stomatal densities, small stomatal pores, restriction of stomatal opening to night time only and a thick boundary layer are characteristic features of CAM plants to avoid a decline in leaf water content. Luttge & Noble (1984) studied the day-night variations in malate concentration, osmotic pressure and hydrostatic pressure in *Agave deserti,* concluding that in addition to the inverted stomatal rhythm, the oscillations of malate markedly affect osmotic pressure and hence water relations of CAM plants. Koller & Rost (1986) reported that characteristic features in *Sensevieria cylindrica* associated with adaptations to a dry environment include a thick cuticle and sunken stomata to help prevent water loss, fibres to resist wilting and specialized mesophyll cells probably for water storage. It was stated by Smith & Nobel (1986) that an approximately linear relationship was found between the number of vascular bundles and leaf surface area in the desert CAM plant *Agave deserti*. The bundles were separate from each other by about 8 water storage cells. It was found by Abd El-Rahman & Hassib (1973) that plants growing in the desert had more vascular bundles and were shorter than those growing elsewhere (i.e. not in the desert).

MATERIALS AND METHODS

Suckers of *Aloe vera* and *Aloe eru* were grown in pots (25 kg soil/pot). The design of the experiment is shown by the end of this section. Leaf water content was obtained by taken 100 g fresh weight of leaves, oven dried at 105°C to a constant weight. The difference between fresh weight and dry weight gave the water content.

Degree of succulence was determined as water content g/leaf area dm^2. Leaf sap E.C. was determined by using conductivity meter GIHYDAC (Fresenius *et al.* 1988). Air and leaf temperature was determined using tiny thermometer. Water use efficiency of production were determined as g dry wt./L The diurnal march of transpiration rate of plants under different water regimes was investigated using the weighing method of pots (Kramer (1969). Leaf sap pH was determined by pH meter.

The diurnal march of stomatal aperture were studied using an infiltration technique (Dale 1961), using a mixture of ethylene glycol and isobutyl alcohol. A study of cross sections of *Aloe* leaves under control and stressed condition was completed and the stomatal frequency and stomatal opening of *Aloe* leaf epidermis were determined using ocular micrometer (CARL ZEISS JENA).

Statistical analysis: Results were statistically analyzed using 4-way Anova for each variable measured.

RESULTS AND DISCUSSION

The variation in the leaf water content on a fresh and dry weight basis is represented in Table 1 (a & b). The water content of *Aloe vera* was slightly lower the water content of *Aloe eru*. Plants grown under diffused sunlight gave slightly higher values than those grown under direct sunlight. Plants irrigated weekly with fresh water and plants treated with green manure exhibited higher values than for water and saline stressed plants.

Degree of succulence (water content g/leaf area dm^2): The degree of leaf succulence of the two *Aloe* species (2 months old) of control (Treatment 1) and water stressed plants (irrigated every 3 weeks) during September are shown in Table 2. *Aloe vera* leaves had a lower degree of succulence than *Aloe eru* leaves and plants under shade had a higher degree of leaf succulence. Water stressed plants exhibited lower leaf succulence than control plants.

Table 1a: Leaf water content (% fresh wt.) of *Aloe vera* and *Aloe eru* (2 years old).

Treatment	*Aloe vera*				*Aloe eru*			
	Direct sunlight		Diffused sunlight		Direct sunlight		Diffused sunlight	
	Loamy sand	Sandy loam	Loamy sand	Sandy loam	Loamy sand	Sandy loam	Loamy sand	Sandy loam
1	93.9	93.7	94.6	94.5	94.9	95.2	95.4	95.5
2	92.5	92.9	93.2	93.6	94.3	94.5	95.0	95.3
3	88.9	90.3	90.8	91.6	92.5	93.9	93.7	94.6
4	92.2	91.7	93.3	93.4	94.9	95.3	95.4	95.3
5	93.8	93.7	94.3	94.4	94.9	95.3	95.4	95.3

Table 1b: Leaf water content (g/g dry wt.) of *Aloe vera* and *Aloe eru* (2 years old).

Treatment	*Aloe vera*				*Aloe eru*			
	Direct sunlight		Diffused sunlight		Direct sunlight		Diffused sunlight	
	Loamy sand	Sandy Loam	Loamy sand	Sandy loam	Loamy sand	Sandy loam	Loamy sand	Sandy loam
1	15.4	14.9	17.5	17.2	18.5	19.7	20.8	21.1
2	12.4	13.1	13.7	14.5	16.6	17.1	18.8	20.2
3	8.0	9.3	9.9	10.9	12.4	15.5	14.9	17.7
4	11.8	11.0	13.9	14.2	18.7	20.3	20.5	20.5
5	15.1	14.8	16.6	16.7	18.7	20.1	20.8	20.5

1=Irrigated weekly with fresh water; 2= every 2 weeks with fresh water; 3= every 3 weeks with fresh water; 4= Irrigated with saline water 1g NaCl+1g $CaCl_2$+1g $MgCl_2$/L weekly; 5= Irrigated with fresh water+250 g/pot *Acacia saligna* leaves weekly.

Source	df	F-Value	Prob
Factor A	1	287.5	0.0000*
Factor B	1	41.1	0.0000*
AB	1	4.2	0.0467*
Factor C	1	6.3	0.0155*
Factor D	4	71.1	0.0000*
AD	4	13.4	0.0000*
BD	4	1.3	0.2675*
CD	4	3.2	0.0210*

Statistical analysis for the leaf water contents (significant values only).
A: Plant species (*vera, eru*);
B: Sun light (direct, diffuse);
C: Soil (loamy sand , sandy loam);
D: Watering treatment (1-5).
* Significant

Table 2: Degree of succulence (water content g/leaf area dm^2) of *Aloe vera* and *Aloe eru* (2 months old).

Treatment	*Aloe vera*		*Aloe eru*	
	Direct sunlight	Diffuse sunlight	Direct sunlight	Diffuse sunlight
1	18.6	19.7	21.9	23.7
2	17.8	18.5	17.8	18.9

1=Irrigated weekly with fresh water; 2=Irrigated every 3 weeks with fresh water.

Source	df	F -Value	Prob
Factor A	1	1300.672	0.0000*
Factor B	1	.00	0.0000*
Factor C	1	0.16	0.0008*
AC	1	13.3	0.0046*
Factor D	4	1.89	0.0000*
ABD	4	1.79	0.0231*

Statistical analysis for the Degree of succulence (significant values only).
A: Plant species (*vera, eru*);
B: Sun light (direct, diffuse);
C: Soil (loamy sand , sandy loam);
D: Watering treatment (1-5).
* Significant

Leaf sap electrical conductivity ($mScm^{-1}$): Table 3 shows that E.C. is lower for *Aloe vera* than *Aloe eru* . Leaf sap E.C. of plants grown under direct sunlight are higher than for plants grown under diffused sunlight. Plants irrigated weekly with fresh water exhibited lower E.C., while stressed plants showed the highest records. Plants irrigated with brackish water showed higher E.C. than those irrigated weekly.

Table 3: Leaf Sap E.C. ($mScm^{-1}$) of *Aloe vera* and *Aloe eru* as affected by different studied treatments.

Treatment	*Aloe vera*				*Aloe eru*			
	Direct sunlight		Diffused sunlight		Direct sunlight		Diffused sunlight	
	Loamy sand	Sandy loam	Loamy sand	Sandy loam	Loamy sand	Sandy loam	Loamy sand	Sandy loam
1	3.0	3.2	2.4	2.6	5.1	5.5	4.8	5.0
2	3.4	3.4	2.9	3.0	5.2	6.3	5.0	5.6
3	4.0	4.2	3.5	3.8	6.5	6.7	5.6	5.9
4	3.8	4.0	3.6	3.9	6.4	6.5	5.2	5.6
5	3.2	3.1	2.5	2.5	5.1	5.5	4.8	5.2

1=Irrigated weekly with fresh water; 2= every 2 weeks with fresh water; 3= every 3 weeks with fresh water; 4=Irrigated with saline water 1g NaCl+1g $CaCl_2$+1g $MgCl_2$/L weekly; 5=Irrigated with fresh water+250 g/pot *Acacia saligna* leaves weekly.

Source	df	F-Value	Prob
Factor A	1	208.92	0.0000*
Factor B	1	7.42	0.0095*
Factor C	1	181.44	0.0000*
AC	1	15.25	0.0004*
Factor D	4	0.04	0.0000*
AD	4	0.25	0.0005*
CD	4	78.95	0.0003*

Statistical analysis for the the leaf sap data. (significant values only).
A: Plant species (*vera, eru*);
B: Sun light (direct, diffuse);
C: Soil (loamy sand , sandy loam);
D: Watering treatment (1-5).
* Significant

Air and leaf temperature: *Aloe* leaf temperature by day were higher than ambient air temperature. The difference between air and leaf temperature may reach 6°C. The difference was greater for plants under direct sunlight than those under diffused sunlight.

Water use efficiency of production: Dry weight per unit water used and water use efficiency of production (g/l); were calculated for 2 years old for *Aloe* plants in all treatments (Fig. 1).

Aloe vera exhibited higher WUE of production than *Aloe eru.* Plants grown under diffused sunlight recorded higher WUE than plants grown under direct sunlight. Maximum WUE of production was attained by water stressed plants. Addition of *Acacia saligna* to *Aloe* raised WUE of production to about 50% over the control, which indicated that *Aloe* plants are being very economic in their water use. The WUE of production rises with water stress.

Figure 1: Water use efficiency of production of Aloe vera and Aloe eru (2 years old)
1=Irrigated weekly with fresh water; 2= every 2 weeks with fresh water; 3= every 3 weeks with fresh water; 4= Irrigated with saline water 1g NaCl+1g $CaCl_2$+1g $MgCl_2$/L weekly; 5= Irrigated with fresh water+250 g/pot *Acacia saligna* leaves weekly.

Transpiration rate, leaf sap pH and stomatal behaviour (Figs. 2-5):

Transpiration rate was recorded for two successive days during September. Observations of plants irrigated every week as well as water-stressed plants were conducted. The leaf sap pH of the *Aloe* plant is acidic. The acidity of leaf sap rose by night and decreased by day, i.e. pH was lower by night than by day. This is a known characteristic of Crassulacean Acid Metabolism plants (CAM plants). This acid rhythm results from fixation of CO_2 by night when the stomata are open, resulting in nocturnal malic acid and malate accumulation.

The stomatal opening index march shows opening of stomata by night and closure by day (Plate Ia, b). In drought stressed plants opening of stomata by night is, however, markedly reduced, while closure is maintained throughout the daytime. The transpiration rate of *Aloe* plants is notably low. Transpiration occurs by night when the stomata are open, and transpiration loss is negligible or absent by day when the stomata are closed and ambient air temperature is at its maximum level. It has been pointed out that leaf temperature attains higher values than ambient air temperature by day under direct sunlight. The transpiration rate attains a maximal peak at mid night and is maintained at minimal rates through out the whole daytime, with the result that the amount of water loss for the two *Aloe* species studied is very low.

Transpiration rate by night is lower for *Aloe vera* than *Aloe eru*, for plants grown under sunlight, at midnight it amounted to 1.25 g/h/100 g fresh wt.; while under shade lower values were recorded. The behaviour of drought stressed plants is clear (Figs. 2-5). Transpiration rates are greatly reduced and reached very low levels by night and day in *Aloe vera*. Water losses during the night by *Aloe eru* were rather higher than that record for *Aloe vera*.

This striking behaviour of stomata, leaf pH and transpiration shows how *Aloe* plants are capable of enduring the harsh environment of raised air temperature and decreased soil water potential under severe drought conditions by virtue of their CAM rhythm. By day the malate previously accumulated during the night is broken down, CO_2 concentration increases and as a result stomata close. During long-lasting periods of drought little CO_2 is taken up during the night so that only CO_2 arising from respiration can be used for photosynthesis by day.

Anatomical adaptive characteristics: Adaptive characteristics of *Aloe vera* and *Aloe eru* leaf anatomy are presented in Plates 1&2. Plate 1 illustrates stomatal behaviour of *Aloe vera* opened by night (A) and closed by day (B). Stomatal density (frequency) is rather equal for both the upper and lower epidermis in *Aloe vera* (20 mm^{-2}) (Plate 1 C, D), while the stomatal density for *Aloe eru* amounted to 40 mm^{-2} for the upper epidermis and 25 mm^{-2} for the lower epidermis (Plate 1 E, F).

Thick outer epidermis cell walls in both *Aloe* species are noticed (Plate 2 A, B), differentiated to cuticle, cutinized and cellulose layers. Plate 2 B reveals the structure of two sunken stomata of *Aloe eru*. Mesophyll tissue appears in Plate 2 for *Aloe vera* (C) and *Aloe eru* (D), consisting of 10-12 rows of large chlorenchymatous palisade cells with large storage spaces and narrow intercellular spaces.

The leaf anatomy of control compared to water-stressed (irrigated every 3 weeks) plants of *Aloe vera* are represented in Plate 2 E, F, respectively. Inner to the palisade tissue lies the multi-layered water storage tissue of large parenchymatous cells. Vascular bundles lie in one row between the mesophyll and water storage parenchyma cells. The water stressed leaf shows large numbers of vascular bundles than does the nonstressed plant and they are nearer one to the other than in the control.

Plate 2 (G, H) reveals the leaf anatomy of control and water-stressed plants of *Aloe eru*. It is noticed that the vascular bundles in *Aloe eru* leaf are more numerous than in *Aloe vera*, becoming still more numerous under the response of water stress (Plate 2 H) quite close to each other i.e. the venation becomes more dense.

In conclusion, results recorded here for transpiration, leaf sap pH, stomatal behaviour, ambient air temperature and leaf temperature under direct sunlight and shade on two successive days in September indicate that the two succulent CAM plants *Aloe vera* and *Aloe eru* have the characteristics peculiar to CAM plants (the Crassulacean Acid Metabolism plants).

The acid rhythm results from accumulation of CO_2 by night and accumulation of malate while by day malate consumption occurs and pH rises by day. Stomata open by night and close by day. *Aloe vera* and *Aloe eru* transpiration rates were notably reduced to zero by day and reached low values by night when the relatively large and low-frequency stomata are open.

Large stomata allow considerable gas exchange by night, their low frequency helping to minimize or prevent water loss during day when the stomata are closed. The two *Aloe* species studied are water savers, water economic or thrifty and of higher water use efficiency of production. WUE was notably higher in drought stressed and shaded plants. Addition of green manure to desert soil raised the WUE to 50% compared with the control, due to increase the fertility and higher soil nutrients (especially nitrogen) and improved soil moisture status.

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الملخص العربى

العلاقات المائية والمعدل اليومى لفقد الماء وحركة الثغور ودرجة حموضة العصير في نوعى الصبار *ألوى فيرا وألوى إيرو*

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إهتمت هذه الدراسة بتأثير معاملات رى مختلفة وكذلك رى بماء قليل الملوحة (عادم) وكذلك إضافة مخصب عضوى تحـت ظـروف الظـل وضوء الشمس المباشر على المحتوى المائى ودرجة العصيرية وحموضة العصير والمعدل اليومى للنتح وحركة الثغور والتركيب التشـريحى لصنفى الصبار ألوى فيرا وألوى إيرو وهما من النباتات العصيرية التابعة للنباتات الكراسيولية حمضية الأيض CAM والتى تتبـع إسـتخداماً إقتصادياً للماء نتيجة للأيض الحمضى الكراسيولىالإيقاعى، بفتح الثغور ليلاً وإغلاقها نهاراً وبذلك ينخفض فقد الماء للحد الأدنى.

أظهرت الدراسة ملائمة نوعى الصبار المدروسين الفسيولوجية والتشريحية والتىنتج عنها درجة عالية لكفاءة إستخدام الماء للإنتاج كذلك أظهر ألوى فيرا كفاءه ومقاومة لظروف الجفاف أكثر من ألوى إيرو.