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Anatomical Basis for Optimal Use of Water for Maintenance of Three Xerophytic Plants

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Abstract

Three xerophytic plant species namely *Agave americana* Linn., *Aloe vera* Tourn. and Linn. and *Euphorbia milii* Des Moul. were propagated in a greenhouse each with 5 varying soil moisture contents i.e. 1.25%, 2.5%, 5%, 10%, and 20% and subjected to 4 watering frequencies i.e. daily, weekly, biweekly and monthly. *Euphorbia milii* was the most xerophytic species having relatively lower rate of transpiration than *Aloe vera* and *Agave americana.* It was suggested that the high rate of transpiration in *Aloe vera* and *Agave americana* may be due to the large tetracytic stomata as compared to the small paracytic stomata of *Euphorbia milii.* It was also observed that *Aloe vera* was least tolerant of high soil moisture in daily watering as well as low soil moisture in monthly regime. *Agave americana* and *Euphorbia milii* were species that were more robust with capacity to cope well with low and high watering regimes than *Aloe vera.*

Keywords: anatomical features, conservation of water, plant leaf, transpiration rate, xerophytic plants, water stress

Introduction

Water cycle, water stress, transpiration and indeed plants with their stomatal opening and closing potentials are inseparable. Many empirical explanations have been postulated on the four. Plants are producers on whose other organisms depend for survival especially for food supply. Production of foods especially sugars is through photosynthesis which involves absorption of carbon dioxide (CO_2) through openings (i.e. stomata) in the leaves but as CO_2 comes in to the leaves water vapour also escapes outward to the atmosphere (Boyer *et al*., 1997; Xu and Zhou, 2008). Therefore, water loss is an inevitable consequence of stomatal opening for photosynthetic carbon gain (Caird *et al*., 2007), and this contributes greatly to water stress in plants and consequently to poor growth and development of plants. Transpiration is at the same time known to participate actively in water cycle in the planet Earth because about 2/3 of water in the water cycle passed through the plants. Because of problems of inadequate water supply and unavoidable water loss through transpiration most plants thus become water-stressed. There must always be adequate supply of water to plants in term of quantity, quality and frequency for them to perform better. With current water scarcity in the world where water availability per head is too low, there may be no room for water wastage on irrigation. Therefore there must be ways of judiciously using the available water maximally to achieve optimal yield while still conserving water. Meanwhile, water use efficiency (WUE) of a plant becomes predominant in lieu of water scarcity in the world today, and this will thus depend on water saving capacity (WSC) of plant.

Due to the fact that more water get lost through transpiration at higher rate leads to wilting and eventual death of many plants, makes some researchers contest the acclaimed benefit of transpiration. Tanner and Beevers (2001) reported that there is no possible interrelationship between transpiration and long-distance transport of mineral ions along xylem tissues of plants. This was the result of an experiment performed with *Helianthus anuus.* They found that convective water transport in the xylem, brought about by root pressure and resultant guttation and Munch's phloem counter flow is in itself sufficient for long-distance mineral supply and that transpiration is not required for this function. Therefore, researchers are studying ways to reduce evapotranspiration as a means of meeting the increasing demand for water in agricultural, industrial, and general use. In this situation, understanding adequate watering frequencies, amount and time, is paramount to avoiding wastage of water on irrigation by preventing irregular and indiscriminate watering.

However, there are no clear cut amount of water requirements and frequencies of watering to be used for irrigating most plants in the literatures. Terms such as constantly moist, allow to dry between watering, moderately moist, frequent watering, infrequent watering, water in a week or weeks or a month and tolerant to drought are often used. These terms can be confusing. They are not precise and can be interpreted in many ways which result in plant injury. Few plants are able to grow in constantly soggy soil. Few plants can last long in soil which has dried out completely. Between these two extremes is plenty of room for watering mistakes.

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Unfortunately, studies describing how stomatal features (e.g. stomatal density, stomatal index, stomatal size) of plants respond to different water stresses, relationships with gas exchange especially transpiration are few so far. The objective of this study was to determine the responses of stomatal features to different water status, and to develop the relationship of stomatal features with transpiration rate of *Agave americana, Aloe vera* and *Euphorbia milii*, based on a greenhouse experiment with a soil moisture gradient.

Materials and methods

The study materials namely *Agave americana*, *Aloe vera* and *Euphorbia milii* were propagated using offsets and stem cuttings, placed in plastic pots of oven-dried loamy soil, given watering regimes as shown in Tab. 1 (Walter, 1979).

The experiments were conducted in a greenhouse at University of Ilorin, Ilorin, Nigeria, for 6 weeks using 4 watering intervals as shown in Tab. 2. Number of replicates was 15 and a total of 300 pots per species were used.

Tab. 1. Soil and water regimes used for raising the study materials

Soil (g)	Water (g)	%Moisture content (Water regime)
1600	400	20
1800	200	10
1900	100	5
1950	50	2.5
1975	25	1.25

Tab. 2. Watering frequencies and regimes used for raising seedlings of the study materials

Watering intervals	Soil moisture content (%)
Daily	1.25
	2.5
	5
	10
	20
Weekly	1.25
	2.5
	5
	10
	20
Biweekly	1.25
	2.5
	5
	10
	20
Monthly	1.25
	2.5
	5
	10
	20

After six weeks, the seedlings were taken to the laboratory for morphological and anatomical studies, leaf area measurement and determination of transpiration rate.

The leaf area (*LA*) was determined by using the formula: L × B × K (Franco, 1939).

Where L = Length; B = Breadth; K = Franco's constant = 0.79

Samples of leaves used were taken from different parts of the plant body i.e. upper, middle and lower parts. A sample size of 35 leaves was used for each species.

A cobalt chloride paper method was used to determine the transpiration rate of each specimen (Dutta, 2003; Obiremi and Oladele, 2001). Strips of filter paper of 2 cm x 6 cm dimension were cut and immersed in 20% cobalt chloride solution. The strips were thoroughly dried in an oven. The property of cobalt paper is that they are deep blue when dried, but in contact with moisture they turn pink. The blue, dried strips were placed in a sealed, airtight polythene bag and weighed (W1) using mettler balance. It was transferred quickly to the plastic containers and affixed with a string to the plants. Two dried cobalt papers were placed on the leaf, one on the upper and the other one on the lower surface of a thick healthy leaf, and were covered completely with glass slides (Dutta, 2003). The time (in seconds) taken for the strips to turn pink was noted. Once turned pink, the bag was quickly untied and sealed again and weighed (W2). Weight of water transpired was determined as W2 minus W1. The surface area of leaves used was measured. Transpiration rate was expressed as mol m^{-2} sec^{-1}.

Leaf segment of an area of 1cm square from each specimen was cut and immersed in concentrated solution of nitric acid or trioxonitrate (v) acid for maceration. The upper (adaxial) and lower (abaxial) surfaces were separated with dissecting needle and forceps, and rinsed with clean water. Using 35 fields of view at X40 objective as quadrats, the number of subsidiary cells per stoma was noted to determine the frequency of the different complex types present in each specimen. Frequency of each complex type was expressed as percentage occurrence of each stomatal complex type based on occurrences of all stomatal complex types. Terminologies used followed those of Dilcher (1974). The stomatal density was determined as the number of stomata per square millimeter (Stace, 1965).

Stomatal index (SI) was determined as follows:

SI = S/E+S X 100

Where: SI = stomatal index; S = number of stomata per square millimeter; E = number of ordinary epidermal cells per square millimeter.

The mean stomatal size of a species was determined as product of length and breadth of guard cells. A sample of 35 stomata was used per seedlings of a watering regime. From both upper and lower surfaces, presence or absence of stomata was noted to determine the leaf type whether it is epistomatic, hypostomatic or amphistomatic.

All data generated were analyzed using Analysis of Variance (ANOVA) and significant differences or means were separated with Duncan's Multiple Range Test (DMRT). A probability value of <0.05 was used as bench mark for significant difference between parameters.

Results and discussion

Leaf emergence

Leaf emergence was observed first in 7 days in offsets watered with 10cc monthly watering regime in *A. americana* while the least germination occurred in 34 days in offsets watered with 20 cc monthly and 20 cc weekly watering regimes of *A. americana* and *A. vera* respectively (Tab. 3). Rate of mortality of offsets and cuttings was high in monthly and biweekly watering regimes than in daily and weekly watering regimes. This corroborates the results of experiments on tomato (Dahal *et al*., 1996), *Artemisia sphaerocephala* (Zheng *et al*., 2005a), *Hedysarum fruticosum* (Zheng *et al*., 2005b), *Anthemis cotula* (Rashid *et al*., 2007) and *Omphalea oleifera* (Sanchez-Coronado *et al*., 2007) where water stress resulted in total reduction of seed germination.

Tab. 3. Leaf emergence/germination period in three xerophytic species raised with percentage moisture contents

Watering regime (%)	*Agave americana*	*Aloe vera*	*Euphorbia milii*
Daily			
1.25	21b	13d	20b
2.5	11d	9e	15d
5	24b	8e	--
10	15c	--	13e
20	12d	--	15d
Weekly			
1.25	--	--	11f
2.5	--	--	17c
5	--	--	17c
10	16c	22b	18c
20	24b	34a	13e
Biweekly			
1.25	--	--	--
2.5	--	--	20b
5	--	--	--
10	14cd	9e	20b
20	13d	17c	--
Monthly			
1.25	--	--	--
2.5	--	--	26a
5	--	--	--
10	7e	--	11f
20	34a	--	13e

Means with same letters along the rows are not significantly different at $p<0.05$

Stomatal complex types

Agave americana and *Aloe vera* consistently showed tetracytic stomata (Fig. 1 and 2) while *Euphorbia milii* had paracytic stomata (Fig. 3) under all watering regimes. Variations were, however, observed in the values of stomatal size, stomatal density and stomatal index (Tab. 4 and 6). In *Agave americana*, the range of variation for stomatal density was 1.55-4.38, that for stomatal index was 1.38-5.39 and that for stomatal size was 91.95-628.84 (Tab. 4). In *Aloe vera*, the range of variation in stomatal density was 0.38-2.50, that in stomatal index was 0.91-4.46 and that for stomatal size 144.87-534.39 (Tab. 5). In *Euphorbia milii*, the range of variation in stomatal density was 9.25-21.00; that of stomatal index was 5.10-10.41 while that of stomatal size was 48.13-86.74 (Tab. 4). Stomatal features such as its density had been correlated positively with water use efficiency (WUE) of plants (Klooster, 2004; Shan, 1991; Shi, 1999; Wang *et al*., 2007; Xu and Zhou, 2008). Similarly, several reports have shown that the stomatal density and its index increase with water stress (Yang and Wang, 2001; Zhang *et al*., 2006), but the number of stomata per leaf decrease (Quarrie and Jones, 1977). AbdulRahaman and Oladele (2003), Oyeleke *et al*. (2004) had earlier observed similar pattern of stomatal density, index and size on the

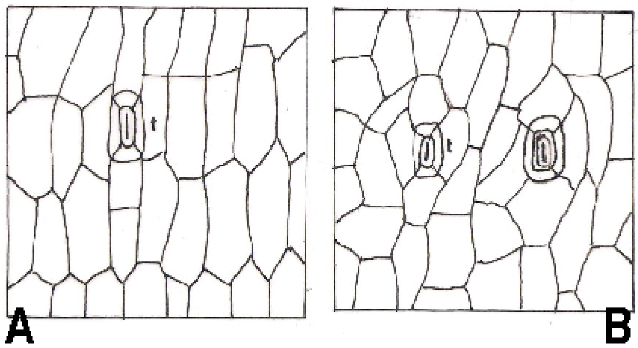

Fig. 1. Surface view of leaf epidermis, abaxial (A) and adaxial (B) of *Agave americana* propagated with 1.25 cc daily watering regime showing tetracytic (t) stomata x1200

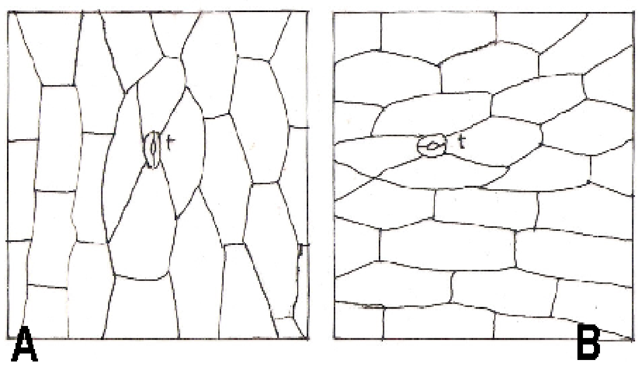

Fig. 2. Surface view of leaf epidermis, abaxial (A) and adaxial (B) of *Aloe vera* propagated with 2.5 cc daily watering regime showing tetracytic (t) stomata x1200

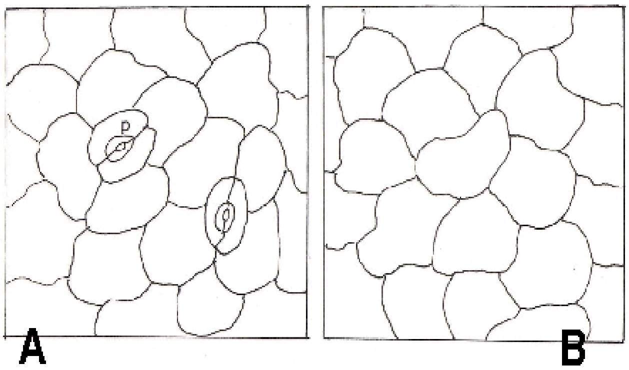

Fig. 3. Surface view of leaf epidermis, abaxial (A) and adaxial (B) of *Euphorbia milii* Propagated with 1.25cc daily watering regime showing paracytic (p) stomata x1600

Tab. 4. Stomatal anatomy of *Agave americana* raised with different percentage moisture contents

Watering regimes (%)	Leaf surface	Stomatal density (mm^{-2})	Stomatal index (%)	Stomatal size (um)
		Daily		
1.25	Abaxial	2.91c	3.54e	499.50b
	Adaxial	4.13a	5.15b	360.56c
2.5	Abaxial	2.05cd	2.85ef	557.62a
	Adaxial	3.85b	5.35b	331.94c
5	Abaxial	2.45c	4.47d	475.34b
	Adaxial	2.50c	3.56e	222.33d
10	Abaxial	1.55d	1.38	381.77c
	Adaxial	2.00cd	2.33ef	360.93c
20	Abaxial	3.13bc	3.92e	504.32ab
	Adaxial	4.38a	6.12a	336.16c
		Weekly		
10	Abaxial	1.57d	2.34ef	338.30c
	Adaxial	1.73d	2.21ef	275.50d
20	Abaxial	2.75	5.39b	440.59bc
	Adaxial	2.11cd	4.94d	309.17c
		Biweekly		
10	Abaxial	3.71b	4.07d	252.86d
	Adaxial	4.38a	5.26b	91.95e
20	Abaxial	2.63c	3.73e	628.84a
	Adaxial	3.88b	5.11b	521.25a
		Monthly		
10	Abaxial	2.67c	4.10d	99.56e
	Adaxial	2.00cd	3.01e	93.75e
20	Abaxial	2.00cd	2.58ef	446.28b
	Adaxial	3.86b	4.80d	341.19c

Means with same letters along the columns are not significantly different at $p<0.05$

abaxial and adaxial leaf surfaces of some vegetable species and some afforestation species respectively.

Transpiration rate

In daily watering regime, *Aloe vera* had higher rate of transpiration than *Agave americana* and *Euphorbia milii* at 1.25-5% soil moisture (Tab. 7). But the species was less tolerant of high soil moisture in daily watering and low soil

Tab. 5. Stomatal anatomy of *Aloe vera* raised with different percentage moisture contents

Watering regimes (%)	Leaf surface	Stomatal density (mm^{-2})	Stomatal index (%)	Stomatal size (μm)
		Daily		
1.25	Abaxial	0.38c	0.91d	195.64d
	Adaxial	0.50c	1.54c	253.12c
2.5	Abaxial	0.60c	1.55c	236.90c
	Adaxial	1.00b	2.66b	287.83c
5	Abaxial	2.50a	3.65a	261.67c
	Adaxial	0.75c	1.86c	249.75c
		Weekly		
10	Abaxial	2.63a	4.46a	534.39a
	Adaxial	2.13a	3.36a	334.95b
20	Abaxial	0.80c	1.82c	215.00c
	Adaxial	0.70c	1.51c	284.66c
		Biweekly		
10	Abaxial	0.63c	1.36c	144.87d
	Adaxial	0.38c	1.04c	195.58d
20	Abaxial	0.80c	1.82c	215.00c
	Adaxial	0.70c	1.51c	284.66c

Means with same letters along the columns are not significantly different at $p<0.05$

Tab. 6. Stomatal anatomy of *Euphorbia milii* raised with different percentage moisture contents

Watering regimes (%)	Leaf surface	Stomatal density (mm^{-2})	Stomatal index (%)	Stomatal size (μm)
		Daily		
1.25	Abaxial	10.33e	6.55c	65.33c
	Adaxial	--	--	--
2.5	Abaxial	15.00b	9.12ab	86.74a
	Adaxial	--	--	--
10	Abaxial	10.67e	4.82d	85.75a
	Adaxial	--	--	--
20	Abaxial	13.75bc	8.74b	80.50a
	Adaxial	--	--	--
		Weekly		
2.5	Abaxial	12.33d	6.03c	85.68a
	Adaxial	--	--	--
5	Abaxial	21.00a	10.41a	80.29a
	Adaxial	--	--	--
20	Abaxial	13.22cd	5.95c	81.04a
	Adaxial	--	--	--
		Biweekly		
2.5	Abaxial	10.50e	5.10d	76.51b
	Adaxial	--	--	--
10	Abaxial	9.25e	5.47cd	48.13d
	Adaxial		--	--
		Monthly		
2.5	Abaxial	10.50e	5.10d	76.50b
	Adaxial	--	--	--
20	Abaxial	12.00d	6.12c	73.47b
	Adaxial	--	--	--

Means with same letters along the columns are not significantly different at $p<0.05$

Tab. 7. Transpiration rate of some ornamental plant species propagated with different percentage moisture contents

Watering regimes (%)	Transpiration rate (mol/m²/sec⁻¹)					
	Agave americana		*Aloe vera*		*Euphorbia milii*	
	Abaxial	Abaxial	Abaxial	Abaxial	Abaxial	Abaxial
Daily						
1.25	$2.91x10^{-5}$a	$3.01x10^{-5}$a	$8.66x10^{-5}$a	$1.13x10^{-4}$a	$2.23x10^{-3}$c	--
2.5	$1.68x10^{-5}$a	$1.70x10^{-5}$a	$8.64x10^{-5}$a	$1.48x10^{-4}$b	$8.38x10^{-4}$b	--
5	$3.46x10^{-5}$a	$3.23x10^{-5}$a	$2.22x10^{-4}$b	$1.49x10^{-4}$b	--	--
10	$4.93x10^{-5}$a	$5.05x10^{-5}$a	--	--	$2.56x10^{-3}$c	--
20	$1.05x10^{-5}$a	$1.22x10^{-5}$a	--	--	$2.03x10^{-3}$c	
Weekly						
2.5	$2.82x10^{-5}$a	$3.00x10^{-5}$a	--	--	$2.23x10^{-4}$b	--
5	$1.28x10^{-5}$a	$1.32x10^{-5}$a	--	--	$8.19x10^{-4}$b	--
10	$4.21x10^{-5}$a	$4.44x10^{-5}$a	$1.47x10^{-4}$b	$1.11x10^{-4}$b	--	--
20	$7.67x10^{-5}$a	$1.14x10^{-4}$b	$8.67x10^{-5}$a	$6.17x10^{-5}$a	$1.78x10^{-4}$b	--
Biweekly						
2.5	--	--	--	--	$7.93x10^{-4}$b	
10	$3.50x10^{-5}$a	$3.65x10^{-5}$a	$1.93x10^{-4}$b	$2.74x10^{-4}$b	$3.61x10^{-4}$b	--
20	$3.55x10^{-5}$a	$2.30x10^{-5}$a	$3.39x10^{-4}$b	$.70x10^{-4}$b	--	--
Monthly						
2.5	--	--	--	--	$4.40x10^{-3}$c	--
10	$6.61x10^{-5}$a	$6.44x10^{-5}$a	--	--	--	--
20	$2.42x10^{-5}$a	$2.59x10^{-5}$a	--	--	$2.70x10^{-3}$c	--

Means with same letters along the rows are not significantly different at $p<0.05$

moisture in monthly watering regime (Tab. 7). The data on monthly watering regime suggested that *Euphorbia milii* was the most xeromorphic species followed by *Agave americana* and *Aloe vera* even though the latter species transpired less in weekly and biweekly watering regimes at 10%-20% soil moisture (Tab. 7). Hence *Euphorbia milii* requires less frequent watering than the other two species. *Agave americana* was the most robust in coping with all the watering regimes while *Aloe vera* was the least robust in this regard. The higher transpiration rate in *Agave americana* may be related to the tetracytic stomata and high stomatal size. While the low transpiration rate in *Euphorbia milii* and *Aloe vera* may be related to paracytic stomata and smaller size of stomata. There are therefore, significant differences at $p<0.05$ in the rate of transpiration across the soil moisture contents among the three species. Earlier work of Carr and Carr (1990) revealed that large number of subsidiary cells per stoma may be responsible for a more precise and rapid regulation of stomatal opening. This claim was confirmed by Obiremi and Oladele (2001), AbdulRahaman and Oladele (2003), Oyeleke *et al*. (2004) and Saadu *et al*. (2009) in stomatal complex types of some species of *Citrus,* vegetables, afforestation tree and tuber species where those stomata with large number of subsidiary cells transpired faster than those with small subsidiary cells. Meanwhile, the variation in rates of transpiration among the watering frequencies is explained by Awad and Castro (1992) that when the water supply is reduced, the stomatal guard cells lose solutes, increasing their water potential, and increasing their abscisic acid (ABA) level thus, causing reduction in their pressure potential and subsequently stomatal closure.

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