Response of Trichomes to Water Stress in Two Species of *Jatropha*

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Abstract: Background: The need to reduce water wastage on irrigation of plants in view of global water crisis was the basis for this study. **Context and purpose of the study:** Thus two *Jatropha* species namely *Jatropha gossypifolia* Linn. and *Jatropha curcas* Linn. were propagated in a greenhouse and subjected to 4 watering frequencies i.e., daily, weekly, biweekly and monthly, each with 5 varying soil moisture contents i.e., 1.25, 2.5, 5, 10 and 20%. After six weeks of watering in the greenhouse, the leaves of surviving seedlings were studied anatomically. Aim of the study was to determine anatomical features which may be adaptations to water stress and thus can serve as indices for frequency of watering. **Results:** Water stress results in high trichome density and high trichome index. In *J. gossypifolia*, unicellular and multicellular trichomes are present while in *J. curcas* trichome types are long branched unicellular, long branched multicellular, long unbranched unicellular, long unbranched multicellular short unbranched unicellular and scale. Trichome density and index were higher in *J. gossypifolia* than in *J. curcas*. **Conclusion:** The relationship between water stress, trichome density and index as well as likely influence of trichomes on transpiration was discussed.

Key words: Irrigation, *Jatropha* species, trichomes, transpiration and water stress

INTRODUCTION

Trichomes are one of the epidermis fine outgrowths or appendages on plants and certain protists. These are of diverse structures and functions. Examples are hairs, glandular hairs scales and papillae (Esau, 1965; Yu *et al.*, 2010). The trichomes (pubescences) that often cover the plant body are the result of divisions of epidermal cells. They may be either unicellular or multicellular and are either glandular, consisting of a stalk terminating in a glandular head, or nonglandular, consisting of elongated tapering structures (Encyclopaedia Britannica, 2009). The plant epidermis is a multifunctional tissue playing important roles in water relations, defense and pollinator attraction (Glover, 2000). With regard to water economy, among the possible indicators of water requirements for plants are stomatal complex types and trichome types and densities Abdulrahaman, 2009; Saadu *et al.*, 2009). Trichomes are prominent in water economy, according to the (Esau, 1965; Straburger, 1975; Newman, 1976; Encyclopaedia Britannica, 2009) leaf and stem trichomes increase the reflection of solar radiation, thereby reducing internal temperatures and thus reduce water loss in plants growing under arid conditions. Plants are prone to loss of water during the hot sunny days via a process called transpiration. Transpiration is the process where water contained in liquid form in plants is converted to vapour and released to the atmosphere. Much of water taken up

by plants is released through transpiration (Tanner and Beeves, 2001; Oladele, 2002; Burghardt and Riederer, 2003; George *et al.*, 2007; Metselaar and Lier, 2007). About 99% water uptake by plants is lost to the atmosphere in form of water vapor (Oladele, 2002). To prevent too much water loss plant epidermis is equipped with wax cuticle, stomata and trichomes.

Two *Jatropha* species were selected for the purpose of this study based on the economical importance. These are *Jatropha curcas* and *Jatropha gossypifolia*. *Jatropha gossypifolia* is a bushy, gregarious shrub of about 1.8 m in height. The leaves are about 3-5 lobed, palmately. The flowers are red-crimson or purple in corymbs, with greenish seeds in capsule (Oudhia, 2001). This plant is believed to originate from Central to South America and Caribbean. Different parts of *J. gossypifolia* are used in different countries in many ways. *J. gossypifolia* is a multipurpose plant with many attributes and considerable potential. The plant has traditional uses such as emetic, emmenagogue, itches, blood purifier, antibacterial, antifertile, anticancer, antiulcer, intermittent fevers, carbuncles eczema, sores on the tongues of babies, swollen mammae, stomachache, bathing of wounds and venereal diseases. Various chemical constituents such as terpenoids, flavonoids, alkaloids, lignan, fatty acids, amino acids have been isolated and identified (Balee, 1994; Morton, 1968; Kakade *et al.*, 2008). The bark contains the alkaloid

jatrophine and a lignin (jatroiden) is found in its stem. Seeds are emetic, purgative and used for body pain. Plant is antibiotic, insecticidal and used for toothache and as blood purifier (Oduola *et al.*, 2005). The stem latex of *J. gossypifolia* is routinely used in southern Nigeria by herbalists, rural dwellers and some people in urban centers to stop bleeding from nose, gum and skin (Oduola *et al.*, 2005). Also, Kumar *et al.* (2006) reported that crude extract of *Jatropha gossypifolia* exhibited significant antimicrobial activity and properties that support folkloric use in the treatment of some diseases. If, however, the seed and the plant is ingested without proper processes they are poisonous. *J. gossypifolia* is an ornamental and hedge plant (Iwu, 1993) used in beautification of many residential houses, recreation centers and offices. The contrast between the purple-leaves and/or red-leaves and green fruit is something special.

Jatropha curcas is a multipurpose plant with many attributes and considerable potential. It is a tropical plant that can be grown in low to high rainfall areas and can be used to reclaim land, as a hedge and/or as a commercial crop. Thus, growing it could provide employment, improve the environment and enhance the quality of rural life (Openshaw, 2000). *Jatropha curcas*, the physic nut, is frequently grown in tropical and subtropical areas throughout the world, as an ornamental plant and occasionally for medicinal purposes. The physic nut as typical of many members of Euphorbiaceae, has a caustic, milky sap and toxic seed. There apparently are varieties in the toxicity of the seeds, some varieties are considered toxic and others are harmless. The toxic and harmless varieties cannot be distinguished visually. *J. curcas* is an annual shrub or short-lived tree up to about 15ft (5 m) tall. Stems are thick, green, glabrous, mostly herbaceous or somewhat succulent, becoming woody at the base. Leaves are alternate, long petioled, palmately veined, cordate to truncate at the base, about 6 in (1.50 m) wide, margin irregular or with 3-5 shallow lobes, points acute or obtuse. Flowers are small, yellow, unisexual, in clusters in leaf axils, mostly hidden in foliage. Fruit an ovoid, 3-locular capsule, at first green and fleshy, becoming brownish or almost black and dry at maturity, containing up to 3 black seeds about ¾ in (20 mm) long.

Usefulness of this plant is enormous and cut across almost all sectors. These include: young leaves may be safely eaten, steamed stewed; pounded leaves are applied near horses' eyes to repel flies in India; *J. curcas* leaves help in dressing the wound; flowers of the species are listed as a honey plant; nuts are sometimes roasted and eaten, although they are purgative; nuts can be burned like candlenuts when strung on grass; also nuts are used

as contraceptive in south Sudan; seeds are also used as a contraceptive in south Sudan; seed can be used as bio-diesel for any diesel engine without modification; the oil obtained from seeds has been for illumination, soap, candles, cooking, in engines, varnishes, pest control and medicinal for skin diseases, as purgative; pounded seeds are used in tanning; press cake of *J. curcas* becomes organic fertilizer and soil improver; root ashes are used as a salt substitute; root can be used to kill mollusks, has been listed for homicide, piscicide and raticide as well; *J. curcas* roots help in making yellow dye; bark is used as a fish poison; dark blue dye and wax can be produced from the bark of the *J. curcas* plants; latex strongly inhibits the watermelon mosaic virus; sap stains linen. Sometimes used for marking; Mexicans grow the shrub as a host for the lac insect, which is used in medicine as hepatoprotective and antiobesity drug (Openshaw, 2000; Pramanik, 2003; MacIntyre, 2007).

In country such as India, *J. curcas* is extensively exploited in afforestation to check desertification. Government is seriously spending on its propagation and people are encouraged to participate in this laudable programme. Aside checking desert encroachment, some pilot biodiesel plants are built to produce biodiesel oil from *J. curcas* seeds for moving engines.

In this study, the response of trichomes to water stress as well as relationship between the trichomes and rate of transpiration were investigated in two species of *Jatropha* namely *J. gossypifolia* and *J. curcas*.

MATERIALS AND METHODS

Five seeds each of *J. curcas* and *J. gossypifolia* were placed in 5 sets of plastic pots, filled with loamy soils of 5 regimes of moisture contents namely 1.25, 2.5, 5, 10 and 20%. A total of 300 pots per species were maintained in a greenhouse at University of Ilorin, Ilorin, Nigeria. The plants in soils of each moisture content were subjected to 4 regimes of watering namely daily, weekly, biweekly and monthly (Table 1, 2). The experiments were conducted for a period of 4 months.

Times (days) taken for emergence of leaves were noted. Leaves were harvested for anatomical study. Leaf cuticles were macerated in concentrated nitric acid or trioxonitrate (v) acid, rinsed in distilled water, stained in 1% aqueous safranin solution and mounted in dilute

Table 1: Soil and water regimes used for raising the study materials

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

Table 2: Watering frequencies and regimes used for raising seedlings of the study materials

Watering intervals	Soil moisture content (%)
Daily	1.25
	2.50
	50.00
	10.00
	20.00
Weekly	1.25
	2.50
	5.00
	10.00
	20.00
Biweekly	1.25
	2.50
	5.00
	10.00
	20.00
Monthly	1.25
	2.50
	5.00
	10.00
	20.00

glycerine. Parameters on trichome determined were trichome density, index and type (Dilcher, 1974) and frequency of each trichome type was expressed as percentage occurrence of such complex type based on all occurrences. Statistical analysis of data consisted of Analysis of Variance and Duncan Multiple Range Test (Bailey, 1995; Duncan, 1955).

RESULTS

Trichomes: *Jatropha gossypifolia*: Trichome types present were unicellular and multicellular (Fig. 1) in all seedlings of all watering regimes (Table 3). High trichome density (700.12 and 400.00 mm^{-2}) on abaxial and adaxial surfaces of 5 cc daily watering regime plant and low trichome density (41.25 mm^{-2}) on adaxial surface of 20 cc monthly watering regime plant. High trichome index

Table 3: Some correlations between trichome and transpiration rate in *Jatropha gossypifolia* propagated with different % moisture contents

Watering regimes (%)	Leaf surface	Trichome type	Trichome frequency (%)	Trichome density (mm^{-2})	Trichome index (%)
Daily					
1.25	Abaxial	Unicellular, Multicellular	100.00	150.00 ^d	67.06 ^b
	Adaxial	Unicellular, Multicellular	100.00	110.00 ^f	58.72 ^{bc}
2.5	Abaxial	Unicellular, Multicellular	100.00	120.02 ^f	37.05 ^d
	Adaxial	Unicellular, Multicellular	100.00	124.00 ^f	34.06 ^d
5	Abaxial	Unicellular, Multicellular	100.00	700.12 ^a	92.69 ^a
	Adaxial	Unicellular, Multicellular	100.00	400.00 ^b	86.33 ^a
10	Abaxial	Unicellular, Multicellular	100.00	130.52 ^d	59.15 ^{bc}
	Adaxial	Unicellular, Multicellular	100.00	146.00 ^d	57.54 ^c
20	Abaxial	Unicellular, Multicellular	100.00	100.21 ^f	80.31 ^a
	Adaxial	Unicellular, Multicellular	100.00	180.00 ^d	58.69 ^c
Weekly					
5	Abaxial	Unicellular, Multicellular	100.00	100.00 ^f	63.00 ^b
	Adaxial	Unicellular, Multicellular	100.00	150.01 ^d	68.51 ^b
10	Abaxial	Unicellular, Multicellular	100.00	231.00 ^c	74.06 ^b
	Adaxial	Unicellular, Multicellular	100.00	170.05 ^d	68.22 ^b
20	Abaxial	Unicellular, Multicellular	100.00	305.00 ^{bc}	70.11 ^b
	Adaxial	Unicellular, Multicellular	100.00	220.00 ^c	76.00 ^{bb}
Biweekly					
20	Abaxial	Unicellular, Multicellular	100.00	60.24 ^e	82.75 ^a
	Adaxial	Unicellular, Multicellular	100.00	72.03 ^e	84.38 ^a
Monthly					
20	Abaxial	Unicellular, Multicellular	100.00	55.55 ^e	84.86 ^a
	Adaxial	Unicellular, Multicellular	100.00	41.25 ^e	17.19 ^c

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

Fig. 1: Surface view of leaf epidermis abaxial (A) and adaxial (B) of *Jatropha gossypifolia* showing paracytic (p) and brachyparacytic (b) stomata and unicellular trichomes (u) x2000

Table 4: Some correlations between trichome and transpiration rate in *Jatropha curcas* propagated with different % moisture contents

Watering regimes (%)	Leaf surface	Trichome type	Trichome frequency (%)	Trichome density (mm ⁻²)	Trichome index (%)
Daily					
1.25	Abaxial	LBU, LBM, LUU, LUM, SUU and Scale	2.25 and 97.75	80.67 ^a	9.68 ^g
	Adaxial	LBU, LBM, LUU, LUM, SUU and Scale	4.00 and 96.00	45.00 ^g	27.84 ^d
2.5	Abaxial	LBU, LBM, LUU, LUM, SUU and Scale	1.60 and 98.40	124.25 ^c	42.40 ^e
	Adaxial	LBU, LBM, LUU, LUM, SUU and Scale	1.00 and 99.00	90.00 ^d	41.03 ^e
5	Abaxial	LBU, LBM, LUU, LUM, SUU and Scale	6.25 and 93.75	100.00 ^e	32.96 ^d
	Abaxial	LBU, LBM, LUU, LUM, SUU and Scale	3.00 and 97.00	320.00 ^a	78.29 ^a
10	Abaxial	LBU, LBM, LUU, LUM, SUU and Scale	3.00 and 97.00	30.50 ^g	15.63
	Abaxial	LBU, LBM, LUU, LUM, SUU and Scale	2.00 and 98.00	80.00 ^e	31.24 ^d
20	Abaxial	LBU, LBM, LUU, LUM, SUU and Scale	2.00 and 98.00	62.51 ^f	27.75 ^d
	Abaxial	LBU, LBM, LUU, LUM, SUU and Scale	2.00 and 98.00	100.37 ^e	53.65 ^b
Weekly					
10	Abaxial	LBU, LBM, LUU, LUM, SUU and Scale	3.04 and 96.96	68.00 ^f	40.04 ^e
	Abaxial	LBU, LBM, LUU, LUM, SUU and Scale	3.00 and 97.00	62.02 ^f	29.16 ^d
20	Abaxial	LBU, LBM, LUU, LUM, SUU and Scale	2.50 and 97.50	101.34 ^e	50.77 ^b
	Abaxial	LBU, LBM, LUU, LUM, SUU and Scale	2.00 and 98.00	82.08 ^e	41.73 ^c
Biweekly					
10	Abaxial	LBU, LBM, LUU, LUM, SUU and Scale	5.00 and 95.00	10.65 ^h	16.98 ^f
	Abaxial	LBU, LBM, LUU, LUM, SUU and Scale	5.00 and 95.00	60.00 ^f	38.97 ^d
20	Abaxial	LBU, LBM, LUU, LUM, SUU and Scale	5.00 and 95.00	128.00 ^e	48.66 ^{bc}
	Abaxial	LBU, LBM, LUU, LUM, SUU and Scale	5.00 and 95.00	41.00 ^g	37.82 ^d
Monthly					
10	Abaxial	LBU, LBM, LUU, LUM, SUU and Scale	2.05 and 97.95	82.50 ^e	20.23 ^e
	Abaxial	LBU, LBM, LUU, LUM, SUU and Scale	2.05 and 97.95	201.00 ^b	47.82 ^{bc}
20	Abaxial	LBU, LBM, LUU, LUM, SUU and Scale	5.00 and 95.00	205.00 ^b	47.92 ^c
	Abaxial	LBU, LBM, LUU, LUM, SUU and Scale	5.00 and 95.00	310.00 ^a	70.48 ^a

LBU: Long, branched unicellular, LBM: Long, branched multicellular, LUU: Long, unbranched unicellular, LUM: Long, unbranched multicellular, SUU: Short, unbranched unicellular. Means with same letters along the columns are not significantly different at $p > 0.05$

Fig. 2: Surface view of leaf epidermis, abaxial (A) and adaxial (B) of *Jatropha curcas* showing paracytic (p) stomata and long-branched multicellular (c), long-unbranched unicellular (d), long-unbranched multicellular (e), short unicellular and scale (s) trichomes x2000

(92.69%) on abaxial surface of 5 cc daily watering regime and low trichome index (17.19%) on adaxial of 20 cc monthly watering regime plant. This indicated that occurrence of trichomes increased as water supplied for irrigation reduced except in 5 cc daily watering regime plants. There were many trichomes on the adaxial surface than on the abaxial.

***Jatropha curcas*:** Trichome was conspicuously present in all leaves of all seedlings across the watering regimes and intervals. Trichome types included long-branched uniseriate, long-branched multicellular, long-unbranched

unicellular, long-unbranched multicellular, short-unbranched unicellular and scale (Fig. 2) on both leaf surfaces with scale type being the more frequent of the six (Table 4). Trichome density was high (320.00 mm⁻²) on the adaxial surface of 5 cc daily watering regime seedling, it was low (10.65 mm⁻²) on the abaxial surface of 10 cc biweekly regime. Generally, trichome density was higher in water-stressed plants of monthly watering regimes than in all other watering regimes. High frequency of long, branched and unbranched unicellular and multicellular and short unbranched unicellular (7.02%) and least frequency of scale (92.98%) occurred on the adaxial

surface of 20 cc biweekly watering regime seedling while the least frequency of long, branched and unbranched unicellular and multicellular and short unbranched unicellular (1.00%) and high frequency of scale trichomes (99.00%) occurred on the adaxial surface of 2.5 cc daily watering regime seedling. High trichome index (78.29%) occurred on the adaxial surface of 5 cc monthly watering regime seedling and low trichome index (9.68%) occurred on the abaxial surface of 1.25 cc daily regime (Table 4). High trichome density and trichome index on the adaxial surfaces and low density and trichome index on the abaxial surfaces were adaptations to water conserving and/or water stress. Similarly increase in trichome frequency, trichome density and trichome index occurred as the water supplies decreases. The occurrence of more trichomes on the adaxial surface than abaxial is to prevent water lost through the transpiration process since the adaxial surface is exposed to direct sunlight.

DISCUSSION

Among the possible indicators of water requirements for plants are stomata complex types and trichome types and stomatal densities (Esau, 1965; Straburger, 1975; Newman, 1976). Trichomes are specialized unicellular or multicellular structures derived from the epidermal cell layer which may have various functions depending on the plant species and organs (Rodriguez *et al.*, 1983; Manetas, 2003; Sarret *et al.*, 2006).

In this present study trichomes were conspicuously seen in the two species of *Jatropha*, namely *J. gossypifolia* and *J. curcas* (Table 3, 4). These trichomes can be broadly classified into three types: unicellular, multicellular and scale trichomes (Metcalf and Chalk, 1988) in the two species. Unicellular and multicellular trichome types occurred in *J. gossypifolia* with 100.00% on both abaxial and adaxial surfaces while in *J. curcas* long-branched unicellular, long-branched multicellular, long-unbranched unicellular, long-unbranched multicellular, short-unbranched unicellular and scale trichomes occurred on both surfaces with 3.02% long and short unicellulars and multicellulars and 96.98% scale on abaxial and 3.00% long and short unicellular and multicellular trichomes and 97.00% scale on adaxial (Table 4). Trichome density and trichome index were high on abaxial surface (141.99 mm⁻²-density; 75.98%-index) in *J. gossypifolia* and low (94.30 mm⁻²-density; 34.50%-index) in *J. curcas*. While on adaxial surface, trichome density was high in *J. curcas* (126.28 mm⁻²) and low (12.33mm⁻²) in *J. gossypifolia*; trichome index was high (57.89%) in *J. gossypifolia* and low (44.85%) in *J. curcas*.

In the earlier study by Abdulrahman (2009) shown that relative low transpiration in *J. curcas* than in *J. gossypifolia* could be due to low stomatal density and low stomatal index which covered the leaf surfaces and thus reduced loss of water to the atmosphere. Meanwhile, higher rate of transpiration in *J. gossypifolia* could as well be due to the fact that high trichome density and trichome index also themselves transpired. According to Shields (1950), living trichomes, which themselves lose water do not protect the plants from excessive transpiration as do dead trichomes which form protective layers. Dead trichomes are believed to reduce transpiration and at the same time act as a protection against direct sunlight (Straburger, 1975) and are very common on xerophytes (Fahn, 1990). This result is however, contradictory to the earlier study of Abdulrahman and Oladele (2004) and Oyeleke *et al.* (2004) where presence of trichomes in some Nigerian vegetable species and some afforestation tree species respectively played major role in water economy by reducing transpiration rate.

Plant trichomes commonly serve a role in mechanical and chemical defense against herbivores. Trichomes also have potential to alter physiology by reducing the amount of light absorbed by leaves, lowering temperatures and reducing water loss (Levizou *et al.*, 2005; Galmes *et al.*, 2007; Franks and Farquhar, 2007; Holeski, 2007; Kato *et al.*, 2007). For example, Woodman and Fernandes (1991) and Bubbleman and Vandermeer (2009) reported that trichomes in *Verbascum thapsus* L. and *Cannabis* plants function both in herbivore defense and in reducing transpiration. In many species dense leaf hairs are beneficial because they reflect light and thus, shade the leaf which reduce temperature and water loss, rather than significantly increasing the boundary layer (Ehleringer, 1983). Trichomes also contribute to longevity of leaves. Sandquist and Ehleringer (1997, 1998) reported that *Encelia farinosa* Gray ex Torr plants from drier habitats had greater pubescence. Plants with more pubescence were able to retain their leaves longer into periods of drought and tended to have lower leaf light absorptance, stomatal conductance and photosynthetic rates. However, the role of trichomes in controlling rate of transpiration depend on whether they are dead or alive (Straburger, 1975).

High trichome density and high trichome index on the adaxial surface and low density and index of trichomes on the abaxial surface of leaves of *J. curcas* and *J. gossypifolia* are adaptations to water conservation and/or water stress. Abdulrahman (2009) observed that the two species of *Jatropha* transpired lesser than species such as *Canna indica*, *Agave americana* and *Aloe vera* without trichome presence on their leaf

surfaces. More trichomes on the adaxial surface than abaxial is to prevent water loss through the transpiration process since the adaxial surface are exposed to direct sunlight.

This, therefore, suggests that occurrence of trichomes on the surfaces of leaves discouraged high rate of transpiration in plants (Quarrie and Jones, 1977; Karabourniotis *et al.*, 1995; Smith and Hare, 2004; Levizou *et al.*, 2005; Galmes *et al.*, 2007; Franks and Farquhar, 2007; Holeski, 2007; Kato *et al.*, 2007). And in essence it complements the water economy role of stomata. This trend had earlier been reported in some selected vegetable species in Nigeria and in some ornamental plant species (Abdulrahman and Oladele, 2004; Abdulrahman, 2009).

CONCLUSION

Occurrence of trichomes on leaf surfaces especially on adaxial surface may serve as an index for level of frequency of watering plants. In these two species, however, the influence of trichome density and index was not felt as far as rate of transpiration is concern.

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