



PHYSIO-ANATOMICAL EVALUATION OF SOME TREE SPECIES FOR AFFORESTATION IN DRY REGION OF KOGI STATE, NIGERIA

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ABSTRACT

The stomatal features of plant species have ability to release water vapour into the air. Hence, correlations between the stomatal features and transpiration rate of five tree species namely *Daniellia oliveri*, *Delonix regia*, *Piliostigma thonningii*, *Azadirachta indica* and *Tectona grandis* was studied to evaluate their capacity for afforestation. The leaf epidermal layers were isolated using nail polish; they were observed under the light microscope to examine their stomatal features. The transpiration rate was evaluated using the cobalt chloride method. The results revealed that *Delonix regia* and *Piliostigma thonningii* are amphistomatic; while the remaining three species are hypostomatic. The stomatal complex types observed are anomocytic, brachyparacytic and paracytic. The stomatal density ranged from 14.41 mm⁻² to 93.61 mm⁻²; the stomatal index ranged from 7.15% to 28.23%; while the stomatal size ranged from 11.19 μm² to 29.36 μm². The study revealed that stomatal traits such as hypostomatic leaf nature, stomatal complex types (i.e. paracytic, brachyparacytic), low stomatal index, small stomatal size possessed by the plant species may be responsible for their lower rate of transpiration; which in turn might be suitable for their afforestation in dry areas. Therefore, *Tectona grandis* which released the lowest amount of water (2.49 × 10⁻⁶ mol m⁻²s⁻¹) into the atmosphere might be the most suitable for afforestation, followed by *Piliostigma thonningii*, *Daniellia oliveri*, *Delonix regia* and lastly *Azadirachta indica* (2.97 × 10⁻⁶ mol. m⁻²s⁻¹). Conclusively, the stomatal features showed positive correlations with transpiration rates; thereby enhancing the potentials of the studied species for afforestation in dry region.

Keywords: Afforestation, Transpiration rate, Stomatal complex types, Correlations, Anatomical traits

INTRODUCTION

Trees play significant role in the water cycle as more than 98% of water absorbed by plants are lost to the atmosphere through stomatal, lenticular and cuticular transpiration; thereby humidifying the atmosphere (Oladele, 2002; AbdulRahaman *et al.*, 2013; Omolokun, 2019).

Trees can serve as windbreaks or shelter belts which reduce wind speed, thereby preventing loss of properties by windstorms (Obiremi and Oladele, 2001; Fadamiro *et al.*, 2004; Fuwape *et al.*, 2018). They provide habitat for all kind of wildlife; improve the quality of life as they purify the air, land and water of pollutants including dust and chemicals; reduces heat build-up and noise pollution and many other social benefits (Maji *et al.*, 2010; Kumar *et al.*, 2013; Agbelade *et al.*, 2016; Lawal *et al.*, 2018).

The exploitation of trees for domestic, agricultural and industrial purposes over the years has been indiscriminate, thus resulting in deforestation, which is a progressive removal of trees and other vegetation cover from natural forest ecosystem due to human activities (Omolokun and Oladele, 2010). The process of afforestation to fix atmospheric carbon (iv) oxide has been proposed as a major policy response to climate change (Sedjo, 1989; Obiremi and Oladele, 2001; Oladele and AbdulRahaman, 2008; AbdulRahaman *et al.*, 2013).

The assessment of leaf epidermis in surface view shows that there are wide variations in the distribution, size, shape, type and frequency of stomata, trichomes and epidermal cells (Metcalf and Chalk, 1988; AbdulRahaman, 2009). Therefore, plants growing in different habitats exhibit various anatomical adaptations which enable them to survive in a particular environment (Gostin, 2009; Erdal and Demirtas, 2010; Omolokun and Oladele, 2010).

Stomatal features influence the rate of transpiration through stomatal conductance (i.e. the rate of gas exchange, which is carbon dioxide uptake and water loss through the stomata). Greater stomatal conductance indicates potentially higher influx

of carbon dioxide into the leaves for photosynthesis and higher rate of water loss from the leaves through transpiration (Oyeleke *et al.*, 2004; Omolokun, 2019).

The number of subsidiary cells having direct contact with the guard cells (i.e. stomatal complex types) may be related to their level of transpiration rate (Obiremi and Oladele, 2001). Therefore, the more the subsidiary cells surrounding the guard cells, the faster the opening of the stomata and vice-versa (AbdulRahaman and Oladele, 2003; Omolokun, 2019).

Stomatal features such as nature of stomata on the leaf surfaces (i.e. hypostomatic and amphistomatic), composition of stomatal complex types (i.e. heterogenous or homogenous), stomatal density, stomatal index and stomatal size influence transpiration rate (Saadu *et al.*, 2009; AbdulRahaman *et al.*, 2013; Omolokun, 2019).

The correlation between the leaf anatomical features and transpiration rates had been reported to be essential in the selection of plant species for afforestation in dry location (Obiremi and Oladele, 2001; Oyeleke *et al.*, 2004; AbdulRahaman *et al.*, 2013).

The current emphasis and concern in environmental issues, especially desertification and climate change necessitates prompt response. Therefore, this study is conducted to elucidate the physiological and anatomical capacity of some tree species for water conservation, with a view to determining their level of suitability for afforestation in Lokoja metropolis.

MATERIALS AND METHODS

Collection and Identification of Plant Species

The leaves of the studied plant species were collected in June, 2015 from Salem University Campus, Lokoja, Kogi State, North Central Region of Nigeria (Table 1); which is a relatively dry ecological zone (i.e. Guinea savanna). The identification of the plant species was authenticated at Lagos State University Herbarium.

Table 1: Information on the Studied Species

S/N	Plant Species	Common Name	Family	Voucher Number
1.	<i>Daniellia oliveri</i> (Rolfe) Hutch & Dalz.	Africa copaiba, balsam	Fabaceae	LSH 001173
2.	<i>Delonix regia</i> (Boj. Ex Hook) Raf	flamboyant tree, flame tree	Fabaceae	LSH 001174
3.	<i>Piliostigma thonningii</i> (Schum) Milne-Red head	poinciana, gulmohar tree	Fabaceae	LSH 001175
4.	<i>Azadirachta indica</i> A. Juss.	neem tree	Meliaceae	LSH 001176
5.	<i>Tectona grandis</i> L. f.	teak, Indian oak	Verbenaceae	LSH 001177

LSH – Lagos State University Herbarium

Sampling and Isolation of Leaf Epidermal Layers

Three (3) leaves of each of the plant species were collected randomly for anatomical study. The nail polish method was used for the isolation of the leaf epidermal layers. It was carried out by rubbing transparent finger nail polish on the abaxial and adaxial surfaces of each leaf and allowed to dry. After drying, a short clear cellophane tape was firmly pressed over the dried nail polish on the leaf surfaces. The tape were carefully peeled from the leaf and affixed on a clean slide for microscopic studies (Mbagwu *et al.*, 2007).

Microscopic Observation of Leaf Surfaces

Observations were made on Olympus binocular light microscope (at a magnification of $\times 40$ objectives) to determine stomatal complex types and their frequencies, stomatal density, stomatal index and stomatal size. Measurements were taken with the aid of micrometer eye-piece graticule and final figure obtained with ocular constant. Sample size of 30 was used for each of the parameters. Photomicrographs of good preparations were taken using binocular light microscope fitted with Amscope Camera (Model MU 1000) at a magnification of $\times 2000$.

Identification and Determination of Frequency of Stomatal Complex Type

Stomatal complex types were identified based on the number of subsidiary cells per stoma (Omolokun and Oladele, 2010). The frequency of each stomatal complex type was determined as percentage occurrence of each stomatal complex type relative to all occurrences using thirty fields of view at $\times 40$ objective as a quadrat (Omolokun, 2019).

Determination of Stomatal Density

The mean stomatal density was determined as the number of stomata per square millimeter (0.152mm^2 field of view) based on the entire leaf surface (Omolokun and Oladele, 2010).

Determination of Stomatal Index

The mean stomatal index was determined as number of stomata per square millimeter divided by number of stomata plus number of ordinary epidermal cells per square millimeter multiplied by 100. It was expressed mathematically according to Omolokun *et al.*, (2023) using the formula below:

$$SI = S/E + S \times 100$$

Where: SI = stomatal index; S = number of stomata per square millimetre.

E = number of ordinary epidermal cells per square millimetres.

Determination of Stomatal Size

The mean stomata size of each of the species was determined by measuring the length and breadth of guard cell (using an eye-piece micrometer) multiplied by the Franco's constant. It was expressed mathematically as: $SS = L \times B \times K$; Where: SS = Stomatal size; L = Length; B = Breadth; K = Franco's constant = 0.78524. This method followed those of Franco (1939) and adopted by Omolokun (2019).

Determination of Transpiration Rate

The cobalt chloride method paper was used to determine the transpiration rate of each species (Dutta, 2003; AbdulRahaman *et al.*, 2013). The strips of filter paper of 2cm by 6cm dimension was cut and immersed in 20% cobalt chloride solution. The strips was thoroughly dried in an oven. The property of cobalt paper is that it is deep blue when dried, but in contact with moisture, it turns pink. The blue dried strips were placed in a sealed, air tight polythene bag and weighed (W_1) using mettler balance. It was transferred quickly to the field and affixed with a string to the marked small branch of the plant with three leaves. The time (in seconds) taken for the strips to turn pink was noted. Once turned pink, the bag was quickly untied, sealed again and weighed (W_2). The weight of water transpired was determined as W_2 minus W_1 . The leaf area of the leaves used determined using the leaf area meter. The transpiration rate was expressed as mole per square meter per second (i.e. $\text{mol/m}^2/\text{sec}^{-1}$).

Statistical Analysis

The data collected were analyzed using Statistical Packages for Social Sciences (SPSS) version 20.0 software. Means were calculated using one way analysis of variance. The means with significant difference were separated using Duncan's Multiple Range Test (DMRT). A probability level of 0.05 was used as a bench mark for significant difference among parameters.

RESULTS AND DISCUSSION

Stomatal Features and Transpiration Rate

The leaf epidermal structures are presented in Plates 1 -5; while the stomatal features (i.e. stomatal density, stomatal index) and transpiration rates are presented in Table 2. Two of the studied species (*Delonix regia* and *Piliostigma thonningii*) are amphistomatic; while the remaining three species (*Daniellia oliveri*, *Azadirachta indica* and *Tectona grandis*) are hypostomatic. The stomatal types present are paracytic, brachyparacytic, and anomocytic.

The stomatal density ranged from 14.41mm^{-2} to 93.61mm^{-2} . The highest stomatal density (93.61mm^{-2}) was found in the abaxial surface of *Azadirachta indica*; while the lowest stomatal density (52.63mm^{-2}) was found in the abaxial surfaces of *Daniellia oliveri*.

The stomatal index ranged from 7.15% to 28.23%. The highest stomatal index (28.23%) was found in the abaxial surface of *Azadirachta indica*; while the lowest stomatal index (7.15%) was found in the adaxial surface of *Delonix regia*.

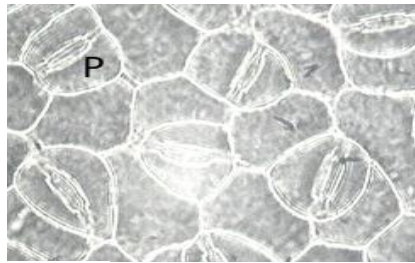
The stomatal size ranged from $11.19\mu\text{m}^2$ - $29.36\mu\text{m}^2$. The largest stomata ($29.36\mu\text{m}^2$) were found in the abaxial surface of *Azadirachta indica*; while the smallest stomata ($11.19\mu\text{m}^2$) were found in the abaxial surface of *Piliostigma thonningii*.

The transpiration rate ranged from $1.06 \times 10^{-6}\text{mol m}^{-2}\text{s}^{-1}$ to $2.97 \times 10^{-6}\text{mol m}^{-2}\text{s}^{-1}$. The highest transpiration rate ($2.97 \times 10^{-6}\text{mol m}^{-2}\text{s}^{-1}$) was found in the abaxial surface of *Azadirachta indica*; while the lowest transpiration rate ($1.06 \times 10^{-6}\text{mol m}^{-2}\text{s}^{-1}$) was found in the adaxial surface of *Piliostigma thonningii*.

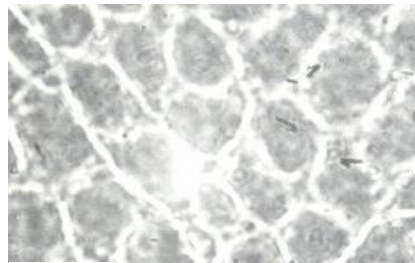
Table 2: Stomatal Features and Transpiration Rate of some Tree Species in Lokoja Metropolis

S/N	Plant Species	Family	Leaf Surface	Stomatal Complex Type	Frequency (% age)	Stomatal Density (mm ⁻²)	Stomatal Index (% age)	Stomatal Size (µm ²)	Transpiration Rate (mol m ⁻² s ⁻¹)
1.	<i>Daniellia oliveri</i>	Caesalpinia-ceae	Abaxial Adaxial	Paracytic -	100.00 -	93.61 ^a -	25.42 ^a -	25.44 ^{ab} -	2.61 × 10 ⁻⁶ ^{ab} -
2.	<i>Delonix regia</i>	Caesalpinia-ceae	Abaxial Adaxial	Brachyparacytic Brachyparacytic	100.00 100.00	65.55 ^{abc} 14.61 ^a	20.22 ^a 7.15 ^a	23.62 ^{ab} 24.44 ^a	1.56 × 10 ⁻⁶ ^b 1.11 × 10 ⁻⁶ ^a
3.	<i>Piliostigma thonningii</i>	Caesalpinia-ceae	Abaxial Adaxial	Brachyparacytic Paracytic Brachyparacytic	60.58 39.42 100.00	51.74 ^{bc} 14.41 ^a	16.78 ^a 11.09 ^a	11.19 ^b 12.23 ^b	1.45 × 10 ⁻⁶ ^b 1.10 × 10 ⁻⁶ ^a
4.	<i>Azadirachta indica</i>	Meliaceae	Abaxial Adaxial	Anomocytic -	100.00 -	81.08 ^{ab} -	28.23 ^a -	29.36 ^a -	2.97 × 10 ⁻⁶ ^a -
5.	<i>Tectona grandis</i>	Verbenaceae	Abaxial Adaxial	Brachyparacytic -	100.00 -	41.97 ^c -	18.76 ^a -	12.88 ^b -	2.49 × 10 ⁻⁶ ^{ab} -

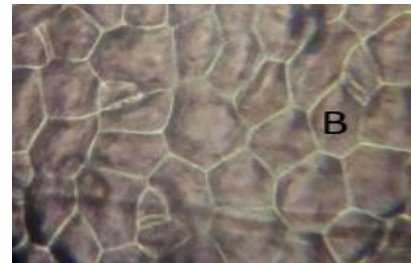
Means with same letters along the column are not significantly different at $p \leq 0.05$



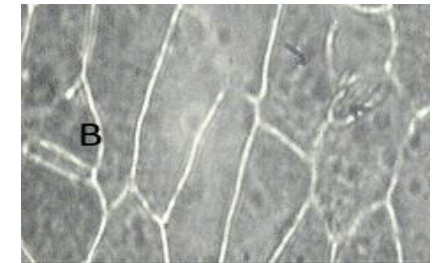
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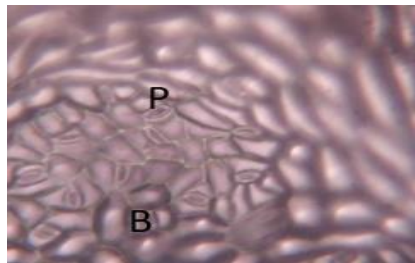
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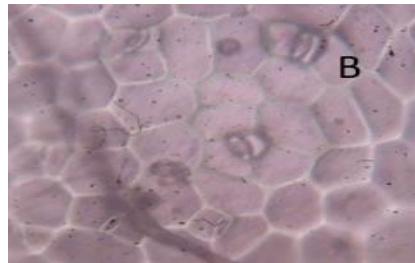
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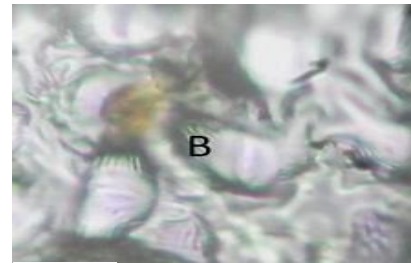
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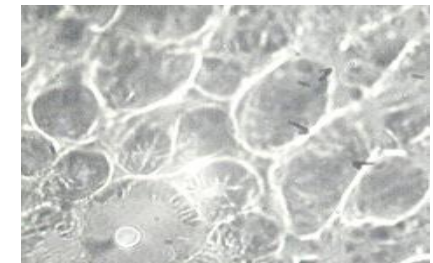
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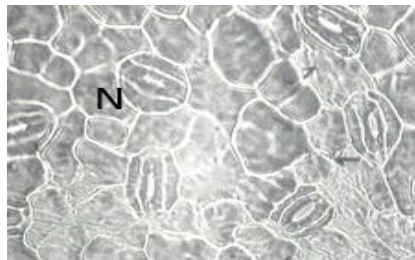
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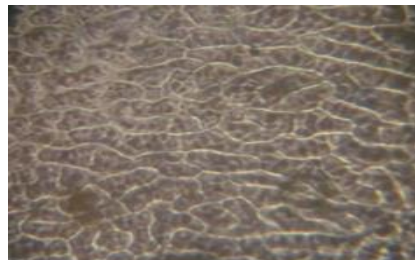
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Plate 1: Photomicrographs of abaxial and adaxial surfaces of *Daniellia oliveri* (a & b); *Delonix regia* (c & d); *Piliostigma thonningii* (e & f); *Tectona grandis* (g & h) and *Azadirachta indica* (i & j) showing B-brachyparacytic SCT, P-paracytic SCT, and N-anomocytic SCT respectively. Abaxial surface (a, c, e, g, i); Adaxial surface (b, d, f, h); SCT - stomatal complex type. All magnifications at $\times 2000$.

Correlation between Stomatal Features and Transpiration Rate of the Studied Species

Correlation between abaxial and adaxial stomatal features (i.e stomatal density, stomatal index, stomatal size) and transpiration rates are shown in Tables 3 and 4 respectively.

The abaxial surface revealed that there are positive correlations between stomatal features and transpiration rates. The adaxial surface revealed that there are positive strong significant correlations between the stomatal features and transpiration rates.

Table 3: Correlation Coefficient between Abaxial Stomatal Features and Transpiration Rates of Studied Plant Species

CHARACTERS	Stomatal Density	Stomatal Index	Stomatal Size	Transpiration Rate
Stomatal Density	1			
Stomatal Index	.875**	1		
Stomatal Size	.881**	.830**	1	
Transpiration Rate	.560*	.697**	.528*	1

*. Correlation is significant at the 0.05 level.

Table 4: Correlation Coefficient between Adaxial Stomatal Features and Transpiration Rates of Studied Plant Species

CHARACTERS	Stomatal Density	Stomatal Index	Stomatal Size	Transpiration Rate
Stomatal Density	1			
Stomatal Index	.951*	1		
Stomatal Size	.901*	.746**	1	
Transpiration Rate	.108	.097	.098	1

*Correlation is significant at 0.05 level

**Correlation is significant at 0.01 level

Discussion

The correlation between the stomatal features and transpiration rate of the studied species were examined to assess their potentials for afforestation. The studied species have hypostomatic and amphistomatic leaf nature. Plant species having stomata on only the abaxial surface of the leaf (i.e. hypostomatic) tend to have lower rate of transpiration; while those having stomata on both abaxial and adaxial surfaces (i.e. amphistomatic) are likely to have higher rate of transpiration (Oyeleke *et al.*, 2004; AbdulRahaman *et al.*, 2013; Omolokun, 2019). This investigation did not support this assertion, because the species *Azadirachta indica* that is hypostomatic had the highest rate of transpiration. This might probably be due to stronger influence of other factors such as the stomatal complex type and stomatal density.

This study portrayed that number of subsidiary cells surrounding the stomata (i.e. stomatal complex type) of each species may be a factor to its transpiration rates. For instance, *Azadirachta indica* having stomatal complex with five or more subsidiary cells (i.e. anomocytic) transpired faster than the remaining four species possessing stomatal complex types with two subsidiary cells (i.e. brachyparacytic, paracytic). This suggests that the former opens more quickly to allow water vapour to escape to the atmosphere, thereby encouraging higher rate of transpiration than the latter. This is in line with the findings of AbdulRahaman and Oladele (2009) and Omolokun (2019) on some palms and wastelands species respectively.

Stomatal density showed positive correlation with transpiration rate in this study. This is due to the fact that *Daniellia oliveri* and *Azadirachta indica* with higher stomatal density had higher rate of transpiration. This is in line with the findings of Oyeleke *et al.* (2004) and Saadu *et al.* (2009) on some *Polyalthia* and tuber species respectively.

Stomatal index portrayed positive correlation with transpiration rate. This implies that *Tectona grandis* with the lowest proportion of stomata (18.76%) on the abaxial surface had the lowest rate of transpiration ($2.49 \times 10^{-6} \text{ mol m}^{-2}\text{s}^{-1}$); while *Azadirachta indica* with the highest proportion of stomata (28.23%) on the leaf surface gave rise to the highest

rate of transpiration ($2.97 \times 10^{-6} \text{ mol m}^{-2}\text{s}^{-1}$). Similar pattern was reported by Saadu *et al.* (2009).

Pataky (1969) stated that stomata whose guard cells are less than $15 \mu\text{m}$ long are termed "small"; while those whose guard cells are more than $38 \mu\text{m}$ long are termed "large". However, stomata whose guard cells are within the range of $15\mu\text{m}$ - $38 \mu\text{m}$ could be described as "moderate"(AbdulRahaman and Oladele, 2009). Based on this information, the species *Tectona grandis* had small stomata ($12.85\mu\text{m}^2$); *Piliostigma thonningii* had small stomata on both the abaxial surface ($11.19\mu\text{m}^2$) and adaxial surface ($12.23 \mu\text{m}^2$) respectively. However, the species *Delonix regia* had moderate stomata on both the abaxial surface ($23.62 \mu\text{m}^2$) and adaxial surface ($24.44 \mu\text{m}^2$) respectively; *Daniellia oliveri* had moderate stomata ($24.44 \mu\text{m}^2$); while *Azadirachta indica* also possessed moderate stomata ($29.36 \mu\text{m}^2$). Stomatal size had been shown to be positively correlated with transpiration rate in this investigation; where the species *Tectona grandis* and *Piliostigma thonningii* with small stomata had low transpiration rate; while *Azadirachta indica* with the largest stomata on the abaxial surface had the highest transpiration rate. Similar trend was observed by some researchers (Oyeleke *et al.*, 2004 and AbdulRahaman and Oladele, 2009). Stomatal size had been reported to show correlation with stomatal density, where small stomata gave rise to high stomatal density and large stomata resulted to low stomatal density (Metcalf and Chalk, 1988; Beerling and Woodward, 1997; AbdulRahaman and Oladele, 2003). There was no such relationship in this work because *Tectona grandis* which had small stomata ($12.88 \mu\text{m}^2$) gave rise to low stomatal density (41.97 mm^{-2}); while *Daniellia oliveri* with moderate stomata ($24.44 \mu\text{m}^2$) resulted to high stomatal density (93.61 mm^{-2}). Similar trend was reported by Omolokun and Oladele (2010) on some timber species respectively.

This study revealed stomatal features in the five studied species that may be of relevance to their lower rate of transpiration; thereby indicating their conservation status. Therefore, *Tectona grandis* possessed brachyparacytic stomatal complex type, low stomatal density, low stomatal index, small stomata, hypostomatic leaf nature and transpiration rate of $2.49 \times 10^{-6} \text{ mol m}^{-2}\text{s}^{-1}$, *Piliostigma*

thonningii possessed brachyparacytic stomatal complex type, paracytic stomatal type, low stomatal density, low stomatal index, small stomata and transpiration rate of 2.55×10^{-6} mol. $m^{-2}s^{-1}$. *Daniellia oliveri* had paracytic stomatal complex type, low stomatal index, hypostomatic leaf nature and transpiration rate of 2.61×10^{-6} mol. $m^{-2}s^{-1}$. *Delonix regia* possessed brachyparacytic stomatal complex type, low stomatal index, hypostomatic leaf nature and transpiration rate of 2.67×10^{-6} mol. $m^{-2}s^{-1}$. Finally, the species *Azadirachta indica* had low stomatal index, hypostomatic leaf and transpiration rate of 2.97×10^{-6} mol. $m^{-2}s^{-1}$.

CONCLUSION

The study revealed that stomatal traits such as hypostomatic leaf nature, stomatal complex type with few subsidiary cells (i.e. paracytic, brachyparacytic), low stomatal index, small stomatal size possessed by the plant species may be responsible for their lower rate of transpiration; which in turn may be suitable for their afforestation in dry areas. However, *Tectona grandis* was the most suitable for afforestation, followed by *Piliostigma thonningii*, *Daniellia oliveri*, *Delonix regia* and the least was *Azadirachta indica*. Therefore, the stomatal features showed positive correlations with transpiration rates; thereby enhancing their potentials for afforestation in dry region.

RECOMMENDATION

It is suggested that the anatomical information from this research should be used to complement the morphological features for more effective appraisal of their afforestation status.

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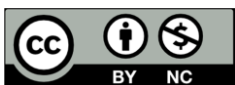
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