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 Danielli oliveri, Delonix regia, Piliostigma thomningii, 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 thomningii 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 × 10⁶ mol m⁻²s⁻¹) into the atmosphere might be the most suitable for afforestation, followed by Piliostigma thomningii, Daniellia oliveri, Delonix regia and lastly Azadirachta indica (2.97 × 10⁶ 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 habitats 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; Agbede 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. heterogeneous 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

<table>
<thead>
<tr>
<th>S/N</th>
<th>Plant Species</th>
<th>Common Name</th>
<th>Family</th>
<th>Voucher Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td><em>Daniellia oliveri</em></td>
<td>Africa copiba, balsam</td>
<td>Fabaceae</td>
<td>LSH 001173</td>
</tr>
<tr>
<td>2.</td>
<td><em>Delonix regia</em> (Boj. Ex Hook) Raf</td>
<td>flamboyant tree, flame tree poinciana, gulmohar tree</td>
<td>Fabaceae</td>
<td>LSH 001174</td>
</tr>
<tr>
<td>3.</td>
<td><em>Piliostigma thonnigii</em> (Schum) Milne-Red head</td>
<td></td>
<td>Fabaceae</td>
<td>LSH 001175</td>
</tr>
<tr>
<td>4.</td>
<td><em>Azadirachta indica</em> A. Juss.</td>
<td>neem tree</td>
<td>Meliaceae</td>
<td>LSH 001176</td>
</tr>
<tr>
<td>5.</td>
<td><em>Tectona grandis</em> L. f.</td>
<td>teak, Indian oak</td>
<td>Verbenaceae</td>
<td>LSH 001177</td>
</tr>
</tbody>
</table>

**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 fingernail 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 ×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. Photographs of good preparations were taken using binocular light microscope fitted with Amscope Camera (Model MU 1000) at a magnification of ×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 ×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 (0.152mm² 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 stoma 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:

\[ SI = \frac{S}{E + S} \times 100 \]

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. mol/m²·s).

**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 thonnigii*) are amphistomatic; while the remaining three species (*Danielli oliveri, Azadirachta indica* and *Tectona grandis*) are hypostomatic. The stomatal types present are paracytic, brachyparacytic, and anomocytic.

The stomatal density ranged from 14.41 mm⁻² to 93.61 mm⁻². The highest stomatal density (93.61 mm⁻²) was found in the abaxial surface of *Azadirachta indica*; while the lowest stomatal density (52.63 mm⁻²) was found in the abaxial surfaces of *Daniellia oliveri*. The stomatal index ranged from 7.15% to 28.23%. The highest stomatal density (93.61 mm⁻²) was found in the abaxial surface of *Azadirachta indica*; while the lowest stomatal density (52.63 mm⁻²) was found in the abaxial surfaces of *Daniellia oliveri*. The stomatal type 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 µm² to 29.36 µm². The largest stomata (29.36µm²) were found in the abaxial surface of *Azadirachta indica*; while the smallest stomata (11.19 µm²) were found in the abaxial surface of *Piliostigma thonnigii*. The transpiration rate ranged from 1.06 × 10⁻¹ mol m⁻²·s⁻¹ to 2.97× 10⁻¹ mol m⁻²·s⁻¹. The highest transpiration rate (2.97× 10⁻¹ mol m⁻²·s⁻¹) was found in the abaxial surface of *Azadirachta indica*; while the lowest transpiration rate (1.06 × 10⁻¹ mol m⁻²·s⁻¹) was found in the adaxial surface of *Piliostigma thonnigii*.
<table>
<thead>
<tr>
<th>S/N</th>
<th>Plant Species</th>
<th>Family</th>
<th>Leaf Surface</th>
<th>Stomatal Complex Type</th>
<th>Frequency (% age)</th>
<th>Stomatal Density (mm²)</th>
<th>Stomatal Index (% age)</th>
<th>Stomatal Size (µm²)</th>
<th>Transpiration Rate (mol m⁻²s⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td><em>Daniellia oliveri</em></td>
<td><em>Caesalpinia-</em></td>
<td>Abaxial</td>
<td>Paracytic</td>
<td>100.00</td>
<td>93.61a</td>
<td>25.42a</td>
<td>25.44ab</td>
<td>2.61 × 10⁻⁶ab</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>ceae</em></td>
<td>Adaxial</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td><em>Delonix regia</em></td>
<td><em>Caesalpinia-</em></td>
<td>Abaxial</td>
<td>Brachyparacytic</td>
<td>100.00</td>
<td>65.55abc</td>
<td>20.22a</td>
<td>23.62ab</td>
<td>1.56 × 10⁻⁶b</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>ceae</em></td>
<td>Adaxial</td>
<td>Brachyparacytic</td>
<td>100.00</td>
<td>14.61c</td>
<td>7.15c</td>
<td>24.44a</td>
<td>1.11 × 10⁻⁶a</td>
</tr>
<tr>
<td>3.</td>
<td><em>Piliostigma thonningii</em></td>
<td><em>Caesalpinia-</em></td>
<td>Abaxial</td>
<td>Brachyparacytic</td>
<td>60.58</td>
<td>51.74c</td>
<td>16.78a</td>
<td>11.19b</td>
<td>1.45 × 10⁻⁶b</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>ceae</em></td>
<td>Adaxial</td>
<td>Paracytic</td>
<td>39.42</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Brachyparacytic</td>
<td>100.00</td>
<td>14.41c</td>
<td>11.09a</td>
<td>12.23b</td>
<td>1.10 × 10⁻⁶a</td>
</tr>
<tr>
<td>4.</td>
<td><em>Azadirachta indica</em></td>
<td><em>Meliaceae</em></td>
<td>Abaxial</td>
<td>Anomocytic</td>
<td>100.00</td>
<td>81.08ab</td>
<td>28.23a</td>
<td>29.36c</td>
<td>2.97 × 10⁻⁶a</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Adaxial</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td><em>Tectona grandis</em></td>
<td><em>Verbenaceae</em></td>
<td>Abaxial</td>
<td>Brachyparacytic</td>
<td>100.00</td>
<td>41.97c</td>
<td>18.76a</td>
<td>12.88b</td>
<td>2.49 × 10⁻⁶ab</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Adaxial</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Means with same letters along the column are not significantly different at p ≤ 0.05
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 ×2000.
Correlation between Stomatal Features and Transpiration Rate of the Studied Species

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

<table>
<thead>
<tr>
<th>CHARACTERS</th>
<th>Stomatal Density</th>
<th>Stomatal Index</th>
<th>Stomatal Size</th>
<th>Transpiration Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stomatal Density</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stomatal Index</td>
<td>.875**</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stomatal Size</td>
<td>.881**</td>
<td>.830**</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Transpiration Rate</td>
<td>.560*</td>
<td>.697**</td>
<td>.528*</td>
<td>1</td>
</tr>
</tbody>
</table>

*Correlation is significant at the 0.05 level.

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

<table>
<thead>
<tr>
<th>CHARACTERS</th>
<th>Stomatal Density</th>
<th>Stomatal Index</th>
<th>Stomatal Size</th>
<th>Transpiration Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stomatal Density</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stomatal Index</td>
<td>.951*</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stomatal Size</td>
<td>.901*</td>
<td>.746**</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Transpiration Rate</td>
<td>.108</td>
<td>.097</td>
<td>.098</td>
<td>1</td>
</tr>
</tbody>
</table>

*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 Daniella 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 × 10^{-6} mol m^{-2}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 × 10^{-6} mol m^{-2}s^{-1}). Similar pattern was reported by Saadu et al. (2009).

Pataky (1969) stated that stomata whose guard cells are less than 15 µm long are termed “small”; while those whose guard cells are more than 38 µm long are termed “large”. However, stomata whose guard cells are within the range of 15µm -38 µm could be described as “moderate”(AbdulRahaman and Oladele, 2009). Based on this information, the species Tectona grandis had small stomata (12.85µm²); Piliostigma thonningii had small stomata on both the abaxial surface (11.19µm²) and adaxial surface (12.23 µm²) respectively. However, the species Delonix regia had moderate stomata on both the abaxial surface (23.62 µm²) and adaxial surface (24.44 µm²) respectively; Daniella oliveri had moderate stomata (24.44 µm²); while Azadirachta indica was the largest stomata (29.36 µm²). 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 (McAuliffe 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 µm²) gave rise to low stomatal density (41.97 mm²); Daniella oliveri with moderate stomata (24.44 µm²) resulted to high stomatal density (93.61 mm²). 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 × 10^{-6} mol m^{-2}s^{-1}, Piliostigma
Possessed brachyparacytic stomatal complex type, paracytic stomatal type, low stomatal density, low stomatal index, small stomat and transpiration rate of $2.55 \times 10^6$ mol. m$^{-2}$s$^{-1}$. **Daniellia oliveri** had paracytic stomatal complex type, low stomatal index, hypostomatosic leaf nature and transpiration rate of $2.61 \times 10^6$ mol. m$^{-2}$s$^{-1}$. **Delonix regia** possessed brachyparacytic stomatal complex type, low stomatal index, hypostomatosic leaf nature and transpiration rate of $2.67 \times 10^6$ mol. m$^{-2}$s$^{-1}$. Finally, the species **Azadirachta indica** had low stomatal index, hypostomatosic leaf and transpiration rate of $2.97 \times 10^6$ mol. m$^{-2}$s$^{-1}$.

CONCLUSION

The study revealed that stomatal traits such as hypostomatosic 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, **Teconota 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.

REFERENCES


