African Journal of Plant Science Vol. 3 (5), pp. 107-112, May 2009
Available online at http://www.academicjournals.org/AJPS
ISSN 1996-0824 © 2009 Academic Journals

Full Length Research Paper

Stomatal complex types and transpiration rates in some tropical tuber species

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Accepted 28 April, 2009

Anatomical study of the leaf epidermis in 6 tuber species namely *Manihot esculenta* Crantz, *Cyperus esculentus* Linn. *Ipomoea batatas* Linn., *Xanthosoma sagittifolium* Schott, *Colocasia esculenta* Schott and *Caladium hortulanum* Vent was conducted. Among these species, only *C. esculenta* has epistomatic leaves with stomata occurring only on the adaxial or upper surface of the leaf. The remaining 5 species have amphistomatic leaves, with stomata on both surfaces of the leaf. *M. esculenta*, *C. esculentus* and *I. batatas* have paracytic stomatal complex type with a frequency of 100%, while *X. sagittifolium*, *C. esculenta* and *C. hortulanum* have brachy-paracytic stomatal complex type with a frequency of 100%. Species with stomatal density range of 22 - 26 stomata per square millimetre, namely *I. batatas*, *X. sagittifolium*, *C. esculentus* and *C. esculenta* were the most transpiring with high potentials for humidification of the atmosphere. Those species with the density range of 16 - 21 stomata per square millimetre, namely *C. hortulanum* and *M. esculenta* were the least transpiring with low humidification potentials. There was no positive correlation between stomatal size and transpiration rate as *C. hortulanum* with the highest stomatal size had the least rate of transpiration. However, stomatal index, that is, the % spread of stomata was positively correlated with transpiration as *I. batatas* with the highest stomatal index of 20.94 had the highest rate of transpiration. Massive cultivation of these tuberous species through intercropping with tree species may help in combating drought and desertification processes. Since these tubers with the exception of *C. hortulanum* are edible, there is added advantage of increased food production, through this suggested cropping system. *I. batatas* being a creeping plant can also be a useful cover crop as part of conservation measures for desertified or exposed areas.

Key words: Tuber species, stomatal complex types, transpiration.

INTRODUCTION

Root and stem tuber crops consist of all carbohydrate-rich underground storage structures that are consumed by man and/or domestic animals. Strictly speaking, from botanical point of view, root tubers are the edible underground storage structures developed from modified roots, while the stem tubers are those crops in which the edible carbohydrate-rich storage organs are developed wholly or in part from underground stems (Wickens et al., 1989). Edible underground storage organs thus consists of diverse variously modified roots such as cassava (*Manihot esculenta*), sweet potato (*Ipomoea batatas*) and carrot (*Daucus carota*) and stem tubers as in cocoyam (*Xanthosoma* spp. and *Colocasia* spp.)

The tropical root and stem tuber crops are staple foods in many parts of the tropics being one of the major sources of carbohydrates human and animal populations (Kay, 1973). Starch is the main type of carbohydrate found in storage organs such as enlarged roots, corms, rhizomes and tubers. The potentials of the crops as a rich source of carbohydrate could go a long way in contributing to feed the millions of Africans who are affected by hunger. The potentials of these crops also include the ability to grow in humid and subhumid tropics with marginal soils thus serving as good revegetation plants in environments where drought is a threat, minimal release of methane to the atmosphere compared to cereals, occurence of broad leaves that can serve as soil cover, minimal requirements of agro-chemicals, relative freedom from pests and diseases and relative high productivity. Harvesting, transportation and storage are constraints that

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Table 1. List of root tuber species studied.

Species	Common name	Type of tuber	Family
Manihot esculenta Crantz	Cassava	Root	Euphorbiaceae
Cyperus esculentus Linn.	Tiger nut, Imumu	Stem	Cyperaceae
Ipomoea batatas Linn.	Sweet potato, Odoku	Root	Convolvulaceae
Xanthosoma sagittifolium Schott	New cocoyam, Koko	Stem	Araceae
Colocasia esculenta Schott	Old cocoyam, Kokodo	Stem	Araceae
Caladium hortulanum Vent	Spotted caladium, Jesus blood	Stem	Araceae

limit the cultivation of the crops, which if overcome, could boost production and environmental sustainability hence improving the lives of the local populace (Tafon, 2003). The activities of farmers however expose the soil surface during tillage leading to desertification and des-truction of virgin forests. Some of the strategies suggested for combating desertification includes afforestation, revegetation and reforestation through planting of trees. These efforts can be complemented using tuberous, non-tree species.

In plants, water in form of vapour gets lost to the atomsphere through a process called transpiration via openings or stomata in the leaf surfaces (Meinzer et al., 1997). This water influences the atmospheric humidity. Hence this study was conducted so as to determine the capacity of some tuberous species to humidify the atmosphere through the stomatal transpiration process in relation to the stomatal complex types. This relationship earlier studied in some *Citrus* species by Obiremi and Oladele (2001) and in some afforestation species by Oyeleke et al. (2004).

MATERIALS AND METHODS

Collection of specimens

The specimens were collected from the Kwara state ministry of agriculture, Ilorin and from flower garden, reservation road, Ilorin, Kwara state, Nigeria. 12 seedlings of each of the study materials were used for this work. The specimens comprise 6 species of root and stem tubers (Table 1).

Isolation of leaf epidermal layers

Leaf segment of an area of 1 cm 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.

Staining, mounting and observation of leaf surfaces on microscope

A portion of each macerated cuticle was taken for microscopic studies. It was stained in 1% aqueous solution of safranin for about 3 - 5 min. Excess stain was rinsed off with clean water. The stained cuticle was mounted in glycerin. Observations were made on the microscope to determine, stomatal complex types and their frequencies, stomatal size, stomatal density and stomatal index. All observations were recorded with drawings or figures and tables.

Determination of frequency of stomatal complex types

Using 35 fields of view at X 40 objective as quadrats, the number of subsidiary cells per stoma was noted to determine the types of stomatal complex present in each specimen. Frequency of each stomatal complex type was expressed as % occurrence of each stomatal complex type based on all occurrences of stomatal complex types (Carr and Carr, 1990; Obiremi and Oladele, 2001). Terminologies used for stomatal complex types followed those of Dilcher (1974) and Metcalfe and Chalk (1988).

Determination of stomatal density and index

The stomatal density (SD) was determined as the number of stomata per square millimeter (Stace, 1965).

Stomatal index (SI) was determined as follows:

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

Where: SI = Stomatal index
S = Number of stomatal per square millimeter
E = Number of ordinary epidermal cells per square millimeter.

Determination of stomatal size

The mean stomatal size or area of a species was determined by measuring length and breadth using a micrometer of a sample of 35 stomata using eye-piece micrometer.

Determination of transpiration rate

A cobalt chloride paper method was used to determine the transpiration rate of each specimen (Obiremi and Oladele, 2001; Dutta, 20 03). Strips of filter paper of 2 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 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 marked small branch (of the plant) with leaves. 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 transferred to the laboratory and weighed (W2). Weight of water transpired (WT) was determined as W2 - W1. The surface area of leaves used was measured using graph. When the leaf is amphistomatic, the upper and lower surfaces of the leaf were measured. Transpiration rate was expressed as $mol/m^{-2}/s^{-1}$. Data from the microscopic studies and transpiration rate were subjected to statistical studies of analysis of variance (ANOVA).

Table 2. Stomatal features and transpiration rate in some root tuber species.

Species	Leaf Surface	Stomatal Complex Type	Frequency (% age)	Stomatal Density (mm^{-2})	Stomatal Size (µm)	Stomatal Index (%)	Transpiration Rate ($mol/m^{-2}/s^{-1}$)	Trichome
Manihot esculenta	Adaxial	Paracytic	100.00	16.45a	52.30a	3.03a	1.47 x 10^{-4}a	Present
	Abaxial	Paracytic	100.00					
Cyperus esculentus	Adaxial	-	-	28.95b	104.77b	17.16b	2.68 x 10^{-4}b	Absent
	Abaxial	Paracytic	100.00					
Ipomoea batatas	Adaxial	Paracytic	100.00	35.75b	97.97b	20.94b	3.07 x 10^{-4}b	Absent
	Abaxial	Paracytic	100.00					
Xanthosoma sagittifolium	Adaxial	Brachyparacytic	100.00	32.23b	79.10a	10.26a	1.82 x 10^{-4}a	Absent
	Abaxial	Brachyparacytic	100.00					
Colocasia esculenta	Adaxial	Brachyparacytic	100.00	22.59a	26.87b	5.55a	2.67 x 10^{-4}b	Absent
	Abaxial	Brachyparacytic	100.00					
Caladium hortulanum	Adaxial	Brachyparacytic	100.00	21.05a	128.57a	14.08b	0.34 x 10^{-4}a	Absent
	Abaxial	Brachyparacytic	100.00					

Means with same letters along the columns are not significantly different at p = 0.05.

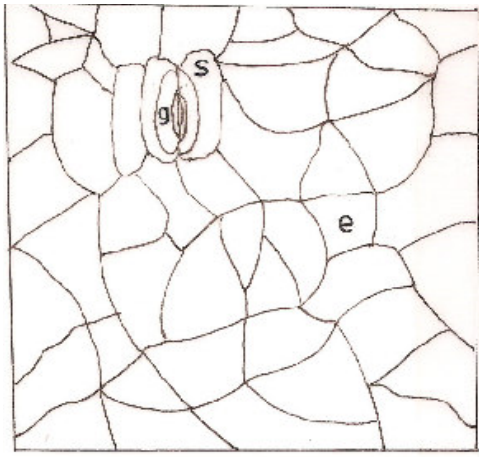

Figure 1. Leaf surface view of *M. esculenta*. Showing paracytic stomata (g = Guard cell, s = Subsidiary cell) and epidermal cell (e) x600.

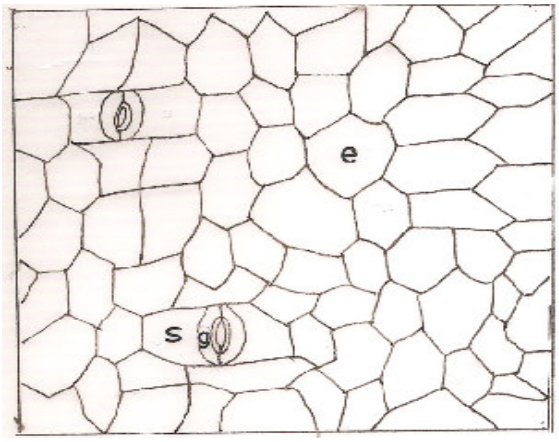

Figure 2. Leaf surface view of *C. esculenta*. Showing brachyparacytic stomata (g = Guard cell, s = Subsidiary cell) and epidermal cell (e) x600.

RESULTS

Based on the presence of stomata on the leaf surface, leaves are of 2 types in the 6 tuber species (Table 1) studied in this work, namely amphistomatic (that is, stomata on both leaf surfaces) and epistomatic (that is, stomata on upper surface only) were observed.

Types and frequency of stomatal complex

2 types of stomatal complex were observed in the species namely paracytic stomata and brachyparacytic stomata (Table 2; Figures 1 - 6). These stomatal types were found to occur at a frequency of 100% (Table 2).

Stomatal density and transpiration rate

Ipomoea batatas was found to have the highest stomatal density followed by *Xanthosoma sagittifolium*, *Cyperus esculentus*, *Colocasia esculenta*, *Caladium hortulanum* and *Manihot esculenta*. With respect to transpiration rate, *Ipomoea batatas* also had the highest value, followed by *Cyperus esculentus*, *C. esculenta*, *X. sagittifolium*, *M. esculenta* and *C. hortulanum* (Table 2). *I. batatas* turned out to be species with the highest stomatal density and the most transpiring species. *M. esculenta* having the least stomatal density was penultimate the least transpiring species. *C. hortulanum* had the lowest transpiration rate (Table 2).

Stomatal size

The largest stomata were found in *C. hortulanum*, followed by *C. esculentus*, *I. batatas*, *X. sagittifolium*, *M. esculenta* and *C. esculenta*. The stomatal size has some

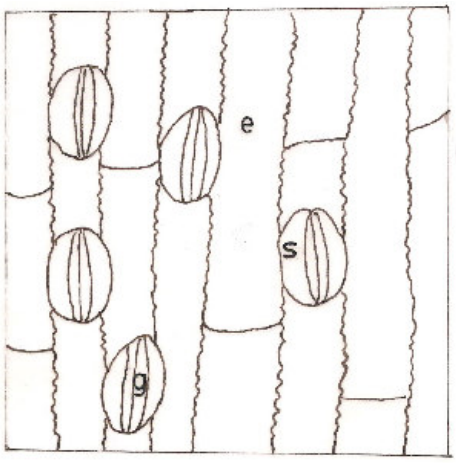

Figure 3. Leaf surface view of *C. esculentus*. Showing paracytic stomata (g = Guard cell, s = Subsidiary cell) and epidermal cell (e) x600.

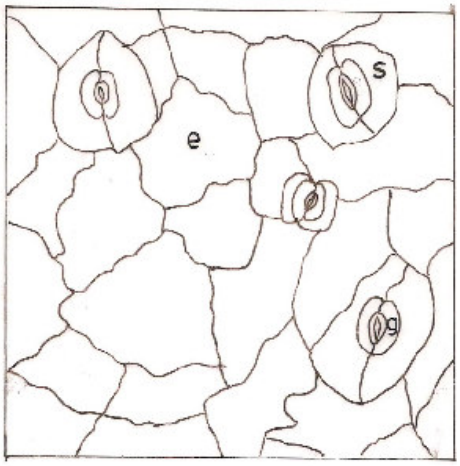

Figure 4. Leaf surface view of *I. batatas* showing paracytic stomata (g = Guard cell, s = Subsidiary cell) and epidermal cell (e) x600.

correlations with transpiration as observed in *Manihot esculenta* whose stomata are small (52.30 µm) transpired very low (1.47 x 10^{-4} mol/m^{-2}/s^{-1}) and in *C. esculentus* whose stomata are large (104.77 µm) transpired very high, 2.68 x 10^{-4} mol/m^{-2}/s^{-1} (Table 2).

Stomatal index

The highest index was found in *I. batatas*, followed by *C. esculentus*, *C. hortulanum*, *X. sagittifolium*, *C. esculenta* and *M. esculenta*. At least there are some relationships between the stomatal index and rates of transpiration in *I. batatas*, *C. esculentus*, *X. sagittifolium* and *M. esculenta* (Table 2).

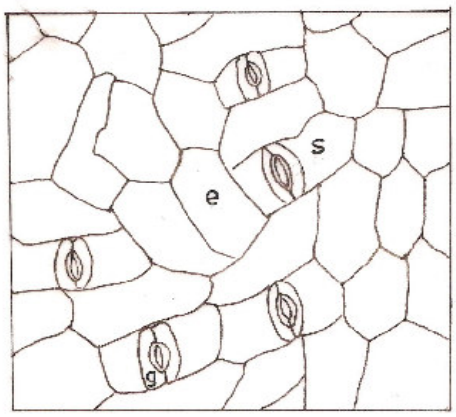

Figure 5. Leaf surface view of *C. hortulanum*. Showing brachyparacytic stomata (s = Subsidiary cell) and epidermal cell (e) x600.

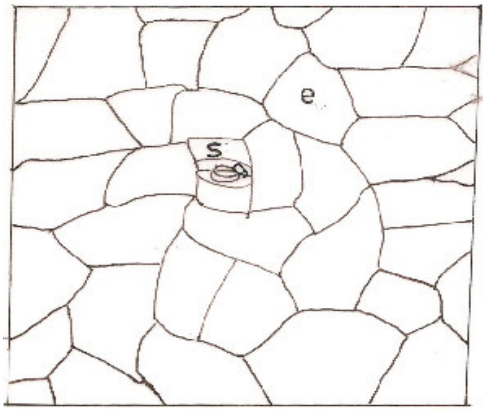

Figure 6. Leaf surface view of *C. esculenta* showing brachyparacytic stomata (s = Subsidiary cell) and epidermal cell (e) x600.

Trichomes

All the 6 species except *M. esculenta* were without trichomes. The trichomes observed in *M. esculenta* were found to be unicellular trichomes (Table 2).

DISCUSSION

Stomata in the 5 species namely *M. esculenta*, *C. esculenta*, *I. batatas*, *X. sagittifolium* and *C. hortulanum*-can be described as large while in *C. esculenta* they can be described as small because according to Pataky (1969) stomata whose guard cells are less than 15 µm are called ''small'' while those in which guard cells are more than 38 µm are known as ''large''. Stomata in the former species are more than 38 µm while they are less than 15 µm in the latter. There was no correlation between stomatal density and stomatal size. For instance, *I. batatas* has large stomata (97.97 µm) and high stomatal

density (35.75 mm^{-2}) compared with *C. esculenta* whose stomata are small (26.87 µm) and yet with low stomatal density of 22.59 mm^{-2} (Table 2). Earlier study by Metcalfe and Chalk (1988) and Beerling and Woodward (1997) showed that large stomata resulted in low stomatal density while small stomata gave high stomatal density. The work of AbdulRahaman and Oladele (2003) also showed this pattern where large stomata actually gave low stomatal density and small stomata gave high density in some vegetable species. Observation of different rates of transpiration among the 6 species may be due to variations in their stomatal features such as stomatal complex types, density, index and size and presence or absence of trichomes, as well as occurrence of stomata either on 1 surface or both surfaces of leaves (AbdulRahaman and Oladele, 2004; Oyeleke et al., 2004).

Some correlations do occur between the stomatal features (like density, index and size) and rate of transpiration in each species studied. Stomatal density has been identified to play major role in water use efficiency of plants thus, its numerical strength on the leaf surface is essential (Wang et al., 2007). The work of Spence et al. (1986), Spence (1987), Royer (2001) and Nejad and van Meeteren (2005) also showed that the geometry and resulting mechanical properties of small stomata, enhanced the capacity of opening or maintaining open pores with lower guard cell turgor pressures, relative to the turgor of the surrounding epidermal cells. In *C. esculentus* and *I. batatas*, high stomatal density, high index and large stomata give high transpiration rate. In *M esculenta* and *C. hortulanum*, low stomatal index and small stomata, and low stomatal density respectively result in low transpiration rate (Table 2). This finding corroborates the works of Evenari (1938) and Oyeleke et al. (2004) that high rate of transpiration occurs with high stomatal density and vice versa. There were exceptions to these correlations in *C. esculenta* and *X. sagittifolium*. There was high rate of transpiration (2.67 x 10^{-4} $mol/m^{-2}/s^{-1}$) in *C. esculenta* in spite of relatively low stomatal density, index and small stomata. Relatively high stomatal density, index and large stomatal size in *C. hortulanum* did not proportionately reflect in its rate of transpiration which was very low (0.34 x 10^{-4} $mol/m^{-2}/s^{-1}$). There was low transpiration rate of 1.82 x 10^{-4} $mol/m^{-2}/s^{-1}$ in *X. sagittifolium* in spite of despite its having high stomatal density and relatively large stomata.

Lack of trichomes in *I. batatas*, *C. esculentus*, *C. esculenta* and *X. sagittifolium* may be responsible for their high rate of transpiration than in *M. esculenta* which possessed unicellular trichomes on its leaf surface. This is in corroboration with earlier findings of Quarrie and Jones (1977), Karabourniotis et al. (1995), Smith and Hare (2004) and Franks and Farquhar (2007) that the presence of trichomes on the leaf surfaces reduced water loss through transpiration. Ability of the trichomes to reduce rate of transpiration was also discussed by AbdulRahaman and Oladele (2004) in some Nigerian vegetable species and Oyeleke et al. (2004) in *Eucalyptus camaldulensis*.

With reference to transpiration in each of the 6 tuber species, *I. batatas* has the highest rate of transpiration of 3.07 x 10^{-4} $mol/m^{-2}/s^{-1}$, followed by *C. esculentus* with transpiration rate of 2.68 x 10^{-4} $mol/m^{-2}/s^{-1}$, *C. esculenta* with transpiration rate of 2.67 x 10^{-4} $mol/m^{-2}/s^{-1}$, *X. sagittifolium* with transpiration rate of 1.82 x 10^{-4} $mol/m^{-2}/s^{-1}$, *M. esculenta* with transpiration rate of 1.47 x 10^{-4} $mol/m^{-2}/s^{-1}$ and lowest rate of transpiration of 0.34 x 10^{-4} $mol/m^{-2}/s^{-1}$ in *C. hortulanum*. Since the higher the transpiration, the higher the water vapour in the atmosphere (Ferree and Hall, 1980; Tanner and Beever, 2001; Oladele, 2002; Burghardt and Riederer, 2003; Caird et al., 2007; Howard and Donovan, 2007; George et al., 2007; Metselaar and Lier, 2007), *I. batatas* that transpired faster than other 5 tuber species thus has the highest potential to humidify the atmosphere, followed by *C. esculentus*, *C. esculenta*, *X. sagittifolium*, *M. esculenta* and lowest potential is in *C. hortulanum* that transpired slower.

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