OBAFEMI AWOLOWO UNIVERSITY

STUDIES ON MORPHOLOGICAL AND ANATOMICAL CHARACTERS INDICATING C_3 AND C_4 PHOTOSYNTHETIC METABOLISM IN THE GENUS *BOERHAVIA* L. IN NIGERIA

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CERTIFICATION

This is to certify that the research study was carried out by ABDULWAKEEL AYOKUN-NUN AJAO as part of the requirement for the award of Master of Science degree in Botany of the Obafemi Awolowo University, Ile-Ife.

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DEDICATION

To ***ELEDUMARE*****,** The ***Akoda*** (The beginning), ***Aseda*** (The author of Being), **Parents and Siblings**, ***Awon Oluko mi*** (Teachers) and to all those who make me to understand the order of great subject of life.

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LIST OF ABBREVIATIONS

cm	Centimetre
cm^2	Square centimetre
µm	Micrometre
mm^{-2}	Per square millimeter
$µm^2$	Square micrometre
BS	Bundle sheath
CAM	Crassulacean Acid metabolism
CO_2	Carbon dioxide
NAD-ME	Nicotinamide–Adenine Dinucleotide-MalicEnzyme
PEPC	Phosphoenolpyruvate carboxylase
Rubisco	Ribulose bisphosphate carboxykinase
S.E	Standard error
ppm	Parts per million
PCK	Phosphoenolpyruvate carboxykinase
NADP	Nicotinamide–Adenine Diphosphate
PGA	Phosphoglycerate Acid
NaCl	Sodium Chloride

ABSTRACT

This study investigated leaf morphological and anatomical characters of four species of the genus *Boerhavia*, known to contain C_3 and C_4 photosynthetic groups. This was with a view to establishing characters that may be useful in the grouping of the Nigerian species into their respective photosynthetic groups, and also to documentig characters that may be peculiar to dicotyledons.

The species investigated were *Boerhavia coccinea* Mill., *B. repens* L., *B. diffusa* L. and *B. erecta* L. collected from different locations in the southwestern Nigeria. Both qualitative and quantitative morphological as well as anatomical characteristics like inflorescence form and arrangement, stem shape, leaf morphology and venation pattern, plant height, stem length, leaf length, leaf width and petiole length were observed and documented. Other characters observed and documented included stomata index, stomata size, interstomatal distance, stomata density, interveinal distance, vein density and vein distance, intercellular air spaces, leaf thickness, mesophyll thickness, Kranz tissue, one cell distant count and maximum lateral cell count.

The results revealed that paniculate inflorescence was a morphological character which delimited C_3 from C_4 species with cymose inflorescence. All anatomical characters investigated were found to be useful in the grouping of these species along photosynthetic pathways. However, among all the characters examined, interveinal distance of less than 166 µm for C_4 and which was greater than 166 µm for C_3 was recorded. Maximum lateral count ranging from 2 to 6 for C_4 and 5 to 10 for C_3 was found to be peculiar to the dicotyledons.

The study concluded that with the combination of morphological and anatomical characters, *B. erecta, B. coccinea and B. repens* were C_4 species while *B. diffusa* was a C_3 species.

CHAPTER ONE

INTRODUCTION

1.1 Background to the Study

Family Nyctaginaceae

The family Nyctaginaceae is a relatively small comprising about 30 genera and 400 species (Douglas and Manos, 2007). They are mainly distributed in the tropical and subtropical area of the new world (Brittrich*etal*.,1993), with some genera extending into the temperate regions such as Southern Africa (Jordan 2000).The family is commonly known as four-O'clock family, as most of the species have flowers that open in the late afternoon or early evening (Levin *et al*., 2001). The most interesting feature of the family Nyctaginaceae is its flowers which are arranged in inflorescence (Cyme) and are collectively enclosed in three brightly coloured bracts (Dequan and Gilbert, 2003). The fruit which is an achene, is enclosed by the perianth that persists during its formation (Bogle, 1974). Accessory fruit can vary in sculpturing, either forming ribs, which can elongate into swings, or covered in sticky, glandular hairs and warts (Spellenberg, 2003). Its accessory fruit also aids in dispersal (Douglas and Manos, 2007). Many members of the family are used as medicine in Asia, Brazil and Mexico to treat dysentery, diarrhea, muscular pain, abdominal colic, and as poultices for boils, abscesses and scabies(Aoki *et al*. 2008). In Southern Africa roots of some indigenous species are with large viscid and mucilaginous glands (Stannard, 1988).Five genera which include *Mirabilis*,*Phaeoptilum*, *Pisonia*,*Commicarpus* and *Boerhavia*are distributed throughout southern Africa, except for the extreme southern part(Madeleen and Stefan, 2010).

1.2 Genus *Boerhavia*

The genus*Boerhavia*, consists of 40 species worldwide, they are distributed in tropical and sub-tropical regions. It is found in Ceylon, Australia, Sudan and Malay Peninsula, China, Africa, America and Islands of the Pacific (Fosberg, 1978). In Taiwan, three species*, repens* L., *B. crispa* Heyne and *B. diffusa* L. is recorded for the genus (Fosberg, 1978). However, only *B. diffusa* was represented in the flora of Taiwan (Liu, 1976; Yang and Lu, 1996). More recently, *B.erecta L.* and *B. coccinea* Mill.werereported as being newly introduced (Chou *et al*., 2004; Chen and Wu, 2005).*Boerhavia diffusa* is a very broad collective species and renowned as a difficult species in regions of the Old World and New World in both hemispheres (Whitehouse, 1996; Spellenberg, 2003).

In the study of wide range of specimens, Fosberg (1978) divided the species into two groups, *B. diffusa* group and *B. repens* group, on the basis of the mode of inflorescence, viz. in *B. diffusa* group the inflorescences are strictly paniculate, while in *B. repens* group the inflorescences are axillary and pedunculate cymes . Both of these groups are complex and several distinct species are included. In Nigeria, four species are known to be present and they are *B. coccinea, B. diffusa, B. erecta* and *B.repens* (Hutchinson and Dalziel, 1972). There are many genera of plants which contains species with diverse photosynthetic pathways including*Boerhavia*, *Euphorbia*, *Amaranthus*, *Cyperus* and *Cleome*(Pinto –Escobar and Mora-Osejo 1966; Gentry 1993; Jorgensen and Ulloa 1994; Ehleringer *et al.,* 1997; Muhaidat *et al*., 2007).

1.3 Photosynthetic Pathways in Plant

There are three different types of photosynthetic pathways in plants. These are Crassulacean Acid Metabolism(CAM), C_3 and C_4 photosynthetic pathways (Ehleringer *et al*., 1997; Wang, 2004). Themost important and commonly encountered of them are the C_3 and C_4, however, Crassulacean Acid Metabolism (CAM) has been reported in some succulent plants (Ranson and Thomas, 1961).CAM plants which are typically found in desert environment where the conditions for growth are generally harsh. They demonstrate pronounced variation in structure, adaptation to stressful environment, and expression of CAM photosynthesis (Robert*et al.,* 1997; Borland *et al.*, 1998). Despite these variations, CAM species share anatomical traits that may reflect functional constraint (Gilson, 1982; Noble, 1988). A general feature of CAM anatomy is leaf succulence, characterized by undifferentiated mesophyll cells with high enlarged vacuoles (Gilson 1982). These anatomical traits provide capacitance for C_4 acid accumulation and water storage in CAM photosynthetic tissue (Osmond *et al.,* 1999). They arealternative photosynthetic pathway and a variation of the C_3pathway, and they contain plants adapted to survive extreme conditions.

1.3.1 Crassulacean Acid Metabolism (CAM)

Plants that followed CAM photosynthetic pathway are modern day desert plants, but many are epiphytic while some submerged aquatic plants have been found to use this pathway(Winter and Smith, 1996). If we exclude the aquatic plants, the modern day CAM plants are specialized to survive conditions of extreme water shortage (Winter and Smith, 1996). They can fix the CO_2 just like the C_3 plants do by using theRibulose bisphosphate carboxykinaseenzyme (Rubisco enzyme) from the Calvin cycle (Boom, 2004). However, instead of atmospheric CO_2 that the Rubisco fixes, CO_2 comes from an internal storage pool of malic acid that has been accumulated during the night. In the light, decarboxylation of this malic acid pool releases an internal CO_2 source. Thus, these plants are able to fix atmospheric CO_2 in complete darkness using a dark regulated Phosphoenolpyruvate carboxylase (PEPC) system and store this in the form of malate (Kluge and Ting, 1978). This allows CAM plants to keep their stomata completely closed during the day avoiding water loss and photorespiration. Thus CAM plants have separated carbon fixation from carbon assimilation in time (Boom, 2004). Water lost due to evaporation is kept to a minimum, since the stomata are shut during the warm day, photorespiration is reduced to a minimum because at time of Rubisco fixation, there is no contact with the atmosphere the plant generates its own CO_2 instead of using the diffused CO_2 from the oxygen containing air. Thus,