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EFFECTS OF ALTITUDINAL GRADIENTS ON MORPHO-ANATOMICAL CHARACTERS OF *CHROMOLAENA ODORATA* (L.) KING & ROBINSON

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ABSTRACT

Understanding the adaptive modifications of plants in relation to changes in environmental conditions, especially altitudes are very important. This work investigates foliar morphological and anatomical characters of *Chromolaena odorata* (L.) King & Robinson growing in three different elevations (280m, 312m and 360m) with the aim of establishing its response to varying elevations. Our results showed that morphological characters such as mean leaf area reduced significantly ($p < 0.05$) from $66.768 \pm 2.107 \text{ cm}^2$ to $33.036 \pm 1.346 \text{ cm}^2$ with increase in altitude and mean petiole length reduced significantly ($p < 0.05$) from $1.723 \pm 0.260 \text{ cm}$ to $1.331 \pm 0.063 \text{ cm}$ only at lower altitude when compared to the data for higher altitudes. Anatomical characters such as mean stomata index significantly ($p < 0.05$) decrease with increase in altitude and both mean leaf and mesophyll thickness increased significantly ($p < 0.05$) from lower altitude only to the mid altitude and not to the higher altitude. In conclusion, this study has revealed that plants and in this case, *C. odorata*, generally responds to changes in altitudinal gradient with reduction in morphological characters (leaf area and petiole length) and anatomical character (stomata index) while there appeared to be a general increase in anatomical characters such as leaf and mesophyll thickness studied. This study has increased our knowledge on the response of plant to altitudinal gradients.

Keywords: Altitude, *Chromolaena odorata*, Leaf Anatomy, Stomata Index, Leaf Thickness, Mesophyll Thickness

INTRODUCTION

Acclimatization studies in plants are important in understanding the adaptive modifications that occur in relation to changes in environmental conditions. Changes in altitudinal gradient along mountain regions are known to be associated with variations in so many biotic and abiotic factors. These include air, temperature, humidity, light intensity, radiation, wind exposure, soil fertility among others (Gönüz

and Özörgücü, 1999; Kofidis *et al.*, 2003). A combination of all these factors subjects plants to a lot of environmental pressure which now becomes expressed as changes not only on their physiology and productivity levels, but on their general appearance – morphology and internal structures – anatomy (Kofidis *et al.*, 2003).

It is established that plant morphological parameters such as height, leaf area and petiole length vary with different altitudes (Kofidis *et al.*, 2003), with progressive

decrease in plant height with increasing altitude. These responses are presumably associated with reduced temperature as well as nutrient and water limitations (Cordell *et al.*, 1998; Kao *et al.*, 1998). It may also be an adaptive strategy against the damaging effect of strong wind that normally blows at high altitude or an attempt to be closer to the warmer soil surface thereby improving photosynthetic activities of plant (Korner and Chochrane, 1983; Kofidis *et al.*, 2003). In addition to reduced height, many plants have been found to have smaller leaves at high elevation (Morecroft and Woodward, 1996; Cordell *et al.*, 1998; Venema *et al.*, 2000). This has been reported in a considerable number of species and the response is attributed to low air temperature which is a feature of higher altitudes (Woodward, 1983; Cordell *et al.*, 1998; Kao and Chang, 2001).

As expected, the internal tissues of plants are equally affected by changes in altitudinal gradients. For example, the leaf blade is reported to be thicker in higher than in lower altitudes (Cordell *et al.*, 1998; Weih and Karlsson, 1999). This is attributed to increase in sizes of the epidermal and mesophyll cells rather than their number. However, a common observation is that it usually results in thinner leaves (Suzuki, 1998). Thicker leaves are reported to occur only in few cases (Cordell *et al.*, 1998). The volume of leaf mesophyll cells is generally higher at middle altitude than at higher altitude and this was suggested to be due to greater chlorenchymatic biomass of plants in that region (Cordell *et al.*, 1998; Weih and Karlsson, 1999). Altitude also affects the density of leaf stomata which are reported to increase in number per unit area from lower altitude to higher altitude (Kao and Chang,

2001; Kofidis *et al.*, 2003; Qiang *et al.*, 2003). The increase in stomatal number has been attributed to higher solar intensity (Furukawa, 1997; Apel, 1989), because low stomata density at low altitude presumably reflects the arid conditions (higher temperature and lower humidity) inherent in the lower part of the mountain (Kofidis *et al.*, 2003).

Most literature in the past usually focused on the characteristics of the essential oils of some aromatic plants along altitudinal gradients (Putievsky *et al.*, 1986; Muller-Riebau *et al.*, 1997; Boira and Blanquer, 1998). Few studies focused on morphological and anatomical changes, especially in higher plants (Kofidis *et al.*, 2003). Therefore the present study investigated changes in the foliar morphology and anatomy of *Chromolaena odorata* – a species that is known to inhabit different altitudinal gradient in South-Western Nigeria – in relation to changes in altitudinal gradient. This was to establish the strategies employed by this species to cope with the changes in altitudinal gradient.

MATERIALS AND METHODS

Plant material and collection sites

The plant material used for this work is *C. odorata* and they were collected at three different altitudes along Hill 2, Obafemi Awolowo University, Ile-Ife, Nigeria. The plant was identified and confirmed at the Herbarium in the Department of Botany, Obafemi Awolowo University, Ile-Ife.

Description of study sites

The study sites were located on Hill 2 within the Obafemi Awolowo University Campus Ile-Ife, Nigeria. The altitudes of the three sites where plants were collected are as shown in Table 1.

Table 1: Collection sites, altitudes and GPS coordinates

Sites	Altitudes	GPS coordinates
1	280m	N 07 ⁰ 27.636' E 004 ⁰ 33.453'
2	312m	N 07 ⁰ 31.813' E 004 ⁰ 31.685'
3	360m	N 07 ⁰ 31.919' E 004 ⁰ 31.717'

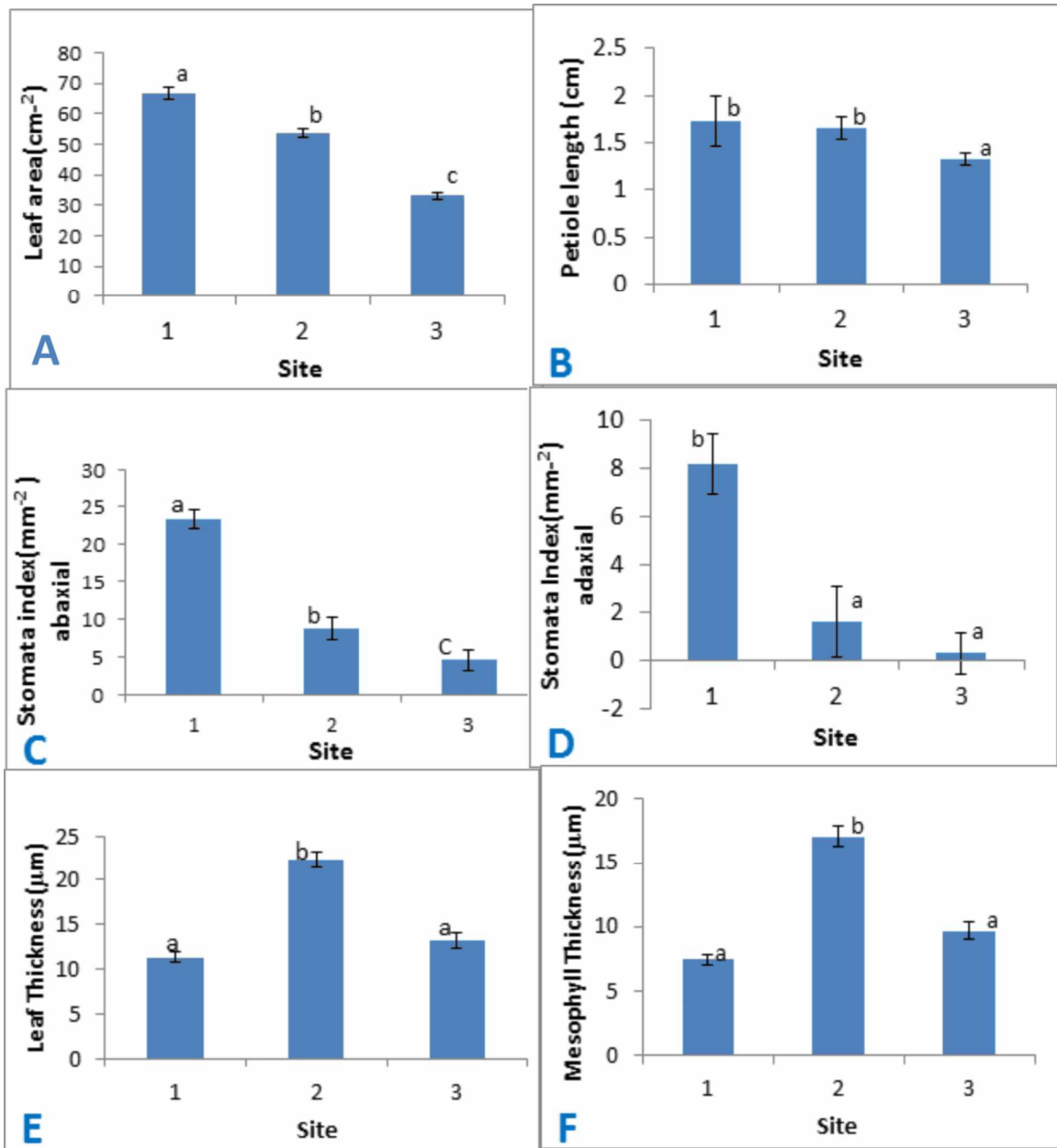


Figure legend

Figure 1: Effect of altitudinal gradients on the morpho-anatomical characters of *C. odorata* (Vertical bars are standard error; N=20). Similar letters on tops of corresponding bars indicate statistically non-significant differences at $p < 0.05$ for each sites

Morphological study

The qualitative morphological characters such as leaf colour, hairiness of stem and stem colour were observed and noted on the field while quantitative morphological characters such as leaf area (length and width of leaves), and petiole length were measured with a metric ruler in the

laboratory. Plants of the same chronological age (at the same level of branching) were sampled. The first five leaves from the tenth branch of the selected plants were measured. This was done in order to ensure that the leaves measured were of the same age. Twenty plants each were sampled at each of

the sites and the average leaf area and petiole length were calculated.

Anatomical study

For the purpose of studying the detailed anatomical structures, fresh mature leaves of all the species were used. For stomatal index studies, epidermal peels of the leaf were obtained either manually using forceps and dissecting needle, while difficult materials were obtained using standard procedures previously described (Adedeji and Jewoola, 2008; Saheed and Illoh, 2010). Transverse sections of the leaves were cut using a sledge microtome set at 20 μ per thickness. Specimens were processed using standard anatomical procedures as described previously (Illoh, 1995; Adedeji and Jewoola, 2008; Saheed and Illoh, 2010). Images of sections were viewed and captured with a Celestron compound Microscope fitted with a JVC KYF70B digital camera. Quantitative characters were measured with the aid of calibrated ocular micrometer inserted in the eye-piece of the microscope; these measurements were later multiplied by the ocular constant with respect to the power under which they were taken. The average values of all measurements taken were recorded. The stomata index was calculated by expressing the number of stomata per unit area as a percentage of the total number of epidermal cells in the same area. This has been previously described (Illoh, 1995; Adedeji and Jewoola, 2008; Saheed and Illoh, 2010).

Statistical Analysis

One way analysis of variance (ANOVA) was used to test for significant differences in the observed effects of the altitudinal gradient on both foliar morphology and anatomical characters at the probability level of $p < 0.05$.

RESULTS

Foliar Morphology – The effect of different altitudes on the mean leaf area of *C. odorata* is shown in Fig. 1A. It was observed that there was a significant decrease ($p < 0.05$) in the mean leaf area from the lower altitude (Site 1) to the higher altitude (Site 3). The

result of the effect of altitudinal gradient on the mean petiole length of *C. odorata* is shown in Fig. 1B. There was no significant difference ($p < 0.05$) in the mean petiole length from the lower altitude (Site 1) to the mid altitude (Site 2). However, the mean petiole length in site 1 and site 2 are significantly higher ($p < 0.05$) than Site 3.

Leaf anatomy through epidermal surface

– The mean stomata index on the abaxial surface (Fig. 1C), showed a significant reduction ($p < 0.05$) from Site 1 and to Site 3. The mean stomata index on the adaxial surface (Fig. 1D), also reduced significantly ($p < 0.05$) from the lower altitude (Site 1) in comparison to the mid (Site 2) and higher altitude (Site 3). However, there was no significant difference ($p < 0.05$) between site 2 and site 3.

Leaf anatomy through transverse section

– The transverse section of the leaf shows that the epidermal cell is one layered, made up of parenchyma cells which are irregular in shape and covered with non-striated cuticle. The mean leaf thickness (Fig. 1E) significantly increases ($p < 0.05$) from lower (site 1) and higher (site 3) altitudes compared to the mid-altitudes (site 2). The mean mesophyll thickness (Fig. 1F) significantly increases ($p < 0.05$) from lower (site 1) and higher (site 3) altitudes compared to the mid-altitudes (site 2).

DISCUSSION

Plants growing along altitudinal gradients exhibit differences in a number of morphological characteristics which generally become expressed by reductions or sometimes increase at high elevations (Körner and Chochrane, 1983; Woodward, 1983; Cordell *et al.*, 1998; Kao and Chang, 2001). This is eventually expected to result in corresponding reduction or increase in anatomical characters (Codignola *et al.*, 1987; Cordell *et al.*, 1998; Kofidis *et al.*, 2003). Morphological parameters that are affected include leaf area and petiole length which in the *C. odorata* plants studied show that the leaf areas were reduced significantly ($p < 0.05$) with increase in altitudes, while petiole length was also significantly

($p < 0.05$) reduced from site 1 compared to the other two higher altitudes while there is no significant reduction ($p < 0.05$) from site 2 and site 3 (Fig. 1B). This clearly indicates that the higher the altitude the smaller the size of the foliar organs of the plant. This is because considerable number of species growing in higher altitudes were reported to possess smaller leaves which has been attributed to the low air temperature at such altitudes rather than the photosynthetic process itself (Woodward, 1983; Cordell *et al.*, 1998; Kao and Chang, 2001). According to Körner and Diemer (1987), photosynthetic process alone does not appear to be associated with the reduction of leaf size at high altitude in plants. Such plants have been measured to have photosynthetic rates close to those of the low altitude plants. However, there are reports of some plant species with larger leaf area with increase in altitudes (Weih and Karlsson, 1999). Another report on *Nepeta nuda* showed that there was no significant difference in leaf area with respect to changes in altitude (Kofidis and Bosabaldis, 2008). However, leaves of this plant were shown to undergo major alterations in relation to seasons by having the largest leaf area by the middle of summer (usually in July).

Stomatal density and stomatal index decrease with increasing atmospheric carbon dioxide level both in geological time and under laboratory conditions (Lockheart *et al.*, 1998). It was reported that CO_2 can directly affect stomatal differentiation (Lockheart *et al.*, 1998) and that stomatal density was negatively correlated with atmospheric CO_2 below 3000m (Qiang *et al.*, 2003). This is because CO_2 level decreases with increasing elevation. Previous studies have shown increases in stomatal density, with elevation acting as a limiting factor in photosynthesis. Increases in stomatal density resulting in increasing stomatal conductance should offset the decreases in CO_2 , but it is reported that such CO_2 availability is not a limiting factor (McElwain, 2004). In *C. odorata* studied, we observed that the leaves at lower

elevation had higher stomata index which was significantly higher ($p < 0.05$) than the leaves in mid and higher altitudes. Our result clearly indicates that the lower the altitude, the higher the stomata index. This observation finds support in the work of Schoettle and Rochelle (2000) who also reported that the stomata density decreased with increasing elevation. In contrast, Körner (1988), Körner *et al.*, (1989) and Körner (1999), reported a general trend towards increased stomatal density at high elevation. This pattern is consistent in many mountain ranges around the world. They however agreed that there are nevertheless a number of locations where the pattern is reversed or nonexistent. For example, in *Lobelia teleki* from Mt. Kenya, stomatal density increased by 20% between 3750m and 4190m, then decreased by nearly 50% between 4190m and 4740m some wet tropical mountains, such as Mt. Wilhelm in Papua New Guinea, there did not appear to be any elevational trend in stomatal density (Körner *et al.*, 1989). Holland and Richardson (2009) concludes that the apparently contradictory results demand a discussion of the various theories to explain the observed relationships between stomatal traits and elevation.

The reported variation in morphological response observed in *C. odorata* along the three study sites can also be seen in anatomical data. The mean leaf thickness showed that there was a significant ($p < 0.05$) increase from lower altitude to the mid altitude, whereas, there is no significant increase ($p < 0.05$) in comparison to the higher altitude. This perhaps indicates that there is no correlation between leaf thickness and altitude. The lack of clear pattern recorded in this study has been previously reported in many plant species. For example, there have been cases where leaves of plants at higher altitudes were found to be thicker than leaves of lower altitude plants (Codignola *et al.*, 1987; Cordell *et al.*, 1998; Kofidis *et al.*, 2003) while the reverse have been reported in some other plant species (Morecroft and Woodward, 1996; Suzuki, 1998). The reason

for this has been attributed to the species specificity which is associated with genetical make up of individual plants and environmental conditions at various elevation habitats. We hasten to add however that increases in leaf thickness may be a disadvantage for CO₂ uptake. Diffusion of CO₂ may be reduced due to an increase in length of the pathway from the stomata to the carboxylation site (Vitousek, 1992).

In conclusion, this study confirmed earlier reports that plants generally respond to changes in altitudinal gradient and in this case, *C. odorata* responded to changes in altitude with observed changes in foliar morphology as well as anatomical parameters. This study has increased our knowledge on the response of this plant to altitudinal gradients.

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