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Full Length Article

Moringa oleifera (Horseradish Tree) Leaf Adaptation to Temperature Regimes

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ABSTRACT

Geographical distribution and growth of plants are to a great extent governed by temperature. As *Moringa oleifera* trees are mainly found throughout the tropics around the world, the extent of their physiological adaptability to lower temperature was the main objective of this study. The successful cultivation of *M. oleifera* trees in cooler climates would greatly increase their production areas. A total of 264 trees were randomly assigned to three temperature-controlled greenhouses, each with a different fluctuating night/day temperature regime namely; 10/20°C, 15/25°C and 20/30°C. Throughout the trial period, stomatal index, leaf conductance as well as leaf sampling for microscopical analysis were conducted to identify morphological adaptations to lowers temperatures in the leaves across all three temperature regimes. Leaves from the 10/20°C treatment had an average thickness of 0.239 mm, compared to 0.136 mm at 20/30°C. This is a 43.1% increase in leaf thickness, as a result of a mere 10°C decrease in temperature. Leaves were thicker mostly due to a broader spongy mesophyll layer. Despite larger stomata observed at the 20/30°C TR, the lower stomatal index resulted in a significant 18.9% reduction in leaf conductance compared to the 10/20°C TR. Although higher temperatures generally favoured tree growth, plants acclimatized to lower temperatures through physiological adaptations.

Key Words: Leaf thickness; Stomatal index; Stomatal density; Photoinhibition; Biofuel

INTRODUCTION

Moringa oleifera also known as Horseradish or Drumstick-tree is a fast growing, drought tolerant tree that is able to tolerate poor soil conditions. *M. oleifera* originates from the sub-Himalayan regions of north-western India, but is currently found in tropical regions throughout the world. The tree is particularly renowned for its great versatility; its uses being a food source for humans and animals alike, coagulant for water purification, remedy for numerous ailments as well as a source for biofuel production (Anwar *et al.*, 2007; Rashid *et al.*, 2008). Although *M. oleifera* is grown in numerous African countries, no evidence of large-scale commercial plantings has been reported, possibly as a result of the limited scientific data that are currently available on both the propagation and cultivation of the tree. As neither soil type nor rainfall, seem to considerably deter the growth of *M. oleifera* trees, temperature seemingly is the key factor influencing their dispersal and productivity. Temperature is one of the most important uncontrollable climatic factors governing the respiration rate, which has a direct effect on photosynthesis as well as the growth and development of plants (Munir, 2004). This in turn has an effect on the natural geographical plant distribution, tree performance, physiology and productivity (Sakai & Larcher, 1987; Grace, 1988). According to Anwar *et al.* (2007) *M. oleifera* production should be increased in climatically suited areas due to the multitude of beneficial uses it offers to mankind. As *M. oleifera* trees favour hot and humid environments, trees are mainly found in tropical regions around the world (National Research Council, 2006). In order to increase the production areas of *M. oleifera* beyond the tropics, the effect of cooler growing temperatures have on trees needs to be thoroughly understood. The main aim of this study was thus to determine the effect of lower growing temperatures on leaf morphology and productivity.

MATERIALS AND METHODS

All trials were conducted in temperature controlled glasshouses at the Phytotron section on the Experimental Farm of the University of Pretoria (25°45′ S, 28°16′ E) at an altitude of 1372 m above sea level.

Trees used for this study were cultivated from seed, originating from wild *M. oleifera* trees in Malawi. Seeds were planted and germinated in seedling trays containing Hygromix™, a sterile, soilless growing medium for seedlings, containing peat and polystyrene manufactured by Hygrotech Seed (Pty) Ltd. Five weeks after planting, 264 seedlings were transplanted into 10 L black plastic bags filled with a commercial bark potting medium manufactured

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by Braaks (Pty) Ltd. and then equally divided and randomly assigned to three temperature-controlled glasshouses. The three glasshouses were set at 10/20°C±2°C, 15/25°C±2°C and 20/30°C±2°C, respectively simulating night/day temperature fluctuations, while plants were exposed to natural daylight. The average photosynthetic active radiation (PAR) at 12:00 in the afternoon on a clear day, inside the temperature-controlled glasshouses was measured at 1350 μmol m^{-2} s^{-1}. Trees were irrigated to field-capacity three times a week.

Leaflet segments for anatomical examination were randomly collected from the three temperature treatments and prepared for light microscopy before being imbedded according to Coetzee and van der Merwe (1996). Segment specimens were fixed in 2.5% gluteraldehyde in a 0.075 M phosphate buffer (pH 7.4-7.6) for two hours, before being rinsed 3X in the same 0.075 M phosphate buffer (10 minutes each). Specimens were then fixed in 0.5% aqueous osmium tetroxide (OsO_4) for 2 h and rinsed 3X with distilled water (10 min each), followed by dehydration of the specimens in a range (30%, 50%, 70%, 90%) of ethanol: water dilutions, followed by three times in 100% ethanol. Leaf samples were then impregnated with 50% Quetol epoxy resin for 1 h followed by 4 h in 100% Quetol and then polymerized at 60°C for 39 h. Sections of 0.5 to 1 μm thick were cut with a Reichert Ultracut E ultramicrotome and placed onto microscope slides before being stained with toluidine blue. Sections were then mounted in immersion oil and viewed with a Nikon Optiphod light microscope. Photographs were taken digitally using a Nikon DXM 1200 digital camera.

Randomly collected leaflets from the three different temperature regimes (TRs) were prepared for SEM according to Coetzee and van der Merwe (1996). A 3 mm X 5 mm square leaf sample was sectioned from the centre of a pinnule (leaflet) (between the midrib and the pinnule margin) collected from a central pinna. The fixation and dehydration procedures of the leaf samples used for the SEM were identical to those discussed in the preceding light microscopy section. Following dehydration, some of the leaf samples were transferred and dried in a Bio-Rad E3000 critical point drier with liquid CO_2, before being mounted on aluminium stubs and coated with gold in a Polaron E5200C sputter coater. Leaf sections were viewed with a JOEL 840 scanning electron microscope, while photographs were taken digitally.

The PAR (μmol m^{-2} s^{-1}) was measured using a ceptometer (AppPAR LP80 Series, manufactured by Decagon Devices Inc. 950 NE Nelson Ct. Pullman, WA 99163. USA).

Leaf conductance (mmol m^{-2} s^{-1}) measurements at the three TRs were conducted using a Porometer (Model SC-1, manufactured by Decagon Devices Inc. 950 NE Nelson Ct. Pullman, WA 99163. USA).

The stomatal index (SI), rather than stomatal density (SD) of the various treatments was determined according to the formula below developed by Salisbury (1927) for comparison, as the SD might be affected by the expansion of the surrounding epidermal cells due the factors such as light, temperature, leaf position and water status (Royer, 2000).

$$SI\ (\%) = \frac{\text{stomatal density}}{\text{stomatal density + epidermal cell density}} \times 100$$

Data collected over the 32-weeks trial period were statistically analyzed using the Statistical Analysis System (SAS Version 9.1) program for Microsoft Windows, by the Statistics Department at the University of Pretoria. The Analysis of Variance (ANOVA) was performed, together with F-test (Steele & Torrie, 1980) to enable the comparison between treatment means.

RESULTS AND DISCUSSION

The difference in temperature regime (TR) visibly influenced leaf thickness, as leaf thickness increased with a decrease in temperature. Leaves randomly collected from the plants grown under the 20/30°C treatment, were on average 0.136 mm thick, while the average leaf thickness under the 10/20°C regime was 0.239 mm (Fig. 1 & 2). The average leaf thickness increased by 43.1%, caused by a 10°C decrease in the night/day TR. Leaves from the plants grown at 10/20°C not only had more spongy mesophyll tissue, but also longer palisade cells, compared to the leaves from the 20/30°C treatment.

Anatomical modifications observed in plants are often in response to changing environmental conditions (Shao *et al*., 2008). Progressive decreases in leaf thickness with an increase in TR were also observed in cherimoya (*Annona cherimola* Mill.) (Higuchi, 1999), macadamia (*Macadamia integrifolia*) (Trochoulias and Lahav, 1983), avocado (Persea americana Mill.) (Lahav and Trochoulias, 1982) and mangosteen (*Garcinia mangostana* L.) (Wiebel *et al.,* 1994) trees. Reduced temperatures however, result in increased development of mesophyll tissue in the leaves, consequently increasing their thickness. This is a response of plants to minimize the damaging effects of photoinhibition. Photoinhibition, also known as light-induced damage to PSII, occurs when the rate of light energy absorption exceeds the rate of its consumption in chloroplasts, resulting in damage to PSII (Melis, 1999). Exposure of plants to low temperatures can instigate photoinhibition, even under low to normal light intensities (Öquist & Huner, 1990; Schöner & Krause, 1990; Somersalo & Krause, 1989). Boese and Huner (1990) presume that the increase in mesophyll tissue responsible for leaf thickening at lower temperatures may be a mechanism to counteract photoinhibition. As the added palisade cells absorb an additional fraction of the light, thereby reducing the proportion of cells exposed to the high

Table I: Average leaf dry mass (g), area (cm^2) and conductance (mmol $m^{-2}s^{-1}$) at trial termination after 32 weeks. Different letters indicate significant differences at $P \leq 0.05$ according to the F-test

Temperature regime	Average leaf dry mass (g)	Average leaf area (cm^2)	Average leaf conductance (mmol $m^{-2}s^{-1}$)
10/20°C	37.5^{b}	1031.57^{e}	328.3^{f}
15/25°C	63.13^{a}	1875.50^{d}	315.6^{f}
20/30°C	68.44^{a}	1980.34^{c}	266.4^{g}

Fig. 1: Differences in *Moringa oleifera* leaf anatomy, caused by the different temperature treatments. A=10/20°C, B=15/25°C and C=20/30°C. a - upper (adaxial) epidermis, b - palisade parchenchyma, c - spongy mesophyll, d - lower (abaxial) epidermis

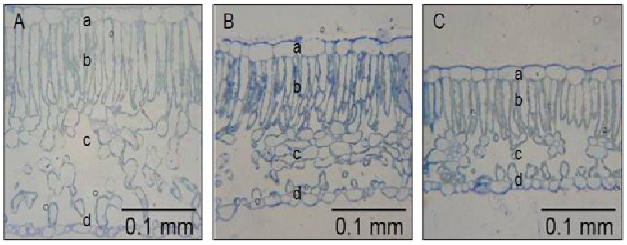
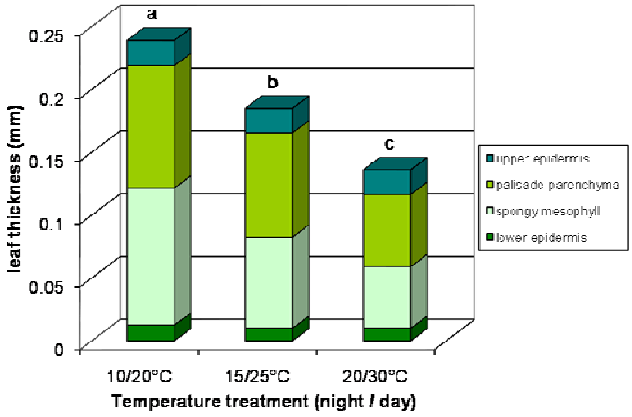


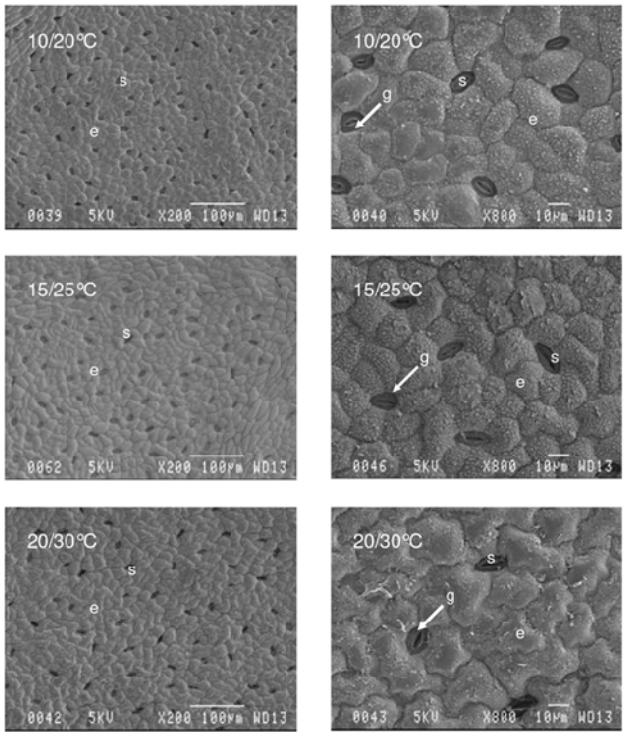
Fig. 2: Temperature effect on *Moringa oleifera* leaf thickness and the various leaf components. Different letters indicate significant differences at $P \leq 0.05$ according to the F-test



light intensity. The significantly thicker leaves of the 10/20°C TR was probably an adaptation to counteract photoinhibition, however photoinhibition was not measured. According to Terashima *et al.* (2001) an added advantage of thicker leaves is the larger intracellular chloroplast surface area and potentially higher CO_2 assimilation rates.

Further physiological differences were also revealed after a closer look at the abaxial leaf surfaces of plants from the three temperature treatments. SEM photographs of the abaxial leaf surfaces from the 10/20°C, 15/25°C and 20/30°C temperature treatments at 200X and 800X magnification are given in Fig. 3. The number of stomata per unit leaf area is significantly higher at the lower 10/20°C

Fig. 3: Illustration of differences in stomatal number and size between the 10/20°C, 15/25°C and 20/30°C temperature treatment at a 200X (left) and 800X (right) magnification. s–Stomata, e–Epidermal cells and g–Guard cells



temperature incubation, however the individual stomata are smaller in size. Although the stomata were larger at the higher 20/30°C temperature treatment, the lower stomatal density (SD), resulted in a reduction in leaf conductance.

The average stomatal index of the three temperature treatments was 11.56%, 10.21% and 8.49% for the 10/20°C, 15/25°C and 20/30°C treatments, respectively. With a significant difference in stomatal index between the high 20/30°C and low 10/20°C TR, no significant differences in the stomatal index could, however be found between the 10/20°C and 15/25°C as well as the 15/25°C and 20/30°C treatments. The lower 10/20°C treatment regime had more stomata and epidermal cells per unit area, compared to the higher 20/30°C temperature treatment regime. Although the guard cells of the stomata and epidermal cells were sizably larger at the higher temperature 20/30°C treatment, the stomatal index was still lower, confirming the reduced leaf conductance measured at higher temperatures (Table I).

The reduction in stomatal conductance with an increase in temperature, is partially the result of the lower stomatal index at the higher TRs. However, Menzel and Simpson (1986) found the increase in air temperature to raise the leaf to air vapour pressure deficit, thus lowering stomatal conductance in lychees. The decrease in stomatal conductance is thus due to both the difference in leaf physiology as well as environmental circumstances. The

reduction in leaf conductance lowers photosynthesis, as photosynthesis is positively related to stomatal conductance (Kelly & Latzko, 1993). Considering the reduced stomatal conductance under the high 20/30°C TR, expectably plants at this regime would demonstrate reduced growth due to sub-optimal photosynthesis. However, the significantly greater leaf area (Table I) of plants at this TR, increased the total number of stomata per plant, resulting in increased vegetative growth and dry matter accumulation (Bañon *et al.*, 2006). Thus although the individual stomata might have had a lower conductance, the greater number of stomata per plant gave the 20/30°C TR the advantage over the lower TRs.

From Table I it is also evident that the reduction in temperature between the 20/30°C TR and 15/25°C TR, did not significantly affect leaf dry matter production.

CONCLUSION

From general observations, the higher growing temperatures favoured growth of *M. oleifera.* The thickening of the leaves at the lower 10/20°C is thus symptomatic of an adaptation against temperature stress, resulting in reduced growth. Although tropical climates therefore seem best for the cultivation of *M. oleifera*, reduced but satisfactory growth should still be achievable in below optimal climates, as trees seem to tolerate lower growing temperature through physiological adaptations.

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