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# Wide-Band Tracheids from a Southern African Succulent and Their Responses to Varying Light Intensities: A Pre-Adaptation for Future Water Stress?

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**Abstract:** Examination of leaf and stem tissues in a broad range of genera resulted in the discovery and analysis of a novel tracheid type termed Wide-Band Tracheids (WBTs; the term is derived from the comparatively wide secondary wall) in derived genera of Aizoaceae, Cactaceae and Portulacaeae (Caryophyllales). In Aizoaceae, WBTs are only found in genera of Ruschioideae; in Cactaceae, WBTs are found in xylem of leaves and stems in genera of Opuntioideae and Cactaceae. However, in the genus *Anacampseros* (Portulacaceae), WBTs are found in leaf xylem, but not as part of the xylem of the stems and instead, WBTs are found in piths and rays. It was hypothesized that the wide secondary wall prevents primary wall contact during extreme water stress and thus WBTs were thought to differentiate in response to water-stress. In order to determine what factors cause WBT initiation and differentiation, seedlings of *Anacamperos rufescens* (Portulacaceae) were exposed to varying light intensities that mimicked spring and summer light levels found in southern Africa. In this experiment, results show that WBTs are generally formed in advance of probable water-stress event times. Furthermore, the number of WBTs are directly correlated to the intensity of light received as a seedling; however, the mean WBT size remained relatively unchanged, presumably due to a rigid genetic control. Results suggest that, in *Anacampseros rufescens*, the later a seedling germinates, the greater the number of WBTs differentiate, which pre-adapts the plant for future water-stress events.

**Key words:** *Anacampseros*, light, portulacaceae, tracheid, wide-band

## INTRODUCTION

Wide-Band Tracheids (WBTs) have very wide annular or helical secondary cell walls (Fig. 1). These cells were first discovered in cacti (genus *Mammillaria* L.) by Schleiden (1845) (of cell theory fame). Recently, WBTs have been discovered in three related families of the Order Caryophyllales: Aizoaceae (only in subfamily Ruschioideae), Cactaceae (all but Pereskioideae) and Portulacaceae (four genera *Anacampseros* L., *Ceraria* H. Pearson and Stephens, *Grahamia* Gill. and *Talinaria* Brandegee) (Mauseth *et al*., 1995; Mauseth and Landrum, 1997; Landrum, 2001, 2002, 2006). Their systematic occurrence implies that only derived genera were capable of evolving these cells.

Most of these derived genera are associated with arid environments (water-stress environments). Thus, the proposed function of wide secondary wall of WBTs is to keep the primary cell walls of these tracheids from hydrogen bonding under water stress, which would permanently incapacitate the cells (Landrum, 2006).

In addition, another feature of arid-adapted plants is an intense light exposure for seedlings. The largest WBTs in genera of Portulacaceae are found in species of *Anacampseros*, which grows in or near the Namib and Kalahari Desert regions of southern Africa. Light intensity levels have been measured up to 500 μmol $m^{-2}$ $sec^{-1}$ in these deserts (Martin and Cox, 1984; Rossa and von Willert, 1999; Egbert and Martin, 2000). Earlier, preliminary experiments (unpublished data) on light intensity and WBT expression indicated that, regardless of species of *Anacampseros*, light intensity had a significant effect on WBT expression. In this study, germinating seeds of *Anacampseros rufescens* (Haw.) Sweet were exposed to various light intensity treatments for 60 days to confirm previous preliminary data which indicated that light levels have a role in the initiation and differentiation of WBTs.

## MATERIALS AND METHODS

Twenty germinating seeds of *Anacampseros rufescens* (Haw.) Sweet, obtained from field-collected seeds (Mesa Gardens No. 7083.2), were grown for 60 days under five different light intensities: 67.8 μmol $m^{-2}$ $sec^{-1}$, 114.9, 232.8, 314.6 and 349.2 μmol $m^{-2}$ $sec^{-1}$ (measured with an Extech© light meter). Seeds were germinated in petri dishes on moist filter paper until cotyledons appeared, after which seedlings were transferred to plastic pots with a mixture high in sand and vermiculite. Light sources consisted of four 40 watt Sylvania Grolight©

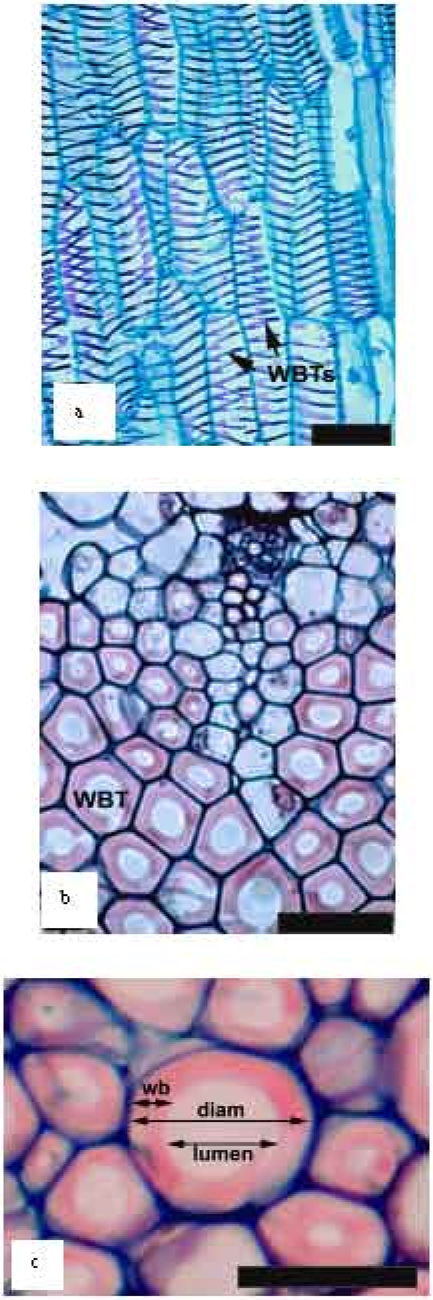


Fig. 1: Wide-Band Tracheids (WBT) in stems of *Anacampseros rufescens* (a) and *A papyracea* (a). (a) Longitudinal section of pith WBTs (X400); bar = 25 µm, (b) Transverse section showing pith WBTs (X400); bar = 50 µm and (c) Pith WBTs from *Anacampseros papyracea* showing how measurements were determined for WBT diameters, lumen area and wide-band (wb) area (X600); bar = 50 µm

bulbs and two 75 watt incandescent bulbs placed in a Percival Intellus© environmental growth chamber, Model No. E30BHO. Temperatures ranged from 18-25°C and photoperiod was 15 h.

Light intensities were chosen by results of two previous experiments, by using net radiation flux values gathered in the Namib Desert (von Willert *et al*., 1992) and by using the values of Martin and Cox (1984) for range grasses of the Kalahari Desert. Lux values were converted to µmol $m^{-2}$ $sec^{-1}$ of PAR by using a conversion table supplied by Sylvania, Inc. In order to obtain the intensities described above, variations in light intensity were created by using tissue filters placed above the seed containers and by using distance from the light sources. Great care was taken to insure that each treatment received the correct perpendicular illumination and that no lateral light entered a treatment. All plants received 10 mL water aliquots per week.

After 60 days, the plant specimens were harvested, fixed with Navashin's solution, dehydrated in an increasing ethanol/tertiary butyl alcohol series (50-100%) and then embedded in Paraplast Plus. Standard safranin and fast-green staining methods were used (Mauseth *et al*., 1984). Stem sections were cut at 15 or 25 µm. Slides were viewed using bright-field microscopy and images were obtained using a Nikon Eclipse E400 microscope with a Nikon DXM1200 digital camera (Fig. 1). Wide-band tracheids were measured using the Motic© Images 1.3 software. Measurements were gathered (Fig. 1c) by subtracting the lumen area from the whole cell area to give the wide-band area; these data were converted into percentages for easier comparison. The mean number of WBTs was calculated by examining and counting pith and ray WBTs in at least two (but usually four) stems. Any statistical calculations were performed using SPSS version 14.0.

## RESULTS

The number of WBTs did increase greatly as light intensity increased over the 60 day period (Fig. 2a-e) and plant size diminished (Fig. 3). Where the cell area occupied by the wide-band remained fairly constant whereas WBT number increased as light intensified. Although various size classes did exist, no statistically significant differences were found in percent area of the wide-bands between treatments.

Examination and measurement of Wide-Band Tracheids (WBTs) in the stem transverse sections (Table 1) show two significant findings. First, as light intensity increased from 67.8 to 314.6 µmol $m^{-2}$ $sec^{-1}$, the number of WBTs increased tenfold from 20.7 to 208,

Table 1: Measurement results of wide-band tracheids by light treatment

| Light treatment ($\mu$mol m$^{-2}$ sec$^{-1}$) | Mean WBT cell number measured | Mean WBT area | Mean cell area | Mean lumen area | Mean % cell area occupied by the wide-band/SD |
|---|---|---|---|---|---|
| 67.8 | 20.7 | 101.9 | 27.9 | 74.0 | 72.4/9.4 |
| 114.9 | 15.5 | 93.2 | 24.3 | 68.9 | 74.4/9.4 |
| 232.8 | 196.0 | 119.9 | 30.6 | 89.3 | 75.0/9.5 |
| 314.6 | 208.0 | 116.1 | 39.2 | 77.0 | 67.0/8.9 |
| 349.2 | 149.0 | 100.4 | 30.0 | 70.4 | 71.9/9.9 |

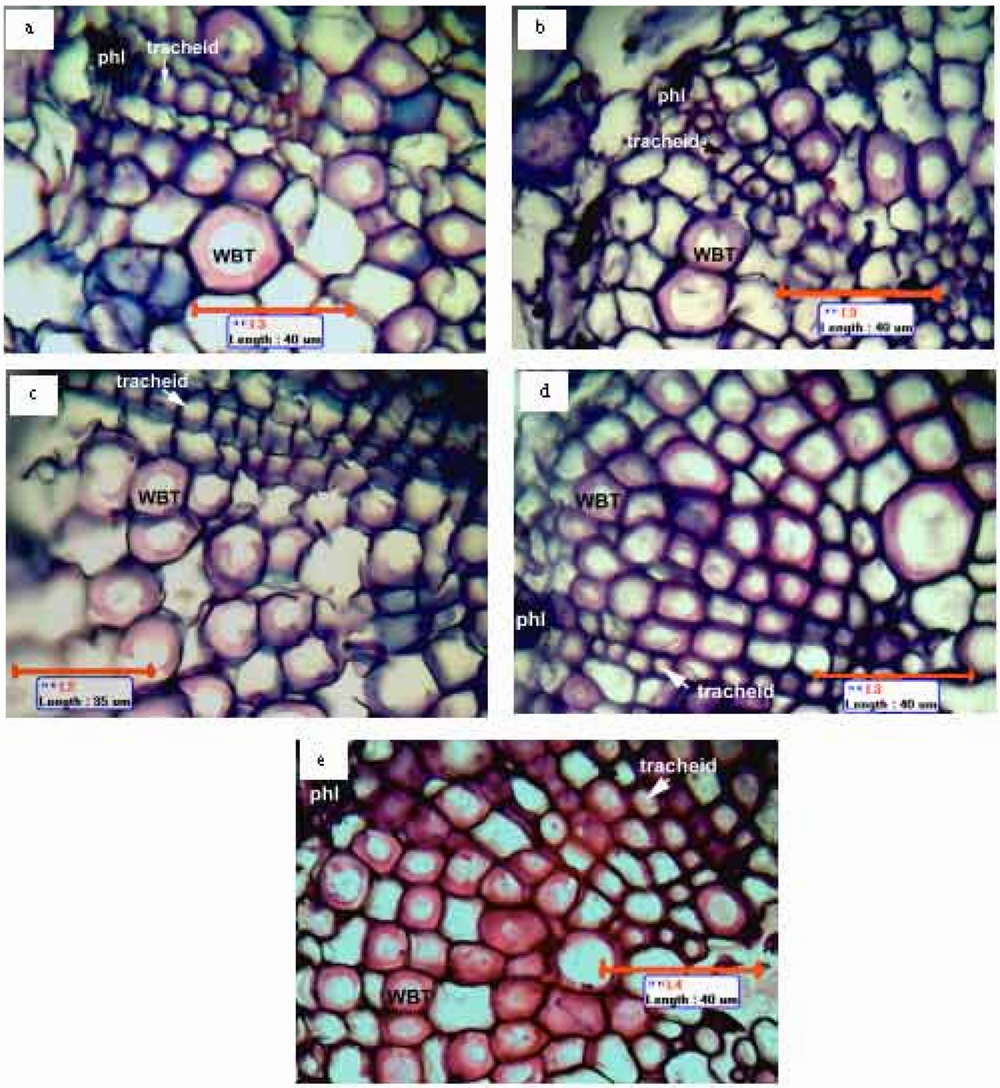


Fig. 2: Stem transverse section of *Anacampseros rufescens*. (a) Wide-band tracheids from the 67.8 $\mu$mol m$^{-2}$ sec$^{-1}$ treatment; wide-band tracheids (WBT), common tracheids and phloem cells (phl) are noted (X400), (b) Wide-band tracheids from the 114.9 $\mu$mol m$^{-2}$ sec$^{-1}$ treatment; wide-band tracheids (WBT), common tracheids and phloem cells (phl) are noted (X400), (c) Wide-band tracheids from the 232.8 $\mu$mol m$^{-2}$ sec$^{-1}$ treatment; wide-band tracheids (WBT), common tracheids and phloem cells (phl) are noted (X400), (d) Wide-band tracheids from the 314.6 $\mu$mol m$^{-2}$ sec$^{-1}$ treatment; wide-band tracheids (WBT), common tracheids and phloem cells (phl) are noted (X400) and (e) Wide-band tracheids from the 349.2 $\mu$mol m$^{-2}$ sec$^{-1}$ treatment; wide-band tracheids (WBT), common tracheids and phloem cells (phl) are noted (X400)


Fig. 3: Plants of *Anacampseros rufescens* from the five light treatments; from left to right, 67.8, 114.9, 232.8, 314.6 and 349.2 μmol $m^{-2}$ $sec^{-1}$ (x2). Note the color change as light intensity increased, from green to maroon, indicating an increase in anthocyanins, possibly for protection from ultraviolet light (UV); also, the size of stems and leaves decreased as light levels increased, however, the stem width increased as light levels increased

respectively; however, at 349.2 μmol $m^{-2}$ $sec^{-1}$, the number decreased by 27.9% to 149 WBTs. Secondly, the area of the cell occupied by the wide-band secondary wall stayed relatively unchanged (well within one standard deviation; Table 1 as light intensity increased. Figure 2a-e shows the transition from few WBTs to many WBTs as light intensity increased. Although various size classes of WBTs were present, depending on the cell location (e.g., later ray WBTs were slightly larger than near-pith WBTs; Fig. 2), no statistically significant differences between treatments were found in percent area occupied by the wide-bands.

As light intensity increased, the seedlings became shorter and wider, especially at the stem-root interface (Fig. 3). Leaves became shorter as well and the presence of betalains (for protection from ultraviolet light) increased, as can be seen by the increase in color from green to maroon. Lastly, there was a decrease in overall plant size and morphology as light intensity increased to 349.2 μmol $m^{-2}$ $sec^{-1}$ (Fig. 3).

All of the above findings support earlier experiments on light intensity and WBT expression, which indicated that light intensity has a profound effect on WBT expression.

## DISCUSSION

The results clearly show a direct correlation between light intensity and WBT expression. The logical conclusion is that this association is selectively advantageous for these plants, as the presence of additional WBTs should help with water-stress events later on; thus the greater the light intensity (to a point around 314.6 μmol $m^{-2}$ $sec^{-1}$), the greater the number of WBTs. Above 314.6 μmol $m^{-2}$ $sec^{-1}$, the light intensity causes a decrease in WBT number; this intensity, in nature, may be too high for the plants to function normally.

Wide-band tracheids (WBTs) have only been found in arid-adapted plants in three succulent families (Aizoaceae, Cactaceae and Portulacaceae) and only in the more derived genera of these families (Mauseth *et al*., 1995; Mauseth and Landrum, 1997; Landrum, 2001, 2002, 2006). The more derived genera inhabit the more arid areas of their ranges and logically, WBTs possibly evolved as a mechanism for coping with persistent drought events; leafy cacti species (e.g., species of *Pereskia* Miller) and species of the less succulent portulacs (e.g. *Talinum* Adans., *Calandrinia* Kunth) live in less drought-stressed habitats and have not evolved WBTs (Mauseth and Landrum, 1997).

One factor in this experiment was determining the appropriate light levels. Rossa and von Willert (1999) found that several geophytes in semi-arid regions of Namaqualand reached photosynthetic net saturation at light intensities of greater than 500 μmol $m^{-2}$ $sec^{-1}$. Martin *et al*. (1999) found that CAM bromeliads were more efficient in photosynthesis at light intensities around 100 μmol $m^{-2}$ $sec^{-1}$ but could function at levels up to 800 μmol $m^{-2}$ $sec^{-1}$. Martin and Cox (1984) found that a light intensity of 216 μmol $m^{-2}$ $sec^{-1}$ produced optimal results in their study on germination rates of native Kalahari lovegrasses. Egbert and Martin (2000) used mean light intensities of 325-550 μmol $m^{-2}$ $sec^{-1}$ for a photosynthetic rate study of three succulent species, one of which (*Lithops olivacea*; Aizoaceae) has wide-band tracheids (Landrum, 2001); Egbert and Martin's results indicated that the lower light intensities were more efficient for overall photosynthetic rates.

The results from this study clearly show a direct correlation between light intensity and WBT expression; thus the greater the light intensity (to a point around 314.6 μmol $m^{-2}$ $sec^{-1}$), the greater the number of WBTs. The logical conclusion is that this association is selectively advantageous for these plants. Hypothetically, seeds that germinate in the spring would have time to grow, produce more seeds and finish their reproductive cycles by the time that water availability is threatened in later hotter days of their season. However, seeds that germinate later are faced with higher light intensities than normal and would have to survive decreasing water availability as summer approached. Survival of these water stress events would be helped by the differentiation of pith and ray parenchyma cells into wide-band tracheids, which could prevent collapse of the water conduction system during water stress and serve as water storage cells as well.

Thus, the data supports the hypothesis that seedling exposure to higher light intensities would pre-adapt these plants to the coming water stress events. Above 314.6 μmol $m^{-2}$ $sec^{-1}$, the light intensity causes a decrease in WBT number; this intensity, in nature, may be too high for the plants to function normally. The mechanism for such relationships between light intensity and number of WBTs is unknown and this presents an area for active research in the near future.

The recruitment of pith and ray parenchyma into wide-band tracheids is another area of much-needed research. In addition, results from the measurements of WBT size imply a tight genetic control over the mean sizes of WBTs as they are being produced; other succulent species with similar wide-band tracheids are being studied for clues to the signaling pathways for this rigid control mechanism.

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