



Water relations and drought tolerance of young African tamarind (*Tamarindus indica* L.) trees



N. Van den Bilcke*, D.J. Simbo, R. Samson**

Department of Bioscience Engineering, Faculty of Science, University of Antwerp, Groenenborgerlaan 171, 2020 Antwerp, Belgium

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ABSTRACT

Tamarindus indica L. is an important multipurpose tree, indigenous to Africa, now introduced worldwide and known for its drought tolerance. The effects of drought on tamarinds, especially at seedling stage, are hardly investigated. However, this information is important for its conservation and domestication. In a growth chamber experiment we investigated the water relations of African tamarind seedlings under short-term soil drought stress. Initially tamarind seedlings can be considered as drought-tolerant at the expense of internal water storage reserves as they keep on transpiring (sap flow) and growing (diameter fluctuations). They finally spent 20% of their stem and root water storage reserves and experienced stem water potentials near -3 MPa. Therefore, they can be classified as anisohydric. Their risk-taking behavior led to a high rate of seedling mortality (50%) because of whole plant hydraulic failure. They were not hydraulically efficient and they possessed low water storage capacity in stem and root (45%) due to high tissue density. When re-irrigated, remaining seedlings recovered slowly as a consequence of non-stomatal limitations and partial shoot dieback. Although tamarind seedlings show traits related to drought tolerance, we suggest that the species contains some water saving mechanisms. Contrasts with the co-occurring water-conserving tree species baobab (*Adansonia digitata* L.) are also discussed.

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1. Introduction

Tamarind (*Tamarindus indica* L.; family Fabaceae) is a semi-evergreen multi-purpose species with numerous food and non-food uses. It is mainly valued for its fruits, especially the pulp, which is used for a wide variety of domestic and industrial purposes (e.g. Nordeide et al., 1996; El-Siddig et al., 1999; Lockett and Grivetti, 2000; El-Siddig et al., 2006; De Caluwé et al., 2010; Havinga et al., 2010; Van der Stege et al., 2011). The species is pantropical (occurring in sub- and semi-arid tropics) and introduced worldwide, naturalized and cultivated in over 50 countries (El-Siddig et al., 2006). The major production areas can be found in Asia (Thailand and India) and Central America (Mexico and Costa Rica), where trees are grown in plantations and high-yielding cultivars with fruit pulp having specific tastes (sweet/sour) are being developed (De Caluwé et al., 2010). Africa, where tamarind is assumed to be indigenous (El-Siddig et al., 1999; Diallo et al., 2007), does not produce

tamarind on a commercial scale (it still serves as a subsistence crop), though it is widely used to meet local communities' requirements (El-Siddig et al., 2006). Except in West Africa where the tree species appears commonly in Sahelian and northern Sudanian climatic zones (but less toward the more humid Guinean zone; Havinga et al., 2010), participative domestication programs have commenced in certain areas. In all surveys carried out in Sahelian countries, tamarind appeared among the top five species with the greatest potential value for domestication (Akinifesi et al., 2008). El-Siddig et al. (2006) describe *T. indica* in more detail.

Although tamarind trees are commonly saved when land is cleared for agricultural purposes (Boffa, 1999), recent studies recorded poor recruitment and low tree density, particularly in populations close to farmland (Fandohan et al., 2010). These facts in combination with the extensive local use and demand for tamarind fruits and leaves may threaten tamarind populations, especially in habitats subjected to drought stress and human pressure (Maranz, 2009; Bourou et al., 2012).

In this study we focus on tamarind planting material originating from the Sahelian zone of Mali, West Africa. The Sahelian zone in Mali is characterized by harsh drought events leading to the degradation of agroforestry parkland systems, in which most of the regions' tamarind trees are found. Only a few studies have been performed on drought tolerance of (adult) tamarind trees (Zhong-hua et al., 2004; Zhixian et al., 2008). So far as we know, no studies have been carried on drought tolerance of tamarind seedlings. However, understanding the impact of drought stress on tamarind seedlings is important for the conservation (e.g. recruitment success in the field) and domestication of the species (e.g. good water resource management and practices for plantings).

Abbreviations: D, stem diameter fluctuations (mm); DG, daily stem growth (mm d^{-1}); DN, day number; $DW_{\text{plant part}}$, oven-dry mass (g) with plant part l = leaves, s = stems, r = roots and sr = small roots; E, transpiration rate ($\text{mmol H}_2\text{O m}^{-2} \text{s}^{-1}$); F, sap flow rate (g h^{-1}); $FW_{\text{plant part}}$, fresh mass (g) of each plant part; LA, total leaf area (cm^2); MDS, maximum daily stem shrinkage (mm); nL, number of leaves; PAR, photosynthetic active radiation ($\mu\text{mol m}^{-2} \text{s}^{-1}$); P_n , net photosynthesis ($\mu\text{mol m}^{-2} \text{s}^{-1}$); RH, relative humidity (%); T, air temperature ($^{\circ}\text{C}$); $WC_{\text{plant part}}$, water content of each plant part (%); WD, wood density (g cm^{-3}); WUE, water use efficiency; Ψ_s , stem water potential (MPa).

* Corresponding author. Tel.: +32 3 265 36 82.

** Corresponding author. Tel.: +32 3 265 34 37; fax: +32 3 265 32 25.

E-mail addresses: Nina.VandenBilcke@ua.ac.be (N. Van den Bilcke),

Roeland.Samson@ua.ac.be (R. Samson).

Therefore, the aim of this study was to answer the following question: How are the water relations of tamarind seedlings influenced by a short-term soil drought event? To answer this question, the ecophysiological, morphological and anatomical traits related to the water status of tamarind seedlings were unraveled. Based on the fact that tamarind is a semi-evergreen hardwood species, we hypothesized that tamarind seedlings tolerate short-term soil drought by:

- (1) continuing water use and growth;
- (2) keeping most of its leaves;
- (3) lowering stem water potential; and
- (4) depleting the limited amount of stem water storage.

The results of this study were compared with data obtained in a previous study, investigating sap flow and water use in African baobab (*Adansonia digitata* L.) seedlings in response to drought (Van den Bilcke et al., 2013). Baobab is a deciduous lightwood species, growing in the same environment as tamarind, and showing a conservative water use strategy.

2. Materials and methods

2.1. Plant material and experimental design

Tamarind (*T. indica*) seedlings were established from seeds under well-watered conditions in a greenhouse at the University of Antwerp in April 2007 and 2010. Seeds originated from Hombori, a northern provenance in the Sudano-Sahelian zone of Mali (15°4'N, 0°53'W, West-Africa), which is characterized by a mean annual precipitation of 375 mm (World Climate database of Hijmans et al., 2005). In order to improve germination, seeds were scarified by soaking in 50% H₂SO₄ for 60 min (Muhammad and Amusa, 2003), followed by sequential rinses in distilled water. In April 2011, a batch of young tamarind trees (n = 26) were transplanted in a growth chamber (Snijders Scientific B.V., Economic Delux, Tilburg, The Netherlands) in containers, which were filled with a mixture of riverine sand (grain size 0.2 mm) and a controlled release fertilizer (Scotts International B.V., Osmocote Exact Standard 5–6 M, Geldermalsen, The Netherlands; 15% N, 3.9% P and 9.9% K). The size of the containers was 3.5 dm³ (12 cm diameter × 40 cm height) and 7 dm³ (21 cm diameter × 27 cm height) for the one-year and four-year old tamarind trees, respectively.

The growth chamber allowed for control of radiation and air temperature regime. The daytime period was selected to last from 6 am until 6 pm. Photosynthetic active radiation (PAR) reached 200 μmol m⁻² s⁻¹ during daytime and was measured with a quantum sensor (PAR logger, LI-190 Quantum Sensor, LI-COR, Lincoln, NE, USA). Air temperature (T, °C) was set at 27.5 °C during daytime and at 21 °C during night time. Temperature and relative humidity (RH, %) were continuously recorded using a mini data logger (Helios mini, Skye Instruments Ltd., Powys, UK). Vapor pressure deficit (VPD, kPa) of the air was calculated from the measured T and RH as the difference between saturation vapor pressure at air temperature (e_v⁰, kPa) and ambient vapor pressure (e_v, kPa). During daytime, VPD increased to an average of approximately 1 kPa, while during night time it decreased to 0.5 kPa.

Before the start of the experiment, the young trees were watered close to field capacity (which corresponds with a soil moisture content of 17.5%), every two days. The experiment comprised two parts:

- 21 one-year-old tamarind seedlings were used to investigate the effects of drought and recovery on a number of morphological and physiological parameters (see Sections 2.2.1, 2.2.2, 2.2.5, 2.3 and 2.4). The seedlings were divided into three groups. Each group was subjected to a different treatment: 7 seedlings remained well-watered (designated as control seedlings) close to field capacity, 7 seedlings were exposed to drought by withholding water from the soil completely from day number (DN) 4 until DN 29 (i.e. drought-stressed seedlings), from DN 4 to DN 29 water was withheld from

7 seedlings and then from DN 29 to DN 43 these seedlings were rewatered to allow for recovery from stress (i.e. recovered seedlings). Measurements on control and drought-stressed seedlings were performed between DN 25 and DN 26, and on recovered seedlings between DN 41 and DN 43. Soil moisture content in the containers was monitored gravimetrically during the course of the experiment (Fig. 4), by weighing the filled containers and correcting for seedling biomass to obtain mass of the wet soil. At the end of the experiment the oven dry mass of the soil was determined to be able to calculate soil moisture content.

- Two one-year-old tamarind seedlings (seedlings I and II) were selected for dynamic water flow measurements at the stem level (see Sections 2.2.3 and 2.2.4). Both seedlings were continuously monitored from DN 1 to DN 43 and from DN 63 to DN 84. Drought stress was applied by interrupting the water supply from DN 4. After the drought period (DN 29), both seedlings were re-watered close to field capacity until DN 84. Note that some preliminary measurements of sap flow and diameter fluctuations were performed on three four-year-old tamarind trees, planted in April 2007.

2.2. Ecophysiological measurements

2.2.1. Stomatal conductance and water use efficiency

Stomatal conductance to water vapor diffusion (g_s, mol H₂O m⁻² s⁻¹) and also transpiration rate (E, mmol H₂O m⁻² s⁻¹) were measured using a portable infrared gas analyzer (LI-6400, LI-COR, Lincoln, NE, USA). Leaf temperature was set at 28 °C and relative humidity of the reference air was kept around 60% (VPD around 1 kPa). The CO₂ concentration of the reference air (C_a) was adjusted to 400 ppm and the light intensity in the chamber was set to 200 μmol m⁻² s⁻¹ (similar PAR as in the growth chamber). The air flow was kept at 500 mL min⁻¹. As leaves of tamarind are pinnate and composed of little leaflets, leaf area (LA) in the chamber, which is normally fixed at 6 cm², was corrected by destructively determining LA of each measured leaf sample with a leaf area meter (LI-3100C, LI-COR, Lincoln, NE, USA). Furthermore, g_s and net photosynthesis rate (P_n, μmol m⁻² s⁻¹) were also measured at saturating light intensity (>1000 μmol m⁻² s⁻¹) (other settings remained the same) to investigate the intrinsic water use efficiency (WUE). The slope of the relationship between P_n and g_s represents WUE (P_n/g_s) (Mediavilla et al., 2002). Measurements were made between 9 am and noon on at least two leaves of three individuals each of the control, drought-stressed and recovered seedlings.

2.2.2. Stem water potential

Midday stem water potential (Ψ_s, MPa) was measured with a pressure chamber (PMS Instrument Co., Corvallis, OR, USA). Attached leaves were enclosed in aluminum foil and thus prevented from transpiration about 2 h prior to the measurements. Measurements were conducted between 12 and 2 pm. By assuming zero sap flow in the petiole of the leaf, the measured value corresponded with midday Ψ_s (McCutchan and Shackel, 1992).

2.2.3. Sap flow rate

Sap flow rates (F, g h⁻¹) of two tamarind seedlings were continuously measured with sap flow sensors (Model SGA5, Dynamax Inc., Houston, TX, USA), based on the constant power heat-balance principle (Baker and Van Bavel, 1987). The sap flow sensors were installed at ~15 cm above soil surface on both tamarind seedlings. For more information about the installation of sap flow sensors and the calculation of F we refer to the operation manual of Dynamax Inc. (2005), Steppe and Lemeur (2004) and Van den Bilcke et al. (2013).

2.2.4. Diameter fluctuations

Stem diameter fluctuations (D, mm) were continuously measured with Linear Variable Displacement Transducers (LVDTs, Model DC-SC 250, Schaevitz, Hampton, VA, USA), fixed by a custom-made stainless

steel holder at the stem base, ~10 cm above the soil surface on the seedlings, where the sap flow sensor was attached. D can be divided into two components: (1) a reversible component which is related to changes in hydration of extensible tissues (i.e. the depletion of water storage pools during the day and the replenishment of water storage pools during the night) and (2) an irreversible component which is related to tissue growth (Kozłowski, 1972). Measurements of D were performed to evaluate the daily depletion and replenishment of internal water storage pools and to calculate maximum daily stem shrinkage (MDS, mm) and daily stem growth (DG, mm d⁻¹). MDS corresponded to the difference between the daily maximum and minimum stem diameters. DG was defined as the difference between two successive daily maximum stem diameters.

2.2.5. Plant water content

Fifteen tamarind seedlings (five per treatment) were divided into leaves, stem, taproot and fine roots. Fresh weights (FW, g) and dry weights (DW, g) of the different plant parts were determined and the water content of each plant part ($WC_{\text{plant part}}$, %) was calculated as $100 \times (1 - DW_{\text{plant part}} / FW_{\text{plant part}})$.

2.3. Morphological measurements

2.3.1. Leaf phenology

Leaf shedding and the formation of new leaves were monitored on a daily basis by counting the number of leaves (nL) on each seedling.

2.3.2. Wood density

Wood density (WD, g cm⁻³) of the control seedlings was calculated as the ratio between DW_s and fresh volume (measured by the displacement of water). WD was considered as a proxy of the vulnerability to xylem cavitation (Pineda-García et al., 2011). Higher WD should lead to less vulnerability to embolism (Hacke et al., 2001; Choat et al., 2005).

2.4. Anatomical measurements

2.4.1. Light microscopy

Transverse sections of fresh leaves, stems and roots of the control seedlings were made by hand or by using a sliding microtome. Some fresh samples were stained with thionine to detect the possible presence of mucilage (a polysaccharide mixture with a high water-binding capacity) (Ghanem et al., 2010). Stomatal imprints of both adaxial and abaxial leaf sides of the control seedlings were made, using the replica

method (Wuytack et al., 2010). Transverse sections and stomatal imprints were viewed by light microscopy images at different magnifications. Stomatal length and stomatal density (number of stomata per unit area) of leaves, and vessel diameters of stem and root were estimated using CellP software (Olympus Soft Imaging Solutions, Münster, Germany).

2.4.2. Scanning electron microscopy

Specimens of fresh leaves and stems of the control, drought-stressed and recovered seedlings were cut into small pieces. Samples were preserved in FAA-solution (formaldehyde–acetic acid–alcohol) until they were dehydrated through ethanol series and critical point dried (Leica EM CPD030, Wetzlar, Germany). The pieces were mounted on aluminum stubs, sputter coated with gold (Cressington 108 auto, Watford, UK) and transferred to the cold stage of the scanning electron microscope (SEM) (SEM 515, FEI Company, The Netherlands). Both adaxial and abaxial leaf surfaces and stem surface were visualized by SEM photographs at different magnifications.

2.5. Data acquisition and analysis

Signals from sap flow sensor and LVDTs were logged at 10 s intervals and averaged every 5 min using a data logger (DL2e, Delta-T Devices Ltd., Cambridge, UK).

All statistical tests were demonstrated with R statistical software (R Development Core Team, 2009). Normality of the data and homogeneity of the variances were tested with the Shapiro–Wilk normality and Bartlett tests, respectively. Differences between treatments were analyzed using linear mixed models in case of nested data (nlme package, Pinheiro et al., 2009; Zuur et al., 2009), with treatment as fixed factor and leaf samples as random factor nested within plant. Otherwise, differences between treatments were performed assessing one way ANOVA with factor treatment and a Tukey Honestly Significant Difference (HSD) approach.

3. Results and discussion

In the present study, the water relations' data suggested that tamarind seedlings could be considered as initially drought-tolerant. However, after severe drought stress (25 days without water), tamarind seedlings exhibited signs of water limitation and when the minimum threshold (~–3 MPa) (Table 1) at stem level was exceeded (DN 26) some of the tamarind seedlings succumb to drought while some others showed partial dieback.

Table 1
Mean values (\pm standard deviation) of the leaf, stem and root variables of seedlings subjected to different treatments (control, drought, recovery). Measurements on control and stressed seedlings were performed between DN 25 and DN 26, and on recovered seedlings between DN 41 and DN 43. Different characters indicate significant differences between treatments and P-values in bold are statistically significant ($P < 0.05$).

Measured variables	Symbol	Unit	Control	Drought	Recovery	P-value
<i>Leaf</i>						
Number of leaves	nL	–	23 \pm 2 ^a	23 \pm 1 ^{*a}	25 \pm 23 ^{*a}	0.997
Stomatal conductance (at 200 $\mu\text{mol PAR m}^{-2} \text{s}^{-1}$)	g _s	mol H ₂ O m ⁻² s ⁻¹	0.098 \pm 0.061 ^b	0.003 \pm 0.001 ^a	0.0362 \pm 0.000 ^{ab}	<0.001
Transpiration (at 200 $\mu\text{mol PAR m}^{-2} \text{s}^{-1}$)	E	mmol H ₂ O m ⁻² s ⁻¹	1.220 \pm 0.690 ^b	0.043 \pm 0.015 ^a	0.561 \pm 0.003 ^{ab}	<0.001
Water use efficiency (at >1000 $\mu\text{mol PAR m}^{-2} \text{s}^{-1}$)	WUE	$\mu\text{mol CO}_2 \text{ mol}^{-1} \text{H}_2\text{O}$	4.12 \pm 0.85 ^b	3.01 \pm 1.99 ^a	4.37 \pm 1.03 ^b	<0.001
Leaf water content	WC _l	%	66.8 \pm 8.3 ^c	12.9 \pm 4.3 ^a	39.9 \pm 18.5 ^b	<0.001
Leaf dry weight	DW _l	g	1.18 \pm 0.22 ^a	1.20 \pm 0.05 ^a	0.38 \pm 0.45 ^a	0.307
<i>Stem</i>						
Stem water potential	Ψ _l	MPa	–1.35 \pm 0.34 ^{ab}	–3.16 \pm 1.23 ^a	–1.21 \pm 0.49 ^b	0.024
Stem water content	WC _s	%	45.8 \pm 9.0 ^a	37.5 \pm 4.8 ^a	47.1 \pm 7.8 ^a	0.259
Stem dry weight	DW _s	g	0.84 \pm 0.17 ^a	0.97 \pm 0.11 ^a	2.19 \pm 0.76 ^b	0.003
<i>Root</i>						
Root water content	WC _r	%	45.1 \pm 5.3 ^{ab}	39.0 \pm 9.5 ^a	48.1 \pm 1.4 ^b	0.032
Root dry weight	DW _r	g	4.70 \pm 1.04 ^a	4.66 \pm 2.42 ^a	5.86 \pm 1.09 ^a	0.336
Fine root dry weight	DW _{sr}	g	0.11 \pm 0.05 ^a	0.19 \pm 0.11 ^{ab}	0.35 \pm 0.15 ^b	0.023

* Including wilted leaves still attached to the plant.

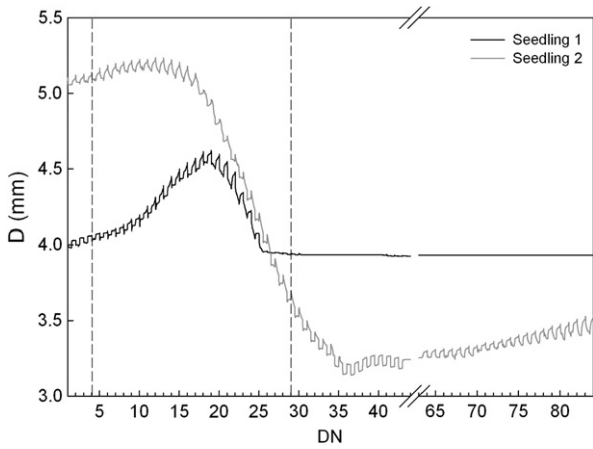


Fig. 1. Diameter fluctuations (D , mm) of two tamarind seedlings as a function of day number (DN). The vertical dashed lines correspond with the beginning and the end of the drought period.

3.1. Non-conservative water use strategy

Leaf transpirational water losses (and consequently sap flow) lower stem xylem water potential (it becomes more negative) during daytime, which is followed by a water recharge from both water storage and transport tissues during night time (Goldstein et al., 1998), which is reflected in daily stem diameter fluctuations (Fig. 1). At the onset of the drought period, the overnight water recharge of both storage and transport tissues in the tamarind seedlings was still complete, as on a daily basis water uptake by the roots exceeded leaf transpirational water loss. This was also manifested in an increasing trend in the daily use of stem water storage (MDS) to buffer sap flow (Fig. 2) and in the strong investment in tissue growth (DG) (i.e. stem swelling) (Fig. 3). However, after 10 days (seedling II) and 16 days (seedling I) of drought, the overnight water recharge of stem storage pools became incomplete as on a daily basis less water could be taken up by the roots (less increase in stem diameter) than was lost by leaf transpiration during the day (steep decrease in stem diameter). This incomplete recharge was expressed in a gradual decrease in sap flow (Fig. 4), a sharp decrease in the overall diameter fluctuations (i.e. stem shrinkage) (Fig. 1) and a decline in MDS and DG (DG even became negative) (Figs. 2 and 3). Therefore, we might conclude that the first hypothesis, which stated that tamarind seedlings are able to continue water use and growth during short-term drought, is not strictly confirmed. If we compare our results with the observed trends in baobab seedlings, then we notice that

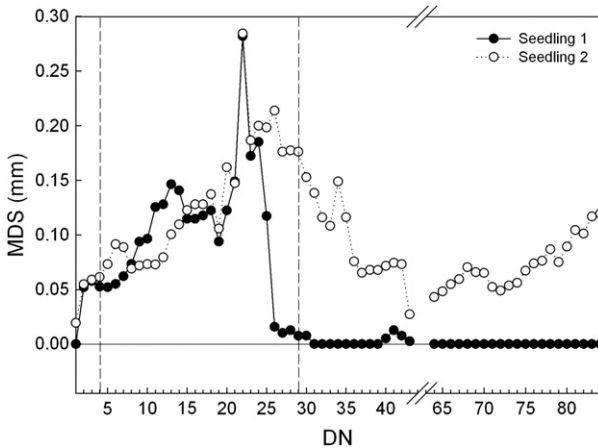


Fig. 2. Maximum daily shrinkage (MDS, mm) of two tamarind seedlings as a function of day number (DN). The vertical dashed lines correspond with the beginning and the end of the drought period.

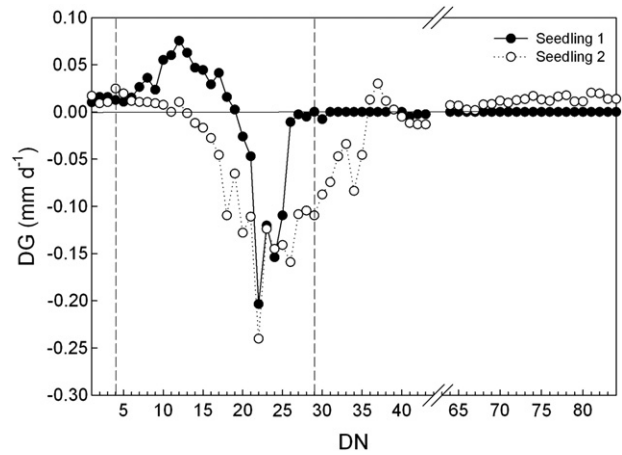


Fig. 3. Daily growth (DG, mm d⁻¹) of two tamarind seedling as a function of day number (DN). The vertical dashed lines correspond with the beginning and the end of the drought period.

soon after the onset of a drought period, MDS and DG of baobab seedlings rapidly decline to zero, which indicated no water was taken up by the roots and no (or little) water was lost by transpiration (Van den Bilcke et al., 2013). Tamarind seedlings seem to be less sensitive to drought compared to baobab seedlings, as they gradually decrease sap flow within three weeks of drought, while sap flow in baobab seedlings decreased to zero after one week of drought because of stomatal sensitivity to water stress (Van den Bilcke et al., 2013).

After ~one month (25 days) of soil drought, stomatal conductance in the tamarind seedlings was significantly reduced by >95% (Table 1) and consequently leaf transpiration (sap flow) ceased to zero (Fig. 4). Tamarind seedlings seem to be more sensitive to drought compared to *Ziziphus mauritiana* (another evergreen hardwood species) and *Parkia biglobosa* (a deciduous hardwood species also belonging to the family Fabaceae), both species growing in the same environment as tamarind, as their transpiration rate was only lowered by ~55% after three weeks of drought and stomatal conductance by ~80% after one month of drought, respectively (Osonubi and Fasehun, 1987; Teklehaimanot et al., 1998; Kulkarni et al., 2010).

The reduction in stomatal conductance in the tamarind seedlings was first reflected in an increase in WUE subsequently followed by a decrease in WUE (ratio of P_n/g_s following Mediavilla et al., 2002) (Fig. 5;

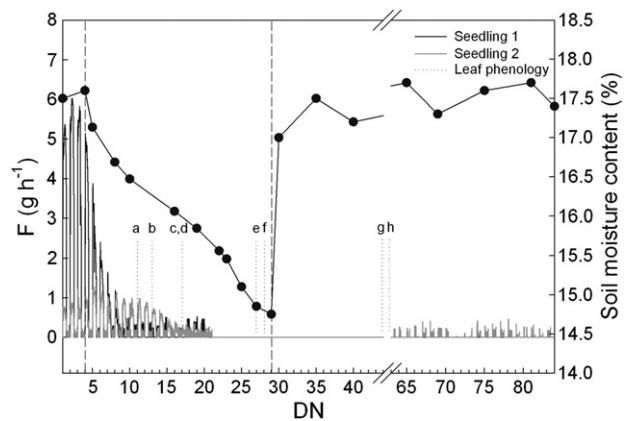


Fig. 4. Sap flow (F , g h⁻¹) of two tamarind seedlings and soil moisture content (%) as function of time. DN represents day number. The vertical dashed lines correspond with the beginning and the end of the drought period. The vertical dotted lines represent leaf phenology. Seedling 1: (a) some leaves are turning yellowish, (c) half of the leaves are wilted and (f) all leaves are wilted. Seedling 2: (b) some leaves are turning yellowish, (d) half of the leaves are wilted, (e) all leaves are wilted, (g) development of buds at the stem base and (h) development of first leaves on twigs.

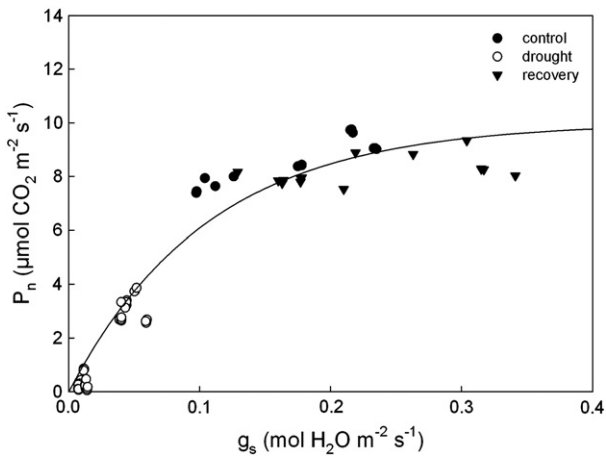


Fig. 5. Relationship between net photosynthesis (P_n , $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) and stomatal conductance (g_s , $\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$). The regression $P_n = a(1 - e^{-b g_s})$ with a and b as parameters, was fitted to the data. Each symbol is a single measurement for light intensities above $1000 \mu\text{mol PAR m}^{-2} \text{ s}^{-1}$. The slope of the curve represents the water use efficiency (WUE) or the ratio of P_n/g_s ($\mu\text{mol CO}_2 \text{ mol}^{-1} \text{ H}_2\text{O}$).

Table 1), indicating that metabolic (non-stomatal) limitations are becoming a more important limitation for photosynthesis than stomatal limitations (Rouhi et al., 2007). De Smedt et al. (2012) and Randriamanana et al. (2012) observed in baobab seedlings subjected to drought a significant increase in WUE at lower values of stomatal conductance indicating that stomatal closure mainly limits photosynthesis. Due to leaf desiccation (appearing at a soil moisture content of 14.75%), WC_i of the drought-stressed tamarind seedlings was very low (~10–15%), while the tamarind leaves normally contain the highest amount of water (70%) of all plant parts. Drought-stressed baobab seedlings initially maintain a high WC_i (~80%) before leaf loss, likely because of the abundant presence of mucilage, a polysaccharide that might play an important role in water absorption or osmotic regulation to buffer leaf water status (Van den Bilcke et al., 2013). In contrast, we did not detect any mucilage in tamarind leaves.

Furthermore, after ~one month (25 days) of soil drought, the drought-stressed tamarind seedlings experienced stem water potentials near -3 MPa (Table 1) compared with control values of about -1 MPa , which confirms the third hypothesis which stated that tamarind seedlings lower water potential under short-term drought, even far below the general defined permanent wilting point for most plants (-1.5 MPa). *P. biglobosa* and *Z. mauritiana* seedlings might

also decline water potentials below -2.5 MPa when drought intensifies (Osonubi and Fasehun, 1987; Kulkarni et al., 2010). The high suction tension allows tamarind seedlings (i) to withdraw excessive amounts (20%) of limited stem and root water storage (see further), which confirms the fourth hypothesis, and (ii) to extract as much water as possible from the coarse and sandy soil used in our study, since it was characterized by a very low available water content of 3%. In contrast, baobab seedlings only spend 2% of their stem and root water storage and maintain their water potential near -1 MPa , thereby avoiding xylem cavitation (De Smedt et al., 2012; Van den Bilcke et al., 2013).

The hydraulic architecture of the (semi-) evergreen tamarind seedlings allows them to withstand greater xylem pressure to maintain water transport during drought. Both stem and taproot were characterized by high wood density ($\sim 1.5 \text{ g cm}^{-3}$) and likely high root density because they contained smaller xylem vessels (~ 25 and $\sim 35 \mu\text{m}$ diameter resp.), an abundant presence of fibers and little parenchyma (Fig. 6) compared to baobab seedlings (see further). Wood density was higher in tamarind seedlings compared with adult trees, in the current study. Nygård and Elfving (2000) reported a wood density of $\sim 0.7\text{--}0.75 \text{ g cm}^{-3}$ in adult tamarind trees of Burkina Faso. Given the trade-off between high wood density and water storage (Hacke et al., 2001), stem and root of tamarind seedlings consequently possess lower water storage capacity ($WC \sim 45\%$) and less vulnerability to xylem cavitation than coexisting deciduous trees, like baobab. Baobab seedlings are characterized by low wood and root density ($\sim 0.2\text{--}0.25$ and $\sim 0.095\text{--}0.25 \text{ g cm}^{-3}$ resp.), high stem and root water storage capacity ($WC_s \sim 75\%$; $WC_r \sim 90\%$) and the abundant presence of parenchymatic stem and root tissue ($\sim 80\%$ and $\sim 90\%$ resp.). They are more hydraulically efficient than tamarind seedlings because of wider xylem vessels (~ 58 and $\sim 55 \mu\text{m}$ diameter resp.) but more vulnerable to xylem cavitation (Choat et al., 2005; Van den Bilcke et al., 2013).

Based on these findings, tamarind seedlings can be classified as anisohydric species, as they allow midday stem water potential to decline as soil water potential declines, by keeping their stomata open and maintaining relatively high transpiration rates, which might be beneficial when soil water is abundant or under moderate drought stress. In response to severe drought stress, this risk-taking behavior might eventually lead to hydraulic failure by xylem dysfunction (through cavitation and embolism) and even mortality. In our experiment, half of the tamarind seedlings succumb to drought. The slow recovery (e.g. the postponed development of new leaves and sap flow) of some tamarind seedlings after ~one month upon re-watering indicates that the reduction in stomatal conductance is a result of both stomatal closure and non-stomatal factors, leaf wilting and xylem cavitation/embolism in

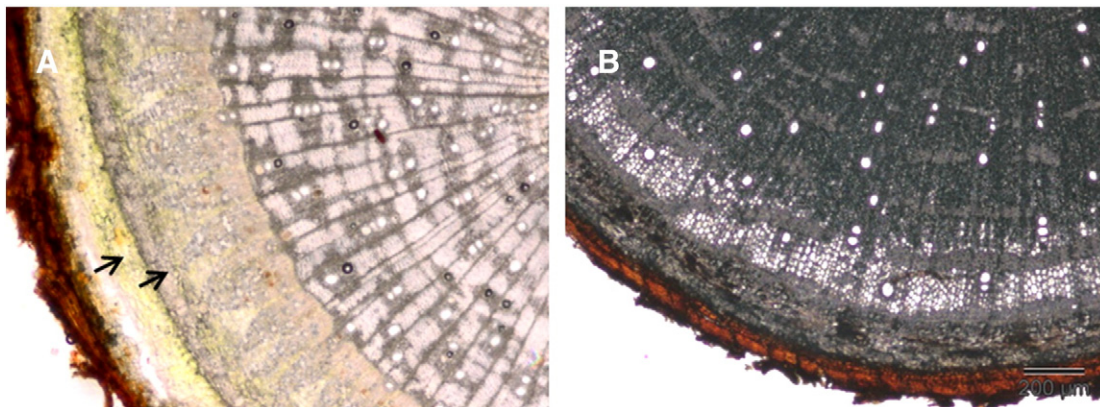


Fig. 6. Light microscopy images of stem and root transverse sections of tamarind seedlings. Scale bar is represented. (A) Stem with small vessels surrounded by paratracheal parenchyma sheaths (dark gray) and fibers (light gray). Two green layers in the inner bark are indicated by arrows. (B) Root with small vessels, parenchyma permeated by fiber sheaths.

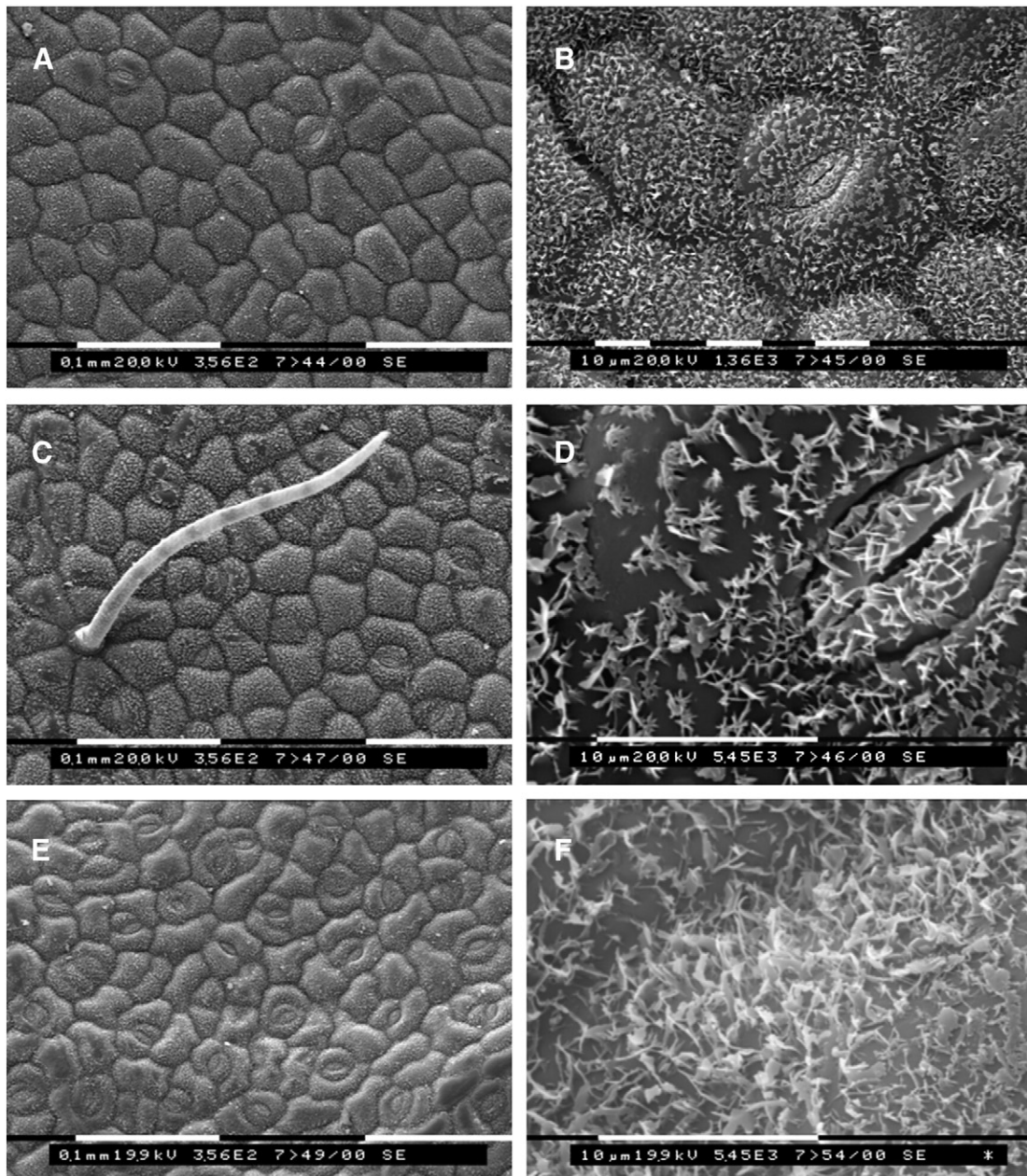


Fig. 7. Scanning electron microscopy images of leaf surfaces of tamarind seedlings. Scale bars are represented. (A) Adaxial leaf surface of a control seedling, showing few little stomata. (B) Adaxial leaf surface covered with wax platelets or crystals. (C) Abaxial leaf surface of a control seedling and the presence of a trichome. (D) Detail of B. (E) Abaxial leaf surface of a drought-stressed seedling with higher stomatal density. (F) Detail of E showing the coverage with wax platelets.

some of the twigs. However, baobab (*A. digitata* L.) seedlings fall into the category of isohydric species as was found by Chapotin et al. (2006a,b), De Smedt et al. (2012) and Van den Bilcke et al. (2013) as they maintain a relatively high (less negative) midday leaf water potential and consequently avoid hydraulic failure by xylem cavitation when confronted with soil drought, by rapid stomatal closure (Tardieu and Simonneau, 1998; Franks et al., 2007; McDowell et al., 2008) and by (partially) shedding their leaves and by finally going into drought-enforced dormancy (De Smedt et al., 2012; Van den Bilcke et al., 2013). For these reasons all baobab seedlings survived short-term drought. Also, baobab seedlings rapidly recovered, indicating that the decrease in stomatal conductance is mainly a result of stomatal closure to keep xylem vessels safe from cavitation. We observed that some baobab seedlings kept part of their leaves during a short-term drought period, serving as a stand-by to photosynthesize as soon as soil water became available (Van den Bilcke et al., 2013).

3.2. Some water saving mechanisms?

Although plant water storage is depleted during drought stress, tamarind seedlings seem to have developed some mechanisms to minimize water loss and maximize water uptake to tolerate temporally drought.

3.2.1. Leaf surface structure

Tamarind leaves show several possible morphological adaptations, more specific, xeromorphic characteristics to minimize transpirational water loss (according to Koch et al., 2009): (i) small leaf size; (ii) small stomata (stomatal length was $<20\ \mu\text{m}$ as was also found by Hunsche et al. (2010) \leftrightarrow stomatal length of leaves of baobab seedlings $>35\ \mu\text{m}$), amphistomatous leaves (stomata confined to both leaf sides) with a lower stomatal density at the adaxial leaf side (Fig. 7A, E) (as also reported by Hunsche et al., 2010); (iii) cellular trichomes (hairs) present on leaf veins and margins (and also shoot) (Fig. 7C), as Teklehaimanot

et al. (1998) observed on leaflets of *P. biglobosa*, and (iv) wax crystals (platelets) present on both leaf sides (Fig. 7A–F), which was also reported by Hunsche et al. (2010).

3.2.2. Stomatal oscillations

In this paragraph we will focus on a special ecophysiological feature we observed. Note that this feature was only found in two four-year-old tamarind trees on which several preliminary measurements of dynamic water flow (sap flow and diameter fluctuations) were carried out before the start of the actual measurements.

The oscillations in sap flow measured in some young tamarind trees were accompanied by oscillations in the stem diameter and were most probably caused by stomatal oscillations (Fig. 8). The latter are defined as the phenomenon of cyclic opening and closing movements of stomata (Barrs, 1971). This author proposed that short-term stomatal oscillations (<10 min) with small amplitudes are associated with control of CO₂ status and appear to depend on the external CO₂ concentration. Slower oscillations (at least 30–50 min), which we observed in some young tamarind trees, are associated with the control of the plant-water status (Steppe et al., 2006). In the young tamarind trees, the increase (decrease) in sap flow coincides with a decrease (increase) in stem diameter. Four clear oscillations in sap flow and diameter are observed in a time interval of approximately 12 h, giving a mean period of about 3 h per oscillation. Steppe et al. (2006) and Dzitiki et al. (2007) found seven oscillations in sap flow (time interval was 8 h) in orange trees, growing under natural climatic conditions in Zimbabwe. A possible explanation why this feature occurred in some young tamarind trees and not in others might be that the used planting material originates from different genotypes as cross pollination within the species occurs (Diallo et al., 2007). Another explanation might be plant age since we did not observe this feature in one-year-old seedlings. We expect that stomatal oscillations (and consequently oscillations in sap flow and diameter) might also occur in young tamarind trees growing under natural conditions. The advantage of stomatal oscillations is that the plant is still able to gain CO₂ for photosynthesis through incompletely closed stomata, while reducing transpirational water loss, most probably resulting in an improved WUE (Yang et al., 2005). Further research is needed to support this statement.

3.2.3. Leaf reduction and twig dieback

Leaves started wilting after ~one week of withholding water but still remained attached to the seedlings (Table 1 and Fig. 4). At the same time new leaves developed, but after three weeks of drought (soil moisture content decreased by circa 3%) all leaves wilted. This finding did not confirm the second hypothesis which stated that tamarind seedlings tolerate short-term drought by keeping most of its leaves. Leaf wilting reduces leaf area and thereby minimizes transpirational water losses. We noticed a lot of the seedlings did not recover after the drought

period, as no new buds sprouted. In this case, seedling mortality is caused by whole plant-hydraulic failure. In the other seedlings we observed some twig dieback due to hydraulic failure (cavitation and embolism). The advantage of twig mortality might result in an improvement of the water status in the rest of the plant (Davis et al., 2002) and a possible explanation why part of the seedlings (50 to 60%) survived drought.

3.2.4. Non-foliar photosynthesis

Stems of tamarind seedlings are characterized by a double green layer under the periderm (Fig. 6A). The primary and green stem surface (epiderm/periderm) is covered with cellular trichomes (Fig. 9A, B), while the secondary woody stem surface (periderm) is characterized by lenticels and/or cracks (Fig. 9C, D). On both surfaces, occasionally some stomata are observed (Fig. 9B), but we believe that functional stomata are only present on the primary stem. We presume that cortical photosynthesis might occur through the observed lenticels and/or cracks, which might be an important strategy to gain CO₂ for photosynthesis without much water loss, especially when the leaves are wilted during drought stress (Rouhi et al., 2007; Wittmann and Pfanz, 2008; Eyles et al., 2009). Simbo et al. (2013) reported in baobab seedlings, also characterized by a chlorenchyma layer underneath the periderm and lenticels and/or cracks on the stem surface (Van den Bilcke et al., 2013), the occurrence of cortical photosynthesis during drought.

3.2.5. Fine root formation

The recovered tamarind seedlings contained more fine roots than the control seedlings in order to increase access to soil water and enhance water uptake to support plant recovery (Table 1).

4. Conclusions

The collection of traits exhibited by tamarind seedlings allows them to tolerate moderate drought stress. Tamarind seedlings continue to transpire and grow at the onset of drought. When drought intensifies WUE of the leaves is reduced, leaves desiccate and the limited amount of stored water in stem and root (due to high tissue density) is being depleted. At severe (complete water withholding) but short-term drought the risk taking behavior (i.e. negative water potentials through spending water and/or trying to extract water from the soil) of the species eventually leads to whole plant hydraulic failure in some seedlings and in partial hydraulic failure (shoot dieback) in others. This risk taking behavior and the high risk for partial hydraulic failure might also explain the long time needed for the seedlings to recover when re-irrigated. Besides traits related to drought tolerance, tamarind seedlings contain some water saving properties (xeromorphic leaves) and mechanisms such as leaf reduction, twig dieback, non-foliar photosynthesis and fine root formation. Further research on some of these traits is recommended. We expect that at severe and long-term drought, all seedlings finally succumb to drought. The latter should be explored more thoroughly in other (longer) drought stress experiments. The high rate of tamarind seedling mortality and the slow recovery of the remaining seedlings, after a short-term drought period, are facts that should be taken into account when managing tamarind plantings in the field to reduce dry season mortality, to increase productivity and efficient use of limited water resources. This work also demonstrated that co-occurring species might have different hydraulic traits and water-use strategies to cope with short-term drought stress. Baobab seedlings, given as example in this study, are drought avoidant and conserve their internal water storage reserves. As a consequence, there is a 100% survival rate and a fast recovery of the species for comparable drought spells as simulated in this study. The actual survival of tamarind and baobab seedlings in the field will depend – among other factors – on the characteristics of the drought spells. However, both contrasting strategies seem to be successful as both species co-occur in the same environment.

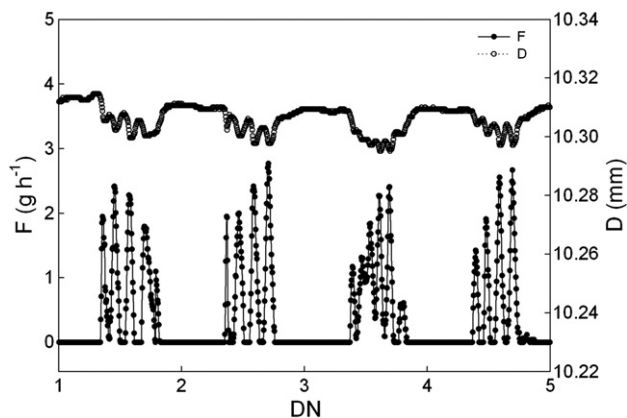


Fig. 8. A five day sample of the sap flow (F , $g\ h^{-1}$) and diameter fluctuations (D , mm) during the irrigation period, showing oscillations. F oscillates in anti-phase to D .

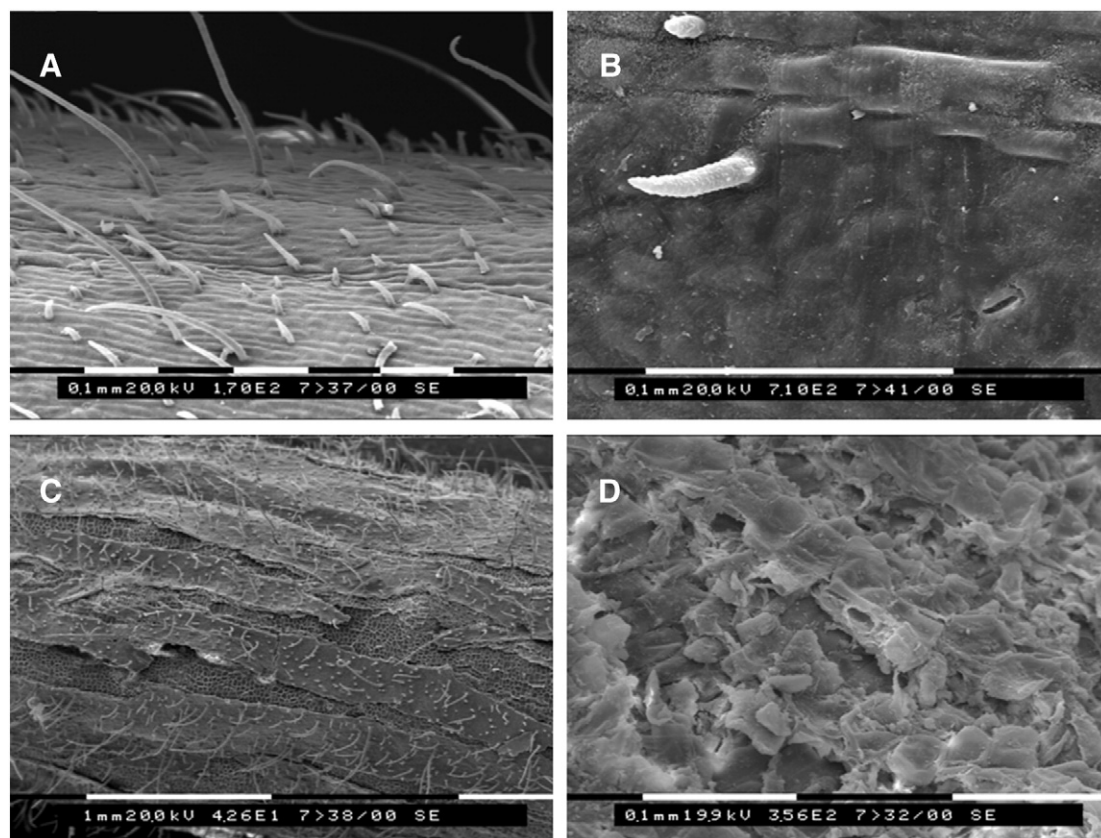


Fig. 9. Scanning electron microscopy images of stem surfaces of tamarind seedlings. Scale bars are represented. (A) Young green stem surface covered with hairs. (B) Detail of (A) showing hairs and a stoma. (C) Woody stem surface still covered with hairs and showing cracks. (D) Stem wood surface showing lenticels.

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