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Bud development, flowering and fruit set of *Moringa oleifera* Lam. (Horseradish Tree) as affected by various irrigation levels

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Abstract

Moringa oleifera is becoming increasingly popular as an industrial crop due to its multitude of useful attributes as water purifier, nutritional supplement and biofuel feedstock. Given its tolerance to sub-optimal growing conditions, most of the current and anticipated cultivation areas are in medium to low rainfall areas. This study aimed to assess the effect of various irrigation levels on floral initiation, flowering and fruit set. Three treatments namely, a 900 mm (900IT), 600 mm (600IT) and 300 mm (300IT) per annum irrigation treatment were administered through drip irrigation, simulating three total annual rainfall amounts. Individual inflorescences from each treatment were tagged during floral initiation and monitored throughout until fruit set. Flower bud initiation was highest at the 300IT and lowest at the 900IT for two consecutive growing seasons. Fruit set on the other hand, decreased with the decrease in irrigation treatment. Floral abortion, reduced pollen viability as well as moisture stress in the style were contributing factors to the reduction in fruiting/yield observed at the 300IT. Moderate water stress prior to floral initiation could stimulate flower initiation, however, this should be followed by sufficient irrigation to ensure good pollination, fruit set and yield.

Keywords: floral initiation, pollen viability, pollen tube growth, biofuel, miracle tree

1 Introduction

As a member of the *Moringaceae* family, *Moringa oleifera* Lam. also known as the miracle, horseradish or drumstick tree is one of the most useful trees currently found throughout the tropics of the world (Jahn, 1988). This fast growing, small to medium sized tree is used as animal forage, source of nutrition, medicine, water purification, cosmetics and even as biofuel (Anwar *et al.*, 2007; Rashid *et al.*, 2008; Fuglie, 2001). Flowers are white to cream coloured and zygomorphic. The tree bears 20 to 30 cm long fruit that once mature, change colour from green to brown, revealing numerous round or triangular seeds with three papery wings (Folkard *et al.*, 1999). Despite *M. oleifera* being grown throughout the world and the continent of Africa, the limited scientific data that is currently available on its cultivation could be the reason for the absence of large-scale commercial *M. oleifera* plantations. *Moringa oleifera* is known to tolerate sub-optimal growing conditions (Palada & Chang, 2003; Morton, 1991), but a reduced seed weight in conjunction with lower oil content as a result of drought stress has been reported for *M. oleifera* trees in Punjab, Pakistan (Anwar *et al.*, 2006). Due to the potential uses of *M. oleifera* fruit as a food/fuel source, the main objective of this study was thus to evaluate the effect of three irrigation levels on flowering, pollination and consequent fruit set. A further objective was the identification of an irrigation regime that would favour flower initiation and fruiting.

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2 Materials and methods

Trials were conducted on six year old *Moringa oleifera* trees at the field trial 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 and an average annual rainfall of 674 mm. Trees for the purpose of this trial were grown from seeds sourced in India and transplanted into the field. A total of 12 trees were then divided into three groups of four trees each, each of these three groups were subject to a different irrigation treatment. Irrigation water was applied through a surface drip irrigation system at three levels. Three dipper lines were installed at the 900IT, two dripper lines at the 600IT, while the 300IT had a single dripper line at the base of the tree trunks. The in-line dripper spacing was 30 cm, with an application rate of 2.1 litres/hour/dripper. According to Palada & Chang (2003) the minimum annual rainfall requirement for *M. oleifera* is 250 mm/year. The three administered irrigation levels were thus based on the minimum (300 mm/year; 300IT) amount for the tree, average (600 mm/year; 600IT) annual rainfall for the research site and a higher (900 mm/year; 900IT) treatment, simulating supplement irrigation under field conditions. The irrigation amounts were administered, simulating total annual rainfall (mm/year). Plastic sheeting was then placed over the dripper irrigation, underneath the trees covering an area of four meters on either side of the trunks. With this rainfall exclusion method, irrigation can be administered with greater accuracy without having to compensate for rainfall. Organic mulch was placed on top of the plastic sheeting so as to not adversely affect the energy balance of the soil. Semi-weekly soil water content measurements were conducted using a neutron probe (Campbell Pacific Nuclear, 503DR Hydroprobe), while continuous soil moisture measurements were conducted using theta probes (Delta-T, UK) to verify differences in soil water levels between treatments. Neutron probe measurements were calibrated against soil samples from each measurement depth and finally expressed as volumetric water content (m^3/m^3), which is the volume of water within a given volume of soil (Brady & Weil, 2002).

Trees were subjected to the irrigation treatments for nine months prior to floral assessment. During bud development, four random inflorescences on each tree were tagged and monitored until fruit set. Mathew & Rajamony (2004) classified *Moringa oleifera* flowers into seven developmental stages. Besides the seven stages described by Mathew & Rajamony (2004), two additional stages, namely fertilized flower and fruit set were added to total nine developmental stages (Table 1).

2.1 Pollen viability test

The *in vitro* pollen germination test was performed using the hanging drop method (Shivanna & Rangaswamy, 1992). According to Bhattacharya & Mandal (2004) a 10 % sucrose solution with a 200 µg/ml boric acid (H_3BO_3) concentration yielded the highest pollen germination percentages for *M. oleifera*. Consequently, the abovementioned concentration was used for the pollen germination trials across all three irrigation treatments. Pollen collected from freshly opened flowers at each of the three irrigation treatments was immersed in a drop of the sucrose solution using a needle. Pollen was left to germinate at room temperature (20°C) for two hours. The cover slip containing the pollen was then placed onto a microscope slide and observed under a Leitz Biomed light microscope, while digital pictures were taken with a Canon PowerShot A630 camera. A hundred randomly selected pollen grains per slide were assessed for their viability. Pollen was considered viable once the pollen tube length was equal or greater than the diameter of the individual pollen grains.

2.2 Fluorescence microscopy

Flower pistils were collected from trees subjected to the three irrigation treatments and prepared for fluorescence microscopy according to Martin (1959). Pollinated pistils were removed from the flower using a scalpel and fixed in FAA (80 % Ethanol : 37 % Formaldehyde : 100 % Acetic acid, in proportions 8:1:1) for 24 hours. After rinsing the pistils in distilled water they were softened in 5M NaOH solution for 24 hours and again rinsed three times in distilled water. Pistils were then stained in 0.1 % ABF (Aniline Blue Fluorochrome) and 0.1 N K_3PO_4 for 24 hours in complete darkness. After incubation, samples were removed and placed in a drop of 50 % Glycerin on a microscope slide and covered with a cover slip that was gently pressed to flatten the pistil. Samples were then viewed using a Zeiss Inverted Fluorescence microscope, while digital pictures were taken using an AxioCamMR5 Camera.

2.3 Data analysis

Data were subjected to analysis of variance (ANOVA) using Proc GLM (SAS) and normalized using the Blom method to correct for heteroscedasticity in the data. Data were statistically analysed using Statistical Analysis System (SAS, 2008), by the Statistics Department at the University of Pretoria.

Table 1: *Stages of Moringa oleifera bud and flower development adapted from Mathew and Rajamony (2004).*

Stage		*Description*	*Picture*
1	Globular	Buds were greenish and inconspicuous	
2	Slightly globular	The colour changed to light green and bud was bulged. Ridges and furrows visible.	
3	Elongated	Colour became greenish white. The bud enlarged and ridges and furrows more prominent. Tip of the bud became creamy white.	
4	Slightly elongated	Colour creamy white. The bud was enlarged.	
5	Much elongated	Colour yellowish white all over, calyx green. Centre portion bulged out, both ends tapering.	
6	Elongated upper portion roundish	Yellowish white colour. One side of the bud split opened and one of the petals exposed. The smallest petal exposed first.	
7	The flower bud had fully opened	Five sepals and petals, yellowish white. Anthers yellow, dorsifixed filaments were of different lengths, style creamy white and pitted stigma.	
8	Fertilized flower	Senescence of sepals, petals and anthers. Ovary appears swollen.	
9	Fruit set	Sepals, petals and anthers have senesced and visible fruit has been formed.	

3 Results

The volumetric water content (m^3/m^3) for each of the three irrigation treatments (900IT, 600IT and 300IT) at five different profile depths (0–200 mm, 200–400 mm, 400–600 mm, 600–800 mm and 800–1000 mm) showed substantial differences (Figure 1).

Results revealed that the number of flower buds formed per inflorescence increased with a decrease in irrigation treatment during both the 2011 and 2012 season (Figure 2). The 900IT had a total of 195 initiated flower buds, while the total number of flowers at the 300IT was 592 in 2011. In 2012 a total of 248 flower buds were initiated at the 900IT compared to 529 at the 300IT. The decrease in irrigation between the 900IT and 300IT increased flower numbers by 67.1 %, during 2011 and 53.1 %, during 2012.

Although *Moringa oleifera* flower initiation seemingly benefited from lower irrigation levels (water stress), increased instances of bud abortion as well as a reduction in the number of fertilized flowers were observed with a decrease in irrigation. A total of 68 flowers were fertilized at the 900IT, compared to 65 for the 600IT and 53 of the 300IT during 2011 (Figure 3). This was a 4.4 % and 22.1 % reduction in fertilized flowers between the 900IT and 600IT, as well as the 900IT and 300IT respectively. During 2012, 50 flowers were fertilized at the 900IT, compared to 78 at the 600IT and 51 at the 300IT (Figure 3). Between the 900IT and 600IT there was a 35.6 % reduction in fertilized flowers, while both the 900IT and 300IT had similar numbers of fertilized flowers. When comparing the bud-to-fruit ratio during 2011, for every 2.9 buds initiated at the 900IT, one fruit was produced, whereas the bud-to-fruit ratio for the 600IT and 300IT were 6.1 and 11.2 respectively. During 2012 however, the bud-to-fruit ratio for the 900IT was 4.9, 5.3 for the 600IT and 10.6 for the 300IT.

The majority of flower abortion at both the 600IT and 300IT during 2011 and 2012 occurred between stage one and four whereafter flower numbers stabilized and reached similar levels to that of the 900IT (Figure 2).

To ascertain whether reduced fertilization and fruit set was attributable to inferior pollen, pollen viabilty tests were performed for all three irrigation treatments. Results revealed that pollen viabilty decreased with a decrease in irrigation amount. The average pollen viablity was siginficantly different ($P \leq 0.05$) between all three irrigation treatments. For the 300IT it was found to be 45.4 % (±3.1) compared to 57.4 % (±3.2) at the 600IT and 76.6 % (±3.1) at the 900IT.

Flourescence microscopy of the flower style and ovary was used to detect the presence of pollen tube growth. A significant number of pollen tubes were detected in the styles of flowers from the 900IT, suggesting that conditions in the style were conducive to pollen tube growth. However, pollen tube growth in flowers from the 300IT was severly reduced (Figure 4).

4 Discussion

Although low (300IT) irrigation levels resulted in a higher number of flower buds, proportionally less flowers were fertilized and produced fruit, which can be attributed to flower abortion caused by lower pollen viability and moisture stress in the styles, inhibiting pollen tube growth and fertilization. Instances of increased flower initiation under moderate water stress have also been observed in *Litchi chinensis* (Stern *et al.*, 1993), *Averrhoa carambola* (Salakpetch *et al.*, 1990), *Garcinia mangostana* (Poonnachit *et al.*, 1996), *Anacardium occidentale* (Nambiar, 1977), *Mangifera indica* (Singh, 1977), *Theobroma cacao* (Alvim, 1977) and *Coffea arabica* (Alvim, 1960; Maestri & Barros, 1977; Schuch *et al.*, 1992). Although short periods of water stress seemingly increase flower initiation in certain species, prolonged water stress is reported to usually lead to bud abscission and decreased flowering/fruiting. The maximum potential number of seed (yield) is fixed at fertilization (Alqudah *et al.*, 2011). However, further post-fertilization water stress could cause abortion of developing ovules and/or reduce photosynthetic rate, thereby lowering assimilate supply, which will result in smaller seed (Simpson, 1981) and consequently lower yield. Reduced pollen viabilty at the lower irrigation treatments (300IT and 600IT) possibly explains the poor fertilization and fruit set at these treatments. However an abundant supply of viable pollen from adjacent well-watered irrigation treatments would rule out this as the sole reason for reduced fruit set. According to Simpson (1981) and Fang *et al.* (2010), moisture stress in the style contributes significantly to increased instances of flower abortion as fewer pollen tubes reach the ovary. Reduced fruit set observed in the 300IT can thus not merely be attributed to lower pollen viability but also due to moisture stress creating unfavourable conditions within the style for pollen tube growth. Despite *Moringa oleifera* being tolerant to low soil water levels, stress during the reproductive phase can have severe implications on the consequent yield. From an agronomic point of view, the identification of drought sensitive stages in the growth cycle is not only important from a water-use perspective, but also because water is often in short supply. If the aim is thus to maximize yield from limited resources, trees should not be stressed during the reproductive stages. Moderate water stress prior to floral initiation could be beneficial by stimulating increased flower initiation, while ample irrigation thereafter would ensure better fruit set and greater yield.

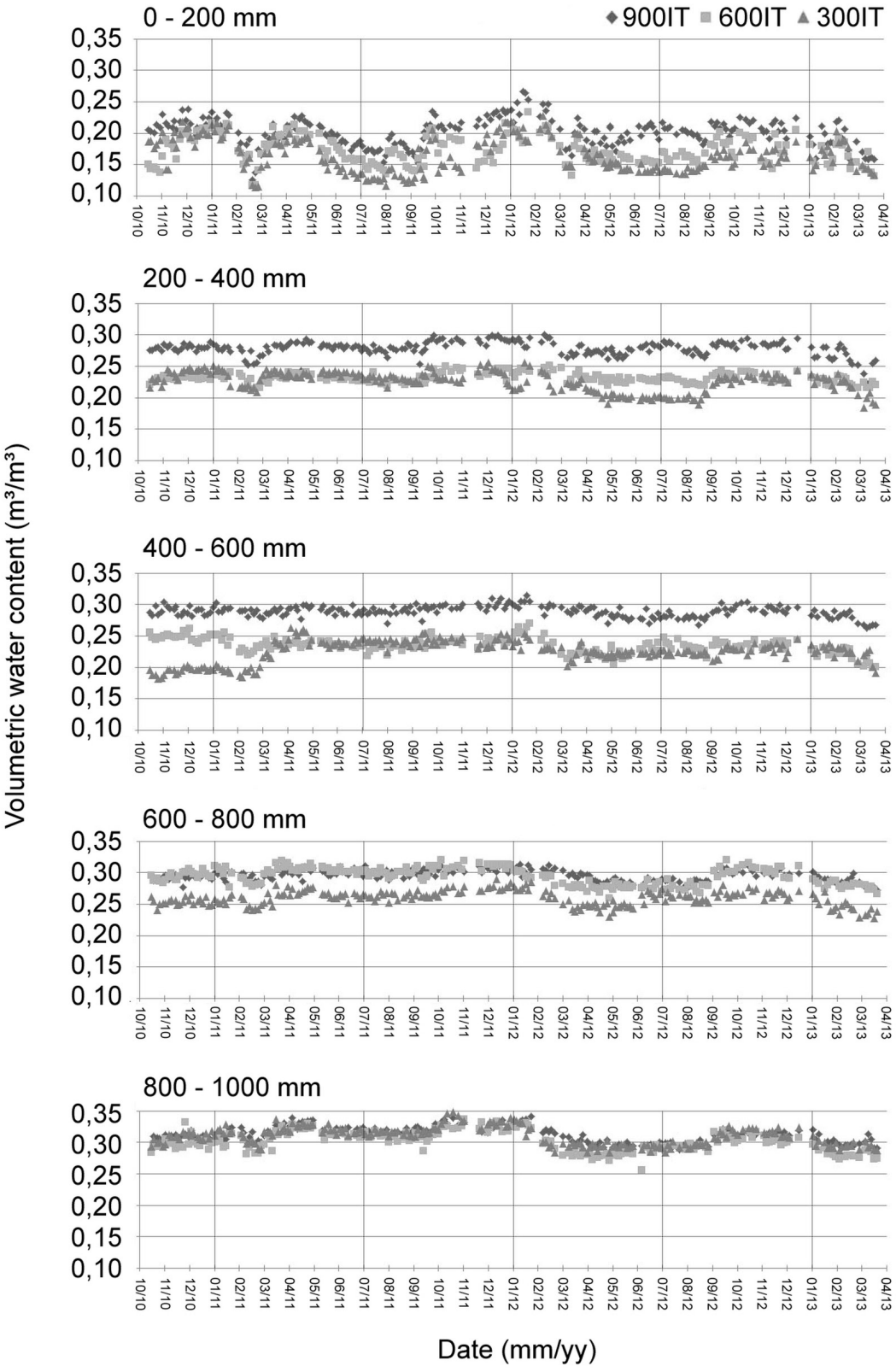

Fig. 1: *Soil volumetric water content (m^3/m^3) measurements throughout the soil profile (at five depths) within each of the three irrigation treatments (300IT, 600IT and 900IT) during 2011 and 2012.*

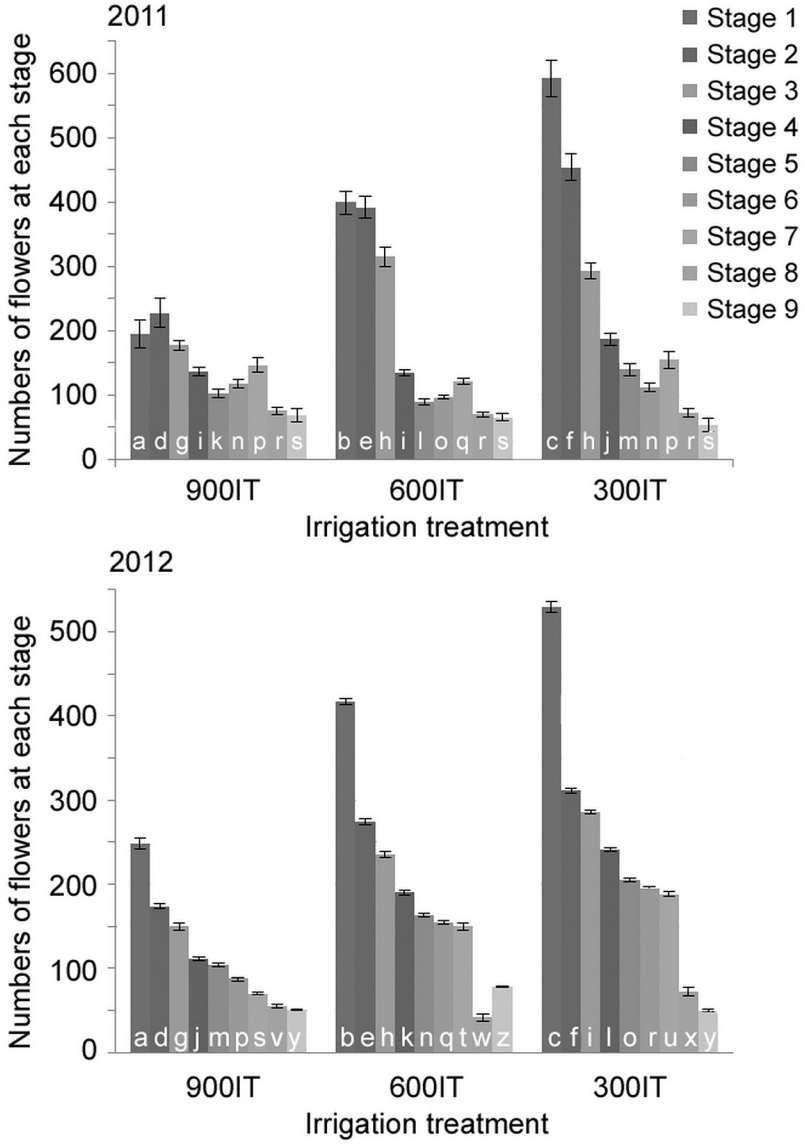

Fig. 2: *Number of flowers at each flowering stage (Table 1) at three irrigation treatments (300IT, 600IT and 900IT) for both the 2011 and 2012 season. Different letters indicate significant differences at $P \leq 0.05$, while error bars represent standard error of the mean (SEM).*

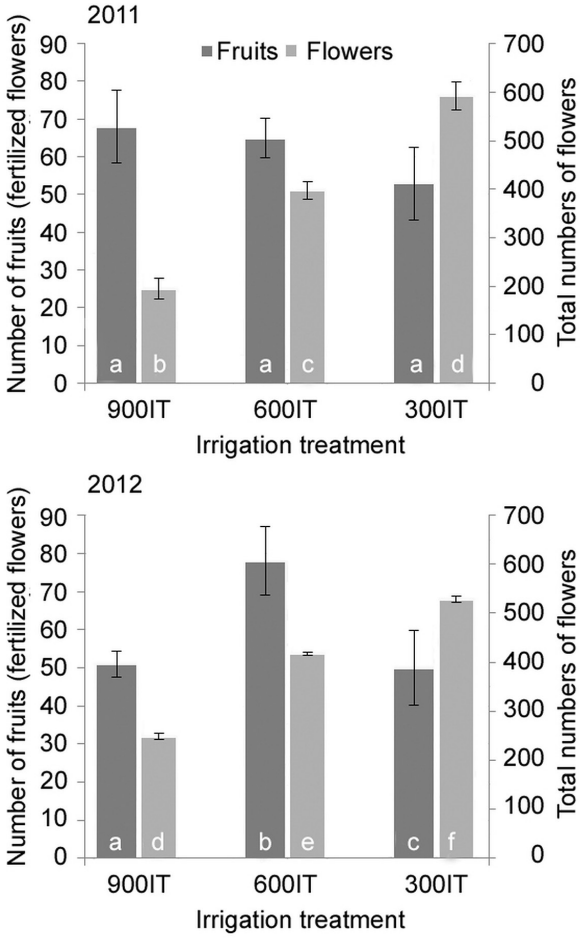

Fig. 3: *Total number of flower buds (stage 1) vs. total number of fruit (stage 9) at each of the three irrigation treatments (300IT, 600IT and 900IT) for both the 2011 and 2012 season. Different letters indicate significant differences at $P \leq 0.05$, while error bars represent standard error of the mean (SEM).*

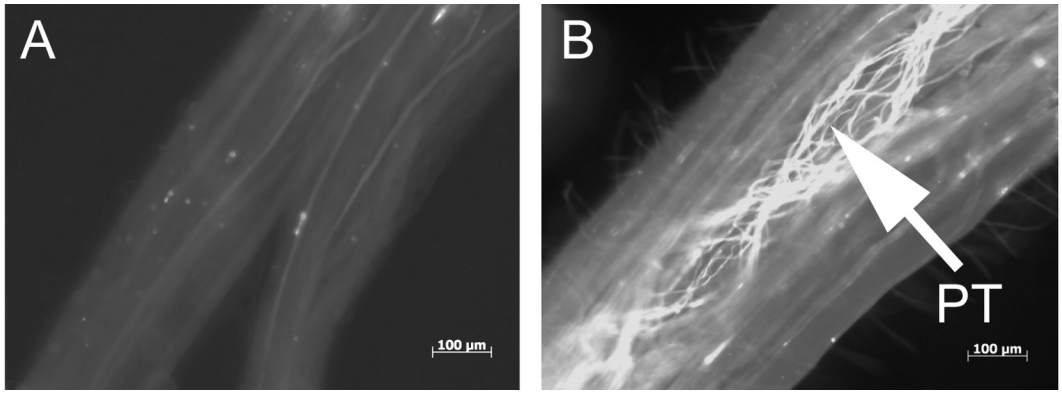

Fig. 4: *Fluorescence microscopy of Moringa oleifera styles without (A) and with (B) pollen tube growth. A – 300IT; B – 900IT; PT – Pollen tubes.*

Acknowledgements

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