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The embryo, endosperm and seed coat structure of developing *Moringa oleifera* seed

Quintin E. Muhl [1], Elsa S. du Toit *, Joachim M. Steyn [1], Petrus J. Robbertse [1]

Department of Plant Production and Soil Science, Faculty of Natural and Agricultural Sciences, University of Pretoria, 0002, South Africa

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ABSTRACT

In order to comprehend storage compound synthesis and accumulation throughout seed growth, the integral processes of embryogenesis and endosperm development need to be described. Micro-anatomical studies of developing *Moringa oleifera* seed have revealed the endosperm to be primarily nuclear, becoming cellular from the chalazal end towards the developing embryo at a fruit diameter of ±6 mm. At a fruit diameter of ±8 mm the cellular endosperm had covered the entire inner integument, which coincides with the developing embryo reaching the globular stage. Cotyledon development commenced at a fruit diameter of ±12 mm and continued up until ±24 mm. It was also during this phase that the majority of storage compounds were synthesized and stored, making it the most sensitive stage to environmental stresses. At the end of this phase the cotyledons had filled the entire space covered by the seed coat, while the unicellular epidermal layer of the inner integument remained distinctly visible between the cotyledons and the testa. The mature seed coat had layers of thickened as well as fibrous cells while being roughly triangular with three wings. Prior to studying the physiological and biochemical processes during seed growth and development, the post-fertilization anatomy of the endosperm and embryo needed to be understood.

1. Introduction

As a member of the family Moringaceae, *Moringa oleifera* Lam. also known as the miracle, horseradish or drumstick tree, is one of the most useful trees currently found throughout the tropics worldwide (Jahn, 1988). Lately there has been growing interest in *M. oleifera*, and more specifically their seed, due to its multitude of uses. Seed can be used as a food/fodder source, for oil extraction, water purification as well as the treatment of various ailments (Oliveira et al., 1999; Fuglie, 2001; Anwar et al., 2007; Santos et al., 2012). Seed growth and storage compound biosynthesis are fundamental processes affecting both final yield and reproductive potential. By studying the post-fertilization seed anatomy, greater insight can be gained into how physiological and biochemical processes throughout seed growth and development might be affected, once trees are subjected to drought stress (Bewley and Black, 1994). Due to discrepancies in the literature regarding the structure of the seed coat, special attention was given to its development and structure. By following seed growth throughout post-fertilization development, insight into the formation of the various seed structures could be obtained, as opposed to only examining seed at maturity. In addition, the effect of environmental stresses during specific developmental stages can be better understood.

2. Materials and methods

Seed from different developmental stages based on the fruit diameter (mm), were randomly sampled from mature six-year-old *M. oleifera* trees at the field trial section on the Hatfield 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 (Fig. 1). Trees for the purpose of this trial were grown from PKM1 variety seeds, sourced in India and transplanted into the field in a single row, spaced ±2 m apart. Fruit diameters (mm) were simply measured using a calliper while the fruit remained attached to the tree.

Seed development throughout the various post-fertilization phases was studied using both dissection- and light microscopy, while seed structures are described using the terminology of Corner (1976a).

2.1. Light microscopy

Seed preparation for light microscopy was done according to O'Brien and McCully (1981). After harvesting, seeds were removed from the outer protective pericarp and immediately fixed in FAA (80% ethanol: 37% formaldehyde: 100% acetic acid, in proportions 8:1:1 *v*/v/v) for

* Corresponding author. Tel.: +27 12 420 3227.
E-mail addresses: quintin.muhl@up.ac.za (Q.E. Muhl), elsa.dutoit@up.ac.za (E.S. du Toit), martin.steyn@up.ac.za (J.M. Steyn), hannes.robbertse@up.ac.za (P.J. Robbertse).
[1] Tel.: +27 12,420 3227.

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Fig. 1. A — *Moringa oleifera* trees at the field trial section on the Hatfield Experimental Farm of the University of Pretoria, B — monarch butterfly (*Danaus plexippus*) pollinating a *Moringa oleifera* flower, C — immature *Moringa oleifera* fruit, leaves and flowers, and D — mature *Moringa oleifera* fruit.

24 h, before being dehydrated in an ethanol in water series (30%, 50%, 70%, 100%, 100% *v*/v) for 24 h at each concentration. Subsequently, ethanol was extracted through a series of xylene in ethanol concentrations (30%, 50%, 70%, and 100% *v*/v) and specimens impregnated with paraffin wax (60 °C). Embedded seed samples were cut into 10 μm thick sections using a Reichert–Jung semi-thin rotary microtome, and mounted onto microscope slides. After sections had been de-waxed in a series of xylene concentrations, they were stained in both 1% aqueous safranin and 0.5% fast green (95% ethanol) according to O'Brien and McCully (1981).

Sudan III was used for all intracellular lipid staining according to Culling (1974). Sections were submerged in propylene glycol (2 min.) and then transferred into Sudan III solution (10 min.) Thereafter, excess Sudan III was removed by rinsing sections in two changes of 85% and 50% propylene glycol. Lipids stained red/orange with Sudan III.

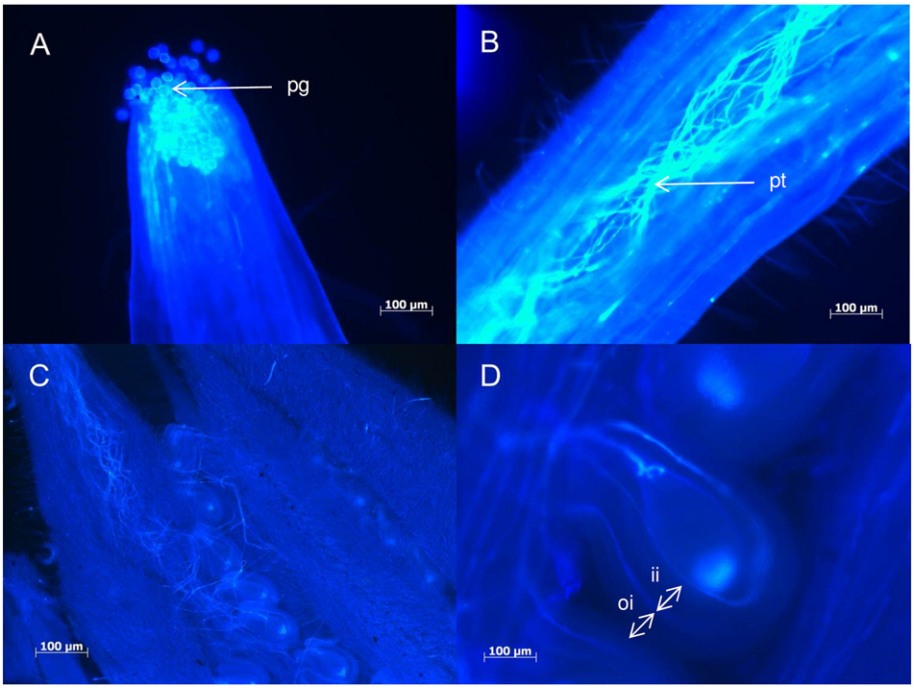

Fig. 2. A — Pollen grains on the stigma, B — pollen tube growth through the style, C — pollen tubes entering the ovary, containing numerous ovules, and D — pollen tubes entering an ovule through the micropyle. pg — pollen grains, pt — pollen tubes, oi — outer integument, and ii — inner integument.