Response of radicle cells of fungicide treated and untreated maize seed subjected to stress conditions

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ABSTRACT

Ultrastructural changes within cells are influenced by stress such as increased temperature due to improper storage, lack of oxygen and blockage in pathways responsible for water uptake. The objective of this study was to assess the effect, if any, of fungicide treatments on the ultrastructure of radicle cells of maize (*Zea mays*) after seeds had been subjected to stress conditions. Maize seeds were treated with Celest® XL (fludioxonil + mefenoxam) and Apron® XL (metalaxyl–M). The control consisted of untreated seeds. Following treatment, seeds were subjected to 2 d AA and 48 h rapid imbibition and thereafter prepared for transmission electron microscopy. Initial percentage germination (81%) of the seed lot was reduced after 2 d AA, with the untreated control having the lowest percentage germination (61%) followed by Apron® XL (65%) and Celest® XL (69%). The most obvious ultrastructural difference between the untreated control and the two fungicide treatments was the position of the lipid bodies. These formed a layer in close association with the cell wall in fungicide treated seeds, but in the untreated control they appeared more concentrated in the cytoplasm. Treated and untreated seeds may use different mechanisms, namely numerous vauoles and/or the movement of lipid bodies from the cell wall, to tolerate the stress conditions during rehydration of the seed after accelerated ageing and rapid imbibition.

Keywords: accelerated ageing, electron microscopy, embryonic root, rapid imbibition, *Zea mays.*

INTRODUCTION

Seed treatments are used to protect the seed from pests and pathogens and have been used with much success (Bradley et al., 2001). The success of such treatments is measured by the seed germinating and developing normally (Bradley et al., 2001), however, occasionally seed treatments may also cause phytotoxicity especially after seed storage. Germination starts with the imbibition of water. The earliest stage of imbibition of water by a dry seed involves rapid hydration of the desiccated tissues of the embryo. This is a prerequisite for the resumption of processes of growth and development at an ultrastructural level. Seeds leak solutes (organic and inorganic ions, sugars, amino acids and proteins) into the surrounding medium during the early stages of imbibition. Intracellular constituents are lost often resulting in extensive embryo damage and even its death (Copeland and McDonald, 2001). In a study on pines (*Pinus* sp.), De Castro and Martinez-Hounduvilla (1984) found that after imbibition, vacuoles are present in metabolically active cells as this replaced protein bodies and that during imbibition lipolysis takes place slower than proteolysis.

The way in which seeds react to stress is reflected through ultrastructural changes. The accelerated ageing (AA) test exposes unimbibed (dry) seeds for a short period to high temperature (45ºC) and high relative humidity (~95%). During the stress test, the seeds absorb moisture from the humid environment and the raised seed moisture content, along with the high temperature, causes rapid seed ageing (Rice and Dyer, 2001). The decline in respiratory activity and increased electrolyte leakage after accelerated ageing of soybeans has been interpreted as the result of membrane damage (Parrish and Leopold, 1978). Ultrastructural examination of the root tips of monocotyledons (Berjak and Villiers, 1972) confirmed that membrane systems of aged seeds suffer deteriorative changes during imbibition, including abnormalities in mitochondrial and plastid membranes, and the fusion of lipid droplets to form larger bodies or irregular pools in the cytoplasm. Mitochondria in dry seeds are only partially functional and functionality develops during germination (Hodson et al., 1987). Investigations on the mitochondrial activity in maize seeds showed that impaired mitochondrial activity was detected in moderately aged kernels (Dreyer and van de Venter, 1992).

In this study fungicide treated and untreated maize seeds were subjected to stress conditions of high humidity and high temperature following 2 d AA and rapid water imbibition. Radicle cells of these seeds were examined using transmission electron

microscopy (TEM) to investigate the difference in ultrastructural responses in fungicide treated and untreaded maize seeds following stress conditions.

MATERIAL AND METHODS

Treatment of seeds

Agricol (Pty) Ltd, Silverton, Pretoria, South Africa supplied untreated maize seed, cultivar 'Maverik' of predetermined medium vigour and 81% germination (Aveling et al., 2012). Syngenta South Africa (Pty) Ltd, Midrand, South Africa supplied the following fungicides: Celest® XL [*fludioxonil (25 g aiL^{-1}) + mefenoxam (10 g aiL^{-1})*] and Apron® XL [*metalaxyl – M (350 g aiL^{-1})*]. Seeds were mixed with the recommended dosage rate of fungicide in water for 5 min until all the seeds were covered with the fungicide. The control (untreated seeds) was treated in the same manner using water alone. After treatment, the seeds were left to air dry on paper towels in a laminar flow cabinet.

Preparation of the seeds

For the accelerated ageing (AA) test, treated and untreated pathogen free seeds were subjected to high relative humidity (90-100% RH) and temperature (45ºC). Four replicates of 50 seeds per treatment (for germination test) and two replicates of 50 seeds per treatment (24 for imbibition test and remainder for TEM studies) were placed on a grid above water in an AA chamber and incubated at 45ºC in the dark. After 48 h incubation (2 d AA), standard germination tests were done using a modified method of the International Seed Testing Association (ISTA) (2017). The four replicates of 50 seeds of all treatments were placed on germination paper [Anchor Paper 54x30 cm, (Agricol (Pty) Ltd, South Africa)] according to ISTA rules, rolled up and incubated in polythene bags in an upright position at 25 ± 1°C. Percentage germination was determined after 11 days and expressed as the percentage germinated seedlings. The two replicates of 24 seeds per treatment of the 2 d AA seeds were subjected to rapid imbibition. Seeds were individually placed in individual wells of a 24 well ice-cube tray containing 4 mL water per well and incubated at 25ºC in the dark for 48 h. Seed coats were removed. The seeds were then dissected and the embryos were separated from the rest of the seed with the aid of a stereo-microscope (Nikon/SMZ-1, Japan). Small sections (1 mm^2) of the radicle area of the embryo were dissected.

Statistical analysis using the SYSTAT 12.0 statistical program (SYSTAT, 1990) was performed on all data and least significant differences ($P< 0.05$) were determined according to the Student t-test.

Transmission electron microscopy (TEM)

The dissected radicle samples were fixed for 8 h in 2.5% glutaraldehyde in 0.075 M phosphate buffer (pH 7.4). They were rinsed in 0.075 M phosphate buffer and post-fixed in 1% aqueous osmium tetroxide for 2 h. Samples were then rinsed again and dehydrated in an ethanol series (30, 50, 70, 90 and 100%) and embedded in Quetol 651 resin (van der Merwe and Coetzee, 1992) at 60ºC for 48 h. Ultra-thin sections of eight radicles per treatment were cut using a Reichert Ultracut E ultramicrotome (Vienna, Austria) and stained with 4% aqueous uranyl acetate (10 min) and lead citrate (5 min) (Reynolds, 1963) for viewing with a Philips EM301 transmission electron microscope (Eindhoven, The Netherlands) at 60 kv.

RESULTS AND DISCUSSION

The initial percentage germination of the maize seed used in the experiments was 81% for untreated seed and 83% and 80% for Apron® XL and Celest® XL treated seed, respectively (Aveling et al., 2012). After 2 d AA the percentage germination of seed treated with Celest® XL (69%) was significantly (p<0.05) higher than the untreated control (61%) but did not differ from that of seed treated with Apron® XL (65%), which in turn did not differ significantly from the untreated control.

The characteristic changes in the ultrastucture of the radicle of seeds that have imbibed water are a breakdown of numerous protein bodies, with formation of vacuoles, appearance of the endoplasmic reticulum, the appearance of better defined elongated mitochondria, with more cristae and an increased number of golgi bodies (Bliss et al., 1984; Crèvecoeur et al., 1976, 1983). Ultrastructural micrographs in our study showed intact cells with clearly defined nuclei and organelles (vacuoles and mitochondria) in both the treated seeds and the untreated control (Figures 1 A, B, and C).

Cells were densely packed with ribosomes. The regularly shaped nuclei with clearly defined membranes possessed compact nucleoli. Fransolet et al. (1979) reported that it was the nucleolus in maize seeds exposed to a heatshock of 5 h at 46°C that underwent the most dramatic ultrastructural changes with total loss of the granular component and formation of electron opaque corpuscles. However, after 19 h of a return to 16°C these ultrastructural changes were reversed. Changes to the nucleolus were not evident in our study. This may be due to the fact that after the 48 h at the elevated temperature of 45°C (2 d AA) the seeds were imbibed in water at 25°C for 48 h before embedding in resin, which may have reversed the ultrastructural changes, as suggested by Fransolet et al. (1979).

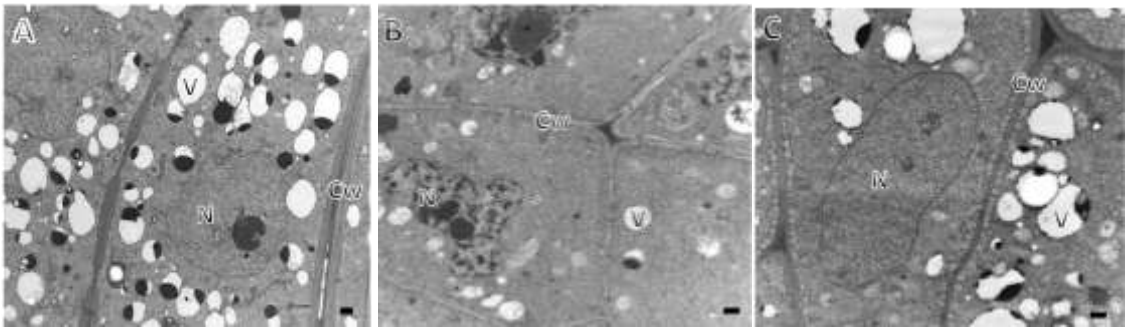

Figure 1. TEM micrographs of radicle tissue of untreated and fungicide treated maize seeds following 2 d accelerated ageing and 48 h rapid imbibition, (Bar = 1 μm) A) Untreated control with numerous vacuoles, B) Apron® XL with few vacuoles and C) Celest® XL with numerous vacuoles (CW = cell wall, N = nucleus, V = vacuole).

There were, however, differences between the untreated control and the Apron® XL and Celest® XL treated samples. The most noticeable difference was in the structure and number of the vacuoles. Vacuoles were visible and densely present in the untreated control (Figure 1 A) and Celest® XL (Figure 1 C) treated samples in comparison to Apron® XL. The increased number of vacuoles in maize root cells is important for osmotic adjustment under water or salt stresses (Hajibagheri et al., 1985). It is possible that the manner of response of cells of the radicle tissue in the untreated maize seeds, and to a certain extent in the seed treated with Celest® XL, to the stress of rapid water imbibition involved the formation of numerous vacuoles. There were electron opaque structures within most of the vacuoles in the untreated control (Figure 1 A) and Celest® XL (Figure 1 C). According to the literature, the black segment of the vacuoles could be electron opaque remnants of protein bodies (Hodson et al., 1987). De Castro and Martinez-Hounduvilla (1984) provided proof that the breakdown of proteins takes place at a faster rate than the breakdown of lipids during imbibition, which could explain the numerous lipid bodies compared to protein bodies in our study.

The lipid body layer in the untreated control seemed to be present as lipid droplets scattered in the cytoplasm (Figure 2 A), whilst, in contrast, the lipid layer was closely associated with the cell walls in the Apron®XL and Celest® XL treatments (Figures 2 B and C, respectively), perhaps involving another response mechanism. According to Cordova-Tellez and Burris (2002), the compact alignment of the lipid bodies along the inner surface of the plasma membrane occurs during natural drying of maize seeds.

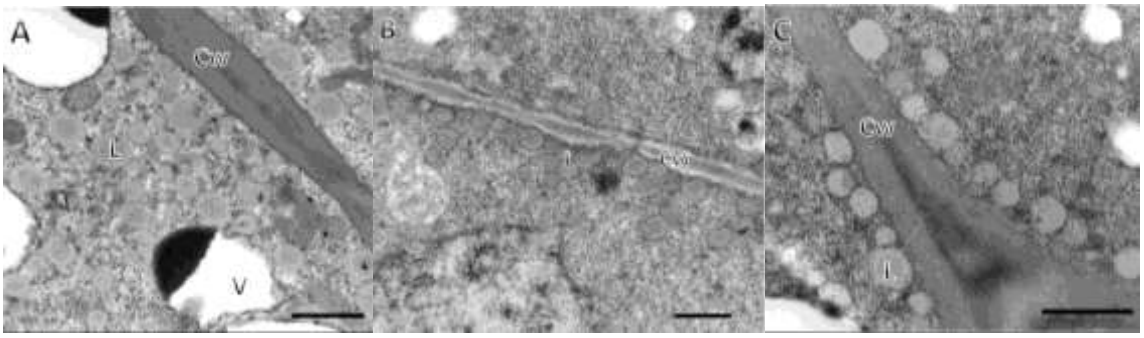

Figure 2. TEM micrographs of radicle tissue of untreated and fungicide treated maize seeds following 2 d accelerated ageing and 48 h rapid imbibition showing differences in the lipid body arrangement, (Bar = 1 μm) A) Untreated control with lipid bodies scattered throughout the cytoplasm and, B) Apron® XL and C) Celest® XL with lipid bodies closely associated with the cell walls (CW = cell wall, L = lipid, V = vacuole).

Deltour and Bronchart (1971) reported that in the root of the germinating maize seed the dispersal of these peripheral lipid bodies in the cell cytoplasm is not complete until 72 h after imbibition. Wen et al. (2009) stated that this peripheral alignment reduces the hydrophilic surface of the cytoplasm which will limit the rate of water imbibition into the cytoplasm during rehydration, essential for the maintenance of membrane integrity. It is possible that the fungicide treatments maintained the peripheral alignment of the lipid bodies to tolerate the stress conditions during rehydration of the seed after accelerated ageing and rapid imbibition which may have resulted in the minimal increase in germination when compared to the control.

Vartapetian et al. (1987) found nearly all mitochondria were fully formed in maize seeds imbibed for 48 h and that they had an oval shape, electron-dense matrix and elongated cristae randomly distributed inside the mitochondria. This was confirmed in the radicle cells of both treated and untreated seeds in our study (Figure 3 A, B, and C).

CONCLUSIONS

Results from our study showed some differences between the untreated control and the two fungicide treatments following rapid imbibition after 2 d accelerated ageing. The untreated seed had the lowest germination after 2 d AA and it is possible that this is associated with the position of the lipid bodies scattered in the cell. This movement of lipid

bodies from the cell wall may be indicative of an inability to reform functionally competent membranes during rehydration of the seed after rapid imbibition, resulting in loss of vigour and lack of germination as reported by Parrish and Leopold (1978).

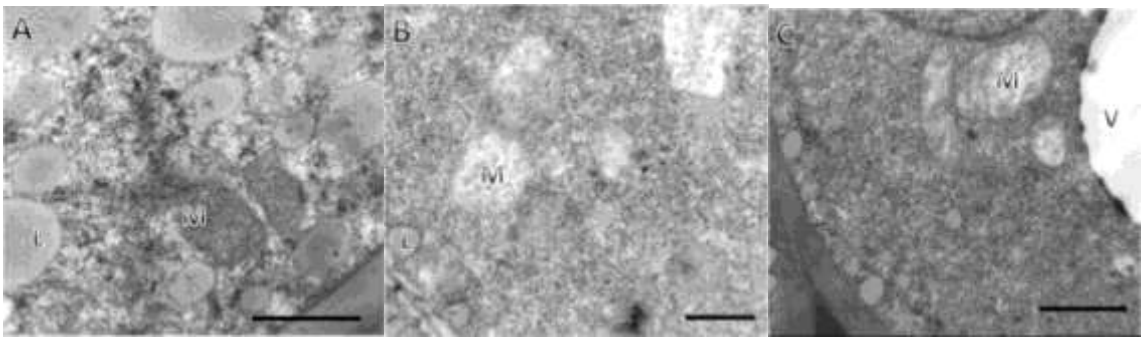

Figure 3. TEM micrographs of radicle tissue of untreated and fungicide treated maize seeds following 2d accelerated ageing and 48 h rapid imbibition showing no major differences in the mitochondrial structure, (Bar = 1 µm) A) Untreated control, B) Apron® XL and C) Celest® XL. (L = lipid, M = mitochondrium, V = vacuole).

The fungicide treatments to the seeds played a positive role by maintaining the peripheral alignment of the lipid bodies. Although the AA test simulates storage under poor conditions, these results will need to be confirmed with further TEM studies once the seed has been stored for several months as fungicide seed treatments can result in reduced germination after seed storage.

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