CHAPTER 4

PACLOBUTRAZOL INDUCED LEAF, STEM, AND ROOT ANATOMICAL MODIFICATIONS IN POTATO

4.1 ABSTRACT

Plants of potato cultivar BP 1 were treated with 67.5 mg of PBZ per plant as a foliar spray. A month after treatment leaf, stem and root histological observations were made. PBZ treatment resulted in reduced shoot growth, thicker leaves, and increased stem and root diameters. Leaves of treated plants showed increased chlorophyll *a* and *b* contents, had a thicker epicuticular wax layer, and elongated and thicker epidermal, palisade and spongy mesophyll cells. The thickness of the stems was associated with an increase in cortex thickness, enlarged vascular bundles, and larger pith with bigger pith cells. An increase in the width of the cortex and the induction of more secondary xylem vessels in response to PBZ treatment increased the root diameter. PBZ resulted in the accumulation of starch granules in the stem pith cells and cortical cells of the stem and root. Increased leaf thickness, and increased stem and root diameters following application of PBZ has been reported before but the underlying anatomical modifications have not been reported previously.

Keywords: chlorophyll; cortex cell; mesophyll tissue; pith cell; starch granules

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4.2 INTRODUCTION

The regulation of plant growth with synthetic plant growth regulators has become a common agricultural practice. Of the available synthetic plant growth regulators, the triazoles are potent at low concentrations to inhibit shoot growth (Davis *et al.*, 1988). PBZ is a triazole derivative known to interfere with *ent*-kaurene oxidase activity in the *ent*-kaurene oxidation pathway leading to a decrease in endogenous GA levels and ABA catabolism (Rademacher, 1997).

PBZ suppressed growth in a wide range of plant species, and treated plants exhibited a dark green colour, were shorter and more compact in appearance (Terri & Millie, 2000; Sebastian *et al.*, 2002). Plant morphological and anatomical modifications in response to PBZ treatments have been reported in various plant species. Berova & Zlatev (2000) reported a reduced height and an increased stem thickness of tomato in response to PBZ treatment. Treating *Chrysanthemum* plants with PBZ as a soil drench resulted in thicker leaves, reduced stem diameter, and roots with an increased diameter (Burrows *et al*., 1992). Sopher *et al.* (1999) observed thicker and broader maize leaves having more epicuticular wax, enlarged vascular elements, and enlarged epidermal, mesophyll and bundle sheath cells due to PBZ treatment. In wheat, PBZ increased thickness of the leaves by inducing additional layers of palisade mesophyll cells (Gao *et al.*, 1987).

Greenhouse and field experiments on the response of potato grown under non-inductive conditions to PBZ showed that PBZ treatment resulted in compact plants with thicker and dark green leaves. PBZ treatment prevented flower formation. No reports dealing with PBZ

induced anatomical changes in potato are available. The objective of this investigation was to determine the effect of PBZ on leaf, stem, and root anatomy.

4.3 MATERIALS AND METHODS

4.3.1 Plant culture

In a greenhouse experiment on the experimental farm of the University of Pretoria the effect of PBZ on the anatomy of potato leaves, stems and roots was investigated during 2003. Plants of cultivar BP1 were grown in 5-liter plastic containers with a mixture of sand and coconut coir (50:50 by volume) as growing medium. During the growing period diurnal temperatures ranged between 17 and 35 ºC and the average relative humidity was 54%. Plants were fertilized with a standard Hoagland solution and watered regularly to avoid water stress.

4.3.2 Treatments

One month after planting, during early stolon initiation, the plants were treated with PBZ at rates of 0, 45.0, 67.5 and 90.0 mg active ingredient (a.i.) per plant as a foliar spray. (Cultar formulation, 250 g a.i. PBZ per liter, Zeneca Agrochemicals SA (PTY.) LTD., South Africa). The solution was applied as a fine spray using an atomizer and the control plants were treated with distilled water.

4.3.3 Chlorophyll content

Two weeks after treatment, crude leaf chlorophyll extracts were made using 80% acetone. Spectrophotometer (Pharmacia LKB, Ultrospec III) readings were recorded at 663 and 645 nm, and the concentrations of chlorophyll *a* and *b* determined using the specific absorption coefficients recommended by MacKinney (1941).

4.3.4 Morphology and anatomy

Plant height was measured from the base of the stem to the apex. One month after treatment leaf, stem, and root material were collected from the 67.5 mg a.i. PBZ treated plants and control plants. Leaf material was taken from the mid portion of the third youngest fully expanded leaves. Internode samples were taken from the mid portion of the main stem, and the root samples were taken 1 cm below the points of attachment to the stem.

Sections of the leaves, stems, and roots were fixed in formalin/acetic-acid/alcohol (FAA), dehydrated in increasing ethanol concentrations and embedded in paraffin wax (melting point, 58 ºC) after substituting the alcohol with xylene (O'Brien & Mc Cully, 1981). Sections of about 8 µm were made with a rotary microtome and stained in Safranin 0, counter stained in Fast Green, and mounted in Clear Mount (O'Brien & Mc Cully, 1981). Images were made using a Kodak camera (Nikon DXM 1200, Nikon, Japan) fitted on a light microscope (Nikon Opti. Photo, Nikon, Japan). Measurements of leaf anatomical structures were made using image analyser (UTHSCSA Image Tool for Window 3.00).

4.4 RESULTS

PBZ treated leaves were dark green due to high concentrations of chlorophyll *a* (0.82 mg g^{-1} FW) and *b* (0.26 mg g^{-1} FW) (Table 4.1). Leaves of the control treatment contained 0.54 and 0.17 mg g^{-1} FW chlorophyll *a* and *b*, respectively. PBZ treated plants exhibited thicker epicuticular wax layers, larger epidermal cells, a single layer of large and elongated palisade mesophyll cells, and a thicker spongy mesophyll tissue (Figure 4.1B) compared to the control (Figure 4.1A).

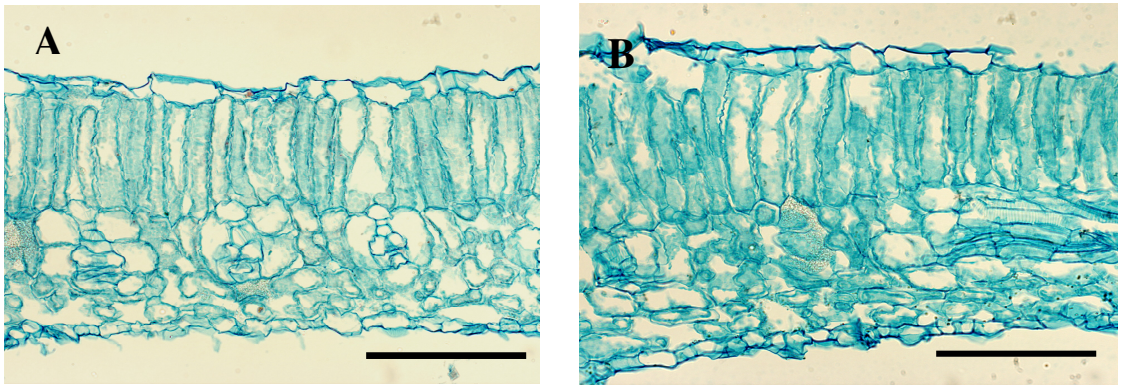

Figure 4.1 Light micrographs of transverse sections of leaves showing enlarged epidermal, palisade mesophyll and spongy mesophyll cells of PBZ treated (B) compared to the control (A). Thicker epicuticular wax deposition can be seen on PBZ treated leaf (B). Scale bar 100 µm

Leaf thickness increased from 215 µm to 268 µm in response to PBZ treatment (Table 4.1). PBZ increased the length and diameter of epidermal cells by about 24 and 14%, respectively over the control (Table 4.1). PBZ treatment increased leaf palisade mesophyll cell length and width (Table 4.1). The mean palisade mesophyll cell length and diameter of the treated leaves were respectively about 116 µm and 21 µm, compared to 88 µm and 15 µm for the untreated leaves. PBZ treatment increased the thickness of spongy mesophyll by about 15% over the control, 96 µm thick.

Table 4.1 Effect of PBZ on leaf, stem and root characteristics. Mean value ± standard deviation

Plant part	Control	PBZ treated	Increase over the control (%)
Leaf			
Chlorophyll a (mg g^{-1} FW)	0.54± 0.05	0.82 ± 0.09	51.8
Chlorophyll b (mg g^{-1} FW)	0.17 ± 0.03	0.26 ± 0.08	52.9
Total thickness (µm)	215.4 ± 5.1	267.8 ± 6.7	24.3
Epidermal cell length (µm)	34.2 ± 13.9	42.3 ± 12.5	23.7
Epidermal cell width (µm)	12.3 ± 3.4	14.0 ± 3.0	13.8
Palisade cell length (µm)	87.6 ± 5.8	116.3 ± 6.4	32.7
Palisade cell width (µm)	14.9 ± 2.6	21.1 ± 3.1	41.6
Spongy mesophyll thickness (µm)	95.6 ± 7.9	110.3 ± 8.0	15.4
Stem			
Stem length (cm)	76.4 ± 1.7	43.5 ± 2.3	-43.1
Stem diameter (mm)	6.6 ± 0.5	10.4 ± 1.2	63.5
Root			
Root diameter (mm)	2.9 ± 0.2	4.4 ± 2.1	51.7

PBZ treatment resulted in shorter and thicker stems compared to the control plants (Table 4.1 and Figure 4.2). The mean plant height was reduced from 76.4 cm to 43.5 cm in response to PBZ treatment while stem diameter was increased by 58% (Table 4.1). This is attributed to the induction of a thicker cortex, well-developed vascular bundles, and a larger pith diameter in response to the treatment (Figure 4.3B). The stem of PBZ treated plants had larger symmetrical pith cells containing numerous starch granules (Figure 4.3D) while the control plants exhibited smaller irregularly shaped pith cells almost devoid of starch granules (Figure 4.3C).

Figure 4.2 Potato plant height reductions in response to PBZ treatment: A = untreated, B = 45 mg a.i. PBZ, C = 67.5 mg a.i. PBZ, and D = 90 mg a.i. PBZ

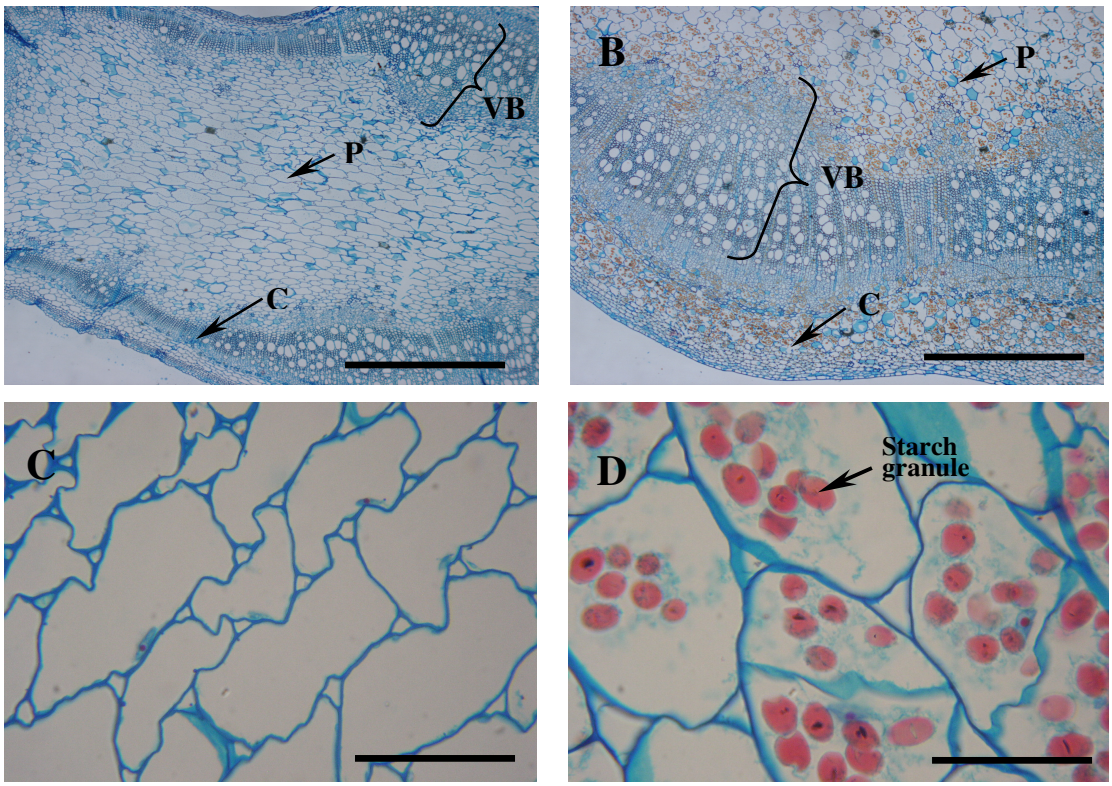

Figure 4.3 Transverse micrographs of sections from the stems of the control and PBZ treated potato plants. The treated stem (B) is characterised by increased cortex thickness (C), well-developed vascular bundles (VB), and wider pith diameter (P) compared to the control (A). Treated plants developed larger, oval shaped pith cells containing starch granules (D) compared to the smaller and irregularly shaped pith cells without starch granules (C). Scale bar 100 µm

The average root diameter of PBZ treated plants was 4.4 mm, 52% thicker than the 2.9 mm of the control (Table 4.1). PBZ increased the width of root cortex and the number of vascular vessels compared to the control (Figure 4.4A and 4.4B). Roots of treated plants developed larger cortical cells containing numerous starch granules (Figure 4.4D) while the untreated plants possessed thin and elongated cortical cells with few starch granules (Figure 4.4C).

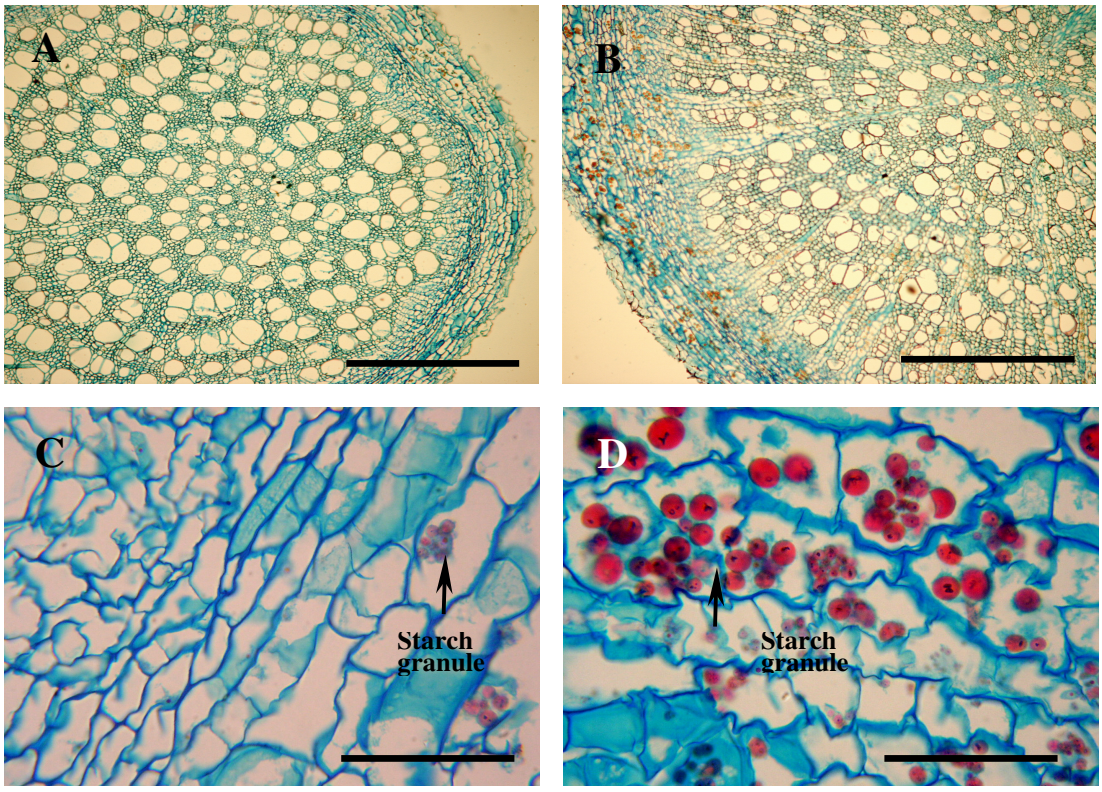

Figure 4.4 Transverse sections of roots of the control and PBZ treated potato plants. Treated plants (B) had larger root diameters due to an increase in the width of the cortex and the induction of more secondary xylem vessels compared to the control (A). Larger root cortical cells of treated plants contained numerous starch granules (D) compared to the smaller cortical cells of the control plants with few starch granules (C). Scale bar 100 µm

4.5 DISCUSSION

PBZ treated potato plants exhibited a dark green colour due to high chlorophyll *a* and *b* contents. The increase in chlorophyll content may be attributed to enhanced chlorophyll synthesis and/or more densely packed chloroplasts per unit leaf area. Sebastian *et al.* (2002) reported enhanced chlorophyll synthesis in *Dianthus caryophyllus*, and Khalil (1995) observed more densely packed chloroplasts per unit leaf area in response to PBZ treatment. Increased chlorophyll content in potato due to PBZ treatment was observed by Balamani & Poovaiah (1985) and Bandara & Tanino (1995). The higher chlorophyll content of treated potato leaves may be related to the influence of PBZ on endogenous cytokinin levels. It has been proposed that PBZ stimulates cytokinin synthesis that enhances chloroplast differentiation, chlorophyll biosynthesis, and prevents chlorophyll degradation (Fletcher *et al.*, 1982). GA biosynthesis inhibitors increased cytokinin content in soybean (Grossman, 1992) and *Dianthus caryophyllus* (Sebastian *et al.*, 2002).

The observed higher epicuticular wax deposition on treated leaves may be related to the increase in endogenous ABA levels in response to PBZ treatment (Rademacher, 1997). An increase in ABA stimulates the synthesis of lipid transfer proteins in barley that play an important role in the formation of epicuticular waxes, a process that affects the water relation of the leaves (Hollenbach *et al.*, 1997). PBZ treatments caused an increase of 10% in total wax load and change the proportion of certain wax constituents in potted rose cultivars within 11 days of application (Jenks *et al*., 2001). The development of a thicker epicuticular wax layer provides better protection against some plant pathogens and minor mechanical damage (Kolattukudy, 1987).

The observed increase in leaf thickness is attributed to an increase in epidermal cell diameter, palisade cell length and spongy mesophyll depth. Burrows *et al.* (1992) reported that increased *Chrysanthemum* leaf thickness in response to PBZ treatment was due to thicker spongy mesophyll, and the induction of additional layers of palisade parenchyma, although individual cells were shorter, of small diameter and more tightly packed. In maize PBZ treated leaves showed more epicuticular wax deposition and were thicker and broader owing to enlarged vascular elements, epidermal, mesophyll, and bundle sheath cells (Sopher *et al.*, 1999). Hawkins *et al.* (1985) reported a 15-24% increase in soybean leaf thickness due to the elongation of the palisade cells without affecting the number of palisade rows and spongy parenchyma thickness. Dalziel & Lawrence (1984) reported that PBZ induced a 100% increase in sugar beet leaf thickness due to a three to four fold increase in palisade cell length, without affecting the number of rows.

PBZ treated potato plants were shorter and had thicker stems than the control. Reduced internode length caused height reduction. Davis & Curry (1991) reported that shoot growth reduction in response to PBZ treatment occurs primarily due to a decrease in internode length, and the effective dose varies with species and cultivar. This response may probably be explained by the reduction in the endogenous GA level. GA enhances internode elongation of intact stems (Salisbury & Ross, 1992). Liu & Loy (1976) showed that GA promote cell division by stimulating cells in the G_1 phase to enter the S phase and by shortening the duration of S phase. They concluded that increased cell numbers lead to more rapid stem growth. Similar reductions in shoot growth were reported in Scaevola (Terri & Millie, 2000) and *Dianthus caryophyllus* (Sebastian *et al.*, 2002) in response to PBZ treatment. More recently, Suzuki *et al.* (2004) reported that the presence of PBZ in the medium strongly inhibited etiolated and non-etiolated longitudinal shoot growth of *Catasetum fimbriatum.*

PBZ treatment increased cortex thickness, size of the vascular bundles, and pith diameter and resulted in thicker stems. This modification may be attributed to radial expansion of cells due to reduced endogenous GA activities in response to the treatment. Wenzel *et al.* (2000) reported that GA limits the extent of radial expansion of plant organs. In dicot stems, cell shape alterations are apparently caused by a more longitudinal orientation of cellulose microfibrils being deposited in the cell walls, preventing expansion parallel to the these microfibrils but allowing expansion perpendicular to them (Eisinger, 1983). The non-uniform distribution and arrangement of the vascular elements in the potato stems resulted in irregularity in the shape of the stems. Various authors reported different results in various plant species with respect to PBZ induced stem anatomy modifications. PBZ reduced both cell number and length in safflower stem (Potter *et al.*, 1993). Burrows *et al*. (1992) reported that PBZ treatment brought about a 50% reduction in *Chrysanthemum* stem diameter because of an enhanced development of secondary xylem and a marked reduction in the number of sclerenchyma bundle caps. In peach shoots, PBZ reduced the proportion of xylem and increased that of phloem and cortex, and increased xylem density (Aguirre & Blanco, 1992). In an investigation on poinsettia, McDaniel *et al.* (1990) found that PBZ application suppressed cell wall thickening in the phloem fiber caps, decreased the width of xylem ring, and disfavoured the differentiation of interfasicular supporting tissues.

It was observed that untreated plants had more, thinner and longer roots compared to the treated plants. PBZ increased root diameter by increasing the width of the cortex and by favouring the formation of more secondary xylem vessels. Depending on the plant species and the concentration, PBZ either stimulated or inhibited root growth. PBZ caused thickening of maize roots and increased their starch content (Baluska *et al.*, 1993). Treating primary roots of pea inhibited root extension but promoted radial cell expansion (Wang & Lin, 1992). Increased root

diameter has been correlated with larger cortical parenchyma cells in soybean and maize (Barnes *et al.*, 1989). Increasing root diameter in *Chrysanthemum* was due to an increase number of rows and diameter of cortical cells (Burrows *et al.*, 1992). A stimulatory effect of PBZ on root growth has also reported in English ivy (Geneve, 1990) and mung bean (Porlingis & Koukourikou-Petridou, 1996).

PBZ increased the accumulation of starch granules in the pith cells of the stem, and in the cortical cells of the stems and roots. It is postulated that the increase in the number of starch granules may be attributed to PBZ stimulated reduction in the GA activity. Under favourable conditions for tuberization (GA content below threshold level), the activities of enzymes involved in potato tuber starch biosynthesis such as ADPG-pyrophosphorylase, starch phosphorylase and starch synthase increase (Visser *et al.*, 1994; Appeldoorn *et al.*, 1997). Mares *et al.* (1981) observed that exogenous application of GA_3 on growing tubers substantially reduced the activity of ADPG-pyrophosphorylase, while the activity of starch phosphorylase remained more or less constant. Booth & Lovell (1972) reported that application of GA_3 to potato shoots reduced starch accumulation in the tubers. PBZ treatment increased root starch content in maize plants (Baluska *et al.,* 1993). PBZ treatment increased starch accumulation in the leaves, stems, crowns and roots of rice seedling while GA_3 treatment decreased starch accumulation in the leaves and crowns of the seedlings (Yim *et al.*, 1997).

4.6 CONCLUSION

PBZ modified the morphology of the potato plant in such a way that treated plants appeared to be dark green, and short and compact. Darkness of the leaves was due to an increase in chlorophyll *a* and *b* concentrations. The induction of elongated and thicker epidermal, palisade and spongy mesophyll cells in response of PBZ treatment increased leaf thickness. The thickness of the stems was correlated with an increase in cortex thickness, enlarged vascular bundles, and larger pith with bigger pith cells. An increase in the thickness of cortex and the induction of more secondary xylem vessels increased the root diameter. PBZ enhanced starch synthesis in the pith cells of the stem and cortical cells of the stems and roots. This study confirms that PBZ treatment can induce morphological and anatomical modifications in potato similar to those reported in a wide range of plant species.