CHAPTER 4

ANATOMY OF WILD GINGER ENLARGED ROOTS IN RESPONSE TO NITROGEN NUTRITION, FERTIGATION FREQUENCY AND GROWING MEDIUM

4.1 Introduction

Wild ginger is an indigenous forest floor plant of southern Africa scientifically known as *Siphonochilus aethiopicus* (Schwerf) B.L. Burt., and belongs to the family Zingiberaceae. The generic name *Siphonochilus* is derived from the Greek name siphono meaning tube and chilus meaning lip in reference to the shape of the flower and the specific name *aethiopicus* means from southern Africa (Van Wyk & Gericke, 2000). The plant is highly prized for its medicinal value and as a result, it has been over harvested from the wild to a point just short of total extinction (Arnold & de Wet, 1993; Hutchings, 1996; Van Wyk, Outdshoorn & Gericke, 1997; van Wyk & Gericke, 2000).

Rhizomes are chewed fresh to treat asthma, hysteria, colds and flu as well as to treat malaria and also chewed by women during menstruation. The highly aromatic roots have been reported to be used by Zulu people as a protection against lightning and snakes (Van Wyk *et al.*, 1997).

Little is known about the effect of nitrogen, fertigation frequency and growing medium on the enlarged root of wild ginger. Hence, this trial was established to determine the effect of nitrogen nutrition, fertigation frequency and growing medium on the anatomical structure of wild ginger enlarged root.

4.2 Materials and methods

The experiment was conducted in a Laboratory at the Department of Plant Production and Soil Science, University of Pretoria. To determine the effect of N nutrition on the enlarged root anatomy, wild ginger plants were grown in pine bark under a glasshouse. Treatments used were six levels of nitrogen viz. 0, 50, 100, 150, 200 and 250 kg·ha^{-1}. Thus, to determine the response of enlarged root anatomy of wild ginger to fertigation frequency and growing medium, wild

ginger plants were grown in either sand or pine bark with five fertigation frequencies (0.25L/day, 1L/day, 2L/day, 2L/2nd day and 2L/week). Plants were harvested at 224 days after emergence (DAE) for sectioning. Thereafter, enlarged roots were immersed in small test tubes and fixed in a solution of F.A.A [(100 ml acetic acid (CH_3COOH), 100 ml formaldehyde (HCHO) and 1800 ml 50% ethanol (CH_2H_2OH), thereafter were put on a mechanical shaker for 2 to 3 hours. Samples were dehydrated with 30, 50, 70 and 100% ethanol. Samples were dehydrated with 30, 50, 70 and 100% ethanol and each concentration was changed after 2 to 3 hours on a mechanical shaker and the procedure was repeated once for 30, 50 and 70% and twice for 100% alcohol.

Ethanol was extracted from the samples with 30, 50, 70 and 100% xylene and also each and every concentration was changed after 2 to 3 hours, except for the 100% xylene where, after 2 to 3 hours of agitation, 2 small wax granules were added into the small test tubes with samples. Another 2 wax small granules were added only when the previously added granules had dissolved. Wax granules were added up until liquid xylene in the test tube looked milky. Samples were put in an oven at 50 °C and the test tubes were filled with wax granules up to the top. Other wax granules were added only after wax in the samples had dissolved and this procedure was repeated three times.

Wild ginger enlarged root samples were mounted on a Thermolyne Histo – Centre II (Getan, England). Specimens were prepared on silver plates with a protrusion like extended to the outside. Silver plates were spread with glycerin to enable easier removal of the specimens from the plates. A small amount of liquid wax was poured into the protrusion of the plate where the specimen was to be placed. The procedure was done on a hot plate at 63.4 °C and later specimens were put on a cold plate at –9 °C for 2 hours for the wax and the samples to be in contact. Thick wax from the samples was removed with a razor blade so that an adequate part of the sample could be available for cutting.

Samples were cut at 5 – 7 microns in a microtone 2040 Autocut Sterea star Zoom, Reichert Jung – 0.7x to 42x 570 (Leica, Johannesburg, South Africa). Water was poured into an electrothermal E7 9QN (Instec, USA) and was kept at 40 °C with lights on (GEC 30/250V 40W). Cut samples were put in water to stretch in order to enable easier collection with micro slides. Micro slides

were smeared with 5 microlitre of Haupt solution, so that samples could stick on them. Micro slides were labelled with a diamond stick 1 (MafTek, Britain). After putting the samples on the micro slides they were taken to the microscope to check if the specimens came out clear after which micro slides with specimens were put on a hot plate (Kunz instrument aps HP 3, Denmark) at 2.5 °C.

4.2.1 **Staining**

Nine staining beakers were prepared. The first beaker contained saffranin (water base), the second beaker had toludien blue solution (alcohol base), the third beaker had 100% distilled water, the fourth beaker had 50% distilled water and 50% ethanol, the fifth beaker had 30% distilled water and 70% ethanol, the sixth beaker contained 100% alcohol (to remove all the available water from the specimens), the seventh beaker had 50% xylene, the eighth and ninth beakers contained 100% xylene (to remove available wax from the samples). Each and every slide was inserted in all the beakers, starting with the beaker with saffranin and ending with the one with 100% xylene. In each solution, samples were inserted for two minutes and about ten seconds in 50, 70 and 100% ethanol as well as 50% xylene. However, samples were kept for five minutes in two beakers with 100% xylene to ensure that all the wax had been removed from the samples.

Slides were removed from the staining bath and wiped to remove water and then taken to the microscope to detect whether the cells were clearly visible and that no air bubbles were present on the slides. Samples were observed with a microscope at 100X magnification. Thereafter, the slides were taken together with specimens and put on a hot plate at 2.5 °C so that the mount could dry off.

4.2.2 **Mounting**

Samples were mounted with a clear mount and taken to a midi hot plate (CJB glassware, Halfway House, South Africa) at 2.5 °C to enable the mount to dry off so that samples could be preserved for years. Samples were mounted slowly so as to get the air bubbles off the slides.

Slides were mounted with 15 microlitres (μL) of a clear mount and samples on the slides were closed with Menzel glasses and thereafter, put on the midi hot plate.

4.3 Results

4.3.1 Nitrogen nutrition effect on the anatomy of enlarged roots of wild ginger

Differences were detected for the anatomy of roots across nitrogen treatments. Differences were observed based on the number of primary xylems, number of cells between two primary xylems, the size of the pith, number of glandular cells, presence of starch, number of pericycle layers, the size of the endodermis and the surface area of the cortex. Wild ginger enlarged roots had a solid core of xylem, surrounded by a ring of phloem which was surrounded by ground tissue (cortex). The innermost layer of the cortex is the endodermis.

With respect to roots of plants that received no nitrogen, they had 16 primary xylems and ten cells between two primary xylems as well as phloem cells behind the metaxylem. There was a big pith and two glandular cells surrounded by primary xylems. There was a single layer of pericycle protecting the vascular bundles to the inside followed by an endodermis with very thick casparian strips and from the outside followed a big surface area of cortex with starch grains (Table 4.1 and Fig. 4.1).

In roots of plants fertilized with 50 $kg \cdot ha^{-1}$ N, there were 12 primary xylems and 10 cells between two primary xylems and surrounded by a ring of the phloem cells. There was a small pith and two glandular cells as well as a single layer of pericycle followed by an endodermis with very thick casparian strips from the outside followed by a big surface area of cortex with starch grains (Table 4.1 and Fig. 4.2).

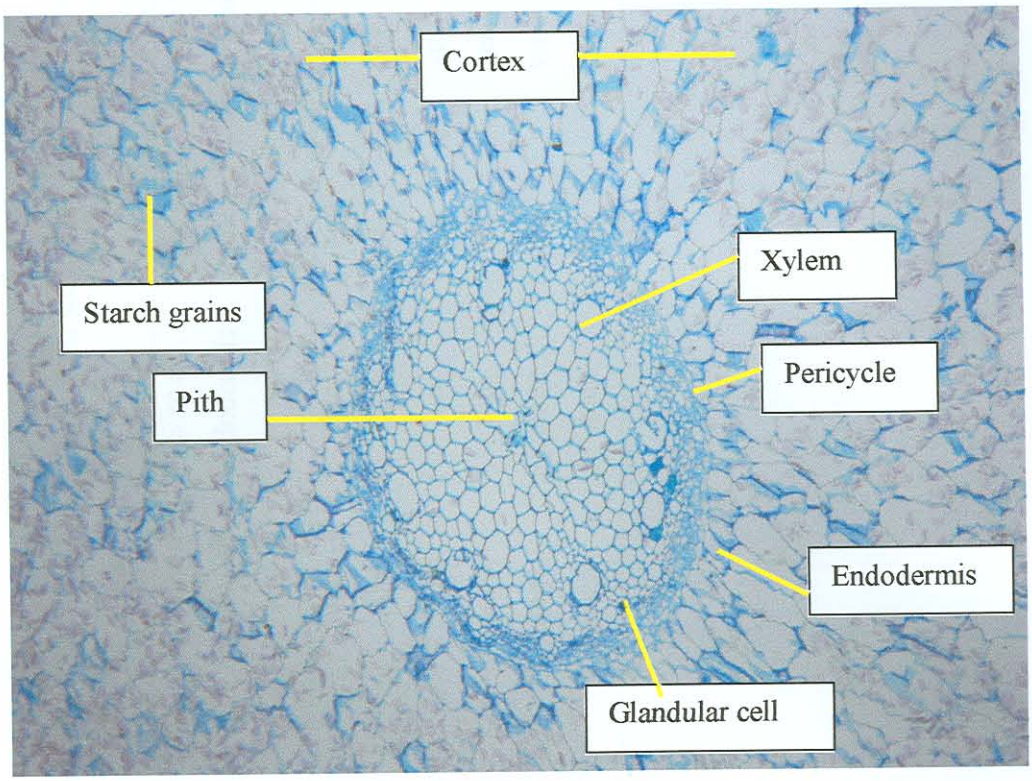

Fig. 4.1 Cross section of wild ginger enlarged root that received no nitrogen

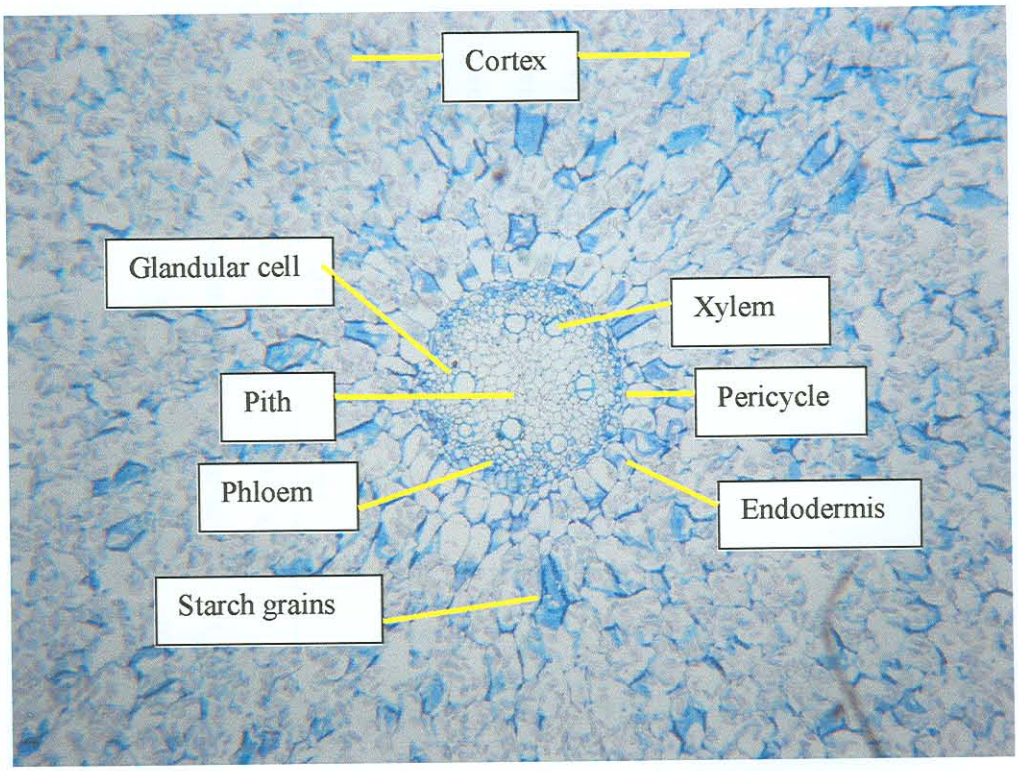

Fig. 4.2 Cross section of wild ginger enlarged root fertilized with 50 $kg \cdot ha^{-1}$ N

Table 4.1 Anatomical structures of wild ginger enlarged root as affected by N nutrition during 2001/2002 seasons

Nitrogen ($kg \cdot ha^{-1}$)	Number of primary xylem	Number of cells between primary xylems	Size of the pith	Number of glandular cells	Number of pericycle layer	Size of endodermis	Size of cortex	Presence of starch grains
0	16	10	big	two	single	thick	big	yes
50	12	9	small	two	single	thick	big	yes
100	19	10	small	four	single	thin	big	yes
150	10	10	small	four	single	thin	big	yes
200	13	undifferentiated	small	six	single	thick	big	yes
250	10	10	big	eight	single	thick	big	yes

For plants that received 100 kg·ha^{-1} N, their enlarged root had 19 primary xylems and 10 cells between two primary xylems and surrounded by a ring of phloem cells. There was a small pith and four glandular cells. Furthermore, there was a single layer of pericycle and endodermis with a thin casparian strip. There was a big surface area of cortex with starch grains (Table 4.1 and Fig. 4.3).

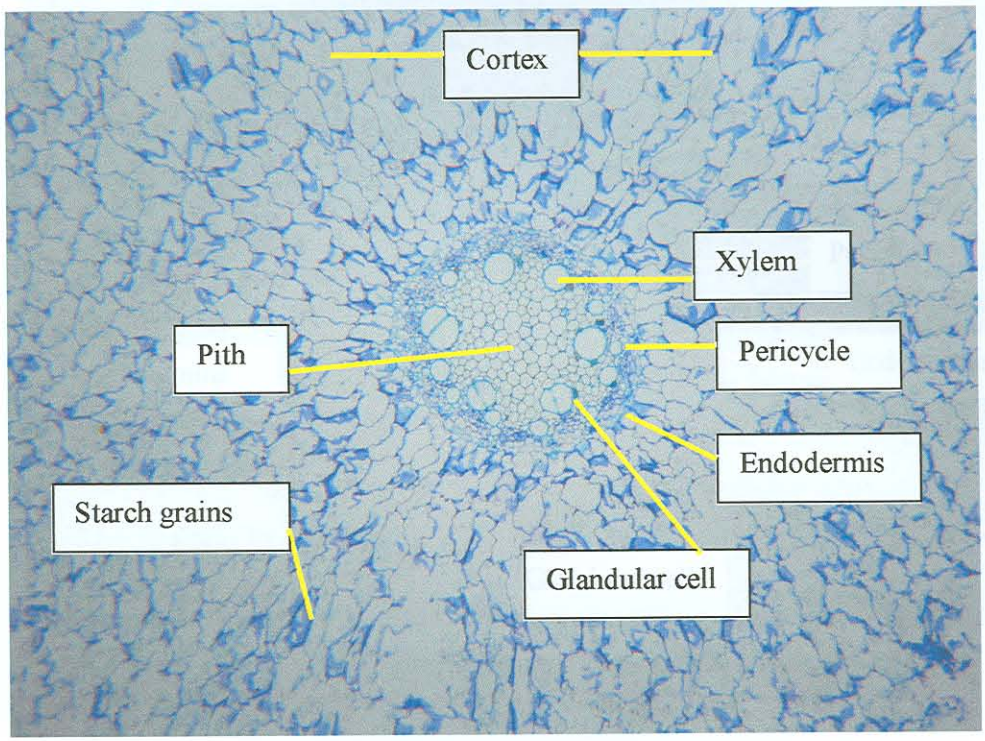

Fig. 4.3 Cross section of wild ginger enlarged root fertilized with 100 kg·ha^{-1} N

For plants that received 150 kg·ha^{-1} N, their root had 10 primary xylems and 10 cells between two primary xylems. There was a small pith and four glandular cells formed on the forefront of the metaxylem. There were many cell layers of pericycle and endodermis with thin casparian strips. There was a big surface area of cortex with starch grains (Table 4.1 and Fig. 4.4).

With respect to plants that received 200 kg·ha^{-1} N, the anatomy of the root had 13 primary xylems and undifferentiated cells between two primary xylems as well as phloem cells behind the metaxylem. Polarization allowed the xylem's thick walls to stand out while the phloem was discreetly tucked within. There was a small pith and six glandular cells. There was a single layer of pericycle protecting the vascular bundles to the inside followed by endodermis with very

thick casparian strips. There was also a big surface area of cortex with starch grains (Table 4.1 and Fig. 4.5).

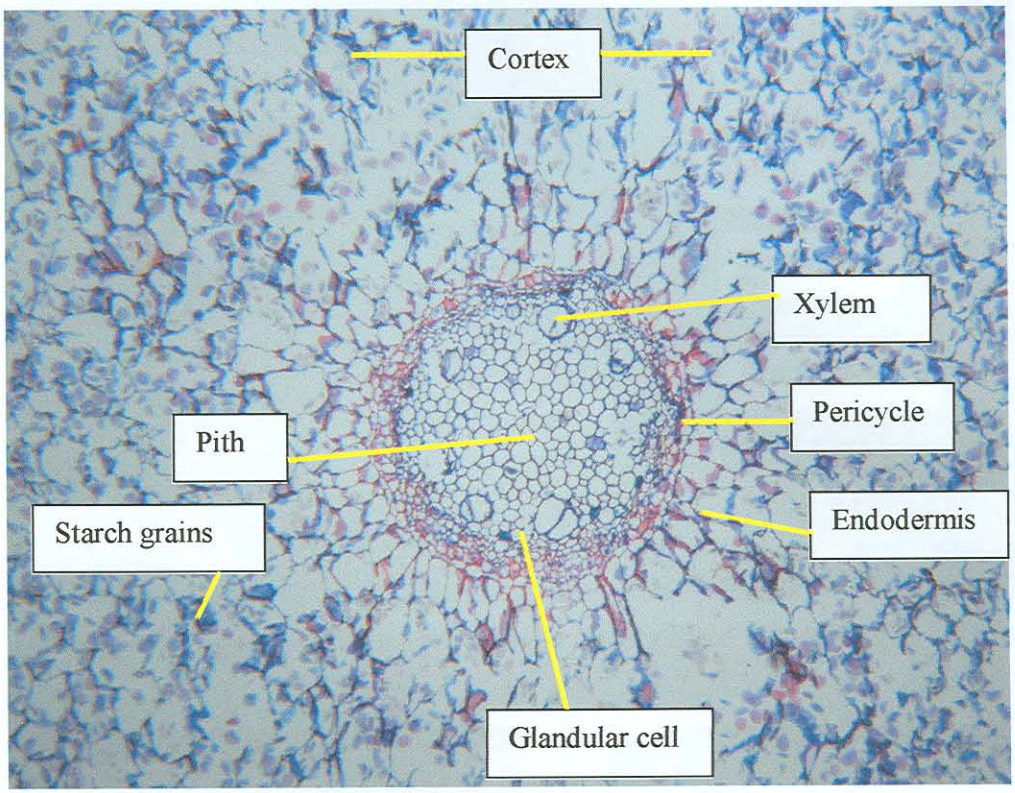

Fig. 4.4 Cross section of wild ginger enlarged root fertilized with 150 kg·ha^{-1} N

For plants fertilized with 250 kg·ha^{-1} N, their root anatomy had 10 primary xylems and 10 cells between two metaxylems. There was a big pith and eight glandular cells as well as the interior was the pericycle of about one cell layer thick followed by an endodermis with very thick casparian strips. There was a big surface area of cortex with starch sheath (Table 4.1 and Fig. 4.6).

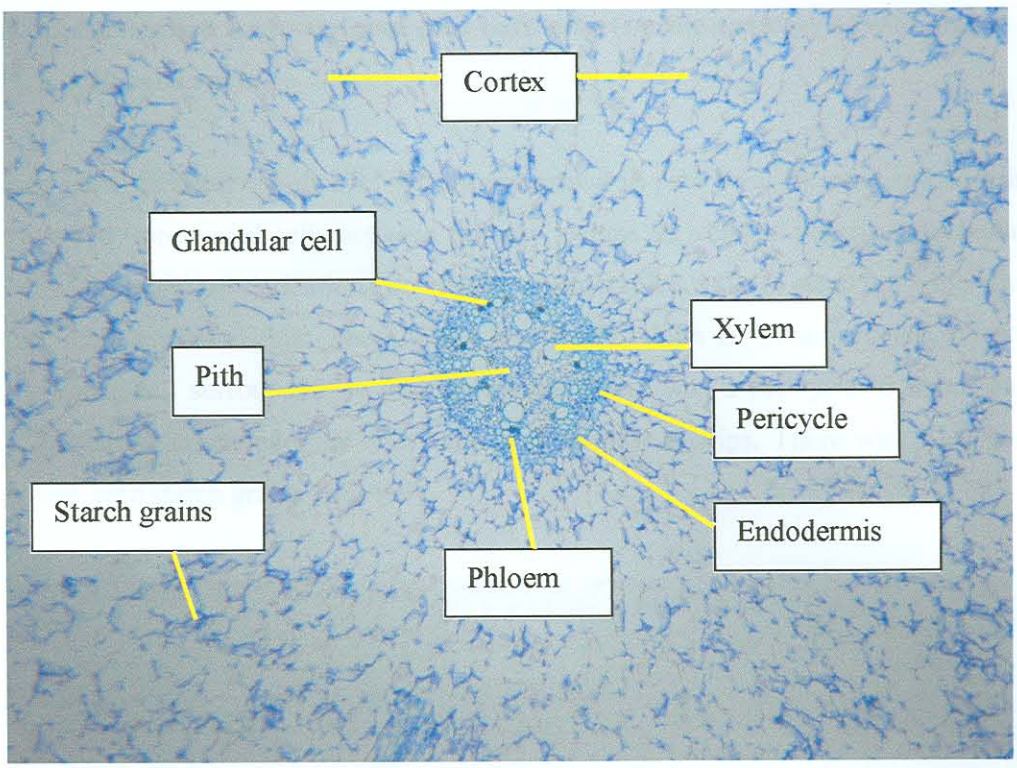

Fig. 4.5 Cross section of wild ginger enlarged root fertilized with 200 kg·ha^{-1} N

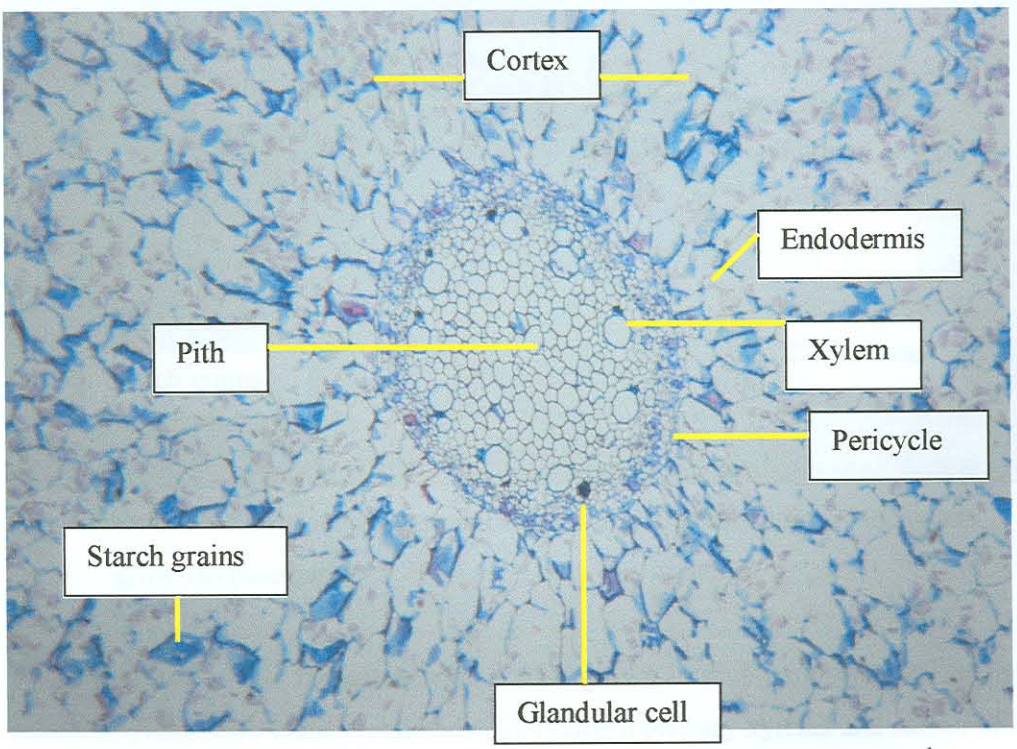

Fig. 4.6 Cross section of wild ginger enlarged root fertilized with 250 kg·ha^{-1} N

4.3.2 Fertigation frequency and pine bark effect on the anatomy of wild ginger enlarged roots

For plants fertigated with a 0.25L/day and grown in pine bark, the root had 13 primary xylems with undifferentiated cells between primary xylems as wells as phloem cells behind the xylem cells (Table 4.2). Polarization allowed the xylem's thick walls to stand out while the phloem was discreetly tucked within. There was a small pith which is the most interior feature, and one glandular cell surrounded by primary xylem. There was a pericycle of about one layer thick followed by an endodermis with very thick casparian strips. There was a big surface area of cortex with starch grains (Fig. 4.7).

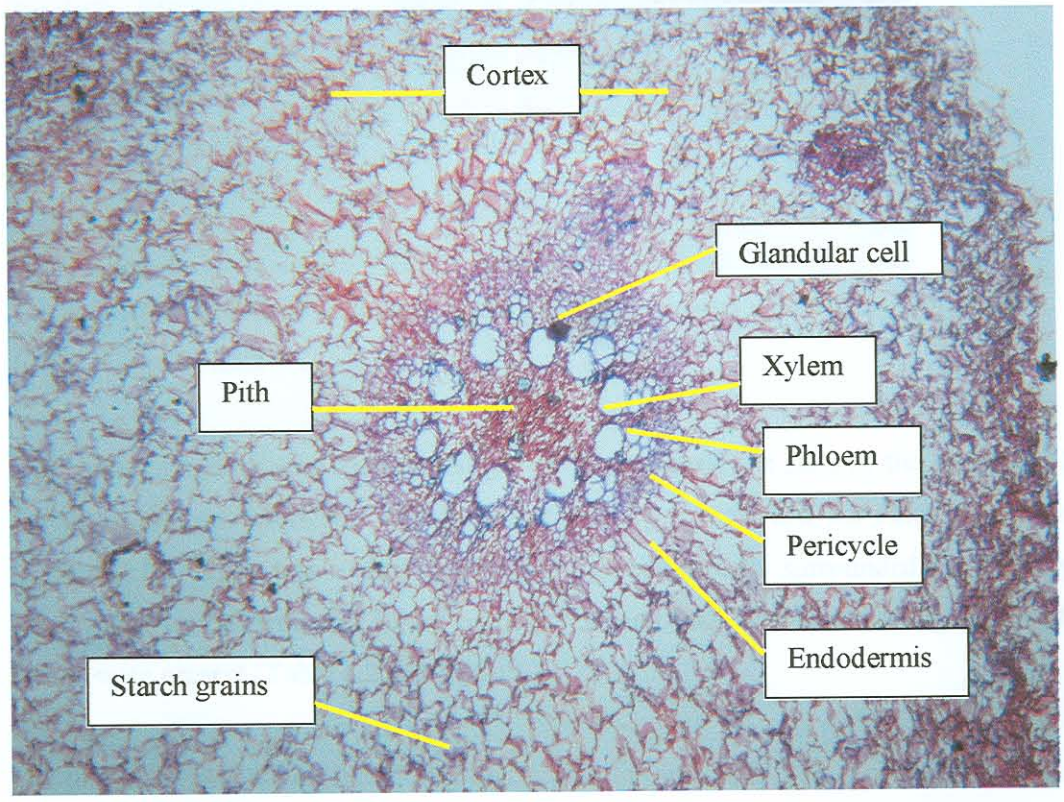

Fig. 4.7 Cross section of wild ginger enlarged root fertigated with 0.25L/day and grown in pine bark

With respect to plants fertigated with 1L/day grown in pine bark, the stele of the root had 14 primary xylems with undifferentiated cells between primary xylems and surrounded by a ring of the phloem cells (Table 4.2). There was a small pith and two glandular cells surrounded by

primary xylem. There was a single layer of pericycle followed by a non-differentiated endodermis and a small cortex. No starch grains were present (Fig. 4.8).

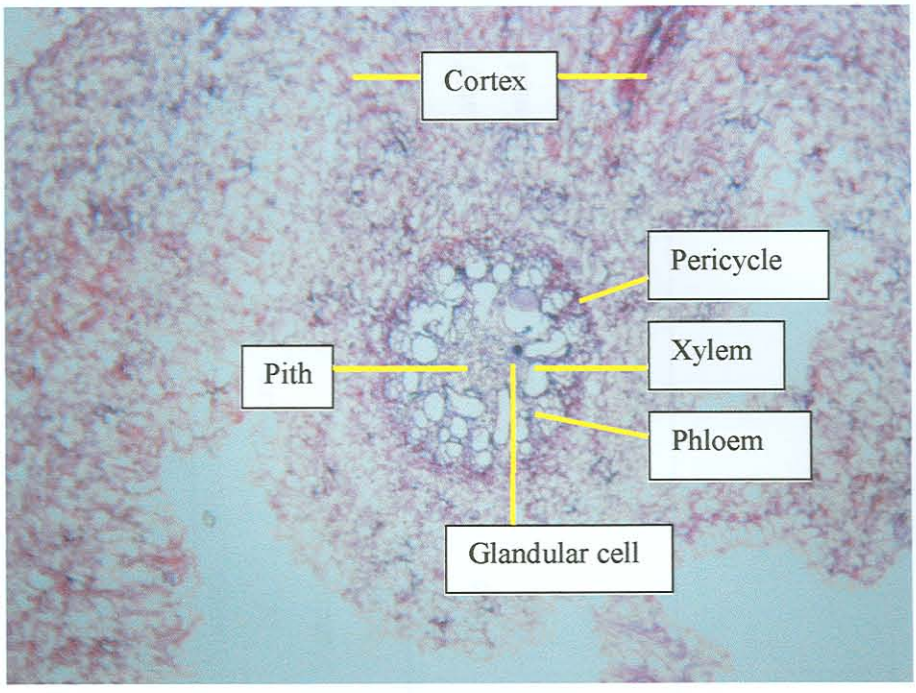

Fig. 4.8 Cross section of wild ginger enlarged root fertigated with 1L/day and grown in pine bark

For plants fertigated with 2L/day and grown in pine bark, the stele of the root had 12 primary xylems with undifferentiated cells between primary xylems and surrounded by a ring of the phloem cells. There was a small pith and no glandular cells surrounded the primary xylems. There were undifferentiated layers of pericycle and endodermis and a big cortex. No starch grains were present (Table 4.2 and Fig. 4.9).

For plants fertigated with 2L/2nd day and grown in pine bark, their root had 18 primary xylems and 10 cells between two primary xylems. There was a big pith and no glandular cells surrounded the primary xylems. There was a small pith which is the most interior feature. There was a pericycle of about one layer thick followed by endodermis with very thick casparian strips. There was a big surface area of cortex with the presence of starch grains (Table 4.2 and Fig. 4.10).

Table 4.2 Anatomical structures of wild ginger enlarged root grown in pine bark as affected by fertigation frequency during 2002/2003 seasons

Fertigation frequency	Number of primary xylem	Number of cells between primary xylem	Size of pith	Number of glandular cells	Number pericycle layers	Size of endodermis	Size of cortex	Presence of starch grains
0.25L/day	13	Undifferentiated	small	one	single	thick	big	no
1L/day	14	Undifferentiated	small	three	single	undifferentiated	small	no
2L/day	12	Undifferentiated	small	no	single	undifferentiated	big	no
2L/2nd day	18	10	small	no	single	undifferentiated	big	no
2L/week	13	Undifferentiated	small	no	double	undifferentiated	small	no

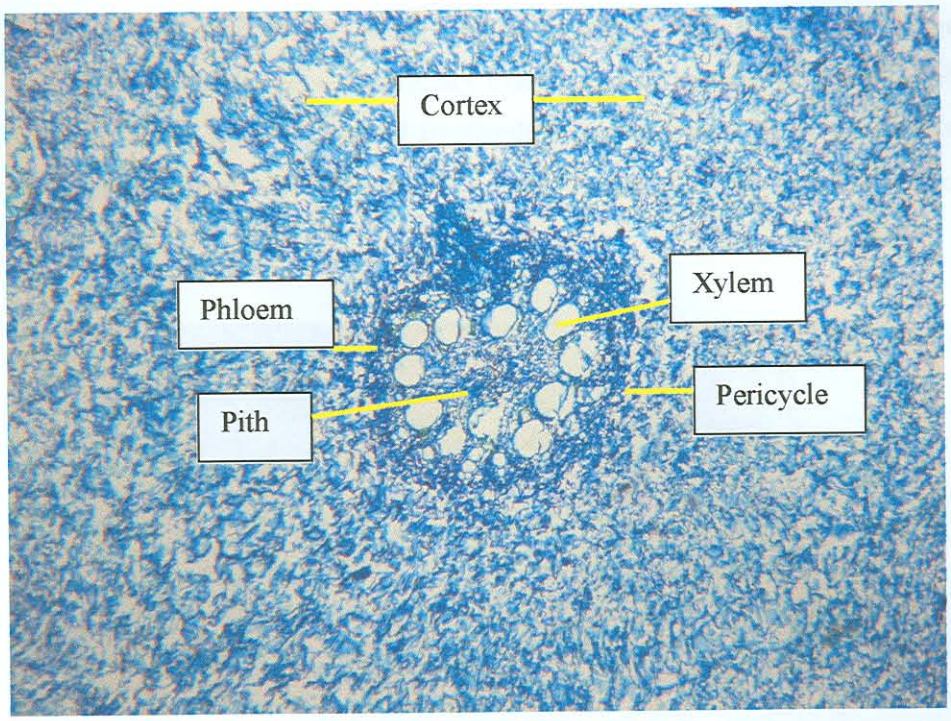

Fig. 4.9 Cross section of wild ginger enlarged root fertigated with 2L/day and grown in pine bark

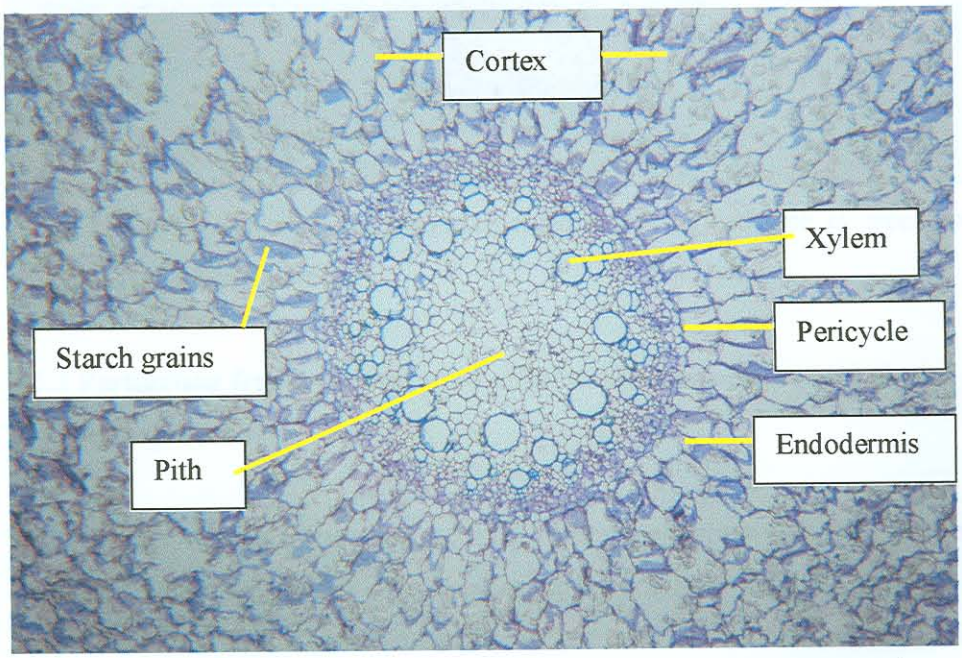

Fig. 4.10 Cross section of wild ginger enlarged root fertigated with 2L/2nd day and grown in pine bark

For plants fertigated with 2L/week and grown in pine bark, the root had 13 primary xylems and undifferentiated cells between primary xylems and surrounded by a ring of phloem cells (Table 4.2). There was a small pith and no glandular cells surrounded the primary xylem. There were two layers of pericycle followed by a thick endodermis and a small cortex. No starch grains were present (Fig. 4.11).

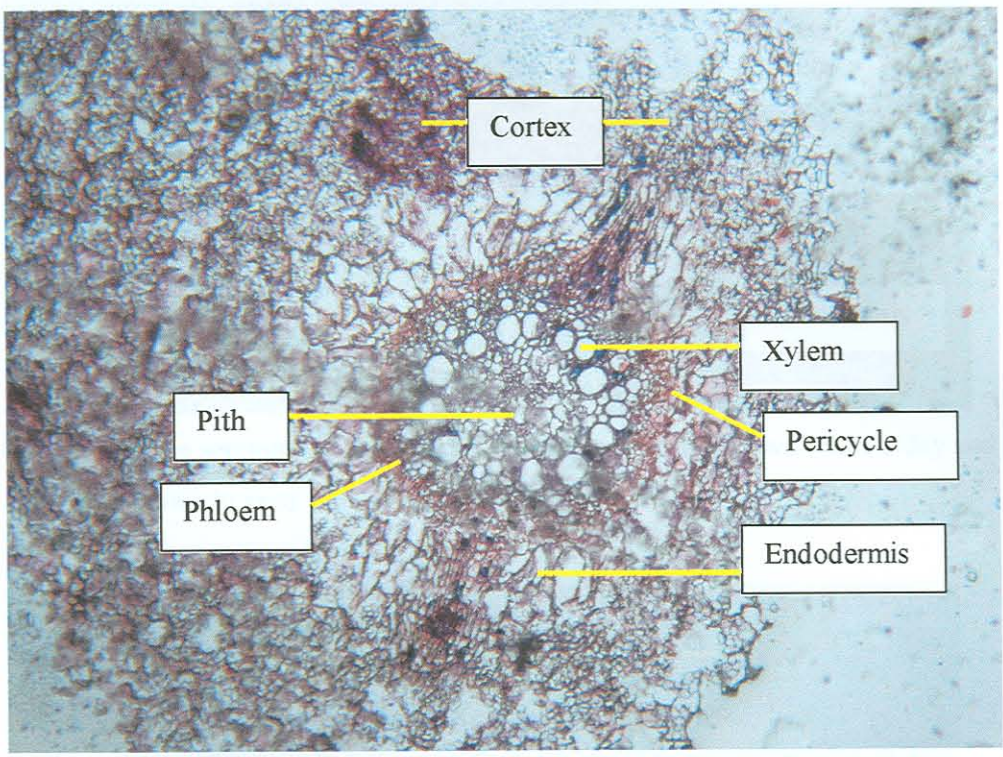

Fig. 4.11 Cross section of wild ginger enlarged root fertigated with 2L/week and grown in pine bark

4.3.3 **Fertigation frequency and sand effect on the anatomy of wild ginger enlarged roots**

With respect to plants fertigated with 0.25L/day and grown in sand, the root had 16 primary xylems and undifferentiated cells between primary xylems with phloem cells behind the xylem cells (Table 4.3). There was a small pith and no grandular cells surrounded the primary xylems. There was a single layer of pericycle followed by a thick endodermis and a big cortex with scattered starch grains present (Fig. 4.12).

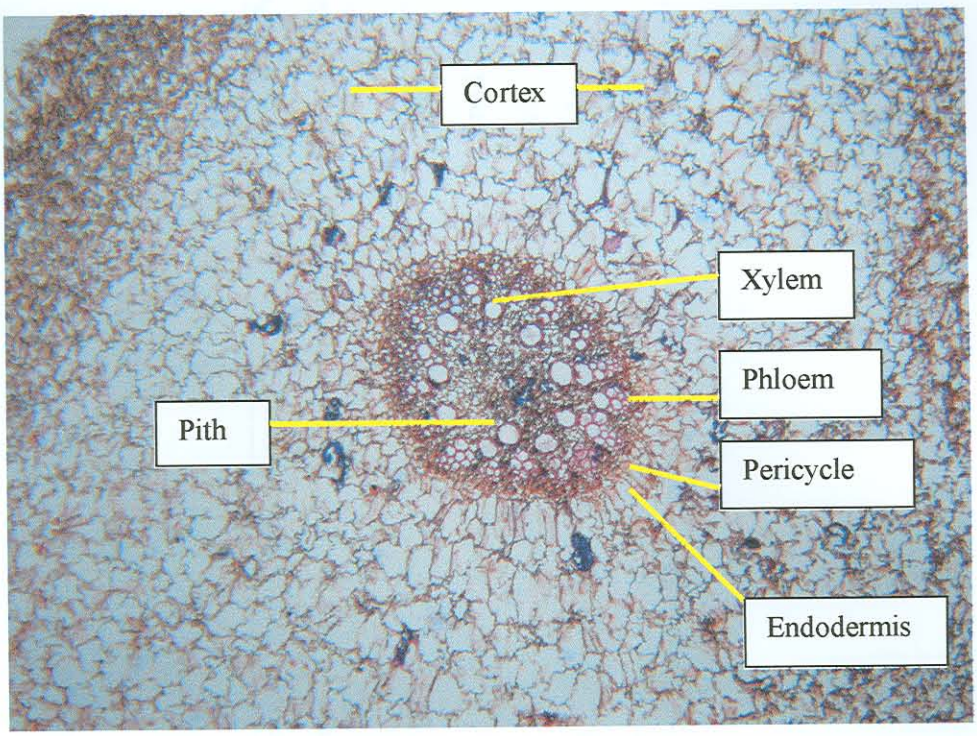

Fig. 4.12 Cross section of wild ginger enlarged root fertigated with 0.25L/day and grown in sand

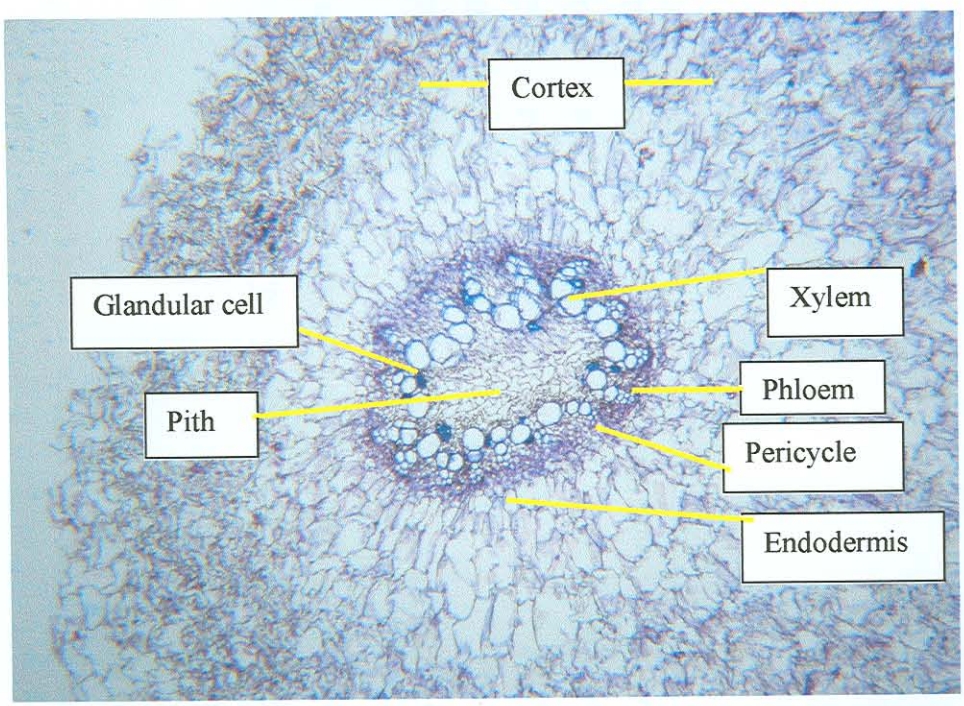

Fig. 4.13 Cross section of wild ginger enlarged root fertigated with 1L/day and grown in sand

Table 4.3 Anatomical structures of wild ginger enlarged root grown in sand as affected by five fertigation frequency in sand during 2002/2003

seasons

Fertigation frequency	Number of primary xylem	Number of cells between primary xylem	Size of pith	Number of glandular cells	Number of Pericycle layers	Size of endodermis	Size of cortex	Presence of starch grains
0.25L/day	16	undifferentiated	small	no	Single	thick	big	yes
1L/day	14	undifferentiated	small	four	single	thick	small	yes
2L/day	15	undifferentiated	small	no	double	thick	big	no
2L/2nd day	14	undifferentiated	small	eight	undifferentiated	undifferentiated	big	no
2L/week	undifferentiated	undifferentiated	small	sixteen	double	undifferentiated	small	no

For plants that received 1L/day and grown in sand, the stele of the root had 14 primary xylems with undifferentiated cells between primary xylems with phloem cells behind the xylem cells (Table 4.3). There was a small pith and four glandular cells surrounded the primary xylem. There was a pericycle of about one layer thick followed by an endodermis with very thick casparian strips. There was a small surface area of cortex with starch grains (Fig. 4.13).

With plants that received fertigation frequency of 2L/day and grown in sand, the root had 15 primary xylems and undifferentiated cells between primary xylems with phloem cells behind the xylem cells (Table 4.3). There was a small pith which is the most interior feature and no glandular cells surrounded the primary xylems. There was a pericycle of about two layers thick followed by endodermis with very thick casparian strips. There was a big surface area of cortex with the absence of starch grains (Fig. 4.14).

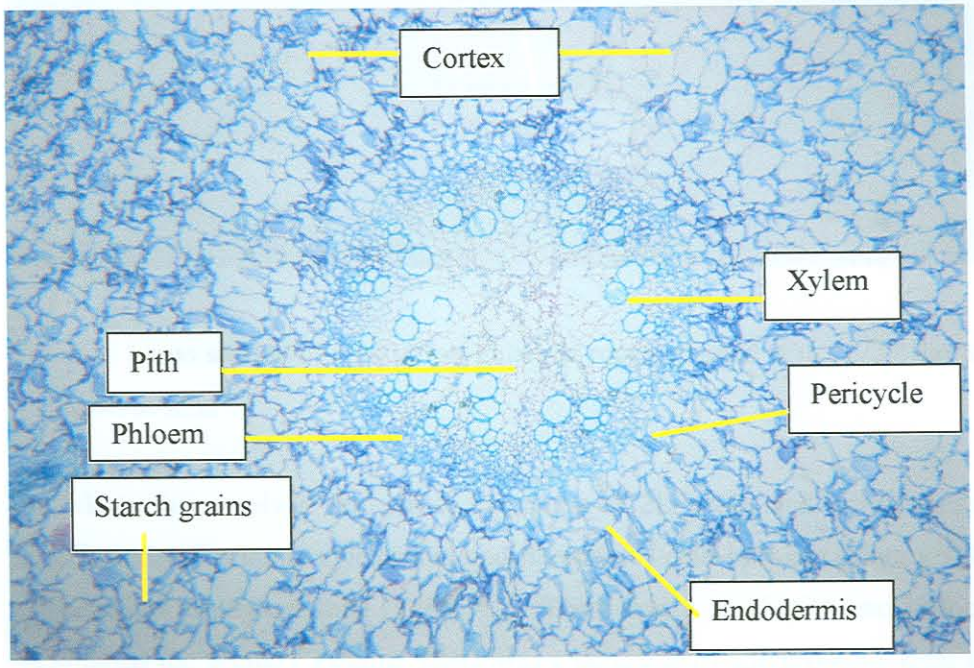

Fig. 4.14 Cross section of wild ginger enlarged root fertigated with 2L/day and grown in sand

For plants that received fertigation frequency of 2L/2nd day and grown in sand, the root had 14 primary xylems and undifferentiated cells between primary xylems and surrounded by a ring of phloem cells (Table 4.3). There was a small pith and eight granular cells surrounded the primary

xylem. There were undifferentiated layers of pericycle and endodermis and a big cortex. No starch grains were present (Fig. 4.15).

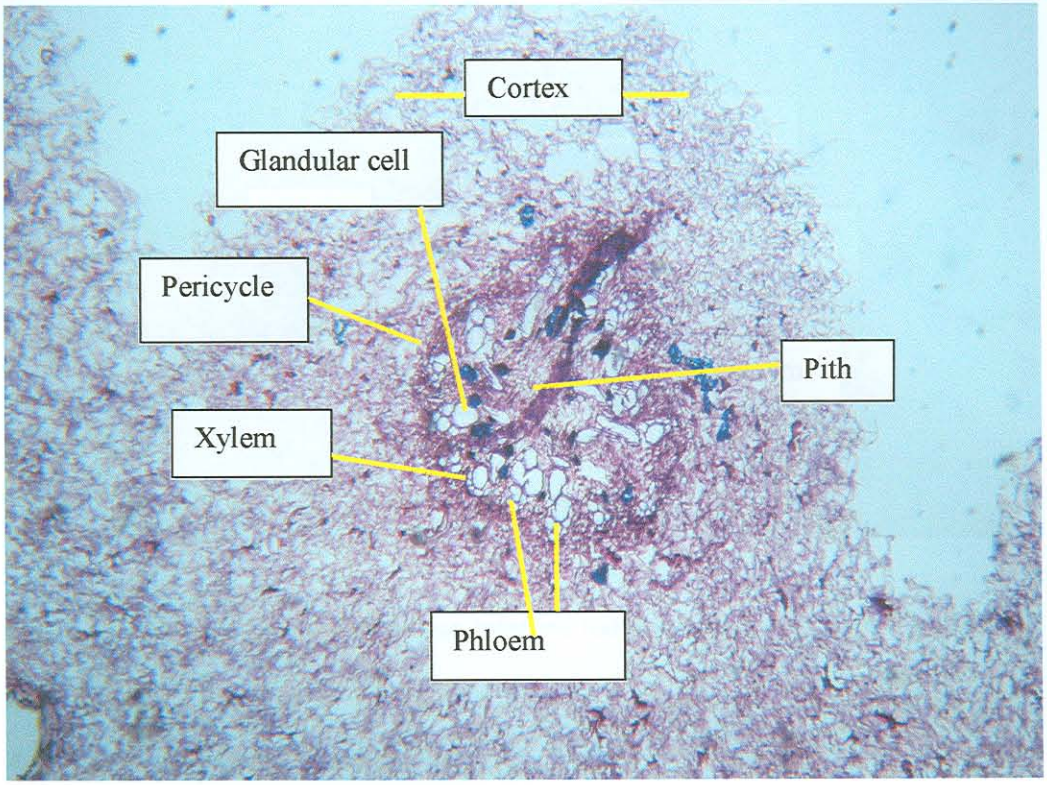

Fig. 4.15 Cross section of wild ginger enlarged root fertigated with 2L/2nd day and grown in sand

For plants that received fertigation frequency of 2L/ week and grown in sand, the root had undifferentiated primary xylems and undifferentiated cells between primary xylems with phloem cells behind the xylem cells (Table 4.3). There was a small pith and sixteen glandular cells surrounded the primary xylems. There was a pericycle of about double layer thick followed by an undifferentiated endodermis. There was a small surface area of cortex with no starch grains present (Fig. 4.16).

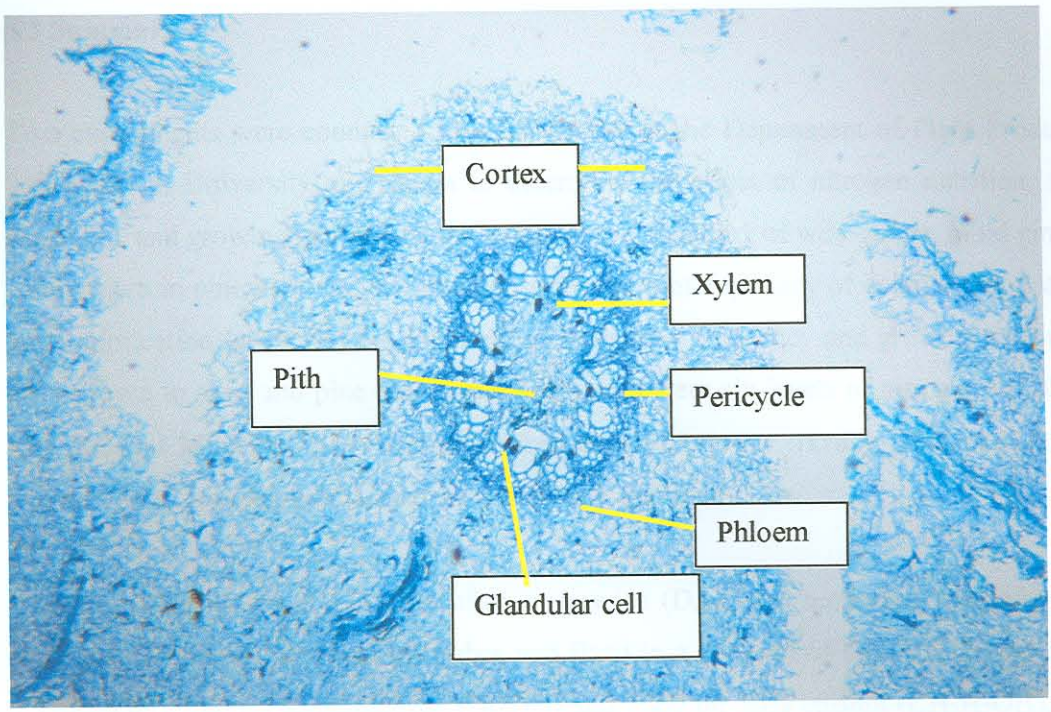

Fig. 4.16 Cross section of wild ginger enlarged root fertigated with 2L/week and grown in sand

4.4 Discussion and Conclusions

Siphonochilus aethiopicus (Schweif.) B.L. Burt, when compared with the typical monocot, such as the normal ginger (*Zingiberaceae officinale*) showed a lot of similar characteristics. The root showed the typical arrangement of the amphivasal vascular bundles and the protective cell layers such as the pericycle and endodermis. More interesting was the presence of glandular cells.

This study demonstrated that N nutrition for wild ginger is important for increasing glandular cells that are important for essential oil production. More glandular cells were, therefore, produced in plants grown in sand with the least fertigation frequency (2L/week).

4.5 Summary

Two experiments were conducted in a Laboratory at the Department of Plant Production and Soil Science, University of Pretoria to determine the effect of nitrogen nutrition, fertigation frequency and growing medium on the enlarged root anatomy of wild ginger. Wild ginger plants were grown in pine bark under a glasshouse for the anatomy study of enlarged roots as affected by N application levels and for the effect of fertigation frequency and growing medium, plants were grown in sand and pine bark. Treatments used were six levels of nitrogen viz. 0, 50, 100, 150, 200 and 250 kg·ha^{-1}, five fertigation frequency (0.25L/day, 1L/day, 2L/day, 2L/2nd day and 2L/week) and growing media used were pine bark and sand.

Plants were harvested at 224 days after emergence (DAE) for sectioning thereafter, enlarged roots were immersed in small test tubes and fixed in a solution of F.A.A [(100 ml acetic acid (CH_3COOH), 100 ml formaldehyde (HCHO) and 1800 ml 50% ethanol (CH_2H_2OH), thereafter were put on a mechanical shaker for 2 to 3 hours. Samples were dehydrated with 30, 50, 70 and 100% ethanol and ethanol was extracted from the samples at 30, 50, 70 and 100% xylene. Samples were mounted so that they could be preserved for years and stained with toluidine solution. Anatomical structures of wild ginger enlarged root were observed with a light microscope at 100X magnification. Anatomical structures observed in wild ginger enlarged roots were primary xylems and the cells between them, glandular cells, pericycle layer, cortex, endodermis and pith.

The number of glandular cells increased from two with zero nitrogen to eight with 250 kg·ha^{-1} N, indicating improved capacity for the production of essential oil. Enlarged roots from all the nitrogen treatments had a single pericycle layer, a thick endodermis, and a large cortex containing starch grains. In all the treatments, there were 10 cells between the primary xylems. The number of primary xylems varied from 10 to 19, but there was no clear relationship with the level of nitrogen applied. This anatomical study has demonstrated that N nutrition of wild ginger is important for increasing the number of glandular cells that are important for essential oil production.

With plants grown in pine bark, a fertigation frequency of 1L/day increased glandular cells. Glandular cells increased from one with plants that received 0.25L/day to three with plants that

received 1L/day, indicating improved capacity for production of essential oils by wild ginger roots. For plants grown in sand, fertigation frequency of 2L/week increased the number of glandular cells in the root anatomy of wild ginger. Growers should fertigate wild ginger more frequently when grown in pine bark and with a low frequent fertigation when grown in sand in order to increase glandular cells essential for oil production, which will then saves water and nutrients. More glandular cells were produced, therefore, produced in plants grown in sand with the least fertigation frequency (2L/week).