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Comparative analysis of electromagnetic field effects on root structure and starch accumulation in cassava cultivars Kasetsart 50 and Rayong 72

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Abstract
This research investigated the effects of electromagnetic fields on the structure and starch content of cassava (*Manihot esculenta* Crantz) roots, varieties Kasetsart 50 and Rayong 72. The internal root structure of both unstimulated (control) and stimulated (treated) roots was examined using microscopy, and the quantity of starch accumulation in these roots was measured. The objective was to study the impact of electromagnetic fields on the internal structure and starch accumulation in cassava roots. Microscopic examination of root internal structures revealed the presence of druse crystals and exarch protoxylem in both cassava varieties. The storage roots exhibited food storage in various layers, with parenchyma displaying large intercellular spaces. As the roots aged, sclerenchyma accumulated starch grains. Stimulation of roots with electromagnetic fields increased the formation of storage parenchyma and starch grains, consistent with reports of fibrous roots developing into storage roots. This observation was made in the treated plants, as opposed to the control (unstimulated) plants. The mechanism of action of electromagnetic fields is hypothesized to involve alterations in ion dynamics and molecular movement within plant cells, potentially accelerating various chemical reactions. The results provide fundamental information on the effects of electromagnetic fields on cassava roots, which will be beneficial for developing technologies to enhance cassava yield and quality.

Keywords: Electromagnetic field, Root structure, Storage accumulation, Cassava (*Manihot esculenta* Crantz).

Introduction

Cassava (*Manihot esculenta* Crantz) is an economically important tuber crop in tropical and subtropical regions worldwide (FAO, 2020), serving as a primary carbohydrate source for a large population and raw material in various industries. Therefore, enhancing its yield and quality is essential. The storage roots of cassava are a major carbohydrate sink, primarily in the form of starch. The internal root structure, particularly the parenchyma tissue, plays a crucial role in determining starch quantity and quality. Understanding the internal root structure helps elucidate the mechanisms of starch accumulation and facilitate crop improvement (Yang et al, 2018). Numerous studies have examined the impact of various factors on cassava growth and yield, including genetics, environment, and management practices. However, the effects of electromagnetic fields on cassava roots remain poorly understood (Howeler, 2012).

Electromagnetic fields are physical factors that influence living organisms, including plants (Aydın and Genç, 2018). They have been reported to stimulate growth, enhance nutrient uptake, and improve product quality (Shabrangy, 2024; Grzelka et al, 2023). The underlying mechanisms are not fully clear, but they are thought to involve alterations in ion dynamics and molecular movement within plant cells (Ureta-Leones et al, 2021). Internal root structure analysis of cassava generally reveals the presence of druse crystals and exarch xylem protoxylem. Storage roots exhibit food storage across various layers, characterized by parenchyma with abundant intercellular spaces. As roots mature, sclerenchyma accumulates starch grains. Electromagnetic field stimulation of roots results in enhanced storage parenchyma and starch grain formation. This observation aligns with research indicating that the development of fibrous roots into storage roots is contingent upon the development of fibrous roots and parenchyma, both of which are crucial components in cassava root development (Chaiyarak et al., 2022; Kaur et al., 2021; Adu, 2020).

When plant cuttings are subjected to electromagnetic fields, positively charged ions move in the direction opposite to the negative direction of the field. This induces the movement of charged ions and molecules, which can increase the frequency of molecular collisions and accelerate chemical reactions. For example, it might enhance amylase activity, breaking down stored starch into sugars. With more available nutrients, the plant can generate energy for root development and cell division. Thus, studying the impact of electromagnetic fields on the internal root structure and starch accumulation in cassava is crucial for developing technologies to enhance cassava yield and quality (Hafeez et al, 2023; Suárez-Rivero et al., 2023; Nyakane et al, 2019).

The selection of cassava varieties Kasetsart 50 and Rayong 72 for this study is based on several reasons: 1. Economic Importance: Cassava is a significant economic tuber crop in Thailand. Using widely cultivated and economically important varieties ensures the relevance and direct applicability of the research findings for farmers and related industries. 2. Varietal Differences: Kasetsart 50 and Rayong 72 exhibit variations in genetic traits, morphology, or starch quantity and quality. Comparing the effects of electromagnetic fields on both varieties provides a comprehensive view of how these effects vary across different genotypes and their specific characteristics. 3. Existing Baseline Data: Previous research or baseline data on biological features, structure, or chemical composition may exist for these varieties, facilitating comparisons, references, and further exploration. 4. Material Availability: Kasetsart 50 and Rayong 72 are readily available, widely cultivated, or supported by institutions with readily available planting materials for research purposes, making the research implementation more feasible (Yuanjt et al., 2023; Phoncharoen et al., 2020) In addition, these cultivars have been previously acknowledged for their superior yield potential and exhibit robust adaptability to the prevailing adapted to growing conditions in Thailand (Tappiban et al., 2020).

Results and discussion

For clarity and comprehensive understanding, the results and their implications are divided into two main parts: Internal Root Structure and Nutrient Accumulation and Effects of Electromagnetic Fields on Root Development and Starch Accumulation. The first part will detail the observed anatomical features of cassava roots and their nutrient storage mechanisms (Internal Root Structure and Nutrient Accumulation). The second part will then delve into how exposure to electromagnetic fields specifically influenced these structural and physiological aspects (Effects of Electromagnetic Fields on Root Development and Starch Accumulation).

Internal Root Structure and Nutrient Accumulation

In studies examining the internal structure of cassava roots from Kasetsart 50 and Rayong 72 varieties through transverse sections, the presence of druse crystals was observed within root cells. The primary root structure revealed distinct layers: epidermis, cortex, endodermis, pericycle, primary phloem, primary xylem, and parenchyma tissue. Protoxylem was found dispersed in points near the pericycle, while metaxylem developed towards the root center, resulting in an exarch xylem arrangement with protoxylem located externally and metaxylem internally. Transverse root sections exhibited an arc-like structure of the protoxylem (Fig 1-8 not provided). In storage roots, nutrient accumulation primarily occurs in the cortex due to its greater width compared to the stele. Secondary root growth showed nutrient storage in parenchyma tissue, secondary xylem, and secondary phloem. Abundant intercellular spaces were noted in the parenchyma tissue, particularly within the cortex. In mature roots, sclerenchyma largely stores nutrients such as starch granules. This anatomical transformation is crucial for storage root development, with Chaweewan and Taylor (2015) highlighting the significant role of secondary growth driven by the vascular cambium, leading to extensive formation of secondary xylem parenchyma. These enlarged parenchyma cells serve as the primary storage sites for carbohydrates (as starch) and other nutrients, causing root expansion into storage roots. Similarly, Ruscher et al. (2021), while focusing on nutrient transport and accumulation mechanisms (specifically symplastic phloem starch loading), also described storage root development from fibrous roots via secondary growth and the formation of starch-accumulating parenchyma cells.

Effects of Electromagnetic Fields on Root Development and Starch Accumulation

Cassava roots exposed to electromagnetic fields demonstrated increased formation of storage parenchyma tissue and starch granules (when stained with iodine) across all tested field strengths compared to unstimulated roots. This aligns with Siebers et al. (2017), who indicated that fibrous roots develop into storage roots, a process contingent on the development of fibrous roots and parenchyma tissue, both vital for cassava root growth. Fibrous roots predominantly develop within 30-60 days after planting, subsequently differentiating into storage roots (Carluccio et al., 2022; El-Sharkawy, 2004). Cassava root system development typically initiates between 45 and 90 days after planting, contingent upon various factors. Among these, the spatial orientation of the meristem is particularly influential, as it plays a pivotal role in the process of root differentiation, leading to increased root diameter and length. When planting material is exposed to electromagnetic fields, positive ions move towards the negatively charged region of the field, inducing the movement of ions and charged molecules. This increases molecular collision frequency, accelerating internal chemical reactions such as enhanced amylase activity to hydrolyze stored food into usable sugars. The increased nutrient availability fuels root growth from meristematic tissues and promotes cell division (Maffei, 2014). Furthermore, studies such as that by Radhakrishnan and Kumari (2012) on legumes found that pulsed magnetic field pre-treatment of soybean seeds has the potential to enhance soybean production efficiency through mechanisms involving improved protein and mineral accumulation and altered enzyme activity, leading to better growth and yield.

Fig 1. Anatomical features of Cassava cultivar Kasetsart 50 roots stimulated with varying electromagnetic field intensities at 4x magnification.

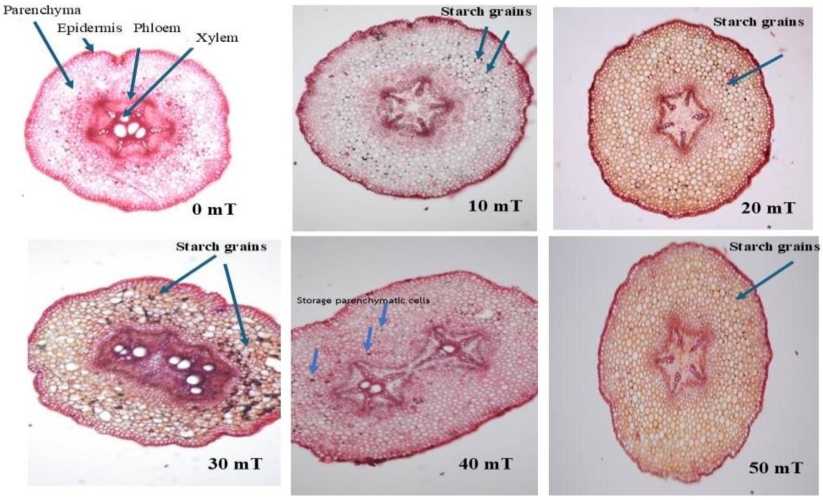

Fig 2. Anatomical features of Cassava cultivar Kasetsart 50 roots stimulated with varying electromagnetic field intensities at 10x magnification. Arrows indicate specific structures such as epidermis, parenchyma, phloem, xylem, storage parenchymatic cells, and starch grains within the root cross-sections.

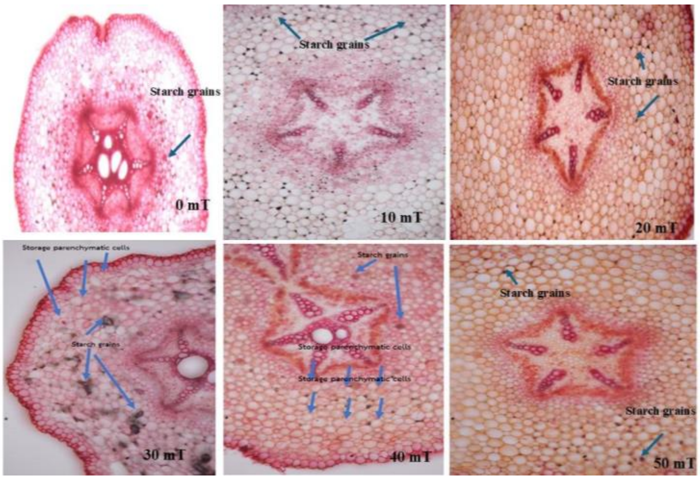

Fig 3. Anatomical features of Cassava cultivar Kasetsart 50 roots stimulated with varying electromagnetic field intensities at 20x magnification. Arrows point to starch grains and storage parenchymatic cells within the root tissues.

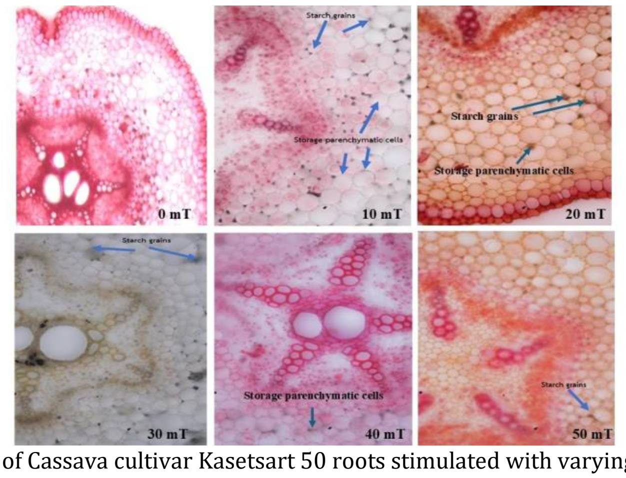

Fig 4. Anatomical features of Cassava cultivar Kasetsart 50 roots stimulated with varying electromagnetic field intensities at 40x magnification. Arrows highlight the presence of starch grains and storage parenchymatic cells within the root tissues.

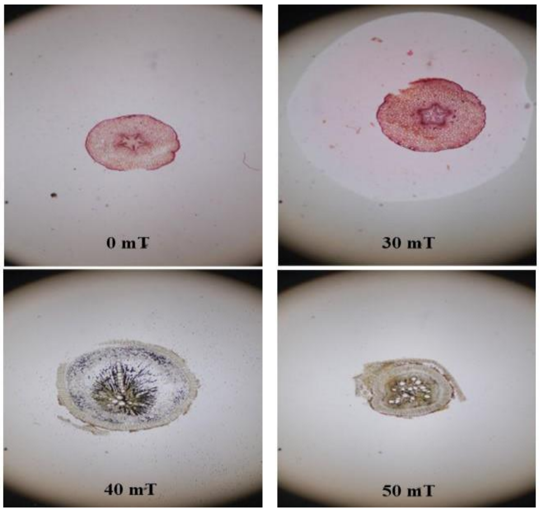

Fig 5. Anatomical features of Cassava cultivar Rayong 72 roots stimulated with varying electromagnetic field intensities at 4x magnification.

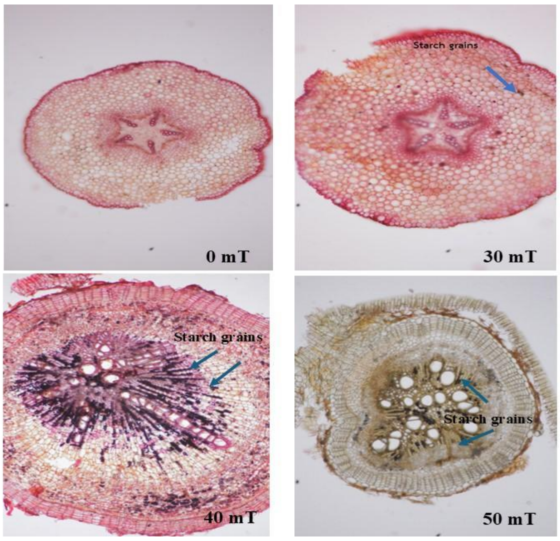

Fig 6. Anatomical features of Cassava cultivar Rayong 72 roots stimulated with varying electromagnetic field intensities at 10x magnification. Arrows point to the presence and distribution of starch grains within the root tissues.

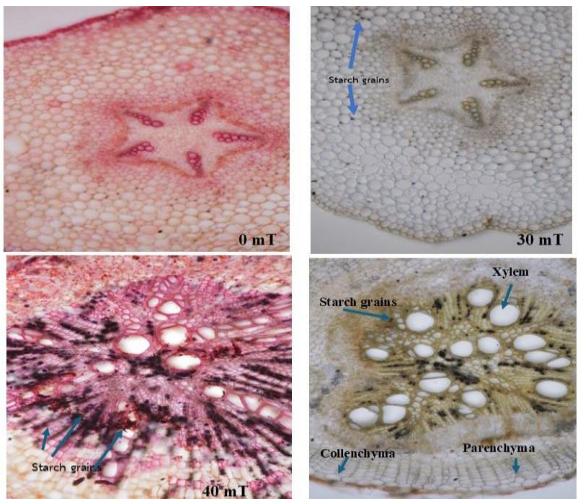

Fig 7. Anatomical features of Cassava cultivar Rayong 72 roots stimulated with varying electromagnetic field intensities at 20x magnification. Arrows highlight the presence of starch grains, xylem, collenchyma, and parenchyma within the root tissues.

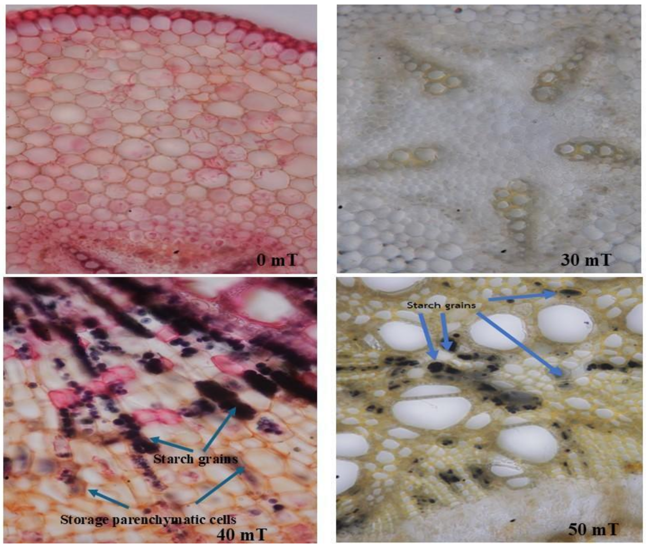

Fig 8. Anatomical features of Cassava cultivar Rayong 72 roots stimulated with varying electromagnetic field intensities at 40x magnification. Arrows point to starch grains and storage parenchymatic cells within the root tissues.

Materials and Methods

Materials and Equipment:

1) Cassava roots, cultivars Kasetsart 50 and Rayong 72.
2) Microscope
3) Safranin stain
4) Iodine solution
5) Razor blade
6) Petri dishes and water
7) Sharp-pointed needle
8) Microscope slides and coverslips
9) Tissue paper

Methods

The anatomical features of cassava roots were investigated as follows:

1) Cassava samples from Study 2 (cultivars Kasetsart 50 and Rayong 72) were used to examine the internal root structure using the free hand section technique. Cross-sections of cassava roots were made with a razor blade. A small amount of water was added to a Petri dish. The razor blade was moistened with water to reduce friction during sectioning. Thin sections of cassava root were cut and then submerged in the water.
2) A fine brush was used to select floating plant tissue sections, which were then placed onto microscope slides. A small amount of water was added to prevent drying. Slides were observed under a light microscope at the lowest magnification to select sections with uniform thickness and no tears.
3) The selected sections were then stained with Safranin O for approximately 20-30 seconds. The stain was removed by adding water to the tissue sections and blotting with tissue paper, or by transferring the sections to a Petri dish with distilled water until no further color was released.
4) A small drop of water was added, and a coverslip was carefully placed to avoid air bubbles. The coverslip was positioned at a 45-degree angle, with one edge touching the water drop and the opposite edge resting on a sharp-pointed needle. The needle was slowly lowered while simultaneously moving its tip away from the coverslip until the coverslip was flat against the slide. Excess water and stain were blotted with tissue paper, and the underside of the slide was dried.
5) The prepared slides were observed under a light microscope, starting with the lowest magnification and progressing up to 40x. The arrangement of tissue layers was compared, and images were captured.
6) The Safranin O stain was then gently washed off the samples with running distilled water. Iodine solution was added to the tissue samples and they were again observed under a light microscope at magnifications from the lowest up to 40x. The arrangement of tissue layers was compared, and images were captured.

Conclusions

In this study, the effects of electromagnetic fields (EMFs) on the root structure and starch content of cassava varieties Kasetsart 50 and Rayong 72 were investigated. The study revealed that cassava root cells exhibit druse crystals, and the primary root structure comprises epidermis, cortex, endodermis, pericycle, primary phloem, primary xylem, and parenchyma, with exarch xylem development. In storage roots, food is stored in the cortex, parenchyma, secondary xylem, and secondary phloem, with parenchyma containing numerous intercellular spaces, and sclerenchyma accumulating starch grains as the root matures.

EMF stimulation was observed to induce the formation of storage parenchyma and starch grains in cassava roots. Furthermore, EMFs influence the movement of ions and molecules within plant cuttings, potentially accelerating chemical reactions such as amylase activity, thus promoting root growth and cell division. These findings suggest that EMF stimulation can modify root development and starch accumulation in cassava.

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