ROOT MORPHOLOGY SENSING

Abstract

Root system architecture (RSA) is a critical determinant of plant performance and productivity, playing a pivotal role in water and nutrient uptake. However, investigating root morphology presents considerable challenges due to its concealed nature and intricate complexity. There have been recent developments in root phenotyping and genetics leading to the emergence of precise terminology to describe diverse root phenotypes, thus unlocking new opportunities for enhancing agriculture and plant sciences. The importance of RSA traits in crop breeding, particularly in species with limited genetic diversity of major crops are irreplaceable. Effective incorporation of RSA traits necessitates a thorough exploration and characterization of genetic and phenotypic diversity. High-throughput phenotyping (HTP) methodologies, including imaging, computer vision, and machine learning (ML), enable non-destructive assessment of diverse root trait data on a large scale.Advancements in imaging protocols, computer vision algorithms, and ML techniques have significantly improved the resolution and accuracy of root trait data. Advanced software tools, such as ARIA, DART, GiA Roots, RootNav, and SmartRoot, facilitate efficient feature extraction while ML models minimize measurement variability and biases.However, challenges persist in bridging the technological gap for root trait phenotyping, including the standardization of protocols and reduction of costs. The urgent need for low-cost, scalable, and robust methodologies is highlighted to enable precise root morphology sensing across diverse phenotypes and experimental conditions. The significance of accurate root morphology sensing for understanding RSA and its potential applications in agriculture

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and crop improvement is ushering in a new era of sustainable and optimized plant productivity.

Keywords-Root; morphology; root architecture; phenotyping; imaging; machine learning

I. INTRODUCTION

The investigation of root system morphology, anatomy, and spatial distribution, commonly known as root architecture, poses considerable challenges due to the 'concealed nature' and intricate complexity of these below-ground organs. However, the growing interest in root phenotyping and genetics has facilitated the development of novel terminology to precisely describe various root phenotypes, structures, and functions. This emerging knowledge opens up new avenues for studying and understanding root systems, paving the way for advancements in agriculture and plant sciences.

Root system architecture (RSA) plays a vital role in enhancing plant performance and seed yield. It comprises various traits like root growth habit, length, thickness, number, angle, and surface area. However, RSA traits are rarely utilized in breeding programs due to measurement difficulties and root plasticity influenced by environmental variation. Further genetics research is needed to identify genes controlling RSA traits. Crop species like maize, soybean, common bean, rice, and wheat exhibit diverse RSA. Incorporating RSA traits in breeding could be rewarding, particularly for soybeans with limited genetic diversity. To implement RSA traits effectively, researchers must explore and characterize genetic and phenotypic diversity. Fortunately, high-throughput phenotyping (HTP) methods, including computer vision, automation[1], remote sensing[2], machine learning (ML; [3]), and deep learning (DL; [4]), enable non-destructive assessment of diverse phenotypic data on a large scale. Although HTP efforts have primarily focused on above-ground traits, continued research is required to successfully implement root trait-driven breeding, one must understand the genetics of root traits. Scientists will be able to tailor phenotypes for certain climatic conditions and agronomic techniques by integrating phenomic data on both root and shoot attributes. The root structure of plants is linked to various environmental advantages, such as nutrient acquisition, drought tolerance, flood resistance, and lodging resilience. However, root phenotyping and research face challenges due to the technological gap in collecting, observing, and quantifying essential root trait data. Factors contributing to this challenge include the genetic complexity of traits, the intricate phenotypic expression, the morphometric nature of expression, and interactions with the environment, including soil structure, nutrient availability, temperature, water, other plants, and microbes.

Plant structure displays significant variation under diverse growing conditions, even among plants sharing the same genotype[5]. Environmental factors like temperature and light intensity strongly influence plant architecture. Consequently, conducting plant phenomic studies poses more challenges compared to animal phenomic studies. While breeding has improved shoot traits to enhance crop yield, plant roots have been relatively neglected, despite their importance in water and nutrient uptake. The first green revolution centered on growing crops in soil and using high rates of chemical fertilizers. The potential for a second green revolution lies in developing crop tolerance to low soil fertility, which is a significant cause of low crop yield in developing countries. Enhancements in root architecture, encompassing the shape and spatial arrangement of root systems within the soil, could play a pivotal role in this revolution as plant roots extensively explore the soil and play a crucial role in nutrient acquisition. Technological challenges in RSA trait phenotyping can be divided into two major components: (1) root extraction from soil, and (2) imaging and computer aided feature (trait) extraction [6].

Researchers have adopted three main approaches for root sampling: controlled laboratory methods, moderately controlled greenhouse methods, and minimally controlled field methods. While field methods offer greater relevance to real-world production and physiological significance, controlled laboratory methods allow large-scale phenotyping and higher throughput. Bridging the gap between lab and field methods remains a key goal for researchers. The high labour and time costs associated with field root trait phenotyping have prompted the exploration of automation to increase throughput and enable the study of larger sample sizes. Such advancements offer exciting prospects for understanding the role of root system architecture (RSA) and its application in future research endeavours. Existing techniques that characterize the morphology and function of roots are extremely significant in understanding the below-ground mechanisms of crops that ranges from soil penetration to nutrient uptake. These techniques have crossed the limits of conventional destructive sampling and characterizing the roots (root excavation and analysis) and promises an advanced technological intervention to the root phenotyping.

The need for advances in imaging protocols, computer vision, and machine learning (ML) is evident for efficient trait extraction in root phenotyping [6]. Field extractions, soil coring, and minirhizotrons are common methods for root investigation but more recent methods including X-ray computed tomography, MRI, PET, and 3D imaging have produced data on root traits with higher precision. Despite these developments, their inclusion in extensive genetic material screening is frequently constrained by their high cost and low throughput. Therefore, standardized protocols and reduced cost and time are crucial for scaling plant phenotyping methodologies. Several software tools are available for image-based root phenotyping, making use of advanced computer analysis of high-resolution digital images. These tools, including ARIA, DART, GiA Roots, RootNav, and SmartRoot, offer efficient and accurate feature extraction, potentially without causing damage to the samples. The integration of computer vision with ML has further improved image processing and feature extraction, reducing measurement variability and removing subjectivity and biases. ML models have been trained to recognize and differentiate root tips from 2D images, and random forest-based approaches have been used to handle missing trait values in noisy root images [7]. However despite the progress in software, data processing, and phenotyping protocols, there is a need for a low-cost, scalable, and robust methodology that can standardize RSA trait acquisition across diverse phenotypes and experimental conditions. Such advancements will be crucial for furthering research in root system architecture and its potential impact on plant performance and crop improvement.

The advanced technologies covering both the destructive and non-destructive sampling include root imagine techniques, root scanning and digitization, rhizotrons, mini-rhizotrons, root electrical capacitance and conductivity, isotopic techniques etc. Many physiological and genetic strategies in this regard include fluorescent dyes and markers, fluorescent proteins to easy visualize and track the root growth and interactions as well as obtaining an insight into specific root characters and developmental processes.

II. ROOT MODELLING

Root modelling is a valuable tool in plant science that aims to simulate and understand the complex structure and function of root systems[8]. The root ideotype, which represents the ideal root system architecture tailored to specific environmental conditions and

plant breeding objectives, plays a pivotal role in guiding root modelling studies. Root ideotype refers to the set of root architectural traits that are deemed optimal for a particular research context or environment. Developing an ideal root system is essential for enhancing plant performance, as it influences nutrient and water uptake, stress tolerance, and overall plant productivity. However, the ideal root system can vary depending on factors such as soil type, nutrient availability, water availability, and other environmental conditions. Therefore, root ideotype design needs to be context-specific and carefully tailored to the desired objectives of the study. Root modelling techniques encompass a range of computational approaches, including functional-structural root models and image-based root phenotyping, which contribute to understanding the intricate interactions between root architecture, environmental conditions, and plant growth dynamics. Functional-structural root models simulate the three-dimensional distribution of root structures and their interaction with the surrounding soil environment. These models integrate developmental processes, such as root elongation, branching, and root-soil interactions, to predict the root system's behavior in different environmental scenarios**[8]**.

Innovative soil-free techniques, such as hydroponics, aeroponics, gel plates, and growth pouches, present a unique advantage in root system architecture analysis by providing enhanced contrast between roots and the growth substrate. This contrast allows for more accurate and efficient extraction of root characteristics. However, it is important to consider that root systems of plants grown in artificial media may exhibit variations compared to those grown in traditional soil environments. Despite this, pouch systems have shown promising results, especially when plants are grown vertically on germination paper, making them suitable for seedling screens across various plant species, including beans, maize, wheat, oilseed rape, and pearl millet. While these techniques have their limitations, their widespread use is due to their compatibility with high-throughput root phenotyping platforms, making them valuable tools for advancing root research and understanding plant growth dynamics.Image-based root phenotyping methods provide valuable data for constructing root models by taking detailed root architectural traits from 2D or 3D root images**[9]**. These techniques enable the extraction of quantitative data on root length, diameter, branching pattern, and other relevant traits that contribute to root ideotype definition. By combining root modelling with image-based phenotyping, researchers can evaluate and optimize root ideotypes for specific conditions, such as drought, nutrient deficiency, or soil types. This interdisciplinary approach helps bridge the gap between theoretical root system design and practical application in crop improvement and sustainable agriculture. Overall, root modelling provides a powerful framework for understanding root system behavior and guiding the development of root ideotypes tailored to specific environmental conditions and breeding objectives. This integrated approach holds great promise for advancing our knowledge of root systems and improving crop performance and resilience in diverse and challenging growing environments.

III. ROOT IMAGING TECHNIQUES

Mark up Language format, shared by various root phenotyping programs (**Lobet*et al.*, 2011**) **[10]**, Advanced imaging methods like X-ray computed tomography (CT), magnetic resonance imaging (MRI), various forms of microscopy allow researchers to visualize and analyze the internal structure of roots non-destructively.The complete root system architecture (RSA) can be automatically or semi-automatically captured using root

phenotyping image analysis software like Smart Root and Root Nav **[11]**. The Root System enables the generation of RSA data in a standard XML format, encompassing root topology, geometry, and properties obtained from 2D or 3D images at one or more time points. However, capturing the entire RSA of real soils is labor-intensive and low in throughput. Despite this, certain root traits can be evaluated relatively quickly through manual measurements or using image analysis tools such as the Digital Imaging of Root Traits platform **[12]**. These methods offer viable options for analyzing specific root traits efficiently.

1. **X-ray Computed Tomography (CT):** X-ray computed tomography (CT) allows researchers to gain unprecedented insights into the complex and hidden world of plant root systems. The principle of X-ray CT involves the projection of X-rays through the plant material, and the detection of the transmitted X-rays on the opposite side**[13]**. By rotating the sample and collecting a series of X-ray projections from multiple angles, a comprehensive 3D image of the root system is reconstructed using sophisticated algorithms. The resulting high-resolution images enable detailed visualization and quantification of root traits, such as root length, diameter, branching patterns, and spatial distribution within the soil.One of the key advantages of X-ray CT for root imaging is its non-destructive nature. Unlike traditional methods that often involve excavation and destructive sampling, X-ray CT allows researchers to study intact root systems without disturbing their natural environment. This non-invasive characteristic is particularly beneficial for longitudinal studies, where researchers can track root growth and development over time, capturing dynamic responses to changing environmental conditions.X-ray CT also provides valuable information about root-soil interactions. By imaging the roots within their soil environment, researchers can observe root penetration, root-soil contact, and explore how roots respond to different soil structures and nutrient distributions. This knowledge is essential for understanding nutrient uptake efficiency and water acquisition strategies, which are critical factors influencing plant growth and performance.X-ray CT has facilitated the development of sophisticated root phenotyping platforms and software tools**[13]**. Automated image analysis algorithms enable the extraction of quantitative root traits from large datasets, significantly improving the efficiency and accuracy of root phenotyping studies. These platforms, such as RhizoScan and RootNav, allow researchers to process a vast amount of root image data, contributing to a more comprehensive and systematic understanding of root phenotypes**[14; 11]**.The integration of X-ray CT with other imaging techniques, such as MRI and fluorescence microscopy, has further expanded the capabilities of root phenotyping. Combined approaches offer multi-modal imaging, providing complementary information on root structure, function, and physiology, enhancing the richness of root phenotype data and enabling more comprehensive analyses. It has been a game-changing tool in root imaging and phenotyping, revolutionizing the study of root systems in plant science. Its non-invasive nature, high-resolution imaging, and compatibility with automated image analysis have greatly advanced our understanding of root architecture and function. By elucidating the complex interactions between roots and their environment, X-ray CT contributes to the development of more resilient and resource-efficient crops, enhancing agricultural sustainability and productivity in the face of global challenges.

2. **Some Prominent Applications of X-ray CT in Agricultural and Horticultural Fields:**

- **Agricultural Crops**

➢ **Maize (Corn):** X-ray CT has been extensively used for root phenotyping in maize. Researchers have employed this technique to study root architecture traits, such as root length, diameter, and branching patterns, to understand how different root systems influence nutrient and water uptake efficiency. This knowledge aids in breeding maize varieties with improved root traits for better resource utilization and higher yields.

➢ **Wheat:** X-ray CT has been applied in wheat research to investigate the interaction between wheat roots and soil. By imaging wheat roots in different soil conditions, researchers gain insights into root penetration and growth patterns, which influence nutrient availability and water uptake. This information contributes to the development of wheat varieties with enhanced drought tolerance and nutrient use efficiency.

➢ **Rice:** In rice, X-ray CT has been utilized to study water uptake dynamics in flooded paddy fields. Researchers use X-ray CT to monitor the movement of water within rice root systems, which is critical for understanding how rice plants adapt to anaerobic conditions and water stress. This knowledge helps in breeding rice varieties with improved waterlogging tolerance.

- **Horticultural Crops**

➢ **Tomato:** X-ray CT has been employed in tomato root phenotyping to study root architecture and its response to different soil environments. By non-invasively imaging tomato root systems, researchers can quantify root traits that influence nutrient and water uptake efficiency, contributing to the development of more efficient irrigation strategies for tomato crops.

➢ **Citrus:** In citrus crops, X-ray CT has been used to study root growth and development in response to various rootstocks and soil conditions. By visualizing citrus root systems, researchers can assess the impact of different rootstocks on nutrient uptake and root health, leading to improved rootstock selection for enhanced citrus production.

➢ **Grapevine:** X-ray CT has been applied in grapevine research to study the root distribution in different soil layers and its influence on water and nutrient uptake. By understanding the root system architecture in grapevines, researchers can optimize irrigation and fertilization practices to improve grape quality and yield.

3. **Positron Emission Tomography:** Positron Emission Tomography (PET) is a powerful and non-invasive imaging technique that has emerged as a valuable tool in root phenotyping [15]. Originally developed for medical applications, PET has been adapted for use in plant science to investigate root structure, dynamics, and function [16]. The fundamental principle behind PET lies in the detection of positron-emitting radionuclides, commonly labeled with isotopes like carbon-11 or fluorine-18, which are introduced into the plant's root system. As the isotopes decay, they emit positrons, which subsequently interact with surrounding electrons, leading to the generation of gamma rays. The PET scanner then detects these gamma rays to create 3D images of the radionuclide distribution within the roots [17].

PET offers unique advantages in root phenotyping. Unlike other imaging techniques, PET allows researchers to study root metabolic processes in real-time [18]. By introducing radioisotopes tagged to specific nutrients, such as carbon or nitrogen, scientists can track the uptake and transport of these elements in living roots. This capability provides insights into nutrient allocation, utilization, and transport mechanisms within the root system, shedding light on plant nutrient acquisition strategies. Additionally, PET enables the study of root-microbe interactions by labeling microbial activities with appropriate radionuclides. Researchers can explore how rhizosphere microorganisms interact with roots and contribute to nutrient cycling, symbiosis, and disease resistance. Understanding these intricate root-microbe interactions is crucial for developing sustainable agricultural practices that harness beneficial microorganisms to enhance plant health and productivity. Integrating PET with the other imaging methods provides a comprehensive understanding of root architecture, nutrient uptake, and water dynamics. By complementing one another, these imaging modalities offer a holistic view of root system functionality.

4. **Magnetic Resonance Imaging (MRI):** Plant root systems possess intricate three-dimensional (3D) structures with numerous features that are challenging to quantify accurately in two dimensions (2D) as stated by [9]. Specific characteristics, such as the arrangement of seminal roots at the root crown of cereals, often distributed asymmetrically, and the angle and number of roots and root whorls in maize crowns, are better studied and understood in a 3D context. Additionally, dynamic growth responses, like gravitropism and circumnutating, can be more effectively analysed when considering the full 3D architecture. To create 3D representations of root systems, researchers utilize techniques such as multiple-viewpoint imaging of plants grown in optically transparent media or hydroponic systems with support. Remarkably, such methodologies have successfully revealed the genetic basis for various 3D root architectural traits in rice, which were not apparent through conventional 2D phenotyping. Advancements in non-destructive 3D phenotyping have enabled root analysis in soil using medical imaging techniques like magnetic resonance imaging (MRI).

Magnetic Resonance Imaging (MRI) is an advanced and non-destructive imaging technique that has found increasing applications in characterizing plant roots. Unlike X-ray computed tomography (CT) or positron emission tomography (PET), MRI does not use ionizing radiation, making it a safe and highly versatile tool for studying living plant tissues, including roots. In MRI, plants are placed in a strong magnetic field, and radiofrequency pulses are applied, which leads to the excitation and relaxation of hydrogen nuclei in plant tissues. The emitted signals are then detected and processed to generate high-resolution images of the root systems. MRI allows for the visualization of root growth and architecture in real-time, providing valuable insights into root development and responses to various environmental stimuli. It is particularly useful in investigating root-soil interactions, as it can be performed directly in soil, allowing researchers to study roots in their natural and undisturbed environment. By analysing MRI data, researchers can extract quantitative information about root traits, such as root length, diameter, branching patterns, and spatial distribution. Moreover, MRI's capability to monitor water movement within roots enables the study of water uptake processes and hydraulic functioning, providing valuable information on root water transport.

Several studies have demonstrated the efficacy of MRI in characterizing plant root systems. For instance, studies used MRI to visualize the spatial distribution of water in roots and xylem vessels, gaining insights into water transport mechanisms in plants[19]. Many have employed MRI to study the growth dynamics of roots and root hairs, shedding light on their development and responses to different soil conditions[20]. MRI's ability to provide detailed 3D images of living root systems makes it a valuable tool for root phenotyping and can complement other imaging techniques in comprehensive root studies. As MRI technology continues to improve, it holds great promise for advancing our understanding of root biology and its crucial role in plant growth and adaptation. The application of MRI has been observed in the agricultural crops like maize, rice, wheat, soybean, potato etc. and horticultural vegetable crops like tomato, carrot and citrus.

IV. ROOT SCANNING AND DIGITIZATION THROUGH MICROSCOPY

High-resolution scanners are used to digitize root systems, converting them into three-dimensional models that can be analyzed for various root traits.Root scanning and digitization are essential techniques in modern plant science, enabling non-destructive and high-throughput analysis of root systems. These methods involve the use of advanced imaging technologies to capture detailed images of roots, followed by digital processing to extract quantitative root traits. Laser scanning confocal microscopy and light-sheet fluorescence microscopy are employed for subcellular root imaging, providing high-resolution images of root tissues and cellular structures [21]. Following root scanning, sophisticated image analysis software, such as SmartRoot[10] and RootNav[11], enables the extraction of root traits, including length, diameter, branching patterns, and volume, from the digital images. These digital phenotyping techniques facilitate large-scale root phenotyping studies and contribute to a better understanding of root biology and its implications for plant growth and development.

V. RHIZOTRONS AND MINI-RHIZOTRONS

Rhizotrons are transparent or semi-transparent containers placed in the soil to observe root growth in real-time without disturbing the roots. They allow researchers to study root development over a continuous period of time.Mini-rhizotrons, on the other hand, are narrow tubes inserted into the soil that offers a view of root growth at various depths. They enable researchers to monitor root dynamics and interactions with the surrounding soil.

Rhizotrons and mini-rhizotrons are innovative tools that have revolutionized root research in agriculture [22]. These specialized devices allow researchers to observe and study the intricate world of plant roots in real-time without disturbing the soil. The term "rhizotron" encompasses a wide range of experimental setups designed to facilitate root observation, while "mini-rhizotron" specifically refers to a downscaled version of the rhizotron technology. Both these systems provide unique opportunities to gain insights into root growth, architecture, and interactions with the surrounding environment. A rhizotron typically consists of transparent walls, allowing direct visualization of root systems as they develop in the soil. Researchers can install cameras or imaging devices outside these walls to capture images of roots over time, creating a time-lapse view of root growth and interactions. Rhizotrons are particularly useful for studying large and deep-rooted plants such as trees, where traditional root excavation would be impractical or destructive. With rhizotrons,

researchers can monitor the behavior of roots in response to various factors like soil conditions, nutrient availability, and water stress. Mini-rhizotrons, on the other hand, are portable and compact versions of rhizotrons that can be installed in both laboratory and field settings. Mini-rhizotrons offer similar benefits to their larger counterparts but on a smaller scale. They are especially useful for studying smaller plants and crops, enabling high-throughput root phenotyping. Mini-rhizotrons are equipped with high-resolution cameras and imaging systems, allowing for frequent and non-destructive monitoring of root growth dynamics [23]. Researchers can easily move mini-rhizotrons from one location to another, making them ideal for conducting comparative studies across different growth conditions and genotypes.

The information gathered from rhizotrons and mini-rhizotrons has significantly advanced our understanding of root biology, including root architecture, spatial distribution, and interactions with soil microbes. The data obtained from these systems provide critical inputs for crop breeding, precision agriculture, and sustainable land management. By gaining insights into root responses to environmental challenges, researchers and agronomists can develop crop varieties with improved root traits, enhancing nutrient and water use efficiency, and ultimately, ensuring global food security in the face of changing climates and growing populations.

In conclusion, rhizotrons and mini-rhizotrons have become indispensable tools in modern agricultural research, offering a window into the hidden world of plant roots and unlocking new possibilities for crop improvement and sustainable farming practices. As technology continues to evolve, these imaging systems will continue to play a vital role in advancing root research and shaping the future of agriculture.

VI. ROOT ELECTRICAL CAPACITANCE AND CONDUCTIVITY

Electrical measurements are used to study root traits like capacitance and conductivity, providing insights into root water content and nutrient uptake.Plant roots are not only vital for nutrient and water uptake but also play a crucial role in signalling and responding to environmental stimuli. Root electrical capacitance and conductivity have emerged as valuable tools for probing the health and function of plant roots[24]. Electrical capacitance refers to the ability of a root to store electrical charge, while electrical conductivity is a measure of the root's ability to conduct electrical current. These electrical properties are closely related to root physiological processes, including cell membrane integrity, water uptake, nutrient absorption, and root-microbe interactions. The electrical capacitance of roots reflects their overall size, surface area, and cellular structure. When roots encounter changes in soil conditions, such as the availability of water or nutrients, the capacitance can fluctuate, providing real-time insights into root responses. Capacitance measurements can help identify root zones that are actively absorbing water and nutrients, offering clues to the plant's nutrient uptake efficiency and water stress tolerance. On the other hand, electrical conductivity is influenced by the presence of ions and electrolytes in the root tissues and surrounding soil. Changes in conductivity can indicate alterations in ion uptake and transport, as well as potential stress conditions[25]. Monitoring electrical conductivity over time allows researchers to investigate the dynamics of ion movements within the root system and assess the impact of various environmental factors on root health.

Various techniques are used to measure root electrical capacitance and conductivity. One approach involves the use of electrodes that are inserted into the soil or nutrient solution to record electrical signals generated by the roots. Advanced sensors and data acquisition systems enable high-resolution measurements and real-time monitoring of root electrical properties[26]. Additionally, non-invasive methods, such as electrical impedance spectroscopy, can provide valuable information without damaging the root system.This approach has been studied in a wide range of crops, including rice, maize, wheat, and Arabidopsis and some vegetables.

VII. ISOTOPE TECHNIQUES

Isotopic labeling is used to trace the movement of nutrients in the root system helping to understand nutrient uptake pathways and root functionality. Isotopes have become indispensable tools in root morphology studies, offering unique insights into root architecture, nutrient acquisition, and rhizosphere interactions. Isotopes are atoms of the same element with different numbers of neutrons, and their incorporation into root studies enables researchers to trace the fate of specific elements and compounds within the root system[27].

Stable isotopes, such as ^{15}N, ^{13}C, ^{18}O, are commonly used to investigate nutrient uptake and allocation in plant roots[28]. By labelling the soil or nutrient solution with stable isotopes, researchers can track the movement of nutrients from the soil into the roots and subsequent allocation to different plant tissues. This approach helps to identify the efficiency of nutrient uptake pathways and understand how root architecture influences nutrient acquisition.Radioisotopes, such as ^{32}P, ^{33}P, and ^{45}Ca, offer a powerful means to study nutrient transport and distribution in real-time. These isotopes emit radiation that can be detected using specialized instruments, allowing researchers to quantitatively measure nutrient flow in roots[29]. Radioisotopes are particularly useful for studying nutrient transport dynamics and identifying transporters responsible for nutrient uptake and distribution in roots.

In addition to nutrient studies, isotopes are invaluable for exploring root-microbe interactions. By labelling root exudates or microbial cells with isotopes, researchers can investigate the exchange of nutrients and signals between roots and rhizosphere microorganisms. Understanding these interactions is essential for harnessing beneficial microorganisms to enhance nutrient availability and improve plant health. Isotopic techniques have also been employed to study root water uptake patterns and root responses to water stress. Isotopes, such as ^{2}H and ^{18}O, can be used to label soil water, allowing researchers to trace the movement of water into roots under different soil moisture conditions. This approach sheds light on the mechanisms of water uptake and helps in designing water-efficient root systems for sustainable agriculture.

VIII. FLUORESCENT DYES AND MARKERS

These are used to label specific roots or regions of roots enabling easy visualization of growth and interactions of roots.Fluorescent dyes are molecules that emit light of a specific color (wavelength) when exposed to light of a different wavelength. In root morphology studies, fluorescent dyes are often applied to the roots through the growth medium or root-soil interface. Once taken up by the root, these dyes travel through the vascular system, illuminating the entire root structure. Dyes such as propidium iodide and calcofluor white are

commonly used to stain root cell walls and provide detailed information on cell organization and growth patterns[30].

Fluorescent markers, on the other hand, are genetically encoded proteins that emit fluorescence when expressed in plant tissues. With the advancement of genetic engineering techniques, researchers can introduce fluorescent marker genes into plants to specifically label root tissues, such as root tips, lateral roots, or root hairs. This targeted labeling allows for the visualization of specific root structures and processes in real-time. Confocal microscopy, a widely used imaging technique, complements the use of fluorescent dyes and markers by providing high-resolution 3D images of the root system[31]. Confocal microscopy uses a pinhole to block out-of-focus light, enabling precise imaging of fluorescent signals from different depths of the root. Their applications range from investigating root growth patterns and branching to studying root-microbe interactions, nutrient uptake, and water transport.

The advancements in root morphology sensing driven by innovative imaging techniques, computer vision algorithms, and machine learning methodologies have revolutionized our understanding of root system architecture (RSA). The concealed and intricate nature of roots, once a significant challenge in plant research, is now being unveiled with unprecedented precision and non-destructive assessment. These breakthroughs have opened up exciting avenues for studying RSA traits, previously underutilized in breeding programs due to measurement difficulties and environmental influences on root plasticity. It holds immense promise for agricultural and plant sciences, enabling researchers to explore and characterize the genetic and phenotypic diversity of root traits across diverse plant species. Incorporating RSA traits in breeding programs could have transformative effects on crop performance and yield especially in addressing the challenges of low soil fertility in developing countries. The ability to optimize phenotypes for specific environmental conditions and agronomic practices will play a vital role in enhancing crop resilience and sustainability. In the pursuit of a second green revolution, where sustainable and efficient agriculture is paramount, these strategies emerge as a cornerstone technology with the potential to shape the future of crop improvement and global food security.

IX. CONCLUSION

In conclusion, the complex world of root system architecture (RSA) is a key area that supports the fundamentals of plant production and performance. The importance of RSA in water and nutrient uptake cannot be overstated even if it is concealed from view. Recent advances in genetics and root phenotyping have revealed a precise language, giving us the tools to explain the variety of root phenotypes in depth and with clarity. The remarkable significance of RSA traits in crop breeding, particularly in species with little genetic variation, continues to be an indispensable asset. The skillful integration of these features into the plant's genetic makeup, however, depends on a thorough investigation and characterization of the genetic and phenotypic components. Thanks to the constantly improving imaging protocols, computer vision algorithms, and ML approaches, the canvas itself has advanced significantly. The skill of feature extraction is refined by software tools like ARIA, DART, GiA Roots, RootNav, and SmartRoot, which efficiently breathe life into data. In the meantime, the conductor of ML models ascends to the podium and orders the ensemble to reduce any biases and variability that can skew our interpretation.

Standardization and cost-cutting are necessary to build the bridge between the world of technological competence and the world of root trait phenotyping. It is an urgent call because low-cost, scalable, and reliable procedures are the cornerstones that will allow us to explore the range of various phenotypes and experimental data.

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