

Article

Rice Root Reactions to Soil Amendments and Enhanced Soil Water Retention: A Scanner-Based Rhizotron Approach for Optimizing Semi-Dry Cultivation

Mohammad Wasif Amin ^{1,2,*} , Naveedullah Sediqui ^{3,4} , Shafiqullah Aryan ⁵ , Safiullah Habibi ⁶ , Khalid Joya ⁷ , Atsushi Sanada ³, Shinji Suzuki ¹, Irie Kenji ³ and Machito Mihara ¹

¹ Faculty of Regional Environment Science, Tokyo University of Agriculture, Tokyo 156-8502, Japan; s4suzuki@nodai.ac.jp (S.S.); m-mihara@nodai.ac.jp (M.M.)

² Department of Agronomy, Faculty of Agriculture, Parwan University, Charikar 1102, Afghanistan

³ Faculty of International Agriculture and Food Studies, Tokyo University of Agriculture, Sakuragaoka 1-1-1, Setagaya-ku, Tokyo 156-8502, Japan; naweedone@gmail.com (N.S.); a3sanada@nodai.ac.jp (A.S.); k3irie@nodai.ac.jp (I.K.)

⁴ Department of Horticulture, Faculty of Agriculture, Alberoni University, Mahmud Raqi 1254, Afghanistan

⁵ Faculty of Agriculture, Nangarhar University, Jalalabad 2601, Afghanistan; shafiqaryan@gmail.com

⁶ Faculty of Agriculture, Tokyo University of Agriculture and Technology (TUAT), Saiwai-cho 3-5-8, Fuchu-shi, Tokyo 183-8509, Japan; safiullahhabibi23@gmail.com

⁷ Agricultural and Food Marketing, Consumer Psychology, Kiel University (CAU), Wilhelm-Seelig-Platz 6/7, 24098 Kiel, Germany; khalid.joya@ae.uni-kiel.de

* Correspondence: 12624001@nodai.ac.jp; Tel.: +81-9037650029

Abstract

Drought reduces soil moisture and impairs root function, posing a significant threat to rice production in arid regions. The influence of soil amendments on early rice root development under semi-dry cultivation remains insufficiently characterized, especially when assessed using non-destructive rhizotron techniques. This study employed a scanner-based rhizotron system to evaluate early root responses of rice seedlings to six amendments under semi-dry irrigation: vermicompost and peat moss, spirulina powder, gypsum, rice husk biochar, zeolite, and an unamended control. The vermicompost plus peat moss (VC+PM) treatment demonstrated the highest water-holding capacity (26%), root projected area (9.60 cm² plant⁻¹), and root surface area (84.79 cm² plant⁻¹). VC+PM also promoted extensive lateral branching (233 secondary and 1709 tertiary roots) and the greatest total lateral root length (363.09 cm plant⁻¹), resulting in superior biomass (shoot: 140.00 mg plant⁻¹; root: 56.70 mg plant⁻¹) and the lowest root-to-shoot ratio (0.90). These improvements are attributed to the enhanced moisture retention of peat moss and the nutrient and phytohormone contributions of vermicompost. In contrast, rice husk biochar exhibited the lowest water-holding capacity (14%), while other amendments produced moderate or limited effects. The results establish a direct relationship between improved soil water retention and early-stage drought-avoidant root development. The combination of VC and PM emerges as a promising approach to enhance root plasticity and seedling establishment in water-saving rice systems. As this study was conducted under controlled rhizotron conditions and limited to the seedling stage (20 days after sowing), future research should prioritize multi-season field trials to assess yield translation and economic feasibility assessments to support farmer adoption.

Keywords: drought; root system architecture; scanner based rhizotron; vermicompost; rice seedling; soil amendment; water holding capacity



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1. Introduction

A major outcome of climate change is the intensification of drought stress. Soil drought stress is characterized by a considerable reduction in soil water content (SWC) or humidity, typically occurring alongside elevated atmospheric temperatures. These conditions emerge from an imbalance between soil evaporation and plant transpiration, which ultimately causes a decline in SWC [1]. Drought stress negatively influences soil attributes and, as a result, disrupts plant metabolic processes and growth [2]. Furthermore, it significantly modifies soil physical properties, thereby affecting root-soil interactions. As highlighted by Reinsch et al. [3], drought episodes transform the interrelationships among biological, chemical, and physical soil processes, leading to diverse effects on soil properties. Notably, when clay loam soils dry, they contract due to water depletion. This phenomenon leads to soil cracking, which subsequently alters soil mechanical and hydraulic characteristics [4]. This results in increased bulk density, lowered porosity, and limitations on root penetration [3].

Soil water retention (SWR) capacity is a critical factor in semi-dry rice cultivation, where water availability is both intermediate and variable. Soil amendments (SAs) are commonly applied to improve soil physical properties, including porosity, aeration, structure, and water retention [5]. These amendments reduce the permanent wilting point and enhance hydraulic conductivity, thereby improving rice root access to soil water. Recent research indicates that organic amendments enhance SWR through multiple mechanisms. For example, treatments with corn straw juice, fulvic acid, and humic acid increase soil saturated water content, field capacity, and plant-available water (PAW) capacity [6]. SAs are generally classified as either organic or inorganic materials that must be incorporated into the soil [7]. To mitigate drought stress, the use of natural amendments such as vermicompost (VC), biochar, spirulina powder, peat moss (PM), manure, and superabsorbent has been proposed [8–11].

SAs promote plant growth and establishment by creating a favorable root environment and enhancing nutrient availability through increased soil organic matter [5,12–14]. These amendments also improve SWC and facilitate more effective use of limited water resources. As a result, water stress exerts a reduced impact on the soil's biochemical and chemical properties, leading to more efficient water use by plants [15,16].

In addition, parallel research on inorganic amendments highlights the efficacy of materials such as gypsum, which enhances porosity and deep rooting. Zeolites are also valued for improving SWR, infiltration, and cation exchange capacity [17,18].

Although both organic and inorganic SAs have demonstrated efficacy in enhancing crop performance, the selection of amendments for this study was guided by a defined rationale. The six SAs were chosen to represent distinct mechanisms: gypsum and zeolite (inorganic, modify soil structure); biochar, VC, and PM (organic, improve water retention); and spirulina powder (novel, nitrogen-rich biomass). Application rates (Table 1) were determined through preliminary trials and a literature review to enhance soil properties while avoiding nutrient imbalance or hydrophobicity [19].

Despite recent advancements, substantial research gaps persist. Previous studies have primarily assessed the effects of these SAs on the growth and yield of shallow-rooting rice cultivars under water deficit conditions. The present study distinguishes itself by focusing on deeper-rooting rice genotypes cultivated using the semi-dry bed method. In this system, the potential interactions between SAs and root architecture have not yet been investigated. Additionally, the examination of root morphological traits in this context has been limited by the labor-intensive and destructive nature of conventional methods such as soil coring and excavation [20].

Recent research employs rhizotron systems and image analysis to enable non-destructive, repeated assessments of root morphology. Multiple image processing and analysis programs are available for root measurements, including WinRHIZO Tron, Root-Snap, ImageJ, and RootNav 2.0 [21]. Seethepalli et al. [22] recently introduced RhizoVision Analyzer, an open-source software tool designed for high-throughput root crown phenotyping. Adu et al. [23] assessed root morphological traits of *Brassica rapa* genotypes using a scanner-based rhizotron system (SBRs). Nagel et al. [24] investigated root geometry and growth in *Arabidopsis* and barley with an automatic rhizotron phenotyping system. Sanada and Agehara [25] developed an SBRs suitable for non-destructive root evaluation in soybean seedlings. These advanced root analysis methods have been widely adopted in recent studies.

SBRs represent a valuable non-destructive technology for observing and quantifying root system (RS) architecture, allowing continuous visualization of development without disturbing the growth environment [26]. Since the development of an SBRs for soybean seedlings by Agehara and Sanada [25], these methods have been widely adopted. Typically, SBRs employ high-resolution scanning in combination with image analysis software to measure key morphological traits, including root length, surface area, diameter, and tip number [27].

Several programs are available for root image analysis. ImageJ is an open-source image processing program utilized across various scientific disciplines. In contrast, WinRHIZO and WinRHIZO Tron are commercial programs specifically developed for root image analysis. WinRHIZO enables automatic and interactive root measurements for washed root images, while WinRHIZO Tron facilitates manual root measurements for rhizotron or in situ root images [21]. Building on rhizotron system and root image analysis protocols established in recent studies, the objectives of this study were to: (1) quantify the effects of six contrasting SAs on SWR under semi-dry conditions; (2) characterize the corresponding early root architectural responses using an SBRs; and (3) identify the amendment or amendments that most effectively promote root traits associated with drought avoidance in rice seedlings.

2. Materials and Methods

2.1. Soil Amendment Treatments and Rhizotron Experiment

A glasshouse experiment was conducted at Tokyo University of Agriculture, Japan, from June to July 2025. The research implemented a completely randomized design (CRD), utilizing three biological replicates per treatment ($n = 3$ independent rhizotron systems), yielding a total of 18 rhizotrons. Six SA treatments were tested: (i) unamended soil (S+WA), (ii) soil with vermicompost and peat moss (S+VC+PM), (iii) soil with spirulina powder (S+SP), (iv) soil with gypsum (S+GS), (v) soil with rice husk biochar (S+RHB), and (vi) soil with zeolite (S+ZL). The composition of these treatments was informed by Amin et al. [19]. Application rates (Table 1) were selected based on optimal values identified through initial trials and relevant literature [19]. The goal was to enhance soil structure and nutrient supply while avoiding nutrient imbalances or hydrophobic responses. SAs were chosen for their contrasting nutrient compositions and physical-chemical characteristics. This approach enabled a systematic evaluation of amendment impacts on both root development and soil quality under semi-dry conditions (Table 1). Characterization data for the amendments—including nitrogen, phosphorus, potassium concentrations; pH; soil organic matter; soil organic carbon; and bulk density—are provided in Table 2. The rice variety employed, DRO-1 IR64 (deep-rooting), was developed by Uga et al. [28] at the International Rice Research Institute (IRRI). Red Akadama, a soil with low fertility and limited organic matter, served as the growth substrate. Rhizotrons (22.8 cm length $\times$ 23.8 cm depth $\times$ 3.8 cm

width; 2036 cm³) were filled with 2.07 kg of appropriately amended soil, according to each treatment (Figure 1). This mass was selected in preliminary tests to achieve a bulk density of approximately 0.95 g cm⁻³, reflecting field conditions for clay loam soils. The chosen mass also provided sufficient rooting volume for the 20-day growth period and ensured even moisture distribution and consistent packing across all rhizotrons. SAs were thoroughly mixed seven days before sowing to achieve uniformity. For each rhizotron, amendment quantities were calculated relative to 2.07 kg of air-dried soil. Details for each treatment are listed in Table 1. Rice seeds underwent disinfection by soaking in 0.1% Benlate solution for 24 h, followed by rinsing in running water for two days to encourage pre-germination. Three seeds were sown 2 cm deep along the lower scanning window of each rhizotron. After emergence, seedlings were thinned to one uniform plant per unit. Plants were cultivated in rhizotrons for 20 days after sowing (DAS). Each rhizotron was set at a 30-degree incline to maximize root contact with the lower scanning window (Figure 1). Daily irrigation maintained soil moisture at near-optimal levels. Water was added to replenish 70% of the soil's field capacity, keeping soil water content between 60% and 80% of field capacity. This regimen simulated semi-dry conditions while avoiding severe water stress.



Figure 1. The rhizotron system was designed to facilitate the observation of rice seedling root growth under semi-dry bed conditions. In this setup, seedlings were grown adjacent to a transparent panel, allowing direct visualization of their RSs. To prevent light exposure and minimize the risk of photoinhibition, the transparent surfaces were shielded with a protective foam layer throughout the experiment. This arrangement enabled precise, non-invasive imaging and analysis of root architecture.

Table 1. Soil amendments, with their abbreviation forms, were used in this experiment.

Soil Amendments	Treatment Combinations with Abbreviation Forms
Soil without amendment	S+WA
Vermicompost + peatmoss (VC = 1.55% + PM = 7.80%)	S+VC+PM
Spirulina powder (SPP = 0.5%)	S+SPP
Gypsum (GS = 1.2%)	S+GS
Rice husk biochar (RHB = 1.7%)	S+RHB
Zeolite (ZL = 2%)	S+ZL

Source: Adapted from Amin et al. [19].

Table 2. Initial physical and chemical properties of the soil amendment treatments used in the experiment.

Soil Amendment Treatments	N (mg kg ⁻¹)	P (mg kg ⁻¹)	K (mg kg ⁻¹)	pH	SOM%	SOC%	BD (g cm ⁻³)
S+SW	15.21 ± 0.18 ^c	10.01 ± 0.18 ^c	126.62 ± 4.03 ^c	6.83 ± 0.12 ^{ab}	5.08 ± 0.08 ^d	2.95 ± 0.53 ^c	1.17 ± 0.00 ^a
S+VC+PM	30.76 ± 4.80 ^a	16.41 ± 0.18 ^a	152.12 ± 1.18 ^b	6.58 ± 0.27 ^b	8.65 ± 0.18 ^a	5.02 ± 0.1 ^a	0.84 ± 0.05 ^c
S+SPP	17.26 ± 0.22 ^b	12.07 ± 0.21 ^b	127.12 ± 1.64 ^c	6.80 ± 0.27 ^{ab}	6.33 ± 0.14 ^b	3.67 ± 0.08 ^b	1.11 ± 0.05 ^a
S+GS	15.08 ± 0.43 ^c	10.70 ± 0.18 ^c	126.37 ± 1.50 ^c	7.22 ± 0.21 ^a	5.22 ± 0.09 ^d	3.02 ± 0.05 ^c	1.05 ± 0.04 ^{ab}
S+RHB	18.51 ± 0.18 ^b	13.41 ± 0.18 ^b	168.62 ± 6.36 ^a	6.91 ± 0.09 ^{ab}	5.91 ± 0.06 ^c	3.42 ± 0.03 ^b	0.92 ± 0.03 ^{bc}
S+ZL	15.66 ± 1.18 ^{bc}	12.71 ± 0.18 ^b	148.62 ± 3.06 ^b	6.95 ± 0.09 ^{ab}	6.24 ± 0.05 ^b	3.62 ± 0.31 ^b	0.93 ± 0.03 ^{bc}
<i>p</i> -value	***	***	***	ns	***	***	***

Results are expressed as mean ± standard deviation (SD) from five replicates (n = 5), with each pot treated as an independent replicate. Means sharing the same letter are not significantly different, as determined by analysis of variance (ANOVA) followed by Tukey's test at the 5% significance level. *** *p* < 0.001; ns: not significant. SOM: Soil organic matter; SOC: Soil organic carbon; BD: Bulk density. Source: Adapted from Amin et al. [19].

2.2. Soil Water Retention Analysis

Akadama soils were amended and placed in rhizotrons, after which samples for SWR analysis were collected two days before seeding. Control samples without amendments were also incorporated into the analysis to provide a baseline for comparison. For SWR assessment, five analytical replicate soil cores (n = 5) were extracted from each of three biological replicate rhizotrons per treatment. Water retention characteristics were evaluated using both the suction and centrifuge methods, following the protocols outlined by Suzuki et al. [29]. In the suction method, soil samples were packed into glass cylindrical vessels with an internal diameter of 30 mm and a height of 30 mm [29]. For the centrifuge method, stainless-steel vessels with an internal diameter of 27 mm and a height of 30 mm were utilized. Bulk density for all soil samples was determined using the reference values reported by Amin et al. [19] (see Table 2). Amended and control soil samples were packed into each vessel using an identical procedure, ensuring minimal compaction across treatments. Following capillary saturation with tap water, the matric potential was adjusted to −63 cm (field capacity) via the suction method and to −1000 cm (optimal moisture content for growth) via the centrifuge method. Volumetric water content at each matric potential was determined by oven-drying samples at 110 °C for 24 h. Water holding capacity (WHC) was subsequently calculated as the difference in volumetric SWC between the two matric potentials.

2.3. Rhizotron Root Image Analysis

Development of RSs within the rhizotrons was assessed by capturing images at 5, 10, 15, and 20 days after sowing (DAS) using a flat-bed scanner (Perfection V800; Epson, Nagano, Japan) (Figure 2). All image files were saved as JPEGs with a resolution of 300 dpi. Only the lower window of each rhizotron was imaged since root growth was absent in the upper window throughout the study. Root image analysis was performed using ImageJ software (ImageJ 1.54g, National Institutes of Health, Bethesda, MD, USA). To improve visual clarity, the images were converted from 24-bit color to 8-bit grayscale. A uniform, manually adjusted threshold was then utilized for all images to accurately separate root pixels from background pixels, supporting precise root diameter estimation. After thresholding, the images were transformed into binary form, with roots and background represented as black and white pixels, respectively. The total black pixel area was quantified as the root projected area.

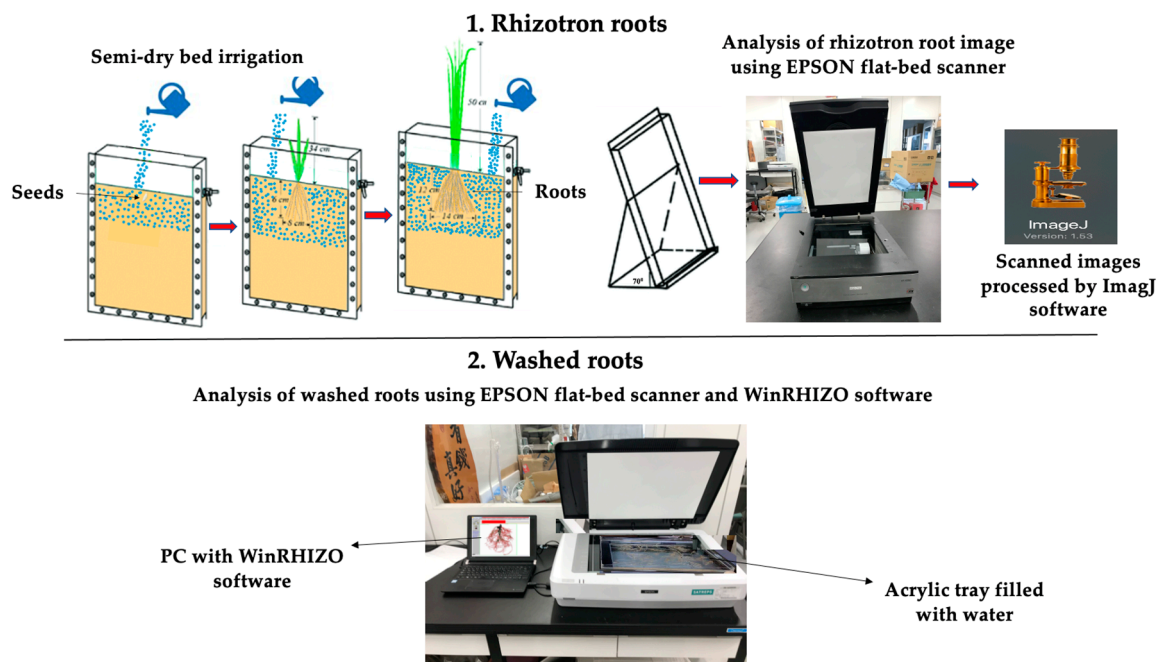


Figure 2. Illustration of seedlings' growth in rhizotrons and their image analysis by ImageJ and WinRHIZO software.

2.4. Root and Shoot Biomass

At 20 DAS, plants were separated at the root–shoot boundary. Roots were gently washed to remove any remaining soil. The fresh weight of shoots was taken immediately after harvest, while dry weight was measured after oven-drying the samples at 65 °C for 48 h. Roots were preserved in 60% ethanol before being scanned. Following imaging, root fresh weight was recorded, and dry weight was determined after oven-drying at 65 °C for 48 h.

2.5. Image Analysis of Washed Roots

After washing, root samples were arranged in an acrylic tray containing water to minimize overlap between roots (Figure 2). Scanning was performed on the roots using a flat-bed scanner (EPSON 10000 XL; Epson, Nagano, Japan) set to 400 dpi, and images were saved as JPEG files. Root traits were analyzed with WinRHIZO software (WinRHIZO Arabidopsis 2019a; Regent Instruments, Quebec, QC, Canada). The software's automated global thresholding was applied to convert images to a binary format, optimizing root segmentation. Quantitative features measured included root diameter, tip number, total root length, and surface area. Roots were classified by diameter into two groups: greater than 0.2 mm and 0.2 mm or smaller. Primary roots were visually identified and measured for length and surface area using SmartRoot, an ImageJ plugin, following the protocol described by Sanada and Agehara [21]. The lengths and areas of secondary roots were determined by subtracting primary root measurements from the WinRHIZO data for roots with diameters above 0.2 mm. WinRHIZO was also used to directly analyze tertiary root length and surface area for roots with diameters of 0.2 mm or less. Root images were first converted to binary images using the software's automated global thresholding algorithm that optimized root segmentation.

2.6. Statistical Analysis

Statistical analysis involved conducting a one-way analysis of variance (ANOVA), with 'Treatment' set as a fixed factor to test its effects on each parameter measured. Tukey's honest significant difference (HSD) test was applied for pairwise post hoc comparisons,

using a significance level of 5%. Linear correlation assessments were carried out to identify relationships between key variables. Visualization of the data took place in Python 3.7.4 within Jupyter Notebook (<https://api.anaconda.org>, accessed 15 October 2025), and all statistical analyses were performed using R version 3.6.2.

3. Results

3.1. Characterization of Soil Amendments

Table 2 summarizes the initial physical and chemical properties of the SAs used in this study, showing clear differences among treatments. The S+VC+PM mixture had the greatest concentrations of N, P, SOM, and SOC, while the S+RHB amendment had the highest K content. The S+VC+PM treatment also exhibited the lowest bulk density. All parameters except pH varied significantly between treatments ($p < 0.001$). These baseline differences are essential for interpreting subsequent plant root growth responses throughout the experiment.

3.2. Soil Water Retention Analysis Across Treatments

The SAs caused notable changes in soil physical properties, especially SWR, which strongly affected plant-available water (PAW) and root growth under semi-dry conditions (Figure 3). The combination of PM and VC amendments was most effective in creating drought-resilient soil, achieving the highest WHC (26%) and thus the largest PAW reservoir. Analysis of the water retention curve clarifies this effect: although S+PM+VC had the greatest water content at saturation (pF 0), it also lost water more rapidly as tension increased, resulting in the lowest retention at the permanent wilting point (pF 3.0). In contrast, soil amended with RHB provided the least favorable conditions for early drought tolerance, with the lowest WHC (14%). Such a limited water supply would be depleted quickly, leaving plants in the S+RHB treatment more susceptible to water stress as the soil dries and hardens, restricting root growth. The remaining amendments—S+SPP (WHC: 19%), S+GS (WHC: 21%), S+WA (WHC: 18%), and S+ZL (WHC: 17%)—produced intermediate WHC values. Although these treatments improved on S+RHB, their smaller water reservoirs compared to S+PM+VC would result in a shorter period of PAW during dry spells. As a result, RSs in these intermediate treatments would encounter moisture stress sooner than those in S+PM+VC, likely leading to lower root biomass and less extensive development as plants use up available water more rapidly.

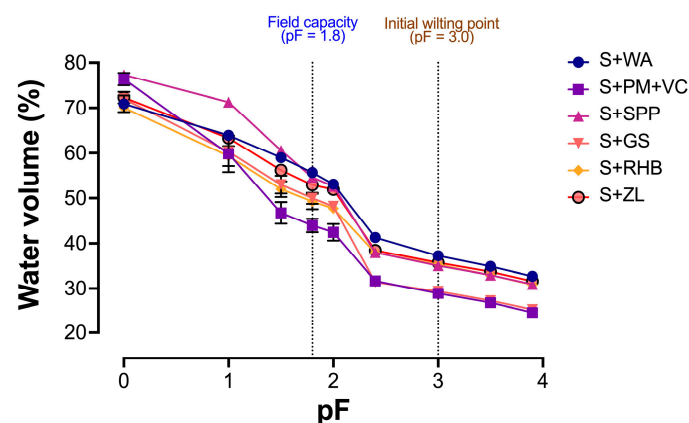


Figure 3. Soil water retention curves of various amended soils used in this experiment.

3.3. Root Projected Area (Rhizotron Image Analysis)

RS development, a critical factor for early-stage drought avoidance in semi-dry systems, was significantly influenced by SAs, with performance directly correlating with their

water-retention properties (Table 3). The S+VC+ PM treatment consistently produced the most extensive RSs, achieving a final root area of $9.60 \text{ cm}^2 \text{ plant}^{-1}$ at 20 DAS, which was significantly greater than all other treatments (Figure 4). This superiority, evident from the earliest measurement at 5 DAS, is a direct phenotypic response to the favorable soil moisture environment created by its high-WHC (26%). In contrast, the S+WA, S+SPP, and S+GS treatments resulted in the most restricted root development, with the smallest final root areas (approximately $6.4 \text{ cm}^2 \text{ plant}^{-1}$). The S+RHB and S+ZL amendments showed intermediate root growth.

Table 3. Root projected area of rice seedlings as affected by soil amendment treatments under semi-dry bed conditions.

Soil Amendment Treatments	Root Projected Area ($\text{cm}^2 \text{ Plant}^{-1}$)			
	5 DAS	10 DAS	15 DAS	20 DAS
S+WA	1.05 ± 0.94^b	2.43 ± 0.09^c	4.10 ± 0.19^d	6.51 ± 0.51^c
S+VC+PM	1.46 ± 0.23^a	3.27 ± 0.20^a	6.46 ± 0.24^a	9.60 ± 0.55^a
S+SPP	1.09 ± 0.09^b	2.34 ± 0.11^c	4.64 ± 0.37^{cd}	6.43 ± 0.28^c
S+GS	1.03 ± 0.11^b	2.37 ± 0.10^c	4.01 ± 0.20^d	6.34 ± 0.44^c
S+RHB	1.20 ± 0.05^{ab}	2.89 ± 0.07^{ab}	5.49 ± 0.39^b	7.90 ± 0.50^b
S+ZL	1.14 ± 0.03^{ab}	2.65 ± 0.08^{bc}	5.02 ± 0.15^{bc}	7.07 ± 0.23^{bc}
<i>p</i> -value	*	***	***	***

Results are shown as mean values with standard deviations ($n = 3$). Different letters denote statistically significant differences among the six SA groups. Asterisks indicate significance levels: *** for $p < 0.001$ and * for $p < 0.05$. Details of the SA treatments are provided in Table 1.

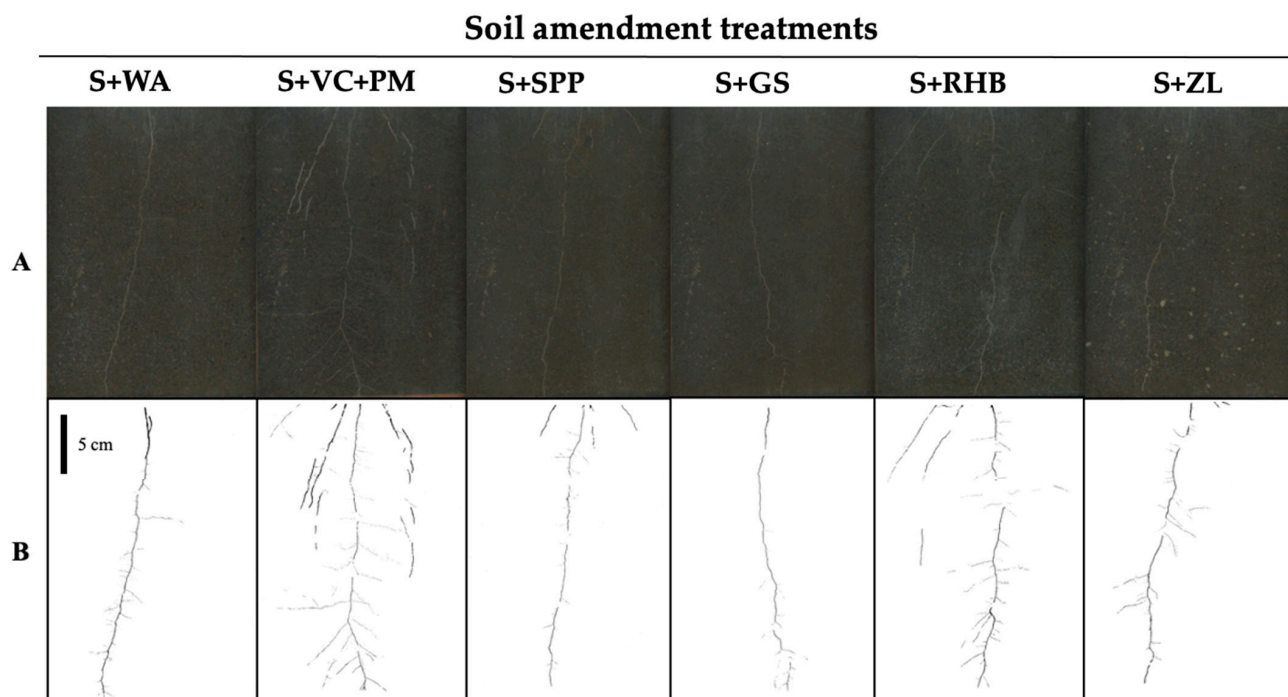


Figure 4. Rhizotron root images of rice seedlings at 20 days after sowing: (A) color images; (B) binary images processed by the ImageJ software. SA treatments are as described in Table 1.

3.4. Plant Biomass Production and Allocation

The SAs substantially increased biomass production under semi-dry cultivation conditions (Table 4). The S+VC+PM treatment resulted in the highest total biomass, with shoot and root dry weights of 140.00 and $56.70 \text{ mg plant}^{-1}$, respectively, both significantly greater than those observed in all other treatments. This enhanced growth demonstrates

the treatment's capacity to alleviate moisture stress, thereby improving seedling vigor and biomass accumulation in water-limited environments. The S+VC+PM treatment also exhibited the lowest root-to-shoot ratio (0.90), suggesting a resource allocation strategy that prioritizes shoot development. In contrast, the S+WA, S+GS, and S+RHB treatments displayed considerably higher root-to-shoot ratios (2.23–2.77).

Table 4. Shoot and root growth of rice seedlings as affected by soil amendments under semi-dry bed conditions.

Soil Amendment Treatments	Fresh Weight (mg Plant ⁻¹)		Dry Weight (mg Plant ⁻¹)		Root:Shoot Ratio (wt/wt)
	Shoot	Root	Shoot	Root	
S+SW	166.66 ± 5.77 ^b	413.65 ± 14.76 ^b	50.00 ± 5.23 ^b	33.45 ± 5.50 ^c	2.48 ± 0.21 ^{ab}
S+VC+PM	736.20 ± 15.29 ^a	670.00 ± 12.78 ^a	140.00 ± 7.56 ^a	56.70 ± 5.78 ^a	0.90 ± 0.07 ^c
S+SPP	186.23 ± 14.56 ^b	300.00 ± 10.00 ^b	56.67 ± 4.67 ^b	33.79 ± 5.68 ^c	1.64 ± 0.30 ^{bc}
S+GS	163.34 ± 10.59 ^b	443.35 ± 9.76 ^b	53.25 ± 0.16 ^b	46.56 ± 5.29 ^{ab}	2.77 ± 0.66 ^a
S+RHB	196.78 ± 13.56 ^b	423.68 ± 14.25 ^b	56.56 ± 6.05 ^b	50.45 ± 0.00 ^{ab}	2.23 ± 0.51 ^{ab}
S+ZL	170.00 ± 10.24 ^b	296.67 ± 11.60 ^b	50.00 ± 0.45 ^b	40.00 ± 0.00 ^{bc}	1.74 ± 0.71 ^{abc}
<i>p</i> -value	****	****	***	***	**

Values are expressed as mean ± standard deviation for three replicates. Distinct letters identify statistically significant differences among the six SA groups. Significance thresholds are: **** $p < 0.0001$, *** $p < 0.001$, and ** $p < 0.01$. Refer to Table 1 for details of the SA treatments.

3.5. Root Diameter and Lateral Root Number (WinRHIZO Image Analysis)

Root architecture analysis identified traits essential for early-stage drought adaptation in semi-dry systems (Table 5). The S+VC+PM treatment produced an RS with a significantly greater average diameter (631 µm) and the highest numbers of secondary (233) and tertiary (1709) roots. In semi-dry cultivation, this configuration is advantageous because thicker roots can penetrate drier, more complex soils. Extensive lateral branching forms a dense foraging network that efficiently exploits soil moisture over a larger soil volume. In contrast, the S+WA and S+SPP treatments resulted in less developed architectures, characterized by sparse lateral branching that limits soil exploration capacity. The remaining amendments exhibited intermediate levels of branching.

Table 5. Root diameter and lateral root number of rice seedlings as affected by soil amendments under semi-dry bed conditions.

Soil Amendment Treatments	Root Diameter (µm)	Root Number per Plant	
		Secondary Roots	Tertiary Roots
S+SW	543.33 ± 16.50 ^b	145.33 ± 10.50 ^c	1326.66 ± 16.04 ^f
S+VC+PM	631 ± 12.05 ^a	233.34 ± 9.50 ^a	1709.00 ± 7.50 ^a
S+SPP	532.34 ± 10.59 ^b	132.00 ± 10.00 ^c	1365.00 ± 5.00 ^e
S+GS	549.00 ± 6.00 ^b	209.66 ± 8.02 ^{ab}	1569.00 ± 9.00 ^b
S+RHB	566.00 ± 6.55 ^b	217.00 ± 9.00 ^{ab}	1540.00 ± 10.00 ^c
S+ZL	543.00 ± 18.00 ^b	202.00 ± 8.54 ^b	1456.66 ± 10.50 ^d
<i>p</i> -value	***	****	****

Values are shown as mean ± standard deviation (n = 3). Statistically significant differences between the six SA treatments are denoted by different letters. Significance levels are indicated as **** for $p < 0.0001$ and *** for $p < 0.001$. See Table 1 for detailed descriptions of the treatments. Mean root diameter was determined from all roots identified with WinRHIZO.

3.6. Distribution of Root Length Across Root Orders

Analysis of root length by order showed that the S+VC+PM amendment conferred architectural advantages (Figure 5). While primary root length remained statistically

similar across all treatments, significant differences emerged in lateral root development. The S+VC+PM treatment produced the highest total lateral root length (363.09 cm plant⁻¹), due to significantly increased lengths in both the ≥ 0.2 mm and < 0.2 mm diameter classes (Table 6). As a result, this treatment achieved the highest cumulative RS length (444.21 cm plant⁻¹), reflecting a greater capacity for soil exploration through enhanced lateral branching rather than primary root elongation. Other amendments formed a statistically lower-performing group for both total lateral and cumulative root length. The S+WA and S+SPP treatments consistently yielded the lowest values, whereas S+GS, S+RHB, and S+ZL exhibited intermediate performance. Limited lateral root growth in treatments such as S+WA and S+SPP likely restricts the plant's ability to access water in a semi-dry bed. This increases vulnerability to rapid drought stress as the soil dries.

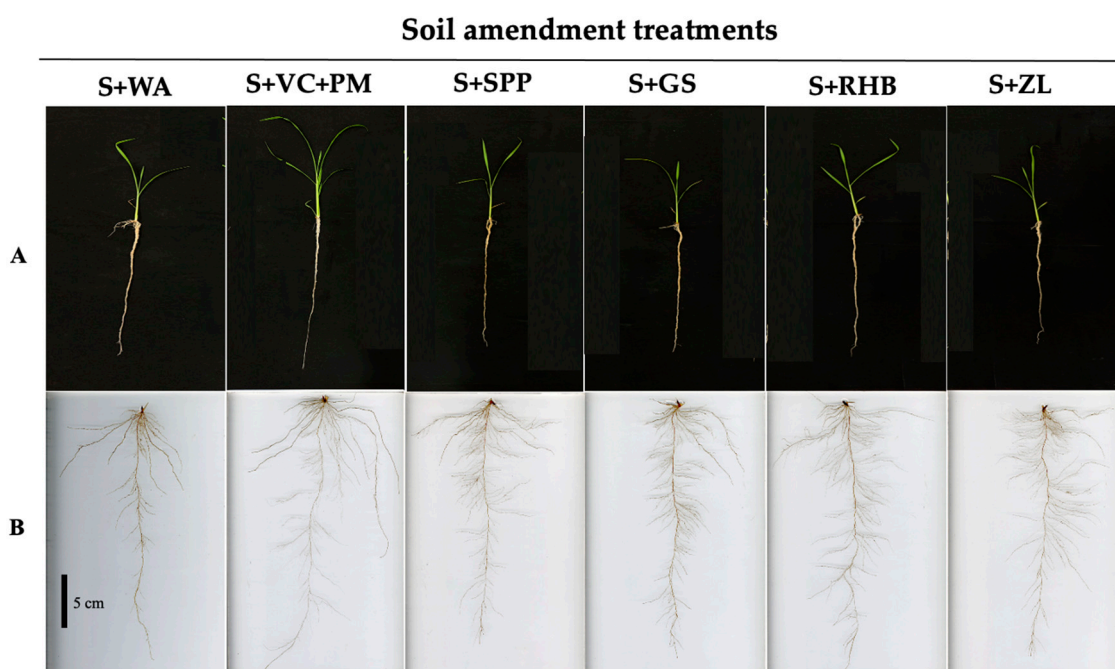


Figure 5. Rice seedlings sampled at 20 days after sowing: (A) photographs; (B) scan images of washed roots. SA treatments are as described in Table 1.

Table 6. Root length of rice seedlings as affected by soil amendments under semi-dry bed conditions.

Soil Amendment Treatments	Root Length (cm Plant ⁻¹)				
	Primary Roots	Lateral Roots		Total	Primary + Lateral
		≥ 0.2 mm	< 0.2 mm		
S+SW	73.66 $\pm$ 7.50 ^a	197.66 $\pm$ 8.02 ^c	122.33 $\pm$ 5.50 ^b	320.00 $\pm$ 13.5 ^{bc}	393.67 $\pm$ 21.01 ^b
S+VC+PM	81.00 $\pm$ 5.00 ^a	224.66 $\pm$ 7.50 ^a	138.23 $\pm$ 6.50 ^a	363.09 $\pm$ 1.89 ^a	444.21 $\pm$ 6.23 ^a
S+SPP	75.00 $\pm$ 7.00 ^a	202.34 $\pm$ 4.04 ^{bc}	124.62 $\pm$ 4.55 ^{ab}	327.00 $\pm$ 5.56 ^b	402.00 $\pm$ 6.55 ^b
S+GS	76.66 $\pm$ 11.50 ^a	215.65 $\pm$ 6.50 ^{ab}	119.43 $\pm$ 4.04 ^b	335.45 $\pm$ 10.57 ^b	412.32 $\pm$ 3.45 ^b
S+RHB	77.33 $\pm$ 4.72 ^a	212.56 $\pm$ 5.03 ^{abc}	127.00 $\pm$ 2.00 ^{ab}	339.61 $\pm$ 3.05 ^b	417.70 $\pm$ 7.54 ^{ab}
S+ZL	74.32 $\pm$ 7.51 ^a	205.00 $\pm$ 4.35 ^{bc}	128.00 $\pm$ 6.00 ^{ab}	333.04 $\pm$ 2.64 ^b	407.34 $\pm$ 9.60 ^b
<i>p</i> -value	ns	**	*	***	**

Data are presented as mean $\pm$ standard deviation ($n = 3$). Significant differences among the six SA treatments are indicated by letters. Statistical significance is denoted as follows: *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, and ns indicates not significant. SA treatments are described in Table 1. Lateral roots were categorized into two diameter classes: roots with diameters ≥ 0.2 mm were classified as secondary roots, while those < 0.2 mm were classified as tertiary roots.

3.7. Distribution of Root Surface Area Across Root Orders

Analysis of root surface area data indicated significant differences in the physical interface for water and nutrient uptake among treatments (Table 7). The S+VC+PM treatment resulted in an RS with the largest total surface area ($84.79 \text{ cm}^2 \text{ plant}^{-1}$), exceeding all other treatments. This superiority was observed across all root orders, with S+VC+PM exhibiting the highest surface area for primary roots (9.66 cm^2), lateral roots $\geq 0.2 \text{ mm}$ (61.79 cm^2), and lateral roots $< 0.2 \text{ mm}$ (13.34 cm^2). The S+RHB treatment produced the second-largest total root surface area (77.67 cm^2), representing a distinct intermediate statistical group. In contrast, the S+WA, S+SPP, S+GS, and S+ZL treatments yielded the lowest total root surface areas, each clustering around $74 \text{ cm}^2 \text{ plant}^{-1}$. A significant correlation was observed between root projected area measured non-destructively on rhizotron images using ImageJ software and root surface area measured destructively on washed-root images with WinRHIZO software in rice seedlings (Figure 6).

Table 7. Root surface area of rice seedlings as affected by soil amendments under semi-dry bed conditions.

Soil Amendment Treatments	Root Surface Area ($\text{cm}^2 \text{ Plant}^{-1}$)				
	Primary Roots	Lateral Roots		Total	Primary + Lateral
		$\geq 0.2 \text{ mm}$	$< 0.2 \text{ mm}$		
S+SW	7.58 ± 0.79^c	56.20 ± 1.25^{bc}	11.09 ± 0.94^b	67.29 ± 0.60^{bc}	74.87 ± 1.01^c
S+VC+PM	9.66 ± 0.11^a	61.79 ± 1.16^a	13.34 ± 0.50^a	75.13 ± 0.70^a	84.79 ± 1.59^a
S+SPP	7.85 ± 0.03^{bc}	56.02 ± 1.48^{bc}	10.42 ± 0.42^b	66.47 ± 1.05^c	74.32 ± 1.08^c
S+GS	7.93 ± 0.05^{bc}	55.57 ± 1.22^{bc}	10.22 ± 0.16^b	65.80 ± 1.06^c	73.73 ± 1.11^c
S+RHB	8.58 ± 0.32^b	57.61 ± 0.81^b	11.47 ± 0.39^b	69.08 ± 0.55^b	77.67 ± 1.24^b
S+ZL	8.25 ± 0.11^{bc}	54.51 ± 0.60^c	10.63 ± 0.34^b	65.14 ± 0.93^c	73.39 ± 0.82^c
<i>p</i> -value	***	***	***	****	****

Data are presented as mean $\pm$ standard deviation ($n = 3$). Statistically significant differences among the six SA treatments are indicated by different letters. Significance is marked as **** for $p < 0.0001$ and *** for $p < 0.001$. Details of the SA treatments are provided in Table 1. Lateral roots were classified into two diameter categories: roots with diameters $\geq 0.2 \text{ mm}$ were considered secondary roots, while those with diameters $< 0.2 \text{ mm}$ were classified as tertiary roots.

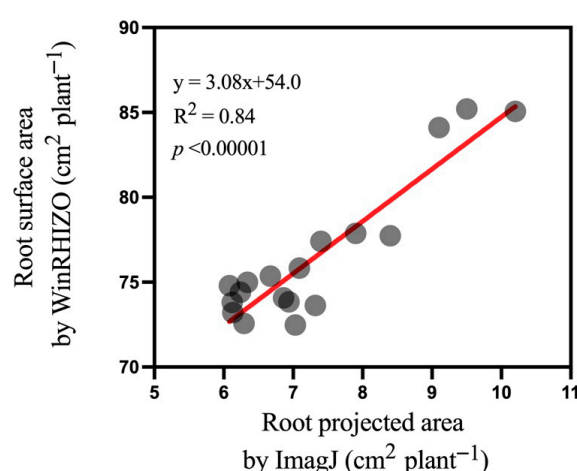


Figure 6. A linear correlation between root projected area measured non-destructively on rhizotron images with ImageJ software and root surface area measured destructively on washed-root images with WinRHIZO software in rice seedlings. Each black ball represents an individual seedling. The red line represents the linear regression fit to the data. All images were collected 20 days after sowing.

4. Discussion

4.1. Interpretation of Amended Soil Characterization

The integration of VC and PM produced the most comprehensive soil improvement, yielding the highest concentrations of N, P, K, SOM, and SOC. This outcome is consistent with the established functions of organic amendments in nutrient provision and in enhancing microbial activity [30]. The marked reduction in bulk density observed with this treatment further confirms improved soil porosity and structure, which facilitates enhanced aeration and root penetration [31]. In comparison, while S+RHB contributed substantial potassium, which is a characteristic feature of biochar, other amendments, such as spirulina, gypsum, and zeolite, produced only moderate increases in nutrient and organic content. These increases were relative to the S+PM+VC treatment [19].

4.2. Inference of Soil Water Retention Across Treatments

The observed SWR and WHC variations among amendments provide a clear mechanistic explanation of the differential root growth during the seedling phase. The superior performance of S+PM+VC, which yielded the highest WHC (26%), is strongly supported by the existing literature. These findings align with reports that PM has high porosity and provides a significant, readily available moisture reservoir for plants [32]. The synergy in S+PM+VC likely stems from the complementary roles of its components. PM contributes superior water retention through its extensive pore network, while VC enhances soil structure and biological activity, collectively creating more favorable growing conditions [33]. The characteristic steep water release curve of S+PM+VC—showing high saturation but lower retention at the permanent wilting point—indicates that a large portion of the stored water is held at tensions that are easily accessible to roots. This creates a more resilient, sustained supply of PAW, which directly supports the sustained root expansion observed in this treatment. The intermediate WHC of the S+SPP treatment suggests that spirulina acts more as a biostimulant than a physical soil conditioner [34]. Its modest effect likely enhances drought resilience through biological pathways rather than creating a significant moisture reservoir. In contrast, the poor performance of S+RHB, which yielded the lowest WHC (14%), contradicts several studies. Previous research has reported that RHB can increase WHC by 26–33% when applied at 1% by mass [35]. This effect is attributed to its porous structure and ability to create intra- and inter-pore spaces that enhance water retention [36,37]. The stark discrepancy in our findings underscores the high context dependency of biochar efficacy. This suggests that factors such as soil texture, initial moisture content, biochar particle size, or production conditions can drastically alter the material's impact on soil hydro-physical properties. The lower WHC observed in our study using RHB with a particle size of <2 mm produced at a pyrolysis temperature of 500 °C highlights the critical context dependency of biochar efficacy. The effect likely results from the interaction of the specific RHB properties with the coarse-textured Akadama soil substrate at our application rate of 1.7%. We posit that this combination may have increased soil macroporosity, enhancing drainage and aeration. However, it may not have generated a sufficient volume of the smaller, capillary pores responsible for retaining plant-available water. This underscores that the net impact on WHC is a balance of pore-size distribution rather than a simple increase in total porosity. The moderate improvement in water retention from the S+GS amendment aligns with its known role as a soil conditioner rather than a primary water-absorbing material. Its efficacy is well documented in problematic soils, where it functions through cation exchange to reduce dispersion and lower bulk density. This process improves soil structure and creates a more favorable pore system for water retention [38,39]. The intermediate results in this study confirm that its benefits are context-specific and not universally applicable across all soil types. Similarly, the

intermediate WHC of the zeolite (S+ZL) amendment aligns with its documented role in soil water management. This role typically shows variable but significant improvements. Research has indicated that zeolite application can increase soil water storage by 3.6–14.7%, with particle size being a key factor in its efficacy [40]. Its function extends beyond simple retention to enhancing the overall soil structure and infiltration rates [41]. However, its greatest potential for optimizing water management in arid environments is often realized when it is strategically combined with other amendments, rather than applied alone [42].

4.3. Elucidation of Root Projected Area

The SBRS approach demonstrated that RS development is a direct phenotypic response to the soil environment. The greater root projected area observed in the S+VC+PM treatment was evident at the early growth stage and maintained through 20 days after sowing (DAS). This can be attributed to the enhancement of both soil physical and biological properties. The principal mechanism involved establishing a favorable hydrological regime. The high WHC (26%) under this treatment provided a stable moisture reservoir, maintaining turgor pressure for cell elongation and reducing early water stress, thereby enabling sustained and vigorous root exploration [43]. This finding supports the study's emphasis on improved SWR as a critical factor in optimizing semi-dry cultivation.

Additionally, the extensive RS in S+VC+PM was likely influenced by factors beyond water availability. The substantial increases in soil organic matter and nutrient content (N, P, K) associated with this amendment have been previously reported. These increases supplied essential components for cell wall synthesis and energy required for root growth [19].

Moreover, the organic composition of VC and PM may support a robust community of beneficial soil microorganisms. These microbes are known to produce phytohormones such as auxins and cytokinins. Such compounds can stimulate root meristem activity and lateral root proliferation, potentially contributing to the increased root projected area observed in this treatment [30,44].

Conversely, the limited root expansion observed with other treatments can be attributed to their reduced water retention, which provided a smaller buffer against drying cycles. These findings suggest that the effectiveness of an amendment in semi-dry cultivation is strongly linked to its ability to comprehensively enhance both the physical and biological properties of the soil.

4.4. Insights into Plant Biomass Production and Allocation

In this study, the roots, shoots, and root-to-shoot ratio of rice seedlings differed significantly in response to SAs. These changes were observed compared to the control treatment that received no SA under semi-dry conditions (Table 4). The superior total biomass and unique resource allocation strategy under the S+VC+PM treatment are characterized by the lowest root-to-shoot ratio. These findings reflect a shift in carbon allocation enabled by improved soil conditions. In this favorable rhizosphere, plants could prioritize carbon for canopy growth rather than investing in extensive defensive root foraging. The latter strategy would be necessary under more limited soil moisture or nutrient availability. This growth pattern aligns with findings that such amendments enhance soil structure, nutrient availability, and microbial activity, thereby supporting more efficient plant development under water-saving irrigation [9,45]. The low root-to-shoot ratio indicates that the plants were not forced into defensive foraging, allowing for greater carbon allocation to shoot growth and, consequently, higher plant biomass. This effect can be directly attributed to the synergistic properties of the amendment blend. The VC component enhances soil structure and stimulates microbial activity, thereby improving nutrient mineralization and root development [8,45]. PM provides significant water-retention capacity and organic

matter, facilitating sustained moisture and nutrient release that buffer against the drying cycles characteristic of semi-dry cultivation [46]. The integration of these properties in the S+VC+PM treatment results in a more favorable water and nutrient environment, optimizing the balance between root investment and shoot productivity. These findings indicate that the strategic application of combined organic amendments represents an effective approach to enhancing biomass production and resource allocation in semi-dry rice cultivation systems.

4.5. Explanation of Root Diameter and Lateral Root Number

WinRHIZO image analysis demonstrated that SAs induce distinct root architectural phenotypes that are essential for drought adaptation in semi-dry systems. The S+VC+PM treatment exhibited superior root architecture, with significantly greater root diameter and the highest number of secondary and tertiary roots—an optimal configuration for water-limited environments. This observation is consistent with established findings on the effects of organic amendments. VC is known to supply phytohormones that can stimulate the initiation of lateral root primordia, while PM may help maintain the stable moisture regime necessary for root emergence and development [47]. The resulting dense foraging network enables efficient exploitation of soil moisture. At the same time, thicker roots penetrate deeper into drier soil layers, collectively accounting for the treatment's sustained hydration and robust growth.

In contrast, intermediate or poor architectural development under other treatments underscores the importance of a hydrated, low-impedance rhizosphere. The sparse lateral branching under S+WA and S+SPP treatments indicates a failure to induce the prolific root branching observed with S+VC+PM. Although previous studies have reported that RHB can increase lateral root development by 20–40% through improved water availability and soil structure [43,48], it showed limited effectiveness in this system. This highlights the context-dependent nature of such amendments. Similarly, the intermediate outcomes for zeolite and gypsum align with their moderate and conditional effects, which often depend on specific soil stressors such as nutrient deficiency or salinity [49]. Overall, the S+VC+PM treatment uniquely provided combined physical and biological conditions that prevented a stress-induced root response. Instead, it promoted an architecture characterized by resilience and high foraging efficiency, which are critical for optimizing semi-dry rice cultivation.

4.6. Interpretation of Root Length

WinRHIZO analysis indicated that the primary architectural advantage of the S+VC+PM amendment was a significant increase in lateral root branching. This amendment produced the highest total lateral root length (363.09 cm plant⁻¹) and cumulative RS length. This outcome is consistent with previous reports that combining VC and PM synergistically increases root elongation by approximately 20–40% under semi-dry conditions [19,47]. The underlying mechanism is likely multifactorial. VC is known to provide phytohormones that can stimulate the initiation of lateral primordia and cell elongation. Concurrently, PM may contribute to stable moisture and nutrient availability, conditions considered necessary for sustained root growth during wetting-drying cycles [30]. This combination produces a finely branched, high-surface-area root network that is optimal for extensive soil moisture acquisition, thereby supporting the study's objective of linking root responses to improved SWR. The intermediate performance of the other amendments further clarifies their roles. Although S+RHB has been shown to enhance root length by 15–35% due to improved water retention and soil structure [43,50], its limited effectiveness in the present study indicates that these benefits are highly context dependent. Furthermore, the advantages

of S+RHB may not match the immediate and synergistic effects observed with combined organic amendments. Similarly, the moderate outcomes observed for S+ZL and S+GS align with previous studies' findings. These studies demonstrate that the primary function of these treatments is to alleviate specific stresses, such as nutrient deficiency or salinity. As a result, these treatments typically produce conditional improvements of 10–25% [51,52]. The persistently low lateral root growth observed under the S+WA and S+SPP treatments demonstrates that their impact on the rhizosphere is insufficient to overcome the hydraulic limitations of semi-dry cultivation.

4.7. Interpretation of Root Surface Area

The results confirm that the S+VC+PM amendment produced an RS with a significantly larger total surface area ($84.79 \text{ cm}^2 \text{ plant}^{-1}$). Such an increase directly reflects a core early-stage drought adaptation mechanism in semi-dry systems. Root architectural traits observed in this context represent precise early-stage adaptive responses that enhance soil exploration. Enhanced surface area also improves water extraction efficiency during seedling establishment. Seedling establishment is a critical and vulnerable phase for final crop performance under water limitation. The advantage provided by the S+VC+PM amendment was observed across all root orders. This observation indicates a comprehensive expansion of the root–soil interface for resource absorption. Superior root surface area development, particularly the proliferation of fine roots ($<0.2 \text{ mm}$), remains critical under semi-dry conditions. Greater surface area maximizes hydraulic contact with the drying soil. It also enables more efficient extraction of scarce water films [19]. The strong correlation between non-destructive rhizotron measurements and destructive WinRHIZO analysis (Figure 6) validates the scanner-based rhizotron approach as an effective tool for phenotyping this key trait, facilitating semi-dry cultivation optimization. The substantial surface area enhancement from the S+VC+PM treatment can be attributed to the synergistic effects of its components. VC is known to supply phytohormones such as auxins and cytokinins. These compounds can stimulate the initiation of lateral root primordia—a developmental event that creates new roots and substantially increases surface area [30,47]. PM may concurrently help maintain the stable moisture conditions considered necessary for the sustained elongation of these initiated roots and the proliferation of water-absorbing fine roots [53]. This synergy between the hormonal stimulation associated with VC and the hydraulic stabilization provided by PM likely contributed to the highly branched, high-surface-area network observed. Such root architecture is regarded as beneficial for resource foraging in water-limited environments. S+RHB produced the second-highest surface area, though its performance remained intermediate. The literature indicates that biochar can increase root surface area by 20–40% through improvements in soil structure and water retention [43,50]. However, its more modest effect in this system highlights the context-dependent nature of biochar efficacy. This may reflect the absence of the additional hormonal stimuli associated with organic amendments such as VC. The lowest-performing amendments (S+WA, S+SPP, S+GS, S+ZL) constituted a distinct group. This finding aligns with previous research indicating that mineral amendments, including zeolite and gypsum, primarily enhance root development by increasing nutrient availability or alleviating specific stresses such as salinity. These amendments provide conditional benefits, resulting in 10–35% increases, rather than comprehensive rhizosphere optimization [19,52].

5. Implications and Limitations

The SBRS produced high-resolution temporal data. This enabled the isolation of soil physical and chemical properties as primary determinants of early root architectural responses to amendments under controlled semi-dry stress. The S+VC+PM treatment

exhibited clear superiority in this context. These findings offer mechanistic insights into how amendments such as PM and VC synergistically promote early drought-avoidance traits. However, the relevance of these results must be interpreted in light of the system's inherent limitations. The two-dimensional growth constraint may influence root architecture and competition differently than three-dimensional field conditions. Additionally, the absence of full-season biotic and abiotic interactions raises uncertainty. This uncertainty concerns the persistence of early advantages. The principal conclusion of this study is the identification of promising SA strategies for stabilizing seedling establishment in semi-dry systems. Nevertheless, their capacity to enhance crop resilience throughout the growing season and their economic viability require rigorous assessment. Such an assessment must be conducted through multi-season field trials in diverse agro-climatic environments.

Furthermore, the methodology demonstrated high reliability, as evidenced by a significant correlation between root projected area measured non-destructively with ImageJ and root surface area measured destructively with WinRHIZO. Although this intensive phenotyping provided robust mechanistic insights, the experimental design was limited to three replicates per treatment. This limitation was due to the labor-intensive nature of the rhizotron and root-washing protocols. This constraint reduces statistical power to detect subtle effects and should be addressed in larger-scale validation studies.

6. Conclusions

- This study demonstrates that SAs can enhance SWR and induce adaptive root architectural responses in rice seedlings under semi-dry cultivation. The combined VC and PM treatment was the most effective under these experimental conditions.
- By employing a non-destructive SBRS, we quantified how amendments differentially affect early root development—a critical phase for early drought adaptation during seedling establishment.
- The S+VC+PM treatment was associated with the largest reservoir of PAW (26% WHC). This amendment promoted an RS characterized by extensive lateral branching, greater diameter, increased length, and larger surface area across all root orders.
- This optimized root architecture facilitated more efficient soil exploration and water extraction. These improvements directly translated to superior biomass production and a favorable root-to-shoot ratio indicative of reduced plant stress.
- The findings suggest that enhancing soil physical properties—particularly water retention—through integrated organic amendments may help mitigate early-stage drought stress. Such improvements appear to foster an RS capable of sustaining growth under water-limited conditions.
- The combination of PM (contributing to hydraulic stability) and VC (associated with nutrient supply and phytohormonal stimulation) was particularly effective in this study. Such a finding underscores the potential benefits of integrated soil management over single-component approaches.
- Other amendments—including RHB, gypsum, and zeolite—showed moderate or context-dependent effects. However, their performance in this study does not preclude their potential value under different soil constraints, such as nutrient deficiency, salinity, or structural degradation.
- In practical terms, these findings support the potential of combined organic amendments like S+VC+PM to stabilize early crop establishment and improve water productivity in semi-dry rice systems. However, the present study was conducted under controlled rhizotron conditions. The focus on the seedling stage (20 DAS), therefore, necessitates cautious extrapolation to field-scale or season-long performance.

- Two priority directions for future research are: (1) multi-season field trials to assess whether these early root benefits translate into sustained yield improvements and (2) comprehensive economic and logistical feasibility analyses to support farmer adoption.

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