Effects of P-dipping on NERICA 4 Rice Resilience to Moisture and Phosphorus Stresses at Early Growth Stages

イネ品種 NERICA 4 の生育初期における土壌水分およびリン酸ストレスに対する P-dipping の効果

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(2024)

要約

干ばつストレスとリン酸欠乏は、サブサハラアフリカにおけるコメの収量を制限する主要な非生物的要因である。 リン酸欠乏は主に、熱帯地方の高度に風化した土壌で発生し、利用可能なリン酸が少なく、酸性度が高いため、Fe および Al 酸化物の含有量が増加する。その結果、リン酸の吸収が阻害される。 肥料養分の施用を行わないで連続的に作物を作付けすることにより、地域においては全体的に土壌のリン酸含有量が顕著に減少する傾向を示されている。イネのリン酸の利用可能性は、土壌の水分条件および拡散、通気および吸着の動態に制限されている。 また、土壌水の利用可能性は根の構造に変化をもたらしており、結果的に土壌水分条件の異なる低地から高地における、作物のリン酸の吸収利用効率に影響を及ぼしている。 地球温暖化により、旱魃の発生が増大している状況を考えると、土壌の水分条件に応じたリン酸の最適施用法について議論することは、サステナブルな稲作を開発する上でも有益である。高度に風化した土壌で利用可能なリン酸の供給を改善するために、農家は様々なのリン酸施用方法を実践している。近年、マダガスカルで開発されたリン酸の根の浸漬方法、いわゆる P-dipping は、灌漑水田において生育および収量を向上することが示唆されている。P-dipping は、移植前に苗の根を土壌にリン酸を溶かした土壌液に浸漬をして、リン酸の吸収及び生育への効果を高めようとする技術である。しかし、土壌の水ストレス条件や異なる土性における P-dipping 処理がイネの生育初期の根の伸長や地上部の生育の関係に及ぼす影響についての知見は極めて少ない。

そこで、まず *O. sativa* と *O. glaberrima* の種間雑種 NERICA 4 を供試して、 異なる土壌水分と P-dipping の 2 元配置分散分析によって交互作用を明らかにした。(第

2 章)。 その結果、地上部の乾物重と草丈は、P-dipping 区間で有意な差が認められ、リン酸施用量40 kg ha^{-1}の処理区が、土壌水分条件にかかわらず最も増加した。葉面積は、適度に乾燥した土壌条件下では、P-dipping 間で有意差は認められなかったが、すべてのP-dipping区は、コントロール（非P-dipping）に比較して増加した。平均根長は土壌水分条件に影響を受けたが、根の平均乾物重は、土壌水分条件にかかわらず、P-dipping 区がコントロールよりも高く、湛水土壌および適度乾燥土壌でそれぞれ平均36%および8%増加した。これらの知見は、NERICA4の生育初期において、天水低地で発生する水ストレスおよび養分ストレスに対して、P-dipping の処理が効果的に生育を改善することを示唆していると結論付けた。

第 3 章では、NERICA4 の生育初期における土壌の土性(砂、粘土ローム、粘土)と P-dipping の複合効果を因子実験で評価した。その結果、異なる土性におけるリン酸処理区において、個体の総乾物重は 1.06～4.63 g pot^{-1}の範囲にあることが示された。P-dipping 処理により、地上部と根の乾物重は、コントロールと比較して、それぞれ1.27～1.98 gおよび0.23～0.38 g pot^{-1}に有意に増加した。平均光合成速度値は、土性に関係なく、コントロールと比較して P-dipping が 42%(20.1 μmol m^{-2} s^{-1})、Brod1（散播１）が36%（19.3 μmol m^{-2} s^{-1})、およびBrod2(散播2)が 37%(19.5 μmol m^{-2} s^{-1})と有意に増加した。また、P-dipping は、粘土質土壌条件で根長を有意に伸長させたが、地上部におけるリン酸の吸収量は有意に増加しなかった。本研究においては、粘土ローム土壌においてのみP-dippingがNERICA 4の成長形質の生理学的および形態学的特性に変化を及ぼした。本研究結果は、サブサハラアフリカ全体で広く適応しているイネ品種 NERICA4 のリン酸吸収と施用効果について、P-dipping 技術を用いて評価したものであり、その結果、リン酸施用の利用効率を改善するための新たな知見を提供することが出来た。本成果は、風化の激しい土壌や

気候変動の多様な影響に直面している地域の小規模稲作農家に、コメの生産向上を通して直接利益をもたらす可能性が期待できる。

Summary

Drought stress and phosphorus (P) deficiency are major abiotic factors limiting rice yields in sub-Saharan Africa (SSA). The P deficiency is mainly attributed to the highly weathered soils in the tropics, which are inherently low in available P and high in P-sorption capacity owing to their acidity and high content of Fe and Al oxides. Continuous and intensive cropping without proportionate nutrient replenishment has further reduced the P content of many soils across SSA. Soil water status and the P availability to rice plants are strongly related through diffusion, aeration, and sorption, and both water and P have specific behaviors and dynamics in the soil. Additionally, water availability has modifying effects on root architecture, which in turn affects P uptake efficiency in both upland and lowland soils. Given that dry spells are becoming more prevalent, the need to optimize water and P use to improve rice production, particularly for smallholder farmers in SSA, cannot be overemphasized. To improve the available P supply in the highly weathered soils of SSA, farmers use several P application methods, one of which is P-dipping, i.e., dipping the root of seedlings into P-enriched slurry before transplanting. However, little is known about the effect of P-dipping on the root distribution of NERICA varieties under soil P and water stress conditions, and in different soil textures at the initial growth stages.

We conducted a split-plot pot research experiment to determine the how localized P application via P-dipping (using four P fertilizer levels) affects NERICA4 rice growth in interaction with two water regimes, including waterlogging and moderately dry (Chapter 2). Results showed that shoot dry weight and plant height differed significantly under the various P-dipping application levels, with an application of 40 kg P ha^{-1} providing the highest mean values under both water treatments. While the mean leaf area did not statistically differ among the P-dipping treatment levels under the moderately dry condition, all P-dipping treatments had increased leaf area relative to that observed without P-dipping. Mean root length differed

significantly between the water treatments, whereas the mean root dry weight was higher with the P-dipping treatments than without P-dipping under both water conditions (36% and 8% mean increases under the waterlogged and moderately dry treatments, respectively). These findings show that P-dipping improves the ability of NERICA 4 rice seedlings to withstand water and nutrient stresses under rainfed lowland at early growth stages.

In Chapter 3, we evaluated the combined effect of soil texture (sand, clay loam, and clay) and P treatments P-dipping (Pdip) and two other broadcasted P fertilizer levels (Brod1 and Brod2) on the growth of NERICA 4 rice in the initial growth stages in a factorial experiment. Findings showed that across all soil textures and P treatments, total plant biomass ranged from 1.06 to 4.63 g pot^{-1}. The Pdip treatment significantly increased shoot and root biomass relative to control from 1.27 to 1.98 and 0.23 to 0.38 g pot^{-1}, respectively. Mean photosynthetic rate values under Pdip (20.1 µmol m^{-2} s^{-1}), Brod2 (19.5 µmol m^{-2} s^{-1}), and Brod1 (19.3 µmol m^{-2} s^{-1}) treatments showed significant 42%, 37%, and 36% increases over control, regardless of soil texture. In striking contrast, P-dipping significantly promoted growth of root length under clay soil, but without a commensurate increase in shoot P uptake. Contrary to our hypothesis, the interactive effect of soil texture and P-dipping influenced NERICA 4 shoot and root physiological and morphological characteristics under clay loam soil texture as opposed to clay. The findings of our studies provide new insights into improving P uptake and use efficiency for the widely adapted NERICA 4 rice variety across SSA, which should directly benefit smallholder farmers in the region who are struggling to improve rice yields in the heavily weathered soils and at the face of the diverse effects of climate change.

Part of this dissertation has been published in the following peer-reviewed international journals:

1. Odama, E., Tsujimoto, Y., Yabuta, S., Akagi, I., & Sakagami, J. I. (2023). P-dipping improved NERICA 4 rice seedling resilience to water and nutrient stresses under rainfed-like conditions. *Rhizosphere*, *26*, 100688. https://doi.org/10.1016/j.rhisph.2023.100688

2. Odama, E., Tsujimoto, Y., Yabuta, S., Akagi, I., Chepkoech, R., Soe, I., & Sakagami, J. I. (2023). Effect of P-Dipping on Growth of NERICA 4 Rice in Different Soil Types at Initial Growth Stages. *Sustainability*, *15*(21), 15402. https://doi.org/10.3390/su152115402

Acknowledgments

First and foremost, glory to God in the highest for keeping and enabling the author to successfully complete the research studies and the preparation of this thesis.

The author is deeply indebted to the main research Supervisor, Professor Dr. Jun-Ichi Sakagami for the technical and fatherly guidance, support, and instruction during the study period, which made the completion of this thesis possible.

The author is so grateful to Dr. Yasuhiro Tsujimoto of the Crop, Livestock and Environment Division, JIRCAS; Professors Dr. Isao Akagi and Dr. Makoto Ikenaga of Kagoshima University; Professors Dr. Daisuke Fujita and Dr. Shoki Tei of Saga University, and Dr. Shin Yabuta of the Faculty of Agriculture, Setsunan University for the insightful review and comments of the author’s work.

Big thanks and appreciation to the author’s parents and siblings for the prayer and moral support during the study period. Special thanks to Mrs. Hope Monica Chandiru and Mr. Gracious Lester Munguni, the author’s wife, and son, respectively.

Finally, the author expresses his gratitude to the Japan International Cooperation Agency for providing the scholarship to study in the United Graduate School of Agricultural Sciences, Kagoshima University, Japan.

Kagoshima, January 2024

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CHAPTER ONE

GENERAL INTRODUCTION

1.1 Background

1.1.1 Global rice production and consumption

Rice (*Oryza* ssp.) is the main staple food crop for more than three billion people worldwide, mainly from Asia and Africa (FAO, 2016); where most of it is produced by smallholder farmers having average farm sizes of 0.5–3 ha in area (Sepat and Rana, 2013). Rice productivity has more than doubled in recent decades, with leading rice producing countries in Asia including India (46.3 M ha^{-1}), China (29.9 M ha^{-1}), Bangladesh (11.6 M ha^{-1}), Indonesia (11.6 M ha^{-1}), Thailand (10.7 M ha^{-1}), and Vietnam (7.2 M ha^{-1}) (IRRI, 2022). The stated rice yields present marked increases in contrast to yields of 1.9 M ha^{-1} in Asia in the 1960s (Sakagami, 2022). The Green Revolution between the 1940s and the late 1960s resulted in the increased rice production, mainly in the Asian continent through transfer of a series of research and technology initiatives (Evenson et al., 2001; Kumar, 2007). Comparatively, rice yields in SSA are about 2.1 M ha^{-1}, far below the average yields in Asia and the potential productivity in the African continent (Tsujimoto et al., 2019). Previously, expansion in rice fields accounted for the increase in total rice production in SSA (Otsuka and Kalirajan, 2006). However recently, this has been attributed to increase in rice yields (Balasubramanian et al., 2007; Seck et al., 2013).

The increased global rice production notwithstanding, the projected increase in the global population, currently having per capita rice consumption at approximately 54 kg $person^{-1}$ (FAO, 2021), demands an increase in world rice production by at least 25% by 2030 (Seck et al., 2012). On the global scale, Asian countries consume the most rice with China leading at about 149,000 thousand metric tons (TM) per annum, followed by India (106,500

TM), Bangladesh (35,900 TM), Indonesia (35,800 TM), Vietnam (21,250 TM), the Philippines (14,400 TM), Thailand (12,500 TM), Myanmar (10,400 TM), Japan (8,250 TM), and Brazil consuming about 7,350 TM of rice (Statista, 2021). Rice consumption in SSA has been increasing rapidly though the increase in rice production has not been commensurate with the significant increase in rice consumption (Sakagami, 2022). However, studies have shown that, owing to a combination of factors such as population growth, dietary changes, and yield improvement on available arable land, the future global demand for rice will be driven by Africa (Van Oort, 2015), as Asia is expected to account for close to 65% of the projected rise in global rice consumption per annum, largely due to population increases rather than per capita gains (OECD/FAO, 2021) (Table 1-1).

Table 1-1. Rice per capita consumption (kg person^{-1} year^{-1})

Region	2018-2020	2030	Growth rate (% p.a.)
Africa	27.4	31.5	1.2
Oceania	13.5	14.2	0.44
North America	6.3	6.6	0.42
Europe	20.7	25.6	–0.08
Latin America and Caribbean	28.0	28.1	–0.14
Asia	77.2	77.5	–0.15

Source: OECD/FAO (2021), “OECD-FAO Agricultural Outlook”, OECD Agriculture statistics (database), http://dx.doi.org/10.1787/agr-outl-data-en.

1.1.2 NERICA rice production in sub–Saharan Africa

Farmers in SSA have grown the traditional and improved indica (*O. sativa*) for long, with diffusion rates ranging between 20 and 100% for improved, introduced cultivars in selected West African countries (Dalton & Guei, 2003; Balasubramanian et al., 2007). Recently, the Africa Rice Center developed the ‘New Rice for Africa’ (NERICA) varieties that have potential yields of 4-7 tons per hectare (Sekiya et al., 2013). The NERICA varieties are interspecific hybrid rice varieties and lines between Asian rice varieties (*O. sativa*) and African rice varieties (*O. glaberrima*), which combine the high-yielding traits of *O. sativa* with the stress-adaptive traits of *O. glaberrima* (Jones et al., 1997). Besides improvement in yield potential, other breeding objectives for the NERICA genotypes included grain quality, broad adaptation to diverse lowlands in this region, tolerance against Rice Yellow Mottle Virus and African Gall Midge (Sie et al., 2008).

Despite its good traits, the yield potential of the NERICA genotypes has not been achieved in SSA, due to biotic and abiotic factors that affect the largely rainfed rice ecosystems in most parts of the region. For example, Kijima et al. (2006) estimated that the average rice yield in farmers' fields in Uganda is only 2.2 ton per hectare. In Guinea, modest applications of fertilizer have boosted the rice yield to as far as 3.5 tons per hectare (Harsch, 2004); while in Kenya, grain yields of about 2.2 tons per hectare have been reported (Atera et al., 2011; Kega and Maingu, 2011). These findings reveal that adequate moisture and soil nutrients are major limiting factors to achieving high rice yield potential in SSA, with P deficiency being a key limiting nutrient (Diagne et al., 2013).

1.1.3 Phosphorus acquisition and uptake for improved rice production

In contrast to the other major nutrients, P is the least mobile and least available to plants in most soil conditions (Hinsinger, 2001). As such, P deficiency is a major limiting factor in crop productivity, especially in the tropics and subtropics (Ismail et al., 2007; Saito et al. 2018). The first line of response to mitigating the P deficiency problem across many SSA countries has been through the promotion of inorganic fertilizer use (Jayne et al., 2018). For instance, countries in SSA imported fertilizers valued at 3.9 billion U.S. dollars in 2020, which was a minimal decrease in comparison to the previous year (Statista, 2023). Despite that considerable foreign exchange drain from SSA, the average fertilizer application rate in the region still averages between 13 and 20 kg of nutrient per hectare (Sheahan & Barrett, 2017), far below the projected 50 kg Nutrient per hectare under the Abuja Declaration on Fertilizer for the African Green Revolution, which the African Union member stated adopted to have been achieved by 2015. However, owing to the inherently low nutrient status of the soils, use of large quantities of organic and inorganic fertilizers have been recommended for improved crop yields (Sileshi et al., 2017). For the most part, the recommendations have not been adopted because of the prohibitively high costs of fertilizers for most farmers to afford. In addition, significant leaching losses have been found with inorganic fertilizer application in Ferralsols and Acrisols (Nakagawa et al., 2012; Nyamangara et al., 2003; Russo et al., 2017).

Physiological and morphological approaches to improve P-acquisition efficiency have also generated considerable root related research with the objectives of modifying of root physiology (Lambers et al., 2006; Prieto et al., 2012; Read et al., 2003), changing root morphology (Desnos, 2008; Williamson et al., 2001), and improving colonization by mycorrhizal hyphae (Javot et al., 2007; Ferrol et al., 2019; Smith et al., 2003). Agronomic research studies on P micro-dozing, and application of small amounts of P in nursery beds before transplanting have also been undertaken (De Bauw et al., 2019b; Garrity et al., 1990; Sarangi et al., 2015; Vandamme et al., 2016; Vandamme et al., 2018). More recently, research studies on P-dipping, which involves dipping the root of rice seedlings into P-enriched slurry before transplanting, have gained traction.

1.2 Statement of the problem and research scope

Improved P uptake and use efficiency play a big role in boosting sustainable agricultural productivity globally. However, adequate P uptake remains a major challenge particularly sub-Saharan Africa, where the predominantly weathered soils are low in available P but high in P-sorption capacities. Considering the growing human and animal population on the African continent and the commensurate increasing demand for food and fiber, current approaches to improve P uptake and use efficiency in rice production are not likely able to keep pace with the demand for food and nutrition security across the region. This is especially true in the wake of the effects of climate change, which have partly been attributed to the increased volumes of inorganic fertilizer production for agricultural use. As such, the opportunity to recommend and use higher P fertilizer rates may not be tenable in the foreseeable future. Thus, P-uptake enhancement approaches such as P-dipping that allow for relatively minimal P fertilizer amounts and employs a localized P application method directly to the roots, boost root growth, reduce time to maturity, and improve rice resilience to drought and P stresses are well poised to sustainably improve rice production and productivity among farmers in SSA and across the world if widely popularized and promoted.

In these studies, firstly, we evaluated NERICA 4 rice shoot and root response to the combined effect of various P levels in the slurry used for P-dipping, and two water regimes including waterlogging and moderately dry. Secondly, we evaluated the combined effect of three soil textures and P treatments including P-dipping and broadcasting on NERICA 4 rice shoot and root physiology and morphology, with a major focus on shoot P uptake in the initial growth stages. Both experiments were conducted in greenhouse environments. The findings of our studies provide new insights into improving P uptake and use efficiency for the widely adapted NERICA 4 rice variety across SSA, which should directly benefit smallholder farmers in the region who are struggling to improve rice yields in the heavily weathered soils and at the face of the diverse effects of climate change.

1.3 Novelties

These studies present the following novelties:

i. P-dipping enhances NERICA 4 to withstand drought and P stresses in soil through developing thick lateral roots, deep roots, and narrow root cone angle.

ii. Soil texture and P-dipping have interactive effects on NERICA 4 rice root morphology.

iii. Increase in NERICA 4 rice root length in clay soil does not necessarily increase P uptake.

iv. P-dipping showed a significant photosynthetic rate in NERICA 4 rice, regardless of soil texture, compared to the broadcasting method.

1.4 Specific Objectives

The research studies addressed the following specific objectives:

a) To evaluate the combined effect of P-dipping and moisture stress on NERICA 4 shoot and root physiological and morphological characteristics.

b) To understand the underlying mechanisms by which NERICA 4 adapts to moisture stress and liming P content in the soil.

c) To assess the effect of moisture stress on the type and size of NERICA 4 rice roots with a focus on P uptake.

d) To evaluate the combined effect of P-dipping and soil texture on NERICA 4 shoot and root physiology and morphology.

e) To examine changes in photosynthetic rate, stomatal conductance, transpiration rate, and intercellular carbon dioxide concentration in response to P-dipping and soil texture variation.

f) To assess the effect of changes in NERICA 4 rice root length on shoot P uptake under P-dipping and varying soil texture conditions.

Specific objectives a, b, and c were addressed in Chapter 2 using root boxes, where four P treatments were factorially combined with two water regimes in three replicates. In Chapter 3, objectives d, e, and f were addressed in a pot experiment, using three soil textures factorially combined with three P treatments in three replicates. Both Chapters 2 and 3 contain an introduction, materials and methods, results, discussion, and conclusion of the research study.

CHAPTER TWO

Effect of P-dipping on NERICA 4 rice seedling resilience to water and nutrient stresses

2.1 Introduction

Drought stress and P deficiency are abiotic factors that limit the yields of upland and lowland rice in sub-Saharan Africa (Chauhan et al., 2017; Diagne et al., 2013; Saito et al., 2019). Soil water status and the availability of P to rice plants are strongly related through diffusion, aeration, and sorption (Kirk et al., 1990), and both water and P have specific behaviors and dynamics in the soil (Bünemann et al., 2011, Lal and Stewart, 2016). Additionally, water availability has modifying effects on root architecture, which in turn affects P uptake efficiency in both upland and lowland soils (De Bauw et al., 2019a). A field study conducted by Kijima et al. (2011) revealed that the adoption of NERICA rice varieties, i.e., an interspecific progeny between *O. sativa* and *O. glaberrima*, was positively affected by its productivity in Uganda, which was directly influenced by the level of rainfall, even when farmers received high quality seeds and prepared their fields for planting in good time.

P deficiency is a major constraint to agricultural production in many regions of SSA and is related to the highly weathered soils in the tropics (Henao and Baanante, 2006), which are low in available P and high in P-sorption capacity owing to their acidity and high content of Fe and Al oxides (Bekunda et al., 2010; Nishigaki et al., 2019). Continuous and intensive cropping without proportionate nutrient replenishment has further reduced the P content of many soils across SSA (Sanchez, 2002). Given that dry spells are becoming more prevalent (Zhao et al., 2015), the need to optimize water and nutrient use to improve rice production, particularly for smallholder farmers in SSA, cannot be overemphasized. To this end, several water and nutrient management strategies have been applied to optimize their use in rice production. For example, approaches in which plants are given access to P via P microdosing, e.g., applying small amounts of P with the seed in directly seeded upland and lowland rice production systems, have been found to have positive effects (De Bauw et al., 2019b; Garrity et al., 1990; Vandamme et al., 2018). However, some studies have shown that microdosing increases the risk of soil nutrient depletion in low-input cropping systems, typical in SSA, and that seasonal balances should be considered (Bagayoko et al., 2011; Blessing et al., 2017).

The application of small amounts of P in nursery beds is another conventional approach to increasing P-use efficiency in transplanted rice production systems (Sarangi et al., 2015; Vandamme et al., 2016). However, this technique increases the risk of P mining from soils, even when large yield gains are achieved, because the additional nutrients transferred to the main fields with rice seedlings are negligible, whereas the large proportion of applied P is retained in the nursery bed (Oo et al., 2020a; Vandamme et al., 2016). Another approach practiced by farmers in China (Lu et al., 1982), India (De Datta et al., 1990; Raju et al., 1980), and Madagascar (Balasubramanian et al., 1994) is P-dipping, which involves dipping the root of rice seedlings into P-enriched slurry before transplanting. P-dipping enables direct and localized P application in the main field during transplanted rice cultivation as the P-enriched slurry is attached to the seedling roots (Rakotoarisoa et al., 2020). P-dipping not only provides rice seedlings with the “start-up” P they require to boost root growth (He et al., 2003) but also enhances plant vigor, enabling plants to withstand water, soil nutrient, and salt stresses (Ibrahim, 2015; Ros et al., 2015; Sarangi et al., 2015; Vandamme et al., 2016). However, little is known about the effect of P-dipping on the root distribution of NERICA varieties under soil nutrient and water stress conditions in the initial growth stages.

In this study, we evaluated the plant growth, biomass, root distribution, and morphology of NERICA 4 rice under combined P-dipping and water regimes. We hypothesized that P-dipping improves NERICA 4 seedling resilience, allowing seedlings to withstand water and nutrient stress under rainfed-like conditions. The findings of this study will directly benefit smallholder farmers in SSA who are struggling to improve rice yields in naturally weathered soils, especially as they are faced with the adverse effects of climate change. The results will also provide new insights into the ongoing research regarding the farmer-preferred NERICA varieties cultivated in SSA.

2.2 Materials and Methods

2.2.1 Physiochemical characteristics of the experimental soil

The chemical and physical characteristics of the study soil are presented in Table 2-1. The mixed soil used for the experiment had a pH of 8.1 and low available P, total N, total C, and exchangeable K concentrations. The experimental soil was predominantly sandy in texture.

Table 2-1. Chemical and physical properties of the experimental soil.

Parameters	**Mixed (Mt.+sandy) soil**[a]	**Mountain soil**	**Sandy soil**
pH (1:2.5 H_2O)	8.1	7.5	9.2
Total N (mg g^{-1})	0.07	0.09	0.02
Total C (mg g^{-1})	0.19	0.57	0.08
Available P (mg kg^{-1})	44.2	84.8	14.9
Exchangeable K (mg kg^{-1})	225.0	150.7	302.4
Sand (%)	96.0	94.3	99.4
Clay (%)	2.4	1.7	0.4
Silt (%)	1.6	4.0	0.2
Textural name	Sand	Sand	Sand

[a] The mixture of mountain (Mt.) and sandy soil used in the experiment.

2.2.2 Experimental design

The experiment was conducted in a greenhouse using root boxes (40 cm L × 40 cm W × 3 cm H) and NERICA 4 rice seeds. The rice was first grown in seedling trays for 14 days. The root boxes were filled with 5 kg of soil (1:1 *w/w*; bulk density: 1.2 g cm^{-3}). The experimental soil was analyzed for pH (H_2O), available P using Truog's method, exchangeable potassium using the 1 mol L^{-1} ammonium acetate extraction method, nitrogen and carbon using the dry combustion method via an NC analyzer (JM1000CN/HCN TOC.TN, J-Science Lab Co., Ltd., Japan), and soil texture using the pipette method. To correct any deficiencies in N and K, the experimental soil in each root box was homogenously mixed with 1.4 g of ammonium sulphate and 0.4 g of potassium chloride, respectively.

Prior to transplanting, rice seedlings were carefully removed from the seedling tray, and the nursery soil on the roots carefully hand-washed using water in a plastic container to avoid root damage and loss. On average, the depth of the root system of each seedling was 10 cm; the seedlings were dipped into P-enriched slurry with a range of P concentrations, i.e., 0% for D0, 0.25% for D1, 0.51% for D2, 0.76% for D3, and 1.02% for D4, for 30 min as recommended by Oo et al. (2020a). The P-enriched slurry was produced by mixing 45 g of air-dried soil, 14 mL of water, and different amounts of single superphosphate fertilizer, i.e., 0 g (D0), 0.65 g (D1), 1.31 g (D2), 1.96 g (D3), and 2.62 g (D4), depending on the different P concentrations. The amount of slurry attached to the seedlings at transplanting was approximately 3 g per box; thus, the P application rate for each treatment was estimated at 20, 40, 60, and 80 kg P ha^{-1}, respectively. We also set up a control treatment in the same manner but without P, N, or K applications. For non-P and Control treatments, the seedlings were transplanted without dipping the washed roots into the slurry. To avoid damage to the roots at transplanting, holes of approximately 15 cm depth and 3 cm width were made in the soil (having field capacity moisture content) inside the root boxes, then seedlings transplanted.

Two different water regimes, namely waterlogging (WL) and moderately dry (MD) water conditions, were applied with the aforementioned fertilizer treatments (D0, D1, D2, D3, D4, and Control) with three replicates. The level of soil in the root boxes was about 4 cm lower than the top edge of the boxes. In the WL treatment, the root boxes were flooded in acetylic tanks (58 cm L × 44 cm W × 44 cm H), and the water level was controlled at 2–3 cm above the soil surface during the entire growing season. In the MD treatment, the volumetric water content was reduced at 0.23 m m^{-3} from 10 days after transplanting to the end of the experiment, which was performed by replacing the rubber stoppers at the bottom of the root boxes with soft, porous sponge to allow limited water movement into the boxes from the bottom.

2.2.3 Data collection and measurements

The shoot parameters plant length and leaf number were measured at 5-day intervals from the start of the water treatments. At 30 days after transplanting, the shoot in each root box was cut, and the leaves removed to measure the leaf area with a digital image analysis machine (LIA32, Nagoya University, Nagoya, Japan). The leaves and stems were oven-dried at 80°C for 48 h to determine the shoot dry weight per root box. Oven-dried plant materials were finely ground, and samples (0.3 g each) were wet digested in 10 mL of di-acid digestion mixture [HNO_3:$HClO_4$ (3:2, *v/v*)]. Total P concentration in plant samples was determined following the vanadate–molybdate method (Chapman and Pratt, 1961) using UV-VIS spectrophotometer (V-530, JASCO Co. Tokyo, Japan). Shoot P uptake was calculated as the product of P concentration and shoot dry weight.

After the shoot was cut, one side of the root box was removed and a pin board was fitted (Kono et al., 1987). The soil was then washed via careful spraying with water at a low pressure until all the soil particles were removed. The washed roots on the pin board were photographed using a digital camera (Nikon D3500 NC, Thailand), and the root cone angle was determined from the images using ImageJ (Version 1.53r, NIH, USA). The root cone angle was considered the angle between the most external left and right nodal roots to the vertical axis (Bettembourg, 2017). From each box, root samples on the pin board were collected from two layers: the above (0–20 cm) and below (>20–40 cm). Samples were labelled, placed in self-sealing plastic bags containing 50% aqueous ethanol solution and stored in a cold room at 4°C in preparation for scanning. Root samples were scanned at 6,400 dpi using an Epson scanner (EPSON GT-X830, Epson American Inc., Los Alamitos, CA, USA), and the images were analyzed using the WinRhizo software (WinRHIZO, Regent Instruments Inc., Québec, Canada; Version 2005b).

During image analysis, pixel classification values of 165–175 were used, and the root diameter was classified into four major types as follows: 0–0.08 mm as fine lateral roots, 0.08–0.2 mm as thick lateral roots, 0.2–1.0 mm as nodal roots, and >1 mm as seminal roots (Gu et al., 2017; Khanthavong et al., 2021; Sandhu et al., 2016). The parameters analyzed included root length within the two soil layers per sample and within the root diameter classifications. Following parameter analysis, the root samples were dried in an oven at 80°C for 48 h to determine the root dry weight.

The daily mean air temperature and daily mean relative humidity in the greenhouse were measured throughout the experiment using a sensor equipped with a data logger (RTR-503, T&D Corporation, Japan). The soil volumetric water content, mean soil temperature, and bulk electrical conductivity (EC) were recorded in three root boxes (2 MD and 1 WL) using sensors fitted at a depth of 11 cm, which were also equipped with a data logger (Em50 Series; METER Group, Inc. USA).

2.2.4 Statistical analyses

Data were analyzed in IBM SPSS Statistics (Version 27.0.1.0) using two-way ANOVA to determine the single and interaction effects of P treatments (D0–D4 and Control) and water treatments (MD and WL). The treatment means from the replicates were compared at the 5% level of probability using Tukey’s HSD test. The mean values from the P-dipping treatments were compared with those of the Control and non–P-dipping treatments using Tukey's HSD test. We used linear correlations to examine the relationship between shoot P uptake and root dry weight. Significance was assumed at the 5% level.

2.3 Results

2.3.1 Greenhouse and experimental soil environments

The daily mean temperature in the greenhouse from transplanting to the end of the experiment was 28.2°C, and the mean relative humidity in the greenhouse was 71.7%. The mean soil volumetric water content at the time of applying the water treatments was at 0.29 m m^{-3} for all root boxes; however, it increased and stabilized at 0.33 m m^{-3} in the WL root boxes immediately after water treatment and reduced and stabilized at 0.23 m m^{-3} in the MD root boxes 10 days after water treatment (Fig. 2-1A). According to the mean EC, the soil environment was nonsaline with mean ECs in the MD and WL conditions of 0.14 and 0.18 mS cm^{-1}, respectively (Fig. 2-1B). The mean soil temperatures throughout the experimental period in the MD and WL conditions were 28.2°C and 28.7°C, respectively.

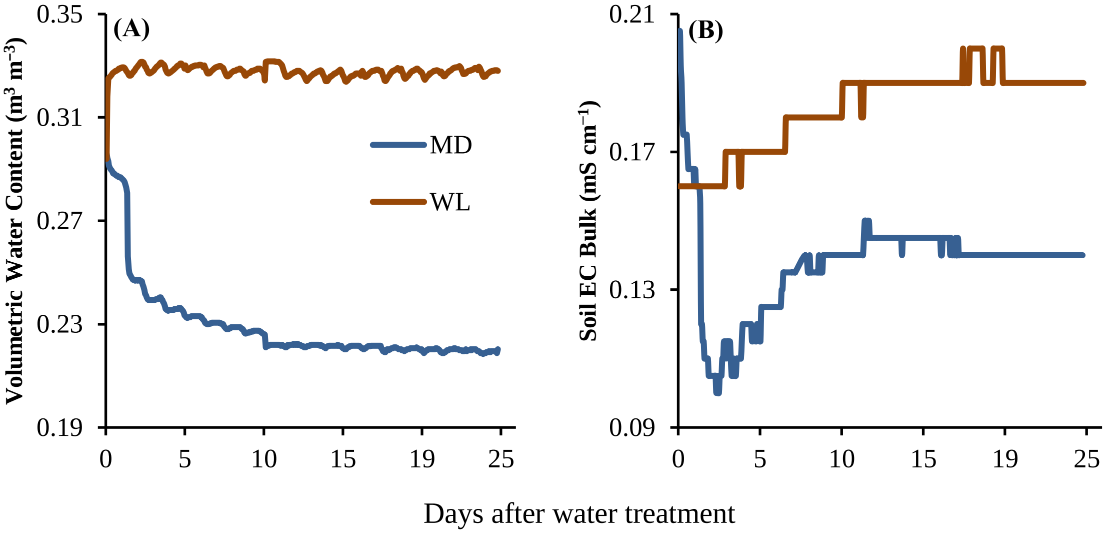

Figure 2-1. Changes in the experimental soil volumetric water content (A) and the electrical conductivity (B) in the moderately dry (MD) and waterlogged (WL) root boxes during the experimental period.

2.3.2 Changes in shoot morphology

The changes in the shoot growth parameters under the MD and WL water treatments and various P treatment levels are shown in Figure 2-2.

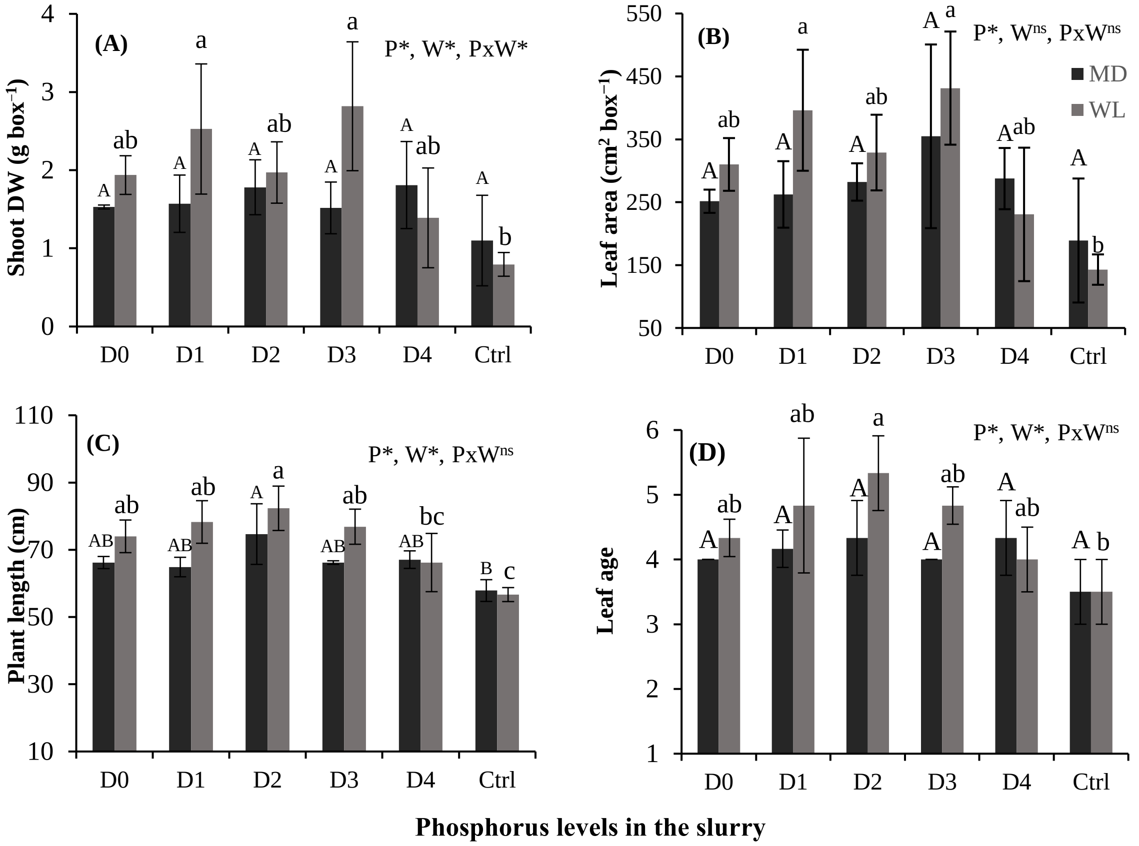

Figure 2-2. NERICA 4 shoot growth parameters under the moderately dry (MD) and waterlogged (WL) treatments. Shoot dry weight (A), leaf area (B), plant length (C), and leaf age (D). D0–D4 and Ctrl indicate the various phosphorus (P) levels in slurry. *, $p < 0.05$; ns, not significant; both according to Tukey's test. Error bars show standard deviations. Different uppercase or lowercase letter combinations indicate significant differences at $p < 0.05$.

The mean shoot dry weights under the WL and MD conditions were 1.9 and 1.6 g, respectively, which were significantly different (p = 0.045; Fig. 2-2A). The mean shoot dry weight also differed significantly according to P treatment levels under the WL condition (p = 0.012; Fig. 2-2A). Significant interaction effects for mean shoot dry weight also existed between the water regimes and P treatments D1 (20 kg P ha^{-1}; p = 0.029) and D3 (60 kg P ha^{-1};

$p = 0.004$). The significant increase in the shoot dry weight under the D1 and D3 treatments relative to the Control within the WL treatment represented 3.2-fold and 3.5-fold increases, respectively. The shoot dry weight under the D0 treatment (without P-dipping) did not differ significantly from that under the D1 and D3 treatments, although 30% and 45% increases, respectively, were observed under the WL condition. Under the WL and MD conditions, the mean shoot dry weight was the lowest under the Control treatment (0.8 and 1.1 g, respectively).

The mean leaf area did not differ under the two water treatments (Fig. 2-2B), although the mean leaf area was higher under WL (310 cm^2) than MD (270 cm^2). Similarly, the mean leaf area did not differ significantly among the P treatment levels under the MD condition, but all P-dipping treatments (D1–D3) led to an increase in the mean leaf area relative to that observed without P-dipping (D0) as follows: D1, 4%; D2, 12%; and D3, 41%. However, D4 showed a 14% decline in the leaf area relative to that observed in D0. Under the WL condition and compared with the Control, the mean leaf area differed significantly under the D1 (p = 0.015) and D3 ($p = 0.006$) P treatments. Compared with the D0 treatment, the D1 and D3 P treatments led to the highest increases in the mean leaf area under the WL condition (28% and 39%, respectively), although these increases were not significant. The mean leaf area for all P treatment levels was higher than that of the Control by 52% and 138% under the MD and WL conditions, respectively. There were no significant interaction effects for leaf area between the P treatment levels and water treatments.

NERICA 4 rice grew significantly higher ($p < 0.001$) under the WL condition than under the MD condition, with mean plant lengths of 72.4 and 66.1 cm, respectively (Fig. 2-2C). The plant lengths also differed significantly under the various P treatments ($p = 0.0005$), with the highest mean plant length achieved under the D2 treatment (40 kg P ha^{-1}) for both water treatments, reflecting mean increases of 37% and 12% relative to the Control and D0 treatments, respectively. The effect of P-dipping on plant length was positive up to D2, but plant length decreased under the D3 and D4 treatments. Control plants were the shortest under both water treatments. No significant interaction effects for plant length were observed between the P treatment levels and water treatments.

As shown in Fig. 2-2D, leaf age exhibited a similar trend to plant length (Fig. 2-2C): (i) there was a significant difference ($p = 0.021$) in the mean leaf age between the WL (4.5) and MD (4.1) water conditions; (ii) under the WL condition, the mean leaf age under D2 differed significantly ($p = 0.004$) from that under the Control; (iii) P-dipping under the WL condition led to an increase in leaf age up to D2, after which leaf age decreased as the P treatment level increased; and (iv) leaf age did not differ significantly among the P treatments under the MD condition. No significant interaction effects were observed for leaf age between the P treatment levels and water treatments.

2.3.3 Changes in root morphology

The changes in root morphology related to P and water treatments are presented in Table 2-2.

Table 2-2. Root architectural changes according to the applied P levels and water treatments.

Water Treatment (W)	**Phosphorus Treatment (P)**	**Root length**	**Root dry weight**	**Root length density**	**Specific root length**	**Root cone angle**	**Root: Shoot ratio**
		(m)	**(g box^{-1})**	**(cm cm^{-3})**	**(m g^{-1})**	**(°)**	
Moderate dry	D0	30.4 ± 5.4	0.23 ± 0.03	0.63 ± 0.11	1.36 ± 0.41	97.7 ± 15.6	0.15 ± 0.02
	D1	30.9 ± 1.8	0.24 ± 0.08	0.64 ± 0.04	1.36 ± 0.42	97.3 ± 17.8	0.15 ± 0.02
	D2	34.5 ± 3.8	0.25 ± 0.04	0.72 ± 0.08	1.42 ± 0.28	101.1 ± 9.5	0.14 ± 0.02
	D3	34.6 ± 8.9	0.23 ± 0.06	0.72 ± 0.18	1.54 ± 0.32	115.6 ± 13.2	0.15 ± 0.01
	D4	34.9 ± 7.7	0.27 ± 0.11	0.73 ± 0.16	1.41 ± 0.62	104.4 ± 5.0	0.15 ± 0.01
	Control	30.1 ± 4.3	0.20 ± 0.05	0.63 ± 0.09	1.56 ± 0.13	97.6 ± 9.7	0.21 ± 0.11
Water logged	D0	25.6 ± 1.4	0.21 ± 0.03	0.53 ± 0.03	1.21 ± 0.11	160.0 ± 7.4	0.11 ± 0.01
	D1	25.3 ± 2.5	0.32 ± 0.06	0.67 ± 0.05	1.01 ± 0.13	165.2 ± 6.1	0.12 ± 0.05
	D2	27.9 ± 8.3	0.33 ± 0.08	0.58 ± 0.17	0.87 ± 0.28	166.6 ± 5.1	0.17 ± 0.01

	D3	31.3 ± 6.5	0.29 ± 0.10	0.65 ± 0.14	1.17 ± 0.48	159.5 ± 4.3	0.10 ± 0.01
	D4	22.2 ± 4.3	0.22 ± 0.07	0.46 ± 0.09	1.03 ± 0.14	143.4 ± 4.4	0.17 ± 0.05
	Control	18.4 ± 7.9	0.18 ± 0.04	0.38 ± 0.17	0.99 ± 0.27	156.0 ± 6.3	0.23 ± 0.02
ANOVA	P	ns	ns	ns	ns	ns	*
(*P*-value)	W	*	ns	*	*	*	ns
	P × W	ns	ns	ns	ns	ns	ns

NERICA 4 root morphology in the moderately dry (MD) and waterlogged (WL) treatments. D0–D4 and Control represent various phosphorus (P) levels in slurry. *, $p < 0.05$; ns, not significant; both according to Tukey's HSD test. Values are means ± standard deviations ($n = 3$).

A significant difference ($p < 0.001$) in total root length existed between the MD (32.6 m) and WL (25.1 m) water treatments. However, the root lengths did not differ significantly according to the D1–D4 P-dipping treatments under the MD condition, although roots were 11% longer than under the D0 treatment. Under the WL treatment and compared with the D0 treatment, the D2 and D4 P treatments led to the highest mean root length increase (22%) and decrease (−14%), respectively. Under both water treatments, Control plants had the shortest roots. Typically, all root morphological parameters remained the same or were reduced under the D4 treatment for both water conditions.

Root dry weight (RDW) did not differ significantly between the two water treatments or among the P levels. However, the mean RDW under the WL treatment (0.26 g) was higher than that under the MD (0.24 g) treatment. For both water conditions, the mean RDWs under the D1–D4 P-dipping treatments were higher than that under the D0 treatment, with 36% and 8% mean increases observed for the WL and MD conditions, respectively. No significant differences in RDW were observed among the P levels under both water treatments.

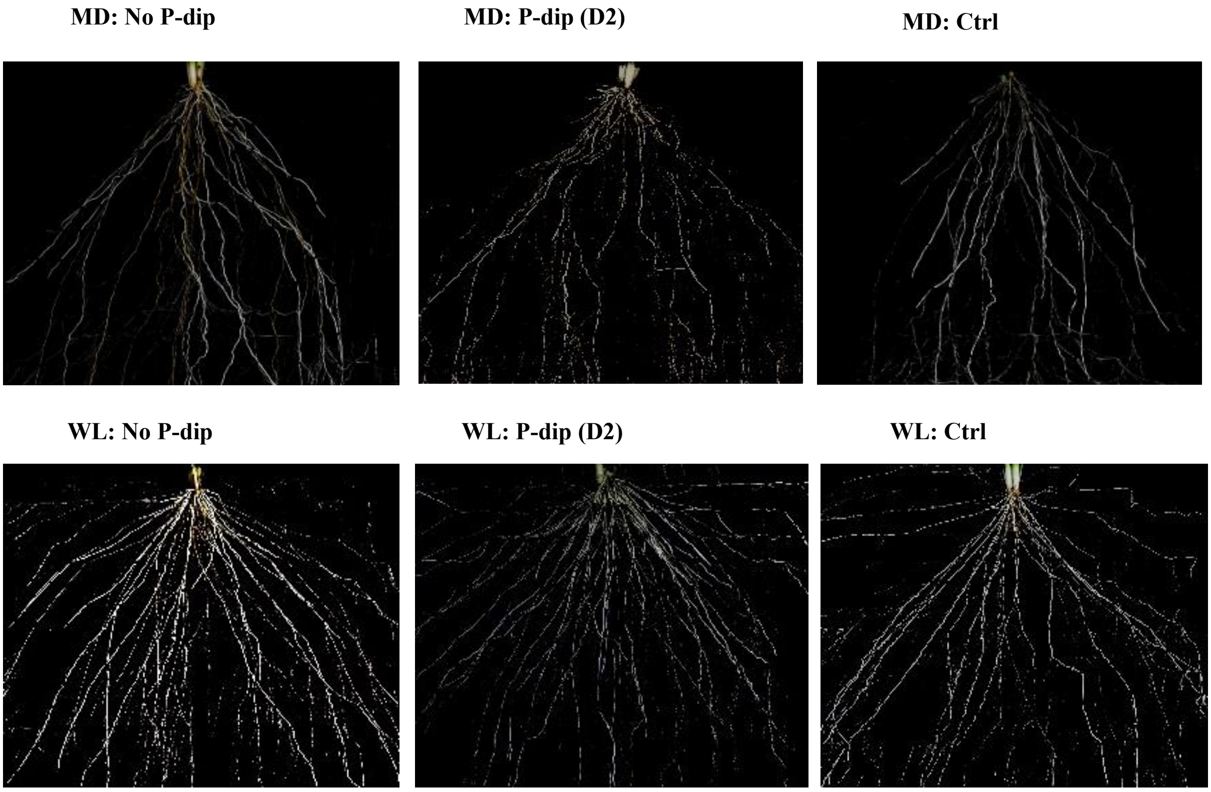
MD: No P-dip
MD: P-dip (D2)
MD: Ctrl
WL: No P-dip
WL: P-dip (D2)
WL: Ctrl

Figure 2-3. Root cone angles under moderately dry (MD) (top row) and waterlogged (WL) (bottom row) water conditions where no P-dipping was performed (left column); P-dipping was performed (center column); and Control (right column). The roots under MD had a narrower cone angle than those under WL condition.

The mean root cone angle under the MD condition (102.3°) was significantly narrower (p = 0.00005) than that under the WL condition (158.4°) (Fig. 2-3). However, the root cone angle did not differ among the various P levels under the two water treatments, although the D1–D4 P-dipping treatments tended to lead to broader root angles than those observed under the D0 and Control treatments for both the MD and WL conditions. The mean root-to-shoot ratio under the water treatments was 0.15, and this ratio was higher in Control plants than plants treated with P under both the MD (0.21) and WL (0.23) conditions.

The distribution of a root system in the soil significantly influences the amount and rate of root nutrient and water uptake. This distribution can be measured by estimating the root length density (RLD: total root length divided by the volume of soil occupied by the roots).

The mean RLD under the MD condition (0.68 cm cm^{-3}) was significantly higher ($p < 0.001$) than that under the WL condition (0.55 cm cm^{-3}) (Table 2-2). In contrast, the RLD did not differ significantly among the P treatments; however, under both water conditions, the RLD was generally higher under the D1–D4 P-dipping treatments than under the D0 and Control treatments. There was no significant interaction effect for RLD between the water and P level treatments. Specific root length (SRL), i.e., the ratio between root length and root weight, which is a measure of root thickness, is considered an important trait for water uptake and nutrient absorption in plants (Paula and Pausas, 2011). SRL differed significantly ($p < 0.001$) between the MD (1.44 m g^{-1}) and WL (1.05 m g^{-1}) water treatments.

2.3.4 Root distribution in soil layers and root classification

The analyses of rice root distribution between the top (0–20 cm) and bottom (>20–40 cm) soil layers are presented in Table 2-3.

Table 2-3. Root length changes between top and bottom soil layers in the root boxes

	Root length under MD (m)				**Root length under WL (m)**			
Phosphorus level	**Top layer (0–20 cm)**	**Bottom layer (>20–40 cm)**	**t-test**	**Bottom RL: TRL**	**Top layer (0–20 cm)**	**Bottom layer (>20–40 cm)**	**t-test**	**Bottom RL: TRL**
D0	13.2a	17.1A	ns	0.56	16.7a	8.9A	*	0.35
D1	15.4a	15.5A	ns	0.50	25.6a	6.6A	*	0.26
D2	16.6a	17.9A	ns	0.52	19.7a	8.2A	*	0.29
D3	18.9a	15.7A	ns	0.45	19.5a	11.8A	*	0.38
D4	18.2a	16.7A	ns	0.48	16.7a	5.4A	*	0.25
Ctrl	11.6a	18.4A	ns	0.61	12.3a	6.1A	*	0.33

NERICA 4 root length distribution within the top (0–20 cm) and bottom (>20–40 cm) layers of the root boxes under moderately dry (MD) and waterlogged (WL) treatments. Bottom RL, root length in the bottom layer; TRL, total root length in the top and bottom layers. D0–D4 and Ctrl represent various phosphorus levels in slurry. *, $p < 0.05$; ns, not significant; both according to a t-test.

Root length did not differ significantly between the top and bottom soil layers and among the P treatment levels under the MD condition (Table 2-3), indicating root growth was active in both MD soil layers. In contrast, the root distribution differed significantly (p = 0.0004) between the two soil layers under the WL condition, with the mean root length found to be higher in the top layer (18.4 m) than in the bottom layer (7.9 m), indicating that root development was shallow under the WL condition. Root lengths did not differ significantly among the P treatment levels in both soil layers.

We categorized the lateral roots (0.01–0.2 mm) into two major sub classes, i.e., fine lateral roots (FLRs: 0.01–0.08 mm) and thick lateral roots (TLRs: 0.08–0.2 mm), under the two water conditions (Fig. 2-4). Under the MD condition, only the TLRs and other large-diameter roots developed. However, under the WL condition, both the FLRs and TLRs developed. The mean root length under the MD condition was significantly higher than that under the WL condition in the TLR (0.08–0.2 mm) classification (p = 0.0009) and general lateral root (0.2–1.0 mm) classification (p = 0.005).

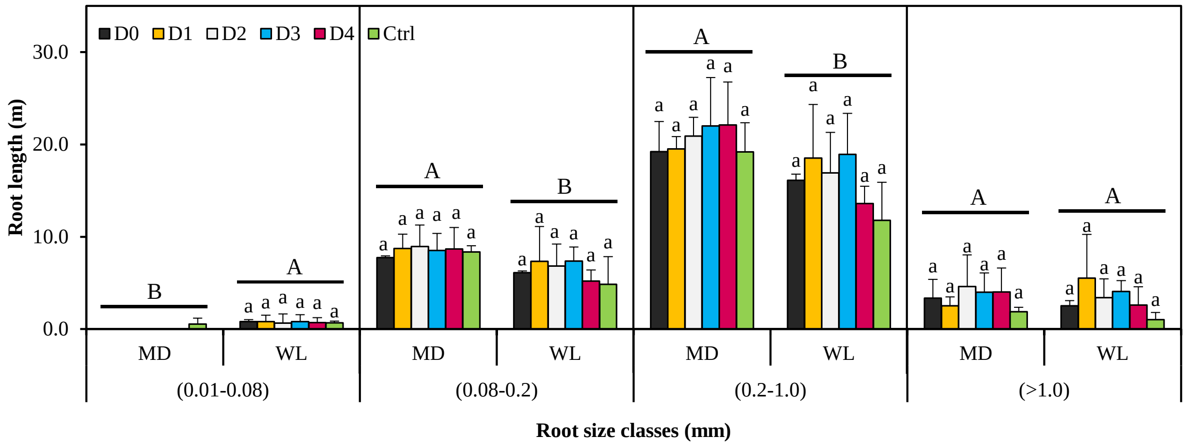

Figure 2-4. Root classes under moderately dry (MD) and waterlogged (WL) conditions. Under the MD condition, thick lateral roots (0.08–0.2 mm) but not fine lateral roots (0.01–0.08 mm) developed. Within the same root size classification, the same uppercase letter indicates that no significant difference existed between the water treatments ($p > 0.05$). Error bars show standard deviations. Root size did not differ significantly among the P treatment levels (D0–D4 and Ctrl) within the water treatments.

A significant difference ($p = 0.008$) in the mean shoot P concentration existed between the WL (3.6 mg g^{-1}) and MD (3.3 mg g^{-1}) water treatments. However, shoot P concentration showed no significant differences between P treatments under WL and MD water regimes (Figure 2-5A). The mean shoot P uptake showed a significant difference ($p = 0.01$) between the WL (6.7 g box^{-1}) and MD (5.1 g box^{-1}) water regimes; and between P treatments under the WL condition ($p = 0.024$) (Figure 2-5B). Compared with the D0 treatment, the P-dipping treatments (D1–D4) led to a 5% and 15% increase in the mean shoot P uptake under the MD and WL conditions, respectively. The mean shoot P uptake for all P treatments (D1–D4) was higher than the Control by 47% and 157% under the MD and WL conditions, respectively. Within the WL treatment, significant interaction effects for mean shoot P uptake existed between the water regimes and P treatments D1 (20 kg P ha^{-1}; $p = 0.01$) and D3 (60 kg P ha^{-1}; $p = 0.008$).

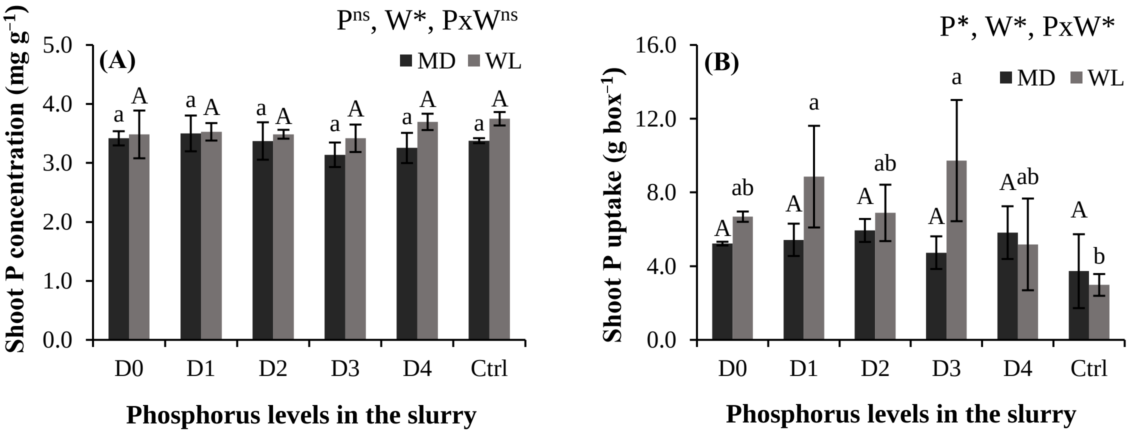

Figure 2-5. NERICA 4 shoot P concentration (A) and shoot P uptake (B) under the moderately dry (MD) and waterlogged (WL) treatments. D0–D4 and Ctrl indicate the various phosphorus (P) levels in slurry. *, $p < 0.05$; ns, not significant ($p > 0.05$); according to Tukey's test. Error bars show standard deviations. Different uppercase or lowercase letter combinations indicate significant differences at $p < 0.05$.

A strong positive linear relationship existed between shoot P uptake and root dry weight (Figure 2-6).

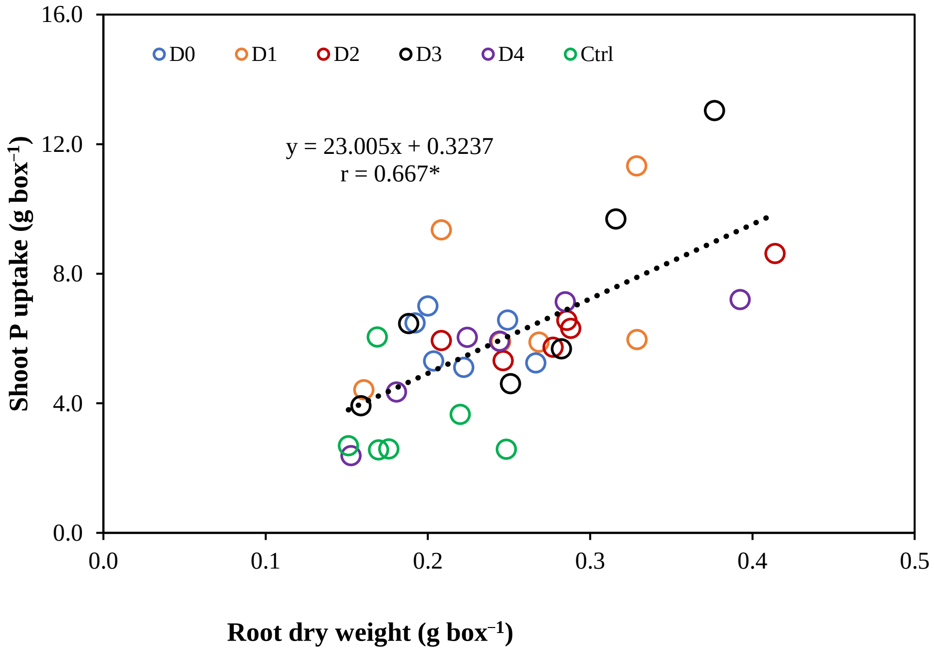

Figure 2-6. Relationship between shoot P uptake and root dry weight. Blue circles represent D0; orange circles, D1; red circles, D2; black circles, D3; purple circles, D4; and green circles represent the Control.

2.4 Discussion

2.4.1 Effects of P-dipping and the underlying mechanisms

Clear effects of P-dipping on shoot dry weight and plant length under the WL condition were observed in this study. The shoot parameters were increased most by the D2 and D3 P-dipping treatments, representing 40 and 60 kg P ha^{-1}, respectively. Generally, the shoot and root parameters either plateaued or were decreased at the D4 P-dipping level representing 80 kg P ha^{-1}. This may have been due to the effect of salt stress caused by the high P fertilizer rate, which likely injured the roots in conjunction with transplanting shock, slowing the overall recovery of plants and denying the benefits of P-dipping (Lu et al., 1982; Oo et al., 2020). Notably, for the measured root parameters, the D2 P level tended to lead to higher mean values under the MD condition, whereas the D3 P level led to higher values under the WL condition (Table 2-2). This might have been due to the increased available P in the soil solution, derived particularly from the soil P fraction under the WL condition. Oo et al. (2020) maintained the same amount of P in slurry but varied the quantity of slurry soil to evaluate the shoot biomass and shoot P uptake, and they recommended P-dipping in 4.3% P_2O_5 slurry for improving the P-use efficiency and biomass production of transplanted rice. Although their recommendation is relatively higher than our case, they used a forest subsoil (20–40 cm) layer for the slurry, which was likely low in available P content. In addition, their recommendation is based on a P concentration in the slurry "trapped" directly on the roots and transplanted along with the rice. However, in our case, mountain soil (Table 2-1) was used to make the slurry and our recommendation is based on the P concentration in the slurry container.

Compared with the MD treatment, the WL treatment led to higher values for shoot parameters such as plant length, leaf area, and shoot dry weight (Fig. 2-2). Optimum rice production is achieved under adequate moisture conditions (IRRI, 2014), and submerging soil under water is known to increase the P concentration in a soil solution (Turner and Gilliam, 1979). The mechanisms underlying increased available P in flooded or WL soil conditions were summarized by Deejay (2017) as follows: the release of P via the microbially-mediated reductive dissolution of Fe^{3+} oxides; the release of P during soil organic matter mineralization; and the solubility of iron phosphate due to increased pH. This is particularly useful in the tropical soils of SSA, where available P content is low owing to P-fixation by active Al and Fe oxides, their compounds, and humus complexes (Shang and Zelazny, 2008). However, NERICA 4 plants under MD conditions developed characteristics to adapt to the reduced moisture condition (discussed below), which allowed the plants to grow and develop under such conditions.

2.4.2 Changes in root morphology and distribution

Rice plants are reported to acclimatize to water stress through the modification of the roots, which become thicker and longer to take up nutrients and water from deeper layers in the soil, and translocation of assimilates to roots instead of shoots in response to drought stress (Yoshida and Hasegawa, 1982). Consistent with this notion, we found that the mean root length was higher under the MD condition than the WL condition (Tables 2-2 and 2-3). In addition, unlike under the WL condition, in which the root distribution between the top and bottom soil layers was significantly different (with fewer roots in the bottom soil layer), we observed active root growth in both soil layers under the MD condition (Table 2-3). We suggest that this root plasticity is one mechanism by which NERICA 4 adapts to water stress.

Root plasticity, defined as the ability of the root system to promote plant growth and development under changing soil conditions and mitigate the effects of stress while maintaining plant productivity (Wang and Yamauchi, 2006; Yamauchi et al., 1996), in terms of the development of deeper roots has been observed in previous studies on rice (Henry et al., 2011; Kano-Nakata et al., 2013; Suralta et al., 2010), millet (Rostamza et al., 2013), maize (Nakamoto, 1993), and bread wheat (Ehdaie et al., 2012; Wasson et al., 2012). The shallow root development under the WL condition could have been due to the effect of P-dipping coupled with the excess available moisture content; conversely, under the MD condition, root development was enhanced to survive under water stress conditions. Superficial rooting, i.e., the concentration of new root growth in the upper layers of the soil, has been reported as a morphological adaptation exhibited by roots in response to anaerobiosis (Jackson and Drew, 1984).

Oo et al. (2021) studied the effect of the synergy between a shallow root system and localized P application on P uptake in lowland rice (*O. sativa* var. IR64), finding that a positive interaction existed between P-dipping and shallow root development and that P-dipping improved P-use efficiency for initial rice growth. In an earlier study, Oo et al. (2020b) noted that P-dipping created hotspots of soluble P, which finding aligns with the P diffusion model of De Bauw et al. (2019a), where they deduced that larger proportions of applied P remain soluble with localized P application given that the binding sites around the application points get saturated. In our study, we also found that P-dipping accelerated shallow root development, which was observed particularly with the D3 treatment relative to the D0 (no P-dipping) or Control treatments under the WL condition (Table 2-2). Khanthavong et al. (2021) reported a similar result but used a NERICA 1 rice variety. Specifically, they observed that soil moisture exerted a significant effect on the total root length distribution across the soil layers in a root box, with total root length decreasing more under continuous soil waterlogging treatment, as compared to the moderate soil moisture and the gradual soil drying treatments.

We noted a higher root-to-shoot ratio in the Control root boxes (in which no external nutrient was added) under both water treatments compared with boxes in which some fertilizer was added (Table 2-2), indicating that the decreases in root-to-shoot ratio might have been caused either by the shortage of nitrogen or potassium in the soil or by the rice plant apportioning more assimilates to the root than the shoot as a nutrient stress survival mechanism. Narang et al. (2000) observed that a major trait of drought-tolerant plants is the enhanced development of their root system, which absorbs more nutrients and improves water uptake, resulting in a higher root-to-shoot ratio, as observed in the present study. Studies have shown that plant roots stimulate soil microorganisms in rhizosphere soil (Badri et al., 2009; Drogue et al., 2012); and that P addition increases microbial biomass in the rhizosphere (Griffiths et al., 2012; Li et al., 2015). While our study did not focus on the microbial biomass in the rhizosphere, it is possible that plant growth promoting rhizobacteria could have generally enhanced the P foraging efficiency of the P-dipping treatments (Bianciotto and Bonfante, 2002; Toro et al. 1997) above the D0 P treatment and Control.

The mean root cone angle under the MD condition was significantly narrower than the mean under the WL condition (Table 2-2). The combination of root length and root growth angle determines how deeply roots penetrate the soil and the spatial distribution of roots in the soil profile, which depends on the soil water level and plant growing conditions (Abe and Morita, 1994). The root cone angle is an important trait for increasing crop production under stress conditions (Uga et al., 2015). Studies in rice (Kato et al. 2006; Nakamoto, 1993) and bread wheat (Oyanagi et al., 1993) showed that plants with a narrow root cone angle exhibited increased root depth, which is consistent with our findings under the MD water condition (Table 2-2). Therefore, we postulate that NERICA 4 adapts to water stress through the narrowing of the root cone angle and development of deeper roots.

The type, size, and distribution of lateral roots directly affect the length and surface area of the root system; therefore, an understanding of the morphology of lateral roots is important for developing rice cultivars with an efficient root system (Casimiro et al., 2003; Robin and Saha, 2015). In the present study, our analysis of root diameter revealed that TLRs (roots of 0.08–0.20 mm in diameter) developed only under the MD condition (Fig. 2-3). Given that lateral roots are the functionally active part of the root system involved in nutrient acquisition and water uptake (Hannan et al., 2020), the development of TLRs under the MD condition may be another mechanism by which NERICA 4 adapts to water and nutrient stress.

The TLRs were more effective in terms of water and nutrient uptake than the FLRs (De Bauw et al., 2020) and even root hairs (Zobel, 1991). TLRs have higher penetration (Clark et al., 2008; Nguyen et al., 1997) and branching (Fitter, 1991; Ingram et al., 1994) abilities owing to the larger radii of xylem vessels and lower axial resistance to water flux (Yambao et al., 1992). These traits improve the resilience of rice crops under soil nutrient and water stress conditions, which is consistent with our findings for NERICA 4 in the present study. The high shoot P uptake could be associated with the root morphological parameters, which allowed exploration of greater soil volume. This is supported by the strong positive correlation that showed between shoot P uptake and root dry weight (Figure 2-6) regardless of the water regime and P treatment.

2.5 Conclusion

This study showed that P-dipping improves the resilience of NERICA 4 to withstand water and nutrient stress under rainfed-like conditions. The NERICA 4 root characteristics that exhibited plasticity to drought stress as adaptative mechanisms included the development of thick lateral roots, deeper root length, and narrower root cone angle. These findings provide new insights into the growth performance of NERICA 4 that will be useful for guiding further research on rice roots and developing water stress– and P stress–resilient varieties for use in SSA and other tropical/sub-tropical regions of the world.

CHAPTER THREE

Effect of P-dipping on growth of NERICA 4 rice in different soil types

3.1 Introduction

Phosphorus deficiency is one of the major constraints limiting sustainable rice production globally (Diagne et al., 2013; Saito et al., 2013; Tanaka et al., 2017). In sub-Saharan Africa, this is exacerbated by both limited mineral fertilizer inputs by smallholder farmers and dominant soil types—such as Ferralsols and Acrisols—within its humid and sub-humid agroecological zones (Bationo et al., 2006; Sileshi et al., 2022). These soil types are inherently low in nutrient contents, low in cation exchange capacities, and low in water-holding capacities (Bationo et al., 2006; Stocking, 2003), strongly leached and deeply weathered, with low pH and high Fe and Al oxide contents that increase the soil P fixing capacity (Balasubramanian et al., 2007; Bationo et al., 2006; Batjes, 2012; Shang & Zelazny, 2008). Large proportions of soil-derived or applied P thus remain unavailable for plant growth, presenting serious agronomic and economic challenges. Improved P acquisition and use by plants are thus of immediate and direct benefit to agriculture in SSA (Sileshi et al., 2022; Vandamme et al., 2018).

Several approaches to coping with the threats of P depletion have been studied, including the use of phosphate rocks (Nakamura et al., 2013; Sharma et al., 2013), breeding of crops that are tolerant to low P conditions (Ahmad et al., 2001; Gunes et al., 2006; Wissuwa & Ae, 2001), recycling P from wastewater (Cornel & Schaum, 2009; Yuan et al., 2012), and releasing fixed P in soil (Rakotoson et al., 2014; Shenker et al., 2005). Among those approaches, given the limited purchasing capacity of smallholder farmers and highly P-fixing soils in SSA, small-dose and localized P application near the root system has shown promise as a management practice (De Bauw et al., 2021; McKenzie & Roberts, 1990; Smith et al., 2001; Tabo et al., 2011). Similarly, the potential of P-dipping for lowland rice production—that is, dipping rice seedling roots into P-enriched slurry just before transplanting—has been found to improve rice seedling resilience to drought and P stresses (Odama et al., 2023), double applied P use efficiency (Oo et al., 2023), shorten days to heading, and increase yield grain (Rakotoarisoa et al., 2020).

On the other hand, soil texture has been widely demonstrated to exert a significant effect on P availability and use efficiency in crop production (Alhaj Hamoud et al., 2019; Azam et al., 2022; Dou et al., 2016; Jabborova, 2022; Martins et al., 2018; Mojid et al., 2020). Improving the opportunity for wider adoption of P-dipping techniques by farmers cultivating rice in diverse soil textures thus implies the importance of understanding the interactive effect of P-dipping and soil texture on rice growth performance. Furthermore, in contrast to excessive chemical fertilizer application rates required for the broadcasting method, which often lead to nutrient losses and cause eutrophication of fresh water, rising nitrous oxide emissions, and degradation of downstream water quality (Lu & Tian, 2017; Vitousek et al., 2009), P-dipping allows for relatively minimal P fertilizer amounts and employs a localized P application method directly to the roots, thereby contributing less to greenhouse gas emissions while contributing to sustainable rice production. The objective of this study was to evaluate the combined effect of P-dipping and soil texture on the initial growth of rice, focusing on shoot P uptake and root morphological development. The hypothesis was that clay soil, owing to its high water and nutrient retention capacities, is most suited to P-dipping.

3.2 Materials and Methods

3.2.1 Physiochemical characteristics of the experimental soils

The experimental soils with a range of textures were collected from Kagoshima (N31.8549 E130.2086), Tanegashima Island (N30.5331 E130.9586), and Tokunoshima Island (N27.8117 E128.8975), Japan. The soils were analyzed for pH (1:2.5 H_2O), available P was determined by Truog's method, and total carbon and nitrogen by the dry combustion method using an NC analyzer (JM1000CN/HCN TOC.TN, J-Science Lab Co., Ltd., Kyoto, Japan), the

1 mol L^{-1} ammonium acetate extraction method was used to determine exchangeable potassium, and soil texture was determined by the pipette method. We determined the acid oxalate extractable aluminum and iron content by ICP-MS (Eran DRC, PerkinElmer, Shelton, Connecticut, USA) after extraction with an acid ammonium oxalate solution (pH 3.0) for 4 h in darkness (Blakemore et al., 1987). We calculated soil organic matter content by multiplying the percentage of organic carbon with the conventional Van-Bemmelen's factor of 1.724 (Piper, 1950). The chemical and physical properties of the three experimental soils are presented in Table 3-1. Briefly, Kagoshima soil was sandy with a pH of 8.8 and low available P content. Tanegashima soil was clay loam with a pH of 4.9 and a relatively high content of available P. Tokunoshima soil was clay with a pH of 5.8 and the lowest content of available P.

Table 3-1. Experimental soil physical and chemical properties.

Property	**Kagoshima** [1]	**Tanegashima** [1]	**Tokunoshima** [1]
WRB classification	Arenosols	Andosols	Acrisols
pH (1:2.5 H_2O)	8.8	4.9	5.8
EC (mS m^{-1})	44.0	16.3	28.9
Total N (%)	0.02	0.19	0.10
Total organic C (%)	0.05	1.62	0.52
C:N ratio	0.9	8.5	5.3
Organic matter content (%)	0.09	2.79	0.90
Available P (mg kg^{-1})	24.5	186.5	18.3
Al oxalate (mg g^{-1})	9.1	25.2	13.2
Fe oxalate (mg g^{-1})	1.7	6.9	1.8
Sand (%)	95.6	30.8	12.6
Clay (%)	2.7	43.4	79.7
Silt (%)	1.7	25.8	7.7
Textural name	Sand	Clay loam	Clay

[1] Locations from which the experimental soil samples were taken.

3.2.2 Experimental design and the environmental condition

The experiment was conducted in a greenhouse using three soil types and three fertilizer treatments factorially combined in 3 replicates. The soil types included sand, clay loam, and clay soil textures, and the fertilizer treatments consisted of control (no P application), two broadcasts, and one P-dipping. We used perforated plastic pots (11 cm high, 9.5 cm bottom diameter, and 12.5 cm top diameter). We filled the pots with 1.5 kg of the three types of soil (bulk density: 1.2 g cm^{-3}) and placed the pots of each soil type in separate plastic containers (48 cm L × 32 cm W × 8 cm H) lined with black plastic sheets. To correct deficiencies in the soil N and K contents, we homogeneously mixed the experimental soil in each pot with 0.43 g of ammonium sulfate (90 mg N pot^{-1}) and 0.12 g of potassium chloride (50 mg K pot^{-1}). We filled the plastic containers with water to allow the soil in the pots to absorb by capillarity to the field capacities—volumetric soil moisture contents at 32% for sand soil, 42% for clay loam soil, and 48% for clay soil. Thereafter, we maintained water in the plastic containers holding the pots at 3–4 cm throughout the experiment.

NERICA 4 rice variety—an interspecific progeny between *O. sativa* and *O. glaberrima*—was grown in seedling trays until the 3–4 leaf stage and with an average of 5 cm of root system length for each seedling. Prior to transplanting, we carefully removed rice seedlings from the seedling tray to avoid root damage, and carefully hand-washed the nursery soil using water in plastic buckets fitted with 1 mm sieves to avoid root loss. For the P-dipping treatment, we dipped the washed seedling roots into the P-enriched slurry for 30 min (Oo, Tsujimoto, & Rakotoarisoa, 2020). To produce the P-enriched slurry, we mixed 45 g of air-dried soil, 14 mL of water, and 1.31 g of single superphosphate (SSP) fertilizer, an equivalent of approximately 68.7 mg P_2O_5 pot^{-1} for the P-dipping (Pdip) treatment.

The rest of the seedlings were transplanted without P-dipping in pots broadcasted with 0.25 g (43.1 mg P_2O_5 pot^{-1} (Brod1)) and 0.49 g (85.9 mg P_2O_5 pot^{-1} (Brod2)) of SSP fertilizer.

To avoid root damage during transplanting, we made holes approximately 6 cm deep and 3 cm wide in the wet soil within the pots before transplanting the rice seedlings. The daily mean air temperature (29.5 °C) and the daily mean relative humidity (70.5%) in the greenhouse were measured using a sensor equipped with a data logger (RTR-503, T&D Corporation, Tokyo, Japan) throughout the experiment. A summary of the P and soil treatments is presented in Table 3-2.

Table 3-2. Overview of the P and soil texture treatments.

Treatments	**Application rate (mg P_2O_5 pot^{-1})**	**Application method**	**Timing**
P application			
Pdip	68.7	P-dipping [2]	At transplanting
Brod1	43.1	Broadcasting	At transplanting
Brod2	85.9	Broadcasting	At transplanting
Ctrl	0	-	-
Soil texture	Quantity (kg soil pot^{-1})	Field condition volumetric moisture content (% *w*/*w*)	
Sand	1.5	32	
Clay loam	1.5	42	
Clay	1.5	48	

[2] The P-enriched slurry for the P-dipping treatment was produced by mixing 45 g of air-dried soil, 14 mL of water, and 1.31 g of SSP fertilizer. To correct deficiencies in the soil N and K contents, 90 mg N pot^{-1} and 50 mg K pot^{-1}, respectively, were homogeneously mixed with the experimental soil in each pot.

3.2.3 Data collection and measurements

At 40 days after transplanting (DAT), we measured the shoot parameters—plant height, leaf age, and Soil Plant Analysis Development (SPAD). We measured plant length from the base of the stem (at the soil surface) to the highest part of the plant. We determined leaf age by counting the number of fully expanded leaves per plant. We conducted gas exchange measurements on the uppermost fully expanded leaf at 38 DAT between 9:00 AM and 1:30 PM, using a portable gas exchange measurement system (LI-6400, Li-Cor Inc., Lincoln, NE, USA) set at a light intensity of 1200 μmol m^{-2} s^{-1}, a block temperature of 32 °C, and an ambient CO_2 concentration of 410 μmol mol^{-1}.

At the same time (40 DAT), the plant shoots in each pot were cut, and the leaves were removed to determine the leaf area using a digital image analysis machine (LIA32, Nagoya University, Nagoya, Japan). The leaves and stems were oven-dried at 80 °C for 48 h to determine the shoot dry weight per pot. The oven-dried plant materials were finely ground, and samples (0.5 g each) were wet-digested in 15 mL of di-acid digestion mixture [HNO_3:$HClO_4$ (3:2, *v*/*v*)]. Thereafter, the total P concentration in plant samples was determined in accordance with the vanadate–molybdate method (Chapman & Pratt, 1962) using a UV-VIS spectrophotometer (V-530, JASCO Co., Tokyo, Japan). We calculated shoot P uptake as the product of shoot dry weight and P concentration.

In preparation for the root analysis, the soil in each pot was carefully removed, placed in a metallic 2 mm gauge sieve, and carefully washed by spraying with low-pressure tap water to rid the roots of all soil particles. The root samples were placed in self-sealing plastic bags containing 50% aqueous ethanol solution and stored in a cold room at 4 °C prior to scanning. Root samples were scanned at 6400 dpi using an Epson scanner (EPSON GT-X830, Epson American Inc., Los Alamitos, CA, USA), and images were analyzed at pixel classification values of 130–150 using the WinRhizo software (WinRHIZO, Regent Instruments Inc., Québec, Canada; Version 2005b) to determine the total root length (RL), root surface area (RSA), and root volume (RV). Following the root morphological analysis, root samples were dried at 80 °C for 48 h in an oven to determine the root dry weight per pot.

3.2.4 Statistical analyses

Data analyses were conducted with IBM SPSS Statistics (Version 27.0.1.0) using two-way ANOVA to determine the single and interaction effects of P treatments (Pdip, Brod1, Brod2, and Ctrl) and soil textures (sand, light clay, and clay). The treatment means were compared from replicates at the 5% level of probability using Tukey's HSD test. Where significant interaction effects existed, we ran pairwise comparisons for each simple main effect, modifying statistical significance with a Bonferroni adjustment.

3.3 Results

3.3.1 Changes in shoot biomass, root biomass, and shoot P uptake

Soil texture and P application methods significantly affected mean shoot biomass, mean root biomass, and mean shoot P uptake (Figure 3-1). Across soil textures and P treatments, total biomass ranged from 1.06 to 4.63 g pot^{-1}. The Pdip treatment significantly increased shoot biomass relative to Control from 1.27 to 1.98 g pot^{-1} (Figure 3-1a). Similarly, amongst the P treatments, Pdip significantly increased mean root biomass by 53% relative to Control (Figure 3-1b). Whereas no statistical difference in mean shoot P uptake existed between Control and Pdip, the Pdip treatment resulted in a 49% increase in shoot P uptake relative to Control (Figure 3-1c). No significant interaction effects between soil texture and P treatments existed for shoot biomass, root biomass, and shoot P uptake.

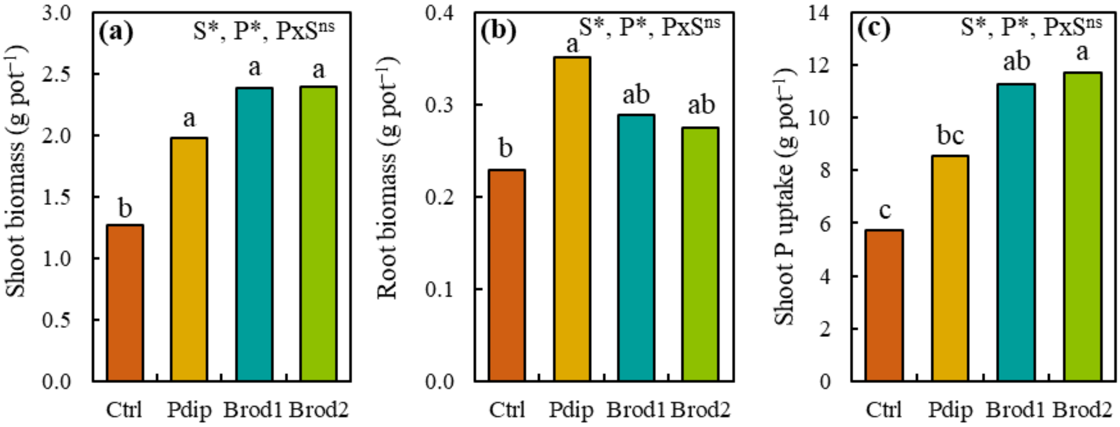

Figure 3-1. Comparison of means from the effect of P treatments (P) on shoot mass (**a**), root mass (**b**), and shoot P uptake (**c**) at 40 days after transplanting. S, soil texture; *, $p < 0.05$; ns, not significant according to Tukey's HSD test. Different lowercase letters above P treatments indicate significant differences between P treatments at $p < 0.05$.

3.3.2 Changes in shoot physiology and morphology

Plant height tended to increase with P application rate under sand and clay soil textures, but under the light clay soil texture, plant height decreased with increased P rate from Brod1 to Brod2 (Table 3-3).

Table 3-3. Shoot morphological changes related to soil texture and P treatments.

Soil Texture (S)	Phosphorus Treatment (P)	Plant Height (cm)	Leaf Age	Leaf Area (cm^2 pot^{-1})	SPAD Value
Sand	Ctrl	55.6 ± 1.8 c	5.0 ± 0.01 b	197.3 ± 10.9 b	14.3 ± 2.4 b
	Pdip	63.1 ± 2.5 b	5.0 ± 0.01 b	238.4 ± 17.4 b	21.7 ± 1.1 a
	Brod1	66.2 ± 2.7 b	7.3 ± 0.58 a	312.0 ± 17.4 a	24.0 ± 1.3 a
	Brod2	72.6 ± 2.1 a	7.2 ± 1.44 a	305.7 ± 27.5 a	17.6 ± 0.1 b
Clay loam	Ctrl	81.7 ± 2.8 b	9.5 ± 0.87 a	447.2 ± 10.7 a	43.5 ± 0.8 b
	Pdip	87.3 ± 3.2 ab	9.3 ± 0.58 a	501.3 ± 16.7 a	47.1 ± 0.3 a
	Brod1	89.6 ± 1.7 a	9.2 ± 0.58 a	499.5 ± 35.6 a	45.6 ± 0.6 a
	Brod2	86.9 ± 0.9 ab	8.8 ± 0.29 a	500.7 ± 12.0 a	46.1 ± 0.6 a
Clay	Ctrl	65.0 ± 5.9 b	4.7 ± 0.58 b	180.1 ± 30.9 c	39.7 ± 1.4 c
	Pdip	81.0 ± 5.5 a	5.0 ± 0.01 b	265.2 ± 28.3 b	43.5 ± 0.6 b

	Brod1	84.8 ± 5.9 a	6.8 ± 0.29 a	368.2 ± 10.3 a	45.8 ± 0.4 a
	Brod2	89.2 ± 1.0 a	7.0 ± 0.50 a	418.4 ± 10.1 a	47.9 ± 0.6 a
Two-way ANOVA	S	*	*	*	*
	P	*	*	*	*
	S × P	*	*	*	*

NERICA 4 shoot morphological changes under sand, clay loam, and clay soil texture and phosphorus treatments including Pdip, Brod1, Brod2, and Ctrl at 40 days after transplanting. *, $p < 0.05$ according to Tukey's HSD test. Values are means ± standard deviations ($n = 3$). Different lowercase letters after parameter values indicate significant differences at $p < 0.05$ within each soil texture.

Mean plant height differed significantly ($p < 0.05$) between clay loam (86.4 cm), clay (79.9 cm), and sand (64.4 cm) soil textures. Mean plant height also differed significantly ($p < 0.05$) between Brod2 (82.9 cm), Brod1 (80.2 cm), Pdip (77.1 cm), and Ctrl (67.4 cm) treatments. Significant interaction effects ($p < 0.05$) between soil textures and P treatments emerged for mean plant height, plant leaf age, leaf area, and SPAD values (Table 3-3). Mean leaf age was significantly affected by clay loam (9.2), sand (6.1), and clay (5.9) soil textures. Plant leaf area was significantly affected by both soil texture and P treatments, with values of 473.4–500.9, 294.2–321.7, and 249.6–277.1 cm^2 pot^{-1} under clay loam, clay, and sand soil textures, respectively. The Pdip treatment showed a significant 47% increase in mean leaf area relative to Control only under clay soil. Under the three soil textures used, the P treatment significantly affected SPAD values, with Pdip showing a 51.7%, 9.6%, and 8.3% increase relative to Control under sand, clay, and clay loam soil textures, respectively.

3.3.3 Gas exchange parameters

In Figure 3-2 we present the changes in the four gas exchange parameters—photosynthetic rate (*A*), stomatal conductance (*gs*), transpiration rate (*E*), and intercellular carbon dioxide concentration (*Ci*)—under soil texture and P treatments, both of which significantly affected all the gas exchange parameters. The effect of soil texture on *A*, *gs*, *E*, and *Ci* showed a consistent tendency where, under clay loam soil texture and sand soil texture, we observed the highest and lowest mean values for all the stated parameters, respectively.

While P treatments did not show such consistent changes across the gas exchange parameters, significant differences existed within each parameter. For instance, mean *A* values under Pdip (20.1 µmol m^{-2} s^{-1}), Brod2 (19.5 µmol m^{-2} s^{-1}), and Brod1 (19.3 µmol m^{-2} s^{-1}) treatments showed significant ($p < 0.05$) 42%, 37%, and 36% increases over the Control, regardless of soil texture (Figure 3-2a). Across both soil textures and P treatments, *gs* values ranged from 0.3 to 1.4 mol m^{-2} s^{-1} while *E* values ranged from 5.4 to 15.5 mmol m^{-2} s^{-1}. Both *gs* and *E* had similar tendencies where under clay loam soil texture, Pdip treatment showed the highest values for *gs* (1.4 mol m^{-2} s^{-1}; Figure 3-2b) and *E* (13.5 mmol m^{-2} s^{-1}; Figure 3-2c).

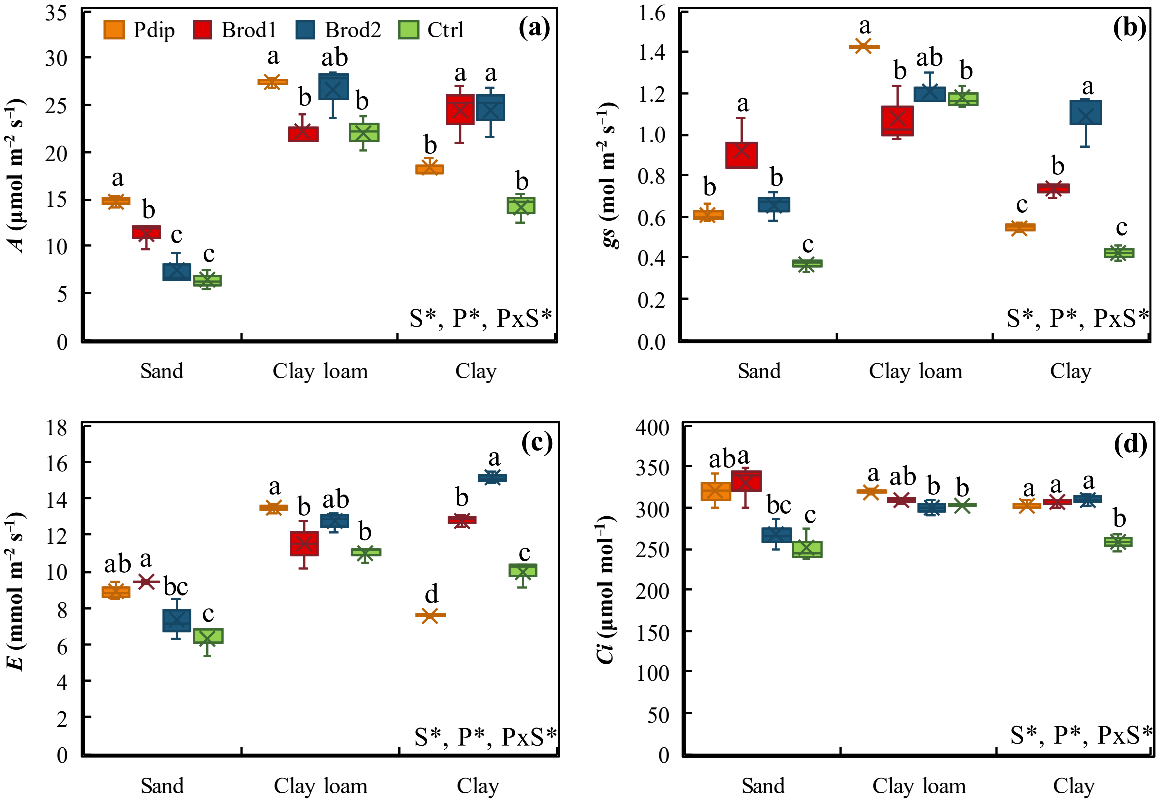

Figure 3-2. Boxplots of the responses of NERICA 4 photosynthetic rate (**a**), stomatal conductance (**b**), transpiration rate (**c**), and intercellular CO_2 concentration (**d**) to sand, clay loam, and clay soil textures planted in P treatments including Pdip, Brod1, Brod2, and Ctrl. *, $p < 0.05$ according to Tukey's HSD

test. Different lowercase letters above P treatments indicate significant differences at $p < 0.05$ within each soil texture.

We observed significant changes in *Ci* between soil textures ($p = 0.017$) and P treatments ($p < 0.05$) (Figure 3-2d). For all the gas exchange parameters, we observed significant interaction effects ($p < 0.05$) between soil textures and P treatments.

3.3.4 Changes in root morphology and shoot P uptake

In Table 3-4 we present the changes in root morphology related to soil texture and P treatments.

Table 3-4. Root morphological changes related to soil texture and P treatments.

Soil Texture (S)	Phosphorus Treatment (P)	Total Root Length	Root Surface Area	Root Volume	Root Length Ratio	Root Mass Ratio	Root to Shoot Ratio
		(m pot^{-1})	(cm^2 pot^{-1})	(cm^3 pot^{-1})	(m g^{-1})	(g g^{-1})	
Sand	Ctrl	33.9 ± 1.3 c	519.7 ± 6.8 c	4.5 ± 0.1 a	29.0 ± 1.1 ab	0.13 ± 0.02 a	0.15 ± 0.03 a
	Pdip	52.0 ± 0.8 a	557.6 ± 8.7 b	4.8 ± 0.3 a	38.2 ± 2.6 a	0.12 ± 0.01 ab	0.13 ± 0.01 a
	Brod1	52.5 ± 0.9 a	588.6 ± 9.9 a	4.9 ± 0.4 a	27.8 ± 5.8 ab	0.11 ± 0.01 ab	0.12 ± 0.02 ab
	Brod2	36.9 ± 0.9 b	361.6 ± 6.4 d	2.8 ± 0.1 b	20.3 ± 5.2 c	0.09 ± 0.02 b	0.10 ± 0.02 ab
Clay loam	Ctrl	27.2 ± 6.0 b	404.2 ± 5.9 d	4.1 ± 0.1 c	13.6 ± 1.2 ab	0.25 ± 0.04 a	0.13 ± 0.06 a
	Pdip	31.9 ± 6.4 b	481.6 ± 9.4 c	4.8 ± 0.1 c	9.8 ± 2.9 b	0.24 ± 0.04 a	0.12 ± 0.04 a
	Brod1	43.0 ± 5.9 b	670.9 ± 29.4 b	6.7 ± 0.4 b	12.6 ± 2.0 b	0.14 ± 0.01 b	0.08 ± 0.01 a
	Brod2	63.7 ± 6.2 a	852.4 ± 29.7 a	8.1 ± 0.9 a	20.2 ± 4.0 a	0.13 ± 0.02 b	0.09 ± 0.02 a
Clay	Ctrl	60.2 ± 0.2 c	717.2 ± 7.2 c	6.7 ± 0.2 b	47.1 ± 8.4 a	0.11 ± 0.05 a	0.33 ± 0.06 a
	Pdip	115.9 ± 3.0 a	1286.3 ± 23.9 a	11.8 ± 0.5 a	53.2 ± 3.9 a	0.10 ± 0.03 a	0.33 ± 0.08 a
	Brod1	66.1 ± 3.7 c	781.8 ± 25.5 b	7.3 ± 0.6 b	25.2 ± 1.0 b	0.08 ± 0.01 a	0.17 ± 0.01 b
	Brod2	75.2 ± 3.5 b	692.5 ± 23.0 c	6.4 ± 0.5 b	27.4 ± 6.3 b	0.08 ± 0.02 a	0.15 ± 0.03 b
Two-way ANOVA	S	*	*	*	*	*	*
	P	*	*	*	*	*	*
	S × P	*	*	*	*	*	ns

NERICA 4 root morphological changes under sand, clay loam, and clay soil texture and phosphorus treatments including Pdip, Brod1, Brod2, and Ctrl at 40 days after transplanting. *, $p < 0.05$ according to Tukey's HSD test. Values are means ± standard deviations (*n* = 3). Different lowercase letters after parameter values indicate significant differences at $p < 0.05$ within each soil texture.

Broadly, the values of all root morphological parameters typically increased with an increase in the P rate from Brod1 to Brod2 under clay and clay loam soil textures but decreased under sand soil texture (Table 3-4). Specifically, we observed a significant difference ($p < 0.05$) in mean total root length (RL) between clay, sand, and clay loam soil textures. There was also a significant difference ($p < 0.05$) in the root length between P treatments, with the highest mean RL under Pdip treatment compared to Brod1, Brod2, and Ctrl treatments. RL showed significant interaction effects between soil texture and P treatment ($p < 0.05$), and analysis of the simple main effects for P treatment showed that Pdip had the highest effect size (partial $\eta^2 = 0.97$). Pairwise comparisons showed the mean RL under Pdip treatment and clay was 83.9 points higher than that under clay loam ($p < 0.05$), and 63.9 points higher than that under sand ($p < 0.05$) soil textures.

In striking contrast, whereas the mean RL under clay (79.4 m pot^{-1}) was significantly higher than that under clay loam (41.5 m pot^{-1}) soil texture, the mean shoot P concentration and shoot P uptake under clay loam soil were significantly higher than those under clay soil texture (Figure 3-3).

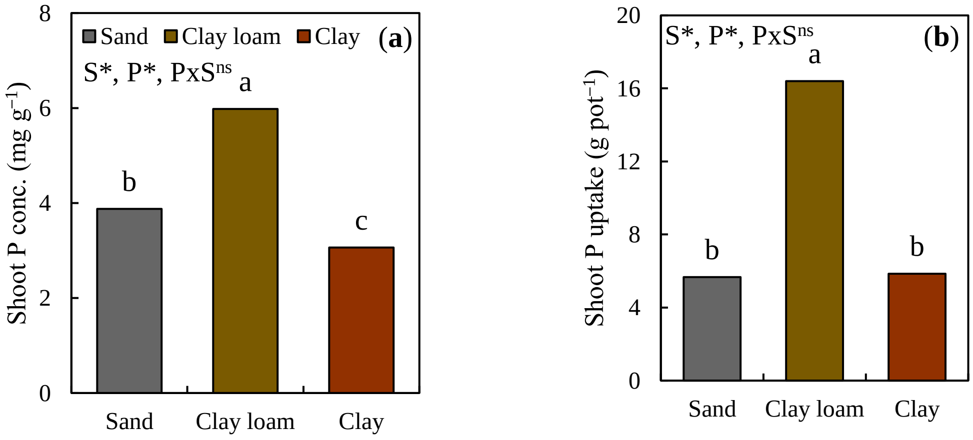

Figure 3-3. Comparison of means from the effect of soil texture (S) on shoot P concentration (**a**) and shoot P uptake (**b**) across P treatments (P) at 40 days after transplanting. *, $p < 0.05$; ns, not significant, both according to Tukey's HSD test. Different lowercase letters above soil textures indicate significant differences between soil textures at $p < 0.05$.

Indeed, we had expected the higher RL under clay soil texture to result in higher shoot P concentration and shoot P uptake values under clay soil texture—but that was not the case. The mean shoot P uptake under clay loam was 180% greater than that under clay soil texture. Root surface area, ranging from 335.6 to 1310.3 cm^2, showed a similar trend to that observed in the root length, where Pdip treatment gave the highest value under clay soil texture (Table 3-4). The mean RSA differed significantly ($p < 0.05$) between that under clay (869.5 cm^2), clay loam (602.3 cm^2), and sand (557.9 cm^2) soil textures. Mean RSA also differed significantly ($p < 0.05$) between P treatments, with mean RSA under Pdip treatment showing 17.8% and 41.7% greater value relative to the combined broadcasting treatments (Brod1 and Brod2) and Ctrl, respectively, with significant interaction effects between soil texture and P treatment for RSA ($p < 0.05$). Among P treatments, Pdip treatment showed the highest simple main effect size (partial $\eta^2 = 0.99$). Pairwise comparisons indicated the mean RSA from the Pdip treatment under clay soil texture was 362.6 and 267.2 points higher than that under sand ($p < 0.05$) and clay loam ($p < 0.05$) soil textures, respectively.

Root volume showed similar morphological changes to RL and RSA, where significant differences in the mean RV under clay soil (8.1 cm^3; $p < 0.05$) were the highest compared to values under the clay loam and sand soil textures. Significant differences ($p < 0.05$) in RV also existed between P treatments, with Pdip treatments showing the highest value (7.1 cm^3) among P treatments. Pairwise analysis of the simple main effects among the P treatments showed that Pdip under the clay soil accounted for the highest (partial $\eta^2 = 0.96$) significant interaction effects in RV.

The changes in root length ratio (RLR)—that is, RL per total biomass—express the root's potential for the acquisition of soil resources. On the other hand, root mass ratio (RMR)—that is, root biomass per total biomass—is an indicator of the biomass allocated to the roots. Soil texture and P treatments significantly affected RLR and RMR (Table 3-4). Significantly, under clay and clay loam soil textures we observed the highest (38.3 m g^{-1}) and lowest (14.0 m g^{-1}) mean RLR values, respectively. Among the P treatments, Pdip was associated with a significant 51.6% increase in the mean RLR relative to the combined broadcasting treatments (Brod1 and Brod2).

Similarly, clay soil showed the significantly highest (0.19 g g^{-1}) mean RMR, and relative to the combined broadcasting treatments, the Pdip treatment also showed a significant 46.9% increase in RMR. Both RLR and RMR were significantly affected by interactions between soil texture and P treatments. The mean root-to-shoot ratio under clay soil (0.25) was significantly higher ($p < 0.05$) than that under sand (0.13) and clay loam (0.10) soil textures (Table 3-4). Among P treatments, we observed the highest and lowest mean root-to-shoot ratios under Ctrl (0.21) and Brod2 (0.11), respectively. The mean root-to-shoot ratio under Pdip (0.19) was equally high but did not differ significantly from that under Control.

3.4 Discussion

3.4.1 Soil texture and P-dipping effects on rice shoot morphology

Our findings demonstrated that P-dipping and soil texture each separately affected rice shoot biomass and shoot P uptake, and they interactively affected plant height, leaf age, leaf area, and SPAD. The mean P-dipping values for above-ground parameters—including plant height, leaf area, SPAD, and shoot biomass—showed significant increases relative to Control across all soil textures. While we had hypothesized that clay soil, owing to its high water and nutrient retention capacities, is most suited to P-dipping the interactive effects between P-dipping and soil texture on plant height, leaf age, leaf area, and SPAD showed that clay loam soil texture exerted the most significant effect.

The higher quantities of available P, organic matter, and nitrogen initially present in clay loam may have accounted for the better shoot growth performance under the clay loam soil. On the other hand, because fertilizer P added to soil rapidly forms insoluble complexes in Acrisols (Bationo et al., 2006; Nziguheba et al., 2002), we postulate that though P fertilizer was added to the Acrisol clay soils it may have been fixed and its effect may have been neutralized in the shoot. Miller et al. (2001) suggested that plant acquisition of P from soil organic matter is enhanced by the secretion of low-affinity enzymes into the soil to provide additional P for plant growth.

Studies have also shown that hydrolysis of organic matter contributes to the amounts of soluble P in the soil solution (Hinsinger, 2001; Matar et al., 1992; Takahashi & Katoh, 2022). Thus, the low organic matter and nutrient contents in sand soil on the one hand, and the possible diffusion away of the applied soil P from the point of application, on the other, may have contributed to the overall low shoot growth response to P application in sand soil (De Bauw et al., 2019; Oo, Tsujimoto, et al., 2020). The high pH in sand soil may have also contributed to the decline in root activity (Kobayashi et al., 2010; Turner et al., 2020), which could in turn have negatively impacted nutrient and water absorption, leading to low shoot growth under sand soil.

3.4.2 Changes in photosynthetic rate under different soil textures

In this study, results of the gas exchange measurements showed that soil texture and P treatments significantly affected the photosynthetic rate of NERICA 4, with the highest mean values obtained under the clay loam soil texture (24.6 μmol m^{-2} s^{-1}) and the P-dipping treatment (20.1 μmol m^{-2} s^{-1}), respectively. With reference to the conclusion by Yang (Yang et al., 2017) that photosynthetic capacity is closely related to the leaf N content, our findings regarding the photosynthetic rate may be explained by the differences in the SPAD values as an estimate of leaf N content, where the highest mean SPAD values were equally obtained under clay loam (45.6) soil texture treatment, and Brod1 (38.5) and Pdip (37.4) P treatments (Table 3-3). The high N content of clay loam soil may have been taken up to the plant leaves, resulting in a high photosynthetic rate under clay loam soil texture. On the other hand, P-dipping may have boosted root growth (Oo, Tsujimoto, et al., 2020), leading to an enhanced P uptake under the Pdip treatment compared to that under Brod1 and Brod2 P application treatments.

3.4.3 Changes in root morphology and the effect on shoot P uptake

Plant roots are directly exposed to the rhizosphere soil, thereby providing the primary channel for nutrient acquisition and its subsequent utilization for plant growth. Root growth and development depend on several soil factors, including texture and density, water and nutrient contents, and concentration of oxygen (Lloret & Casero, 2002; Lynch, 1995; Raven & Edwards, 2001). Our findings here showed that the combined effects of soil texture and P-dipping significantly influenced NERICA 4 root morphology. Specifically, the mean values for RL, RSA, RV, and root biomass under clay soil texture and P-dipping treatment were significantly higher than those for other treatments. The low available P content in the clay experimental soil may have triggered the observed extensive root growth, as the relieved P constraints possibly led to increased soil microbial mass, and consequently an increased microbial utilization of soil carbon for increased root development (Griffiths et al., 2012; Wang et al., 2019).

Increased root morphological characteristics under P-deficient conditions have been reported for enhanced P absorption (He et al., 2003; Lynch & Bates, 2001; Ma et al., 2001). This has further been evidenced by high root-to-shoot ratios, which are generally inversely related to soil nutrient and water availability, as plants allocate more photosynthates to their roots for increased soil exploration (Ho et al., 2005; Prescott et al., 2020; Wang et al., 2021).

While some studies have also shown strong positive linear relationships between root morphological characteristics and P acquisition under P-deficient conditions (Aziz et al., 2011; De Bauw et al., 2018; de Souza Campos et al., 2022), our results showed the opposite—particularly under P-deficient clay soil texture. In our findings, the mean shoot P concentration under the clay texture was −48.8% lower than that under clay loam soil, yet the mean RL under clay texture was 91.4% higher than the mean RL under clay loam soil texture (Table 3-4; Figure 3-3). This suggests that enhanced root morphology does not necessarily enhance P uptake in the initial rice growth stages, and thus, further research needs to be carried out to evaluate the potential of NERICA 4 rice to increase its P acquisition and utilization efficiencies at later stages of the cropping cycle for increased grain yield.

The lower shoot P content of plants under clay soil texture—despite having the most robust root biomass—could be due to the remobilization of the shoot P into the roots. Similar studies by Abdallah et al. (2010) and Irfan et al. (2019) found that in P-deficient soils, shoot P was remobilized or translocated from metabolically inactive to active sites such as the roots; in our study, the clay soil was P-deficient (Table 3-1). On the other hand, we think that the combination of higher root biomass with low plant tissue P concentration in the P-deficient clay soil can be explained by the Piper–Steenbjerg effect (Wikström, 1994), summarized concisely by De Bauw (De Bauw et al., 2021) as follows: Low tissue P concentrations occur when the fast growth of plants grown in an initially higher P medium (locally after placement) eventually leads to a more rapid depletion of external P than the slow growth of plants grown in an initially lower P medium, as was the case in our study.

3.5 Conclusions

We evaluated the combined effect of soil texture and P-dipping on NERICA 4 rice shoot and root physiology and morphology, with a major focus on shoot P uptake in the initial growth stages. Contrary to our hypothesis, the interactive effect of soil texture and P-dipping influenced NERICA 4 shoot and root physiological and morphological characteristics mainly under clay loam rather than clay soil. The clay loam soil examined in our study showed higher shoot morphological characteristics despite the relatively lower root biomass. On the other hand, P-dipping significantly promoted rice root morphology under clay soil, but without a commensurate shoot P concentration and uptake. This suggests that enhanced root morphology does not necessarily enhance P uptake in the initial rice growth stages; thus, further research is necessary to evaluate the potential of NERICA 4 rice to increase its P acquisition and utilization efficiencies at later stages of the cropping cycle for increased grain yield. The findings of our study provide new insights into the existing body of knowledge on the widely adapted NERICA 4 rice variety across SSA, which should ultimately contribute to improving sustainable food security among smallholder farmers in the region.

CHAPTER FOUR

General Discussion

Rice is currently the second largest source of caloric intake in SSA; as such, its demand is anticipated to rise steadily across the region. The high rate of population growth and rapid urbanization in SSA is also expected to result in a shift in consumer preference in favour of rice (Balasubramanian et al., 2007; van Oort et al., 2015). As such, the need to improve the still-relatively low rice yields being obtained in the region keeps increasing. Indeed, much research investment has gone into yield improvement from genetics, which partly has resulted in the release of the upland and lowland NERICA cultivars. The improved cultivars notwithstanding, the average rice yield in SSA remains one of the lowest among rice growing regions (USDA, 2018). In the wake of the declining soil P and increasing cases of drought (Zhao et al., 2015), efforts that can improve P acquisition and use-efficiency can go a long way to improving rice yield since reduced water supply also reduced P mobility in soils due to reduced effective diffusion (Kirk et al., 1990).

Studies have shown that root traits associated with maintaining plant productivity under drought include small fine root diameters, long specific root length, and considerable root length density, especially at depths in soil with available water (Comas et al. 2013). Our study showed similar results (Chapter 2) where a significant difference in total root length existed between the moderately dry (32.6 m) and waterlogged (25.1 m) water treatments. Specific root length under the moderately dry condition was also significantly higher than that under the waterlogged treatment. The findings also revealed that NERICA 4 root plasticity response to moisture stress involved a significant narrowing of the root cone angle under the MD condition (102.3°) in contrast to the WL condition (158.4°). Studies in rice and wheat have demonstrated that narrowing of root cone angel is an important trait for increasing crop production under water stress (Kato et al. 2006; Nakamoto, 1993; Oyanagi et al., 1993; Uga et al., 2015). While such root plasticity of NERICA 4 in response to limited moisture stress gives it the latitude to withstand the drought events during the growing season, enhancing the soil P level equally plays a crucial role in contributing to increased yield.

Phosphorus is responsible for the root development, ripening, early flowering and tolerance to specific biotic and abiotic stresses in rice. Its deficiency contributes to delays in maturity and increased rice vulnerability to diseases (Fageria et al., 2003). Owing to the highly weathered soils in the tropics (Henao and Baanante, 2006) that have low acidity and high content of Fe and Al oxides (Bekunda et al., 2010; Nishigaki et al., 2019), the available P content soils within the SSA is low. While the P deficiency problem is largely being addressed by use of chemical fertilizers, the prohibitively high costs of fertilizers are preventing farmers from applying recommended. Besides, with most farmers using the broadcasting method to apply inorganic fertilizers in the region (Tabo et al., 2006) the environmental risk through runoffs is high. To that end, the P-dipping technique that reduces the risk of runoff, boosts root growth, reduces time to maturity, provides a bright prospect for sustainable and environmentally friendly rice yield improvement in SSA (Oo et al., 2023; Rakotoarisoa et al., 2020; Tsujimoto et al., 2021).

In-depth research into the effect of P-dipping under different soil textures is largely limited, yet studies have revealed that soil texture exerts a significant effect on P availability and use efficiency (Martins et al., 2018; Mojid et al., 2020). That assertion partly instigated the second study we conducted on the combined effect of soil textures and P-dipping on NERICA 4 rice shoot and root physiology and morphology, with a major focus on shoot P uptake in the initial growth stages (Chapter 3). Our findings revealed that soil texture significantly affected total root length, which soil texture had significant interaction effects with P treatments among with P-dipping showed the highest interaction effect size. Mean root surface area also differed significantly between soil textures and P treatments with the P-dipping treatment showing 17.8% and 41.7% greater values relative to the combined broadcasting treatments (Brod1 and Brod2) and control, respectively. Studies conducted by De Bauw et al. (2019a) on P micro-dosing in SSA have shown that localized P placement technologies (such as P-dipping) reduce P diffusion in fine-textured soils and enhance the P uptake under field conditions. Their findings also revealed that localized P placement methods showed higher agronomic efficiency of P fertilizer and led to positive P balance in the subsequent cropping season. In light of these findings, we propose that there is need for further research to understand the effect of P-dipping and soil texture under different water regimes on rice growth and yield particularly at later growth stages.

CHAPTER FIVE

General Conclusion

To gain understanding on how NERICA rice enhances P uptake and use efficiency under P deficient and drought stress conditions for improved yield, we evaluated (i) the combined effect of P-dipping and moisture stress, and (ii) the combined effect of P-dipping and soil texture on NERICA 4 rice shoot and root morphology and physiology at the initial growth stages. For the first study, we hypothesized that P-dipping improves NERICA 4 seedling resilience, allowing seedlings to withstand water and P stress under rainfed-like conditions. In line with the hypothesis, results showed that rice shoot biomass and plant length differed significantly under the various P-dipping application levels, with an application of 40 kg P ha^{-1} providing the highest mean values under both water treatments. We also observed that NERICA 4 develops deep roots, narrows the root cone angle, and develops thick lateral roots as mechanisms to adapt to drought conditions in drought conditions.

In the second study, we hypothesized that clay soil, owing to its high water and nutrient retention capacities, is most suited to P-dipping. Contrary to our hypothesis, the interactive effect of soil texture and P-dipping influenced NERICA 4 shoot and root physiological and morphological characteristics mainly under clay loam rather than clay soil. The clay loam soil examined in our study showed higher shoot morphological characteristics despite the relatively lower root biomass. On the other hand, P-dipping significantly promoted rice root morphology under clay soil, but without a commensurate shoot P concentration and uptake. This suggests that enhanced root morphology does not necessarily enhance P uptake in the initial rice growth stages; thus, further research is necessary to evaluate the potential of NERICA 4 rice to increase its P acquisition and utilization efficiencies at later stages of the cropping cycle for increased grain yield.

These findings provide new insights into the ongoing and relatively infant research int the mechanisms by which the NERICA cultivars adapt to P and moisture stresses, which findings should ultimately contribute to improving sustainable food security among smallholder farmers in the region.

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List of Conference Presentations

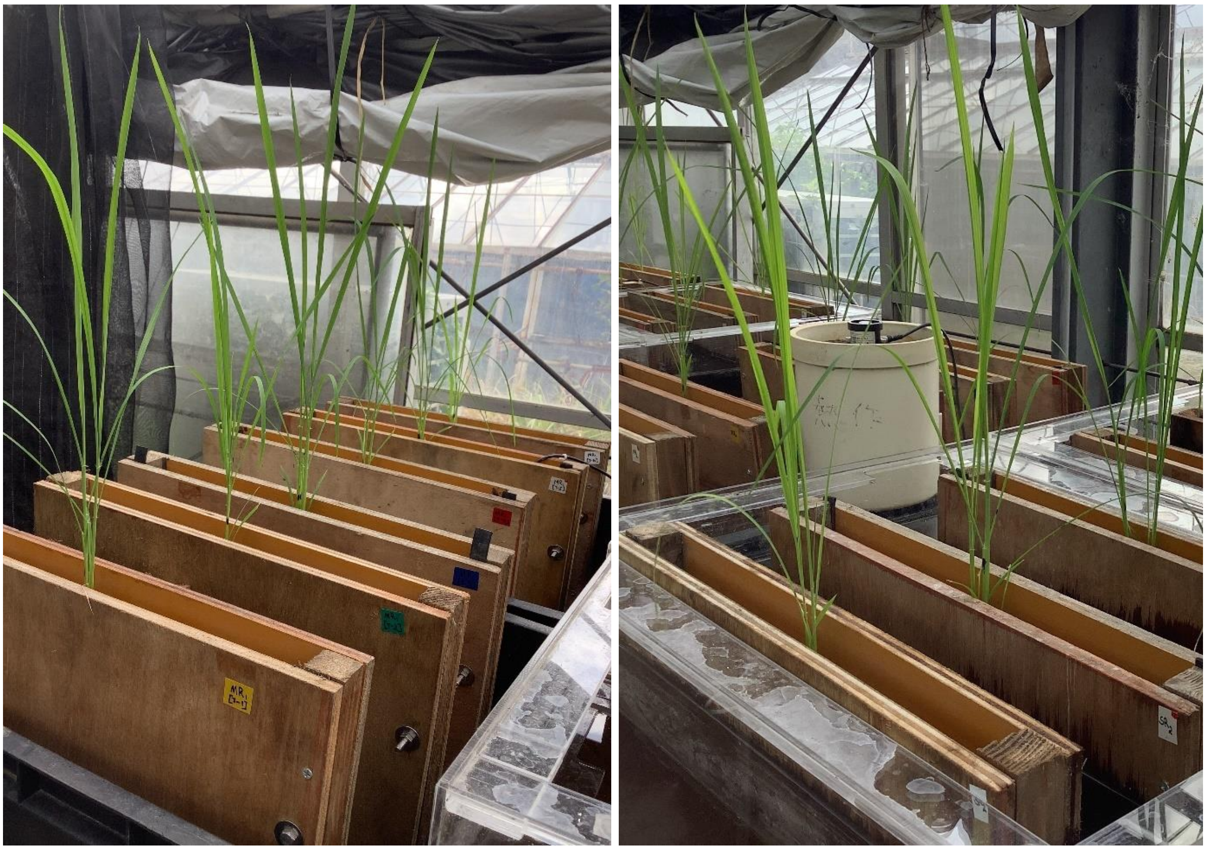
1. Emmanuel ODAMA, Yasuhiro TSUJIMOTO, Shin YABUTA, Isao AKAGI, Rael CHEPKOECH, Ibrahim SOE, and Jun-Ichi SAKAGAMI. Oral Presentation title: *Soil texture influenced the effect of P-dipping on NERICA 4 rice root morphology in early growth stages*; Presented during the 57th Japan Society of Root Research Conference held at Meiji University, May 20-21, 2023.
2. Emmanuel ODAMA, Yasuhiro TSUJIMOTO, Shin YABUTA, Isao AKAGI, and Jun-Ichi SAKAGAMI. Poster Presentation title: *Effect of P-dipping priming on roots of NERICA 4 to withstand drought and nutrient stress under rainfed lowland*; Presented online during the 56th Japan Society of Root Research Conference held online, September 17-18, 2022.
3. Emmanuel ODAMA, Yasuhiro TSUJIMOTO, Shin YABUTA, and Jun-Ichi SAKAGAMI. Oral Presentation title: *Effect of P dipping priming on rice resilience to water and nutrient stress under rainfed lowland*; Presented online during the 131st Japanese Conference on Tropical Agriculture held online, March 15-16, 2022.

List of Awards

1. Won the Outstanding Student Presentation Award at the 131st Japanese Conference on Tropical Agriculture held online on March 15-16, 2022. Title of presentation: *Effect of P-dipping priming on rice resilience to water and nutrient stress under rainfed lowland*.

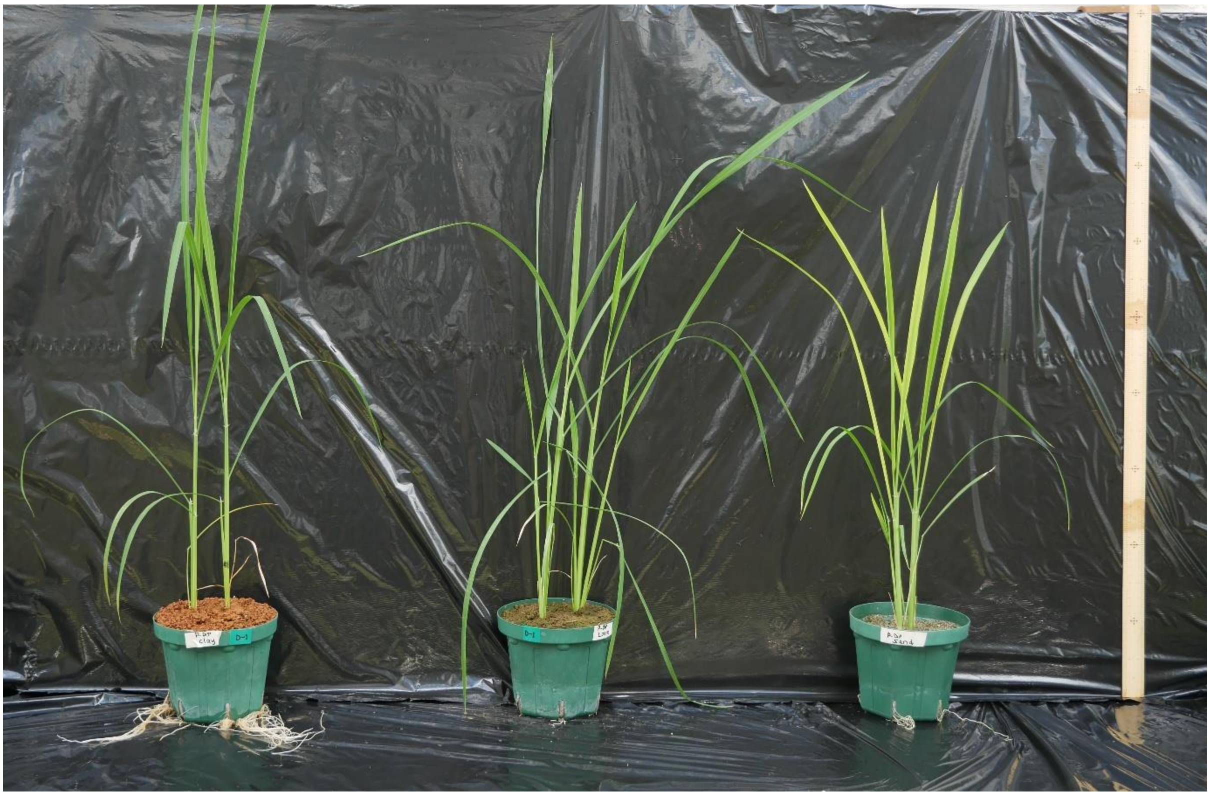
Annexes: Pictorial of Experimental Studies

Annex 1: Figure showing NERICA 4 growth under moderately dry and waterlogged conditions as presented in Chapter Two



NERICA4 growth under moderately dry (*left*) and waterlogged (*right*) conditions. Leaf age and tiller number were more in waterlogged than moderately dry condition.

Annex 2: Figure showing NERICA 4 growth under sand, clay loam, and clay soil textures as presented in Chapter Three



Comparison of NERICA 4 growth from the P-dipping treatment under clay (*left*), clay loam (*middle*), and sand (*right*) soil textures. Clay soil texture showed the most robust root growth; clay loam soil texture showed dense shoot growth; sand soil showed relatively stunted growth with yellowing of leaves.