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Root trait response to drought, recovery, and P deficiency of upland rice

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SUMMARY

Phosphorus deficit and intermittent drought can cause high-yield reductions in rice (*Oryza sativa* L.) production. Large differences in tolerance of low P availability and drought resilience were found in the upland rice, but the underlying mechanisms remain poorly understood, especially the belowground processes. To reveal whether and what root plasticity traits are associated with the tolerance to drought recovery, phosphorus deficit, and their interaction in upland rice, across-scale experiments with bibliometric analysis, greenhouse experiments, field experiments, and mathematic simulations were carried out.

In Chapter 2, I first review the entire landscape of root plasticity during drought and recovery reported in the literature. The knowledge gaps that should be focused on in the future, like regrowth during the recovery phase, root anatomy plasticity, and nutrient homeostasis are pointed out. These contexts are specially addressed with upland rice in my thesis.

Chapters 3 and 4 present the phenotypic dynamics of shoot and root plasticity in phosphorus contrasting genotypes (P-efficient genotype DJ123 and P-sensitive genotype Nerica4) during periods of drought and recovery. These two upland rice genotypes were grown in a greenhouse for six weeks with contrasting water and phosphorus levels. In conclusion, the plant recovery rate after drought is significantly influenced by its phosphorus homeostasis. The P efficient genotype, DJ123, with a better P homeostasis had a better recovery and drought resistance than P sensitive genotype Nerica4. Responses in xylem number and the cortical cell file number of DJ123 explain higher biomass and P accumulation than Nerica4 under phosphorus deficit and intermittent drought conditions. Higher phosphorus acquisition and specific root anatomical plasticity (like the xylem number of nodal root) of DJ123 under drought and phosphorus deficit conditions were associated with its better drought resilience.

To further investigate how root plasticity induces phosphorus efficient acquisition in DJ123 than in Nerica4, a combination of greenhouse, field, and model simulation experiments were carried out in Chapter 5 to test if rhizosphere pH change improves P uptake from phosphorus-deficient soils. In the greenhouse and field experiment, DJ123 had greater P uptake, in total and per unit root length (uptake efficiency), than Nerica4 under low P but not under high P. Rhizosphere pH was increased due to an excess uptake of anions over cations in the DJ123, which contributed to increased phosphorus availability and uptake. In combination with root morphology traits, model simulation with pH-P model can explain the higher uptake of DJ123 compared to Nerica4 by the change in rhizosphere pH. 0.5-unit rhizosphere pH change matters for an efficient P uptake in low-pH phosphorus fixing soil.

Past publications suggested that root dimorphism is important in co-optimizing the acquisition of multiple soil resources. Although these responses are complex, this dissertation demonstrates root trait trade-offs are not always true for optimizing phosphorus and water acquisition. Rhizosphere pH and certain root anatomical plasticity (like the xylem number of nodal root) can be targeted in breeding to increase crop yield under phosphorus deficiency and intermittent drought conditions such as low-input agronomic systems.

Keywords:

Root plasticity; rhizosphere pH; drought recovery; genotype-by-environment interaction; trade-off.

KURZFASSUNG

Phosphordefizit und intermittierende Trockenheit können bei Reis (Oryza sativa L.) zu hohen Ertragseinbußen führen. Bei Hochlandreis wurden große Unterschiede in der Toleranz gegenüber einer geringen P-Verfügbarkeit und der Widerstandsfähigkeit gegenüber Trockenheit festgestellt, aber die zugrunde liegenden Mechanismen sind nach wie vor schlecht verstanden, insbesondere die unterirdischen Prozesse. Um herauszufinden, ob und welche Wurzelplastizitätseigenschaften mit der Toleranz gegenüber Trockenheit, Phosphordefizit und deren Wechselwirkung bei Hochlandreis verbunden sind, wurden skalenübergreifende Experimente mit bibliometrischer Analyse, Gewächshaus- und Feldexperimente sowie mathematische Simulationen durchgeführt.

In Kapitel 2 gebe ich zunächst einen Überblick über die gesamte in der Literatur beschriebene Landschaft der Wurzelplastizität bei Trockenheit und Erholung. Es werden die Wissenslücken aufgezeigt, auf die man sich in Zukunft konzentrieren sollte, wie z. B. das Nachwachsen während der Erholungsphase, die Plastizität der Wurzelanatomie und die Nährstoffhomöostase. Diese Zusammenhänge werden in meiner Dissertation speziell mit Hochlandreis behandelt.

In den Kapiteln 3 und 4 wird die phänotypische Dynamik der Spross- und Wurzelplastizität bei phosphorkontrastierenden Genotypen (P-effizienter Genotyp DJ123 und P-empfindlicher Genotyp Nerica4) während Dürreperioden und Erholungsphasen dargestellt. Diese beiden Genotypen von Hochlandreis wurden sechs Wochen lang in einem Gewächshaus mit unterschiedlichen Wasser- und Phosphormengen angebaut. Die Schlussfolgerung lautet, dass die Erholungsrate der Pflanze nach einer Dürre erheblich von ihrer Phosphorhomöostase beeinflusst wird. Der P-effiziente Genotyp DJ123 mit einer besseren P-Homöostase wies eine bessere Erholungsrate und Dürreresistenz auf als der P-empfindliche Genotyp Nerica4. Die Reaktionen in der Xylemzahl und der Anzahl der Rindenzellen von DJ123 erklären eine höhere Biomasse und P-Akkumulation als bei Nerica4 unter Phosphordefizit- und intermittierenden Trockenheitsbedingungen. Die höhere Phosphorakkumulation und die spezifische anatomische Plastizität der Wurzeln (wie die Anzahl der Xyleme der Knotenwurzeln) von DJ123 unter Trockenheits- und Phosphordefizitbedingungen stehen im Zusammenhang mit seiner besseren Widerstandsfähigkeit gegen Trockenheit.

Um weiter zu untersuchen, wie die Wurzelplastizität eine effiziente Phosphoraufnahme bei DJ123 im Vergleich zu Nerica4 bewirkt, wurde in Kapitel 5 eine Kombination aus Gewächshaus-, Feld- und Modellsimulationsexperimenten durchgeführt, um zu prüfen, ob eine Veränderung des pH-Werts in der Rhizosphäre die P-Aufnahme aus phosphorarmen Böden verbessert. Im Gewächshaus und im Feldversuch hatte DJ123 eine höhere P-Aufnahme, sowohl insgesamt als auch pro Einheit Wurzellänge (Aufnahmeeffizienz), als Nerica4 bei niedrigem P, aber nicht bei hohem P. Der pH-Wert der Rhizosphäre wurde aufgrund einer übermäßigen Aufnahme von Anionen gegenüber Kationen in DJ123 erhöht, was zu einer erhöhten Phosphorverfügbarkeit und -aufnahme beitrug. In Kombination mit den Merkmalen der Wurzelmorphologie kann die Modellsimulation mit dem pH-P-Modell die höhere Aufnahme von DJ123 im Vergleich zu Nerica4 durch die Veränderung des pH-Werts in der Rhizosphäre erklären. Eine pH-Änderung in der Rhizosphäre um 0,5 Einheiten ist für eine effiziente P-Aufnahme in phosphorfixierenden Böden mit niedrigem pH-Wert von Bedeutung.

Frühere Veröffentlichungen legen nahe, dass der Wurzeldimorphismus bei der gemeinsamen Optimierung des Erwerbs mehrerer Bodenressourcen wichtig ist. Obwohl diese

Reaktionen komplex sind, zeigt diese Dissertation, dass Wurzelmerkmale nicht immer für die Optimierung der Phosphor- und Wasseraufnahme ausschlaggebend sind. Der pH-Wert der Rhizosphäre und bestimmte anatomische Eigenschaften der Wurzeln (einschließlich der Anzahl der Rindenzellen und der Anzahl der Xyleme) können in der Züchtung gezielt eingesetzt werden, um die Ernteerträge bei Phosphormangel und intermittierender Trockenheit zu steigern, z. B. bei agronomischen Systemen mit geringem Input.

--Schlüsselwörter:

Wurzelplastizität; pH-Wert der Rhizosphäre; Erholung bei Trockenheit; Wechselwirkung zwischen Genotyp und Umwelt; Trade-off.

LIST OF ABBREVIATIONS

- ABA Abscisic acid
- ABA-GE ABA-glucose ester
- ANOVA Analysis of variance
- AMF Arbuscular mycorrhizal fungi
- C Carbon
- Ca^{2+} Calcium cation
- CCFN Cortical Cell File Number
- DAE Days after emergence
- DJ DJ123
- DR Drought recovery
- DW Dry weight
- E Efflux rate
- G Genotypes (G)
- HCO_3^- Bicarbonate anion
- HP High P
- $H_2PO_4^-$ Dihydrogen phosphate anion
- K Potassium
- LP Low P
- Mg^{2+} Magnesium cation
- N Nitrogen
- NO_3^- Nitrate anion
- NH_4^+ Ammonium cation
- N4 Nerica4
- P Phosphorus
- PAE Phosphorus acquire efficiency
- Pi Phosphate
- PT P transporter
- PUE Phosphorus use efficiency
- RCA Root Cortical Aerenchyma
- RGR Relative growth rate
- RHSA Root hair surface area
- RL Lateral roots
- RLD Root length density
- RN Crown or nodal roots
- RRER Relative root elongation rates
- RS Seminal roots
- RSA Root surface area
- RTD Root tip diameter
- R/S Root/Shoot
- TB Total biomass
- TDR Time Domain Reflectometry
- TP Total P acquisition

- SD Steel diameter
- SO_4^{2-} Sulphate anion
- S. RI Ratio of steel and root area
- WCP Water Conductance Parameter.
- WW Well-watered
- XD Xylem diameter
- XN Xylem number

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This PhD thesis consists of six (6) chapters. It starts with a general introduction, a bibliometric review (Chapters 2), three experimental studies (Chapters 3 to 5), and finally, a general discussion. Each experimental study focuses on specific aims and objectives stated under sections 1.5. Chapters 2 to 5 form the most important parts of this thesis. They have been published as peer-reviewed journal articles (Zheng et al., 2023: Frontiers in plant sci.: doi.org/10.3389/fpls.2023.1084355), or are in preparation to be submitted for publication. My contributions to each paper are specified and listed under the publications section of this thesis. During my thesis I (co)authored seven publications related to other plant species and topics, which I shortly listed in a supplemental chapter at the end of the thesis.

CHAPTER 1: INTRODUCTION

The world's population is set to grow significantly in the foreseeable future, it was estimated that the world population will reach 8.6 billion in 2030 and 9.8 billion in 2050 (United Nations Publication, 2022). Agriculture must produce more food to feed a growing population with a smaller rural labor force, as well as more feedstock for a potentially huge bioenergy market. Additionally, it must contribute to overall development in the many agriculture-dependent developing countries, by implementing more efficient and sustainable production methods, and adapting to climate change.

Rice (*Oryza sativa* L.) is among the important staple food crops, fulfilling food requirements of about half of the world's human population. However, rice production is threatened by many abiotic and biotic stresses such as drought, heat, salinity, and heavy metals, which may lead to unforeseen losses in rice grain quality and productivity (IRRI, 2016; Muehe et al, 2019). Therefore, enhancement in the yield of rice is extremely critical to feed this increased population (Jiang et al, 2012; Li et al, 2018).

1.1 World rice production threatened by drought and P stress

Over 50% of the world’s rice crops are rain-fed, but these non-irrigated lands produce only a quarter of the total global rice output (McLean et al, 2002). Drought stress (both intermittent drought and terminal drought) is a major limitation for upland rice production in rainfed ecosystems, which short- term drought and post-drought recovery by raining usually cycles in the whole life of rice. Concurrently, both historical records and model simulation results suggest the increased risk of drought in the twenty-first century will happen via either decreased precipitation and/or increased evaporation (Dai 2013; Langenbrunner 2021). More frequent and intense drought events are expected to occur globally, particularly in arid and semiarid regions (Davidowitz 2002; Spinoni et al. 2014; Touma et al. 2015). Regions with high seasonable variability will become even more variable and experience more extreme weather events (Konapala et al. 2020). Global rice yield loss to drought is estimated at 18 million tons annually or 4% of total rice production (Evenson and Gollin, 2003). In Asia, approximately one-third of the total Asian rice area is subject to occasional or frequent drought stress (Huke and Huke, 1997). Yield losses in rice production by drought stress are especially severe during the reproductive stage, even from mild drought stress (Venuprasad et al, 2009; Verulkar et al, 2010). Hence, improving the understanding of the rice root system and rhizosphere process after drought stress, and developing rice cultivars with improved drought resilience, are important to meet the demands of global food security.

Besides drought, most natural environments are suboptimal with respect to other multiple soil resources (like N and P) (van der Bom et al, 2020; Hutchings et al, 2003), which will simultaneously restrict plant growth and final production. This is especially true for the upland rice production in developing countries like the sub-Saharan African region, most smallholder farmers cannot afford mineral fertilizer, and the insufficient availability of soil P emerges as a principal and ubiquitous impediment to crop production and food security (Ayaga et al., 2006; Nziguheba et al., 2016). Different with other nutrients, phosphate is relatively immobile in the soil, and P availability in surface soil is generally greater than that in subsoil because of fertilizer placement, the deposition of plant residues over time, and the greater biological activity in surface strata (Lynch and Brown, 2001; Lynch, 2011, 2013). Together, uptake of immobile nutrients like phosphorus is severely reduced in dry soils, due to the reduced effective diffusion rates through dry soil (Hira and Singh, 1977), and the changes in root architecture and function (De Bauw et al, 2020; Ho et al, 2005). A better understanding of upland rice response to drought and phosphorus stress has significant practical relevance to crop production and is the focus of this thesis.

1.2 Recovery after drought matters for rice growth

In both natural and agricultural ecosystems, short- and long-term droughts occur frequently but are typically not permanent. Plants can continue to grow or even grow faster during the later recovery or rewatering period. According to the IPCC, drought is defined as 'a period of abnormally dry weather long enough to cause a serious hydrological imbalance' (Masson-Delmotte et al. 2018). Following this definition, we propose defining plant recovery from drought as 'the period after a drought during which the hydrological balance is restored'. To reduce intermittent drought risk and increase yield stability, the recovery period after the drought is of great importance (Williams and de Vries, 2020). With a growing population and climate change climate change, a better understanding of plant responses to drought and drought and recovery offers the potential to improve plant climate resilience and production.

As a fundamental aspect of plant adaptability and yield, the role of root plasticity in drought tolerance has received increasing attention in recent years (Lynch 1995; Comas et al. 2013; Kashiwagi et al. 2015; Koevoets et al. 2016; Gao and Lynch 2016). Previous pot and field experiments have proved significant variation in the rate of recovery of rice genotypes upon irrigation after the drought. Recovery was associated with root regrowth (Marie 2018; Bochmann 2021). Generally, during the drought phase, the root system growth is reduced or arrested. Soil dries from the top of the profile, exposing the upper part of the root system to water stress. Typically, shallow roots stopped growing, whereas deep root growth might have continued to access water stored in deep soil (Lynch et al, 2014; Lynch and Wojciechowski, 2015; Gao and Lynch, 2016). Additionally, loss of shallow root cortex due to drought-induced senescence may occur. This would mean loss of root hairs, root exudation, and mycorrhizal colonization, which are usually regarded as key elements in phosphorus acquisition by plants (Richardson 2001; Richardson et al, 2009, 2011). This all would lead to a dramatic reduction in the ability to take up nutrients, especially phosphorus, during drought (Fig.1.1). However, a comprehensive understanding of root plasticity during drought and recovery is lacking so far.

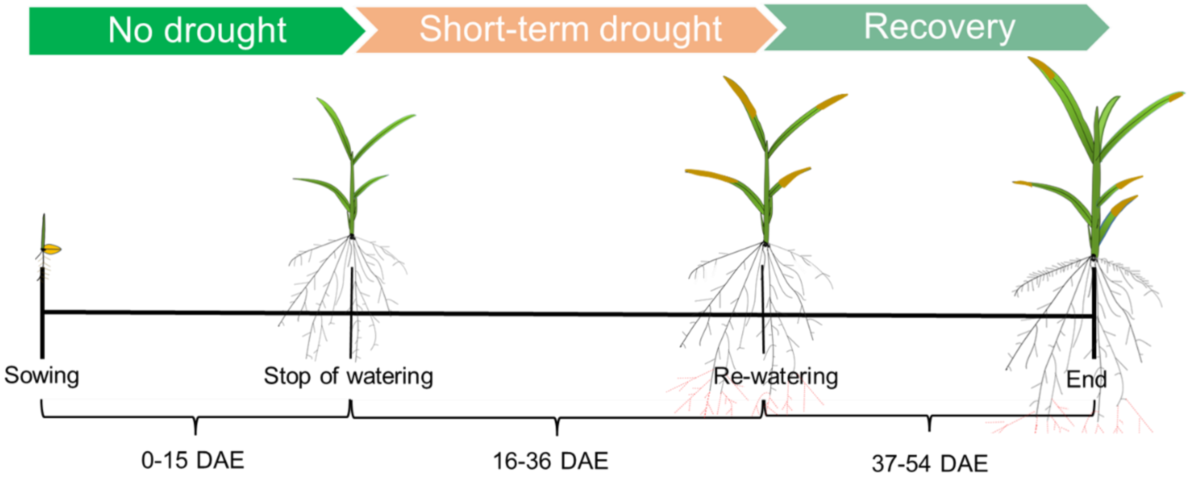

Fig. 1.1 Hypothesized alterations process in rice during and after drought. During drought, reduced water content induces deeper root development, but phosphorus uptake by shallower root maybe be broken down. During rewatering period after drought, different root development may be associated with water and phosphorus uptake, with the potential of affecting plant recovery and further growth performance.

1.3 Balance/trade-off of plant water and P acquisition

While single effects of water, nitrogen and phosphorus to plant growth have been widely studied (Humbert et al, 2013; Cong et al, 2020), the combination of water and other nutrients effects are still largely unknown, more and more recent research gets attention to water& N, and water& P interaction recently (Swift et al, 2019). As soil N&P availability is directly influenced by soil water content (as is soil desiccation), these fundamental questions are of great importance for crop production. Some studies have focused on the relationship between nitrogen nutritional status and drought resistance and the integrated effects of nutrients and water status on leaf characteristics and water relationships of *Coffea arabica* L. (Tesha and Kumar, 1978), *Gossypium hirsutum* L. (Radin and Ackerson, 1989), *Triticum aestivum* L. (Shangguan et al, 2000), *Agrostis palustris* Huds. (Saneoka et al, 2004) and *Oryza sativa* L. (Yang et al, 2012). While some other studies have reported interaction between plants' intermittent drought and phosphorus content, for instance, Zhang et al. (2002) study showed that buffered phosphorus fertilizer improves growth and drought tolerance of 4 woody plants. At the same time, Beebe et al. (2008) found that drought resistance lines also improve yield in phosphorus limited as well as favorable conditions of the common bean.

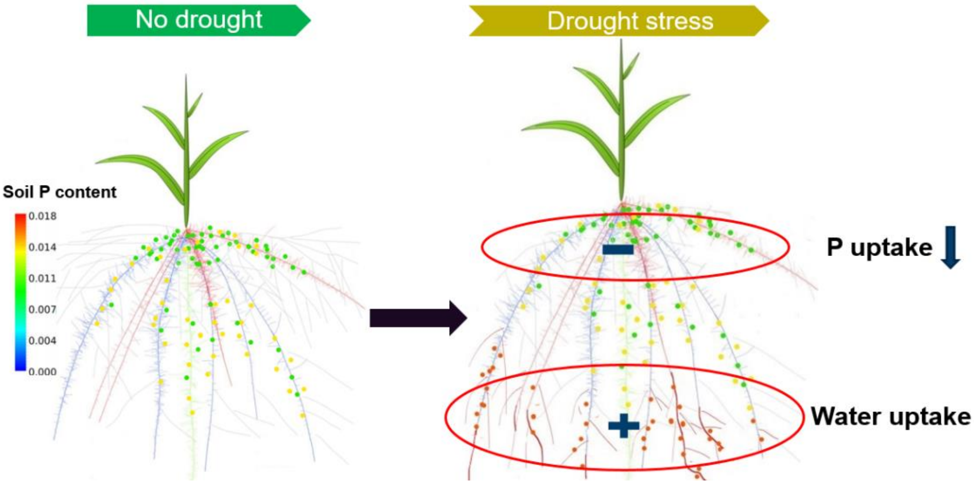

Fig. 1.2 Hypothesized alterations process of rice root morphology during drought. During drought, reduced water content induces deeper root development, but phosphorus uptake by shallower root maybe be broken down with the less available soil P content and loss of shallow root cortex. During the rewatering period after drought, different shallower root development and rhizosphere change happen which affect phosphorus uptake, with the potential of affecting plant recovery and further growth performance.

Yet, roots are vital for the ability of plants to acquire nutrients and water- two functions of fundamental importance to whole-plant growth and performance (Jackson et al, 2000; Chen et al, 2016; Ma et al, 2018). After drought, the plant needs to recover its root system through new growth. Very little is thus far known about this recovery period, what plant traits are associated with a fast recovery, and how these traits are associated with other drought-resistant related traits. Especially, P status and/or efficient absorption in the recovery period is important and largely unknown so far (Fig. 1.2).

To better understand root response to drought from P view, recent progress of key aspects including molecule, root morphology, resource allocation and root-microbiome interaction are summarized as follows:

1.3.1 Plant molecule response

Plett et al. (2020) and Araus et al. (2020) reviewed studies on how plants integrate nitrogen and water signals concurrently. The meta-analysis also supports the idea that key N signaling genes may lead to changes in drought-responsive gene expression. For example, the expression of root-specific rice aquaporin genes OsPIP1.1, OsPIP2.3-2.5, OsTIP1.1-1.2, and OsTIP2.2 is positively associated with N availability (Bao et al, 2009; Ishikawa-Sakurai et al, 2014). Meanwhile, N starvation leads to a reduction in aquaporin gene expression levels, reducing root hydraulic conductivity (Ishikawa-Sakurai et al, 2014). How plants integrate P and water signals is still largely unknown. A recent study on AVP1 gene revealed that a low phosphorus environment can induce its overexpression, which caused subsequently increased rhizosphere acidification and root proliferation mediated by P-ATPase. Using the Arabidopsis AVP1 gene overexpression method, Bao et al. (2009) found that transgenic Medicago sativa (alfalfa) increases its solute and water accumulation, leading to greater photosynthetic activity and less damage to cell membranes under drought and salinity stress. These results indicated that changes in P-related gene expression may affect plant drought-resistant ability straightly and further influence its growth and production, and vice versa.

1.3.2 Root architecture response

As the “hidden-half” of plants, roots are critical to water and nutrient uptake, crop production and food security (Lynch 2007a). Root system architecture refers to the shape (mainly including the morphological structure and topological structure) and spatial distribution of plant roots in the growth medium. Morphological structure refers to the phenotypic characteristics of the root system, mainly including the root thickness, length, root hairs and the number and length of lateral roots (Lynch 1995; Postma et al, 2014). Previous studies have shown that root system architecture regulates water and nutrient uptake and adapts to nutrient & water availability from the soil. As for P and water uptake, generally, water deficit tolerance has been associated with increased rooting depth in rice (Henry et al, 2011; 2012), while greater P acquisition has been linked with increased shallower root, that is, topsoil foraging for immobile nutrient like phosphorus (Wissuwa et al, 2016 and 2020). When plants confront dual or multiple environmental constraints, they must co-optimize their root uptake strategy for limiting resources (Postma et al, 2014). This could be contradictory when P and water are co-limiting, since a deeper root depth that exploits deep resources efficiently may be advantageous in water deficit conditions, which may inadvertently result in decreased P uptake and interference of P homeostasis because immobile phosphorus resource often located in the top layers. This is especially true in the rainfed agroecosystem, in which the rice crop usually experiences intermittent drought. Such a trade-off was shown for common bean (Phaseolus vulgaris L.) (Ho et al. 2005). Whether deep and shallow rooting strategies are a metabolic investment trade-off (in units P or C) is, however, debated as under typical cropping conditions, the larger amount of P and C is allocated to the shoot, and often genotypes that do well under low P may also do well under drought. Further field experiments with rice showed that some certain root architecture traits are highly related to both water and P uptake, like nodal thickness, secondary root branching, and laterals (i.e. S- and L-type), and the relative contribution of each root type largely depend on phosphorous availability and water dynamics (De Bauw et al, 2018 and 2020). Although these studies have revealed the induced root modifications related to drought resilience also affect P uptake efficiency during the drought period, whether P is the limiting factor during the recovery phase and whether P efficient genotypes with higher root uptake efficiency can be associated with a faster regrowth are still largely unknown and worth exploring.

1.3.3 Resource allocation response

In addition to the molecular and root architecture response, resources like carbon and phosphorous allocation may also significantly influence the plant growth rate and further drought resistance and its recovery performance after rewatering. Numerous studies have shown that the allocation of P to leaf fractions is likely related to life-history strategy because these fractions are functionally related to growth, reproduction, and stress tolerance. Shifting P-allocation patterns in leaves is an important mechanism for plants to acclimate to low soil P availability (Hidaka and Kitayama, 2011, Yan et al, 2019). If strong P limitation occurs, plants shift the allocation of P among foliar P fractions, and this might increase plant fitness under the prevailing conditions (Hidaka and Kitayama, 2011). The rapidly-emerging field of ecological stoichiometry have shown plant growth rate are associated with its P investment, fast-growing plants have higher P concentration, low biomass C: P and N: P ratios, this was well supported by the Growth Rate Hypothesis (GRH), an intensively tested theory in the past decades via both theoretical and empirical analysis in grass species and bacteria (Elser et al, 2000; Yu et al, 2012). This is because higher growth rate species needed a proportionally greater requirement for P than for N, higher P allocation to P- rich ribosomal RNA (rRNA) can meet the protein synthesis demands that further support the rapid growth rates (Elser and Hamilton, 2007; Elser et al, 1996 and 2000). Current studies about woody species also showed that the relative growth rate of both species was positively correlated with both foliar nucleic acid P and total N concentrations. Faster-growing *B. sessilis* (Knight) allocated more P to nucleic acids than slow-growing *Banksia attenuata* (Proteaceae) did, but other fractions were similar (Han et al, 2020).

P-allocation and concentration do affect plant growth; however, this is only sustainable if that growth is accompanied by increasing P uptake from the soil. Furthermore, P was absorbed in the expense of carbon (Lynch and Ho, 2005; Lynch 2007b). In low P soils, P acquisition by plants is usually supported by increasing the root mass fraction. Similarly, water-deficient plants typically have higher root mass fractions than well-watered plants, either because of allometric relationships or because of the increased belowground biomass allocation, or decreased shoot growth (Eziz et al, 2017). Increased relative allocation to root growth, especially deep root, is also obviously beneficial for water uptake, since a deep root can help plants capture the water in the deep soil layer, while a reduced shoot size may reduce transpiration rates. But this may slow overall plant growth because of the increased cost (C & P) of root tissue. As, under low P more carbon is allocated to shallower roots while under drought more carbon is allocated to deeper roots there maybe competition for carbon allocation in different root layer under combined water and P limitations (Ho et al, 2005; Nasr Esfahani & Sonnewald, 2024). Concerning the important role of resource allocation in plant growth under stress environment, some outstanding questions towards drought and recovery periods are: What's the C& P dynamics during drought and recovery period? Compared with control, does leaf P content increase, or decrease during drought and recovery period, whether its allocation is associated with plant regrowth rate?

1.3.4 Root-microbiology interaction response

The colonization of arbuscular mycorrhizal fungi (AMF) in the rice is particularly relevant under upland production in the agricultural system. There is evidence showing that AMF can be beneficial for rice yield and nutrition (Maiti et al, 2011). Under drought or phosphorus deficiency condition, arbuscular mycorrhizal fungi (AMF) play an important role in water and nutrient uptake. Evidence in the literature suggests that rice crops will usually increase mycorrhizal colonization to promote water and phosphorus uptake. There is also substantial research on the molecular basis for AMF effects on rice P uptake; the genes, proteins and signaling molecules involved have been relatively well characterized (Paszkowski et al, 2002; Gutjahr et al, 2008). For example, the P transporter (PT) protein OsPT11 is well known for its responsibility for the transport of phosphate (Pi) from the fungus to the plant cytosol (Yang et al, 2012). In non-mycorrhizal rice plants OsPT11 expression is not detected; the expression of gene OsPT11 is therefore a proxy for the mycorrhizal pathway of P uptake while other constitutively expressed PT genes such as OsPT2 represent the direct/root pathway of P uptake (Yang et al, 2012; Chen et al, 2013). Additionally, AMF can improve drought-resistance in rice (Ruíz-Sánchez et al, 2010 and 2011; Schnepf et al, 2011) and thus may confer some stress tolerance to rice genotypes growing under both water and P limitation such as in SSA.

During the drought phase, the root system growth is reduced or arrested. Soil dries from the top of the profile, exposing the upper part of the root system to water stress, the loss of shallow root cortex due to drought-induced senescence may occur. This would mean loss of root hairs, and mycorrhizal colonization, which are usually regarded as key elements in phosphorus acquisition by plants (Richardson 2001; Richardson et al, 2009 and 2011). This all would lead to a dramatic reduction in the ability to take up nutrients, especially phosphorus, during drought (Fig. 1.2). Hence, some outstanding questions like how mycorrhizal colonization changes of new root growth after drought and whether this change is associated with regrowth rate in different genotypes during rewatering period are still unclear, and these will give us a better understanding of rhizosphere process after drought.

1.4 Efficient P uptake by DJ123 than Nerica4

Rice (*Oryza sativa* L.) is a plant belonging to the family of grasses, Poaceae. Large differences in tolerance of low P availability and drought resilience were found in the upland rice, but the underlying mechanisms remain poorly understood. Genotypic variation in upland rice root traits offers a potential genetic resource for plant breeders to develop genotypes with improved water and/or P uptake efficiency (Wissuwa et al, 2016; Wissuwa et al, 2020). In this view, Nerica4 is a popular variety with farmers across the African continent with slightly drought tolerant and good responsiveness to fertilizer application even their performance on P deficiency soil is pool. While the rice gene bank accession DJ123 has been identified as P efficient and can have relatively high grain yields on low-P soils. Both field/greenhouse experiment and mathematical modeling have suggested that DJ123 has an efficient P uptake per unit root biomass or surface area, this may be contributed by not one special root trait but multiple root traits interaction (Gonzalez et al, 2021a; Kuppe et al, 2022) Considering P uptake can be limited due to less diffusion in soil under drought conditions, a better understanding of the mechanisms of phosphorus uptake in upland rice varieties (DJ123 and Nerica4) would provide insight into the limitations of P uptake under drought conditions.

1.5 Study aims

Root development and function during drought recovery has not been widely reported. The overall aim of this thesis was to gain a better understanding of root plasticity during drought and recovery. Root system traits of several upland rice cultivars along with other relevant indices were investigated through greenhouse and field experiments as well as simulation studies. The research objectives in each chapter were listed as follows:

1. To increase plant resilience and maximize plant production, understanding root plasticity during both drought and recovery is necessary (Vilonen et al., 2022). A mechanistic understanding of root plasticity during drought recovery is, however, lacking. In this study, we addressed this knowledge gap through a comprehensive bibliometric analysis (**Chapter 2** of this thesis; Zheng et al. 2022). We mapped the different research areas and trends that focus on the role of roots in plant response to drought and rewatering and asked if important topics were overlooked, potential research trends and hotspots for future studies are provided.

2. During an intermittent period of drought, soil water content usually decreased from surface to the deeper layers, while most P is immobilized and usually distributed in the shallow layers. Very little is thus far known about what plant traits are associated with a fast recovery, especially, whether P status and/or efficient absorption in the recovery period is important for recovery. In this study, we tested whether and how P uptake is associated with its recovery/ regrowth rate, especially under low P condition (**Chapter 3**).

3. Root anatomy is thought to be strongly affected by drought and P stress. We asked if combined stress increases the response for those traits that respond to the individual stresses similarly, and if the response is cancelled out for those that respond opposite. In this study, we tested the effect of soil P and water levels on the anatomy of rainfed rice roots after a period of drought and during recovery period (**Chapter 4**).

4. Suboptimal phosphorus (P) availability is one of the most limiting factors for upland rice production, compared to the more commonly grown cultivars, the genotype DJ123 can efficiently take up P from low P soils. Based on a previous modeling study, we tested whether P efficient uptake in DJ123 is induced by a higher rhizosphere pH caused by greater anion/cation uptake-imbalance from phosphorus-fixing soils compared to Nerica4 (**Chapter 5**).

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CHAPTER 2: Plant root plasticity during drought and recovery: What do we know and where to go?

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Abstract

Aims Drought stress is one of the most limiting factors for agriculture and ecosystem productivity. Climate change exacerbates this threat by inducing increasingly intense and frequent drought events. Root plasticity during both drought and post-drought recovery is regarded as fundamental to understanding plant climate resilience and maximizing production. We mapped the different research areas and trends that focus on the role of roots in plant response to drought and rewatering and asked if important topics were overlooked.

Methods We performed a comprehensive bibliometric analysis based on journal articles indexed in the Web of Science platform from 1900-2022. We evaluated a) research areas and temporal evolution of keyword frequencies, b) temporal evolution and scientific mapping of the outputs over time, c) trends in the research topics analysis, d) marked journals and citation analysis, and e) competitive countries and dominant institutions to understand the temporal trends of root plasticity during both drought and recovery in the past 120 years.

Results Plant physiological factors, especially in the aboveground part (such as "photosynthesis", "gas-exchange", "abscisic-acid") in model plants Arabidopsis, crops such as wheat and maize, and trees were found to be the most popular study areas; they were also combined with other abiotic factors such as salinity, nitrogen, and climate change, while dynamic root growth and root system architecture responses received less attention. Co-occurrence network analysis showed that three clusters were classified for the keywords including 1) photosynthesis response; 2) physiological traits tolerance (e.g. abscisic acid); 3) root hydraulic transport. Thematically, themes evolved from classical agricultural and ecological research via molecular physiology to root plasticity during drought and recovery. The most productive (number of publications) and cited countries and institutions were situated on drylands in the USA, China, and Australia. In the past decades, scientists approached the topic mostly from a soil-plant hydraulic perspective and strongly focused on aboveground physiological regulation, whereas the actual belowground processes seemed to have been the elephant in the room. There is a strong need for better investigation into root and rhizosphere traits during drought and recovery using novel root phenotyping methods and mathematical modeling.

Keywords: Bibliometric analysis; intermittent drought; root dynamics; recovery;

nutrient homeostasis

1 Introduction

Drought undoubtedly represents the most serious hazard to livestock and crops in nearly every part of the world; an estimated 55 million people are affected by droughts globally every year (Vereinte Nationen, 2021). Both historical records and model simulation results suggest the increased risk of drought in the twenty-first century will happen via either decreased precipitation and/or increased evaporation (Dai, 2013; Langenbrunner, 2021). Globally, more frequent and intense drought events are expected to occur, particularly in arid and semiarid regions (Davidowitz, 2002; Spinoni et al., 2014; Touma et al., 2015). Regions with high seasonable variability will become even more variable and experience more extreme weather events (Konapala et al., 2020). Global synthesis analysis predicted that when water supply decreases by approximately 40%, wheat and maize yields will reduce by 21% and 39%, respectively (Daryanto et al., 2016). However, in natural and agricultural ecosystems, short- and long-term droughts happen frequently but are usually not permanent and the plants can continue to grow or even grow faster during the later recovery or rewatering period. IPCC defines drought as “a period of abnormally dry weather long enough to cause a serious hydrological imbalance” (Masson-Delmotte et al., 2018). In accordance with the IPCC drought definition, we suggest defining plant recovery from drought as “the period after a drought during which the hydrological balance is restored”. Since drought can cause lasting damage, recovery might not be 100% compared to well-watered conditions and the definition of ‘restored’ is the point where no significant improvement is further observed, despite sufficient water supply. Considering the growing population, and climate change, a better understanding of plant response during drought and recovery offers the potential to increase plant climate resilience and production.

Water availability limits plant growth and final production nearly in all natural ecosystems, this is especially true in agriculture ecosystems. As a fundamental aspect of plant adaptability and yield, the role of root plasticity in drought tolerance has received increasing attention in recent years (Lynch, 1995; Comas et al., 2013; Kashiwagi et al., 2015; Gao and Lynch, 2016; Koevoets et al., 2016) At the same time, root systems are key to plant growth, water uptake, water perception, and signaling (Lynch, 2007; Hamanishi and Campbell, 2011; Carley et al., 2022). Considerable progress has been made in unraveling the mechanisms of drought responses in plant roots which involve an array of molecular, anatomical, physiological, morphological, and biotic regulations aiming at both tolerance and avoidance of drought stress. For example, during drought plant roots modify aquaporin (AQP) and dehydrin gene expression (Reddy et al., 2017), change metaxylem vessel diameter, root diameter, and crown root number (Gao and Lynch, 2016; De Bauw et al., 2019; Klein et al., 2020), increase ABA levels and change carbon allocation (Zhang et al., 2006; Guo et al., 2021), and alter root microbiota composition (Santos-Medellin et al., 2021). The belowground plasticity is accompanied by aboveground responses, like ABA production in the shoot and aquaporin contributed stomatal closure which are, however, not the focus of this review. During the subsequent recovery period after drought, the hydrological balance restored in plants and soil makes these changes return to normal (e.g. comparable to well-watered conditions), e.g., decrease ABA level, and restored fine roots through root regrowth (Lauenroth et al., 1987; Luo, 2010; Fang and Xiong, 2015; Maurel and Nacry, 2020). Although both drought resistance and post-drought recovery are key determinants of plant growth, some recent studies suggest that recovery may play a more significant role in plant drought adaptation than drought resistance itself (Chen et al., 2016; Gonzalez-Hernandez et al., 2021). Thus, to increase plant resistance and resilience (For definitions see (Enright et al., 2014; Hoover et al., 2021)), and maximize plant production, understanding root plasticity during both drought and recovery is necessary (Vilonen et al., 2022). A comprehensive mechanistic understanding of relevant processes during drought recovery is, however, lacking.

Bibliometric analysis is an effective tool to describe the knowledge status, features, and trends in a certain discipline and is increasingly used to summarize the literature using objective statistics. Specifically, bibliometrics can clarify the current progress of a certain research field and show the temporal trends of research disciplines and research hotspots (Aria and Cuccurullo, 2017). It includes qualitative and quantitative analysis of publications indexed by databases based on statistics and computing technology, which makes the outputs more objective and reliable (van Eck and Waltman, 2014; Liu et al., 2021). After Alan Pritchard proposed the bibliometrics method in 1969, more scientists use this approach to review the subject’s progress, which provides a comprehensive evaluation at various levels; current reviews in nitrogen deposition and soil phosphorus fractions are good illustrations (Alan Pritchard, 1969; Oliveira Filho and Pereira, 2020; Li et al., 2022). To review the entire landscape of root plasticity, including root morphology/ architecture, anatomy, exudation and rhizosphere microbiomes during drought and recovery like shown in Fig. 1, we conducted a comprehensive bibliometric analysis. The objectives of this study include a) understanding the research patterns of root plasticity during drought and recovery research globally, b) developing an accurate overview of the scientific output of root plasticity during drought and recovery over time and space, and c) providing potential research trends and hotspots for future studies.

2 Materials and methods

Bibliometric data collection was carried out on 29 December 2021 based on the Science Citation Index-Expanded (SCI-E) database in the "Web of Science Core Collection" (http://www.webofknowledge.com), considering the SCI-E database could provide comprehensive coverage of the most important publications over the world and include also explicit reference details which enable us to track the intellectual progress trend of our focused topic. Only one database, "Web of Science Core Collection", was used because it is currently not possible to conduct the bibliometric analysis on merged databases. We searched for publication topics with the following search command: ("Root") AND ("Drought" OR "Water stress" OR "Water deficit" OR "Water scarcity") AND ("Recovery" OR "Rewater" OR "Rewet" OR "Legacy effect") NOT ("Submergence" OR "Waterlogging" OR "Flood"). The terms 'AND' and 'OR' were used to maximize the correct selection of interest articles, e.g., the term 'AND' was used to enable the research for all terms of root plasticity during both drought and recovery, the term 'OR' was used to search for at least one of the terms, and the term 'NOT' was used to exclude irrelevant research which appeared in water stress and recovery. Thus, a total of 1102 publications were obtained for all years of publication through Dec. 2021. Publications were screened to ensure main information was included such as title, authors, keywords, ISO source abbreviation, abstract, publication year, volume, and issue, resulting in 1086 proper records (more details can be found in Table 1). Besides "Web of Science Core Collection" database, we also queried the "Scopus" database. Compared with the "Scopus" database, "Web of Science Core Collection" database identified a greater number of publications (1086 vs 880) with more than 80% overlap with the "Scopus" database. We concluded that a more complete result was obtained with the "Web of Science Core Collection" database. Data were then downloaded and converted into a BibTex format for further bibliometric analyses in R (Bibliometrix package in R software).

(Table 1. Here)

We first analyzed a) the number of publications per year and b) the number of scientific productions per country and institute. To better understand the distribution of the output in different journals, we computed the article numbers, the number of citations, and the journal's topical *h*-index. Note that the *h*-index was based on citations acquired in the WoS Core Collection and were different from those published by other databases, notably Google Scholar or Scopus (Hirsch, 2005; Oliveira Filho and Pereira, 2020).

To further investigate trends and advances of the focused topic, keywords frequency and relationship analysis were carried out with the word cloud and co-occurrence analysis. To make the frequency analysis more precise, we merged the common words used in all publications from plural/singular, Latin plant names/common name to the singular and common one firstly, e.g., plants-plant, roots-root, leaves-leaf, *Arabidopsis-thaliana*-Arabidopsis, *Zea-mays*-maize, and *Oryza-sativa*-rice. We used the word cloud to identify the 50 most frequent keywords used in "root plasticity during both drought and recovery research" over the past 120 years. We further drew the keywords co-occurrence network with the 50 most popular keywords to determine the latest research hotspots in root plasticity during the drought and recovery topics. Here, different circle sizes represent keywords' frequency appearance in a cluster; different colors depict different clusters, indicating that these keywords are likely to appear in the same publication. The lines connecting the circles represent the co-occurrence of keywords, with thicker lines, representing stronger relations. To better understand the temporal evolution of research topics, a temporal trend analysis of keywords was carried out and divided the publications of 1975-2022 into four periods (1975–1995, 1996–2005, 2006–2015, and 2016–2022). By dividing the timespan into time slices, the evolution of topics in a specific research field can be shown by the alluvial graph (Aria and Cuccurullo, 2017). Besides, by applying a clustering algorithm to a keyword network, we can highlight certain topics of a given field. We mainly analyzed two themes, namely, basic and motor themes. Basic themes are fundamental concepts that haven't been well-developed. Motor themes represent topics that are both important and well-developed (Cobo et al., 2011). All bibliometric and data analyses, and figures were done with R 4.0.4 and Biorender software.

3 Results and discussion

3.1.Research areas and temporal evolution of keyword frequencies

3.1.1 Most popular keywords

(Fig. 2 Word cloud here)

Plant “growth” (total frequency of 7%) was the most frequently used keyword (Fig. 2). This is likely because drought and recovery represent different periods of plant water status and growth connects them and is also frequently used as an indicator of drought and recovery. Additionally, in contrast to the many shoot-only studies that are not part of this analysis, root-related studies usually take a whole-plant approach. When we ignore common words like “stress”, “drought”, “tolerance”, “response”, “plant” and “water-stress”, the high-frequency keywords in root plasticity during both drought and recovery research can be grouped into 3 types: 1) physiological factors: photosynthesis, gas-exchange, abscisic-acid, (stomatal) conductance, osmotic adjustment, accumulation, and transpiration; 2) different plant species: Arabidopsis, wheat, maize, and trees; 3) abiotic factors: temperature, salinity, nitrogen, and climate change (Fig. 2), which will be discussed below. No words related to biological interactions got into the top 50 list, and the first biological factor ‘fungi’ was found in the top 150 list and appeared only 12 times and thereby had a frequency of 0.2%.

For physiological factors, higher frequency words “photosynthesis”, “gas-exchange”, “stomatal conductance”, and “transpiration” could reflect those aboveground traits received more attention than belowground traits. This shoot-dominated focus continued despite the vital role of roots in determining plant ecology, terrestrial ecosystem functioning, and their designation as the target for the second green revolution (Gašparíková et al., 2002; Lynch, 2007). This is partly because plant water uptake and transport are generally thought to be regulated by the stomata (Li et al., 2020), but probably also due to technological limitations in monitoring root growth dynamics and studying the hydraulic pathways in the root system. Using non-destructive technologies like MRI (Pflugfelder et al., 2017) and SWaP (Dusschoten et al., 2020), a recent study focusing on faba bean and maize has proved that stomatal sensitivity is partly explained by the sensitivity of root hydraulic conductance to soil drying (Müllers et al., 2022a). A better understanding of the role of root conductance in soil drying and rewatering is vital to complete the picture from soil to root to leaf. Associated with the focus on stomatal conductance and morphological leaf traits, “abscisic acid” was another important physiological keyword. For example, Correia et al. (2014) showed that both abscisic acid (ABA) and ABA-glucose ester (ABA-GE) are up-regulated during drought and down-regulated during recovery in the *Eucalyptus globulus*. Besides stomatal closure, ABA controls physiological processes like osmotic regulation, growth inhibition, and transcriptional regulation of stress-responsive gene expression (Zhang et al., 2006; Li et al., 2020).

Of the studied species, the model plant Arabidopsis (total frequency of 81, with a relative frequency of 2%) was the most popular (Fig. 2). Its popularity is associated with its small size, relatively short lifecycle, ease of growing under low-light lab conditions, and small genome which is instrumental for studying processes at the molecular level. In addition, wheat and maize as important cereals and worldwide staple food, were other popular species. From the first three species, it is clear that herbaceous plants are much more often researched than trees. However, as a group, “trees” still had a relatively high frequency (total frequency of 30, with a relative frequency of 1%). Trees are perennial plants with longer life spans and thus are likely to experience temporal drought, some mortality happens when these plants suffer from hydraulic failure; 2) Tree species generally have thicker stems and large enough vessels than tiny plants which make it easier to monitor water transport non-invasively (Brodribb et al., 2017).

Drought stress strongly interacts with other abiotic stresses. Drought stress is often accompanied by heat stress and is aggravated by salt stress. Salinity also can cause similar problems with drought stress due to the high osmotic potential in the soil, which leads to similar response patterns in plants, consequently, some scientists compare salinity and drought stress effects in their research (Sánchez-Blanco et al., 2014; Koevoets et al., 2016; Ma et al., 2020). Concomitantly, nutrient availability and uptake are inhibited by dry soil (Parrondo et al. 1975; Hira and Singh 1977; He and Dijkstra 2014). Climate change may exacerbate these interactions. Although it is challenging to study such interactions, they received relatively much attention as indicated by the frequencies of the words “temperature”, “salinity”, “nitrogen”, and “climate change” (Fig. 2).

3.1.2 Co-occurrence network of popular keywords

(Fig. 3 co-occurrence network here)

The co-occurrence network of the keywords revealed three clusters which we labeled: “plant growth”, “drought tolerance”, and “root hydraulics” (Fig. 3). “Growth” was the most popular keyword in the plant growth cluster (i.e., red cluster). Like the word cloud results, this cluster described the plant growth response in association with aboveground traits like “photosynthesis”, “leaf”, “gas exchange”, “chlorophyll fluorescence”, “stomatal conductance”, and “transpiration”. Even though the initial literature search included the keyword “root”, leaf traits had a higher frequency in this cluster, indicating that the study of plant response to drought and recovery is strongly focused on aboveground parameters. The plant growth cluster included words like ‘‘trees”, ‘‘forest”, and ‘‘yield” hinting at a more agroecological context. For forest ecosystems, climate change has caused more frequent drought events, and scientists have focused on different tree species' growth under drought and other environmental factors like soil nitrogen deficit and higher temperature. In agricultural ecosystems, wheat production has been the main focus of research. Wheat is known to be deep rooting and relatively tolerant to drought compared to other major grains (Fan et al., 2016).

The plant physiology cluster (blue cluster) contains words associated with (molecular) plant physiology such as. “drought”, and “tolerance”, which were the most popular keywords in this cluster and were strongly associated with words like ‘‘gene expression”, ‘‘osmotic adjustment”, ‘‘oxidative stress”, and ‘‘abscisic acid” (ABA). In contrast to the ‘red’ cluster, which mainly focuses on plant growth response, the blue cluster mainly focuses on gene and hormone regulation. The keywords in the blue cluster were associated with the model plant Arabidopsis, indicating that molecular physiology is commonly studied in this plant. “Abscisic Acid” bridges back to the ‘‘red plant growth” cluster through keywords like ‘‘response” and “photosynthesis” (Fig. 3). Mechanistically, Abscisic Acid regulates the stomatal response and thereby directly influences photosynthesis and growth.

The root hydraulics cluster (green cluster) included “root” as the most popular keyword and its relation to “conductance”, “hydraulic conductivity”, “transport”, and “maize” (Fig. 3). This cluster comprises research on water transport from the soil through the roots into the shoot. Hydraulic conductance of soil, rhizosphere, roots, and xylem is of great importance to understanding water “transport”. The word “root” was only weakly connected to leaf and physiological traits, possibly indicating a discrepancy in the research. This is surprising, given that plant hydraulic conductance, transpiration, and CO_2 uptake, are regulated via stomatal opening and closure. The ABA-controlled regulation of stomata is, however, sensitive to soil hydraulic properties (Carminati and Javaux, 2020; Abdalla et al., 2021), interacting with the root length and morphology (Müllers et al., 2022b). The mechanisms are still strongly debated (Li et al., 2020). In addition, it seems that root and water-transport researchers have chosen ‘‘maize” as their favourite model species. Possibly, because maize has a rather sturdy root system which is more easily studied than the fine roots of Arabidopsis or wheat.

3.2.Scientific mapping and trends of the outputs over time

3.2.1 Temporal evolution of the outputs over time

(Fig. 4 Temporal evolution)

The frequently used keywords have changed over the past five decades. From 1975 to 2005, the topics ranged from whole plant physiology to molecular response, but roots were not in focus. Only in recent years, from 2006 to 2022, keywords related to root plasticity to drought and post-drought recovery became more frequent. During this period, the focus shifted to applied aspects such as “yield” and “climate change” as climate change made the need for resilient crop yield more imminent. The keyword “growth” was frequently used in all periods, as it is fundamental to our definitions of drought stress and recovery (Fig. 4).

During 1975-1995, the main focus was on plant physiology as indicated by the frequent use of words such as “osmotic adjustment”, “growth”, (cellular) “injury”, “abscisic acid” and “conductivity”. Some scientists also investigated the impact of microbes on drought response like endophytic fungi and plant growth-promoting rhizobacteria (PGPR), which explain the appearance of keywords like “infection” and “bacteria” (Ruizlozano et al., 1995). With the advancement of molecular technologies, “expression”, and “oxidative stress” increased from 1996-2005, indicating that more scientists devoted themselves to identifying genes involved in drought tolerance. During 2006-2015, research emphasized mechanisms using model plants and an increasing interest in roots, with frequent keywords: “root”, “gas exchange”, “mechanisms”, “Arabidopsis”, and “superoxide-dismutase”. After 2015, the number of studies increased, and more researchers demonstrated that the recovery period matters to the overall plant performance and that is especially true in the field station under climate change (Hagedorn et al., 2016; Guo et al., 2021; Santos-Medellin et al., 2021). During this period, keywords such as “recovery” and “climate change” were used frequently.

3.2.2 Motor and basic themes

(Table 2 basic & motor themes)

The temporal evolution of keywords’ frequency shows keywords that were gradually used less, like “abscisic acid”, ones that remained stable, like “growth”, and those that gained more attention in recent years, like “climate change” and “yield” (Fig. 4). Therefore, we added a thematic analysis to further understand the temporal evolution of keywords. The thematic analysis distinguishes ‘motor’ from ‘basic’ themes. Motor themes are both important, well-developed, and highly cited in recent years, whereas basic themes are the main and driving keywords for the research topic but receive a few citations (Cobo et al., 2011; Liu et al., 2021). To the motor themes belonged words like “water stress”, “stomatal conductance”, “abscisic-acid”, “chlorophyll fluorescence”, “biomass”, “proline”, and “hydraulic conductivity” whereas “drought”, “recovery”, “photosynthesis”, “root”, “climate change”, “growth” and “resilience” were the basic themes (Table 2). Although “water stress”, “abscisic acid”, “proline” (osmotic adjustment), and “conductivity” were motor themes and important, they were studied intensively in the early stage (Fig. 4). The root supports growth through water and nutrient uptake, transport, perception, and signaling. Thematic evolution identified oxidative stress and nitrogen as basic themes, well developed in 1975-2005 (Fig. 4, Table 2). In contrast, “recovery” and “root” (note these were part of our initial search terms), and “climate change” had a stronger development during recent years. This is likely to continue in the near future as climate change demands agro-ecological adjustment to the increasing risk of temporal drought.

We expect motor themes, like root, recovery, climate change, and their relationship to yield and leaf traits, will drive future studies. The challenges of root and rhizosphere dynamic measurement will require the deployment of innovative technologies to accelerate root science. New technologies and methods, like non-invasive root and rhizosphere phenotyping, will be key to understanding root dynamics during drought and rewatering (Wasson et al., 2020). Mathematical modelling will also be important to simulate mechanisms of water transport as well as the discovery of plant traits for greater crop resilience and faster recovery (Hall, 1982; Carminati and Javaux, 2020; Maurel and Nacry, 2020; Javaux and Carminati, 2021; Joshi et al., 2022). Additionally, understanding the interactions with other abiotic stresses will be crucial in the context of “climate change” and “yield”.

3.3.Marked sources and scientific mapping of the outputs

3.3.1 Trends in root plasticity during both drought and recovery

(Fig. 5 Here)

Over the past 120 years, the number of publications on ‘root plasticity during drought and recovery’ increased strongly, but still, the topic seems to be underdeveloped compared to shoot-related research (Fig 5). We distinguish three periods: 1900 to 1990, 1991 to 2004, and 2005 to 2022. For the first period, only 4 publications were found (in 1975, 1983, 1986, and 1988). During this period, few publications were uploaded to WOS and most researchers focused on drought but not recovery. The number of publications during that period was low in all sciences, but especially so in root research as measuring roots was challenging and few technologies were available. Thus, 99% of the analyzed publications were published in the last three decades with an annual increase of 7.20%/a, greater than the annual growth rate in Life Sciences of 5% (Bornmann et al., 2021). The results also revealed that the publications number improved very slightly from 1991-2006 with around 15 papers each year, while they dramatically increased during 2015-2022 (Fig. 5) with 80% of publications found after 2005. The trend of root plasticity during both drought and recovery research was consistent with the trend in the related emerging topic of ‘carbon exchange in global drylands’ (Liu et al., 2021).

3.3.2 Marked countries and dominant institutes

USA and China were the most productive and cited countries. Among the 10 countries with the highest number of publications, the USA (relative frequency=71%) and China (69%) had a similar frequency and were 3 times higher than the third most productive country. Spain, Brazil, and Australia, at position 3-5, had a similar frequency of around 200 (Table 3). Although the most productive are also the most cited countries, USA-based publications were cited 2.2 times more often than those from China, with 14 vs 6 citations per publication. Spain, France, Australia, Italy, India, and Germany were positioned at 3-8 with 12-13 citations per article (Table 3). Three reasons may explain these ranking patterns: all these countries 1) have arid regions with severe drought stress; 2) have advanced technology for root research; 3) have many researchers and a higher GDP that ensures enough human and material resources for related research.

The collaboration map shows a similar ranking: USA (164), China (136), Germany (105), Spain (87), and Australia (74) had the highest collaboration frequency of all countries. Even though Germany's ranking in the most productive and cited countries is not high, it still ranked in the third position according to the collaboration frequency, which indicates that Germany had many collaborations with other countries but less direct ownership in the topic. Germany is well known for its development of technologies, root phenotyping and soil-plant hydrology and as such is a looked-for partner, even though its agriculture is less threatened by drought compared to the other listed countries (Fig. S1).

Our results showed that 1429 institutions all over the world have participated in root plasticity during both drought and recovery. The top ten most productive institutions contributed 27% (294 publications) of the total publications (Table 1, 3). From 1976 to 2021, the Northwest A&F University in China ranked first with the most publications (48), followed by the University of California, Davis (USA), China Agriculture University (China), Khon Kaen University (Thailand), and University of Western Australia (Australia). Consistent with the most productive and cited countries, 9 of the top 10 institutions belong to China, USA, or Australia. Khon Kaen University in Thailand stood out, as Thailand did not rank high in the country ranking.

Some highly productive authors affiliated with Khon Kaen University are A. Patanothai and S. Jogloy. For example, they concluded that during drought and recovery, peanut pod yield was associated with increased root surface area deeper in the soil. (Jongrungklang et al., 2012, 2014). This is the only study we found that proposed a root ideotype for both drought and recovery, although there are other ideotype studies that focused on drought only.

3.3.3 Competitive journals and top-cited publications.

Studies on root plasticity during both drought and recovery were published in 334 journals, most related to botany or agronomy (Table 1). The top ten most productive and cited journals focus on plant research across scales from molecules to ecology. Plant and Soil, Tree Physiology, Frontiers in Plant Science, Agricultural Water

Management, and Journal of Plant Physiology were found to be the five most prominent journals, with a note that Frontiers in Plant science is a relatively new journal. Tree Physiology, Plant Cell and Environment, Journal of Experimental Botany, New Phytologist, and Plant and Soil were the five highest topic-h-index journals. In general, the h-index seems low, indicating that the topic is not receiving much attention despite its societal relevance. Plant Physiology, Tree Physiology, Journal of Experimental Botany, Plant Cell and Environment, and New Phytologist were the five journals that scored highest in total number citations, indicating the greater interest of publications in these journals as a source of bibliographic consultations.

Eight of the top ten journals have a relatively broad scope in plant sciences, except for Tree Physiology and Agricultural Water Management, which have a strong focus on trees and crop water management, respectively. Because drought stress is closely related to soil water content, drought events are easy to appear in a natural ecosystem like a forest ecosystem, so it's not hard to understand why 'Plant and Soil' and 'Tree Physiology' were the most productive journals.

3.3.4 Top-cited publications.

In the SCI-E database, 1086 publications were found when searching across the last 120 years. The oldest publication was published in 1975 by Parrondo et al entitled "Rubidium absorption by corn root tissue after a brief period of water stress and during recovery" published in Physiologia Plantarum, showing that reductions in rubidium uptake during a short period of water deficit only partially recovered during the post-recovery period. The effect of drought and rewetting on nutrient availability remains an important topic today. In the past, the focus was strongly on soil physical effects, such as reduced effective diffusion in dry soil (Hira and Singh, 1977). Recently a more complicated picture emerged involving the microbiota which influences phosphorus concentrations and sorption rates (Chen et al., 2021). Thereby, it still remains a question of how nutrient availability and uptake influences plant growth during drought and recovery and few papers deal with the question after Parrondo's initial work. The study conducted by Xu et al. "Expression of a late embryogenesis abundant protein gene, HVA1, from barley confers tolerance to water deficit and salt stress in transgenic rice" (Xu et al., 1996), published in Plant Physiology in 1996, was the most cited article with 655 citations or 24 citations per year. This study proved the important role of plant LEA (late embryogenesis abundant) proteins under drought and salt stress and its potentia1 for genetic crop improvement toward abiotic stress tolerance. Due to their versatility, LEA-related genes and their function have received much attention as potential drought and salt tolerance genes. Twenty six years later, LEA has been widely studied in the context of drought, still, its functioning is debated and real-world agronomic application is still a promise (Hernández-Sánchez et al., 2022). Among the top ten highly cited papers, two focused on genes (Xu et al., 1996; Swindell et al., 2007), one on proteins (Salekdeh et al., 2002), one on rhizosphere bacteria (Mayak et al., 2004), one on roots and branches hydraulic failure (Anderegg et al., 2012) and one on root-water transport modeling . Surprisingly, despite our root search term, there wasn't any highly cited paper (>295) on root morphology or architecture. Root research on this topic of drought and recovery should get more attention in the future.

4. Ideotypes and genetics

Ideotypes are an important way to identify and select better cultivars in agriculture, but so far we found only one publication that proposed a root ideotype for both drought and recovery. The study conducted by Jongrungklang et al. (2014) proved that greater root surface area of peanut at deeper soil layers contributed to a higher pod yield (2014). As such, a shortlist of key root traits, and their genetics for a faster drought recovery remains speculation. In contrast, several studies have suggested root traits that are beneficial during drought. For example, Fonta et al. (Fonta et al., 2022) observed that a drought-tolerant rice line had, when exposed to drought, deeper and larger diameter roots. A field experiment carried out by Schneider et al (2020) demonstrated that reduced root diameter of maize genotypes under drought can reduce the metabolic costs in soil exploration while penetration into drier soil is more difficult. The authors identified a gene locus (Zm00001d018342) which was also attributed a role in plasticity of root cross-sectional areas (Schneider et al., 2020). Combining field and greenhouse experiments, Liao et al. demonstrated that under drought stress, high, and stable grain yield of 'aus' rice varieties were positively related to 'large-diameter' nodal roots, high and stable deep root growth. Genetically, qRT9 was associated with root thickness regulation (Li et al., 2015). Similarly, the region on chromosome 1 which is located near qDTY1.1, was associated with rice drought-yield, also shoot and root plasticity responses in rice under drought stress, particularly increased deep rooting (Vikram et al., 2011; Wade et al., 2015; Sandhu et al., 2016). However, due to the complexity of drought recovery, root ideotypes may be highly context-specific. Here we suggest that by focusing on the drought period, science might have missed opportunities as there is no clarity if the traits that are proposed to be advantageous during drought also support a strong recovery. Recent studies of maize lines by Chen et al (2016) and Gonzales-Hernandez et al (2021) emphasize that the recovery phase influenced the final plant biomass more than the drought phase. In the context of recovery after drought, many of the traits that have been suggested to enhance growth during drought, still need testing during the recovery period.

We further ask if genetic engineering can improve drought adaptability. Despite significant effort, surprisingly, only one transgenic cultivar (namely Monsanto’s DroughtGard) has been released to farmers so far (Passioura, 2020). The major reasons for the slow progress in the transgenic crop are the complexity of the drought environment, which often results in the lack of clear identification of the target environment, and also due to too much attention being assigned to a single drought process in the laboratory research. While under natural conditions the repeatable drought-rewetting cycle interacts with other (a)biotic factors, these were often ignored in research. Further understanding of root dynamics and the role of roots in plant resilience to both drought and recovery, therefore, should be taken into consideration in the future.

5. Implications and future perspectives

The continued fast growth of ‘root plasticity during drought recovery’ has illustrated the status and importance of this field. We expect this topic to become more prominent in the near future, because of climate change, population growth, and the great need for a stable food supply. Understanding root plasticity during drought recovery is fundamental to increasing plant stress resilience and maximizing production on both the ecological and agricultural sides.

As the key element for plant water uptake and belowground process, how plant-environment interaction like plant hydraulic failure, above-/below- ground biomass allocation, plant/microbiology interaction, and species composition are affected under drought- recovery cycles context should be given more attention in the future. Drought recovery studies will be more helpful in understanding and predicting these processes.

During a drought, growth is reduced and a multitude of other physiological and phenotypic changes occur. Some are easily and quickly restored by rewatering, such as stomatal opening after closure, and others might be permanent ‘damage’. We summarized the current progress on plant and root plasticity during drought and recovery research, even though recovery related research is relatively rare (Table 6). During the recovery phase growth can be accelerated with relative growth rates that are greater than that of the control plants (Xu et al., 2009), but this is not always observed (Steinemann et al., 2015). Besides a rebalanced hydraulics, plants need to alter root traits, including root morphology/ architecture, root anatomy, root exudate and rhizosphere microbiomes, to compensate for the, during drought, lost plant and acclimate to the new soil environment. From the few reports that we found, root plasticity responses to drought recovery were highly species and scenario specific, making it difficult to generalize (Table 6). We hypothesize that the rate of the recovery depends on the performance of the root system. Root performance is influenced by 1) growth substrate condition: soil nutrient (N/P/K) content and soil structure; 2) drought intensity and frequency; 3) species: tree, grass, and crop; 4) growth stage e.g. early vs late season; 5) root physiology traits like ABA, water-soluble carbohydrates, nutrient homeostasis; 6) plasticity of root morphology and anatomy traits; 7) root and microbiome interaction, rhizosphere stability. Restoring root functioning, not in the least soil nutrient uptake, through restoration of root growth, root morphology, and rhizosphere functioning may be the key to fast whole plant recovery after a drought.

6. Concluding remarks

We analyzed the scientific literature on root plasticity during drought and recovery in the past 120 years using bibliometric analysis on the premise that 1) the recovery phase is important as not all droughts are terminal and 2) roots and their responses to drought and rewatering are key to the resilience of both cropping systems and natural vegetations. The rewatering phase received much less attention than the drought periods and the root received much less attention compared to shoots. Aboveground physiological traits of model plants Arabidopsis, crop plants wheat, maize, as well as trees were found to be the most popular study areas. Co-occurrence network analysis showed that three clusters were classified for the keywords including photosynthesis response, physiological traits tolerance, and root hydraulic transport. Further, thematic evolution analysis showed a transition from classical agricultural and ecological research via physiological and molecular response, to root plasticity responding to drought recovery in recent years. Overall, both results showed that root plasticity’s role during drought and recovery is less focused. While progress has been made on leaf traits and root physiology areas, more attention should be given to root morphology and microbiome side using novel root phenotyping methods and mathematical modelling ways, to further understand root plasticity during both drought and recovery.

Author contributions

CZ and JP conceived and designed the experiments and performed the analysis. CZ and JP wrote the first draft; other authors provided reviewing and editing advice. All authors contributed to the article and approved the submitted version.

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Conflict of interest statement

Nothing declared.

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Table

Table 1 Main information in relation to root plasticity during drought and recovery collection. Collaboration index=Authors of multi-authored publications/Multi authored publications.

Description	Data
Timespan	1975:2022
Sources (Journals, Books, etc)	334
Publications	1086
Author's Keywords (DE)	3134
Authors	4112
Average years from publication	10.1
Average citations per publication	34.99
Author Appearances	5091
Authors per publication	3.79
Collaboration Index	3.85

Table 2 Top 10 high-frequency keywords in basic and motor themes of thematic analysis on root plasticity during drought and recovery research.

Basic themes	Occurrences	Motor themes	Occurrences
Drought	172	Water stress	75
Recovery	86	Stomatal conductance	33
Photosynthesis	68	Abscisic acid	22
Root	50	Chlorophyll fluorescence	22
Climate change	36	Biomass	20
Growth	31	Proline	19
Oxidative stress	18	Hydraulic conductivity	15
Nitrogen	17	Conductance	14
Resilience	16	Potential	13
Salt	16	Soil moisture	13

Table 3 Top 10 most productive and cited countries and most productive institutes with the publications of root plasticity during drought and recovery research during the period of 1900–2021.

Country production		**Most cited countries**		**Most productive institutes**	
Country	**Number of publications**	**Country**	**Total Citations (citation/publication)**	**Affiliations**	**Articles**
USA	772	America	10862(14)	NORTHWEST A&F UNIV	44
China	745	China	4830(6)	UNIV CALIF DAVIS	40
Spain	230	Spain	2835(12)	CHINA AGR UNIV	34
Brazil	204	France	2595(13)	KHON KAEN UNIV	31
Australia	180	Australia	2229(12)	UNIV WESTERN AUSTRALIA	29
Germany	180	Italy	1770(10)	GUANGXI UNIV	27
France	133	India	1137(9)	TEXAS A&M UNIV	23
Italy	129	Germany	1135(9)	UNIV CALIF LOS ANGELES	23
Japan	128	Brazil	1017(8)	COLORADO STATE UNIV	22
India	123	Canda	895(7)	UNIV FLORIDA	21

Table 4 Top 10 most productive authors and journals with the publications of root plasticity during drought and recovery research during the period of 1900–2021.

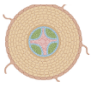
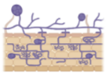
Sources	Articles	h-index	Total citation
PLANT AND SOIL	37	17	801
TREE PHYSIOLOGY	36	24	1748
FRONTIERS IN PLANT SCIENCE	32	15	713
AGRICULTURAL WATER MANAGEMENT	24	14	637
JOURNAL OF PLANT PHYSIOLOGY	23	16	898
PLANT CELL AND ENVIRONMENT	22	20	1384
JOURNAL OF EXPERIMENTAL BOTANY	21	17	1479
NEW PHYTOLOGIST	21	17	925
ENVIRONMENTAL AND EXPERIMENTAL BOTANY	20	12	455
PLANT PHYSIOLOGY	18	16	2461

Table 5 Top 10 high cited papers with the publications of root plasticity during drought and recovery research during the period of 1900–2021.

Title of publications	Year of publications	Journal	Average citation per year	Total citations
Expression of a late embryogenesis abundant protein gene, HVA1, from barley confers tolerance to water deficit and salt stress in transgenic rice	1996	Plant Physiology	24.74	668
Adaptations of Endophyte-infected cool-season grasses to environmental stresses: Mechanisms of drought and mineral stress tolerance	2000	Crop Science	21.96	505
Plant growth-promoting bacteria that confer resistance to water stress in tomatoes and peppers	2004	Plant Science	25.63	487
General mechanisms of drought response and their application in drought resistance improvement in plants	2015	Cellular and Molecular Life Sciences	57.00	456
The roles of hydraulic and carbon stress in a widespread climate-induced forest die-off	2012	Proceedings of the National Academy of Sciences of the United States of America	41.09	452
Transcriptional profiling of Arabidopsis heat shock proteins and transcription factors reveals extensive overlap between heat and non-heat stress response pathways	2007	BMC Genomics	24.25	388
Development of drought-resistant cultivars using physio-morphological traits in rice	1995	Field Crops Research	13.14	368
Proteomic analysis of rice leaves during drought stress and recovery	2002	Proteomics	15.90	334

The crucial role of plant mitochondria in orchestrating drought tolerance	2009	Annals of Botany	22.29	312
Sensitivity of growth of roots versus leaves to water stress: biophysical analysis and relation to water transport	2000	Journal of Experimental Botany	12.83	295

Table 6 Overview of root plasticity during drought and recovery research. References (1) (Xu et al. 2009); (2) (Chen et al. 2016); (3) (Delfin et al. 2021); (4) (He et al. 2022); (5) (Schneider et al. 2020); (6) (Liao et al. 2022); (7) (Chandregowda et al. 2022); (8) (Slette et al. 2022); (9) (Jongrungklang et al. 2014); (10) (De Bauw et al. 2019); (11) (de Vries et al. 2019); (12) (Brunn et al. 2022); (13) (Preece and Penuelas 2016); (14) (de Vries et al. 2020); (15) (Santos-Medellin et al. 2021)

Phases Categories	No drought	Moderate/extreme drought	Recovery
 Whole plant	Plants grow actively	Plant photosynthesis/growth down-regulated or even stopped (1), (2), increased root: shoot ratio (3), more carbon allocated to roots (4)	Plant photosynthesis/growth resumes or is even stimulated (1) (2) (3)
 Root morphology/ architecture	Root normal growth and distribution	Increased root growth at depth (5), 'large-diameter' nodal roots, deep root angle (6), varied specific root length (7)	Increased root biomass (8), decrease root growth at deep layer (9)
 Root anatomy	Normal root anatomy	Fewer but larger cortical cells, higher root cortical aerenchyma, small xylem vessel area (10)	Unknown
 Root exudate	Normal root exudation	Down (11) or up-regulated root exudation (12), (13)	Altered root exudation (11)
 Rhizosphere microbiomes	Plant allocates carbon to rhizosphere bacteria and fungi	Less carbon allocated, changed microbiome composition, reduced heterotrophic microbiome activity or even stopped (14), (15)	Increased activity, altered microbiome composition, and plant-microbe interaction (14), (15)

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Chandregowda M, Tjoelker M, Pendall E, et al (2022) Root trait shifts towards an avoidance strategy promote productivity and recovery in C3 and C4 pasture grasses under drought. Functional Ecology 36:. https://doi.org/10.1111/1365-2435.14085

Chen D, Wang S, Cao B, et al (2016) Genotypic Variation in Growth and Physiological Response to Drought Stress and Re-Watering Reveals the Critical Role of Recovery in Drought Adaptation in Maize Seedlings. Frontiers in Plant Science 6:

De Bauw P, Vandamme E, Lupembe A, et al (2019) Anatomical root responses of rice to combined phosphorus and water stress – relations to tolerance and breeding opportunities. Functional Plant Biology 46: https://doi.org/10.1071/FP19002

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Figure caption

Fig. 1 Schematic representation of root plasticity during drought and recovery. The image is created with BioRender. Created with BioRender.com.

Fig. 2 Top 50 keywords represented by the word cloud; labels are usually single words. and the frequency of each label is shown with font size. The biggest word "growth" appeared 266 times with a frequency of 7%, while the smallest word "forest" only showed up 26 times with a frequency of 1%.

Fig. 3 Co-occurrence Network of top 50 keywords. The size of the circle and the connecting lines represent the frequency and the relationship of the keywords, separately. The larger size the rectangular is, the higher the frequency. Similarly, the thicker the line is, the closer the relationship between keywords. Different colors represent different clusters, indicating that these keywords appear more frequently in the same publication. Red cluster, leaf parameters' response; blue cluster, molecular, and physiological responses; green cluster, root hydraulic and water transport response.

Fig. 4 Thematic evolution of popular keywords in regard to root plasticity during drought and recovery research. The horizontal axis represents the time period, and boxes of different colors represent different keywords. The size of each box represents the frequency in different time periods, and the lines between each box reflect the keywords' temporal evolution, transfer, and inheritance.

Fig. 5 Temporal evolution of outputs on the shoot/root plasticity during drought and recovery research from 1900 to 2021.

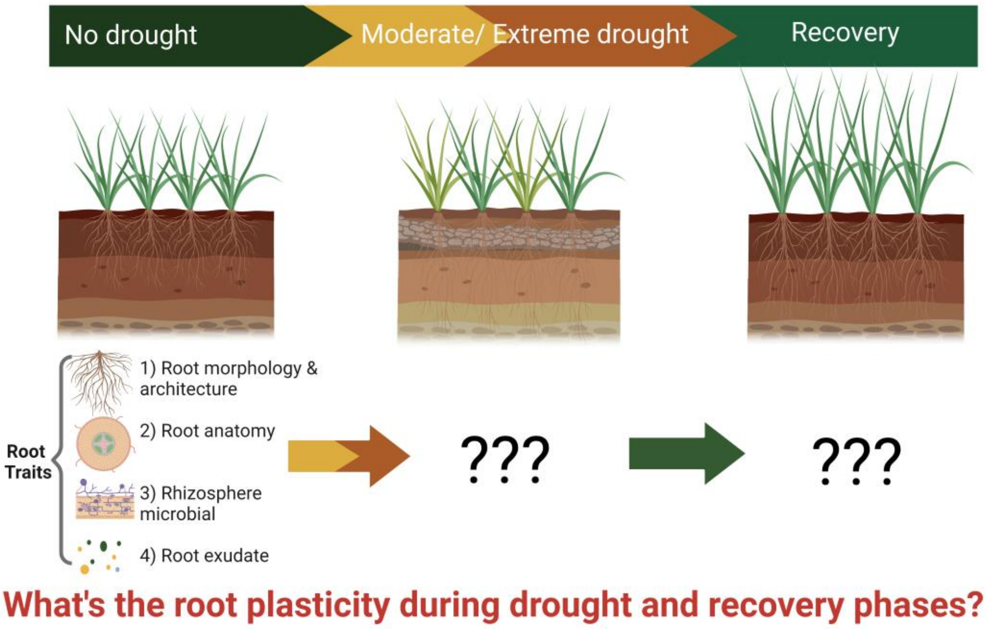
No drought
Moderate/ Extreme drought
Recovery
Root Traits
1) Root morphology & architecture
2) Root anatomy
3) Rhizosphere microbial
4) Root exudate
???
???
What's the root plasticity during drought and recovery phases?

Fig. 1

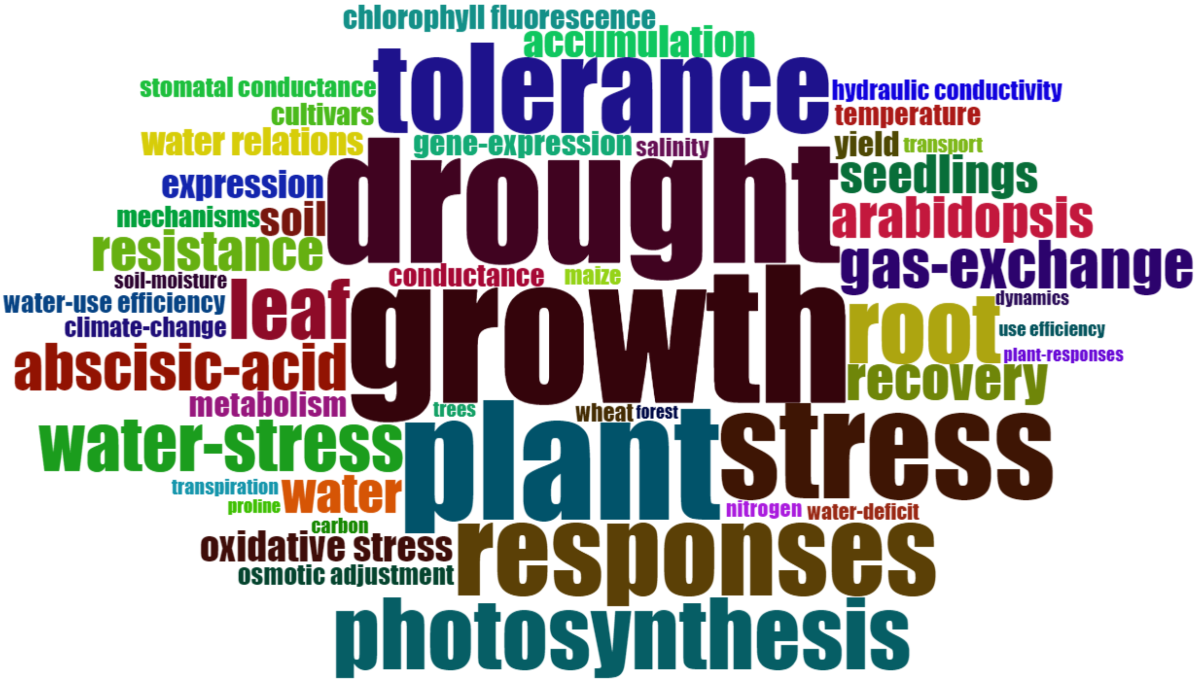
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Fig. 2

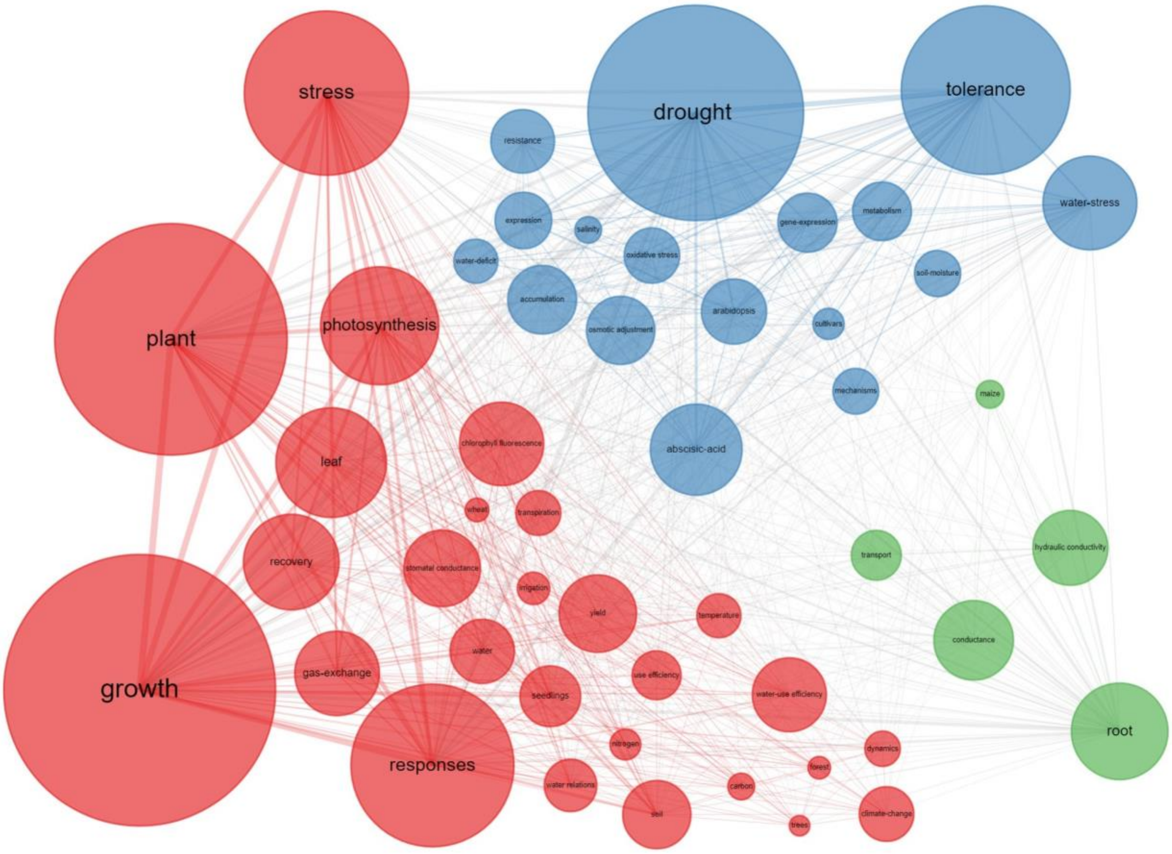
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Fig. 3

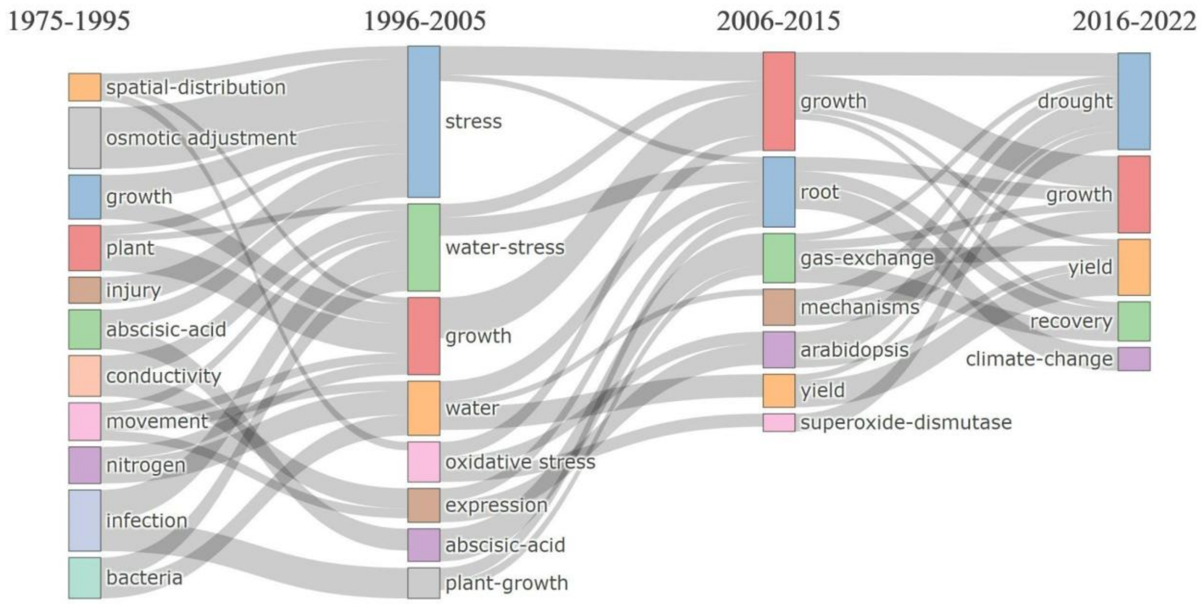
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Fig. 4

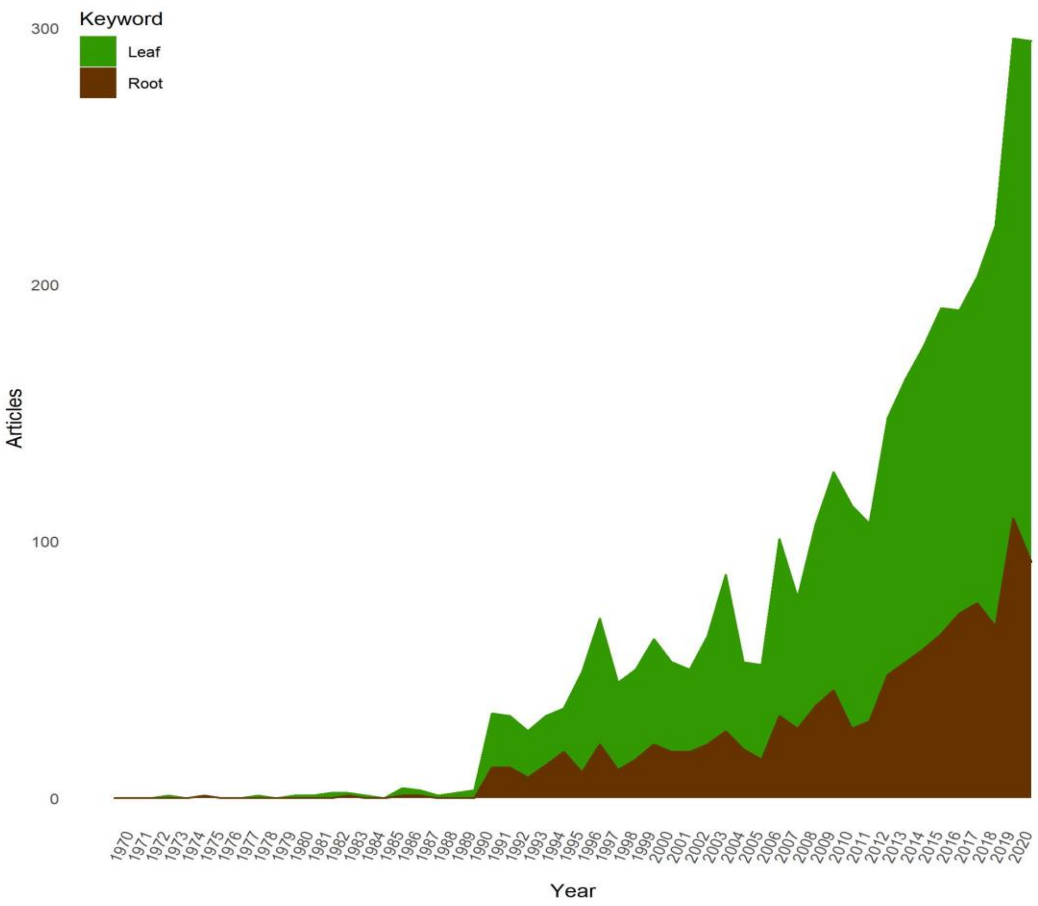
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Fig. 5

Supporting Information

Article title: Plant root plasticity during drought and recovery: What do we know and where to go?

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The following supporting information is available for this manuscript:

Fig. S1 Country collaboration of published articles on root plasticity during drought and recovery topics from 1900 to 2021. The lines between the two countries indicate the collaboration frequency between two countries, the thicker the line is, the higher the frequency.

Table S1 Top 10 high cited papers with the publications of root plasticity during drought and recovery research during the period of 1900–2021 from Web of Science and its availability in Scopus.

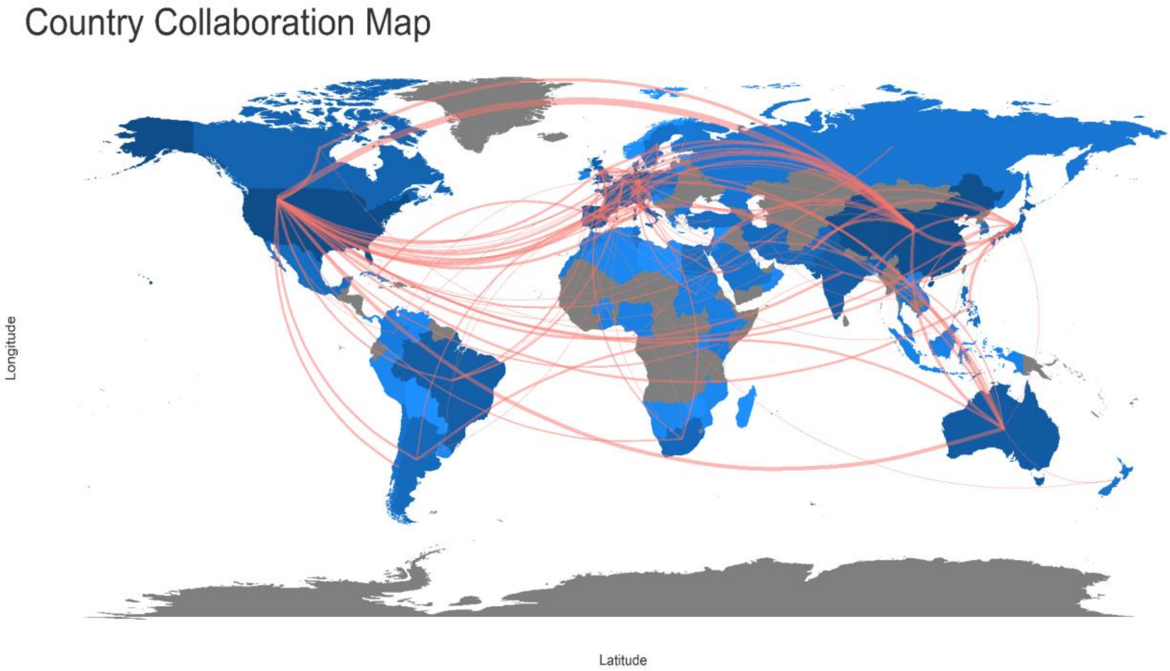

Fig. S1

Table S1

Title of publications	**Year of publications**	**Journal**	**Total citation in Web of Science**	**Availability in Scopus**	**Total citation in Scopus**
Expression of a late embryogenesis abundant protein gene, HVA1, from barley confers tolerance to water deficit and salt stress in transgenic rice	1996	Plant Physiology	668	Yes	804
Adaptations of Endophyte-infected cool-season grasses to environmental stresses: Mechanisms of drought and mineral stress tolerance	2000	Crop Science	505	Yes	588
Plant growth-promoting bacteria that confer resistance to water stress in tomatoes and peppers	2004	Plant Science	487	No	
General mechanisms of drought response and their application in drought resistance improvement in plants	2015	Cellular and Molecular Life Sciences	456	Yes	579
The roles of hydraulic and carbon stress in a widespread climate-induced forest die-off	2012	Proceedings of the National Academy of Sciences of the United States of America	452	No	
Transcriptional profiling of Arabidopsis heat shock proteins and transcription factors reveals extensive overlap between heat and non-heat stress response pathways	2007	BMC Genomics	388	Yes	425

Development of drought-resistant cultivars using physio-morphological traits in rice	1995	Field Crops Research	368	Yes	442
Proteomic analysis of rice leaves during drought stress and recovery	2002	Proteomics	334	No	
The crucial role of plant mitochondria in orchestrating drought tolerance	2009	Annals of Botany	312	Yes	353
Sensitivity of growth of roots versus leaves to water stress: biophysical analysis and relation to water transport	2000	Journal of Experimental Botany	295	Yes	355

CHAPTER 3: Root Plasticity and Phosphate Homeostasis: Key Drivers of Drought Resilience in Upland Rice

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Manuscript under internal review

Abstract

Aims: Phosphorus deficit and intermittent drought significantly restrict upland rice (*Oryza sativa* L.) growth and production, yet the extent to which plant traits contribute to multi stress tolerance, particularly belowground, remains poorly understood. This study aims to identify whether and how P uptake during drought is associated with its resilience to post recovery in upland rice.

Methods: A 30-liter rhizobox experiment was conducted under glasshouse conditions using two upland rice genotypes. The genotypes DJ123 (P-efficient) and Nerica4 (P sensitive but slightly drought tolerant) were sown with 18 seeds per box. The dynamics of biomass, the uptake of phosphorus, and regrowth rate at drought recovery were measured.

Results: The accumulation of biomass is significantly influenced by the availability of phosphorus (P) and water. The performance of the upland rice at the end of a drought was not consistent with performance during post recovery, their P homeostasis, relative growth rate and relative root elongation rate differed under different treatments. Upland rice seedlings with higher phosphorus concentration, lower internal phosphorus use efficiency (PUE), and higher phosphorus accumulation efficiency (PAE) exhibited a higher relative growth rate and biomass accumulation during the recovery phase. The phosphorus-efficient genotype, DJ123, demonstrated superior phosphorus homeostasis and exhibited enhanced recovery and drought resistance compared to the phosphorus-sensitive genotype Nerica4 under low P levels.

Conclusion: This study highlights the critical role of root traits and phosphorus efficiency in upland rice's drought resilience, providing insights for breeding programs aimed at enhancing stress tolerance.

Background

Rice (*Oryza sativa* L.) is a staple food for more than half of the world's population, making it a critical component of global food security (FAO, 2013). With the global population projected to reach 9.7 billion by 2050, the demand for rice is expected to increase substantially. In addition, rice production must adapt to the challenges posed by climate change, including increased frequency of extreme weather events, fluctuating temperatures, and water scarcity. Climate-smart rice varieties, capable of withstanding drought, flooding, and salinity, are essential to maintaining high yields and ensuring food security under these changing conditions (Mackill *et al.*, 2012). Efforts to improve rice production through sustainable agricultural practices and genetic improvements are critical in meeting future food demands while mitigating the impacts of climate change (Godfray *et al.*, 2010). Thus, increasing the resilience and productivity of rice is central to global food systems, underscoring its vital role in feeding the world.

More than 50% of the world's rice is rainfed, but these non-irrigated areas produce only a quarter of the world's rice (RAM, 2003). Upland rice is vital for ensuring food security in many parts of the world, particularly in regions where irrigation infrastructure is lacking, and water availability is inconsistent. Unlike lowland rice, which thrives in flooded paddies, upland rice is grown in rainfed, non-flooded conditions, making it crucial for smallholder farmers in hilly and mountainous regions of Asia, Africa, and Latin America. These areas often experience erratic rainfall and poor soil fertility, conditions under which upland rice varieties, bred for their resilience to drought can perform relatively well (Farooq *et al.*, 2009; Rao *et al.*, 2016; De Bauw *et al.*, 2018; Wissuwa *et al.*, 2020). The ability of upland rice grow without the need for extensive irrigation systems makes it a sustainable option for resource-limited farmers. Furthermore, the genetic diversity within upland rice varieties offers significant potential for breeding programs aimed at improving drought tolerance and nutrient-use efficiency, which are critical traits as climate change intensifies water scarcity and depletes soil nutrients (Bernier *et al.*, 2008; Henry *et al.*, 2012; Wissuwa *et al.*, 2016). Therefore, improving the understanding of root system and rhizosphere process after drought stress, and developing rice cultivars with improved drought tolerance, are important to meet the demands of global food security.

To reduce drought risk and increase yield stability, the recovery period after the drought is of great importance (Chen *et al.*, 2016; Zheng *et al.*, 2023; Sachsenmaier *et al.*, 2024). Generally, during the drought phase, the root system growth is reduced or arrested. Soil dries from the top of the profile, exposing the upper part of the root system to water stress. Typically, shallow roots stopped growing, where as deep root growth might have continued to access water stored in deep soil (Fenta *et al.*, 2014; Lynch & Wojciechowski, 2015; Gao & Lynch, 2016). Additionally, loss of shallow root cortex due to drought-induced senescence may occur. This would mean loss of root hairs, root exudation, and mycorrhizal colonization, which are usually regarded as key elements in phosphorus acquisition by plants (Richardson, 2001; Richardson *et al.*, 2009, 2011). This all would lead to a dramatic reduction in the ability to take up nutrients, especially phosphorus, during drought. Phosphate is relatively immobile in soil, and P availability in surface soil strata is generally greater than that in subsoil strata because of fertilizer placement, the deposition of plant residues over time, and the greater biological activity in surface strata (Lynch & Brown, 2008; Lynch, 2011; York *et al.*, 2013). Together, uptake of immobile nutrients like phosphorus is severely reduced in dry soils, due to the changes in root architecture and function, and the reduced effective diffusion rates through dry soil. As an essential constituent of plants, P is commonly regarded as the determinant factor for its growth. Other studies have shown that relative growth rate and root elongation rate of tree and grass species are closely related to their foliar nucleic acid P and substrate phosphorus concentration, respectively (Borch *et al.*, 1999; Ma *et al.*, 2003; Han *et al.*, 2021). The maintenance of phosphate homeostasis, that is, an appropriate cellular phosphate (Pi) concentration within cells and tissues can maintain a balance that supports plant healthy growth and development (Lin *et al.*, 2009; Prathap *et al.*, 2022), may be vital for the post drought recovery. Recently, many studies have focused on the relationship between nutritional status and drought resistance and the integrated effects of nutrients and water status on leaf characteristics and water relationships (Shangguan *et al.*, 2000; Saneoka *et al.*, 2004; Yang *et al.*, 2012; Zheng *et al.*, 2023). Yet, roots are vital for the ability of plants to acquire nutrients and water- two functions of fundamental importance to whole-plant growth and performance. After drought, the plant needs to recover its root system through new growth. Very little is thus far known about this recovery period, what the plants traits are associated with a fast recovery, and how these traits are associated with other drought resistant related traits. The objective of the research presented here was to clarify the relationship between rice phosphorus status, phosphorus acquisition efficiency and drought recovery in terms of water × phosphorus effects. Furthermore, we wished to test the hypothesis that 1) phosphorus status of the plant at the end of the drought period is a good predictor for the rate of recovery afterwards; 2) P efficient genotype is better at recovery after drought.

Materials and methods

Study area

A pot trial was set up in the greenhouse located at the Laboratoire des Radio-Isotopes (18°91’ S, 47°55’ E, Antananarivo, Madagascar) from April 14 to June 07, 2022. The weather conditions in the greenhouse including light intensity, temperature, and humidity during the experimental period were recorded with WatchDog station (Spectrum Technologies Inc., Plainfield, IL, USA) (Fig. S1). The experiment was a factorial combination of two phosphorus levels: 0 and 25 kg P ha^{-1}, two water regimes: well water, drought recovery, and two upland rice genotypes: DJ123 and Nerica4, in a randomized block design with 4 replicates. The seeds of the two rice varieties were pre-germinated in petri dishes in an oven maintained at 30°C. Nerica4 was initiated two days earlier than DJ123 to ensure both had a similar emergence time. After both radicles had emerged 1-2 mm, both seedlings were sown in each box with 3 rows of 6 seedlings each, spaced 7 cm apart in the lengthwise direction and 10 cm apart in the widthwise direction, one pre-germinated seed was sown per hole (Fig. S2). The experimental containers were 16 wooden rhizoboxes of 30 L (40 cm of length, 30 cm of width and 25cm of height) containing 30 kg of air-dried and 2 mm sieved soil. The soil used was collected from the top 15 cm of an upland rice field in the Commune of Antohobe, District of Betafo in the Vakinankaratra region (19°46’ S, 46°41’ E, 1240 m altitude). Soil is classified as Phaeozem, with pH (H_2O) of 5.05 (soil: water =1: 2.5) and available P of 1.35 mg kg^{-1} (AEM-P: anion exchange resin extraction) (Sibbesen, 1977; Saggar et al., 1990). Soil preparation and the addition of respective fertilizers were carried out according to the P levels. We added the equivalent of nitrogen (N) 50 kg ha^{-1} as NH_4NO_3 and potassium (K) 40 kg ha^{-1} as K_2SO_4 were applied to all soils (Calculation of the per ha equivalents based on the container surface area) firstly, for low P (LP) treatment, the soil was directly mixed with N and K fertilizers and without P then added to the box. For high P (HP) treatment, the equivalent of 25 kg P ha^{-1} was added to the soil while filling top layer (0-10cm) of the experimental containers and deep layer (10-25cm) soils did not receive any P fertilizer to better simulate P distribution under the natural conditions. Regarding the water treatment, soil water levels were monitored using a TDR (Time Domain Reflectometry) every week over a 54-day growth period (Fig. 2). The well-watered (WW) treatment received continuous water supply and maintained relative stable soil volumetric water content around 20-25%; while for the drought recovery (DR) treatment, we stopped watering at 15 days after emerging and started rewatering at 36 days after emerging. The length of the drought treatment was based on the low soil volumetric water content (<8%, Fig. 2) and 30% of rice seedlings showed drought symptom including leaf rolling and leaf tip scorching.

Sample collection and measurement

Plants were harvested at four different time point based on their treatment and growth situation, i.e., 15 days after emergence (DAE), 23 DAE, 36 DAE and 54 DAE. At each harvest, two neighboring rice seedlings were carefully taken from the soil and gently rinsing roots from soil with running water. The roots were then placed in 15% ethanol till they were scanned with an Epson Perfection V800 scanner (Epson America, Inc., USA) at a resolution of 300 dpi. The scans were analyzed with the image-processing software WinRhizo Pro (Regent Instruments, Quėbec, Canada). Dry mass of both root and shoot was recorded separately before and after they were oven-dried at 65°C to a constant weight. All dry shoot parts and root parts were ground together and then analyzed for P content with ICP-OES, and N content with an elemental analyzer in Forschungszentrum Jülich, Germany.

Plant growth and P uptake calculations

The relative growth rate (RGR) of upland rice during the recovery phase (37-54 DAE) was calculated according to Hunt (1982):

$$\mathrm{RGR} = \frac{\ln(\mathrm{W_2}) - \ln(\mathrm{W_1})}{\mathrm{t_2} - \mathrm{t_1}} \quad (1)$$

where W1 and W2 are plant dry weights at times t1 (36 DAE) and t2 (54 DAE). Similarly, the relative root elongation rates (RRER) of upland rice during the recovery phase (37-54 DAE) was calculated as shown in Eq. 2:

$$\mathrm{RRER} = \frac{\ln(\mathrm{R_2}) - \ln(\mathrm{R_1})}{\mathrm{t_2} - \mathrm{t_1}} \quad (2)$$

where R1 and R2 are plant total root length at times t1 (36 DAE) and t2 (54 DAE). The internal P use efficiency for biomass accumulation during drought was calculated according to Rose and Wissuwa (2012):

$$\mathrm{PUE}\left[\mathrm{g\ DW\ mg\ P^{-1}}\right] = \frac{\text{Plant dry weight }\left[\mathrm{g\ plant^{-1}}\right]}{\text{Plant P content }\left[\mathrm{mg\ P\ plant^{-1}}\right]} \cdot 100 \quad (3)$$

The efficiencies to acquire P sources during drought were calculated according to Wissuwa et al. (2020) in Eq. 4:

$$\mathrm{PAE}\ [\text{mg P uptake g root dry weight}^{-1}] = \frac{\text{Total P uptake [mg P plant}^{-1}]}{\text{Root dry weight [g plant}^{-1}]} \cdot 100 \quad (4)$$

Statistical analysis

Data analysis was performed using SPSS 22.0 software (SPSS Inc., Chicago, IL, USA). Normality and homogeneity of variances were tested for all the data with Shapiro-Wilk tests. Data were log-transformed if they didn’t pass the test. The effects of the water, P and genotypes on the P acquisition during drought (at 36 DAE), RGR and RRER during recovery (37-54 DAE) of the two varieties DJ123 and Nerica4 were first evaluated with a three-factor ANOVA. One-way ANOVA analysis was further conducted to evaluate the effects of treatment and significant differences of means were compared with Tukey test. Pearson correlation tests were also performed to illustrate the relationships between the phosphorus status and drought recovery rates. All presented results are reported as means ± standard error of means (± SE). The significance level was set at $P \leq 0.05$.

Results

Biomass accumulation and Root/shoot ratio

The P levels, water treatments and genotypes markedly affected plant growth (Fig. 3). Compared with no P fertilized (LP), P fertilized treatment (HP) increased total biomass accumulation, clearly visible at 36 and 54 days after emergence (DAE). The genotypic differences also increased over time with DJ123 having over 60% more biomass than Nerica4 at 54 DAE (over 60%). During the drought (from 15-36 DAE), drought stress reduced the total biomass of DJ123 and Nerica4 in all P levels. No consistent results were found on root/shoot ratio, except that Nerica4 tended to have greater root/shoot ratios, especially during the early growth stages, and often LP plants had greater ratios than their HP counterparts.

Recovery rates

The relative growth rate during recovery period (36-54 DAE) was significantly affected by P availability, with greater RGRs in HP mesocosms (Table 1, Fig 4a). The P effect depended on genotype: compared with high P treatment, P stress dramatically reduced relative growth rate of Nerica4 by 73%, however no significant effect was found in DJ123 under contrast P levels. Whether plants were drought stressed before this period did not influence their relative growth rates, i.e. the water treatment and its interactions were not significant.

We were especially interested in how the root system recovers from drought. The relative root elongation rate (RRER) during recovery period was influenced by all three factors, water, P, and genotype (Table 1, Figure 4b). Taking the well-watered and P fertilized (WW, HP) treatment as a reference, DJ123 achieved significantly higher RRERs when exposed to a single stress, either drought or P. Exposed to both stresses the mean RRER was still higher than unstress, but the difference was not significant. Nerica4 never achieved significantly higher elongation rates compared to HPWW, but the root elongation rate completely crashed when exposed to both stresses.

P concentration, P acquisition, internal P use efficiency (PUE), and P acquire efficiency (PAE) of upland rice at 36 DAE

At the end of the drought and before the recovery period (36 DAE), no significant three-way interactions among P and water and genotype were found on P concentration, total P acquisition, P internal use efficiency (PUE), and P acquisition efficiency (PAE). P concentration and P internal use efficiency (PUE) were affected by P levels, genotypes and P levels interactions but not by other factors (Table 2). Under high P and drought condition, DJ123 had a lower P concentration than Nerica4, nevertheless, P concentration of DJ123 was significantly higher than Nerica4 by 31% (Fig. 5a). The plant total P acquisition was significantly affected by P, water, genotype, P and water interaction, P and genotype interaction. P fertilization strongly increased P acquisition. Under high P condition, the drought treatment reduced total P acquisition of DJ123 by 21% significantly. For Nerica4, this decline was less drastic and in the Tukey test not significant. Under low P condition, drought didn’t decrease total P acquisition within each genotype and DJ123 showed significantly higher total P acquisition than Nerica4 under same water levels (Table 2 and Fig. 5b).

Different with the P concentration and total P acquisition, the P internal use efficiency was higher under low P than high P condition. Under high P condition, no significant differences were found between two genotypes in well-water and drought treatments, while under low P and drought condition, Nerica4 exhibited higher PUE than DJ123 (Fig. 5c). As for the P acquire efficiency (PAE) at 36 DAE, similar with the P concentration and P acquisition, a dramatic decrease in total P acquisition was observed in low P when compared with high P levels, P and genotype also had a significant interaction on PAE (Table 2 and Fig. 5d).

Relationships between relative growth rate during post recovery and P concentration, internal P use efficiency (PUE), and P acquisition efficiency (PAE)

We related the RGR to the P concentration, internal P use efficiency (PUE) and P acquisition efficiency (PAE) of upland rice at 36 DAE (Fig. 6). This analysis was performed among drought recovery treatments but not well-water treatment, as recovery happens after rice are experienced with drought stress. By including both the differences in different P levels and genotypes, we can examine whether upland rice recovery rate after drought can be explained by its own phosphorus homeostasis.

Both P concentration ($R^2 = 0.506$, $P = 0.002$) and P acquisition efficiency (PAE) ($R^2 = 0.476$, $P = 0.003$) correlated positively to the relative growth rate during post recovery (Fig. 6a and b). Relative growth rate during post recovery were higher when P concentration and P acquisition efficiency were higher at drought. Consequently, PUE, the inverse of the P concentration, was negatively associated with the relative growth rate during post recovery ($R^2 = 0.607$, $P < 0.001$, Fig. 6c).

Discussion

We hypothesized that P-homeostasis at the end of a drought period is important for recovery from drought. This study was set up to test the drought recovery response of a P-efficient and a P-inefficient upland rice genotype in contrasting P levels. With contrast P levels and genotypes under different water treatment, we measured plant growth dynamics during drought and recovery period as well as their P homeostasis. Our results support the hypothesis that a better P homeostasis is a good predictor for the rate of recovery afterwards and the P efficient genotype recovered faster (Fig. 4a, 5 and 6). The performance of the upland rice at the end of a drought was not consistent with performance during post recovery (Fig. 3a), their P homeostasis, relative growth rate and relative root elongation rate differed under different treatments (Fig. 4 and 5); upland rice seedlings with higher P concentration (Fig. 5a), lower internal PUE (Fig. 5c) and higher PAE (Fig. 5d) had a higher relative growth rate and biomass accumulation during recovery (Fig. 3a, 4a and 6). The P efficient genotype, DJ123, with a better P homeostasis had a better recovery and drought resistance than P sensitive genotype Nerica4 (Fig. 3, 4 and 5).

Can recovery period be neglected for drought resilience evaluation in upland rice?

Drought events are expected to increase in both intensity and frequency in the context of global change. Understanding how crops respond to increasing drought events will be important in mitigating the food crisis (Dai, 2013; Langenbrunner, 2021; Zheng *et al.*, 2023). However, most of the studies have focused on plant performance at the drought ends rather than the performance after recovery, which may explain why very few transgenic varieties have been released to farmers to date (Passioura, 2020). Similar to the results of Chen *et al.* (2016) and Gonzalez-Hernandez *et al.* (2021) in the drought recovery study of maize inbred lines, we also found that upland rice performance during recovery is not accordance with the performance during drought, the recovery rate differs among treatments and genotypes (Fig. 3a, 4), which can further contribute to its drought resilience. This suggests that both drought and recovery are key determinants of plant drought resilience and adaptation, post drought recovery may play a more significant role than previously thought. Considering the multiple drought recovery cycles in the field condition and important role of recovery process, the recovery period can't be neglected when evaluating the drought resistance of crops. In addition, the recovery rates were found to be further influenced by P levels (Table 1, Fig. 3a), which serves to highlight the complex interaction between water and nutrients that exists not only during the drought period. This should be given more attention in future studies.

P homeostasis is a good predictor for the post drought recovery

At the end of the drought treatment at 36 DAE, upland rice seedlings exhibited different P homeostasis (including P concentration, P acquisition, internal P use efficiency (PUE) and P acquisition efficiency (PAE)) and post recovery rate. Further correlation analysis showed a significant positive correlation between them (Fig. 6). To the best of our knowledge, this is the first report to attempt to link nutrient status at the end of a drought with subsequent recovery rates. As the main constituent of plant cells, P is essential for cell division and development of the growing tip of the plant. During periods of drought, a reduction in soil P availability can be attributed to a decline in the diffusion capacity of the shallow soil layers, which typically contain most of the P. Meanwhile, more roots may be distributed to the deep layer for water capture and less root activity may occur in shallow layer for P uptake from the plant side, all of which would lead to a dramatic reduction in plant P acquisition ((Ho *et al.*, 2005; Kato *et al.*, 2016). If a plant is unable to maintain its phosphorus homeostasis during a period of drought, it will inevitably suffer a failure to recover. Recent greenhouse and field studies have demonstrated that rice plants are more responsive to P than to the water availability (De Bauw *et al.*, 2018, 2020; Verbeeck *et al.*, 2023), in accordance with these studies, we found that the timing of drought symptoms also varies among treatments (data not shown). The signs of leaf rolling and leaf tip scorching first occurred on Nerica4 under drought and low P conditions, which had the lowest P acquisition, concentration and PAE (Fig. 5a, b, d), while other treatments started to show symptoms when already 40% of the LP-DW-Nerica4 plants were drought stressed. We conclude that maintaining a good P homeostasis is a key aspect of drought tolerance in rice.

Our results demonstrated that phosphorus homeostasis is a good predictor of the recovery rate. Relative growth rate during recovery is significantly influenced by P levels, and the interaction between P and genotype. Under high P conditions, both DJ123 and Nerica4 can maintain high relative growth rates and no significant differences of the relative growth rate between two genotypes were found. However, at low P level and recovery period, biomass accumulation and relative growth rate of DJ123 is higher than those of Nerica4 (Fig. 3a, 4a). We attribute this to DJ123’s higher P acquisition during the drought, which supports greater growth rates. Rewatering after the end of drought can lead to a rapid recovery of plant water uptake. In contrast, reduced phosphorus uptake and root growth recover more slowly and thus can become a limiting factor in the overall recovery rate of the plant. Thus, P homeostasis at end of drought is the key to its recovery and is a good predictor for plant drought resistance.

We hypothesized that the relative growth rate during recovery would be closely related to the relative root elongation rate, as plants may alter their root elongation rate to improve their resource acquisition and grow faster. Previous research demonstrated that root elongation rate depended on water and P levels (Ma *et al.*, 2003; Bengough *et al.*, 2011). Contrary to expectations, our study did not find a correlation between relative growth rate during recovery and relative root elongation rate during recovery. Relative root elongation rate was affected by water, genotypes, and their interactions with P (Table1, Fig. 3b). It is somewhat surprising that DJ123 has the highest relative root elongation rate under low P and well water conditions, while Nerica4 has the lowest value under low P and drought recovery conditions. A much lower relative root elongation rate of Nerica4 may indicate a conservative strategy under dual stress conditions: the plants may need more time to restore nutrient homeostasis and root carbon status (Ouyang *et al.*, 2021). Irrespective of the water levels, DJ123 always has the higher relative root elongation rate than Nerica4 under low P conditions (Fig. 4b), which is consistent with previous results that DJ123 tends to have a higher growth rate than Nerica4 at the early stages of development (Matthias Wissuwa, personal communication). This may also reflect the importance of early vigor in a drought environment (Wissuwa *et al.*, 2020).

Implications of the relationship between plant drought resilience and P acquisition

As two typical rice cultivars with contrasting phosphorus uptake efficiency, DJ123 is known to be P efficient under low P level, while Nerica4 is generally regarded as a high yielding rice variety with a good response to fertilizer application and a better tolerance to drought on fertile soils (Wissuwa *et al.*, 2020; Kuppe *et al.*, 2022; Verbeeck *et al.*, 2023; Mundschenk *et al.*, 2024). The success of DJ123 under low P and moderate drought suggests that P-efficient genotype is better suited for adaptation to dual resource limitation and that DJ123 can be a donor for drought resistance breeding in upland rice. DJ123 maintains comparable P concentration and P uptake relative to Nerica4 under low P and drought conditions, at the same time, DJ123 keeps higher PAE and lower PUE than Nerica4, implying more P can be used for post recovery. In many regions such as sub-Saharan Africa, where drought and phosphorus deficiency occur simultaneously, it is important to screen for more targeted traits and genotypes to adapt to low phosphorus and drought, but to date no rice varieties have been released that can adapt to both P and drought stress. Our results demonstrate that phosphorus is particularly important in short-term drought and post-drought recovery, and that phosphorus homeostasis could be an important indicator of crop drought resilience of the crop. Finally, it should be noted that the intensity of the drought could be critical in determining its post recovery; if the drought intensity exceeds the range of tolerance of the plant especially under extreme drought, the plant may not be able to recover, leading to dramatic yield reductions. This highlights the importance of further drought recovery studies under field conditions to better understand drought resilience and P interaction.

Conclusions

Our findings demonstrate that the performance of the upland rice at the end of a drought is not consistent with performance after a period of recovery, and the relative growth rate during post recovery after drought is intricately linked to its ability to maintain its P homeostasis during drought period in upland rice. Under P stress conditions, P efficient genotype DJ123 can preserve the relative higher P concentration and PAE, thereby a higher relative growth rate and biomass accumulation during post recovery. A better P homeostasis of DJ123 under low P and drought can support recovery of biomass loss during post drought, but P sensitive genotype Nerica4 doesn’t have this ability. Meantime, recovery rate after drought is negatively correlated to its internal PUE. This highlights the importance of P homeostasis for drought recovery in upland rice, phosphorus and water uptake are closely interconnected rather than independent processes, offering insights for a quick recovery and sustainable rice production in both drought and low P conditions. Therefore, we suggest that P homeostasis during drought merits consideration as a potential to improve drought resilience, particularly to intermittent droughts, and this need to be considered in a more targeted breeding for selecting drought resilient upland rice cultivars.

Acknowledgments

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Author contributions

CCZ designed and conducted the experiments, analyzed the results, and led the writing; TR contributed to the design and writing; JAP and MW conceived and designed the study, supervised its execution, assisted with data analysis, and contributed to the writing.

Conflict of interest statement

Nothing declared.

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Table 1 Results of Three-way ANOVA on effects of P levels (P), water treatment, genotypes (G) and their interactions on relative growth rate and relative root elongation rate during the recovery period (37-54 DAE).

Factors	Df	Relative growth rate		Relative root elongation rate	
		F	P	F	P
P	1	74.356	<0.001**	3.570	0.071
Water	1	0.420	0.523	11.777	0.002**
Genotype	1	0.777	0.387	82.323	<0.001**
P × Water	1	0.660	0.425	85.908	<0.001**
P × Genotype	1	27.945	0.000**	43.088	<0.001**
Water × Genotype	1	0.086	0.772	0.112	0.741
P × Water× Genotype	1	0.194	0.663	3.528	0.073

*P≤0.05; ** P≤0.01

Table 2 Results of Three-way ANOVA on effects of P levels (P), water treatment, genotypes (G) and their interactions on total P acquisition, P concentration, P acquisition efficiency (PAE) and P internal use efficiency (PUE) at drought end (36 DAE).

Factors	Df	P acquisition		P concentration		P internal use efficiency (PUE)		P acquisition efficiency (PAE)	
		F	P	F	P	F	P	F	P
P	1	554.432	<0.001**	83.701	<0.001**	90.491	<0.001**	73.108	<0.001**
Water	1	23.192	<0.001**	0.573	0.456	0.058	0.813	3.533	0.072
Genotype	1	16.936	<0.001**	3.159	0.088	0.034	0.856	6.621	0.017*
P × Water	1	5.589	0.027*	3.865	0.061	3.421	0.077	0.002	0.964
P × Genotype	1	9.833	0.004*	24.767	<0.001**	26.217	<0.001**	5.015	0.035*
Water × Gen.	1	0.087	0.771	0.055	0.817	0.113	0.740	0.725	0.403
P × Water× Gen.	1	0.523	0.476	0.834	0.370	1.333	0.260	0.001	0.982

*P≤0.05; ** P≤0.01

Figure caption

Fig. 1. Hypothesized rice plant phenotypes dynamics during and after drought. During drought, reduced water content induces deeper root development, but phosphorus uptake by shallower root maybe be reduced. P might be reallocated from senescing leaves. During the recovery period, root development in response to the rewatering may depend on phosphorus homeostasis, with the potential of affecting plant recovery and further growth performance.

Fig. 2. Soil volumetric water content (10^{-2} m^3 m^{-3}, %) over a 54-day growth period in 20-L containers filled with P-deficient field soil. WW = Well watered treatment; DR = Drought recovery treatment (Drought phase from 16 to 36 DAE, recovery phase from 37 to 54 DAE). Data shown are means of eight replicates ± SE.

Fig. 3. Biomass (a) and root/ shoot ratios (b) over a 54-day growth period in the greenhouse pot experiment. Here and after, HP and LP represent high P and low P, WW and DR represent continuous well-water and drought recovery, DJ and N4 represent genotype DJ123 and Nerica4, respectively. WW = Well watered treatment; DR = Drought recovery treatment (Drought phase from 16 to 36 DAE, recovery phase from 37 to 54 DAE). Data shown are means of four replicates ± SE.

Fig. 4. Effects of P levels, water treatment, and genotypes on relative growth rates (a) and relative root elongation rates (b) of upland rice during the recovery phase (37-54 DAE). The data shown are means ± SE of four replications. Different letters represent significant differences at the level of $\alpha = 0.05$.

Fig. 5. Effects of P levels, water treatment, and genotypes on P concentration (a), P acquisition (b), internal P use efficiency (PUE) (c), and P acquisition efficiency (PAE) (d) of upland rice at 36 DAE in greenhouse mesocosms. The data shown are means ± SE of four replications. Different letters represent significant differences at the level of $\alpha = 0.05$.

Fig. 6. Improved P status is associated with faster recovery. Relationships between relative growth rate during post recovery and P concentration (a), internal P use efficiency (PUE) (b) and P acquire efficiency (PAE) (c). Correlations were determined between P acquisition of upland rice at drought ends (36 DAE) and relative growth rate during post recovery (37-54 DAE) of upland rice in drought recovery treatment.

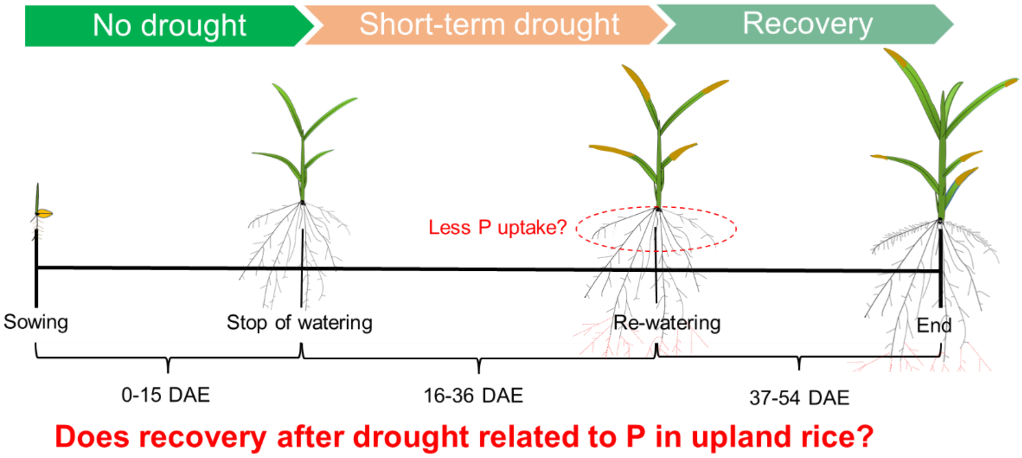
No drought
Short-term drought
Recovery
Less P uptake?
Sowing
Stop of watering
Re-watering
End
0-15 DAE
16-36 DAE
37-54 DAE
Does recovery after drought related to P in upland rice?

Fig. 1.

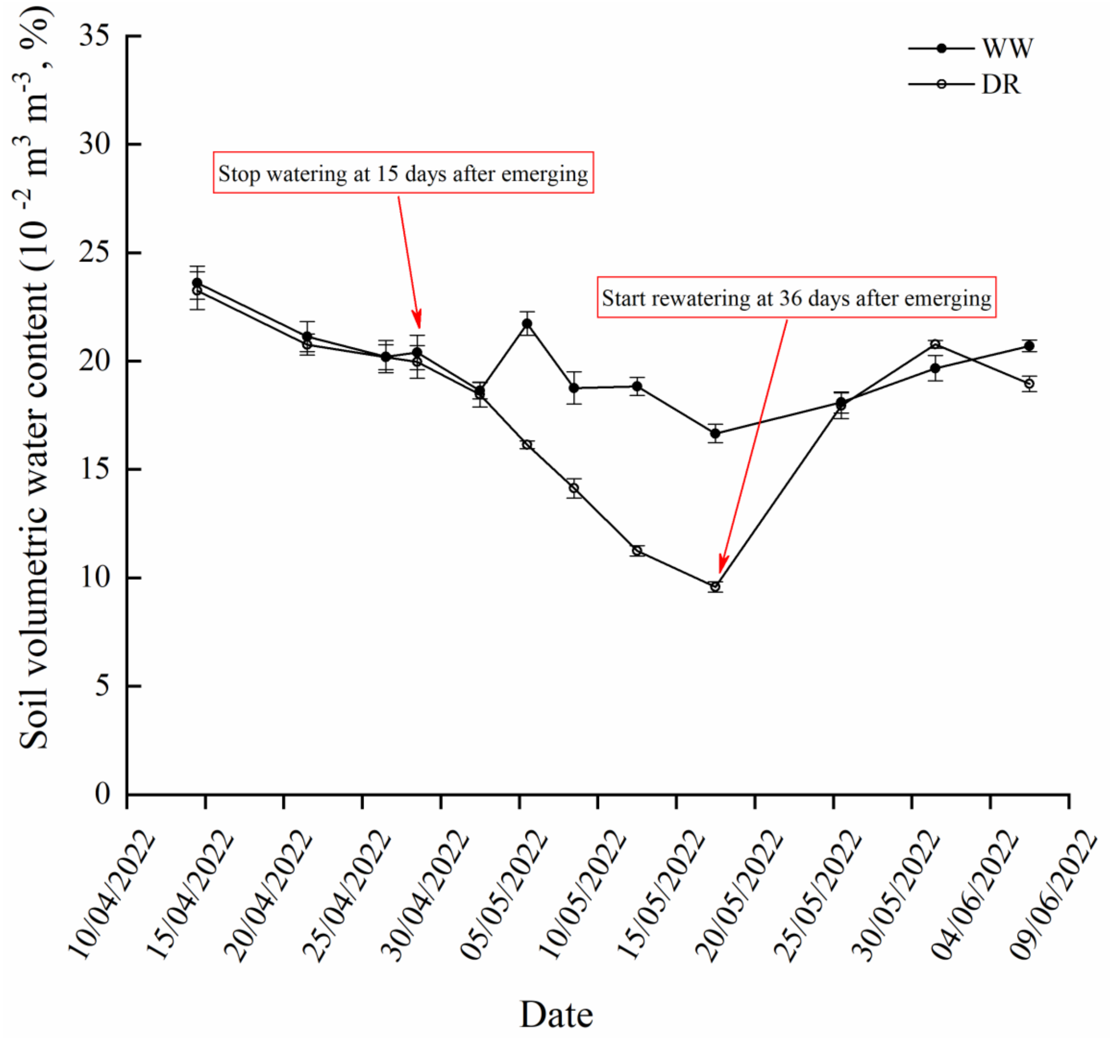
Soil volumetric water content (10 -2 m3 m-3, %)
35
30
25
20
15
10
5
0
WW
DR
Stop watering at 15 days after emerging
Start rewatering at 36 days after emerging
10/04/2022
15/04/2022
20/04/2022
25/04/2022
30/04/2022
05/05/2022
10/05/2022
15/05/2022
20/05/2022
25/05/2022
30/05/2022
04/06/2022
09/06/2022
Date

Fig. 2.

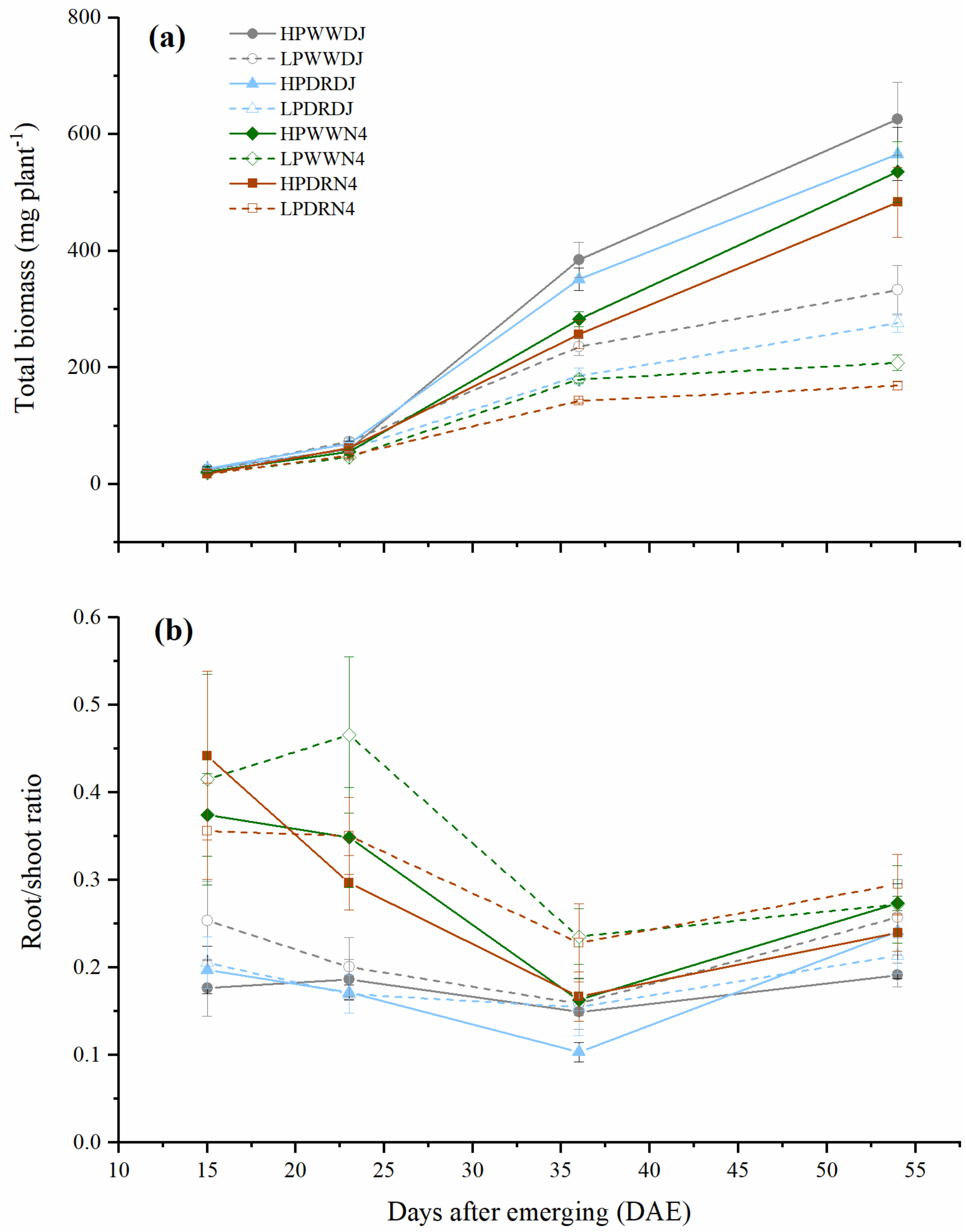
(a)
HPWWDJ
LPWWDJ
HPDRDJ
LPDRDJ
HPWWN4
LPWWN4
HPDRN4
LPDRN4
Total biomass (mg plant^{-1})
800
600
400
200
0
(b)
Root/shoot ratio
0.6
0.5
0.4
0.3
0.2
0.1
0.0
10
15
20
25
30
35
40
45
50
55
Days after emerging (DAE)

Fig. 3.

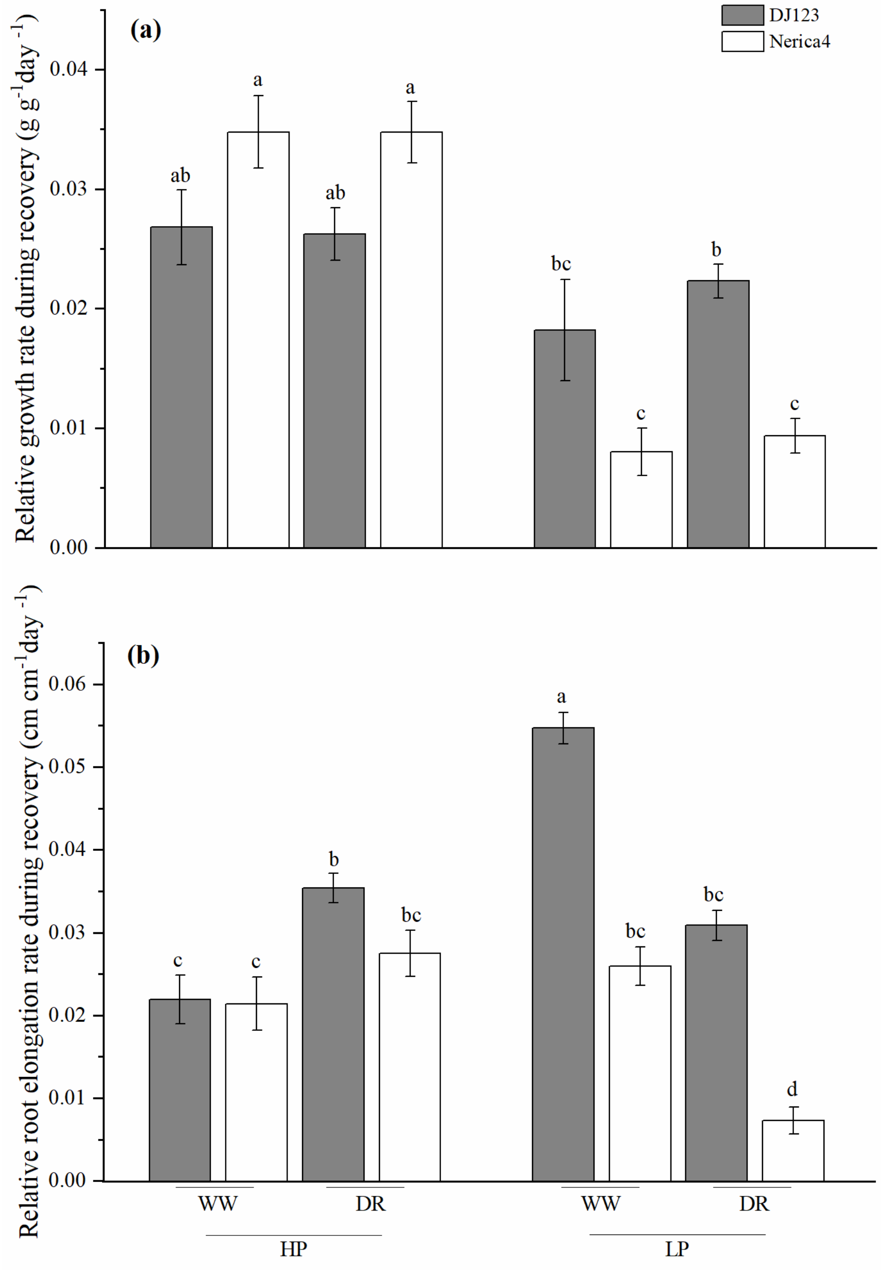
(a)
DJ123
Nerica4
Relative growth rate during recovery (g g^{-1}day^{-1})
0.00
0.01
0.02
0.03
0.04
ab
a
ab
a
bc
c
b
c
(b)
Relative root elongation rate during recovery (cm cm^{-1}day^{-1})
0.00
0.01
0.02
0.03
0.04
0.05
0.06
c
c
b
bc
a
bc
bc
d
WW
DR
WW
DR
HP
LP

Fig. 4.

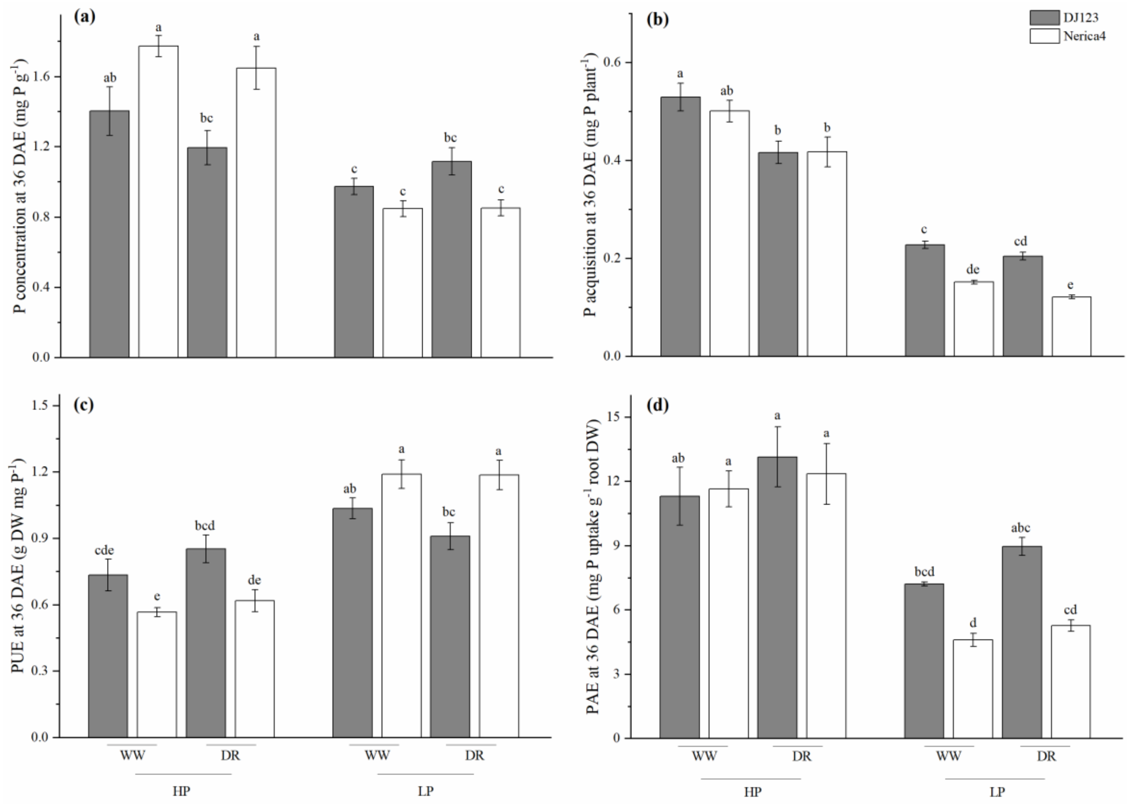
(a)
P concentration at 36 DAE (mg P g-1)
0.0
0.4
0.8
1.2
1.6
ab
a
bc
a
c
c
bc
c
(b)
DJ123
Nerica4
P acquisition at 36 DAE (mg P plant-1)
0.0
0.2
0.4
0.6
a
ab
b
b
c
de
cd
e
(c)
PUE at 36 DAE (g DW mg P-1)
0.0
0.3
0.6
0.9
1.2
1.5
cde
e
bcd
de
ab
a
bc
a
(d)
PAE at 36 DAE (mg P uptake g-1 root DW)
0
3
6
9
12
15
ab
a
a
a
bcd
d
abc
cd
WW
DR
WW
DR
HP
LP
WW
DR
WW
DR
HP
LP

Fig. 5.

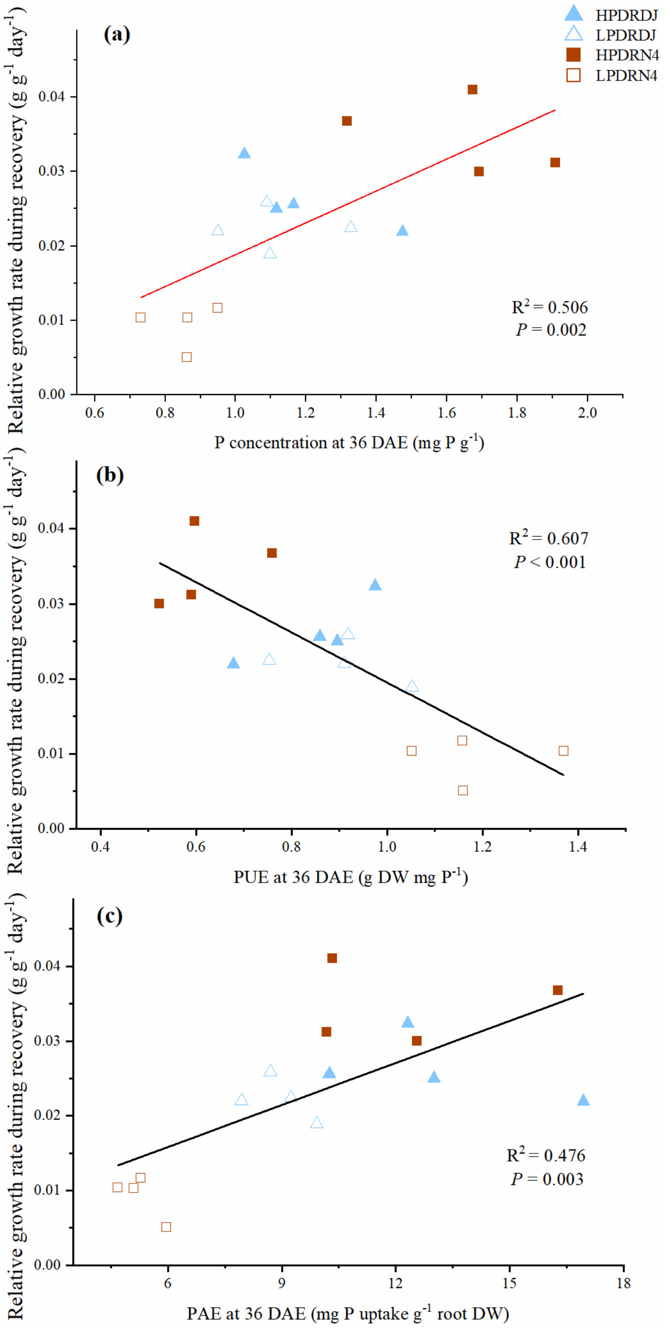
(a)
HPDRDJ
LPDRDJ
HPDRN4
LPDRN4
Relative growth rate during recovery (g g^{-1} day^{-1})
0.00
0.01
0.02
0.03
0.04
0.6
0.8
1.0
1.2
1.4
1.6
1.8
2.0
P concentration at 36 DAE (mg P g^{-1})
R^2 = 0.506
P = 0.002
(b)
R^2 = 0.607
P < 0.001
0.4
0.6
0.8
1.0
1.2
1.4
PUE at 36 DAE (g DW mg P^{-1})
(c)
R^2 = 0.476
P = 0.003
6
9
12
15
18
PAE at 36 DAE (mg P uptake g^{-1} root DW)

Fig. 6.

Supporting Information

Article title: Root Plasticity and Phosphate Homeostasis: Key Drivers of Drought Resilience in Upland Rice

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The following supporting information is available for this manuscript:

Fig. S1 Light intensity (Top), Temperature (Middle), and relative humidity (Bottom) over a 54-day growth period in the greenhouse.

Fig. S2 Three images display the experimental design (Top), growth of the rice seedlings in the greenhouse (Middle) and in one rhizobox (Bottom).

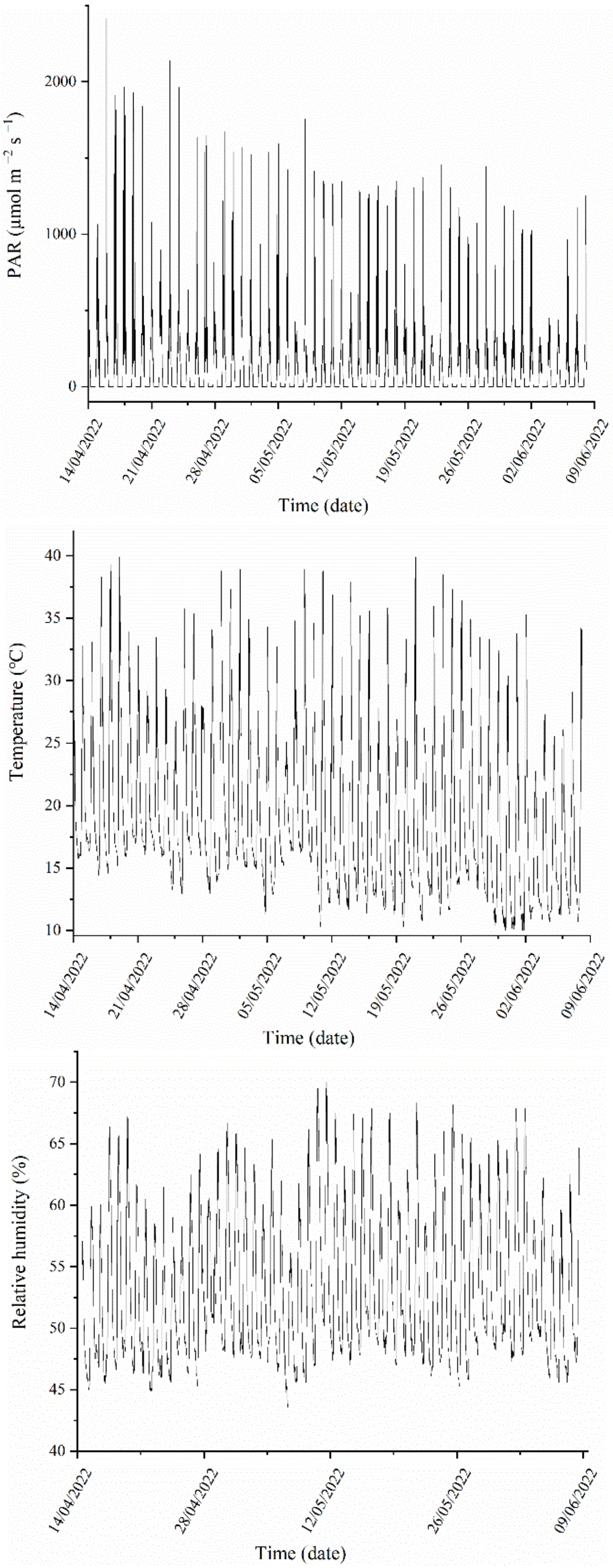
PAR (μmol m $^{-2}$ s $^{-1}$)
2000
1000
0
14/04/2022
21/04/2022
28/04/2022
05/05/2022
12/05/2022
19/05/2022
26/05/2022
02/06/2022
09/06/2022
Time (date)
Temperature (°C)
40
35
30
25
20
15
10
14/04/2022
21/04/2022
28/04/2022
05/05/2022
12/05/2022
19/05/2022
26/05/2022
02/06/2022
09/06/2022
Time (date)
Relative humidity (%)
70
65
60
55
50
45
40
14/04/2022
28/04/2022
12/05/2022
26/05/2022
09/06/2022
Time (date)

Fig. S1

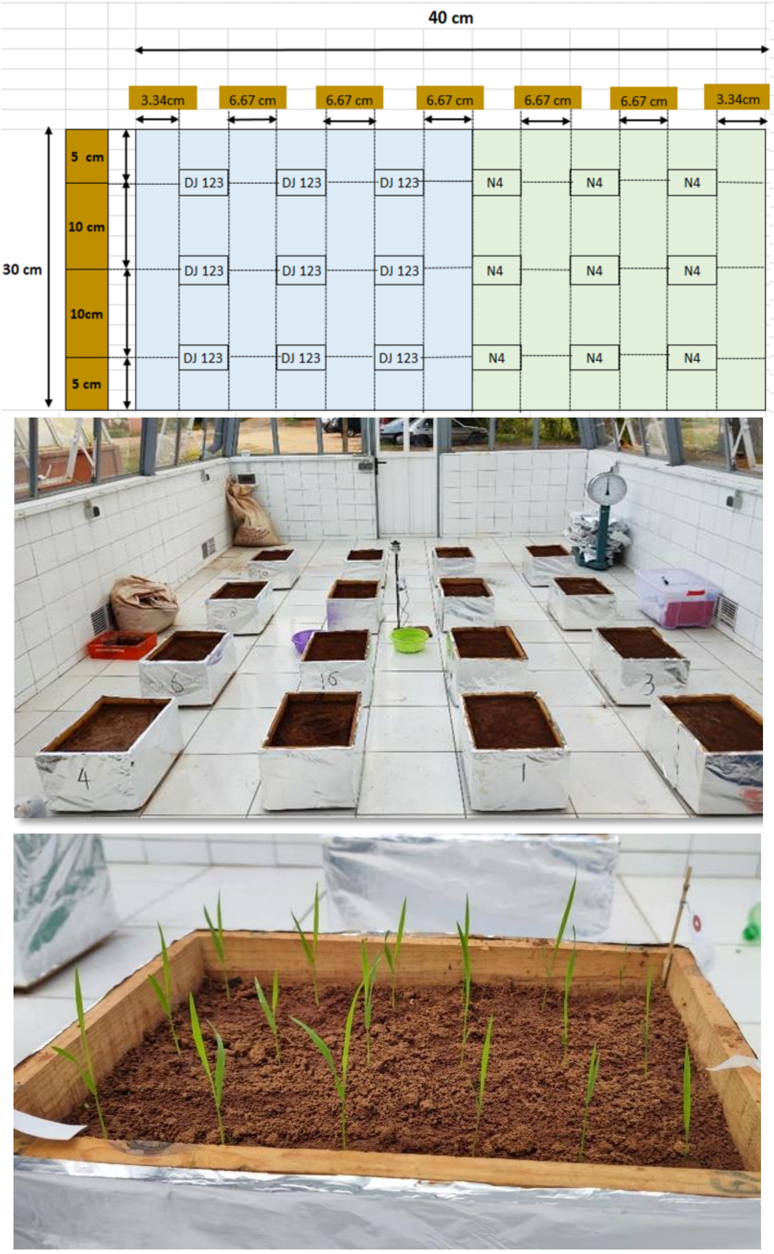
40 cm
3.34cm
6.67 cm
6.67 cm
6.67 cm
6.67 cm
6.67 cm
3.34cm
30 cm
5 cm
10 cm
10cm
5 cm
DJ 123
N4
4
6
16
1
3

Fig. S2

CHAPTER 4: Anatomical root responses of upland rice to concurrent phosphorus and water stress reveal different drought resilience after drought

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Manuscript under internal review

Chapter 4: Anatomical root responses of upland rice to concurrent phosphorus and water stress reveal different drought resilience after drought

Abstract:

Aims: Phosphorus (P) deficit and intermittent drought stress represent significant constraints to upland rice production, particularly in sub-Saharan Africa and numerous other regions. Root functionality plays a pivotal role in resilience to these stresses; however, the root anatomical responses of upland rice to concurrent phosphorus and water stress remain inadequately documented and poorly understood. This study aims to investigate the combined effects of soil P and water stress on root anatomy during two distinct periods: post-drought and post-drought recovery.

Methods: A 30-liter rhizobox experiment was carried out under glasshouse conditions using two upland-rice genotypes: DJ123 (P-efficient) and Nerica4 (P sensitive and slightly drought tolerant). Root anatomy traits response of both seminal roots (SR) and nodal roots (NR) in two genotypes with contrast P levels (high P and low P) and different water treatment (continuous well-water and drought recovery) were evaluated. Root anatomical development was then related to drought resilience.

Results: Intermittent drought and P deficiency lead to a general decrease in most anatomical trait values, except for the formation of root cortical aerenchyma (RCA). The response was more visible on the nodal root during drought treatment and on the seminal roots during recovery. During drought recovery period, DJ123 accumulated greater biomass and phosphorus than Neric4 under low P conditions but not high P conditions. RDA analysis showed that low xylem number (XN) in both seminal root and nodal root explain biomass and P accumulation under intermittent drought conditions.

Conclusions: The anatomical traits of different root types responded to water, P and genotype inconsistently. Specific root anatomy traits (e.g., XN) could be useful target traits for breeding programs aimed at increasing phosphorus deficit and intermittent drought resilience in upland rice.

Keywords: Root anatomy; Phosphorus deficit; Water stress; Recovery; Root cortical aerenchyma number

Introduction

Rice (*Oryza sativa* L.) is one of the world's most important staple crops, meeting the dietary needs of around half the world's population. Over 50% of rice cultivation is rainfed, providing only a quarter of the world's total rice production (McLean et al, 2002). Rainfed ecosystems are easily subject to water deficit (both intermittent and terminal drought). Yield losses are particularly severe during the reproductive phase, even under mild drought stress (Venuprasad et al, 2009; Verulkar et al, 2010), estimated at 18 million tonnes per year, or 4% of total rice production (Evenson and Gollin, 2003). Except for the drought trend, most natural environments are sub-optimal about soil resources such as phosphorus (P) (Hutchings et al, 2003). Phosphorus is a macroelement with vital functions in the pedosphere and biosphere (Su et al., 2019). From the cell to the whole plant, its presence is involved in various processes during plant development, regulating shoot biomass formation and root architecture (Niu et al., 2013; Malhotra et al., 2018). In addition, P deficiency is one of the main production constraints for both natural and agriculture ecosystems, especially in for highly weathered and acidic soils (Ren et al., 2018; Shimamura et al., 2021). In sub-Saharan Africa, the deficit even greater due to the inaccessibility of fertilizer to smallholder producers coupled with generally acidic soils (Wissuwa et al., 2020a; Shimamura et al., 2021). This is particularly true in Madagascar, where highland soils are very low in P (Ramilison, 2004). The dose of P fertilizer commonly used is 3 to 7 kg. ha^{-1} (Ministère de l'Agriculture et de l'élevage, 2015) compared with 10 kg. ha^{-1} in sub-Saharan Africa (Kam, 2016). Thus, enhancing the understanding of rice resilience for both drought and P deficiency is critical for global food security and stability.

Due to their limited mobility and the complexity of the environment, plants have developed a wide variety of mechanisms to cope with these challenges (Meng et al., 2019). In recent years, there has been widespread acceptance of underground adaptation to multi-stress with a "cheaper root" through root anatomical and architectural plasticity. An array of root traits response including increased root hairs, higher arbuscular mycorrhizal fungi (AMF) colonization and root cortical aerenchyma can increase uptake, reduce metabolic costs and improve the plant resilience ((Lynch *et al.*, 2005; Lynch, 2011; Nasr Esfahani & Sonnewald, 2024). As for rice, it has a unique root system architecture includes very fine and short S-type lateral roots, not found in other plant species (Wissuwa et al., 2020). Their root system consists of four root categories: seminal roots (RS), crown or nodal roots (RN), lateral roots (RL) formed by large L-type lateral roots and small S-type lateral roots (Nestler et al., 2016). The seminal root is the first root to develop during germination, and its differentiation starts during embryogenesis (Coudert et al., 2010; Viana et al., 2022). Nodal roots and lateral roots form only after post-embryonic cell differentiation. As for LRs, they originate from the branching of the other two preceding roots in the elongation and maturation zone (Babé et al., 2012; Robbins & Dinneny, 2018). L-type lateral roots (L-type LRs) are the first offshoots from parents (Rebouillat et al., 2009). All seminal, nodal, and L-type roots typically form S-type lateral roots. These are thin (<0.1mm), short (<1 cm) and unbranched (Wissuwa et al., 2020). Other studies have demonstrated that different root types have different functions and that plants can adapt to stress by changing the proportions of the different root types (Nestler and Wissuwa 2016; Wissuwa et al. 2020; Gonzalez et al. 2021). However, whether and how root anatomical characteristics in different root types respond to multiple stresses is still largely unknown.

The root anatomy, that is, the shape and structure of functional parts within the root, has been demonstrated to be a key factor influencing both P uptake and the efficiency of water uptake by crops like rice (De Bauw et al. 2019; Hua et al. 2022; Nasr Esfahani and Sonnewald 2024). The root's internal anatomy comprises two distinct regions: the stele and the surrounding cortex. The stele is a cylinder with xylem and phloem inside. The cortex has larger cells and more space between them. These cells are between the endodermis and the epidermis. They form a barrier to the stele and to the soil. The epidermal and cortical cells absorb water and nutrients from the soil, while the stele is mainly involved in water and solute transport. The xylem is mainly responsible for axial root hydraulic conductivity, while the endodermis and pericycle largely influence radial root hydraulic conductivity. The number of cortical cells and cortical aerenchyma influence the costs of soil exploration, the radial hydraulic conductivity, and the root surface area (Fan et al., 2007; Bailey-Serres & Colmer, 2014; Chimungu et al., 2014; Lynch, 2015). Recent studies have shown the significant roles of different root anatomical traits in plant responses to single stresses like flooding, drought and water deficit, since plant growth and productivity is usually confronted by more than one resource limitations, it is still largely unclear of the root anatomy plasticity under multiple stresses.

In general, the distribution of resources within the soil is subject to temporal and spatial heterogeneous, which are contingent upon prevailing environmental conditions (Freschet et al., 2018). Upland rice production depends, in many developing countries like Madagascar, strongly on the presence of water and the soil phosphorus availability. To increase P uptake under low P availability, plant tends to regulating root growth and development including increased length and density of absorbing hairs, more cortical aerenchyma formation and less cortical cell file number, and faster root elongation and branching (Lynch & Brown, 2008; Marschner, 2012). While for drought, the loss of the root cortex can lead to a reduction in absorbent hairs, root exudates, and mycorrhizae colonization (Shemesh et al., 2010). It has been established that under water stress, root diameter tends to expand to better penetrate the soil (Lynch, 2015). Similarly, the uptake of immobile nutrients such as P is severely reduced in dry soil, due to reduced effective diffusion rates, changes in root architecture and function can be observed (Ho et al., 2005; De Bauw et al., 2019). As drought is not a permanent condition in most agricultural ecosystems, rice is only subjected to it for a limited time, followed by a period of rehydration. Consequently, the return of precipitation can stimulate various traits and/or mechanisms within the root, particularly in relation to drought resistance. Plant growth and final production can be dependent on its behaviour during the recovery phase (Chen et al., 2016; Lawas et al., 2019). The extent of recovery can vary according to various factors, including the duration and intensity of stress, plant genotype, growth stage, and soil nutrient levels (Banda et al., 2014; Yeung et al., 2018). Consequently, a trait-based approach to research would be instrumental in elucidating the mechanisms by which plants allocate resources at the root level to resume growth (Garbowski et al., 2020). It is therefore crucial to enhance comprehension of the root system and rhizosphere processes following drought stress and/or P deficiency, and to cultivate resilient rice cultivars in order to meet the demands of global food security. The objective of this study is to examine the response of each root anatomical element to P and water treatment, as well as their combined effect, following drought (Fig. 1). We hypothesis that: 1). The anatomical traits of the different types of roots differ during the drought and the recovery period; 2). Specific root anatomical characteristics like root xylem number and root cortical aerenchyma contribute to better P stress tolerance and drought resilience.

Materials and methods

Experimental design

A greenhouse experiment was conducted at the Laboratoire des Radio-Isotopes Antananarivo, Madagascar (18°91' S, 47°55' E, 1 222 m altitude) from 14th April to 7th June 2022. The soil used was collected from the top 15 cm of an upland rice field in the Commune of Antohobe, Vakinankaratra region (19°46' S, 46°41' E, 1 240 m altitude). Soil is classified as Phaeozem, with pH (H_2O) of 5.05 (soil: water =1:2.5) and available P of 1.35 mg kg^{-1} (AEM-P: anion exchange resin extraction, Table S1). The experiment was a factorial combination of two water treatments (well water, drought recovery), two phosphorus treatments (0 and 25 kg P ha^{-1}), and two 'Upland Rice' genotypes (DJ123 and Nerica4). The experimental design was a fully randomized block design with 4 replicates.

Plant care & growth conditions

Growth conditions and seed germination were reported in previous study (See Chapter 3, Material and methods part). Nitrogen and Potassium fertilizer were first added to the soil. In each box, 18 seeds were planted in 3 rows 8 cm apart, and each row containing 6 seeds spaced 6 cm apart. For the water treatment, soil water levels were monitored using a TDR (Time Domain Reflectometry) every week over a 54-day growth period (Fig. S1). During the first three weeks of the experiment, all boxes were irrigated with an equal amount of tap water equivalent to 20-25% w/w soil moisture. The well-watered (WW) treatment was maintained in the well-watered till the end of the experiment. The drought recovery (DR) treatment was initiated at 15 DAE by stopping water addition and started rewatering at 36 days after emerging. The length of the drought treatment was based on the low soil volumetric water content (<8%, Fig. S1) and 30% of rice seedlings showed drought symptom including leaf rolling and leaf tip scorching.

Sample collection and measurement

Two different harvests were carried out during the experiment, during which 2 seedlings per genotype were carefully taken from each box for data analysis. The first harvest (36 DAE) marked the end of the drought period, the second and final harvest (54 DAE) was carried out to observe recovery after drought. At each harvest, both plants were carefully taken from the soil and gently rinsing roots with running water. Above- and below-ground biomass were collected along with the rhizosphere soil. A 5 cm sample was taken from the tip of all root types and rinsed with tap water and stored in 50 % alcohol until use for root anatomical analysis. Cross-sections of each root were cut manually following the protocol established by De Bauw et al., (2019), Guo et al., (2008) and Zhou et al. (2022). The images obtained were processed in ImageJ 1.46r (Wayne Rashand, National Institutes of Health, USA), enabling the following parameters to be measured: the number of cortical cells (CCFN), the diameter of the central cylinder (SD), the number of xylems and their diameters (XN, both early and late xylem are considered) and (XD), the number of cortical aerenchymas (RCA). We further calculated S. RI based on the SD and whole root diameter ratio; CCFN. RCA is based on the CCFN and RCA ratio. The axial water conductivity parameter (WCP, μm^4) was subsequently calculated from the Hagen-Poiseuille equation, whereby r represents the average xylem radius, using the following formula:

$$\text{WCP} = \pi \times r^4 \times \text{number of xylems vessels (XN)}$$

Both seminal, nodal and lateral root samples were taken while only seminal and nodal cross-sections were used for further analysis as we cannot get clear images for lateral root. The below-ground biomass and above-ground biomass was recorded separately after they were oven-dried at 65°C to a constant weight. Dry samples were ground together and then analyzed for P content with ICP-OES, and N content with an elemental analyzer in Forschungszentrum Jülich, Germany.

Statistical analysis

Data analysis was performed using R version 4.2.3 with a significance level set at $p < 0.05$. Normality and heterogeneity of variance were tested with Shapiro-Wilk and Levene tests, data that did not meet normality were log-transformed. One-way

ANOVA analysis was further conducted to evaluate the effects of treatment and significant differences of means were compared with Tukey test. To better summarize observed anatomical root responses to P availability, water, varieties and the interaction between these 3 factors, factorial interactions (i.e. Eta-squares(η^2)) were calculated, which is the sum of the squared of a single factor (ssq_{effect}) over the total sum of squares (ssq_{total}).

$$\eta^2 = \frac{\mathrm{ssq_{effect}}}{\mathrm{ssq_{total}}}$$

The reactivity of each factor is noted in percentage and classified as follows: 0–5%, no response; 5–20%, slight response; 20–50%, strong response; >50%, very strong response.

To examine the relationship between root anatomical variation and both total biomass and total phosphorus acquisition, ordination techniques were applied using the international standard software Canoco 5 (Microcomputer Power, Ithaca, NY, USA). The root anatomical variation of the seminal and nodal roots was designated as the response variable, while the biomass and total P acquisition at the recovery period were set as the explanatory variables. A linear model was selected for redundancy analysis (RDA). In total, 36 variables were considered for the analysis of upland root anatomical traits, with the data set comprising data from different periods and root types.

Results

Anatomical response of nodal root at drought (36 DAE) and recovery (54 DAE)

The P levels, water treatments and genotypes markedly affected anatomical traits of nodal root except ratio of steel and root area (S. RI) at 36 DAE (Table. 1, Fig. 2). At drought ends, compared with the well-watered treatment, drought decreased root tip diameter (RTD), steel diameter (SD), xylem diameter (XD) and water conductance parameter (WCP), especially for cortical cell file number (CCFN) (more than 50%). Root Cortical Aerenchyma (RCA) is also markedly improved by drought over 50%, while no other significant effects were found, while it was neither significantly affected by P availability nor by variety. Different with RCA, the number of xylem vessels (XN) were significantly influenced by P availability and P× water× genotype interactions, XN significantly decreased with low P availability, irrespective of water availability or variety (Fig. 4). After 18 days' rewatering at 54 DAE, only two root anatomical traits, XN and CCFN were affected by P levels, water treatments and genotypes, XD and WCP (Water Conductance Parameter) were influenced by P × genotype interactions, while no significant effects were found on other anatomy traits (Table 1, Fig. 2, 3 and 4). Regarding the number of xylem vessels (XN) at 54 DAE, it was significantly influenced by P availability, water and genotype, however, no interactions were found between factors.

Anatomical response of seminal root at drought (36 DAE) and recovery (54 DAE)

The P levels, water treatments and genotypes markedly affected anatomical traits of seminal root at both drought and recovery period except XN, XD, CCFN, RCA and WCP at 36 DAE and XD, CCFN and CCFN.RCA at 54 DAE (Table. 2). At drought ends, most root anatomy traits of DJ123 and Nerica4 were not influenced by P or water or their interactions, exhibited a relatively low plasticity to abiotic stress. Compared with Nerica4, DJ123 had higher RTD, SD, irrespective of water or P treatment. During the recovery period, similar trends were observed in SD, XN, RCA and WCP but not in other traits (Table. 2, Fig. 3). For RTD at 54 DAE, it was significantly influenced by water levels and water × variety interactions. Irrespective of P levels, DJ123 had higher RTD than Nerica4 in drought recovery period. DJ123 had higher WCP than Nerica4 regardless of water and P levels.

Biomass accumulation and P acquisition at 54 DAE

Both the total biomass (TB) and P accumulation (TP) at 54 DAE were significantly affected by P availability, genotypes and their interactions (Table 3). Regardless of water treatment, no significant difference was found between DJ123 and Nerica4 under high P treatment, while DJ123 had higher biomass and P accumulation than Nerica4 under low P treatment. Taking well-watered treatment as a reference, drought recovery treatment significantly decreased total biomass but not total P content. At the end of the recovery period (54 DAE), no significant three-way interactions among P and water and genotype were found on total biomass and P content, there are also no significant effects were found on P concentration among different P, water and genotypes (Table 3).

Responsiveness of root anatomy traits to P genotype and water availability

Comparing the anatomical traits in DJ123 and Neirca4, major differences were observed and were dependent on root types (nodal or seminal root) and treatment (water or P). A summary of the responsiveness or robustness of all analyzed anatomical root traits to P availability, water availability and varying varieties, and their interactions is given in Table 4. For the nodal root anatomy traits, all the traits except XN and CCFN did not respond to P levels (or only slightly responded) during drought and recovery period, noted that XN was the only parameters showed a strong response for both P levels and P× Water× Genotypes interactions (>20%). Besides, CCFN, RCA and CCFN.RCA of nodal root at 36 DAE had strong response to water but no other factors. Different with drought conditions, anatomical traits of nodal root at recovery period showed a weaker response to both P and water treatments. RTD, SD, XN, RCA and XXFN at recovery period (54 DAE) exhibited a slight response to genotypes. Different with nodal root, RTD, SD, XD and WCP of seminal root at 36 DAE had strong response to variety, while very strong response was further observed in RTD and SD of seminal root at 54 DAE.

We were further interested in the relationship between root anatomy plasticity and biomass and P accumulations. RDA results showed that XNN1 (XN: xylem number, S: Seminal root, N: Nodal root, 1: at drought period 36 DAE, 2: at recovery period 54 DAE), XNN2, RTDN1, CCFNN1 and S. RIS2 had positive correlations with biomass and P accumulations (Fig. 5, Table S2), while XNS2 and S. RIS1 were negative correlated with biomass and P accumulations. Root anatomy traits variation could explain 56.74% and 2.8% of the variation for RDA1 and RDA2, respectively. Among all the root anatomy variables, the XNS2, XNN1, S. RIS1 and XNN2 had significant influence on total biomass and total P acquisition of upland rice and can explain 21.1%, 11.6%, 6.8% and 6.5%, respectively (Fig. 5, Table S2).

Discussion

We hypothesized that the anatomical traits differ in different root types during the drought and the recovery period and specific root anatomical characteristics contribute to better P stress tolerance and drought resilience. The objective of this study was to evaluate the drought recovery response of the root anatomy traits in a P-efficient and a P-inefficient upland rice genotype under contrasting phosphorus (P) levels and their relationship with the biomass and P accumulation. With contrasting P levels and genotypes under different water treatments, we measured different root anatomy traits during the drought and recovery periods, as well as plant growth. Our results support the hypothesis that anatomical traits exhibit variation across different root types during both drought and recovery periods (Table 1, 2 and 4, Fig. 2-4). Even though no clear cross section images were got in laterals, response of anatomical traits to P, water and genotypes in seminal root was not consistent with those in nodal root. P efficient upland rice genotype DJ123 tends to have a better drought resilience under low P conditions (Table 3), Specific root anatomical characteristics (i.e. the number of xylem vessels (XN) of nodal root) contribute to better P stress tolerance and drought resilience (Fig. 5).

Do anatomical traits of different root types react differently to drought and recovery?

How plants respond to stress is a matter of how their roots change (Dien et al., 2017), root anatomy traits are affected by both the plant's genes and its environment. Previous studies on root anatomy traits usually focused on certain root types and single factors (De Bauw et al., 2019; Schneider et al., 2020), while nutrient and water uptake strategy are shaped by whole root system and both root types can contribute to its nutrient and water uptake. Similar with the previous study on Barley (Liu et al., 2020), our results also showed that root anatomy traits of nodal root and seminal root are differ in response to P, water and genotypes (Table 1 and 2, Fig. 2 and 3). During the drought period, all the anatomical traits of the nodal root exhibited higher responsiveness to water and phosphorus levels than the seminal root. On the contrary, the anatomical traits of the seminal root during the recovery period are more responsive to water and P levels to nodal root. This differences in seminal and nodal root implies that different root types can contribute to water and P uptake separately, which should be given more attention in future studies. At the same time, although the attempt to obtain a response from the Lateral root was not successful, the results for the nodal root and seminal root responses are also representative, as the nodal root and seminal root are of great importance for soil penetration, root distribution, and the inter-root variation in terms of thickness and branching is less significant in comparison with that of lateral roots. Furthermore, they have a relatively large biomass in comparison with their small share in the uptake of water or P, which highlights potential for the overall root system to become more efficient.

Does the P efficient genotype perform better under drought resilience?

Increased attention has been paid to plant responses to multiple stresses like P and water recently. As in many parts of agriculture and ecosystems, multi-stresses simultaneously limit plant growth. The droughts that plants face under rainfed conditions may be intermittent rather than permanent, and the actual scenario they face is a cycle of drought recovery. Nutrient homeostasis may be the key factors in its drought resilience. Consistent with our previous results in Chapter 3, biomass and phosphorus accumulation after drought recovery were not significantly different between the two genotypes under high-phosphorus conditions, and both DJ and N4 were able to reach to the same biomass and phosphorus uptake as the non-drought treatment after 18 days of rewatering (Table 3). However, the phosphorus-efficient genotype DJ123 performed significantly better than Nerica4 under low-phosphorus conditions, exhibiting higher biomass and P uptake at 54 DAE. Even though Nerica4 is regarded as slightly drought tolerant in past studies, it did not have a higher biomass and phosphorus accumulation under low phosphorus condition, we conclude that the P efficient genotype performs better under phosphorus deficit and intermittent drought conditions.

What root anatomy traits contribute to biomass and P accumulation under drought and P stress?

We were particularly interested in which root anatomical traits could contribute to plant biomass and phosphorus accumulation under drought recovery and low P conditions. The RDA results indicated that XN significantly contribute to plant growth

(Fig. 5). Interestingly, these two factors are not exhibiting very strong response degree to P, water, varieties and their interactions (Table 4). A number of studies have shown that XN plasticity can contribute to water and P uptake (De Bauw et al., 2018; Lopez-Valdivia et al., 2023; Nasr Esfahani & Sonnewald, 2024). We then focused on the response of the XN in DJ123, as they may help to gain more biomass and phosphorus under low P and drought recovery conditions.

The number and size of the xylem vessels are regarded as the crucial factor for water acquisition, exerting a direct influence on the potential axial water conductance of the root (Jackson et al., 2000; Holste et al., 2006). In our study, the xylem vessels number in both seminal root during recovery and nodal root during drought are lower under drought stress. This reduction in response to drought may mitigate the risk of water loss through xylem leakage and was also found in previous studies (De Bauw et al., 2019). However, the underlying physiological mechanism governing this phenomenon remains unclear and warrants further investigation.

In conclusion, the plasticity of specific traits (e.g., XN of nodal root) could be useful target traits for breeding programs aimed at increasing phosphorus deficit and intermittent drought resilience of upland rice. Further anatomical traits response including in lateral root should be explored to better understand plant multi-stress response.

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Author contributions

CCZ and AST conducted the experiments, analyzed the results, and led the writing; TR and MW contributed to the design and writing; CCZ and JAP conceived and designed the study, supervised its execution, assisted with data analysis, and contributed to the writing.

Conflict of interest statement

Nothing declared.

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Table 1: Root anatomical trait data from nodal root at 36 DAE and 54 DAE under two P levels (LP: 0 kg P ha^{-1} P and HP: 25 kg P ha^{-1}) and water treatment (DR: Drought recovery period and WW: Well-watered period) from two rice varieties (DJ: DJ123 and N4: Nerica4). Abbreviations: RTD: Root Tip Diameter; SD: Steel Diameter; S. RI: Ratio of steel and root area; XN: Xylem Number; XD: Xylem diameter; CCFN: Cortical Cell File Number; RCA: Root Cortical Aerenchyma; CCFN.RCA: CCFN/RCA; WCP: Water Conductance Parameter. Data are presented as means with ± SE. The data in the same column followed by the letters show the results of the pairwise comparison after ANOVA and PERMANOVA. Significant differences are indicated: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ns, not significant.

	P	**Water**	**Variety**	**RTD (mm)**	**SD (mm)**	**S. RI (%)**	**XN**	**XD (um)**	**CCFN**	**RCA (%)**	**CCFN.RCA**	**WCP (10^4µm4)**
36 DAE	**LP**	**WW**	**N4**	0.54 (0.05) ab	0.178 (0.016) ab	11.01 (0.63)	11.75 (1.11) c	33.31 (0.43) ab	7.5 (1.19) a	4.75 (4.75) b	80.78 (19.22) b	83.75 (4.02) a
	HP	**WW**	**N4**	0.59 (0.09) ab	0.199 (0.032) ab	11.56 (0.88)	19.5 (0.87) a	30.62 (4.07) b	7.75 (1.6) a	8.25 (5.1) b	58.92 (24.09) b	89.5 (34.73) a
	LP	**DR**	**N4**	0.45 (0.05) b	0.16 (0.019) b	12.93 (1.30)	15.5 (1.85) abc	28.41 (3.97) b	3.75 (0.62) b	28 (2.48) a	446.04 (205.73) a	64.5 (41.71) b
	HP	**DR**	**N4**	0.42 (0.04) b	0.156 (0.019) b	13.58 (2.03)	15 (1.35) abc	23.6 (2.69) b	3.5 (0.95) b	27.25 (3.42) a	451.12 (148.24) a	28.25 (13.81) b
	LP	**WW**	**DJ**	0.62 (0.09) ab	0.211 (0.034) ab	11.66 (2.37)	16.25 (3.64) abc	30.18 (5.58) b	7.75 (0.62) a	12.5 (7.23) b	80.71 (32.46) b	85.75 (42.70) b
	HP	**WW**	**DJ**	0.81 (0.08) a	0.241 (0.008) a	9.34 (1.29)	17 (1.47) ab	39.61 (2.64) a	7 (1.08) a	18.75 (10.87) b	143.30 (65.91) b	254.5 (87.02) a
	LP	**DR**	**DJ**	0.55 (0.08) ab	0.177 (0.024) ab	10.7 (1.09)	12.75 (1.03) bc	28.44 (2.11) b	4.25 (1.65) b	20.5 (6.95) a	265.44 (113;45) a	51.25 (28.47) b
	HP	**DR**	**DJ**	0.58 (0.06) ab	0.194 (0.021) ab	10.98 (0.17)	19.62 (0.625) a	35.26 (0.6) a	5.75 (0.62) b	22.25 (10.33) a	141.66 (72.65) a	93 (18.22) a
	P					ns	**		**			
	Water			*	*					*	**	*
	Variety			**								
	P:Water											
	P:Variety							*				
	Water:Variety											
	P:Water:Variety						**		**			
56 DAE	**LP**	**WW**	**N4**	0.704 (0.037)	0.219 (0.01)	9.69 (0.31)	18 (1.82) abc	37 (2) b	8.5 (0.29) b	21.25 (12.31)	361.06 (203.81)	145.75 (35.05) b
	HP	**WW**	**N4**	0.903 (0.042)	0.278 (0.098)	9.53 (0.31)	23.25 (1.65) a	46.61 (2.77) a	10.25 (0.48) a	13 (10.22)	92.24 (23.49)	474.75 (89.67) a
	LP	**DR**	**N4**	0.732 (0.041)	0.224 (0.011)	9.39 (0.28)	17.75 (1.88) abc	41.96 (1.19) b	9.5 (0.64) b	4 (4)	76.61 (23.39)	217.75 (33.74) ab
	HP	**DR**	**N4**	0.775 (0.11)	0.244 (0.035)	9.94 (0.48)	20.75 (1.43)	51.08 (1.4)	11.25 (0.48)	11.75 (9.27)	143.43 (79.65)	621.5 (103.6)

							ab	a	a			a
	LP	**WW**	**DJ**	0.835 (0.064)	0.271 (0.025)	9.76 (1.03)	16.75 (2.29) bc	46.7 (3.23) a	9 (0.41) b	24.5 (9.36)	110.74 (69.17)	506.75 (131.15) a
	HP	**WW**	**DJ**	0.862 (0.013)	0.275 (0.009)	9.47 (0.91)	22 (2.8) ab	48.29 (2.38) a	7.75 (1.65) b	26.5 (15.73)	372.81 (289.95)	602.75 (110.46) a
	LP	**DR**	**DJ**	0.866 (0.075)	0.261 (0.019)	9.45 (1.51)	14.5 (1.19) c	45.89 (2.77) a	8.75 (0.47) b	22.5 (10.36)	53.89 (18.78)	358.5 (64.92) a
	HP	**DR**	**DJ**	0.85 (0.111)	0.261 (0.041)	9.25 (0.82)	14.5 (2.1) c	39.02 (5.86) b	8.5 (0.87) b	11.25 (11.25)	129.73 (29.73)	349.5 (204.26) a
	P			ns	ns	ns	*		*	ns	ns	*
	Water						*		*			
	Variety						*		*			
	P:Water											
	P:Variety							*				*
	Water:Variety											
	P:Water:Variety											

Table 2: Root anatomical trait data from seminal root at 36 DAE and 54 DAE under two P levels (LP: 0 kg P ha^{-1} P and HP: 25 kg P ha^{-1}) and water treatment (DR: Drought recovery period and WW: Well-watered period) from two rice varieties (DJ: DJ123 and N4: Nerica4). Abbreviations: RTD: Root Tip Diameter; SD: Steel Diameter; S. RI: Ratio of steel and root area; XN: Xylem Number; XD: Xylem diameter; CCFN: Cortical Cell File Number; RCA: Root Cortical Aerenchyma; CCFN.RCA: CCFN/RCA; WCP: Water Conductance Parameter. Data are presented as means with ± SE. The data in the same column followed by the letters show the results of the pairwise comparison after ANOVA and PERMANOVA. Significant differences are indicated: *, P <0.05; **, P <0.01; ***, P <0.001; ns, not significant.

	P	Water	Variety	RTD (mm)	SD (mm)	S. RI (%)	XN	XD (mm)	CCFN	RCA (%)	CCFN.RCA	WCP (10^4 µm4)
36 DAE	LP	WW	N4	0.45 (0.027) bc	0.166 (0.003) b	12.63 (0.81) a	10.25 (0.63)	38.2 (5.99)	5 (0.7)	42.75 (6.8)	244.73 (63.34) b	95 (56.85)
	HP	WW	N4	0.47 (0.031) bc	0.167 (0.007) b	13.12 (0.91) a	13.25 (2.49)	39.22 (4.08)	4.75 (0.25)	38 (8.6)	300.45 (114.97) b	67.75 (18.06)
	LP	DR	N4	0.47 (0.039) bc	0.173 (0.018) b	15.19 (0.83) a	10(1.22)	38.93 (2.61)	4.75 (0.48)	54 (8.43)	666.69 (111.58) a	58.25 (4.5)
	HP	DR	N4	0.43 (0.028) c	0.165 (0.009) b	14.39 (1.01) a	11.5 (2.1)	35.79 (1.84)	4.75 (0.62)	42.25 (5.02)	471.61 (35.71) ab	45.5 (16.88)
	LP	WW	DJ	0.57 (0.019) a	0.205 (0.006) a	12.87(1.15) a	10 (1.47)	46.98 (4)	5 (0.41)	46.75 (6.25)	317.86 (54.5) b	118.5 (31.28)
	HP	WW	DJ	0.53 (0.038) ab	0.188 (0.009) ab	12.46 (0.53) a	11.725 (2.01)	34.43 (4.17)	4.5 (0.5)	44.75 (3.85)	335.41 (97.85) b	56.5 (12.06)
	LP	DR	DJ	0.52 (0.031) abc	0.188 (0.014) ab	13.39 (1.2) a	9 (1.58)	43.03 (4.29)	4 (0.41)	47.25 (5.32)	493.46 (105.6) a	83 (20.55)
	HP	DR	DJ	0.51 (0.011) abc	0.192 (0.009) ab	14.14 (1.32) a	10.25 (1.10)	36.5 (3.49)	4 (0.41)	58 (8.76)	255.2 (31.76) b	63.75 (9.71)
	P						ns	ns	ns	ns		ns
	Water										**	
	Variety			***	**	*						
	P:Water										*	
	P:Variety											
	Water:Variety										*	
	P:Water:Variety											
54 DAE	LP	WW	N4	0.48 (0.033) bc	0.177 (0.016) bc	13.41 (0.75) ab	14 (1.29) a	38.16 (6.19)	5.5 (50.29)	44 (2.12) b	280.16 (64.21)	79.75 (34.96) b
	HP	WW	N4	0.48 (0.021) bc	0.164 (0.007) c	11.79 (0.73) b	11 (0.41) ab	36.23 (4.53)	4.5 (0.64)	41.75 (2.93) b	282.39 (121.89)	44.5 (15.01) b
	LP	DR	N4	0.44 (0.026)	0.156 (0.014)	12.24 (0.94)	14.5 (1.04)	36.72 (2.89)	4.5 (0.64)	37.75 (1.49)	250.13 (48.89)	45.25 (3.42)

				cd	c	ab	a			b		b
	HP	**DR**	**N4**	0.40 (0.023) c	0.15 (0.012) c	13.76 (1.03) a	12.25 (0.94) ab	40.39 (2.92)	4.5 (0.87)	29 (7.41) b	266.78 (77.83)	63.75 (12.69) b
	LP	**WW**	**DJ**	0.54 (0.018) a	0.205 (0.005) ab	13.61 (0.52) ab	11 (1.08) ab	38.85 (3.99)	5.5 (0.5)	42.25 (6.48) a	271.09 (56.23)	84.5 (29.39) a
	HP	**WW**	**DJ**	0.53 (0.019) ab	0.192 (0.006) ab	12.81 (0.17) ab	11 (1.41) ab	46.89 (3.6)	5.75 (0.63)	52 (4.41) a	337.32 (67.04)	133 (41.77) a
	LP	**DR**	**DJ**	0.58 (0.012) a	0.206 (0.006) a	12.38 (0.31) ab	12.75 (2.29) ab	39.94 (8.71)	4.5 (0.64)	41.5 (6.6) a	415.95 (121.53)	104.75 (40.79) a
	HP	**DR**	**DJ**	0.53 (0.011) ab	0.19 (0.003) ab	13.31 (0.23) ab	8.75 (1.75) b	43.86 (3.35)	5.75 (0.75)	51.25 (59.46) a	331.48 (91.55)	86.25 (12.4) a
	P						*	ns	ns		ns	
	Water			***								
	Variety				***		*			*		*
	P:Water					*						
	P:Variety											
	Water:Variety			*								
	P:Water:Variety											

Table 3: Total Biomass, plant P concentration and total P content at 54 DAE. Abbreviations: LP: 0 kg P ha^{-1} P addition and HP: 25 kg P ha^{-1} addition; DR: Drought recovery treatment and WW: Well-watered treatment; DJ: DJ123 and N4: Nerica4. Data are presented as means with ± SE. The data in the same column followed by the letters show the results of the pairwise comparison after ANOVA and PERMANOVA. Significant differences are indicated: *, P <0.05; **, P <0.01; ***, P <0.001; ns, not significant.

Factors			**Parameters at 54 DAE**		
P	**Water**	**Variety**	**Total biomass (mg)**	**P concentration**	**Total P content**
LP	**WW**	**N4**	208.3 (13.88) de	1.42 (0.19)	287.63 (23.97) cd
HP	**WW**	**N4**	535.1 (51.53) a	1.45 (0.14)	759.81 (33.13) a
LP	**DR**	**N4**	168.95 (7.02) e	1.33 (0.17)	220.97 (18.79) d
HP	**DR**	**N4**	483.23 (59.85) ab	1.75 (0.21)	810.38 (19.56) a
LP	**WW**	**DJ**	332.75 (42.13) bc	1.26 (0.13)	401.74 (12.28) b
HP	**WW**	**DJ**	626.53 (62.74) a	1.29 (0.07)	794.25 (44.04) a
LP	**DR**	**DJ**	276.25 (16.45) cd	1.37 (0.13)	372.50 (18.65) bc
HP	**DR**	**DJ**	566.05 (45.65) a	1.56 (0.16)	865.57 (47.81) a
P			***	ns	***
Water			*		
Variety			***		***
P:Water					**
P:Variety			*		***
Water:Variety					
P:Water:Variety					

Table 4: Summary of observed anatomical root responses to P availability, drought recovery, varieties and interactions between these three factors, attributed to factorial interactions (i.e. Et-squares) represented in percentage (%). Observed information is categorized as follows: 0-5%, no response; 5-20%, slight response; 20-50%, strong response; >50%, very strong response.

		RTD	**SD**	**S. RI (%)**	**XN**	**XD**	**CCFN**	**RCA**	**CCFN.RCA**	**WCP**
Nodal root sampling at 36 DAE	**P**	3.55	2.81	0.15	2.82	2.31	0.13	0.93	0.10	5.47
	Water	16.97	13.51	4.51	7.84	9.77	37.85	21.68	26.82	12.90
	Variety	18.32	11.53	8.71	0.31	9.28	1.18	0.20	0.98	8.06
	P:Water	2.64	0.93	1.54	2.82	0.68	0.71	0.50	1.04	4.82
	P:Variety	2.18	0.59	2.23	0.31	17.03	0.13	0.17	0.58	9.80
	Water:Variety	0.12	0.22	2.26	0.31	1.02	2.46	7.19	3.93	2.25
	P:Water:Variety	0.37	0.10	1.32	2.82	0.01	1.76	0.41 (10^2)	5.45	1.22
Nodal root sampling at 54 DAE	**P**	5.16	7.10	0.01	15.94	9.50	22.32	1.92	1.08	16.87
	Water	0.56	1.45	0.14	22.27	0.19	1.70	1.44	5.62	0.84
	Variety	7.25	9.58	0.29	6.46	1.37	5.59	7.70	0.31 (10^2)	3.21
	P:Water	3.20	0.56	0.49	1.19	1.24	0.69	11.92	0.96	0.02
	P:Variety	4.34	4.57	0.60	6.46	15.49	3.82	1.25	9.64	10.47
	Water:Variety	1.16	0.03	0.32	6.46	9.57	3.20	0.39	2.11	9.66
	P:Water:Variety	1.05	1.31	0.29	0.13	0.87	1.93	2.45	1.12	0.81
Seminal root sampling at 36 DAE	**P**	1.45	1.25	0.00	8.79	11.28	1.01	0.53	5.12	5.31
	Water	3.00	0.19	14.91	3.16	0.53	5.52	7.51	18.74	1.30
	Variety	34.00	29.66	2.48	2.50	1.94	5.52	3.42	3.13	7.15
	P:Water	0.16	0.46	0.01	0.63	0.09	1.01	0.29	10.15	0.03
	P:Variety	0.16	0.12	0.17	0.35	7.21	0.11	5.60	0.26	2.00
	Water:Variety	1.61	0.95	1.10	0.04	0.02	2.82	0.03	9.79	0.09
	P:Water:Variety	3.39	2.69	2.49	0.16	2.60	0.11	3.42	0.99 (10^3)	5.47
Seminal root sampling at 54 DAE	**P**	3.85	4.82	0.00	15.24	3.51	5.21	0.77	0.26 (10^4)	0.09
	Water	2.37	1.72	0.01	0.28	0.01	0.22	4.49	0.56	0.89
	Variety	51.96	49.89	0.76	12.12	6.09	9.90	12.71	4.88	15.77
	P:Water	2.58	0.04	20.96	1.88	0.04	3.37	0.45	1.19	0.09
	P:Variety	0.23	0.10	0.04	0.28	1.95	7.91	9.93	0.09	1.12
	Water:Variety	7.01	3.61	2.03	0.90	0.41	0.34 (10^2)	3.27	2.18	0.06
	P:Water:Variety	0.02	0.44	1.70	4.02	1.77	0.15	0.45	1.74	7.48

Responsiveness classes					
	No	Slight	Strong	Very strong	

Figure 1 Schematic representation of the experimental design and root anatomical plasticity during drought and recovery. The image is created with BioRender. Created with BioRender.com.

Figure 2 Cross-section of the nodal root of variety N4 as a function of P levels and water treatment at 36 and 54 DAE (RTD: Root Tip Diameter; HPWW: High P and Well- watered; LPWW: Low P and Well-watered; HPDR: High P and drought recovery; LPDR: Low P and drought recovery).

Figure 3 Cross-section of the nodal root of variety DJ as a function of P levels and water treatment at 36 and 54 DAE (RTD: Root Tip Diameter; HPWW: High P and Well- watered; LPWW: Low P and Well-watered; HPDR: High P and drought recovery; LPDR: Low P and drought recovery).

Figure 4 Boxplots of the Water Conductance Parameter (WCP, $\times 10^4$ μm^4) in nodal root after drought recovery (54 DAE) under two P levels (LP: 0 kg P ha^{-1} P and HP: 25 kg P ha^{-1}) and water treatment (DR: Drought and recovery and WW: Well-watered treatment) from two rice varieties (DJ: DJ123 and N4: Nerica4).

Figure 5 Redundancy analysis (RDA) on root anatomy to investigate the relationship between root anatomical variation and both total biomass (TB) and total P acquisition (TP) at 54 DAE. Anatomical variation of seminal (S) and nodal (N) roots during the drought (1) and recovery periods (2) were designated as response variables, while total biomass (TB) and total P acquisition (TP) during the recovery period were defined as explanatory variables. Abbreviations: SRI: Ratio of steel and root; SD: Steel Diameter; XN: Xylem Number; CCFN: Cortical Cell File Number. XD: Xylem diameter; RTD: Root Tip Diameter; RCA: Root Cortical Aerenchyma; CR: CCFN/RCA.

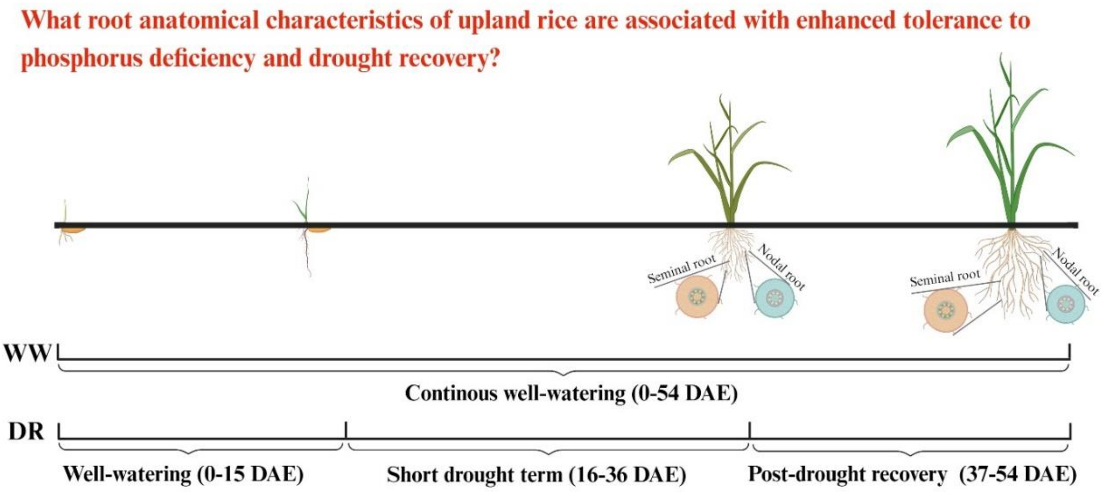
What root anatomical characteristics of upland rice are associated with enhanced tolerance to phosphorus deficiency and drought recovery?
Seminal root
Nodal root
Seminal root
Nodal root
WW
Continous well-watering (0-54 DAE)
DR
Well-watering (0-15 DAE)
Short drought term (16-36 DAE)
Post-drought recovery (37-54 DAE)

Fig. 1

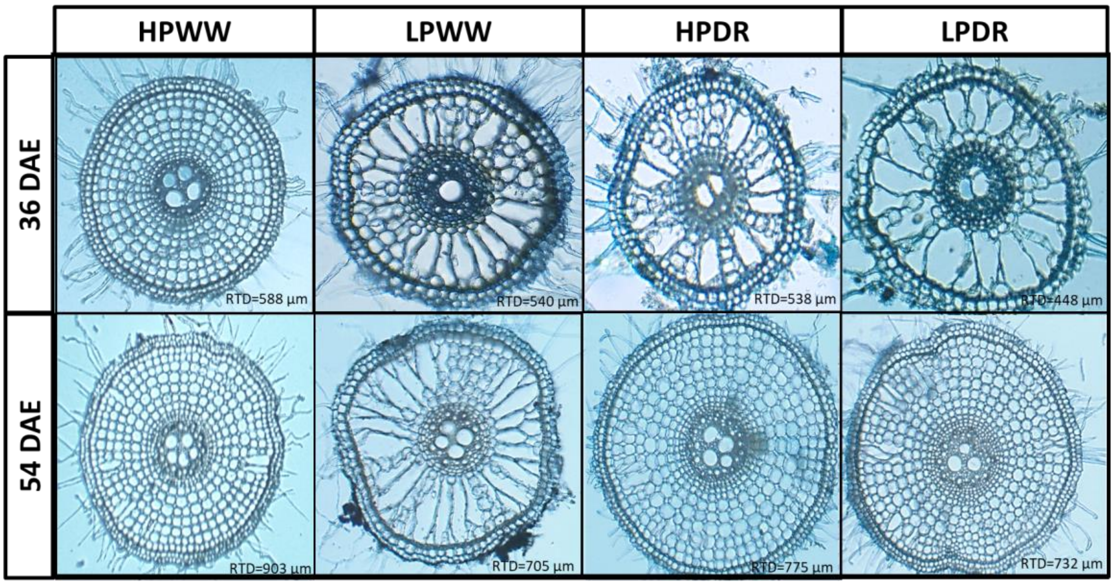
HPWW
LPWW
HPDR
LPDR
36 DAE
RTD=588 µm
RTD=540 µm
RTD=538 µm
RTD=448 µm
54 DAE
RTD=903 µm
RTD=705 µm
RTD=775 µm
RTD=732 µm

Fig. 2

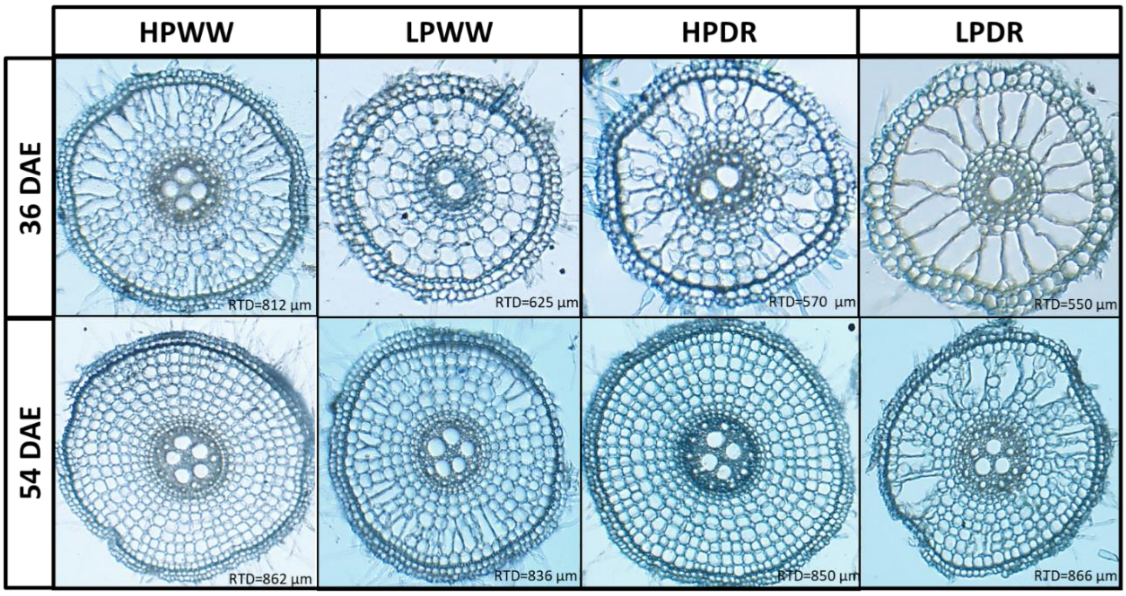
HPWW
LPWW
HPDR
LPDR
36 DAE
54 DAE
RTD=812 µm
RTD=625 µm
RTD=570 µm
RTD=550 µm
RTD=862 µm
RTD=836 µm
RTD=850 µm
RTD=866 µm

Fig. 3

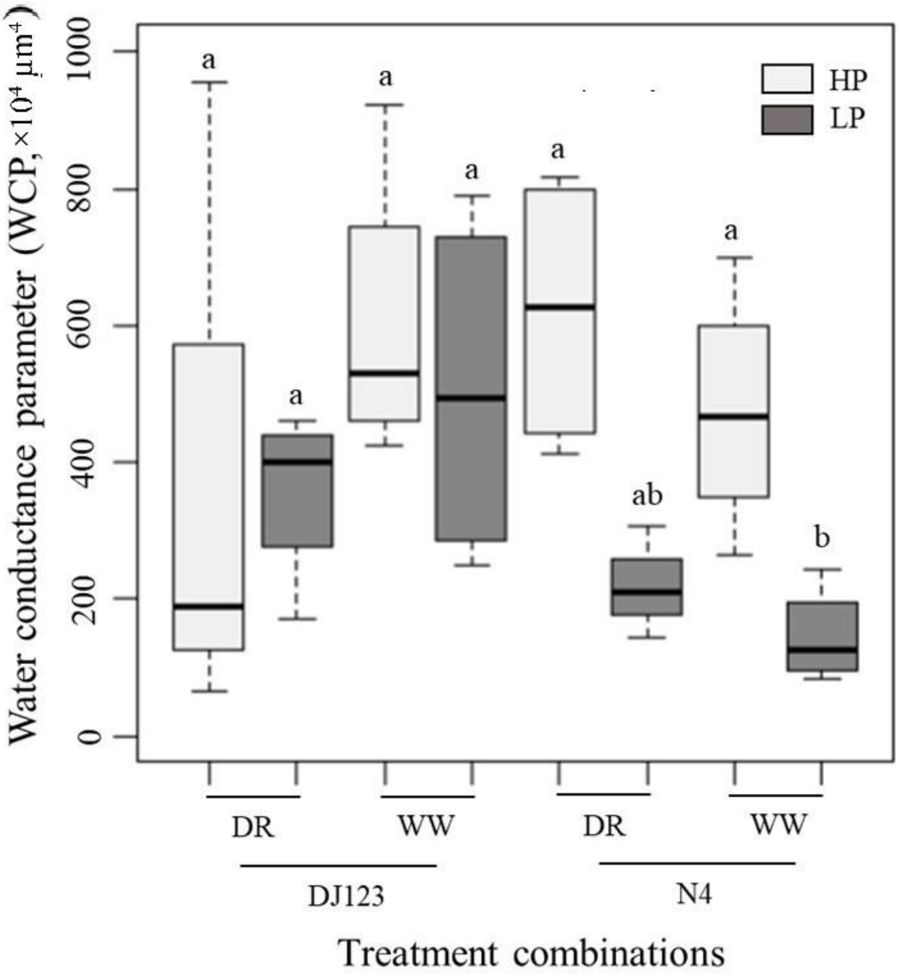
Water conductance parameter (WCP, ×10⁴ µm⁴)
0
200
400
600
800
1000
a
a
a
a
a
ab
a
b
HP
LP
DR
WW
DR
WW
DJ123
N4
Treatment combinations

Fig. 4

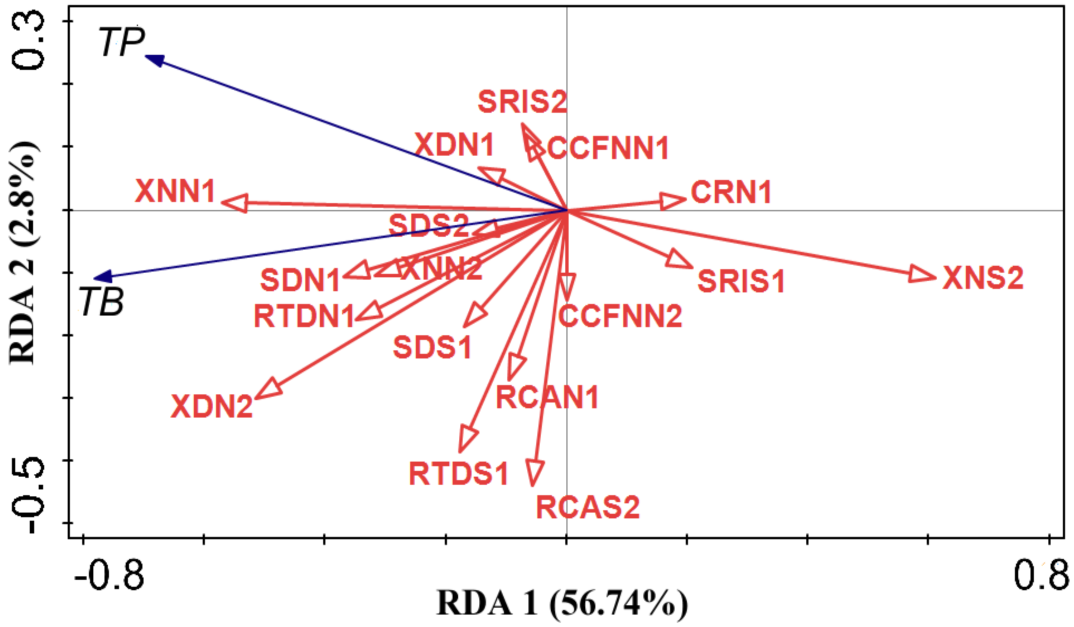
TP
TB
SRIS2
XDN1
CCFNN1
XNN1
CRN1
SDS2
SDN1
XNN2
SRIS1
XNS2
RTDN1
CCFNN2
SDS1
XDN2
RCAN1
RTDS1
RCAS2
0.3
-0.5
-0.8
0.8
RDA 2 (2.8%)
RDA 1 (56.74%)

Fig. 5

Supplementary material

Table S1 Basic proprieties of the soil takend from Antohobe, Madagascar.

Table S2 Results of permutation testing of redundancy analysis (RDA) on predictor variables for biomass (TB) and P accumulation (TP) at 54 DAE under drought recovery and low P conditions. Anatomical variation of seminal (S) and nodal (N) roots during the drought (1) and recovery periods (2) were designated as response variables, while total biomass (TB) and total P acquisition (TP) during the recovery period were defined as explanatory variables. Abbreviations: S. RI: Ratio of steel and root area; XN: Xylem Number; CCFN: Cortical Cell File Number.

Figure S1 Soil water content (g g^{-1}, %) over a 54-day growth period in 30 L containers. DR: Drought/Recovery period and WW: Well Watering period (Drought phase from 16 to 36 DAE, recovery phase from 37 to 54 DAE). Data shown are means of eight replicates ± SE.

Proprieties	**Antohobe soil**
pH Water	5.05
Sand (%)	44.62
Silt (%)	17.37
Clay (%)	38.01
AEM-P (mg kg^{-1})	1.35
SOC (g kg^{-1})	18.13

Table S1

Name	Explains %	Contribution %	pseudo-F	P
XNS2	21.1	35.5	8	0.004
XNN1	11.6	19.5	5	0.036
S.RIS1	6.8	11.4	3.1	0.096
XNN2	6.5	11	3.3	0.056
CCFNN1	5	8.4	2.7	0.114
S.RIS2	1.5	2.5	0.8	0.418
RCAS2	1	1.7	0.5	0.514
XDN1	0.8	1.3	0.4	0.622
SDN1	1.1	1.8	0.5	0.49
RTDN1	0.5	0.9	0.3	0.67

Table S2

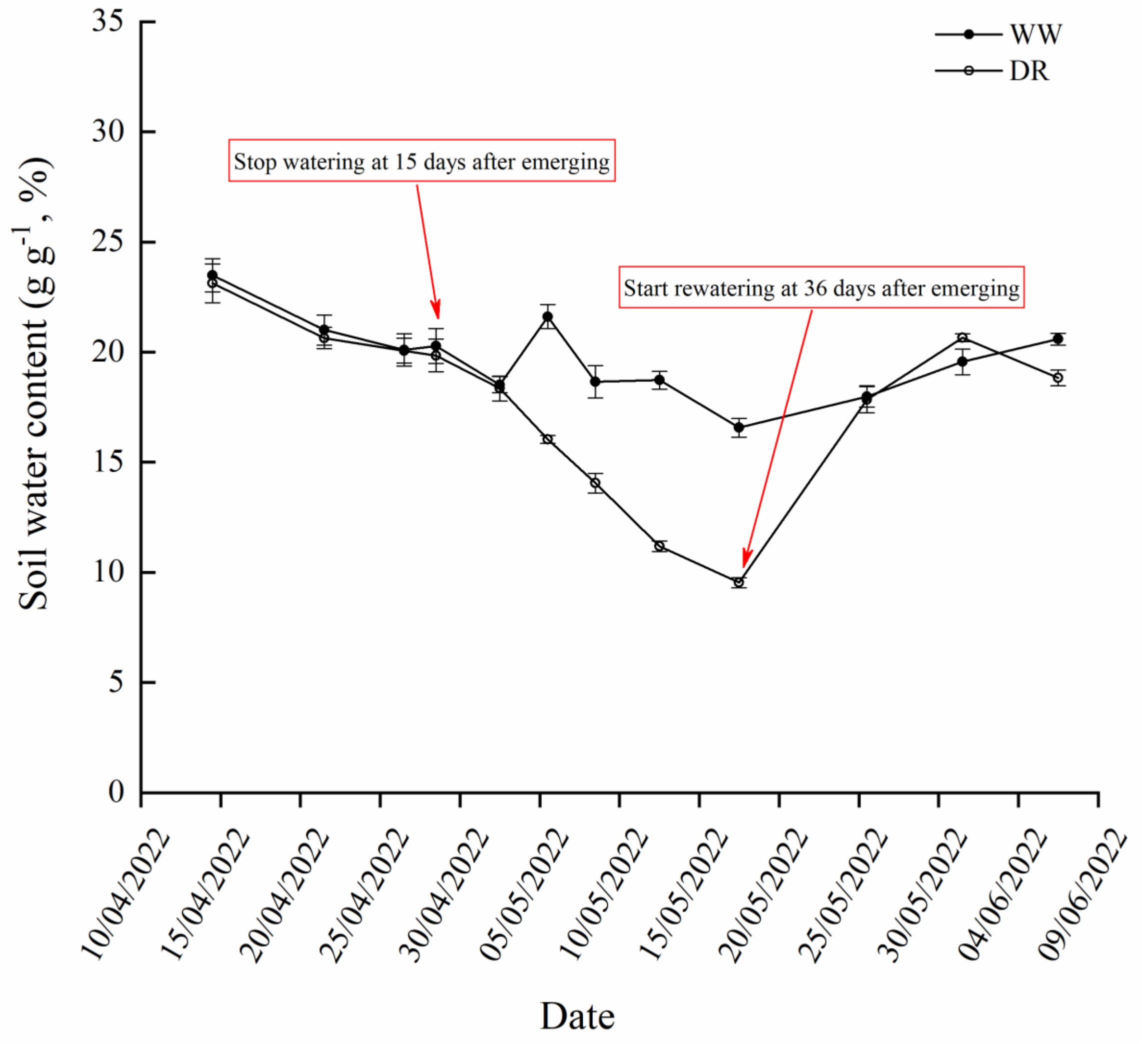
WW
DR
Stop watering at 15 days after emerging
Start rewatering at 36 days after emerging
Soil water content (g g^{-1}, %)
35
30
25
20
15
10
5
0
10/04/2022
15/04/2022
20/04/2022
25/04/2022
30/04/2022
05/05/2022
10/05/2022
15/05/2022
20/05/2022
25/05/2022
30/05/2022
04/06/2022
09/06/2022
Date

Figure S1

CHAPTER 5: Rhizosphere pH-change causes efficient P uptake in upland rice: Evidence from contrasting genotypes in greenhouse, field, and silico studies

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Manuscript under internal review

Chapter 5: Rhizosphere pH-change causes efficient P uptake in upland rice: Evidence from contrasting genotypes in greenhouse, field, and silico studies

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Abstract

Aims: Suboptimal phosphorus (P) availability is one of the most limiting factors for upland rice production. Compared to the more commonly grown cultivars, the genotype DJ123 can efficiently take up P from soils with low P availability. **Based on a previous modeling study, we hypothesized that** P-efficient genotype DJ123 has, compared to the P-sensitive genotype Nerica4, a higher rhizosphere pH caused by greater anion/cation uptake-imbalance and thus improves P availability from phosphorus-fixing soils.

Methods: Across-scale experiments, combining rhizotrons in the greenhouse and optodes in the field, were carried out to measure rice P acquisition, cation- and anion concentrations, root morphology traits, and rhizosphere pH. Mathematical models, accounting for morphology, were used to simulate P acquisition induced by solubilization due to pH change.

Results: In the greenhouse and field experiment, DJ123 showed greater P uptake, in total and per root length (uptake efficiency), than Nerica4 under low P but not under high P. A greater rhizosphere pH of DJ123 than Nerica4 was detected in the field by planar optodes and in the greenhouse by gel plates with pH indicator. Rhizosphere pH was associated with an excess uptake of anions over cations and also a higher root length density in the DJ123, which can explain increased phosphorus availability and uptake. Simulation using Kuppe's rhizosphere model can reason the higher rhizosphere pH and P uptake of DJ123 compared to Nerica4.

Conclusion: These results support that increased rhizosphere pH contributes to P uptake in upland rice varieties from low-P-available soils. In strongly sorbing acidic soils, increased pH improves P acquisition. Thus, the ability to increase soil pH can also be considered a selection target to improve P capture in upland rice and possibly other cereal crops.

Keywords: phosphorus deficiency, upland rice, root efficiency, rhizosphere pH, solubilization

1 Introduction

1.1 P deficit over the world

Phosphorus (P) is one of the primary limitations to plant growth within most agroecosystems. P as a resource is a finite and diminishing (Lynch, 2011; Simpson *et al.*, 2011; Suriyagoda *et al.*, 2014). P as plant nutrient is bound tightly to mineral components in the soils and is also held in soil organic matter, exhibiting a limited fraction amenable to plant uptake (Hou *et al.*, 2018; McDowell *et al.*, 2023). Due to this low soil-P availability to plants, farmers often apply a large quantity of P fertilizer to improve yields. However, in many developing countries like the sub-Saharan African region, most smallholder farmers cannot afford mineral fertilizer, and the insufficient availability of soil P emerges as a principal and ubiquitous impediment to crop production and food security (Ayaga *et al.*, 2006; Nziguheba *et al.*, 2016). Therefore, using P-efficient genotypes is eminent. Upland rice (*Oryza sativa* L.) can grow well on low P soils and is a crucial staple crop for about half the world's human population. Previous studies have found that upland rice germplasms have significant discrepancies in the tolerance of strongly sorbing low P soils and the associated genes have gotten recent attention (Schatz *et al.*, 2014; Mori *et al.*, 2016a; Vandamme *et al.*, 2016; Nestler & Wissuwa, 2016; Wissuwa *et al.*, 2020). The potential mechanisms of efficient P uptake were investigated using mathematical modeling (Gonzalez *et al.*, 2021a, Kuppe *et al.*, 2022). We validated them with experimental results and asked if these mechanisms differ in P-efficient and sensitive genotypes?

1.2 Mechanisms of root traits response to low P

In response to P deficiency, plants have evolved many strategies to improve soil exploration and P exploitation, which can be attributed mainly to the differences in root and rhizosphere traits. Traits that enhance P uptake include increases in root length, surface area, root hair length and density, and nodal and crown root number (Desnos, 2008; Lynch, 2011; Sun *et al.*, 2018). Furthermore, P-uptake is increased through associations with microorganisms, either symbiotic (mycorrhizal fungi) (Smith *et al.*, 2011) or P-solubilizing microorganisms (Zhang *et al.*, 2018; Dipta *et al.*, 2019). Both modifications in root system architecture and mycorrhizal association are effective ways to increase the total surface area available for soil P absorption and thereby improve P acquisition (Lambers *et al.*, 2006; Shen *et al.*, 2011). In addition, plants can enhance P acquisition by altering root physiology (Lambers *et al.*, 2006; Shen *et al.*, 2011; Hinsinger *et al.*, 2011). For example, root-induced rhizosphere acidification and alkalization and release of organic anions can mobilize soil inorganic P by ligand exchange, ligand-induced dissolution, and complexation of cations bound to P, such as Fe, Al, and Ca (Hinsinger, 2001; Rose *et al.*, 2010; Sun *et al.*, 2019). Some plants secrete phosphatases into the rhizosphere, allowing mobilization and utilization of organic P in the soil (Li *et al.*, 2007; George *et al.*, 2008). Even though many traits have been identified, their relative importance is largely unknown. Furthermore, it is not clear if these traits work together synergistically, additive, or antagonistically.

As in other Poaceae, the upland rice root system is composed of several root classes, notably: seminal (primary) roots, crown roots, nodal roots, and lateral roots. Quite uniquely, the lateral roots fall into two classes distinguished by diameter and length: the larger L-type lateral roots (>1 cm; ~0.15mm) and the smaller S-type lateral roots (<1 cm length; ~0.05mm diameter) (Kono *et al.*, 1972; Yamauchi *et al.*, 1987; Rebouillat *et al.*, 2009). In several studies, researchers have tried to associate the uptake of phosphorus from strongly P fixing soil with genetic variation in various root traits, but single trait associations could only explain a small fraction of the variation in P uptake (Nestler & Wissuwa, 2016; Wissuwa *et al.*, 2020; Gonzalez *et al.*, 2021b). Previous simulation models of P uptake from highly P-fixing Ando- and Oxisols typically underestimated P uptake, suggesting that they are missing important mechanisms (Kirk *et al.*, 1999b). Recently, Kuppe et al (2022) published a new rhizosphere model, which was able to simulate the P uptake of the P-efficient genotype DJ123 growing on low-P (P-fixing) Andosol. The model suggested that a synergistic interaction between thicker and thinner roots arises in which thicker roots increase the soil pH such that nearby thinner roots, with their root hairs, provide the necessary surface area into the solubilization zone to take up P. We set out to validate the model with experimental data.

1.3 pH is important for efficient P uptake in upland rice

In the acidic Oxisols, phosphate is relatively immobile as it binds to iron and aluminum ions. Increased rhizosphere pH can improve phosphate availability by solubilizing iron phosphate and aluminum phosphates (Lambers *et al.*, 2008; Penn & Camberato, 2019; Barrow *et al.*, 2020). Besides the solubilization of P, P availability may also increase when other anions, particularly bicarbonates, compete for the positively charged exchange sites. Note that the commonly used Olsen P extraction method is based on addition of sodium-bicarbonate. Rhizosphere pH increases when roots take up more anions (particularly NO_3^-, $H_2PO_4^-$, SO_4^{2-}) than cations (particularly: NH_4^+, K^+, Ca^{2+}, Mg^{2+}). To balance charge differences, roots will take up additional protons, raising the rhizosphere pH and, with it, the bicarbonate concentration in the rhizosphere (Fig. 1; Dijkshoorn *et al.*, 1968; Nye, 1981; Rengel, 2003). Thus, pH changes can increase the P concentration in the soil solution and, thereby, increase uptake in low P soils (Fig. 1). The change in pH can be influenced by various soil factors and plant traits, including soil nutrient levels, For instance, the preference for nitrate over ammonium uptake strongly influences the anion-cation balance, serving as a significant determinant of rhizosphere pH (Nye, 1981; Marschner *et al.*, 1986; Kirk *et al.*, 1999b; Bloom *et al.*, 2002). Besides, experimental and modelling studies has proved that rhizosphere pH change can further influenced by soil characteristics including soil types, soil initial pH and humidity, and root density (Youssef & Chino, 1988, 1989; Kim & Silk, 1999; Kirk *et al.*, 1999a; Custos *et al.*, 2020; Kuppe *et al.*, 2022). However, Exploring the relationship between rhizosphere pH and phosphorus (P) availability, and unraveling the underlying mechanisms, is challenging due to intricate interactions among diverse plant traits and soil parameters. The model of Kuppe et al. (2022) was designed to simulate these processes and predict rhizosphere pH change and P uptake in upland rice. The model predicted that high P uptake depends on high root surface area density and a greater anion over cation uptake by that surface area. However, experimental evidence is thus far lacking, and we asked if the model can predict the differences in P uptake when parameterized for P-efficient and inefficient genotypes in Oxisols.

To validate the model, both the rhizosphere pH-change, biomass and the P accumulation need to be measured. We further measured the relevant model parameter values. These are the uptake of cation and anions, the morphological root traits, the total root surface area, the root hair traits, and several soil characteristics. We conducted experiments in the greenhouse and an agricultural field in Madagascar to measure these parameters on a P efficient and in-efficient genotype. The pH-P model was used to simulate P acquisition and rhizosphere pH change. We tested three hypotheses: (a) the P-efficient genotype DJ123 has a higher rhizosphere pH than the P-sensitive genotype Nerica4 under low P but not high P conditions; (b) higher rhizosphere pH induced by excess anion uptake per root length improves P uptake from P-deficient soils; (c) P-efficient DJ123 has a greater root surface density promoting fast uptake of P.

Material and Methods

We conducted a greenhouse rhizotron and a field experiment to test whether the rhizosphere pH change of rice roots contributes to its P uptake on P-fixing Oxisols. We grew two upland rice genotypes, which, consistently across experiments, differ in P uptake. We estimated their anion-cation uptake imbalance based on the main element contents in plant, which are the main driver for pH change in the rhizosphere. Next, we measured the pH change using both a short-term assay and pH-optodes in the field. Finally, we determined both the total and the root-length-specific P uptake. We used the data to parameterize the mechanistic model developed by Kuppe *et al.* (2022) and simulate P uptake by these contrasting upland rice genotypes.

Plant Material and Preparation

We grew two rice genotypes (DJ123 and Nerica4), belonging to the aus and indica subspecies of rice (*Oryza sativa* L.) respectively. These two genotypes are adapted to the upland growth conditions and contrast in their phosphorous uptake efficiency with DJ123 generally taking up more P per unit root length than Nerica4. In the long run, DJ123 often grows more biomass, a larger root system and has a greater seed mass under P stress (Koide *et al.*, 2013; Mori *et al.*, 2016b; Wissuwa *et al.*, 2020). All seeds were obtained from the Japan International Research Center for Agricultural Sciences, Tsukuba, Japan.

Greenhouse experiment

Experimental design

The greenhouse experiment was a randomized complete block design with two P treatments, two genotypes, and four blocks, each being one replication. Plants were grown in sand-perlite-filled pots and regularly watered with nutrient solution. The factors were two P regimes at 1 µM P (low-P conditions) and 100 µM P (high-P conditions) in the adjusted nutrient solution (see below).

Plant Material and Preparation

To break their dormancy, seeds were incubated at 50°C for 2 days. Afterwards, seeds were surface-sterilized in 0.5% (v:v) NaOCl for 5 min followed by three times washing in distilled water for 10 min. Seeds were germinated on Petri dishes with distilled water at 30°C in the dark. Seedlings with comparable sizes were picked to experiment and were distributed randomly to the 3-liter rhizoboxes (50 * 25 * 3 cm) filled with nutrient-free substrate (a sand and perlite mixture 95: 5 v:v). We planted and grew two seedlings in each rhizobox.

Greenhouse Growth Medium and Conditions for Seedlings

Pre-germinated seeds of rice varieties Nerica4 and DJ123 were planted on April 26, 2021, and harvested after 45 days. During this period, they grew in a greenhouse located on Forschungszentrum Jülich, Germany (50°55'N, 06°21'E). The photoperiod was approximately 14/10 hours day/night. The temperature and relative humidity in the glasshouse were recorded with a data logger (30040_Testo_Logger). The minimum, maximum, and average temperatures were 20, 34, and 26°C respectively and the relative humidity varied between 30-80%, with an average humidity at 65%.

After germination, the seedlings were watered weekly with 60 ml modified Yoshida solution (Yoshida et al. 1976), a tenth-strength Yoshida solution was firstly watered at 7 days-after sowing (DAS) then a third-strength solution were watered at 14 DAS. At 21 DAS the solution was replaced by half-strength Yoshida solution and at 28 DAS until harvest by full-strength Yoshida solution. To minimize ammonium and nitrate differential uptake effect for pH change, a near ammonium-free modified Yoshida solution was used in which NH_4NO_3 was replaced by KNO_3 and $Ca(NO_3)_2$ in the molar ratio 1:0.5. $(NH_4)_6 \cdot Mo_7O_{24} \cdot 4H_2O$ was not replaced. In addition, the phosphorus was added as sodium phosphate at two different concentrations according to the treatment. The first two weeks, the plants received nutrient solution without P. At 14 DAS, the P-treatment ($NaH_2PO_4 \cdot 2H_2O$) started with quantities corresponding to 100 (high) and 1 (low) µM P in full-strength Yoshida solution. The low ammonium and P free modified Yoshida solution contained at full-strength: KNO_3 (N+K: 1.43 mM), $Ca(NO_3)_2$ (Ca: 0.72 mM, N: 1.43 mM), K_2SO_4 (K: 1.02 mM, SO4: 0.5 mM), $CaCl_2 \cdot 2H_2O$ (Ca: 1 mM), $MgSO_4 \cdot 7H_2O$ (Mg: 1.65 mM), $MnCl_2 \cdot 4H_2O$ (Mn: 9.1 µM), $(NH_4)6 \cdot Mo_7O_{24} \cdot 4H_2O$ (Mo: 0.52 µM), H_3BO_3 (B: 18.5 µM), $ZnSO_4 \cdot 7H_2O$ (Zn: 0.15 µM), $CuSO_4 \cdot 5H_2O$ (Cu: 0.16 µM) and EDTA iron(III) sodium salt (Fe: 35.81 µM)).

Rhizosphere alkalization assay

At day 45 after planting, we conducted an assay for the rhizosphere alkalization measurement, modified after Li *et al.* (2007). Rice plants were carefully taken from the substrate and washed in 0.2 M $CaSO_4$ solution for a few minutes and then rinsed with distilled water. Immediately afterward, the intact plants were placed with their roots on a 3-mm-thick agar gel film (5.0 g L^{-1}) with pH 6.0 containing 0.1 g L^{-1} pH indicator (bromocresol purple) and the complete nutrient solution described earlier with P levels identical to those in the rhizobox before. A nipper was used to gently press the roots into the agar till about three-quarters of the root surface was in direct contact with the agar. Then the roots were covered with transparent plastic film with about 3 mm air-gap between film and root to allow some air exchange but avoid drying of the roots. The agar gel film was wrapped with aluminum foil to avoid light penetration into the root zone and placed at 30° for 15 h, while shoots received the same light with the seedlings grow in rhizobox. After 15 hours, the agar gel films were photographed for later color change quantification. Finally, the rice plants were gently removed from the gel for further analysis. The agar gel was melted at 45°C after which we measured the pH with a pH meter.

After the gel pH assay experiment, plants were divided into shoots and roots for further measurements. For the shoots, we recorded fresh- and, after oven-drying at 65°C, dry mass. The roots were rinsed with water and were then preserved in 50% ethanol till they were scanned with an Epson Perfection V800 scanner (Epson America, Inc., USA) at a resolution of 23.6 pixels mm^{-1} (600 dpi). The scans were analyzed with the image-processing software WinRhizo Pro (Regent Instruments, Québec, Canada). Like the shoots, fresh and dry mass was recorded before and after roots were oven-dried at 65°C to a constant weight. All dry shoot parts and root parts were ground together and then analyzed for P, K, Ca, Mg, and S content with ICP-OES, and N content with an elemental analyzer.

Field experiment

Experimental design

The field experiment was laid out according to a randomized complete split-split-plot design with 4 blocks, each split into two fertilizer treatments (no P and 25 kg ha^{-1} P) and each fertilizer treatment split into two genotypic plots. Each sampling plot consisted of five 1.5 m- rows with 20 cm apart, and each planted with 25 rice seeds.

Field Experiment Growth Conditions

The field experiment was conducted at the field station of the University of

Antananarivo, Antohobe, Madagascar (19°78'S, 46°68'E) from April 1st to June 5th, 2022. The soil was a Ferralsol (USDA) of the highlands of the commune of Antohobe. Ferralsols are Oxisols with a high level of iron oxides which strongly bind phosphorus. Based on soil analysis at the beginning of the experiment, the low P field plot was used and fertilized with N-P-K fertilizer at rates of 50-25-40 kg ha^{-1} (high P) or 50-0-40 kg ha^{-1} (low P). Fertilizers were broadcasted by hand after mixing them with about 300 g of soil per subplot. Weeding was done manually as necessary.

Sampling and Measurements

Shoots were harvested 65 days after planting. 4 days before harvest. Rhizosphere pH was measured with planar optodes that were sensitive to pH (product code SF-HP5-OIW; PreSens GmbH). As the planar optode measurement principle has been well described in detail in several previous studies (Holst & Grunwald, 2001; Gansert & Blossfeld, 2008; Blossfeld *et al.*, 2013; Bilyera *et al.*, 2022), therefore, only a brief overview of the main steps is provided here and we refer to the specialized literature for details. Before the experiment, calibration of the planar pH optodes was performed in order to exclude shifts in the sensor response. The calibration procedure for the planar pH optodes was kept same with Blossfeld *et al.* (2013). The fitting response curve was then used to calculate the rhizosphere pH value.

To monitor rhizosphere pH differences in a confined space in the field condition, a simplified “root window” was used because of the limited conditions. Firstly, a 3 cm *8 cm glass was buried vertically to the soil after sowing, with a 5 cm away from the seeds. Three days before harvesting, the soil on the disturbed side of the glass windows was carefully removed, the major (nodal) roots that stick to the glass are exposed. Then two sensor foils (1.5 cm * 1.5 cm) were placed over the nodal root and covered with glass. Thus, the sensor foils were in direct contact with the rhizosphere and the pH value could be read optically through the glass back side. For pH optodes, we used an optimized deployment time of ~18 h overnight to ensure a reliable pH signal under field moist conditions. On the sampling day, the soil on the disturbed side of the glass windows was carefully removed and the glass screens were cleaned with a soft cloth and tissue paper to obtain clear images.

Imaging software (VisiSens AnalytiCal 1; PreSens GmbH) was then used to acquire the images of the pH optodes and to compute the quantitative maps from the raw sensor response images. The results from pilot test runs with this novel system, performed under different environmental conditions and with different plant species, demonstrate the power of this system (Blossfeld *et al.,* 2013; Bilyera *et al.,* 2022).

The initial (bulk) soil pH was measured with a pH meter (pHS-3C, SPSIC Corporation) in a (1:5 w/w) suspension of soil in deionized water (Table 1). Like the greenhouse experiment, the shoot and roots were oven-dried at 65°C to a constant weight, and the dry mass was measured. All dry shoot parts and root parts were then analyzed for P, K, Ca, Mg, and S content with ICP-OES, and N content with an elemental analyzer.

pH-P model simulation

We used the mechanistic pH-P model by Kuppe *et al.* (2022) to simulate rhizosphere changes in pH, the associated P release, and P uptake by rice roots to see if we could explain the observed genotypic differences. We re-implemented the model in R (R code are available by request) using the description in the paper by Kuppe et al (2022). Furthermore, we added fertilizer as an additional optional input to the rhizosphere, parameterized the model based on our data (Table 2) using the same method of calculation as in the original publication. The parameters that were not measured were kept the same as in the original publication. We assumed that the excess uptake of anions over cations is electrically balanced by net proton uptake. This is modeled by efflux of HCO_3^- by the root and root hairs, which increases the pH in the rhizosphere. For the root classes, characteristic of rice, we use same diameter distinction as in Kuppe *et al.* (2022): crown roots, L-type and S-type lateral roots, all with hairs. Note that the data of root classes were obtained from greenhouse rhizotron experiment, root hairs data were further calculated based on the previous publications with DJ 123 and Nerica4 (Nestler *et al.*, 2016; Nestler & Wissuwa, 2016). We treat the S-type lateral roots as HCO_3^--source and P-sink reaction terms in the solute transport equations associated with crown root- and L-type rhizospheres.

In summary, the model considered three processes from soil to plant: (1) Different nutrient uptake and thereby cation/anion balance; (2) rhizosphere soil pH and P availability changes by nutrient anions and cations differential uptake; (3) different root characteristics/classes of DJ123 and Nerica4 rice roots in greenhouse/field experiments. The primary concepts and assumptions include the following:

1). Soil pH gradients in the rhizosphere are caused by the efflux of HCO_3^- from parent roots, S-type later roots, and root hairs.

2). The change in P concentration in soil around a crown root or L-type lateral root depends on diffusion, sorption, solubilization, and the uptake rate of P in solution. The uptake at the root surfaces is realized by Michaelis–Menten kinetics with a markedly steep slope for low concentrations of P, and there is no net diffusion of P across the outer boundary (mirroring of neighboring roots).

Parameterization

Plant growth and P uptake data were obtained from greenhouse experiments. We measured root surface area, root length, root volume, shoot biomass, total biomass, plant nutrient concentration, including anions (NO_3^-, $H_2PO_4^-$, SO_4^{2-}) and cations (NH_4^+, K^+, Ca^{2+}, Mg^{2+}), equations and calculations were as in Kuppe *et al.* (2022). In our study, four scenarios were focused and simulated: genotypes DJ123 and Nerica4 under high and low P conditions. For the genotypes we consider different uptake of nutrients (anions and cations) that influence rhizosphere pH, and differences in root morphological traits (Table 2).

Simulations output

We simulated P uptake from the rhizospheres of unit length roots and whole plant uptake over a growth period of 45 days, using the default parameters in Table 1. Because the efflux per root surface area was assumed constant over time, the total HCO_3^- released to the rhizosphere changed as we varied the root morphological traits (see below). Since the anion and cation demand by the plant is strongly associated with the shoot, we fixed the total HCO_3^- release over time, hence, the efflux per unit root surface area, E, varied with parameters that changed the root surface area. The range in parameter values was such that the pH did not change more than one unit locally. The distance between neighboring roots decreases as the RLD increases. In the model, this also decreases the distances among S-type laterals in DJ123 and Nerica4 under different P levels.

In accordance with the root morphology data obtained from our greenhouse experiment and the previously published data by Matthias Wissuwa (Nestler & Wissuwa, 2016; Wissuwa *et al.*, 2020), we conducted a variation in the length of crown roots and L-type lateral roots while maintaining the length of S-type roots per unit length of parent root and the number of root hairs per unit root surface area as constant variables. Therefore, alterations in the length of crown roots and L-types resulted in changes to the overall S-type length, but not to the S-type branching density. Following Kuppe's pH and P model, the impact of varying the length of S-types per unit length of parent root was tested with two constraints: the root system length and the root surface area. For the sake of simplicity, we fixed the length ratio between L-type and crown roots. In the rhizosphere reaction term of the model, the surface area of S-types and their hairs varies as LSR varies. The total initial P concentration in soil was held constant across all simulations, but we varied the proportion of $P_{s,slow,init}$ and $P_{s,fast\text{-}sol,init}$, the sorption rates, and pH dependencies of sorption.

Calculation of efflux

We first calculated the agar gel OH^- change by comparing the change in agar gel after 15h with rice roots to that of the control.

$$[OH^-]_{change\ speed}=(10^{pH-14}-10^{pHintial})\times V/\ (15\times60\times60)\ s$$

To estimate soil pH change caused by cations and anions balance in the soil, the various ions are concerned in the solutions including:

H_3O^+, OH^-, HCO_3^- and ionic species anions (NO_3^-, $H_2PO_4^-$, SO_4^{2-}) and cations (NH_4^+, K^+, Ca^{2+}, Mg^{2+})), as soil pH is around 5, phosphates in the soil solution are constituted mainly $H_2PO_4^-$, rather than HPO_4^{2-} (Barber, 1995), in our case other nutrient ions like Na^+, Zn^{2+}, organic ions such as organic or amino acids were not taken into account. E value was calculated with

$$E=\frac{\left(\sum_{T=0}^{T=end}\ (cations-anions)\right)mol/g\times(DW_{plant})g}{\left(\sum_{T=0}^{T=end}\ (RSA+RHSA)\right)\times dT_{time}}$$

Data analysis

Statistical analyses were performed using SPSS 22.0 (SPSS). Normality and homogeneity of variances were tested for all the data with Shapiro-Wilk tests. Data were log-transformed if they didn't pass the test. Two-way ANOVA was used to assess the effects of genotypes, P levels, and their interactions. Independent samples T-test was used to evaluate the effects of P levels (low -P conditions (1 µM or no P treatment) vs high-P conditions (100 µM or 25.0 kg ha^{-1} P addition)), one-way ANOVA was used to evaluate the effects of genotypes on gel pH, root traits, biomass and optode pH, shoot dry mass. The significance level was set at $P \leq 0.05$. Model simulation was carried out with Kuppe's pH-P model- Solving the advection-diffusion-reaction equation developed by Johannes Postma in R (Codes are available upon request).

Results

Higher HCO_3^- efflux rate in DJ123 than Nerica4 under P stress

Cations and anions balance in two genotypes & two P levels were measured based on the greenhouse data. HCO_3^- efflux rate was significantly affected by P availability, genotype, and their interactions. Calculated HCO_3^- efflux rate was significantly greater in P efficient genotype DJ123 than P sensitive genotype Nerica4 under P stress, implies a higher potential to change the rhizosphere pH induced by rhizosphere cations and anions balance. However, there was no significant difference between DJ123 and Nerica4 in HCO_3^- efflux under high P (Fig. 2).

Greater rhizosphere pH in DJ123 than Nerica4 under P stress

Under P deficiency condition, root induced rhizosphere pH of P efficient genotype DJ123 was significantly greater than sensitive genotype Nerica4 in both greenhouse and field experiment (Fig. 3 and 4). Under low P, gel-essay pH of DJ123 was significantly greater than that of Nerica4 in the greenhouse, but not in the high P treatment. The rhizosphere pH of DJ123 measured in the field showed a very similar pattern, although the significance was less due to greater variation among replicates. Under low P, the rhizosphere pH of DJ123 was 0.6 units above that of Nerica4. In both greenhouse and field experiment, there was no significant difference in rhizosphere pH change between the two phenotypes under high P condition (Figs. 3 and 4).

Root morphology differences in the greenhouse experiment

Under P deficiency, P efficient genotype DJ123 has a much larger root than the sensitive genotype Nerica4, exhibiting more root tips by 32.30%, larger root surface area and root volume by 18.13%, 18.7% (Fig. 5). However, there was no significant difference between DJ123 and Nerica4 in root diameters. There was also no significant difference in root morphology traits change between the two phenotypes under high P condition (Fig. 5).

Shoot Biomass and P acquisition in the greenhouse and field experiment

P stress reduced shoot biomass and plant P acquisition dramatically in both rhizobox and field, and the reductions in Nerica4 were greater than DJ123 (shoot biomass, 19%

in the greenhouse, 37% in the field; P acquisition, 20% in the greenhouse, 62% in the field) (Fig. 6). Note that the differences of shoot biomass and plant P acquisition in two genotypes were bigger in the field than in the greenhouse. No significant difference was found between DJ123 and Nerica4 in shoot biomass and plant P acquisition under high P (Fig. 6).

Simulated rhizosphere pH and accumulative uptake with pH-P model

Combine with the cations, anion data, root morphology data and biomass data got from both greenhouse and field experiment, a revised pH-P model was used to simulate the rhizosphere pH change and its cumulative uptake in both DJ123 and Nerica4 under contrast P levels. Similar with the greenhouse and field data, although no significant difference was found in rhizosphere pH and cumulative uptake of two genotypes under high P condition, they were significantly higher in DJ123 than in Nerica4 under P stress (Fig. 2). Simulated cumulative uptake in DJ123 was 46% higher than Nerica4 (Fig. 7).

Discussion

Main conclusion

Our results support the hypothesis that the P-efficient genotype DJ123 achieves greater P-uptake per unit root length by increasing the rhizosphere pH more than the P-inefficient genotype Nerica4. The higher rhizosphere pH under P stress (Figs. 3 and 4) in genotype DJ 123 was associated with a higher (estimated) anion (HCO_3^-) efflux rate (mol/length/time). This likely led to greater uptake per unit root length (mol/length/time) and, over time to a greater root length and volume, further increasing the total uptake and biomass production. (Figs. 2, 3, 4, 5 and 6).

Comparable results in greenhouse and field about pH

Soil pH is regarded as the “master variable” of soil chemistry, given its profound impact on countless chemical reactions involving essential plant nutrients, which is especially true for phosphorus nutrients. It has been well documented that rhizosphere acidification and organic anions exudation induced by different plant root can mobilize soil inorganic P in alkaline soil, thereby an efficient phosphorus uptake (Hinsinger, 2001; Li *et al.*, 2007; Rose *et al.*, 2010; Sun *et al.*, 2019). Meantime, increased pH induced by root or liming practice in acid soil can increase phosphate availability by solubilizing iron phosphate and aluminum phosphate (Haynes, 1982; Rose *et al.*, 2010). Our results support the conclusion that higher rhizosphere pH is an effective trait of upland rice for P acquisition under P stress. We obtained comparable results from P stress treatments in two distinct environments, greenhouse rhizotrons and the field. In the greenhouse, we used rhizotrons to create P stress conditions in a simplified and controlled environment, yet minimize the root lose and injury for detailed analyses and allow the further measurement of rhizosphere pH change in gel plate. The field experiment includes many environmental factors include soil humidity and physical properties, rainfall, soil biota (like AM fungal and phosphate solubilizing bacteria) can independently or interactively influence root growth and rhizosphere process, thereby, phosphorus acquisition and final yield. We employed the gel plate with pH indicator and planar optode to explore rhizosphere pH properties under P stress. Under different P levels and genotypes, the whole plant rhizosphere pH change can be reflected by gel pH change, while the certain area of pH change can be shown by the optode pH data. Comparable results from both controlled and natural environment indicate that higher P mobilization for greater P acquisition in DJ123 than Nerica4 is independent of potentially confounding factors of any given environment context.

Model simulation and output, compare with greenhouse and field experiments

Rhizosphere pH is the consequence of numerous, complex processes and is a key factor of the soil-root interface. Previous studies demonstrated that the extent to which the rhizosphere pH can differ from that of the bulk soil depends on various abiotic and biotic factors including soil humidity, soil buffering capacity, initial soil pH, root length density and plant species (Marschner *et al.*, 1986; Youssef & Chino, 1988, 1989; Kirk *et al.*, 1999b; Custos *et al.*, 2020). To better explore the rhizosphere pH changes caused by genetic variation, we validate the pH-P model with the root morphology, plant cation/anions uptake and soil parameters got from our greenhouse experiment. The developed pH-P models can be used to simulate P acquisition and rhizosphere pH change with different genotypes and soil P levels. Similar with the greenhouse and field data, the higher rhizosphere pH by 0.2 units, greater accumulative uptake by 46% in DJ123 than Nerica4 under P stress was found with the model simulation, while there are no significant differences of rhizosphere pH and P between DJ123 and Nerica4 under high P conditions. The well pH maintenance of DJ123 can explain the efficient P uptake under P stress. Furthermore, the developed pH and P model can be used to find out the determining factors which trigger the pH increase. In our study, the greater rhizosphere alkalization of DJ123 than Nerica4 can be ascribed to higher E value and root length density.

E value is associated with N uptake

H^+ or OH^- (or HCO_3^-) proton efflux rate (E) is known as one main pathway to cause an acidification or alkalinization of the root vicinity (Nye, 1981; Jaillard *et al.*, 2002; Hinsinger *et al.*, 2003; Tang & Rengel, 2003; Custos *et al.*, 2020). It was reported that the pH changes across the rhizosphere were ascribed to buffer reaction caused by imbalance between soluble cations and anions in the rhizosphere because the soil pH was controlled mainly by the presence of soluble or exchangeable cations and anions. We validate the E with the anions (NO_3^-, $H_2PO_4^-$, SO_4^{2-}) & cations (NH_4^+, K^+, Ca^{2+}, Mg^{2+}) got from greenhouse experiment. Higher E was found in DJ123 than Nerica4 under P stress but not under P fertilization conditions, which is mainly attributed to the higher cations' accumulation like N of DJ123 under P stress in our study (Data not shown). Compared with other mineral and trace elements, DJ123 has much higher concentration (per gram) and total content than Nerica4 under P stress. As NO_3^- is the only N resource for the upland rice, more NO_3^- uptake is accomplished with more H^+ via charge equilibrium within plant. Furthermore, higher N concentrations represent a higher total anion and thereby a higher potential for the rhizosphere soil pH increase. When there is enough P for upland rice growth, DJ123 and Nerica4 tend to have similar N concentration, leading to a similar rhizosphere pH change.

Except for the plant ionome differences which contributed to rhizosphere pH increase, the root morphology is also vital as it usually interacts with soil solubilization to determine HCO_3^- accumulation. pH-P model developed by Kuppe et al (2022) predicted that the P uptake of DJ123 can be facilitated by longer root hairs and greater root length density. When the efflux is given, more dense and compact root systems will induce greater pH changes. In agreement with these results, our study shown that efficient P uptake of DJ123 than Nerica4 is benefited from its higher root length density (RLD) under P stress conditions, this is because the greater the root length density is, the smaller the neighboring root distance is in DJ123, the HCO_3^- can accumulate more in a relative smaller soil volume, thereby greater P solubilization and uptake. Further results also showed that DJ123 has more S-type laterals and estimated root hair length. As the zone of pH changes and P solubilization around a root is bigger than the zone of P depletion, the short but hairy S-type laterals can spread into the P solubilization zone but outside of P depletion zone to maximize P uptake. Thus, DJ123 can take more P than Nerica4 under P stress through root morphology response.

Small rhizosphere effects cause big effects

Although each variable between DJ123 and Nerica4 is small cannot account for the whole P uptake, there is continuous feedback at the whole plant level that enhanced growth under P stress has an increasing influence over time. Under P stress condition, greater P uptake by DJ123 than Nerica4 can increase its growth and also other nutrients uptake, leading to increased HCO_3^- efflux, P solubilization and finally P uptake. The positive feedback of DJ123 would benefit from greater internal P uptake as well as optimal investment in different root classes.

Rhizosphere pH can be a target for rice breeding

To improve P availability in strongly sorbing acid soil, scientists have tried different ways from farmland land management and breeding sides. Numerous studies have shown that lime applications improved phosphorus availability in P deficiency or unavailability Oxisols, which is mainly contributed by pH increase (Smyth & Sanchez, 1980; Fageria & Baligar, 2008; Muindi *et al.*, 2023). Compared with the lime addition to the field, root induced rhizosphere alkalization can be more economic and environmentally sustainable. However, this trait was not reported by previous studies in DJ123 and Nerica4 in response to P deficiency studies, possibly because of low precision of traditional soil pH measurement by water or $CaCl_2$ extraction method, which minor pH change is often diluted and hard to detect. In-situ rhizosphere pH studies with gel and optode can improve accuracy and reflect rhizosphere pH variations. Higher rhizosphere pH can be achieved by more nitrate uptake, higher root length density and optimized allocation among root classes. We thus found an increased rhizosphere pH needs to be considered in formulating target traits for selecting P-efficient rice cultivars.

Conclusions

Our results across scale, from greenhouse, gel experiment, and field experiment, all confirmed that increased rhizosphere pH contributes to phosphorus uptake in efficient upland rice genotypes DJ123 than Nerica4 from low-P soil but not fertilized conditions. Further model simulations validated by experimental data proved that this kind of positive pH-P relation is accomplished by intra-root facilitation. DJ123 showed better rhizosphere pH modulation due to greater anion uptake in the form ofNO_3^-, greater root length density, and better investigation among root classes. These traits may work self-stimulating and synergistically as the induced increase in rhizosphere HCO_3^- will P solubilization, which affects growth, and consequently P uptake. We thus conclude that increased pH can partially explain efficient P acquisition in strongly P-sorbing acid soil, which can also be considered a selection target to improve P capture in upland rice and possibly other cereal crops.

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Author contributions

CCZ conducted the experiments, analyzed the results, and led the writing; CWK, MB and MW contributed to the design and writing; JAP conceived and designed the study, supervised its execution, assisted with data analysis, and contributed to the writing.

Conflict of interest statement

Nothing declared.

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Tables

Table 1 List of main symbols (variables and parameters) used in Kuppe pH-P model.

Variable name	Description	Units	Value
tc	conversion factor seconds per day	s	60*60*24
r0	root radius of crown root	cm	0.03
r1	mid distance between roots	cm	0.4772
I_{max}	Maximal P uptake rate	mol cm^{-2} s^{-1}	$8 * 10^{-12}$
K_m	Concentration at which half of the maximum rate is achieved	mol cm^{-3}	$1e^{-9}$
Nh	number of root hairs per cm of root		1110
lh	root hair length (cm)	cm	0.0225
rh	root hair radius	cm	0.0008
rs	radius of s-type root	cm	0.0025
lhs	root hairs length on s-types (cm)	cm	0.016
Nhs	number of root hairs on 1 cm s-type segment		900
omegaS	fraction of s-type root length / (lateral root + crown root length)		1.0
b	buffer coefficient		2000
v	velocity of water (needs to be divided by b for sorption reasons)	cm/s	0./b * tc

theta	soil water content in cm3/cm3		0.3
rho	soil bulk density in g/cm3		1.0
thau	soil tortuisity factor		0.24
Dlp	$H_2PO_4^-$ diffusion coefficient in water	$cm^2\ s^{-1}$	8.9×10^{-6}* tc
De	Effective diffusion in soil	cm^2s^{-1}	Dlp*thau*theta/b
Pli	0.1 uM (Cp/31) initial concentration	mol cm^3	0.1e-9 * mc
pHi	pH by water own measurements, initial soil pH		5.0
K1	apparent dissociation constant of H2CO3	mol cm^3	$4.45e^{-10}$
Ks	solubility of CO2 in water	mol cm^3 atm	$3.39e^{-5}$
pCO2	CO2 pressure in soil air atm		$4e^{-3}$
K		$mol^2\ cm^6$	K1*Ks*pCO_2
pH2Bl			function(pH) K*10^pH/0.001
ka	k1 in code absorbtion /s times theta for units Pl to soil volumes		theta * 0.078565240223 *tc /b
kd	desorption /s times rho for units Ps to soil volumes		ka/(10*b)
kan		cm^3 mol s^{-1}	theta * 1.33660e5*tc /b
kdn		cm^3 mol s^{-1}	rho * 9.311873 *tc /b
alpha	0-1 partitioning of Pnaoh over Ps-sol and Ps-slow		0.33

Pnaoh		Mol g^{-1}	$11.29e^{-6}$ * mc
Psslowi	initial pool for slow sorbing P (steady state with Pli)		ka*Pli / kd
pHmax	max ph that can be reached at which 100% of the solubilizable pool is dissolved		7.0
deltaBl		mol cm^{-3}	pH2Bl(pH_{max}) - pH2Bl(pHi)
lambda	ph coupling coefficient		rho * alpha * Pnaoh / (b * deltaBl)
Bli		mol cm^{-3}	pH2Bl(pHi)
bHs		Mol cm^{-3} per pH unit	$1e^{-5}$
Dh		cm s^{-1}	9.55e-5*tc*theta*thau
Db		cm s^{-1}	$1.23e^{-5}$*tc*theta*thau
E	exudation rate	mol cm^{2} per s	$3.3e^{-13}$ * tc
ln10.bhs	simplifying constants		log(10)/bHs
DhK	simplifying constants		Dh*K
Pfertinit		Mol cm^{-3}	$0.118e^{-6}$ * mc

Table 2 List of main variables and parameters got from greenhouse and publications

Parameter	**Symbols**	**Four scenarios**			
		DJ123 with LP	DJ123 with HP	Nerica4 with LP	Nerica4 with HP
Root radius (cm)	r_0	0.00375	0.00375	0.00375	0.00375
mid distance between roots (cm)	r_1	0.5679	0.4843	0.595	0.508
Number of hairs per unit root length (cm^{-1})	N_h	1110	1110	840	1110
Root hair length (cm)	l_h	0.0225	0.018	0.018	0.0175
Root hair radius (cm)	r_h	0.0008	0.0008	0.0008	0.0008
length hairs on s-types (cm)	l_{hs}	0.016	0.011	0.025	0.009
number of root hairs per cm S-type lateral root	N_{hs}	900	1140	750	1200
fraction of s-type length/(l+c length)	ωs	1.38	1.16	1.22	1.02
Total root length (cm)		3354	3799	2659	3023
Total root surface		518. 5	546.7	394. 9	429.5

area (cm^2)					
HCO_3^- efflux mol/cm2/s	*E*	$3.17E^{-13}$	$3.25\ E^{-13}$	$2.12\ E^{-13}$	$3.55\ E^{-13}$

Table 3 Results of two-way ANOVA on effects of P levels (P), genotypes (G) and their interactions (P×G) on root parameters of rice.

Factors	df	R/S ratio		Root length		Root surface area		Root diameter		Root volume		Root tips		Specific root length	
		F	P	F	P	F	P	F	P	F	P	F	P	F	P
P	1	0.05	0.83 NS	37.00	<0.01**	9.80	<0.01**	37.00	<0.01**	14.28	<0.01**	9.08	<0.01**	45.37	<0.01**
G	2	1.07	0.36 NS	22.65	<0.01**	1.78	0.20 NS	22.65	<0.01**	0.70	0.51 NS	7.20	<0.01**	54.20	<0.01**
P×G	2	0.03	0.98 NS	5.97	<0.01**	2.36	0.12 NS	5.97	<0.01**	2.92	0.08 NS	1.67	0.22 NS	1.37	0.28 NS

NS no significant difference ($P > 0.05$); $^{*}P \leq 0.05$; ** $P \leq 0.0$

Figure Captions:

Fig. 1 Schematic representation of how soil solid phase absorbed nutrients uptake by root and relative pH changes. Take anion $H_2PO_4^-$ and cation K^+ uptake as an example. The image was created with BioRender.com.

Fig. 2 Effect of different P levels and genotypes on the root exudation rate in the greenhouse. Different capital letters indicate a significant difference in the genotype in the same p level, and different lowercase letters indicate a significant difference in the P levels in the same genotype. Bars show means ±SE (n=4).

Fig. 3 Effect of different P levels and genotypes on the gel pH after 15 hours. Different capital letters indicate a significant difference in the genotype in the same P level, and different lowercase letters indicate a significant difference in the P levels in the same genotype. Bars show means ±SE (n=4).

Fig. 4 Effect of different P levels and genotypes on the optode pH. Different capital letters indicate a significant difference in the genotype in the same P level, and different lowercase letters indicate a significant difference in the P levels in the same genotype. Bars show means ±SE (n=4).

Fig. 5 Effect of different P levels and genotypes on the rice root parameters in the greenhouse. Different capital letters indicate a significant difference in the genotype in the same p level, and different lowercase letters indicate a significant difference in the P levels in the same genotype. Bars show means ±SE (n=4).

Fig. 6 Effect of different P levels and genotypes on the rice biomass and P acquisition in the greenhouse and the field. Different capital letters indicate a significant difference in the genotype in the same p level, and different lowercase letters indicate a significant difference in the P levels in the same genotype. Bars show means ±SE (n=4).

Fig. 7 The cumulative P uptake and increased $[OH^-]$ of the rice genotypes DJ123 and Nerica4 at two P levels from rhizosphere simulations after 45 days.

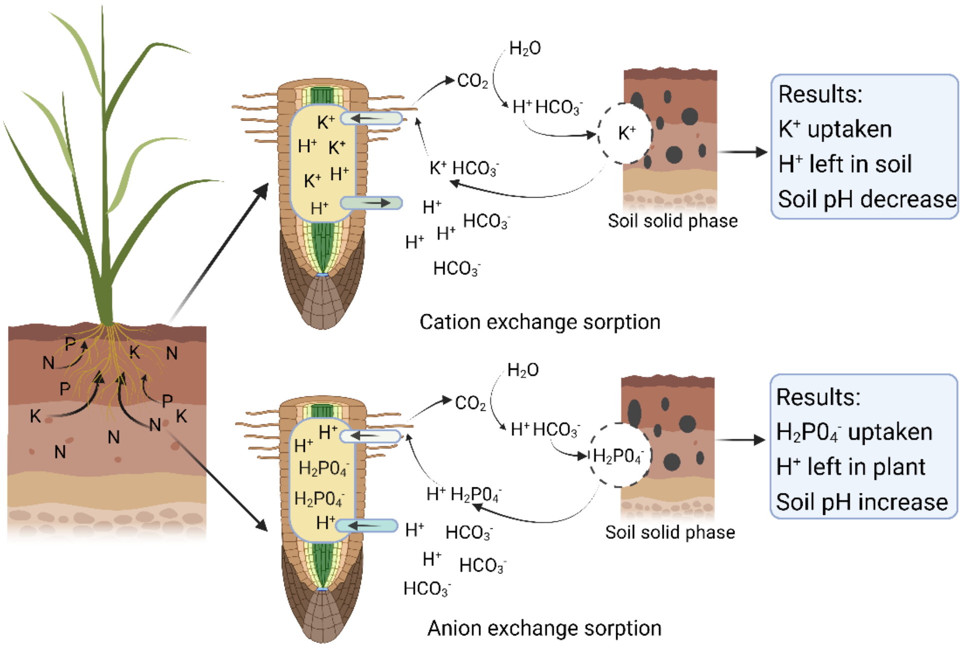
P
N
K
N
P
P
K
N
K
N
N
K+
H+ K+
K+ H+
H+
H2O
CO2
H+ HCO3-
K+
K+ HCO3-
H+
H+ HCO3-
H+
HCO3-
Soil solid phase
Results:
K+ uptaken
H+ left in soil
Soil pH decrease
Cation exchange sorption
H+
H+
H2P04-
H2P04-
H+
H2O
CO2
H+ HCO3-
H2P04-
H+ H2P04-
H+ HCO3-
H+ HCO3-
HCO3-
Soil solid phase
Results:
H2P04- uptaken
H+ left in plant
Soil pH increase
Anion exchange sorption

Fig. 1

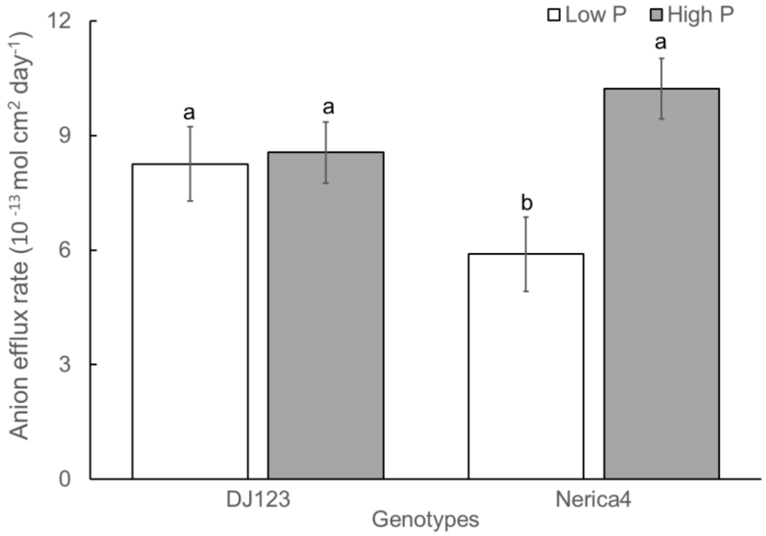
Low P
High P
Anion efflux rate (10^{-13} mol cm^2 day^{-1})
12
9
6
3
0
a
a
b
a
DJ123
Nerica4
Genotypes

Fig. 2

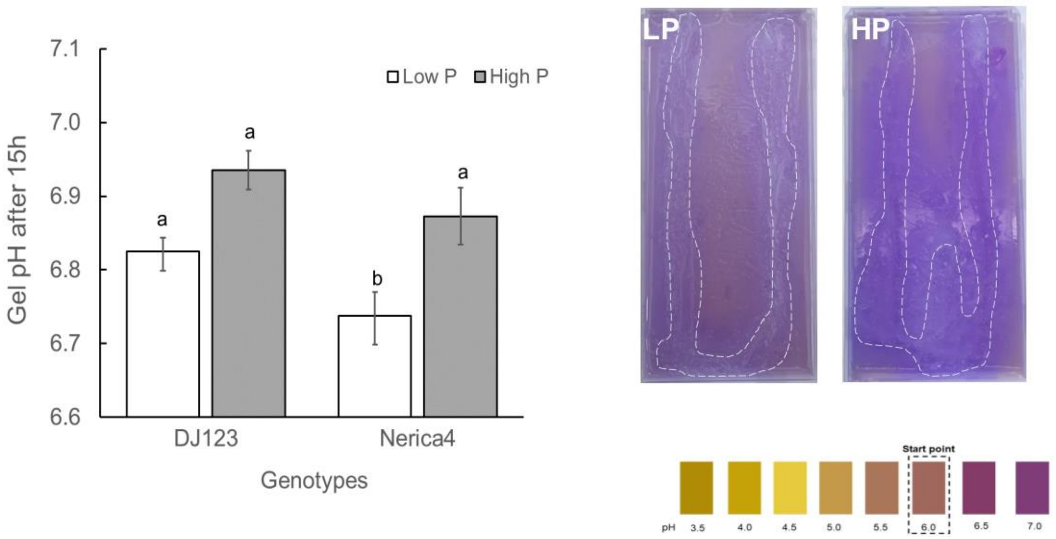
Gel pH after 15h
7.1
7.0
6.9
6.8
6.7
6.6
☐ Low P
▨ High P
a
a
b
a
DJ123
Nerica4
Genotypes
LP
HP
Start point
pH
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0

Fig. 3

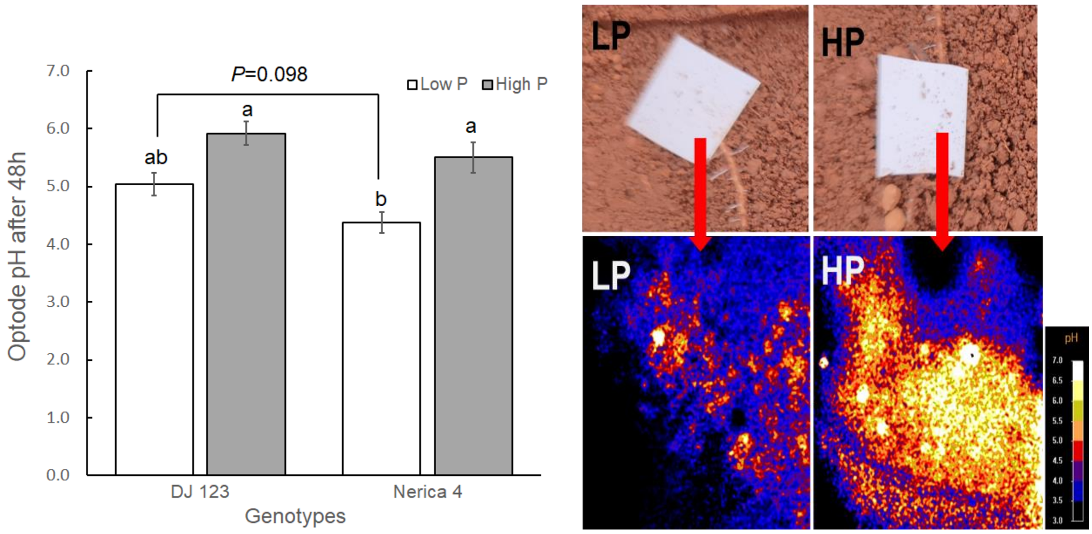
P=0.098
Low P
High P
Optode pH after 48h
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
ab
a
b
a
DJ 123
Nerica 4
Genotypes
LP
HP
LP
HP
pH
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0

Fig. 4

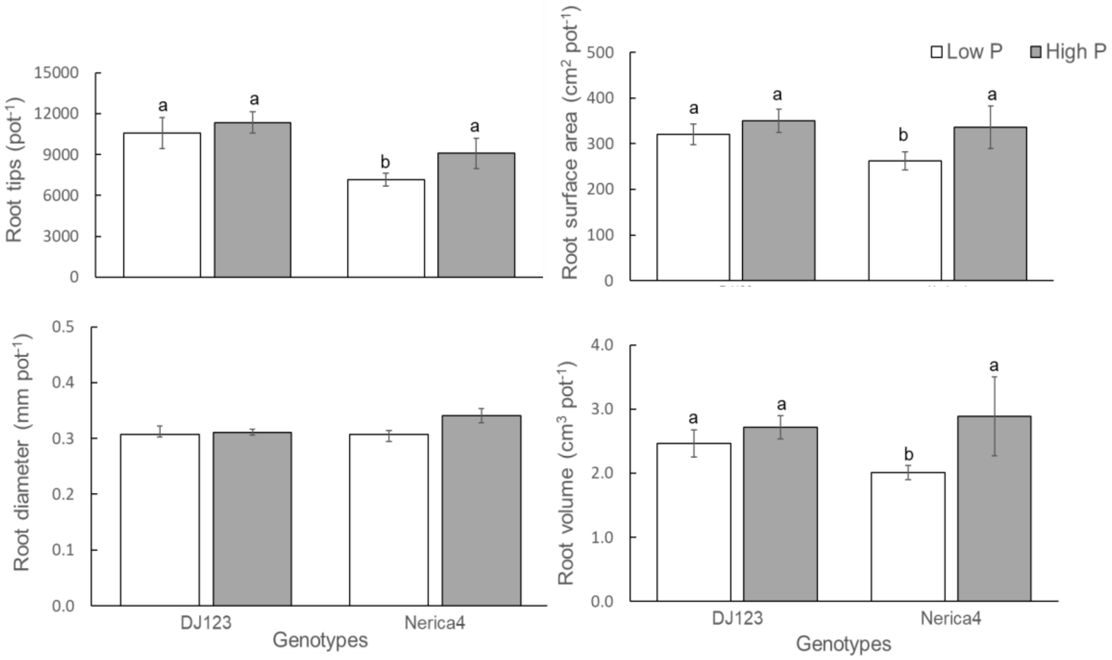
Root tips (pot⁻¹)
15000
12000
9000
6000
3000
0
a
a
b
a
Root surface area (cm² pot⁻¹)
500
400
300
200
100
0
□Low P
▨High P
a
a
b
a
Root diameter (mm pot⁻¹)
0.5
0.4
0.3
0.2
0.1
0.0
DJ123
Nerica4
Genotypes
Root volume (cm³ pot⁻¹)
4.0
3.0
2.0
1.0
0.0
a
a
b
a
DJ123
Nerica4
Genotypes

Fig. 5

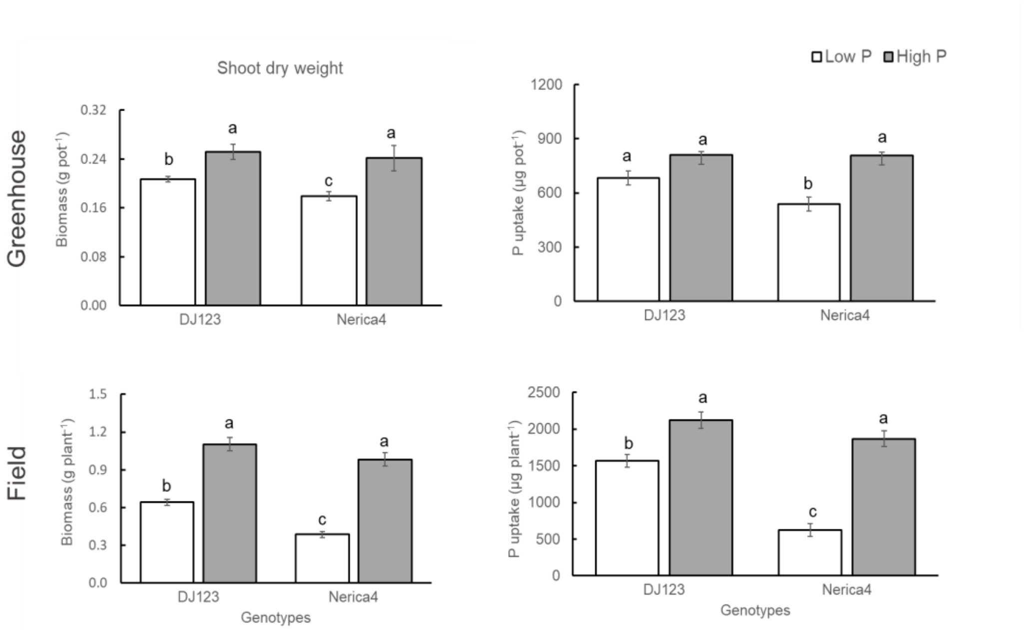
Shoot dry weight
☐Low P ▨High P
Greenhouse
Biomass (g pot^{-1})
0.00
0.08
0.16
0.24
0.32
b
a
c
a
DJ123
Nerica4
P uptake (µg pot^{-1})
0
300
600
900
1200
a
a
b
a
DJ123
Nerica4
Field
Biomass (g plant^{-1})
0.0
0.3
0.6
0.9
1.2
1.5
b
a
c
a
DJ123
Nerica4
Genotypes
P uptake (µg plant^{-1})
0
500
1000
1500
2000
2500
b
a
c
a
DJ123
Nerica4
Genotypes

Fig. 6

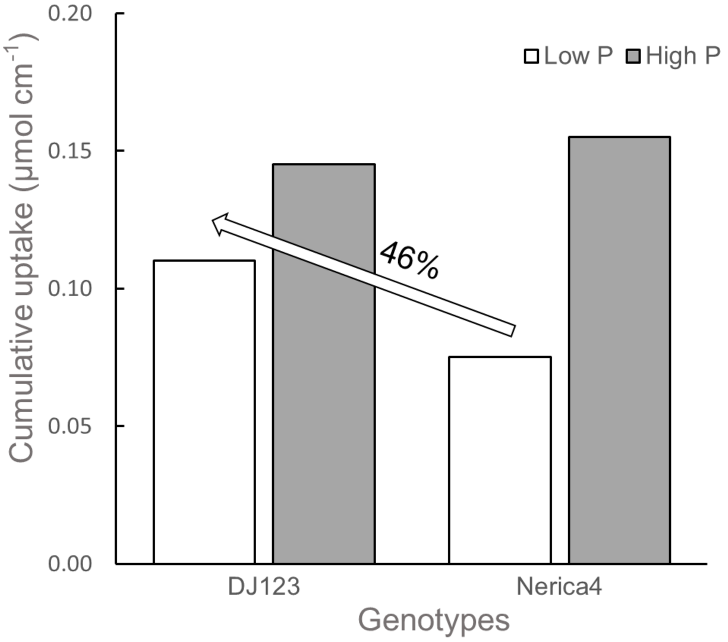
Cumulative uptake (μmol cm-1)
0.20
0.15
0.10
0.05
0.00
□ Low P
▨ High P
46%
DJ123
Nerica4
Genotypes

Fig. 7

Support Material

Fig. S1 Cumulative P uptake by the surface, hairs, and S-types of crown roots and L-type laterals of unit length under different scenarios.

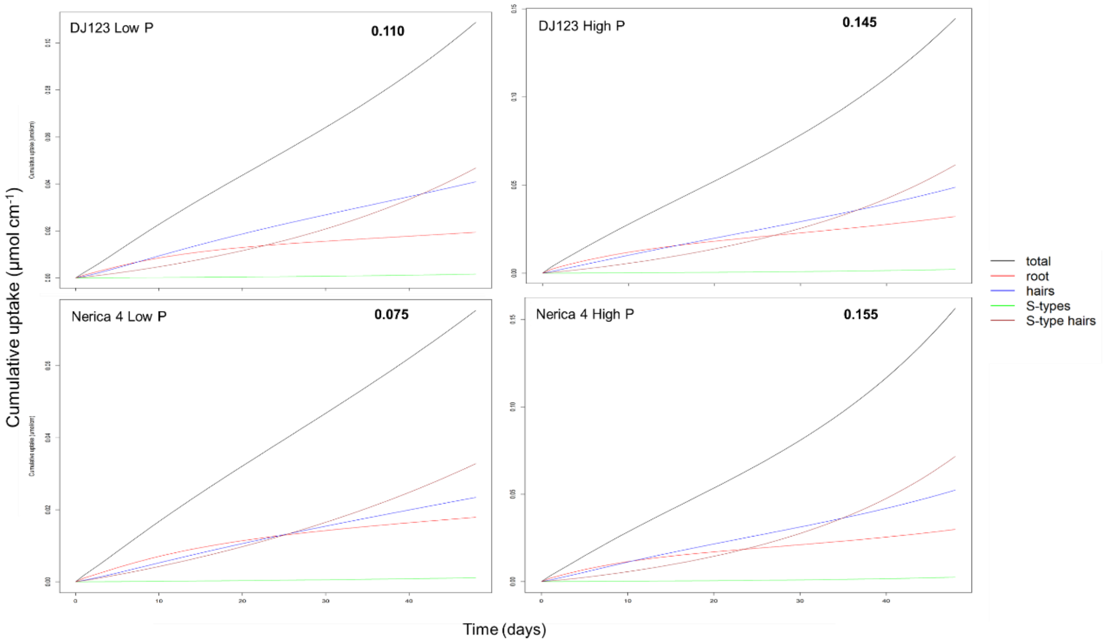

Fig. S1

Chapter 6: Concluding remarks and perspectives

General discussion

This thesis studies root plasticity under drought and post-drought recovery when phosphorus availability is low. I tested whether and what root plasticity traits are associated with the tolerance or resilience to drought recovery, phosphorus deficit, and their interaction in upland rice. For this purpose, in **Chapter 2**, I first addressed this knowledge gap through a comprehensive bibliometric analysis with the aim of achieving a **comprehensive understanding of root plasticity during drought and recovery**. The results showed that root dynamics and root plasticity traits during drought recovery period have received less attention compared with the aboveground traits. We hypothesize that the rate of the recovery depends on 1) soil nutrient level and soil structure; 2) drought intensity and frequency; 3) species: tree, grass, and crop; 4) growth stage e.g. early vs late season; 5) root physiology traits such as ABA, water-soluble carbohydrates, nutrient homeostasis; 6) plasticity of root morphology and anatomy traits; 7) root-microbiome interaction, rhizosphere stability (Zheng *et al.*, 2023). We call for future research that should focus on the above aspects. Given the concurrence of drought and phosphorus deficiency in numerous regions, including sub-Saharan Africa, I focused on answering in **Chapter 3 whether and how P uptake is associated with its recovery/ regrowth rate, especially under low P condition**. The findings revealed that the performance of upland rice at the end of a drought period is not consistent with its performance after a period of recovery. Furthermore, the relative growth rate during the post-recovery period after drought is intricately linked to the ability of upland rice to maintain its phosphorus (P) homeostasis during the drought period. The P-efficient genotype DJ123 can preserve a higher relative concentration of P and P acquire efficiency (PAE) in low P conditions, which in turn allows for a higher relative growth rate and biomass accumulation during the post-recovery period. The superior P homeostasis of DJ123 in low P and drought conditions serves to compensate biomass loss. In contrast, the P-sensitive genotype Nerica4 lacks this capacity when not fertilized. This emphasizes the significance of P homeostasis for drought recovery in upland rice. The uptake of phosphorus and water are closely interlinked processes, rather than independent events. This offers insights for rapid recovery and sustainable rice production in both drought and low P conditions. It is therefore proposed that the maintenance of phosphorus homeostasis during periods of drought may represent a potential means of enhancing drought resilience. This should be integrated into the breeding process for the development of drought-resistant upland rice cultivars. The findings provide an opportunity for developing genotypes that are both drought and low-P tolerant, in contrast to the idea that these stresses require radically different ideotypes, representing trade-offs.

As I showed in **Chapter 3**, the post-drought recovery rate can be influenced by P level. I expected that the root anatomy would respond plastic to the drought and P stresses as well as the rewatering. During the literature review in Chapter 2, I realized that there was no experiment data available. This motivated me to focus on answering in **Chapter 4 whether and what anatomical characteristics of the upland rice roots are associated with a better drought resilience**. The results showed that a general decrease in most anatomical trait values is observed in response to drought and P deficiency, except for the formation of root cortical aerenchyma (RCA). It is established that RCA increases in response to a number of stress factors as part of a general ethylene stress response (Gao *et al.* 2017; Schneider *et al.* 2018). The response was more pronounced in the nodal roots than in the seminal roots. The variation in biomass was more pronounced in response to the P treatment than the drought treatment. P tolerance did not result in enhanced drought tolerance during the drought period. The reduction in the cortical cell file number (CCFN) and the expansion of the root cortical aerenchyma (RCA) persisted in the absence of P, indicating a resistance related to P deficiency. However, DJ123 recovered faster, especially in the presence of P. It was concluded that each of the anatomical traits reacted to drought, but only the cortical cell number (CCFN) and the xylem number responded to P fertilization. The RDA analysis showed that higher total biomass and total P acquisition was associated with the root anatomical response during drought. Given the importance of efficient P uptake by DJ123 compared to Nerica4, further experiments were set to explore the traits that support efficient uptake under both well-watered and drought conditions. Drawing upon the prior theoretical work by Kuppe *et al.* (2022), I asked whether and how rhizosphere pH change contribute to P uptake from P-deficient soils. This is why in **Chapter 5, across-scale experiment from greenhouse to field, and model simulations were**

carried out to test whether a higher rhizosphere pH caused by greater anion/cation uptake-imbalance and thus improves P availability from phosphorus-fixing soils. I found that DJ123 exhibited enhanced phosphorus (P) uptake, both in total and per root length (uptake efficiency), compared to Nerica4 under low P conditions in the greenhouse and field experiments. The rhizosphere pH of DJ123 was found to be higher than that of Nerica4 in the field, as determined by planar optodes, and in the greenhouse, using gel plates with pH indicator. The rhizosphere pH was found to be associated with an excess uptake of anions over cations, as well as a higher root length density in DJ123, which can explain the increased availability and uptake of phosphorus. Simulation using Kuppe's rhizosphere model can reason the higher rhizosphere pH and P uptake of DJ123 compared to Nerica4. I conclude that these results support the hypothesis that increased rhizosphere pH contributes to P uptake in upland rice varieties grown in soils with low P availability. In strongly sorbing acidic soils, increased pH improves P acquisition. Therefore, the ability to increase soil pH can also be considered a selection target to improve P capture in upland rice and possibly other cereal crops. I propose that increase pH works also under soil drying conditions and does not represent the often-mentioned trade-off between shallow and deep rooting phenotypes for P and water acquisition. These results will provide new insights into the mechanisms of plants under the dual stress of water and phosphorus deficits.

Has post-drought recovery received enough attention in drought resilience evaluations?

Increasingly drought events are predicted to occur, both in terms of drought intensity and frequency. Recent studies have demonstrated the potential importance of post-drought recovery, both at the species level and at the ecosystem level (Chen *et al.*, 2016; Schwalm *et al.*, 2017; Gessler *et al.*, 2020; Ouyang *et al.*, 2021; Jiao *et al.*, 2021; Yao *et al.*, 2023; Sachsenmaier *et al.*, 2024). However, recovery related studies are still comparably less than pure drought studies. In agricultural ecosystems, short- and long-term droughts happen frequently but are usually not permanent and the plants can continue to grow or even grow faster during the later recovery or rewatering period. A deeper understanding of the mechanisms of post-drought recovery offers the potential to increase plant climate resilience and production. We systematically summarized the progress of research on post-drought recovery and presented the history and future research hotspots of drought recovery research from a belowground perspective. Restoring root functioning, not in the least soil nutrient uptake, through restoration of root growth, root morphology, and rhizosphere functioning may be the key to fast whole plant recovery after a drought. We thus call for more research to focus on belowground and post drought recovery processes (Zheng *et al.*, 2023).

Phosphorus efficient acquisition for rice drought resilience

Early studies on crops, including common bean and maize, have demonstrated that a balanced uptake of water and phosphorus through the integration of shallow and deep root distribution can optimize plant performance under conditions of water deficit and phosphorus deficiency (Ho *et al.*, 2005; Miguel *et al.*, 2013; Lynch, 2019). A 2.5-year long-term experiment on the tree species downy oak has proved that root non-structural carbohydrate and nutrient homeostasis are the determining factors on its survival and drought recovery. Our results have shown that phosphorus status of the plant at the end of the drought period is a good predictor for the rate of recovery afterwards. Upland rice with higher P concentrations, either due to fertilization or due to genetic variation in P acquisition efficiency, had a higher relative growth rate and biomass accumulation during recovery. The different drought recovery rate in different genotypes could be attributable to 1) Less P is stored in the rice plant, especially under low P level; 2) Rewatering leads to rapid recovery of water status but not of phosphorus homeostasis; 3) Rice growth is more sensitive to phosphorus than water, and drought-induced phosphorus deficiency has a greater impact on regrowth; 4) DJ123 tends to have a higher growth rate than Nerica4 at the early stages of development, this may reflect the importance of early vegetative growth as well as early P acquisition in moderate drought environments.

Given that P uptake efficiency is pivotable to drought recovery, we further ask what root traits caused more efficient P uptake in DJ123 than Nerica4. Considerable research has shown that DJ123 had rapid crown root development, higher proportions of fine lateral roots, longer root hairs and a higher P uptake efficiency (Mori *et al.*, 2016; Nestler & Wissuwa, 2016; Kant *et al.*, 2018; Wissuwa *et al.*, 2020; Mundschenk *et al.*, 2024), we demonstrated increased rhizosphere pH by intra-root facilitation contributes to P uptake from P-fixing oxisols. Furthermore, the response of root anatomical traits, including cortical cell number and xylem number, explained higher biomass accumulation and P acquisition under low P and drought recovery conditions. These results highlight the need for strategic breeding programs and trait selection adapted to specific target environments.

Implications and perspectives

Past publications proved that root dimorphism is important in co-optimizing the acquisition of multiple soil resources (Ho *et al.*, 2005; Lynch, 2019; Burridge *et al.*, 2020; Nasr Esfahani & Sonnewald, 2024). For example, increased basal root whorl number in common bean (Miguel *et al.*, 2013; Rangarajan *et al.*, 2018) and nodal root whorls in maize (Lynch, 2019; Rangarajan *et al.*, 2022) have been reported as dimorphic root phenotypes, these root phenotypes facilitate topsoil exploration for P acquisition through shallower root growth angles in the upper whorls, while the lower whorls develop steeper root growth angles, enhancing deep soil exploration for water acquisition. However, recent studies and this dissertation demonstrates root trait trade-offs are not always true for optimizing phosphorus and water acquisition, at least in rice plant (De Bauw *et al.*, 2018, 2020; Verbeeck *et al.*, 2023; Chapter 3 and 4). No dimorphic root in rice genotypes was found to support optimization of phosphorus and water uptake so far, whereas P efficient genotype DJ123 is better at recovery after drought in upland rice. The efficient P uptake and fast drought recovery of DJ123 may benefit from a lager root, a better intra-root facilitation and an earlier vegetative growth (Wissuwa *et al.,* 2020; Kuppe *et al.,* 2022; Chapter 5). Rhizosphere pH and root anatomical plasticity can be targeted in breeding to increase crop yield under specific conditions such as low-input agronomic systems.

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Extra chapter: Root responses to abiotic and biotic stresses in intercropping and clonal grass systems

Root systems play a critical role in plant adaptation and resilience, particularly in response to biotic (living organisms) and abiotic (environmental) stresses. During my doctoral studies, the global COVID-19 pandemic made me stay in the room, I then spent more time on data analysis and paper writing of already finished experiments. This resulted in 5 publications, two of which I shared first-authorship. In addition, I was root-ambassador at the ISRR 2021 which resulted in two additional co-authored publications. Although these 7 publications were written during my PhD time, they are outside the main topic of my PhD, and thus I decided not to include them as chapters of this thesis. This chapter summarizes the findings from several studies that explore how root morphology, nutrient acquisition, and interspecies interactions influence plant performance under multiple stress conditions.

Nitrogen dynamics in maize/alfalfa intercropping

Intercropping systems, where two or more crops are grown together, are increasingly recognized for their potential to enhance nutrient use efficiency and overall crop productivity. In the maize/alfalfa intercropping system, nitrogen (N) dynamics are particularly interesting because maize is a high N-demanding crop whereas alfalfa is a legume, capable of fixing atmospheric nitrogen. We studied root morphology traits and their contribution to N fixation and N transfer, using ^{15}N-urea leaf labeling and three root separation techniques, which influenced the degree of root contact between maize and alfalfa roots. The results showed a greater N fixation and total N content with no root barrier than that with a mesh barrier or plastic sheet separation. No barrier resulted in greater N transfer from alfalfa to maize. Further redundancy analysis highlighted that specific root traits, such as maize's crown root dry weight and alfalfa's lateral root number, were strongly associated with improved N fixation and transfer. These findings emphasize the critical role of root morphology and interspecies interactions in enhancing the efficiency of N use in intercropping systems.

To explore how the maize and alfalfa intercrop responses to different doses of N-fertilization, we conducted two years of pot experiments with 6 different N levels in maize/alfalfa intercropping systems. The results showed that increasing nitrogen levels significantly enhanced maize root length, surface area, and specific root length, leading to higher N uptake and yield. However, these benefits plateaued at higher N rates, in contrast to maize, alfalfa showed optimal growth and N uptake at lower N levels, with severe competition from maize at higher N rates leading to reduced alfalfa biomass. Combined, the results suggest high dose of N fertilizer can be supraoptimal as excessive N did not further improve maize performance and negatively impacted alfalfa production. This differential response underscores the importance of optimizing nitrogen fertilizer management in intercropping systems to balance the nutrient needs of both crops.

Further details can be found in:

ZQ Shao, **CC Zheng**, JA Postma, WL Lu, Q Gao, YZ Gao, JJ Zhang. Nitrogen acquisition, fixation and transfer in alfalfa-maize intercrops are increased through root contact and morphology responses to interspecies competition. Journal of Integrative Agriculture 2020, 19(0): 2–16

ZQ Shao#, **CC Zheng**#, JA Postma, Q Gao, JJ Zhang. More N fertilizer, more maize, and less alfalfa: maize benefits from its higher N uptake per unit root length. Frontiers in Plant Science 2024 15:1338521. (# Co-first author)

Clonal integration in *Leymus chinensis* under dual stress

Leymus chinensis, a clonal grass species, is well-adapted to the challenging conditions of the in Eurasian Steppe, where saline-alkali stress and heavy grazing are prevalent to constrain *Leymus chinensis* production in northeast China. The ability of this species to tolerate these stresses is partly due to its clonal integration mechanism, where connected ramets (individual shoots of the same plant) share resources such as nitrogen and carbohydrates through connected rhizomes. I first conducted a two consecutive years' field experiment in a natural *L. chinensis* community where plants were exposed to heterogeneous saline-alkali concentrations and varying degrees of biomass clipping. I found that saline-alkali and clipping had an interactive effect on the total biomass of *L.*

chinensis. *Leymus chinensis* can grow well under saline-alkali stress via increased ramet biomass but decreased ramet density. I then developed a simplified graphic model of the limiting resource model based on these results. Ramet compensation and clonal integration were identified to be the main mechanisms of herbivory and saline-alkali tolerance. To further investigate how plant nitrogen allocation strategies affect compensatory growth under saline-alkali stress, a field experiment using two saline-alkali levels, and three clipping levels was conducted in conjunction with the belowground ^{15}N-urea labelling method. Further results revealed that moderate clipping under saline-alkali conditions could stimulate over-compensatory growth, where the plant allocates more biomass and nitrogen to shoots, enhancing its ability to recover from stress. This response was less pronounced under severe clipping, which led to a conservative nitrogen allocation strategy, resulting in under-compensatory growth. The findings suggest that *L. chinensis* has different strategies depending on the intensity of environmental stress, balancing between growth and conservation to optimize survival. To further study how *L. chinensis* ramets support each other via clonal integration under varying environmental conditions, including saline-alkali stress and clipping (simulated grazing). A pot experiment was carried out with a ^{15}N isotope soil labeling method to study clonal integration strategy in the connected mother and daughter ramets of *L. chinensis*, combining homogeneous (both connected ramets were treated) and heterogeneous (only daughter ramets were treated) environments. The results demonstrated that clonal integration allows for resource transfer between mother and daughter ramets, particularly in heterogeneous environments where only one ramet is stressed. For instance, when daughter ramets were subjected to saline-alkali stress, mother ramets enhanced nitrogen uptake and growth, compensating for the stressed daughters. This 'division of labor' enabled the plant to maintain overall biomass and survive under adverse conditions. However, when both mother and daughter ramets were equally stressed, the extent of resource transfer diminished, indicating that clonal integration is more effective in heterogeneous than homogeneous stress environments. Further details can be found in:

H Sun$^{\#}$, **CC Zheng**$^{\#}$, TP Chen, JA Postma, YZ Gao. Motherly care: How *Leymus chinensis* ramets support their offspring exposed to saline-alkali and clipping stresses. Science of the Total Environment 2021, doi: 10.1016/j.scitotenv.2021.149675 (# Co-first author)

HM Ma#, **CC Zheng**#, YZ Gao, CC Baskin, H Sun, HJ Yang. Moderate clipping stimulates over-compensatory growth of *Leymus chinensis* under saline-alkali stress through high allocation of biomass and nitrogen to shoots. Plant Growth Regulation 2020, 92: 95–106 (# Co-first author)

CC Zheng, HM Ma, YZ Gao, H Sun, HJ Yang, CC Baskin. The clonal grass *Leymus chinensis* overcomes salt stress by over-compensatory growth of individual ramets. Crop & Pasture Science 2019, 70: 1004–1014

Implications for agricultural practices

The studies summarized here offer valuable insights into the intricate relationships between root systems and environmental factors in both intercropping and clonal systems. For maize/alfalfa intercropping, understanding the root responses to nitrogen levels and interspecies competition can help optimize fertilizer use, potentially reducing the need for high N inputs while maintaining or even enhancing crop yields. In clonal species such as *L. chinensis*, an understanding of the mechanisms underlying clonal integration and compensatory growth can inform the development of effective grazing management strategies and conservation plans, particularly in ecosystems that are simultaneously threatened by salinization and overgrazing.

In conclusion, these findings highlight the significance of root biology in plant adaptation and resilience. By enhancing the understanding of root systems through advanced root phenotyping technology, agricultural practices can be optimized to improve nutrient use efficiency, enhance stress tolerance, and ultimately, achieve more sustainable crop production systems. Future research should continue to investigate the molecular and physiological mechanisms underlying root responses to biotic and abiotic stresses, paving the way for innovative approaches to crop management in a changing climate.

Further details can be found in:

C Carley, G Chen, KK Das, BM Delory*, A Dimitrova, YY Ding, AP George, LA Greeley, QQ Han, PW Hendriks, MC Hernandez-Soriano*, M Li, JL Pin Ng, L Mau, J Mesa-Marín, AJ Miller, AE Rae, J Schmidt, A Thies, CN Topp, TS Wacker, P Wang, X Wang, LM Xie, **CC Zheng***. Root biology never sleeps. New Phytologist 2022, 235:

2149–2154 (*Co-correspondence author)

BM Delory*, MC Hernandez-Soriano, TS Wacker, A Dimitrova, YY Ding, LA Greeley, JL Pin Ng, J Mesa-Marín, LM Xie, **CC Zheng**, LM York*. A snapshot of the root phenotyping landscape in 2021. bioRxiv 2022, doi.org/10.1101/2022.01.28.478001

Publications

Peer-reviewed publications related to this thesis

CC Zheng, H Bochmann, ZG Liu, J Kant, SD Schrey, T Wojciechowski, JA Postma. Plant root plasticity during drought and recovery: What do we know and where to go? **Frontiers in Plant Science** 2023, 14:1084355. doi: 10.3389/fpls.2023.1084355

CC Zheng, C Kuppe, M Becker, M Wissuwa, JA Postma. Rhizosphere pH-change causes efficient P uptake in upland rice: Evidence from contrasting genotypes in rhizobox, field, and silico studies. (Under internal review).

CC Zheng, T Rakotoson, M Wissuwa, JA Postma. Root plasticity and phosphorus homeostasis: key drivers of drought resilience in upland rice. (Under internal review).

CC Zheng, AS Tiavina, T Rakotoson, M Wissuwa, JA Postma. Anatomical root responses of upland rice to concurrent phosphorus and water stress reveal different recovery rates after drought. (Under internal review).

Publications unrelated to this thesis

ZQ Shao[#], **CC Zheng**[#], JA Postma, Q Gao, JJ Zhang. More N fertilizer, more maize, and less alfalfa: maize benefits from its higher N uptake per unit root length. **Frontiers in Plant Science 2024** 15:1338521. doi: 10.3389/fpls.2024.1338521(# Equal Contribution)

C Carley, G Chen, KK Das, BM Delory*, A Dimitrova, YY Ding, AP George, LA Greeley, QQ Han, PW Hendriks, MC Hernandez-Soriano*, M Li, JL Pin Ng, L Mau, J Mesa-Marín, AJ Miller, AE Rae, J Schmidt, A Thies, CN Topp, TS Wacker, P Wang, X Wang, LM Xie, **CC Zheng***. Root biology never sleeps. **New Phytologist** 2022, 235: 2149–2154 (*Co-correspondence author)

BM Delory*, MC Hernandez-Soriano, TS Wacker, A Dimitrova, YY Ding, LA Greeley, JL Pin Ng, J Mesa-Marín, LM Xie, **CC Zheng**, LM York*. A snapshot of the root phenotyping landscape in 2021. **bioRxiv** 2022, doi.org/10.1101/2022.01.28.478001

H Sun[#], **CC Zheng**[#], TP Chen, JA Postma, YZ Gao. Motherly care: How *Leymus chinensis* ramets support their offspring exposed to saline-alkali and clipping stresses. **Science of the Total Environment** 2021, doi: 10.1016/j.scitotenv.2021.149675 (# Equal Contribution)

ZQ Shao, **CC Zheng**, JA Postma, WL Lu, Q Gao, YZ Gao, JJ Zhang. Nitrogen acquisition, fixation and transfer in alfalfa-maize intercrops are increased through root contact and morphology responses to interspecies competition. **Journal of Integrative**

Agriculture 2020, 19(0): 2–16

HM Ma[#], **CC Zheng**[#], YZ Gao, CC Baskin, H Sun, HJ Yang. Moderate clipping stimulates over-compensatory growth of *Leymus chinensis* under saline-alkali stress through high allocation of biomass and nitrogen to shoots. **Plant Growth Regulation** 2020, 92: 95–106 (# Equal Contribution)

CC Zheng, HM Ma, YZ Gao, H Sun, HJ Yang, CC Baskin. The clonal grass *Leymus chinensis* overcomes salt stress by over- compensatory growth of individual ramets. Crop & Pasture Science 2019, 70: 1004–1014

Conference participation

CC Zheng, C Kuppe, JA Postma. Root-induced pH increase in the rhizosphere utilizes phosphorus from deficient soils: Across-scale evidence from greenhouse to field, and model simulations. International Society of Root Research 12th International Symposium in Leipzig, Germany, June 2-7, 2024 (Oral presentation).

CC Zheng, JA Postma. Explaining genetic variation in P uptake by rice. Rooting 2023-10th International Symposium on Root Development in Gent, Belgium, May 15-18, 2023 (Poster presentation).

CC Zheng, JA Postma. Increased rhizosphere pH contributes to phosphorus uptake from low-phosphorus soil in upland rice. Workshop on rhizosphere traits of upland rice, in ZALF, Germany, March 12-14, 2022 (Oral presentation).

CC Zheng. Root contact and plasticity facilitate nitrogen fixation and transfer in intercrops. 11th Symposium of the International Society of Root Research/ROOTING 2021 in Missouri, US, May 24-28, 2021 (Poster presentation).

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