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**Effects of temperature and vapor pressure deficit on genotypic responses to
nitrogen nutrition and weed competition in lowland rice**

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***“Ở đây một hạt cơm rơi
Ngoài kia bao hạt mồ hôi thấm đồng”***

*(Don't waste a grain of rice from your bowl,
as each comes from the hard labor of a farmer)*

-----*Vietnamese folk verses*-----



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List of abbreviations

A	CO ₂ assimilation rate
AA	Amino acid
AWD	Alternate wetting and drying
CF	Continuous flooding
CH ₄	Methane gas
DM	Dry matter
GS	Glutamine synthetase
g_s	Stomatal conductance
IRRI	The International Rice Research Institute
Mha	Million hectares
Mt	Million tons
N	Nitrogen
NH ₄ ⁺	Ammonium
NO ₃ ⁻	Nitrate
NR	Nitrate reductase
R/S	Root to shoot ratio
RZT	Root zone temperature
SLA	Specific leaf area
SRI	The system of rice intensification
VPD	Vapor pressure deficit
WUE _i	Intrinsic water-use efficiency

SUMMARY

Since rice is the major food for more than half of the world's population, rice production and productivity have significant implications for food security. In adaptation to increasing water scarcity, as well as to reduce greenhouse gas emissions, water-saving irrigation measures (e.g., alternate wetting and drying – AWD) have been introduced in many rice growing regions. Previous studies have shown that AWD increases water use efficiency and reduces methane (CH₄) emissions, while grain yield remains equal or is slightly increased compared to continuous flooding. However, the absence of a ponded water layer in formerly flooded rice fields creates new challenges, such as altered root zone temperature (RZT), enhanced nitrification leading to higher nitrate (NO₃⁻) concentrations in the soil, or stimulated weed germination leading to changes in weed flora. All these factors may affect nutrient uptake and assimilation of rice plants and thus plant growth. Further, vapor pressure deficit (VPD) drives transpiration and water flux through plants, so nutrient uptake and assimilation by plants may be subject to adjustment under varying VPD conditions. As VPD varies largely between rice growing regions and seasons, and is also predicted to continuously increase under global warming, it was included as a factor in this study. The overall objective of the study was to evaluate the response of different rice varieties to arising challenges under water-saving irrigation. Experiments were conducted in the greenhouse and VPD chambers at the University of Hohenheim, where plants were grown in hydroponics. Both during day and night, nutrient uptake rates of rice increased linearly with RZT in the observed temperature range up to 29°C, implying that the optimum temperature for nutrient uptake of rice must be above 29°C. However, the uptake rates of different nutrient elements responded differently to RZT, with the increase in nitrogen (N) uptake per °C being greater than that of phosphorus (PO₄³⁻) and potassium (K⁺), which can potentially lead to an imbalance in plant nutrition. Therefore, the increase in RZT either due to climate change or water management may call for an adjusted fertilizer management. In general, the increase in nutrient uptake per °C was more pronounced during the day than during the night, while the amino acid concentration in the leaves both during the day and night was positively correlated with N uptake during the day, suggesting that plants may benefit more from increased temperature during the day. When both ammonium (NH₄⁺) and NO₃⁻ were supplied, rice plants took up a

higher share of NH_4^+ . However, after depletion of NH_4^+ in the nutrient solution, plants took up NO_3^- without decreasing the total N uptake. The N form taken up by the rice plant had no effect on leaf gas exchange at low VPD, whereas NO_3^- uptake and assimilation increased stomatal conductance in some rice varieties at high VPD, resulting in a significantly higher photosynthetic rate. However, the increase in photosynthesis did not always result in an increase in dry matter, probably due to a higher energy requirement for NO_3^- assimilation than for NH_4^+ . The effect of N form on leaf gas exchange of some rice varieties was only found at high VPD, indicating genotype-specific adaptation strategies to high VPD. However, maintenance of high stomatal conductance at high VPD will only be beneficial at sufficient levels of water supply. Therefore, we hypothesize that with increasing VPD, intensified nitrification under water-saving irrigation may improve leaf gas exchange of rice plants, provided a careful choice of variety and good water management. Furthermore, N form had an effect on the competition between rice and weeds. In mixed culture with rice, a large share of NO_3^- increased the growth and competitiveness of upland weeds but reduced the growth and competitiveness of lowland weeds. Consequently, enhanced nitrification under AWD may reduce the competitive pressure of lowland weeds, but increase the competition of upland weeds. In contrast to rice, growth of the upland weed was not reduced by high VPD, while its nutrient uptake was correlated with water uptake, suggesting that upland weeds will more successfully compete with rice for nutrients as VPD increases. Selection of rice varieties better adapted to NO_3^- uptake will improve rice growth and its competitiveness against weeds under AWD. The cumulative effects of RZT and soil nitrification on rice growth should be considered when evaluating the effects of climate change on rice growth.

ZUSAMMENFASSUNG

Da Reis das Hauptnahrungsmittel für mehr als die Hälfte der Weltbevölkerung ist, haben Reisproduktion und Produktivität des Anbaus erhebliche Auswirkungen auf die Ernährungssicherheit. In Anpassung an die zunehmende Wasserknappheit sowie zur Reduzierung der Treibhausgasemissionen wurden in vielen Reisanbaugebieten wassersparende Bewässerungsmaßnahmen (z. B. alternierende Bewässerung und Trocknung - AWD) eingeführt. Frühere Studien haben gezeigt, dass AWD die Wassernutzungseffizienz erhöht und die Methan (CH₄)-Emissionen reduziert, während der Kornertrag im Vergleich zur kontinuierlichen Überstauung gleich bleibt oder leicht erhöht wird. Das Fehlen einer Wasserschicht in ehemals gefluteten Reisfeldern schafft jedoch neue Herausforderungen, wie z. B. eine veränderte Wurzelraumtemperatur (RZT), eine verstärkte Nitrifikation, die zu höheren Nitrat (NO₃⁻)-Konzentrationen im Boden führt, oder eine stimulierte Unkrautkeimung, die zu Veränderungen in der Unkrautflora führt. All diese Faktoren können die Nährstoffaufnahme und -assimilation der Reispflanzen und damit das Pflanzenwachstum beeinflussen. Darüber hinaus steuert das Dampfdruckdefizit (VPD) die Transpiration und den Wasserfluss durch die Pflanzen, so dass die Nährstoffaufnahme und -assimilation durch die Pflanzen unter variierenden VPD-Bedingungen einer Anpassung unterliegen kann. Da das VPD zwischen den Reisanbaugebieten und den Jahreszeiten stark variiert und außerdem eine kontinuierliche Zunahme unter der globalen Erwärmung vorhergesagt wird, wurde es als Faktor in diese Studie aufgenommen. Das übergeordnete Ziel der Studie war es, die Reaktion verschiedener Reissorten auf die entstehenden Herausforderungen unter wassersparender Bewässerung zu bewerten. Die Experimente wurden im Gewächshaus und in VPD-Kammern an der Universität Hohenheim durchgeführt, wo die Pflanzen in Hydroponik kultiviert wurden. Sowohl tagsüber als auch nachts stiegen die Nährstoffaufnahmeleistungen von Reis linear mit der RZT im beobachteten Temperaturbereich bis 29°C an, was bedeutet, dass die optimale Temperatur für die Nährstoffaufnahme von Reis über 29°C liegen muss. Die Aufnahmeleistungen der verschiedenen Nährstoffelemente reagierten jedoch unterschiedlich auf RZT, wobei die Zunahme der Stickstoff (N)-Aufnahme pro °C größer war als die von Phosphor (PO₄³⁻) und Kalium (K⁺), was möglicherweise zu einem Ungleichgewicht in der Pflanzenernährung führen kann. Daher kann der Anstieg der RZT entweder durch den Klimawandel oder durch das Wassermanagement ein angepasstes Düngemanagement erforderlich machen. Im Allgemeinen war der Anstieg der

Nährstoffaufnahme pro °C am Tag stärker ausgeprägt als in der Nacht, während die Aminosäurekonzentration in den Blättern sowohl am Tag als auch in der Nacht positiv mit der N-Aufnahme am Tag korreliert war, was darauf hindeutet, dass die Pflanzen möglicherweise mehr von einer erhöhten Temperatur am Tag profitieren. Wenn sowohl Ammonium (NH_4^+) als auch NO_3^- zugeführt wurden, nahmen die Reispflanzen einen höheren Anteil an NH_4^+ auf. Nach Verarmung an NH_4^+ in der Nährlösung nahmen die Pflanzen jedoch NO_3^- auf, ohne dass die Gesamt-N-Aufnahme abnahm. Die von der Reispflanze aufgenommene N form hatte bei niedrigem VPD keinen Einfluss auf den Blattgasaustausch, während die NO_3^- -Aufnahme und -Assimilation bei einigen Reissorten bei hohem VPD die stomatare Leitfähigkeit erhöhte, was zu einer signifikant höheren Photosyntheserate führte. Die Zunahme der Photosynthese führte jedoch nicht immer zu einer Zunahme der Trockensubstanz, wahrscheinlich aufgrund eines höheren Energiebedarfs für die NO_3^- -Assimilation als für NH_4^+ . Der Effekt der N form auf den Blattgasaustausch bei einigen Reissorten wurde nur bei hohem VPD gefunden, was auf genotypspezifische Anpassungsstrategien an hohes VPD hinweist. Die Aufrechterhaltung einer hohen stomatären Leitfähigkeit bei hohem VPD ist jedoch nur bei ausreichender Wasserversorgung von Vorteil. Daher stellen wir die Hypothese auf, dass mit zunehmendem VPD eine verstärkte Nitrifikation unter wassersparender Bewässerung den Blattgasaustausch von Reispflanzen verbessern kann, eine sorgfältige Sortenwahl und ein gutes Wassermanagement vorausgesetzt. Außerdem hatte die N form einen Einfluss auf die Konkurrenz zwischen Reis und Unkraut. In Mischkultur mit Reis erhöhte ein hoher Anteil an NO_3^- das Wachstum und die Konkurrenzfähigkeit von Trockenreis-Unkräutern, reduzierte aber das Wachstum und die Konkurrenzfähigkeit von Naßreis-Unkräutern. Folglich kann eine erhöhte Nitrifikation unter AWD den Konkurrenzdruck von Naßreis-Unkräutern verringern, aber die Konkurrenz durch Trockenreis-Unkräuter erhöhen. Im Gegensatz zu Reis wurde das Wachstum der Trockenreis-Unkräuter durch hohes VPD nicht reduziert, wobei ihre Nährstoffaufnahme mit der Wasseraufnahme korreliert war, was darauf hindeutet, dass Trockenreis-Unkräuter mit steigendem VPD erfolgreicher mit Reis um Nährstoffe konkurrieren. Die Selektion von Reissorten, die besser an die NO_3^- -Aufnahme angepasst sind, wird das Wachstum von Reis und seine Konkurrenzfähigkeit gegenüber Unkräutern unter AWD verbessern. Die kumulativen Effekte von RZT und Bodennitrifikation auf das Reiswachstum sollten berücksichtigt werden, wenn die Auswirkungen des Klimawandels auf das Reiswachstum bewertet werden.

TÓM TẮT KẾT QUẢ

Lúa gạo là nguồn lương thực chính cho hơn một nửa dân số toàn cầu, do đó sản xuất lúa gạo có ảnh hưởng lớn đến an ninh lương thực. Để thích ứng với tình hình thiếu hụt nước ngày càng nghiêm trọng cũng như giảm thiểu phát tán khí gây hiệu ứng nhà kính, các kỹ thuật tưới nước tiết kiệm (như tưới ngập ẩm luân phiên) đã được áp dụng ở nhiều vùng sản xuất lúa. Nhiều nghiên cứu trước đây đã chứng minh rằng tưới ngập ẩm luân phiên giúp nâng cao hiệu quả sử dụng nước và giảm phát tán khí mê-tan, trong khi năng suất lúa có thể ngang bằng hoặc cao hơn hệ thống tưới ngập liên tục. Tuy nhiên, do không có lớp nước duy trì liên tục trên bề mặt ruộng, một số vấn đề mới đã nảy sinh trong hệ thống này như: thay đổi nhiệt độ rễ; làm tăng quá trình nitrat hóa dẫn đến tăng nồng độ đạm nitrat (NO_3^-) trong đất; và kích thích hạt cỏ nảy mầm, dẫn đến thay đổi hệ sinh thái cỏ dại trên ruộng. Những sự thay đổi này có thể ảnh hưởng đến khả năng hút và đồng hóa dinh dưỡng và sinh trưởng của cây lúa. Hơn nữa, sự thiếu hụt áp suất ẩm độ không khí (VPD) là yếu tố quan trọng ảnh hưởng đến quá trình thoát hơi nước và vận chuyển nước trong cây, do vậy cây có thể có những điều chỉnh trong việc hấp thụ và đồng hóa dinh dưỡng ở các VPD khác nhau. Có sự đa dạng về VPD giữa các mùa vụ và giữa các vùng sinh thái trồng lúa, trong khi VPD cũng được dự báo sẽ tiếp tục tăng lên ở nhiều nơi hiện tượng ẩm lên toàn cầu, do đó nó cũng là một yếu tố thí nghiệm trong nghiên cứu này. Mục tiêu chính của nghiên cứu này là nhằm đánh giá phản ứng của cây lúa đối với một số vấn đề nảy sinh trong hệ thống canh tác lúa tưới tiết kiệm nước. Các thí nghiệm được tiến hành trong buồng điều khiển VPD và trong nhà lưới ở trường đại học Hohenheim (Đức) và cây được trồng trong dung dịch dinh dưỡng. Tốc độ hút dinh dưỡng của cây lúa tăng tuyến tính với sự tăng nhiệt độ rễ đến 29°C cả ngày lẫn đêm, chỉ ra rằng ngưỡng nhiệt tối ưu cho quá trình hút dinh dưỡng của cây lúa là trên 29°C . Tuy nhiên, tốc độ hút của các nguyên tố dinh dưỡng trong phản ứng với nhiệt độ là khác nhau, theo đó, tốc độ hút đạm tăng mạnh hơn là hút lân và kali, điều này có thể dẫn đến sự mất cân bằng dinh dưỡng trong cây. Do vậy, sự tăng nhiệt độ rễ, do biến đổi khí hậu hoặc do hệ thống tưới, đặt ra yêu cầu cần phải điều chỉnh biện pháp quản lý phân bón. Nhìn chung, tăng nhiệt độ rễ ở ban ngày làm tăng tốc độ hút dinh dưỡng mạnh hơn ở ban đêm, trong khi nồng độ axit amin trong lá tương quan chặt với tốc độ hút đạm ban ngày, điều đó cho thấy cây sẽ có lợi nhiều hơn khi nhiệt độ tăng lên vào ban ngày. Khi đạm được cung cấp ở cả hai dạng amon (NH_4^+) và NO_3^- , cây hút NH_4^+ nhiều hơn. Tuy nhiên, khi nồng độ NH_4^+ giảm, cây tăng cường hút

NO_3^- nhiều hơn, do đó không làm giảm tổng lượng đạm hút. Trong điều kiện VPD thấp, dạng đạm hút không ảnh hưởng đến hoạt động trao đổi khí lá của cây lúa, ngược lại, trong điều kiện VPD cao, NO_3^- làm tăng độ dẫn khí khổng, dẫn đến tăng quá trình trao đổi khí lá ở một số giống lúa. Mặc dù vậy, sự tăng cường độ quang hợp này không phải lúc nào cũng dẫn đến tăng chất khô tích lũy, điều này có thể là do nhu cầu năng lượng cho quá trình đồng hóa NO_3^- cao hơn NH_4^+ . Ảnh hưởng của dạng đạm đến quá trình trao đổi khí lá của các giống lúa chỉ quan sát thấy trong điều kiện VPD cao, cho thấy rằng có sự thích nghi đặc trưng của các kiểu gen trong điều kiện VPD cao. Tuy nhiên, việc duy trì độ dẫn khí khổng ở điều kiện VPD cao chỉ có lợi khi cây được cung cấp đủ nước. Do đó, chúng tôi đưa ra giả thuyết rằng với VPD tăng lên, quá trình nitrat hóa tăng cường trong điều kiện tưới ngập ẩm luân phiên có thể cải thiện sự trao đổi khí của cây lúa, tuy nhiên cần có sự lựa chọn về giống và biện pháp quản lý nước phù hợp. Hơn nữa, dạng đạm có ảnh hưởng đến sự cạnh tranh giữa lúa và cỏ dại. Trong cạnh tranh với lúa, tỷ lệ dinh dưỡng NO_3^- cao làm tăng sự sinh trưởng và khả năng cạnh tranh của cỏ dại ưa ẩm nhưng lại làm giảm sinh trưởng và khả năng cạnh tranh của cỏ dại chịu ngập. Khác với cây lúa, sinh trưởng của cỏ dại ưa ẩm không bị giảm trong điều kiện VPD cao, trong khi tốc độ hút dinh dưỡng của nó có tương quan với tốc độ hút nước, cho thấy rằng cỏ dại ưa ẩm sẽ cạnh tranh tốt hơn với cây lúa về dinh dưỡng khi VPD tăng lên. Việc lựa chọn các giống lúa có khả năng thích ứng tốt hơn với sự hấp thụ NO_3^- sẽ cải thiện khả năng sinh trưởng và cạnh tranh của lúa đối với cỏ dại trong điều kiện tưới ngập ẩm luân phiên. Các tác động cộng gộp của nhiệt độ rễ và quá trình nitrat hóa trong đất đối với sinh trưởng của cây cần được xem xét khi đánh giá tác động của biến đổi khí hậu đối với sự sinh trưởng của cây lúa.

Chapter 1

General Introduction

Rice (*Oryza sativa* L.) is one of the foremost food crops in the world, providing major food for more than 50% of the world's population. With the growing global population, rice demand will continuously increase in the coming decades. However, climate change is challenging rice production worldwide and thus, food security will be under severe threat, if no suitable actions are taken. Moreover, rice production consumes a lot of water, while water scarcity becomes more severe due to climate change, particularly in the dry season or / and in regions with dry climate. Water-saving irrigation measures (e.g., alternate wetting and drying) have been introduced in various rice-growing regions to adapt to increasing water scarcity. However, the shift to the new water management technique also raises concerns, e.g., higher root zone temperatures during drained periods, increased nitrate concentrations due to intensified nitrification, and altered weed population dynamics in the field, requiring adjustments of farming techniques to further improve rice production. This chapter summarizes general information on global rice production and the challenges related to global warming and to the introduction of water-saving irrigation measures.

1.1 Global rice production and demand

In terms of global production, rice is the third most important crop after maize and wheat. Currently, it is grown in more than one hundred countries, providing staple food for more than 50% of the world's population, mainly in Asia, Latin America, and Africa (GRiSP, 2013; Muthayya et al., 2014). Over the last decades, harvested rice area only slightly expanded, while rice yield and production steadily increased. Between 1968 and 2008, the annual growth rates of total rice production and average yield were 3.5 and 2.3%, respectively, meanwhile the harvested area annually increased by 0.6% only (Fig. 1.1) (FAOSTAT, 2020). The sharp increase in rice yield demonstrates the advances of plant breeding, farming techniques, and management strategies. Nevertheless, over the last 10 years, rice production increased more slowly. Between 2008 and 2018, total production and average yield of paddy rice annually increased by 1.4 and 0.9%, respectively, while the harvested area annually increased by 0.4% (FAOSTAT, 2020). However, more than 90% of the global rice is produced in Asia (Kubo and Purevdorj, 2004). In 2018, 705.4 Mt of rice

were harvested on 146.1 Mha in Asia, equaling 90.2 and 87.4% of global rice production and area, respectively. China, India, Indonesia, Bangladesh, Vietnam, Thailand, and Myanmar are the top 7 world's leading rice producers, producing more than 80% of the global rice production (FAOSTAT, 2020).

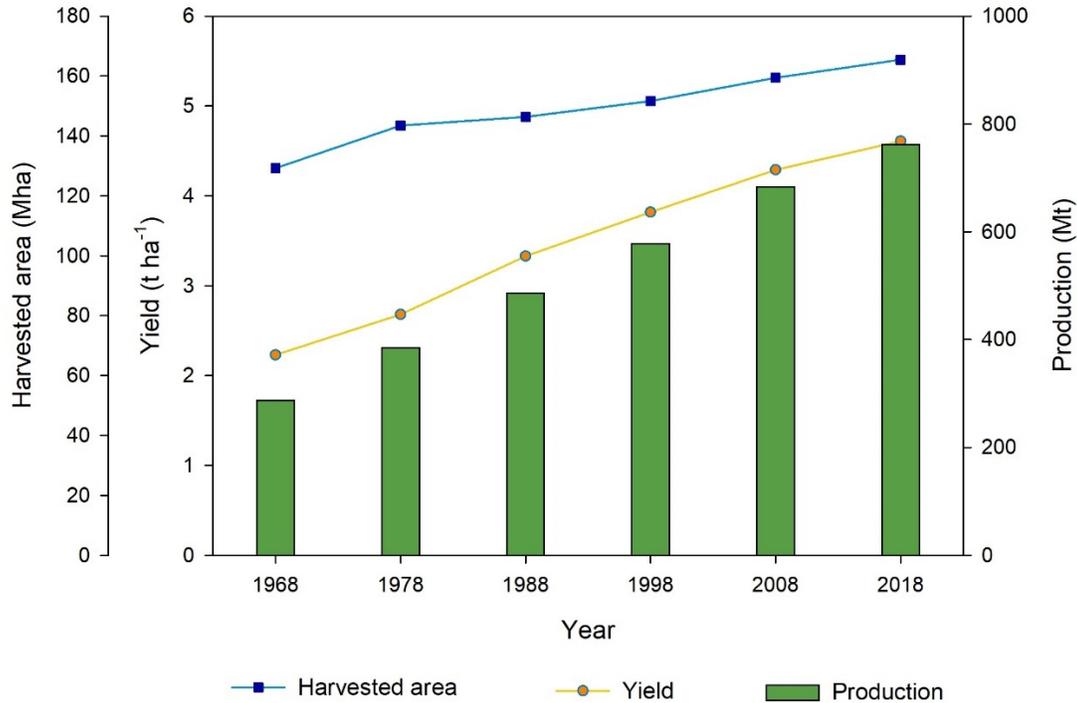


Figure 1.1: Global rice production (Source: FAOSAT, 2021).

Rice is grown in diverse production systems, however, based on the growing environment, it can be classified as irrigated lowland, rainfed lowland, irrigated upland, rainfed upland, and floating rice (Rao et al., 2017). Worldwide, irrigated lowland rice is produced on about 93 Mha, which results in 75% of the global rice production, therefore being the largest and most important rice production system (GRiSP, 2013). The global average yield of irrigated lowland rice is about 5 t ha⁻¹, however, it varies among growing regions and seasons. In the tropics, yields of 7 - 8 t ha⁻¹ can be achieved in the dry season and of 5 - 6 t ha⁻¹ in the wet season (Dobermann and Fairhurst, 2000). The second popular production system is the rainfed lowland rice, which covers about 52 Mha and makes up 19% of the global rice production (GRiSP, 2013). Due to the unpredictability of rainfall, the system often suffers from multiple abiotic stresses, e.g., drought or uncontrolled flooding (Bouman et al., 2007),

leading to low yields, with an average of about 2.3 t ha⁻¹ (Dobermann and Fairhurst, 2000). The irrigated and rainfed upland and floating rice systems contribute about 6% to the total rice production in total. In these systems, rice yields are relatively low because of biotic and abiotic stresses as well as social constraints, and they are not expected to significantly increase in the near future (Bouman et al., 2007; Dobermann and Fairhurst, 2000).

Rice export has been expanded in recent decades. Within 10 years, it increased by more than 47%, from 25.1 Mt in 2008 to 37.0 Mt in 2018 (milled rice only). On average between 2008 and 2018, Thailand, India, Vietnam, Pakistan, and the United State of America were the top 5 leading rice exporters, while the top 5 greatest rice importers were Nigeria, China, Philippines, Saudi Arabia, and Iran (FAOSTAT, 2020). Therefore, most of the leading rice exporters are concentrated in Asia, except for the United State of America. During the last decade, three major exporters, Thailand, Vietnam, and India, contributed more than 60% of the globally traded rice (Sekhar, 2018). However, rice is mostly consumed in the countries / regions where it is produced. Only about 7% of the total production enters the international market (GRiSP, 2013), while about 90% of the world's rice is produced and consumed in Asia (Kubo and Purevdorj, 2004).

Although capita rice consumption in a large part of Asia will continue or start to reduce in the future as a result of rising income and diet diversification, it still increases fast outside Asia, especially in sub-Saharan Africa, where people switch from maize and cassava to rice as their incomes rise. Increases in rice consumption per capita are also predicted to continue in the United States of America and the European Union as an increasing number of people switch from a protein-rich diet to a diet containing more fibers, and also because of immigration from Asia (GRiSP, 2013). With population and economic growth, global rice consumption will remain high, rising to an estimate of 555 Mt milled rice in 2035 (GRiSP, 2013) and about 600 Mt in 2050 (Braun and Bos, 2005). Therefore, rice production must increase by about 1% annually to meet its future demand. Rice production grew by 1.5% annually between 2009/2010 and 2018/2019 (USDA, 2020). If this growth rate can be maintained, it is possible to produce the estimated rice demand in the future. However, with climate change, scarcity of natural resources (e.g., land, water), lack of labor, and frequent occurrence of biotic and abiotic stresses (GRiSP, 2013), it is still a big challenge to maintain the growth rate of rice production in the coming decades.

1.2 Global warming challenging rice production

The adverse effect of global climate change is substantially manifested and continues to be a challenge for agricultural production in the 21st century, particularly global warming, accompanied by an increasing frequency and intensity of extreme weather events (e.g., hot days, hot nights, droughts or heavy precipitation) and sea level rise (*IPCC*, 2018). The Paris Agreement proposed to hold the increase in global mean temperature to well below 2°C above to pre-industrial levels and pursue efforts to limit the increase to about 1.5°C. Based on past and ongoing emissions, temperature is currently increasing at an estimated rate of 0.2°C per decade. If it continues to increase at the current rate, the global temperature will increase by 1.5°C above pre-industrial levels between 2030 and 2052 (*IPCC*, 2018). *Peng et al.* (2004) revealed that annual minimum and maximum temperature at the International Rice Research Institute Farm (Philippines) increased by 1.13 and 0.35°C, respectively, during 25 years (1979 - 2003), indicating that the increase in temperature during the night in the tropics is faster than that during the day.

Global warming may lead to a decline in rice yield because high air temperature may cause a decline in plant biomass and an increase in spikelet sterility (*Jagadish et al.*, 2015). *Peng et al.* (2004) showed that rice grain yield declined by about 15% for each °C increase in growing-season mean temperature.

However, rising atmospheric CO₂ concentrations may enhance the photosynthetic rates of C₃ plants at reduced stomatal conductance (*Ainsworth and Rogers*, 2007). *Lv et al.* (2020) showed that yields of *japonica*, *indica*, and hybrid rice cultivars increased by 13.5, 22.6, and 32.8%, respectively under free-air CO₂ enrichment (about 200 μmol mol⁻¹ above ambient). *Wang et al.* (2015) also demonstrated that elevated atmospheric CO₂ increases rice yields, with the greatest response in hybrid cultivars. Nevertheless, the positive effect of elevated atmospheric CO₂ on yield and water productivity will be offset by the negative effect of temperature increases (*GRiSP*, 2013). *Wang et al.* (2015) revealed that the effect of the elevated CO₂ on rice yield cannot compensate for the decline in grain yield due to a decrease in spikelet fertility and harvest index caused by higher air temperature, with the combined effect of CO₂ and temperature leading to a yield decrease of 9.4 - 10.6%/°C.

Increasing air temperature will result in rising sea levels due to glacier melting, leading to a decrease in agricultural land. The global sea level is projected to rise in the range of 0.2 - 0.8 m and 0.3 - 1.0 m by 2100 relative to 1986 - 2005, if the mean air temperature increases by 1.5 and 2.0°C, respectively (*IPCC*, 2018). Rising sea levels will lead to high risks of salinity intrusion (*GRiSP*, 2013), which is considered as a major threat for rice production in the Asian Mega Deltas (*Schneider and Asch*, 2020). Countries most affected by the decrease in the rice area due to sea level rise are Bangladesh, Japan, Taiwan, Egypt, Myanmar, and Vietnam (*Chen et al.*, 2012).

Water scarcity is projected to increase with global warming, leading to water shortages for agricultural production (*IPCC*, 2018). Further, expansion of irrigated land related to higher evaporative demand (*Dinar et al.*, 2019; *Morriso et al.*, 2009) and increasing urban water demand (*Flörke et al.*, 2018) will intensify water scarcity for agriculture and thus, threaten food security. In irrigated lowland rice, a ponded water layer is usually maintained at a depth of 5 - 10 cm throughout the growing season (Fig. 1.2). It is estimated that irrigated rice receives about 34 - 43% of the global irrigation water and a share of 24 - 30% of the global developed freshwater resources (*Bouman et al.*, 2007). Therefore, increasing crop water productivity to produce more rice from less water, is one of the main goals to assure meeting the rice demand of the future (*Braun and Bos*, 2005; *Zwart and Bastiaanssen*, 2004).

1.3 Water-saving irrigation technologies and new concerns in rice production

1.3.1 Alternate wetting and drying

One solution to improve water productivity in lowland rice production is the implementation of water-saving irrigation measures. Many studies demonstrated that maintaining a ponded water layer throughout the season is unnecessary for achieving high rice yield, thus various water-saving measures were early promoted in various rice-growing regions (*Van der Hoek et al.*, 2001). The system of rice intensification (SRI) which includes principles of water-saving irrigation was developed for transplanted rice. In SRI, young seedlings at an age of less than 15 days should be quickly, shallowly, carefully, and singly transplanted at a wide spacing while the paddy field is kept moist rather than continuously saturated during the vegetative growth period (*Satyanarayana et al.*, 2006), which can be achieved either by minimum daily application of water or by alternate wetting and drying (AWD) (*Thakur et*

al., 2014). The full set of principles of AWD were developed and spread by the International Rice Research Institute (IRRI). In AWD, irrigation water should be applied to re-flood the paddy soil to a depth of about 5 cm only when the ponded water has dropped to about 15 cm below the surface of the soil. However, a water layer of about 5 cm depth should be maintained one week before to one week after flowering to avoid water stress (IRRI, www.knowledgebank.irri.org). Both, SRI and AWD, were adopted and are currently applied in many rice-growing countries (Alauddin et al., 2020; Djaman et al., 2018; Rejesus et al., 2011; Satyanarayana et al., 2006; Yamaguchi et al., 2017; Yang et al., 2017).



Figure 1.2: A ponded water layer under continuous flooding and aerobic soil during drained periods under alternate wetting and drying (Photos taken in Vietnam).

Many studies demonstrated that AWD significantly reduces water inputs in rice production while maintaining or even slightly increasing grain yield compared to continuous flooding (CF) (Carrizo et al., 2017; Tran et al., 2018; Van der Hoek et al., 2001; Yamaguchi et al., 2017). AWD was shown to increase grain yield by 6.1 - 15.2% and reduce irrigation water inputs by 23.4 - 42.6%, leading to an increase in water productivity of 27 - 51% compared to conventional irrigation (Yang et al., 2017). Using WEAP (Water Evaluation and Planning System), Schneider et al. (2019) showed that the application of AWD can strongly reduce water requirements in rice production while it can increase water availability by up to 50% in the entire irrigation system. The increase in grain yield and water use efficiency under AWD has been attributed to altered plant hormonal levels, reductions in redundant vegetative growth, greater root biomass, improved canopy structure, or increased carbon remobilization from vegetative tissue to grain (Yang et al., 2017). However, water use efficiency and grain

yield of rice under AWD vary between seasons, management practices, soil properties, and cultivars (*Carrizo et al., 2017; Howell et al., 2015; Price et al., 2013; Sriphirom et al., 2019; Yang et al., 2017*), and therefore, further studies are needed to improve the efficiency of AWD under certain conditions.

Moreover, AWD has the potential to reduce methane (CH₄) emissions of rice production. Conventional flooded lowland rice is considered as one of the major sources of CH₄, which is a potent greenhouse gas. *Vo et al. (2020)* showed that CH₄ emission factors in irrigated rice fields in Vietnam fluctuate depending on growing-season and region between 1.72 to 3.89 kg CH₄ ha⁻¹ day⁻¹, which is much higher than the IPCC default value estimated for Southeast Asia (1.22 kg ha⁻¹ day⁻¹). Many studies demonstrated that AWD can significantly reduce CH₄ emissions, with a larger decrease being observed in dry seasons (54 - 83%) than in wet seasons (9 - 40%) (*Sander et al., 2020; Sriphirom et al., 2019*).

1.3.2 Root zone temperature in alternate wetting and drying system

Water management in the field can affect soil and root zone temperatures. In conventional flooded fields, the root zone temperature (RZT) is buffered by a ponded water layer. However, in the absence of a ponded water layer under AWD, RZT follows air temperature more closely and roots are exposed to larger daily temperature amplitudes than in flooded fields (*Maruyama et al., 2017; Stuerz et al., 2014*).

Since the daily mean soil temperature was positively correlated with the mean air temperature (*Islam et al., 2015; Zheng et al., 1993*), global warming will lead to increased RZT. With climate change, mean temperature as well as the frequency of hot days and nights are projected to increase (*IPCC, 2018*), however, so far, night temperature increased faster than day temperature (*Peng et al., 2004; Vose et al., 2005*). As RZT plays a crucial role in water and nutrient uptakes and the regulation of plant growth (*Kuwagata et al., 2012; Nagasuga et al., 2011; Setter and Greenway, 1988; Yan et al., 2013, 2012*), improving our understanding on the effects of increasing RZT during day- and night-time on nutrient uptake and assimilation in plants may help to better predict the responses of rice plants to AWD in future climate scenarios.

1.3.3 Nitrogen dynamics in the soil under alternate wetting and drying

Ammonium (NH_4^+) and nitrate (NO_3^-) are the major inorganic nitrogen sources in the soil taken up by plants (*Marschner*, 2011), however, the N forms can be transformed under specific conditions in the soil. Under anaerobic conditions like in paddy fields, NH_4^+ is the dominant and stable N form, whereas in aerated soils, nitrification is favored, leading to higher concentrations of NO_3^- (*Buresh et al.*, 2008; *Ghaly and Ramakrishnan*, 2013; *Miller and Cramer*, 2005). Nitrification is the biological oxidation of NH_4^+ or ammonia (NH_3) to NO_3^- by microorganisms. This process takes place in two steps, in which NH_4^+ is first converted to nitrite (NO_2^-) by ammonia-oxidizing bacteria or ammonia-oxidizing archaea, and then, to NO_3^- via nitrite-oxidizing bacteria or a direct reaction from NH_4^+ to NO_3^- by *Comammox* bacteria (*Beeckman et al.*, 2018; *Norton and Ouyang*, 2019). Various factors influence the nitrification in the soil, with soil moisture, aeration (soil oxygen (O_2) content), pH, and temperature playing dominant roles in this process (*Sahrawat*, 2008).

Several studies demonstrated that nitrification takes place in the rhizosphere of rice plants even in flooded soil due to O_2 release from the roots (*Arth and Frenzel*, 2000; *Kirk*, 2001; *Kirk and Kronzucker*, 2005). *Arth and Frenzel* (2000) found that the O_2 concentration in the rooted soil could be up to 150 μM in a depth of 20 - 30 mm, which is high enough to support activities of microorganism for both NH_4^+ and NO_2^- oxidation (*Laanbroek et al.*, 1994; *Laanbroek and Gerards*, 1993). NO_3^- production could be detected at distances up to 2 mm from the root surface, indicating that the nitrification activity is mainly confined to the root surface (*Arth and Frenzel*, 2000). Because of the nitrification processes in the rhizosphere, rice roots are exposed to both N forms at the root surface and NO_3^- uptake might be in a comparable range as that of NH_4^+ (*Kirk and Kronzucker*, 2005).

Since N forms differ in their assimilation pathway as well as in their demand in photosynthates, leaf gas exchange of rice plants may be subject to adjustment in response to N source. Previous studies demonstrated that uptake and assimilation of NO_3^- require more energy than that of NH_4^+ (*Bloom et al.*, 1992; *Raven*, 1985; *Salsac et al.*, 1987). As most of the NH_4^+ taken up is assimilated into amino acids in the roots (*Marschner*, 2011), leading to a high carbohydrate consumption in the roots (*Bowman and Paul*, 1988), carbohydrate content in the leaves is reduced (*Raab and Terry*, 1995). However, some studies showed that

leaf gas exchange of rice plants did not differ between plants supplied with different N forms, at least under well-water conditions (Guo et al., 2008; Ji and Peng, 2005; Li et al., 2009; Tran et al., 2015; Zhou et al., 2011). On the other hand, NO_3^- acts as osmotic in guard cells and is thus involved in stomatal opening and closure (Guo et al., 2003). Under water deficit, high NO_3^- concentrations in the leaves induced a larger stomatal aperture, which is advantageous for the plant, provided that the leaf water content is not impaired (Zhong et al., 2018). With increased NO_3^- concentration in the soil due to intensified nitrification in AWD (Buresh et al., 2008; Chunmei et al., 2020; Dong et al., 2012), plants will take up a higher share of N as NO_3^- , and thus, physiological responses of rice plants to the different N forms need to be understood when applying water-saving irrigation measures.

1.3.4 Weed dynamics in water-saving rice systems

Weeds are a major constraint in crop production as they lead to reduced plant growth, lowered productivity, and increased production costs. In the field, weeds compete with crops for nutrients, water, and light, and also serve as hosts for pests and diseases, resulting in yield reductions (Zimdahl, 2018). The negative effects of weeds on grain yields were observed to be more severe in direct-seeded than in transplanted rice (Karim et al., 2004), with yield reductions of 30 - 80% or even complete yield failure in direct-seeded rice (Matloob et al., 2015).

Water management significantly influences the weed population and its dynamics, as the water level affects the germination of the weeds and their survival after germination. The most important source of weeds is seedbanks in the soil. Many weed species can maintain their germination capacity for years in the soil and only germinate under favorable conditions (Chauhan and Johnson, 2009; Zimdahl, 2018). Early and deep flooding suppresses the emergence and growth of many weed species, particularly grasses and sedges (Benvenuti et al., 2004; Bhager et al., 1999; Chauhan and Johnson, 2011; Estioko et al., 2014; Kent and Johnson, 2001). Maintaining a floodwater layer is an effective method to control weeds in paddy fields, however, CF becomes increasingly difficult in water-scarce regions (Rodenburg and Johnson, 2009). Water-saving technologies (e.g., AWD) have been introduced as an adaptation to water scarcity, however, as the soil is periodically saturated, weed germination is favored, and changes in the weed population can be expected (Bhager

et al., 1999). *Bhuiyan et al. (2017)* showed that in AWD, sedges and grass species dominate with the highest dominance of *Echinochloa crus-galli*. Among irrigation methods, the largest yield loss caused by weeds was observed under saturated conditions (54.5%) in comparison to flooded conditions (35.2%) (*Juraimi et al., 2009*). The new dynamics of weed species in AWD will alter the competitive relationship between rice and weeds, which arouses the interest of scientists.

1.4 Vapor pressure deficit

The vapor pressure deficit (VPD) describes the difference between the saturation vapor pressure and the actual vapor pressure at a given temperature. Since the saturation vapor pressure of the atmosphere exponentially increases with temperature, global warming increases VPD (*Grossiord et al., 2020*). Global mean VPD increased strongly after the late 1990s and it is projected to consistently increase throughout the current century (*Grossiord et al., 2020; Yuan et al., 2019*). Moreover, several studies revealed a strong reduction of relative air humidity over land during the recent years, particularly over low- and mid-latitude regions (*Simmons et al., 2010; Willett et al., 2014*), enhancing the trend of an increasing VPD on the land surface.

VPD is considered as one of the main factors influencing plant photosynthesis via its effect on stomatal aperture, as plants close their stomata in response to high VPD to minimize water loss (*Grossiord et al., 2020; Yuan et al., 2019*). For instance, stomatal conductance of rice plants decreased with increasing VPD from 1.0 to 2.3 kpa, leading to a decreased CO₂ assimilation rate (*Ohsumi et al., 2008*). Rice is cultivated under a large climatic range, from the wettest to the driest regions (*GRiSP, 2013*), thus it can be exposed to a large range of VPDs. Moreover, during the dry season in arid or semi-arid regions, high VPD is can be a challenge for rice production. As VPD has the potential to alter physiological responses via its effects on stomatal conductance and transpiration, it was included as an experimental factor in this study.

1.5 Research objectives

The overall objective of the study was to evaluate growth and physiological responses of different rice varieties to individual factors that are changing in rice fields subjected to AWD, especially under global warming, such as increasing RZT, increasing NO₃⁻ concentration in

the soil, and altered weed dynamics. The results of the study were used to discuss farming techniques (e.g., water management, cultivar selection) to improve the growth of rice under field conditions. The study concentrated on three specific objectives:

- To study nutrient uptake and assimilation of different rice varieties in response to increasing day and night RZT at different VPDs;
- To study leaf gas exchange and growth responses of different rice varieties to nitrogen source at different VPDs;
- To study the competitiveness of different rice varieties and weed species in response to nitrogen source at different VPDs.

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Chapter 2

Nutrient Uptake and Assimilation

Under Varying Day and Night Root Zone Temperatures in Lowland Rice*

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Abstract

In flooded rice fields, root zone temperatures (RZT) are buffered by the ponded water layer. With global warming, a higher frequency of hot days and hot nights, and the introduction of water-saving irrigation technologies, RZT are likely to vary more widely, particularly between night and day. It is not known how this will affect nutrient uptake of rice, particularly if the climate-driven transpirational demand increases simultaneously, since nutrient uptake at least partly depends on water uptake. We investigated the effects of day and night RZT on water and nutrient uptake and nitrogen (N) metabolism under low and high vapor pressure deficit (VPD). Plants of two rice varieties (IR64 and NU838) were grown hydroponically at three root temperature levels (19, 24, and 29°C). For a period of seven days, fresh weight of the plants, nutrient contents of the nutrient solution (NH_4^+ , NO_3^- , PO_4^{3-} , K^+) and water uptake were measured both at the end of the light period and at the end of the dark period. Nitrate reductase (NR), glutamine synthetase (GS), and amino acid (AA) concentrations in the youngest fully developed leaves were examined on the last day and night of the experiment. The share of day and night uptake of NH_4^+ and NO_3^- depended on RZT, whereas K^+ uptake was higher during the day independent of RZT. Under low VPD, PO_4^{3-} uptake rate did not differ between day and night, however, under high VPD, the uptake of PO_4^{3-} varied between varieties and RZTs. Water uptake of the plants was strongly influenced by VPD, but not by RZT. In contrast, nutrient uptake was hardly influenced by VPD and did not correlate with water uptake, but linearly increased with RZT with an optimum temperature for nutrient uptake above 29°C. This increase was larger for NH_4^+ and NO_3^- than for PO_4^{3-} and K^+ shifting the nutrient requirements of rice. While the increase of nutrient uptake per °C did not differ

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between varieties under low VPD, IR64 showed a greater increase in nutrient uptake to RZT at day-time, whereas NU838 showed a greater increase at night-time under high VPD. The activities of NR and GS seemed to respond to the total daily N uptake rather than to different uptake rates during day or night, while AA concentration was strongly correlated to N uptake during the day. With an optimum RZT for nutrient uptake above 29°C, rice plants could benefit from temperature increase caused by either different water management strategies or climate change if fertilizer management was adapted to the new, shifted requirements.

Key words: fertilizer management, nitrogen assimilation, nitrogen uptake, *Oryza sativa*, vapor pressure deficit.

2.1 Introduction

Rice is one of the most important food crops in the world. The regions where rice is grown, including West Africa, Central and South America, and Southeast Asia, were identified as hot spots of climate change (IPCC, 2018). Rice is cultivated in different growing seasons in both tropical and temperate regions and thus can be exposed to a wide range of temperatures. With climate change, air temperature has increased, and hot days and hot nights are becoming more frequent, especially in the tropics (IPCC, 2018). The increasing air temperature leads to an increase in soil and water temperature and thus, a higher root zone temperature (RZT). In a paddy field, water temperature increased by 0.91°C with each °C increase in air temperature (Pakoktom et al., 2014). In soil, Islam et al. (2015) reported a strong correlation between air and soil temperatures down to 20 cm depth. With the rapid expansion of water-saving rice cultivation systems (e.g., alternate wetting and drying), RZT may be related even more with air temperature, since the layer of ponded water is absent for at least parts of the growing season.

RZT has been linked to water and nutrient uptake, with suboptimal RZT leading to a reduction in relative water content of the shoot (Nagasuga et al., 2011), water uptake capacity and transpiration rate (Ku wagata et al., 2012), nitrogen (N) translocation rate (Engels and Marschner, 1996), and nutrient uptake of shoots (Yan et al., 2012). Moreover, higher RZT increased nutrient uptake and nutrient use efficiency (Hussain and Maqsood, 2015), up to a species-specific optimum temperature (Tindall et al., 1990; Hood and Mills, 1994). The effects of RZT on water and nutrient uptake have been attributed to changes in root growth

and development (Nagasuga et al., 2011), and the adjustment in root properties (BassiriRad and Radin, 1992; Nagasuga et al., 2011) with differences between and within species (BassiriRad and Radin, 1992; Kaspar and Bland, 1992). Therefore, the effect of increasing air temperature on nutrient and water uptake might not only depend on irrigation management, but also depend on the genotype used.

Furthermore, nutrient uptake rates follow diurnal patterns. Terabayashi et al. (1991) showed that plants took up more cations (K^+ , Ca^{2+} , Mg^{2+}) during the day than during the night, whereas phosphate (PO_4^{3-}) uptake showed little diurnal variation and did not change with air or root temperature. Masuda (1989) found higher concentrations of nitrate (NO_3^-) and potassium (K^+) in xylem exudate during the day than during the night, whereas concentrations of PO_4^{3-} were higher during the night. However, the nutrient uptake rates during light and dark periods were not different under constant air and root temperature (Albornoz and Lieth, 2015) while both nutrient uptake and allocation were significantly affected by different RZTs (Yan et al., 2012), reflecting a strong dependence of the diurnal nutrient uptake on RZT. Under suboptimal RZT, translocation of N and K^+ were rather correlated to shoot demand than to RZT, whereas translocation of phosphorus (P) was more dependent on RZT (Engels and Marschner, 1992). Therefore, RZT during day- or night-time may have different effects on the uptake, translocation, and metabolism of nutrients in plants.

Since transpiration drives water uptake, an interaction between vapor pressure deficit (VPD) and RZT on nutrient uptake and transport in the xylem may exist. Houshmandfar et al. (2018) reported a positive correlation between nutrient uptake and transpiration, where the uptake of N, K^+ , Ca^{2+} , Mg^{2+} , Mn^{2+} and SO_4^{2-} increased with increasing transpiration due to high VPD under both ambient and elevated atmospheric CO_2 . Adams (1980) also found a strong correlation between water uptake and the uptake of N and K^+ . In contrast, Tanner and Beevers (2001) found nutrient uptake/transport and transpiration to be independent, since about 70-85% of the total ions are actively taken up and depend on current metabolism (Brouwer, 1956). Also, nutrient uptake during the night has been shown to be an active process and less influenced by water uptake rate (Terabayashi et al., 1991). Although nutrient uptake is an active process, it can depend on water uptake under low evaporative demand (Brouwer, 1956; Shaner and Boyer, 1976). However, the relationship between day and night RZT and water and nutrient uptake under different transpiration rates has not been clarified.

With both, changing cultivation techniques and increasing air temperature, RZT is likely to change to a different extent during day and night. Since this applies in the humid and arid tropics as well as in temperate rice-growing regions, different transpirational demands should be considered when studying the temperature response of water and nutrient uptake. As N assimilation is energy-demanding and depends on assimilates which are easier available during the day, N could be metabolized differently when taken up either during day or night. Therefore, the objectives of this study were (1) to investigate the impact of VPD and RZT on nutrient and water uptake during day and night in order to identify possible problems in rice production related to shifts in soil temperature caused by water-saving irrigation measures and/or climate change; (2) to study nutrient uptake of rice varieties differing in temperature sensitivity at varying day and night temperatures for evaluation of the degree of genetic diversity in the temperature dependence of nutrient uptake; and (3) to explore the effect of different rates of N uptake caused by varying RZTs during day and night on N metabolism with the aim of increasing our knowledge about the relation between N uptake and its assimilation.

2.2 Methodology

2.2.1 Plant material

Two indica rice varieties, namely IR64 and Nhi Uu 838 (NU838), were used. IR64 is an early maturing, improved semi-dwarf *indica* rice variety originating from the Philippines. It has been extensively used in various scientific studies and has been classified as cold-sensitive and relatively responsive to temperature changes (Stuerz et al., 2014). NU838 is a hybrid variety with large leaves and is one of the most popular rice varieties in Vietnam. It is adapted to the different agro-ecological zones of Vietnam and has been classified as cold-tolerant (Pham et al., 2005).

2.2.2 Growth condition and treatments

The experiment was carried out in 2018 in a greenhouse of the Institute of Agricultural Sciences in the Tropics, University of Hohenheim, Germany. For germination, seeds of the two rice varieties were placed on wet filter paper for four days. Germinated seeds were transferred to seeding trays (one seed per hole) containing clean sand under artificial light. For the first four days, seedlings were irrigated with tap water, for another four days with

half strength nutrient solution in the composition proposed by *Yoshida et al.* (1976). Afterward, the seedlings were transplanted in boxes (40 x 30 x 12 cm) containing 8 liters full strength nutrient solution (pH 5.5) with 72 plants per box for the next twelve days. The full-strength nutrient solution contained 1.43 mM NH_4NO_3 (2.86 mM N), 0.32 mM $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$, 0.5 mM K_2SO_4 (1 mM K), 1 mM $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 1.6 mM $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 35.6 μM $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, 9.5 μM $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, 18.9 μM H_3BO_3 , 0.15 μM $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 0.16 μM $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 0.07 μM $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$ (0.52 μM Mo) and 70 μM citric acid. The nutrient solution was renewed once at day 7. During pre-cultivation, plants were grown at 12 h photoperiod and an average day and night air temperature of 32 and 20°C, respectively.

At the age of 24 days (5-leaf-stage, two tillers), plants were transplanted in individual tubes (diameter 5 cm; height 10 cm) containing 160 mL nutrient solution, fixed with a thin foam plug. The tubes with foam plugs were wrapped with parafilm to avoid evaporation and were placed in plastic boxes (40 x 30 x 12 cm) containing water of adjustable temperature. Varieties were randomized within each box, which contained 6 plants of each variety. In three replicates, boxes were placed in chambers connected to either a humidifying or a dehumidifying system to adjust the VPD of the atmosphere. VPD was 0.8 kPa during the day and 0.3 kPa during the night in the chamber of low VPD and 2.3 and 1.2 kPa during day and night in the chamber of high VPD, simulating the conditions during a tropical wet and dry season, respectively.

Temperature adjusted water was continuously circulated between cooling/heating systems and the plastic boxes, where three temperature regimes at day-time or night-time were established: low temperature (19°C), medium temperature (24°C); and high temperature (29°C). To minimize temperature effects on plant growth, which would affect nutrient uptake, those plants that were exposed to low temperature during the day were subjected to high temperature during the night and vice versa. Day and night RZT was controlled in accordance with the 12 h light period between 8 am. and 8 pm. During the treatment period, the average day and night air temperature was 30 and 24°C, respectively and was comparable for both VPD chambers. The air and root temperature of the treatments are described in Fig. 2.1. To provide constant nutrient supply, nutrient solution in the tubes was renewed every twelve hours at the beginning and the end of the day period.

	Day	Night	Day	Night	Day	Night
Above-ground temperature (°C)	30	24	30	24	30	24
Below-ground temperature (°C)	29	19	24	24	19	29
	Treatment 1		Treatment 2		Treatment 3	

Figure 2.1: Temperature scheme for day and night temperature treatments.

2.2.3 Plant biomass and water and nutrient uptake

Before the onset of the treatments, fresh weight of three plants of each treatment was measured. During the experiment, fresh weight and water uptake of these plants were determined twice per day (at time of renewing nutrient solution) by weighing. For fresh weight determination of plants, roots were carefully and quickly dried with tissue paper. The remaining nutrient solution was stored in the fridge (4°C) until nutrient concentration measurement. NH_4^+ , NO_3^- , and PO_4^{3-} concentrations in the fresh nutrient solution (at the beginning) and remaining nutrient solution (after 12 hours) were measured using a Technicon AutoAnalyzer. K^+ was measured with a flame photometer (Jenway, PFP7 Industrial Flame Photometer).

Nutrient uptake rate was calculated based on the following formula:

$$\text{Nutrient uptake rate } (\mu\text{mol g}^{-1} \text{FW h}^{-1}) \equiv \frac{(V1 * C1 - V2 * C2)}{T_g * \text{FW}}$$

Of which:

V1: volume of the fresh nutrient solution at the beginning (mL)

V2: volume of the remaining nutrient solution after 12 hours (mL)

C1: nutrient concentration of the fresh nutrient solution ($\mu\text{mol/mL}$)

C2: nutrient concentration of the remaining nutrient solution ($\mu\text{mol/mL}$)

T_g : time lag (h)

FW: the mean fresh weight of the plant between time lag (g)

Water uptake rate ($\text{mmol g}^{-1} \text{FW h}^{-1}$) was calculated as water uptake per fresh weight and time.

2.2.4 Nitrogen metabolism and enzyme assays

At day 7 after the onset of treatments, the youngest fully developed leaf was harvested on separate plants in each treatment at four different time points: 2 pm (mid of day), 8 pm (end of day), 2 am (mid of night), and 8 am (end of night). Fresh samples were quickly frozen in liquid nitrogen and stored at -80°C until analysis of enzyme activities (Hartmann and Asch, 2019), and free amino acids.

Nitrate reductase (NR) activity

Approx. 0.01 g frozen leaf material was ground and homogenized in 1 mL 100 mM Potassium phosphate (K_2HPO_4) buffer (pH 7.3) with 0.5 mM EDTA using a FastPrep®24 Classic Instrument for 60 seconds. Then the homogenates were centrifuged at 4°C and 12000 rpm for 10 minutes. 80 μL of the supernatant was incubated with 80 μL 30 mM KNO_3 and 10 μL 2.5 mM NADH at 30°C for 30 minutes in the dark with mild shaking. The reaction was terminated by quickly adding 80 μL 1% sulfanilamide and 50 μL 0.02% NEDDH. NR activity was determined based on the formation of nitrite (NO_2^-). The absorbance of NO_2^- was measured at 540 nm using a spectrophotometer with potassium nitrite (KNO_2) as the reference standard. The NR activity was expressed as $\mu\text{mol NO}_2^- \text{g}^{-1}$ leaf fresh weight h^{-1} .

Glutamine synthetase (GS) activity

Methods described by Li et al. (2016) and Peng et al. (2016) were adapted to measure GS activity. Using a FastPrep®-24 Classic Instrument, approximately 0.01 g frozen leaf material was ground and homogenized in 1 mL extraction buffer: 70 mM MOPS (pH 6.8), 10 mM MgSO_4 , 5 mM glutamate, 2 mM dithiothreitol, 10% (v/v) ethanediol and 0.1% (v/v) Triton X-100. Then the homogenate was centrifuged at 4°C and 12000 rpm for 30 minutes. 50 μL of the supernatant was incubated with 50 μL assay buffer (100 mM glutamate, 70 mM MOPS (pH 6.8), 50 mM MgSO_4 , 15 mM NH_2OH , and 15 mM ATP) at 37°C for 30 minutes. The reaction was terminated by adding 100 μL acidic FeCl_3 solution (670 mM HCl, 200 mM trichloroacetic acid and 88 mM FeCl_3). After 10 minutes for color development, the solution

was centrifuged at 4000 rpm for 10 minutes. The absorbance of the supernatant was measured at 540 nm with a spectrophotometer on 96-well-plates. L-glutamic acid- γ monohydroxamate (γ -glutamylhydroxamate, γ -GHA) was used as the reference standard. The GS activity was expressed as $\mu\text{mol } \gamma\text{-GHA g}^{-1} \text{ leaf fresh weight h}^{-1}$.

Total free amino acid concentration

The amino acid (AA) concentration was determined based on the ninhydrin method described by *Moore and Stein* (1954). Approximate 0.01 g frozen leaf material was ground and homogenized in 1 mL 80% Ethanol using a FastPrep®-24 Classic Instrument for 2 minutes. After 10 minutes, the extract was centrifuged at 8000 rpm for 15 minutes. The supernatant was collected and the extraction procedure was repeated with the pellet. 100 μL of the supernatant was incubated with 75 μL of ninhydrin color reagent (0.3 g hydrindantin and 2 g ninhydrin dissolved in 75 mL DMSO and 25 mL 4 M Na-Acetate buffer) at 80°C for 30 minutes. Then the mixture was cooled down and 100 μL of stabilizing solvent (50% ethanol) were added. The absorbance was measured at 570 nm on a 96-well-plate, using L-leucine as the reference standard.

2.2.5 Statistical analysis

Because VPD treatments were not replicated, data were analyzed with analysis of variation (ANOVA) using PROC MIXED in the Statistical Analysis System (SAS, version 9.4) for each VPD level separately. A linear model was used to examine the effect of root zone temperature treatment (TR), variety (V), day / night (DN) and their interaction on enzymes activities and AA concentration in the leaves. The uptake rates of water and nutrients were statistically analyzed using a repeated measures models to determine the effects of V, temperature levels (T), DN and their interactions. Mean values were compared using the least significant difference at $p \leq 0.05$. Pearson's correlation coefficients were calculated with SAS.

2.3 Results

2.3.1 Day and night water and nutrient uptake at different root zone temperatures under low and high VPD

Nutrient and water uptake rates were significantly different between day and night with the exception of PO_4^{3-} uptake under both VPDs (Tab. 2.1). Differences between varieties in nutrient uptake were only found for PO_4^{3-} and K^+ under high VPD, whereas under low VPD, varieties did not differ in nutrient uptake for the measured nutrients. However, significant differences in water uptake were observed between varieties. RZT significantly affected nutrient uptake but not water uptake under both VPD conditions. An interaction effect of V x DN x T on nutrient uptake was only observed under high VPD.

Table 2.1: Analysis of variance (ANOVA) of nutrient and water uptake rates under low and high VPD at day- or night-time on 2 varieties exposed to 3 root zone temperature levels (19, 24, 29°C). All measurements of nutrient and water uptake were performed on 7 consecutive days on 3 individual plants (n = 21).

VPD	Source of variation	d.f	Significance of F ratio				
			NH_4^+ uptake rate	NO_3^- uptake rate	PO_4^{3-} uptake rate	K^+ uptake rate	Water uptake rate
Low	V	1	ns	ns	ns	ns	**
	DN	1	***	***	ns	***	***
	T	2	***	***	**	**	ns
	V x DN	1	ns	ns	ns	ns	***
	V x T	2	ns	ns	ns	ns	ns
	T x DN	2	*	**	ns	ns	ns
	V x DN x T	2	ns	ns	ns	ns	ns
High	V	1	ns	ns	**	***	*
	DN	1	***	***	ns	***	***
	T	2	***	***	**	***	ns
	V x DN	1	**	ns	ns	*	***
	V x T	2	*	ns	ns	ns	ns
	T x DN	2	*	***	***	ns	ns
	V x DN x T	2	***	**	**	*	ns

***, **, * significant at $p \leq 0.001$, $p \leq 0.01$, and $p \leq 0.05$, respectively; ns: not significant

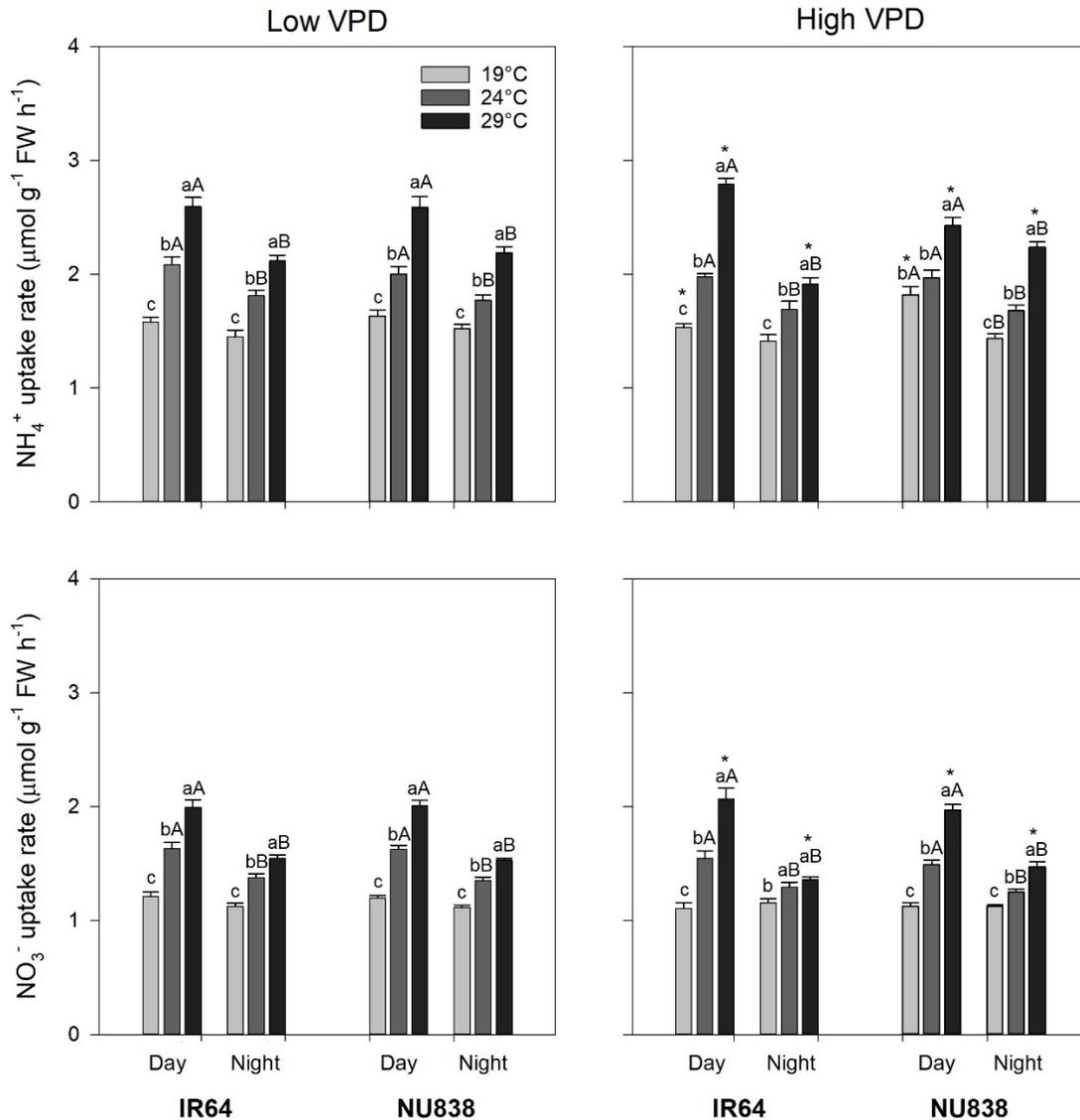


Figure 2.2: NH_4^+ and NO_3^- uptake at 19, 24, and 29°C during day- and night-time of two rice varieties (IR64 and NU838) under low and high VPD. Values presented are means from measurements on 7 consecutive days on 3 individual plants ($n = 21$). Error bars show the standard error of the mean. Small letters indicate significant difference between temperature levels; capital letters indicate significant difference between day and night; asterisks indicate significant difference between varieties, all at $p \leq 0.05$.

Under both VPD conditions and for both varieties, uptake rates of NH_4^+ and NO_3^- significantly increased with RZT during day and night (Fig. 2.2). However, the uptake rate of NH_4^+ was higher than NO_3^- . On average, plants took up $1.93 \mu\text{mol g}^{-1} \text{FW h}^{-1} \text{NH}_4^+$ and $1.44 \mu\text{mol g}^{-1} \text{FW h}^{-1} \text{NO}_3^-$, which means that 57% of the total N was taken up as NH_4^+ . In

general, rice plants took up more N during the day (54% on average) than during the night. In both varieties, N uptake was significantly higher during the day than during the night at 24°C and 29°C but not at 19°C RZT, with an exception of NH_4^+ in NU838 under high VPD. Under low VPD, N uptake rates were not affected by variety whereas at 29°C and high VPD, IR64 showed higher uptake rates of NH_4^+ and NO_3^- than NU838 during the day and lower rates during the night. Furthermore, at high VPD, NH_4^+ uptake rate at 19°C during the day was higher in NU838 than in IR64.

Under low VPD, PO_4^{3-} uptake was neither affected by variety nor by day /night, but increased with RZT (Fig. 2.3). Under high VPD, differences between PO_4^{3-} uptake during day and night were observed with IR64 showing a significantly higher PO_4^{3-} uptake rate during the night at 19°C and a higher uptake rate during the day at 29°C, whereas, NU838 showed a higher uptake rate during the night at 29°C. Higher RZT significantly increased the uptake rate of PO_4^{3-} at high VPD with an exception for IR64 at night-time. Differences between both varieties were only observed under high VPD, with higher PO_4^{3-} uptake rates of IR64 at 24°C and 29°C during the day and at 19°C and 24°C during the night.

Uptake rates of K^+ were higher during the day than during the night with 57% and 60% of K^+ taken up during day under low and high VPD, respectively (Fig. 2.3). Under both VPDs, K^+ uptake rates did not differ significantly between 19°C and 24°C, but at 29°C, strongly increased uptake rates of K^+ were observed at both day and night and for both varieties, with an exception for IR64 at night-time under high VPD. Differences between varieties were found at high VPD, where IR64 showed a higher uptake rate of K^+ than NU838 at 24 and 29°C during the day and at 19°C during the night.

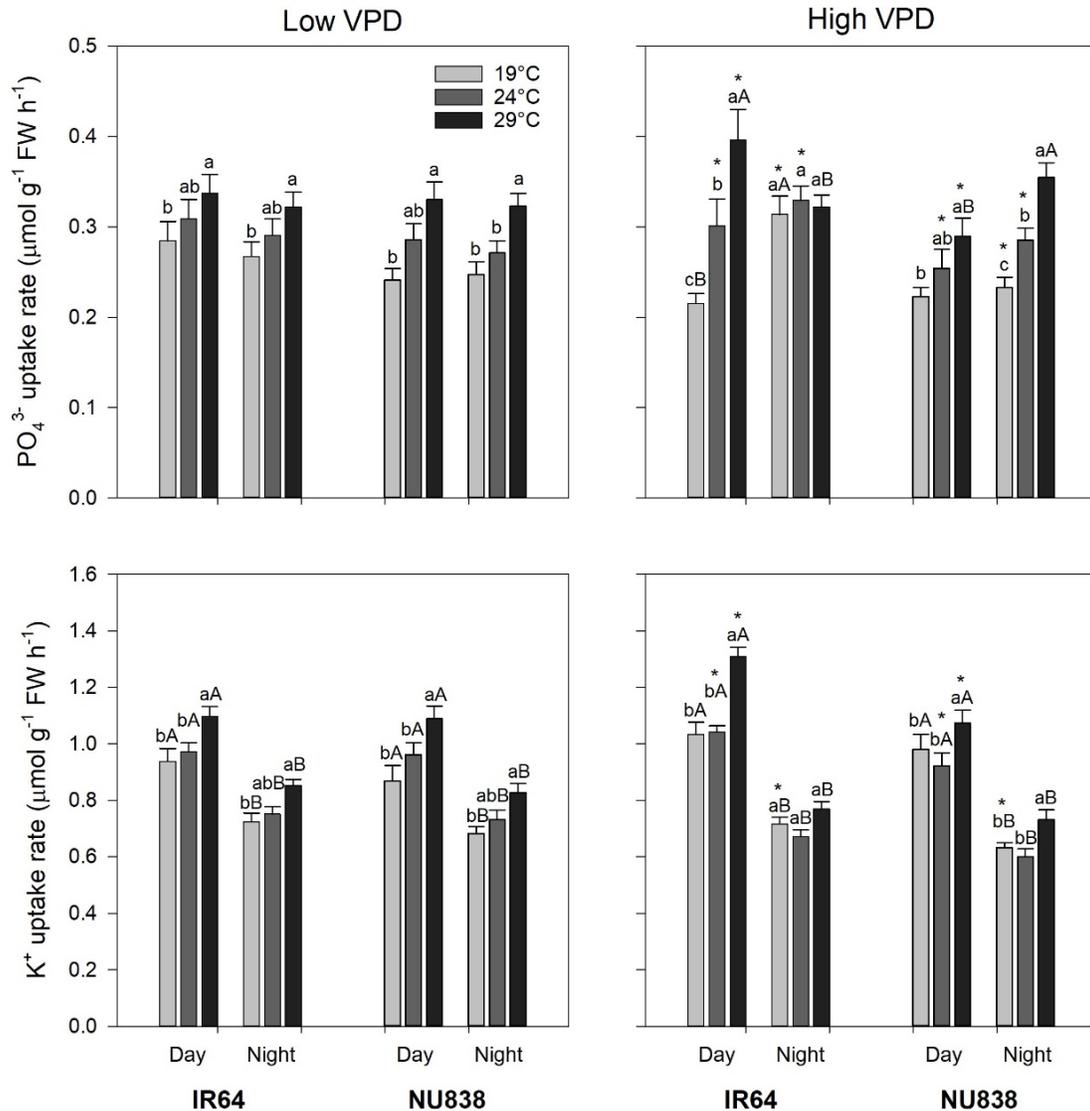


Figure 2.3: PO_4^{3-} and K^+ uptake at 19, 24, and 29°C during day- and night-time of two rice varieties (IR64 and NU838) under low and high VPD. Values presented are means from measurements on 7 consecutive days on 3 individual plants ($n = 21$). Error bars show the standard error of the mean. Small letters indicate significant difference between temperature levels; capital letters indicate significant difference between day and night; asterisks indicate significant difference between varieties, all at $p \leq 0.05$.

Total water uptake more than doubled under high VPD as compared to low VPD (Fig. 2.4). The difference was even larger during the day when water uptake under high VPD exceeded that of low VPD by 144%, while during the night by 49% only. Furthermore, the largest share of the water was taken up during the day, with 80% under low and 87% under high VPD, respectively. IR64 had a significantly higher water uptake rate during the day than NU838

under both VPDs with the exception at 19°C and high VPD. No correlation between water and nutrient uptake was found during the day while during the night, water and nutrient uptake were closely correlated for IR64 under low VPD only (data not shown).

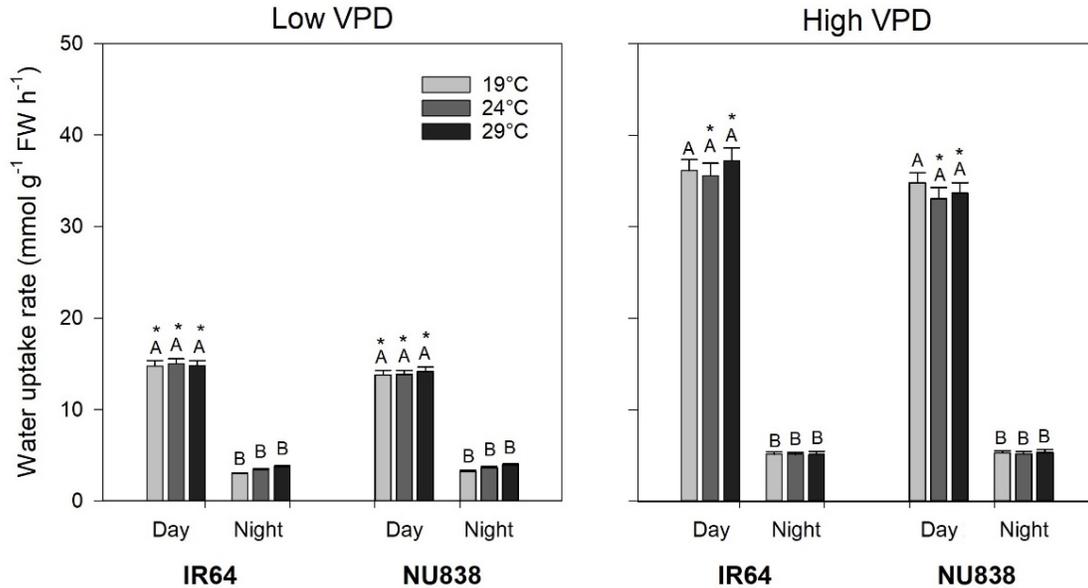


Figure 2.4: Water uptake at 19, 24, and 29°C during day- and night-time of two rice varieties (IR64 and NU838) under low and high VPD. Values presented are means from measurements on 7 consecutive days on 3 individual plants ($n = 21$). Error bars show the standard error of the mean. Capital letters indicate significant difference between day and night; asterisks indicate significant difference between varieties, all at $p \leq 0.05$.

2.3.2 The correlation between root zone temperature and water and nutrient uptake under low and high VPD

To compare the temperature effect on nutrient and water uptake between varieties, day / night and VPDs, linear regression analyses were performed between RZT levels and uptake rates and slopes of the regressions and significance levels are presented in Tab. 2.2. Under low VPD, slopes of both varieties were relatively similar, with larger increases of NH_4^+ and NO_3^- uptake rates with temperature at day-time than at night-time. Under low VPD, NH_4^+ uptake during the day increased by 0.101 and 0.095 $\mu\text{mol g}^{-1} \text{FW h}^{-1}$ per °C in IR64 and NU838, respectively, while it increased by 0.067 $\mu\text{mol g}^{-1} \text{FW h}^{-1}$ per °C only during the night in both varieties. NO_3^- uptake during the night increased by 0.042 $\mu\text{mol g}^{-1} \text{FW h}^{-1}$ per °C in both varieties, while during the day, it increased by 0.078 and 0.081 $\mu\text{mol g}^{-1} \text{FW h}^{-1}$ per °C in

IR64 and NU838, respectively. In contrast, both varieties showed an opposite trend in NH_4^+ uptake in response to RZT under high VPD. Here, the increase in NH_4^+ uptake rate per $^\circ\text{C}$ in IR64 was much higher during the day than during the night (0.126 and 0.050 $\mu\text{mol g}^{-1} \text{FW h}^{-1}$ per $^\circ\text{C}$, respectively), whereas in NU838 it was lower during the day than during the night (0.061 and 0.080 $\mu\text{mol g}^{-1} \text{FW h}^{-1}$ per $^\circ\text{C}$, respectively).

Table 2.2: Slope and level of significance of the linear regression between root zone temperature levels (19, 24, 29 $^\circ\text{C}$) and nutrient and water uptake rates ($\mu\text{mol g}^{-1} \text{FW h}^{-1}$) for two varieties (IR64 and NU838) at day- and night-time under low and high VPD. Uptake rates are mean values of measurements performed on 7 consecutive days. The regression analyses were performed over 3 temperature levels with 3 individual plants as replications, each (n = 9).

Nutrient and water uptake rate	Low VPD		High VPD	
	IR64	NU838	IR64	NU838
Day-time				
NH_4^+	0.101***	0.095***	0.126***	0.061***
NO_3^-	0.078***	0.081***	0.096***	0.085***
PO_4^{3-}	0.005*	0.009***	0.018***	0.007***
K^+	0.016**	0.022**	0.028***	0.009 ^{ns}
Water	5.50 ^{ns}	39.52 ^{ns}	106.71 ^{ns}	-113.82 ^{ns}
Night-time				
NH_4^+	0.067***	0.067***	0.050***	0.080***
NO_3^-	0.042***	0.042***	0.020***	0.035***
PO_4^{3-}	0.006*	0.008***	0.001 ^{ns}	0.012***
K^+	0.013**	0.014*	0.005 ^{ns}	0.010 ^{ns}
Water	71.80**	70.13*	1.39 ^{ns}	8.77 ^{ns}

***, **, * significant at $p \leq 0.001$, $p \leq 0.01$, and $p \leq 0.05$, respectively; FW: fresh weight

The uptake rate of PO_4^{3-} only increased between 0.001 and 0.018 $\mu\text{mol g}^{-1} \text{FW h}^{-1}$ per $^\circ\text{C}$. Varietal differences in PO_4^{3-} uptake between day and night were found at high VPD, where the increase of PO_4^{3-} uptake per $^\circ\text{C}$ in IR64 was large during the day (0.018 $\mu\text{mol g}^{-1} \text{FW h}^{-1}$

PO_4^{3-} per $^\circ\text{C}$) and not significant during the night, while the opposite trend was observed in NU838 (0.007 and 0.012 $\mu\text{mol g}^{-1} \text{FW h}^{-1} \text{PO}_4^{3-}$ per $^\circ\text{C}$, respectively).

Under low VPD, K^+ uptake rate increased with RZT with a larger increase during the day (0.016 and 0.022 $\mu\text{mol g}^{-1} \text{FW h}^{-1}$ per $^\circ\text{C}$ in IR64 and NU838, respectively) than during the night (0.013 and 0.014 $\mu\text{mol g}^{-1} \text{FW h}^{-1}$ per $^\circ\text{C}$ in IR64 and NU838, respectively). In contrast, at high VPD, no correlation was found between RZT and K^+ uptake with the exception for IR64 at day-time.

A correlation between RZT and water uptake rate was only found under low VPD during the night, when water uptake rate increased by 71.80 and 70.13 $\mu\text{mol g}^{-1} \text{FW h}^{-1}$ per $^\circ\text{C}$ in IR64 and NU838, respectively.

Since N uptake increased more with increasing temperature than uptake of PO_4^{3-} and K^+ , the ratios to N of these nutrients decreased with increasing temperature for both varieties and under both VPDs (data not shown). The $\text{PO}_4^{3-}/\text{N}$ uptake ratio significantly decreased with temperature in both varieties at low VPD, and in IR64 at night-time and NU838 at day-time under high VPD. The K^+/N uptake ratio decreased with temperature under both VPD levels and for both rice varieties. While at 19°C on average 0.30 K^+/N was taken up, at 29°C the ratio was on average 0.24 only.

2.3.3 Nitrogen assimilation in rice leaves at different root zone temperature under low and high VPD

To evaluate the effect of N uptake during day and night on N metabolism, the activities of NR and GS and the AA concentration were determined in leaves of plants under different day and night RZT treatments. Since temperature treatments changed between day and night in order to maintain the same average temperature for all treatments, results in Fig. 2.5 are presented depending on day / night temperature treatment.

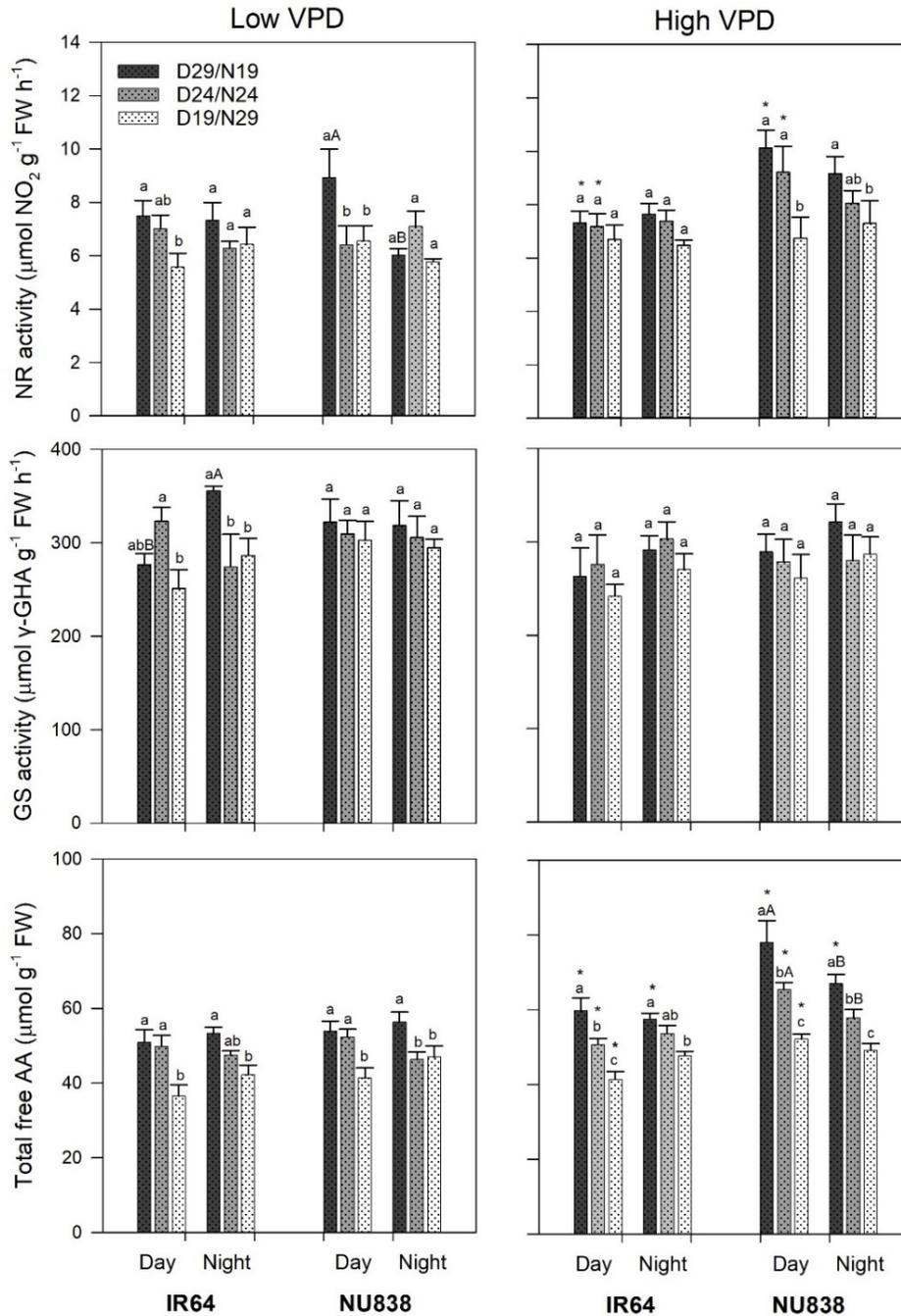


Figure 2.5: Activities of nitrate reductase (NR) and glutamine synthetase (GS), and amino acid (AA) concentration at day- and night-time in the leaves of rice plants at different RZT treatments under low and high VPD. Values presented are means \pm SE (standard error) of leaves sampled at 2 times (mid and end of day or night, respectively) in 3 replications each ($n = 6$). Small letters indicate significant difference between RZT treatments; capital letters indicate significant difference between day and night; asterisks indicate significant difference between varieties, all at $p \leq 0.05$.

Table 2.3: Pearson correlation coefficients between nitrogen (NO_3^- and NH_4^+) uptake rates and amino acid concentration in the leaves for two varieties (IR64 and NU838) at day- and night-time under low and high VPD. Uptake rates are mean values of measurements performed on 7 consecutive days, whereas leaves for measurement of amino acid concentration were harvested at the end this period. The regression analyses were performed for 3 individual plants as replications grown at 3 root zone temperature treatments, each ($n = 9$).

Amino acid concentration	Low VPD		High VPD	
	IR64	NU838	IR64	NU838
<u>NO_3^- uptake rate day</u>				
Day	0.82**	0.80*	0.87**	0.90**
Night	0.76*	0.65*	0.85**	0.94***
<u>NH_4^+ uptake rate day</u>				
Day	0.78*	0.71*	0.85**	0.88**
Night	0.77*	0.72*	0.82**	0.92***
<u>NO_3^- uptake rate night</u>				
Day	-0.69*	-0.74*	-0.81*	-0.79*
Night	-0.83**	-0.74*	-0.71*	-0.86**
<u>NH_4^+ uptake rate night</u>				
Day	-0.81**	-0.82**	-0.88**	-0.84**
Night	-0.75*	-0.62 ^{ns}	-0.75*	-0.89**

***, **, * significant at $p \leq 0.001$, $p \leq 0.01$, and $p \leq 0.05$, respectively; ns: not significant

Under low VPD and for both varieties, a higher NR activity at day-time was found at higher RZT during the day (D29/N19), whereas it did not differ between RZT treatments at night (Fig. 2.5). Under high VPD, RZT treatments did not lead to a significant difference in NR activity in IR64, while in NU838 the highest NR activity at both day and night was observed in D29/N19, followed by plants grown in D24/N24 and D19/N29. Differences in NR activity between varieties were found at high VPD, where leaves of NU838 showed a higher NR

activity than IR64 in D29/N19 and D24/N24 treatments at day-time. Little differences in GS activity between temperature treatments, varieties and day / night were found, with an exception in IR64 under low VPD, where D24/N24 induced a higher GS activity at day and D29/N19 induced a higher GS activity at night.

AA concentrations increased with day temperature / decreased with night temperature in both varieties and both during day and night, with an even larger effect at high VPD (Fig. 2.5). Differences between varieties were observed under high VPD, where NU838 showed a significantly higher AA concentration than IR64 in all temperature treatments during the day and in D29/N19 during the night. Differences in AA concentration between day and night were only observed in NU838, where D29/N19 and D24/N24 led to a higher AA concentration during the day than during the night.

Under both VPD conditions, AA concentrations, both during day and night, were strongly positively correlated with NO_3^- and NH_4^+ uptake rates during the day (Tab. 2.3), but negatively correlated with NO_3^- and NH_4^+ uptake rates during the night.

2.4 Discussion

2.4.1 Day and night nutrient and water uptake under different VPD

Under both VPD conditions, nutrient uptake rates differed between day and night with an exception for PO_4^{3-} . Independent of VPD, rice plants took up more N and K^+ during the day than during the night, which was similar for both varieties. Similarly, *Okuyama et al. (2015)* found higher uptake rates of NO_3^- during the day than during the night for rice. *Matt et al. (2001)* indicated that NO_3^- uptake during the day was 40% higher than during the night, while *Adams (1980)* found a close correlation between light intensity and the uptake of N and K^+ . It has been argued that the decrease of N uptake during the night may be attributed to the reduction of metabolic activity related to the strongly reduced carbohydrate supply from the shoot (*Marschner, 2011*), since the regulation of nitrogen uptake is directly driven by the carbohydrate flux from the shoot to the root rather than carbohydrate concentration of the root (*Rideout and Raper, 1994*). *Delhon et al. (1996)* also reported that NO_3^- uptake during day and night was carbon-dependent since the supply of sugar to the root stimulated NO_3^- uptake and reduction; however, the uptake during darkness was more dependent on sugar availability than in the light. Moreover, carbohydrates synthesized by photosynthesis play an

important role in the expression of nitrogen metabolic enzymes (Vincentz et al., 1993). In the current experiment, the difference in N uptake between day and night in both VPDs was observed at medium (24°C) and high (29°C), but not at low (19°C) RZT. In contrast, the difference between day and night uptake rates of K⁺ was independent of RZT. No significant effects of RZT on water uptake was found, which implies that transpiration rate of rice plants was not affected by different RZTs. In contrast, Kuwagata et al. (2012) found reduced transpiration rates under low RZT, while, Shimono et al. (2004) indicated that cool irrigation water affected photosynthesis and stomatal conductance of rice plants in the reproductive stage, but not in the vegetative stage. Since in our experiment water uptake was not influenced by RZT, we hypothesize that also stomatal conductance and thus assimilation rates and shoot-root carbohydrate fluxes were not affected. Therefore, differences in N uptake between RZTs are rather a direct temperature effect on absorption and transport activities than a result of altered carbohydrate fluxes. Nevertheless, light had a much stronger effect on K⁺ uptake than temperature, which supports the hypothesis of an uptake mechanism dependent on assimilates.

In the current experiment, although the N uptake was lower during the night, night-time absorption still accounted for 46% of the total daily N uptake. In both soil culture and hydroponic experiments, Okuyama et al. (2015) found that plants took up to 40% of the total N during the night regardless of cultivation systems, while Terabayashi et al. (1991) reported that N and K uptake during the night ranged from 30-40% of the daily uptake, depending on season and temperature. In the current experiment, mean RZT between treatments was both 24°C during day and night, resulting in an overestimation of the share of the nutrients taken up during the night in comparison to natural conditions. In combination with the high availability of N in the growth medium, plants maintained a high uptake rate of N during the night.

Under low VPD, PO₄³⁻ uptake did not differ between day and night in the three RZT levels and for both varieties, whereas day and night uptake of PO₄³⁻ under high VPD differed between varieties and RZT levels. Uptake of P in cucumber and tomato was 5 and 6 times higher during the night than during the day (Masuda, 1989). Adams (1980) indicated that the uptake of P was less affected by light intensity but closely related to nutrient solution temperature. Bradbury and Malcolm (1977) found a correlation between the leaf diffusive

resistance and VPD under the combination of high P and K nutrition, which implies that plants with high levels of P and K in the leaves show a more sensitive stomatal response to high VPD. In our experiment, no difference in average PO_4^{3-} uptake was found between VPD levels, but at high VPD. Under high VPD PO_4^{3-} uptake was reduced during the day and increased during the night in IR64 at 19°C and NU838 at 29°C, while IR64 at 29°C induced a higher uptake rate in the day than in the night. Our results show a varietal difference in PO_4^{3-} uptake in response to RZT under high VPD, however, the mechanism is not understood yet.

In the experiment, no correlation between water and nutrient uptake was found during the day in both varieties. Water and nutrient uptake were only correlated in IR64 under low VPD during the night. So far, contrasting results have been reported on the relationship between water and nutrient uptake. *Matsunami et al.* (2013) showed a close correlation between nitrogen and water uptake capacity. While *Houshmandfar et al.* (2018) demonstrated a high correlation between nutrient uptake and transpiration rate, *Mcgrath and Lobell* (2013) reported that a lower transpiration rate decreased the mass flow of nutrients to the roots and thus reduced nutrient uptake. In our experiment, plants were hydroponically grown, and thus, effects of water movement in the soil are excluded. *Shimono and Bunce* (2009) reported a positive correlation between transpiration rate and nitrogen uptake capacity of rice plants grown in hydroponics under both ambient and elevated CO_2 conditions, which has been attributed to the increase in carbohydrate supply for nitrogen uptake due to assimilation, which was correlated with transpiration. In current experiment, the correlation was found in the night, and thus, an increased carbohydrate supply from assimilation can be excluded. *Tanner and Beevers* (2001) found a disassociation between transpiration rate and nutrient uptake of plants grown in hydroponics, which suggests transpiration-independent water flow in the xylem to maintain the long-distance transport of mineral nutrient at low transpiration rates. It has been also claimed that nutrient and water uptake rates are independent, but not at very low transpiration rates (*Brouwer, 1956; Shaner and Boyer, 1976*), a statement that partly matches our results. However, variation among varieties exists, since in NU838 water and nutrient uptake were not correlated even under low VPD during the night. *Houshmandfar et al.* (2018) proposed a mechanism other than mass flow, such as changes in root architecture or function enhancing nutrient uptake into the transpiration stream, which could also explain

the varietal differences in water uptake rate observed in our experiment. However, plants of NU838 were slightly bigger than plants of IR64 and water uptake per plant was higher in NU838. Since the water uptake rate as calculated here decreases with plant size, it is difficult to argue if varietal differences in water uptake rate result from the difference in size or from changes in root characteristics.

2.4.2 Nutrient uptake of two rice varieties as affected by root zone temperature

A significant effect of RZT on nutrient uptake rate was observed for the two varieties, with high correlations between RZT and N uptake rate during both day and night. However, the increase of N uptake with RZT was higher during the day than during the night with an exception of NH_4^+ uptake in NU838 under high VPD. Low water temperature (17°C) significantly reduced N uptake of rice (Jia et al., 2015) while it increased with RZT between 10°C and 20°C in cucumber (Yan et al., 2012). Engels and Marschner (1996) indicated that low RZT (12°C) led to a decrease of N translocation rate in maize. The optimum RZT for nutrient uptake in tomato was found to be 26.7°C (Tindall et al., 1990) and in snapdragon (*Antirrhinum majus*) 22°C (Hood and Mills, 1994). However, in our experiment, the constant increase in N uptake in the observed RZT range ($19\text{-}29^\circ\text{C}$) implies that the optimum RZT for N uptake in rice plants must be above 29°C . With a stronger increase in uptake with temperature during the day, plants benefit more from increasing RZT during the day than during the night, however, genotypic differences were observed.

The difference in nutrient uptake between both varieties was observed at high VPD, with IR64 showing a much stronger temperature response than NU838 during the day, while NU838 showed a stronger temperature response than IR64 during the night. Matsunami et al. (2013) demonstrated a genotypic variation in N uptake capacity of rice plants, but not much is known about the varietal differences in nutrient uptake in response to temperature. The nutrient uptake in higher plants is characterized by selective transport and accumulation, which differs between and within plant species. The composition of root membranes (e.g. lipids and protein) plays a crucial role in the regulation and selectivity of nutrient uptake, however, it is influenced by temperature (Marschner, 2011). Lindberg et al. (2005) indicated the temperature dependence of the lipid composition of the root membrane as well as of ATPase activity. Yan et al. (2012) showed that low RZT significantly reduced nutrient

concentration in shoots but increased nutrient concentration in roots, implying that RZT also affects assimilation and transport of nutrients within the plant. Since the uptake of most of the nutrient is active and energy-dependent (Brouwer, 1956; Marschner, 2011), and further, the varietal difference in both uptake and assimilation of nutrients was only observed at high VPD, while membrane properties are probably not affected by VPD, we hypothesize that it was rather a result of altered nutrient assimilation and transportation capacities than of changes in membranes properties in response to RZT of the two varieties.

In the current experiment, higher RZT significantly increased PO_4^{3-} and K^+ uptake rates, however, the temperature response was less pronounced than for NH_4^+ and NO_3^- . In maize, increasing RZT from 22.4°C to 28.8°C increased P and K shoot uptake by 50% and 40%, respectively (Hussain and Maqsood, 2015), while in cucumber P and K uptake in the shoot were increased by 125% and 26% after increasing RZT from 10°C to 20°C (Yan et al., 2012). However, P and K uptake were not affected by RZT between 10°C and 25°C in barley under limited nutrient supply (Pettersson, 1995). In an experiment on maize using three RZT levels (12, 18 and 24°C), Engels and Marschner (1992) indicated that not only uptake but also translocation of P was rather dependent on RZT than on shoot demand. In our experiment with a RZT range of 19-29°C no cold or heat damage occurred. Hence, it could be clearly shown that under non-stress conditions PO_4^{3-} and K^+ uptake rates were less affected by day or night temperature than N uptake rates that were highly correlated with RZT during both day and night.

Moreover, the ratio of $\text{PO}_4^{3-}/\text{N}$ and K^+/N uptake rates declined with increasing RZT, however, the decrease was higher during the day than during the night. Since a shift in the composition of nutrients taken up could lead to nutritional imbalances within the plant, increasing temperatures could call for adapted fertilizer management strategies in the background of climate change.

2.4.3 Effects of root zone temperature and VPD on N metabolism

In the experiment, plants were subjected to the same daily mean, but different day and night RZTs, which allowed for estimation of the contribution of day and night N uptake on N metabolism of the plants. Under both VPDs and for both varieties, no difference in enzymes activities between day and night was observed, however, there was a significant effect of RZT treatment. In general, the D29/N19 treatment induced higher enzymes activities and

higher AA concentration in the leaves, followed by D24/N24 and D19/N29. Several studies showed the relationship between N uptake and NR and GS activities. While *Eilrich and Hageman* (1973) reported a positive correlation between NR activity and NO_3^- content, *Shaner and Boyer* (1976) showed that the root-shoot NO_3^- flux played a much larger role in regulating NR activity than leaf NO_3^- content. Regarding day and night activity, *Li et al.* (2016) also found a higher level of NR activity during the day, since its activity is regulated by the light/dark cycle. Short-term treatment of rice with cold water led to a decrease of AA, while the GS activity increased, but declined afterward (*Jia et al.*, 2015). In our experiment, NO_3^- uptake increased with RZT both day and night, but no effect of RZT on day and night N metabolism was observed. Furthermore, no correlation was found between N uptake and NR or GS activity (data not shown). N uptake increased with increasing RZT at a higher rate during the day than during the night, resulting in a higher daily N uptake in the D29/N19 treatment than in the D19/N29 treatment. Therefore, the results support the hypothesis of *Fan et al.* (2007), who proposed that NO_3^- can be stored in cellular pools and remobilized later. *Li et al.* (2016) reported no significant difference in activity of GS between day and night. High night temperature increased GS activity in the leaves of rice at high N supply, but decreased GS activity at low N supply, implying that the temperature response of GS activity depends on the N status of the plant (*Chen et al.*, 2013). *Schjoerring et al.* (2002) demonstrated that a significant amount of NH_4^+ absorbed or assimilated in the roots can be translocated via the xylem to the leaves, however, the process is influenced by temperature. Thus, at higher temperature, NH_4^+ concentration in the xylem was decreased while NH_4^+ flux was increased due to higher transpiration, leading to increased GS activity in the leaves. In our experiment, NH_4^+ uptake rate and GS activity did not differ between VPD levels, implying that the NH_4^+ flux in the rice plants was not driven by transpiration. Moreover, GS activities in the leaves, both during day and night, were not significantly different between RZT treatments, indicating that root temperature did not directly impact on NH_4^+ flux. *Kirk* (2001) and *Kirk and Kronzucker* (2005) demonstrated that lowland rice can enhance nitrification and NO_3^- uptake at the root surface due to oxygen transport away from the root. However, when both, NH_4^+ and NO_3^- , were provided, the presence of NO_3^- in the nutrient solution enhanced NH_4^+ flux as well as NH_4^+ assimilation and cytosolic NH_4^+ accumulation within the rice roots (*Kirk and Kronzucker*, 2005). In our experiment, NH_4^+ uptake was not

measured directly, but it was calculated from the reductions of NH_4^+ concentration and nutrient solution volume. However, as both N sources were supplied while the nutrient solution was renewed every 12 hours, we assume that nitrification at the root surface did not affect NH_4^+ flux. Since, in our case, also NH_4^+ uptake did not directly stimulate GS activity, a similar mechanism for NO_3^- seems likely. Furthermore, we suggest that N metabolism does not respond immediately to N uptake during day or night, but rather depends on total daily N uptake.

In contrast, AA concentration in the leaves increased with higher RZT during the day and the high concentration was still maintained during the night in the treatments when RZT declined. Under both VPDs and for both varieties, AA concentration both during day and night was strongly correlated with NH_4^+ and NO_3^- uptake during the day. At high nitrogen supply, excess AA are stored mainly in the vacuole of young leaves (*Tilsner et al., 2005*). Results in our study showed a relatively high AA concentration in the leaves in comparison to previous studies in rice (*Hsu and Kao, 2003; Zhong et al., 2018*), reflecting a high nitrogen uptake and metabolism. Here we hypothesize that N taken up during the day, when more energy is available to the plant, is immediately used for AA built-up and storage, whereas N taken up during the night is mainly stored as NO_3^- .

Differences between varieties were observed at high VPD, where NU838 showed a higher NR activity at D29/N19 and D24/D24 during the day and a higher AA concentration in all temperature treatments during the day and at D29/N19 during the night, which demonstrated a higher N assimilation capacity of the leaves of NU838 at high VPD. It has been demonstrated that rice varieties show a wide variation in NR activity, and low NR activity may lead to an excessive accumulation of NO_3^- in the plant tissues (*Basuchaudhuri, 2016*), but if the lower AA concentration in the leaves of IR64 in comparison to NU838 is a result of lower NR activity and is thus associated with NO_3^- accumulation, cannot be answered here, as NO_3^- concentration has not been measured. Since differences in the preferential site of NO_3^- assimilation, i.e. shoot or root, exist (*Marschner, 2011*), IR64 might preferentially assimilate AA in the roots. However, because NU838 outperformed IR64 in terms of biomass (data not shown), a higher N assimilation capacity of this variety seems likely.

2.5 Conclusion

While nutrient uptake linearly increased with RZT in the observed temperature range and hardly changed with VPD, water uptake mainly depended on VPD and was not influenced by RZT. A correlation between water and nutrient uptake was only found at very low water uptake rates and depended on variety. Since NO_3^- and NH_4^+ uptake increased faster with RZT than PO_4^{3-} and K^+ , the ratio between the nutrients shifted with temperature. Therefore, RZT increases related to water management strategies or climate change will require nutrient management adaptations under field conditions. However, varietal differences exist, but was only exhibited under high VPD. Here, IR64 showed a stronger temperature response than NU838 during the day, while during the night, the opposite was observed. Since with the introduction of water-saving irrigation measures different effects on soil temperature during day and night are expected, choice of variety is an important factor for optimal nutrient uptake. For NR and GS activities rather the total daily N uptake than the uptake during either day or night seems to be relevant, whereas AA concentration both at day-time and night-time was closely related to the N uptake during the day. NU838 showed a higher NR activity and AA concentration the leaves at higher RZT under high VPD than IR64, and a higher N assimilation capacity might explain the higher biomass of this variety. As only root but not air temperature was altered in this experiment, we hypothesize that increases in nutrient uptake with temperature were not a result of changes in carbohydrate fluxes, but rather a direct effect like either root membrane composition and ATPase activity or, more likely, assimilation and transport of nutrients in the roots. Since depending on RZT, a large share of N can be taken up during the night, the question remains how N taken up during the night is transported, stored and used by the plant.

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Chapter 3

Leaf Gas-Exchange of Lowland Rice in Response to Nitrogen Source and Vapor Pressure Deficit*

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Abstract

In anaerobic lowland fields, ammonium (NH_4^+) is the dominant form of nitrogen (N) taken up by rice plants, however, with the large expansion of water-saving irrigation practices, nitrification is favored during drained periods, leading to an increased availability of nitrate (NO_3^-). Since the uptake and assimilation of the two N sources differ in their demand of photosynthates, leaf gas exchange may be subject to adjustments in response to N sources, particularly at high evaporative demand, where stomatal conductance (g_s) is very sensitive. Three experiments were carried out to study leaf gas exchange of various lowland rice varieties in response to N source at low and high vapor pressure deficit (VPD). In the first experiment, seedlings of 12 rice varieties were grown at high VPD for 3 weeks. From this, four rice varieties differing in g_s and CO_2 assimilation rate (A) were selected and grown for 2 weeks at low VPD and after that, they were shifted to high VPD for 1 week whereas in the third experiment the same varieties were grown separately at low and high VPD conditions for 2 weeks. In all three experiments, plants were grown hydroponically in nutrient solution with N sources as sole NH_4^+ or NO_3^- . At high VPD, NO_3^- nutrition led to a higher g_s and A in four out of 12 varieties (IR64, BT7, NU838, and Nipponbare) relative to NH_4^+ nutrition, while no effect was observed at low VPD or after a short-term exposure to high VPD. Further, varieties with a high intrinsic water-use efficiency - WUEi (IR64 and BT7) showed the strongest response to N source. Higher g_s was partially supported by increased root/shoot ratio, but could not be fully explained by the measured parameters. However, higher A in NO_3^- -fed plants did not always result in increased plant dry matter, which is probably related to the higher energy demand for NO_3^- assimilation. Our results suggest that at high VPD,

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NO_3^- nutrition can improve leaf gas exchange in varieties having a high WUE_i , provided a sufficient water supply. Therefore, intensified nitrification under water-saving irrigation measures may improve leaf gas exchange and the growth of rice plants under high transpirational demand. However, choice of variety seems crucial since large varietal differences were observed in response to N source. Further, breeding strategies for genotypes adapted to aerobic soil conditions should consider responses to NO_3^- , potentially using gas exchange measurements as a screening tool.

Key words: assimilation rate, ammonium, nitrate, nitrification, stomatal conductance, water-saving irrigation.

3.1 Introduction

Ammonium (NH_4^+) and nitrate (NO_3^-) are the two main nitrogen (N) forms in the soil. While in anaerobic lowland rice fields NH_4^+ is predominant, nitrification is favored in aerobic soils and, thus, NO_3^- becomes the dominant N form (Buresh et al., 2008). Chunmei et al. (2020) showed that water-saving irrigation significantly increased the NO_3^- content in the soil due to improved soil aeration, while continuous flooding increases the NH_4^+ content. However, some studies demonstrated the existence of substantial quantities of NO_3^- in the rhizosphere even in flooded soils caused by nitrification due to the release of oxygen from the roots (Kirk, 2001; Kirk and Kronzucker, 2005). Therefore, rice plants take up both, NH_4^+ and NO_3^- , with the ratio of the two N forms depending on the root-associated nitrification activity (Briones et al., 2003; Kirk, 2001; Li et al., 2007) and the nitrification in the soil (Buresh et al., 2008; Chunmei et al., 2020). When both N sources are provided in equal concentrations in the nutrient solution, rice plants preferentially take up NH_4^+ but still, up to 43% of total N is taken up as NO_3^- (Vu et al., 2020). Kirk and Kronzucker (2005) showed that rice roots are exceptionally efficient in the uptake and assimilation of NO_3^- , indicating that the uptake of NO_3^- in lowland rice might be comparable to that of NH_4^+ . With the large expansion of water-saving irrigation technologies, nitrification is intensified during drained periods, consequently, plants may take up a larger share of N as NO_3^- compared to NH_4^+ .

The energy costs for uptake, transport, and assimilation differ between the two N forms with higher costs for NO_3^- relative to NH_4^+ . The total costs of NO_3^- uptake, assimilation, and transport are 32 mol photons mol^{-1} N, while the costs for NH_4^+ are only 9.5 mol photons mol^{-1}

^{15}N (Raven, 1985). NH_4^+ supplied to plants would save photo-energy equivalents of 10 mol ATP mol $^{-1}$ N compared to NO_3^- (Salsac et al., 1987). In barley, NO_3^- -fed plants required up to 23% of the energy from root respiration for its absorption and assimilation, while only 14% was needed in NH_4^+ -fed plants (Bloom et al., 1992), demonstrating the greater demand of photosynthates for the uptake and reduction of NO_3^- relative to NH_4^+ . Moreover, NO_3^- and NH_4^+ are assimilated at different sites, which may lead to differences in the partitioning of photosynthates within the plant. Ammonium is predominantly assimilated into organic compounds in the root, whereas NO_3^- is readily transported and distributed throughout the plant (Marschner, 2011), therefore, NH_4^+ nutrition results in greater depletion of carbohydrates in the root (Bowman and Paul, 1988). However, in sugar beets, NH_4^+ treatments also lowered concentrations of starch, sucrose, and maltose in the leaves in comparison to NO_3^- treatments, due to carbon skeleton consumption in NH_4^+ assimilation (Raab and Terry, 1995).

Since N forms differ in their assimilation pathways and their demand of photosynthates, leaf gas exchange of plants may be subject to adjustments in response to N source. Previous studies showed that N source influenced photosynthesis, photorespiration, and water uptake of plants, however, variation among species has been reported (S. Guo, Zhou, Shen, & Zhang, 2007). For instance, NO_3^- nutrition increased CO_2 assimilation rate (A) and stomatal conductance (g_s) compared to NH_4^+ in cucumber (Zhou et al., 2011), whereas the opposite was reported for French bean (S. Guo et al., 2002) and tomato (Horchani et al., 2010). In tobacco, Lu et al. (2005) showed a reduction in g_s and transpiration rate caused by NH_4^+ compared to NO_3^- and $\text{NH}_4^+/\text{NO}_3^-$.

Lowland rice is considered NH_4^+ tolerant and measured photosynthetic parameters did not differ between plants fed with different N sources in previous studies under well-watered conditions (S. Guo et al., 2008; Ji and Peng, 2005; Li et al., 2009; Zhou et al., 2011). However, NO_3^- nutrition combined with secondary water stress, induced with polyethylene glycol (PEG), resulted in reduced g_s , A, and water consumption in comparison to NH_4^+ nutrition (Ding et al., 2015; S. Guo et al., 2008). In contrast, Zhong et al. (2018) showed that the acclimation of photosynthesis to water stress in rice plants could be improved by the uptake and assimilation of NO_3^- , since NO_3^- and amino acids could serve as osmotically active substances maintaining leaf turgor, however, varietal differences were observed. The

effect of N source on photosynthesis has been attributed to the adjustment of g_s with subsequent effects of the CO_2 supply to chloroplasts (S. Guo et al., 2008; Torralbo et al., 2019). However, g_s (Ohsumi et al., 2008) and water uptake capacity (Vu et al., 2020) of rice varieties are highly sensitive to vapor pressure deficit (VPD), hence, the effect of N source on leaf gas exchange of rice may differ with changes in VPD and between varieties. Moreover, global VPD increased strongly after the late 1990s due to global warming (Grossiord et al., 2020; Yuan et al., 2019) and it is projected to consistently increase throughout the current century (Yuan et al., 2019), therefore, the effect of N form on leaf gas exchange of rice plants should be considered under varying VPD conditions.

With the intensification of nitrification during drained periods in water-saving irrigation practices, rice plants take up a larger share of N as NO_3^- , which may increase the demand of photosynthates for the uptake and assimilation of N. Since varietal differences in both CO_2 assimilation and N uptake and assimilation can be expected, particularly at high VPD, the objectives of this study were: (1) to assess the range of varietal responses to N source in terms of leaf gas exchange; (2) to investigate the short- and long-term effect of VPD on the response of leaf gas exchange to N source; and (3) to examine effects of N source on dry matter (DM) production and partitioning in relation to the measured photosynthetic parameters.

3.2 Materials and methods

3.2.1 Plant material

Nine common local varieties differing in genetic background and growth characteristics were selected. Seeds were provided by the Vietnam National University of Agriculture (VNUA) and the Vietnam National Seed Group (Vinaseed group). Additionally, three varieties (IR64, Nipponbare, and Nerica 4) were used as international check varieties in the study. IR64 was provided by the International Rice Research Institute (IRRI), while Nipponbare and Nerica 4 were provided by the Africa Rice Center (AfricaRice). Information on their genetic background and growth characteristics of the 12 varieties are given in Tab. 3.1.

3.2.2 Treatments and growth conditions

Three experiments were carried out in the greenhouse at the Institute of Agricultural Sciences in the Tropics, the University of Hohenheim, Germany.

Table 3.1: Genetic background and growth characteristics of 12 rice varieties used in the study.

N°	Variety	Subspecies	Type	Origin	Characteristic
1	IR64	Indica	Lowland, inbred variety	Philippines	High yield, early maturity, susceptible to abiotic stress and commonly used as an international check variety (<i>Mackill and Khush, 2018</i>)
2	Bac thom 7 (BT7)	Indica	Lowland, inbred variety	Vietnam	Popular in the North of Vietnam, aromatic, good tillering capacity, and moderate lodging resistance (<i>Pham et al., 2009</i>)
3	Khang dan 18 (KD18)	Indica	Lowland, inbred variety	Vietnam	Popular in the North of Vietnam, moderate tillering capacity, thick and large leaves, and moderate lodging resistance (<i>Pham et al., 2009</i>).
4	Jasmine 85	Indica	Lowland, inbred variety	Vietnam	Popular in the South of Vietnam, moderate tillering, susceptibility to drought, and lodging resistance (<i>Pham et al., 2009</i>).
5	Bao thai	Indica	Lowland, local variety	Vietnam	Popular in the mountainous area. Late maturity, photoperiod sensitivity (*).
6	Khau nam xit	Indica	Lowland, local variety	Vietnam	Local variety in the mountainous area, large leaves and tall plant type (*).
7	Seng cu	Indica	Lowland, local variety	Vietnam	Local variety in the mountainous area, large leaves (*).
8	Nhi uu 838 (NU838)	Indica	Lowland, hybrid variety	China	Popular in the North of Vietnam, high yield, good tillering, large leaves, lodging resistance and cold tolerance (<i>Pham et al., 2009</i>).
9	TH3-4	Indica	Lowland, hybrid variety	Vietnam	High yield, good tillering capacity, large leaves, and lodging resistance (<i>Pham et al., 2009</i>).
10	J02	Japonica	Lowland, inbred variety	Vietnam	Newly introduced, high yield, moderate tillering, and moderate lodging resistance (*).
11	Nipponbare	Japonica	Lowland, inbred variety	Japan	Modern, photoperiod sensitive variety. Has been used as japonica check variety (<i>Hirooka et al., 2018</i>).
12	Nerica 4	Interspecific (<i>O. sativa</i> x <i>O. glaberrima</i>)	Upland, inbred variety	Africa Rice	High yield, good tillering, and good response to fertilizers (<i>Kinyumu, 2009</i>).

* information from local Agriculture Extension Centres.

For all 3 experiments, seeds were germinated in the dark. Germinated seeds were transferred to cleaned (rinsed/washed) sand and irrigated with tap water during the first 4 days and then, irrigated with 50% standard YOSHIDA nutrient solution with both N sources as NH_4NO_3 (Yoshida et al., 1976) for the following 4 days. After that, seedlings were transferred to plastic boxes (40 x 30 x 12 cm) containing 8.5 L of 100% standard YOSHIDA nutrient solution at a density of 60 seedlings per box. In this box, seedlings remained for another 10 days.

To provide different N sources, modified nutrient solutions containing 2.86 mM N in the forms of sole NH_4^+ as $(\text{NH}_4)_2\text{SO}_4$ or NO_3^- as $\text{Ca}(\text{NO}_3)_2$ and KNO_3 were prepared. Other macro- and micro-nutrients were provided in equal amounts in both N source treatments in the following concentrations: 0.32 mM P, 1.0 mM K, 1.0 mM Ca, 1.6 mM Mg, 35.6 μM Fe-EDTA (Ferric - Ethylenediaminetetraacetic Acid), 9.5 μM Mn, 18.9 μM B, 0.15 μM Zn, 0.16 μM Cu, 0.52 μM Mo. To inhibit nitrification and ensure identical conditions, 7 μM Dicyandiamide ($\text{C}_2\text{H}_4\text{N}_4$) was added to both N treatments (Duan et al., 2006).

All three experiments were conducted with four replications per treatment and artificial light was provided with 12 h photoperiod. The position of boxes and pots was interchanged every day to eliminate the edge effects. Ambient temperature and relative air humidity (RH) were monitored every 10 min with Tinytag data loggers (Gemini data loggers Ltd., UK), and VPD was calculated. Nutrient solution was renewed every 7 days, while its pH was monitored and adjusted daily to a value of 5.5 ± 0.1 using 1N NaOH or HCl.

Experiment 1 – Genotypic variation

To evaluate the variation in leaf gas exchange of 12 varieties in response to N source, the first experiment was carried out at “standard” greenhouse conditions, without additional humidification or dehumidification. Due to the high air temperature and low RH, it is considered as “high VPD”, with average day and night VPD at 2.0 and 0.9 kPa, respectively. Seedlings were transferred to boxes (20 x 15 x 12 cm; 12 plants, each) containing 4.5 L of modified nutrient solution. Leaf gas exchange was measured 3 weeks after the onset of N source treatment, and then plants were harvested for dry matter determination.

Four rice varieties (IR64, BT7, KD18, and Jasmine 85) showing different responses in leaf gas exchange to the different N sources in experiment 1, were selected and examined in experiments 2 and 3, which were conducted in self-constructed growth chambers with the

possibility to manipulate VPD. After pre-cultivation as described above, individual plants were grown in pots containing 1 L of modified nutrient solution.

Experiment 2 – Acclimation to low VPD

To estimate the effect of a sudden increase in VPD on leaf gas exchange (experiment 2), plants were grown at low VPD (0.7/0.2 kPa day/night) for the first 2 weeks and after, air was dehumidified to increase the VPD (2.4/1.9 kPa day/night) for 1 week. Leaf gas exchange was measured shortly before and 1 week after increasing the VPD.

Experiment 3 – Acclimation to high VPD

The effect of acclimation to high VPD was studied in Experiment 3, where plants were grown in separated chambers differing in VPD. Plants were either exposed to low VPD (0.6 kPa and 0.4 kPa during day and night) or to high VPD (2.1 and 1.8 kPa during day and night) for 2 weeks. After that, leaf gas exchange was measured.

In Experiment 3, the day after gas exchange measurements, the same leaves were sampled during the last hours of the light period. The samples were quickly frozen in liquid nitrogen and stored at -80°C . Starch concentration was determined with the method described in *Pieters et al. (2001)*. Test tubes of 1.5 ml containing 0.47 cm^2 frozen leaf and 0.5 mL of 96% ethanol were incubated at 70°C for 30 min. The ethanolic extract was collected and extraction was repeated once to remove sucrose. The remaining leaf material was rinsed with distilled water and ground to a fine powder in liquid nitrogen using plastic pestles. A 0.5 ml of 50 mM sodium acetate (pH 4.8) containing $1\ \mu\text{l}$ α -amylase and $1\ \mu\text{l}$ amyloglucosidase per sample were added and samples were incubated at 37°C for 36 hours. An extraction of $40\ \mu\text{l}$ was loaded in 96 well-plates with $200\ \mu\text{l}$ assay buffer (100 mM imidazole pH 6.9, 10 mM MgCl_2 , 33 mM ATP, 12.5 mM NADP). Finally, 1.0 unit of hexokinase was added. Quantification of starch was based on the production of nicotinamide adenine dinucleotide hydrogen phosphate (NADPH) at 340 nm using a microplate reader Infinite (Tecan).

Roots, dead leaves, and green leaf sheaths and blades were separated and oven-dried at 70°C until constant weight for dry matter determination. Specific leaf area (SLA) was calculated as total green leaf area divided by its dry weight.

3.2.3 Gas exchange measurement

g_s and A were measured on the youngest fully expanded leaf of the main tiller the following day after replacement of the nutrient solution. Measurements were performed with a portable gas exchange system GFS-3000 (Heinz Walz GmbH, Germany). In the cuvette, the temperature was set to 30°C in all three experiments, while light intensities were set at 1500 and 1200 $\mu\text{mol m}^{-2} \text{s}^{-1}$ photosynthetic photon flux density (PPFD; using LED light) in the first and second experiments, respectively, or followed ambient light (700 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD) in experiment 3. Humidity in the cuvette was adjusted to create a similar VPD as outside the cuvette. Growth environment and cuvette environment are summarized in Tab. 3.2. Intrinsic water-use efficiency (WUEi) was defined and calculated as A/g_s ($\mu\text{mol mmol}^{-1}$) (Rashid et al., 2018).

3.2.4 Data analysis

Data were analyzed with Statistical Analysis System (version 9.4). In experiment 2 and 3, data analysis was performed for each VPD separately, as VPD was not included as a factor due to the lack of degree of freedom. A two-way analysis of variance (ANOVA) in a linear model (PROC MIXED) was used to examine the effect of variety, N source, and their interaction on measured parameters. Means were separated using least significant difference test at $p < 0.05$.

3.3 Results

In Experiment 1, g_s ranged from 247 $\text{mmol m}^{-2} \text{s}^{-1}$ in Khau nam xit fed with NH_4^+ to 446 $\text{mmol m}^{-2} \text{s}^{-1}$ in KD18 fed with NH_4^+ (Fig. 3.1A). NO_3^- nutrition led to significantly higher g_s than NH_4^+ in IR64, BT7, NU838, and Nipponbare, while for the other varieties, N form did not affect g_s . The largest difference in g_s between plants fed with different N sources was observed in BT7, which showed a 31% higher g_s fed with NO_3^- than with NH_4^+ , while the smallest differences were observed in KD18 and Bao thai.

Table 3.2: Growth conditions and gas exchange measurement conditions.

Experiment		Growth conditions					Measurement conditions			
		Air temp. (°C)		VPD (kPa)		Light ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	VPD (kPa)	Temp. (°C)	Light ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	CO ₂ (ppm)
		Day	Night	Day	Night					
1 st experiment	High VPD	29.6	21.1	2.0	0.9	1200	2.0	30	1500	400
2 nd experiment	Low VPD	31.7	23.7	0.7	0.2	700	1.1	30	1200	400
	High VPD	31.1	25.5	2.4	1.9	700	2.5	30	1200	400
3 rd experiment	Low VPD	30.0	24.5	0.6	0.4	700	0.9	30	700	400
	High VPD	28.8	25.0	2.1	1.8	700	2.5	30	700	400

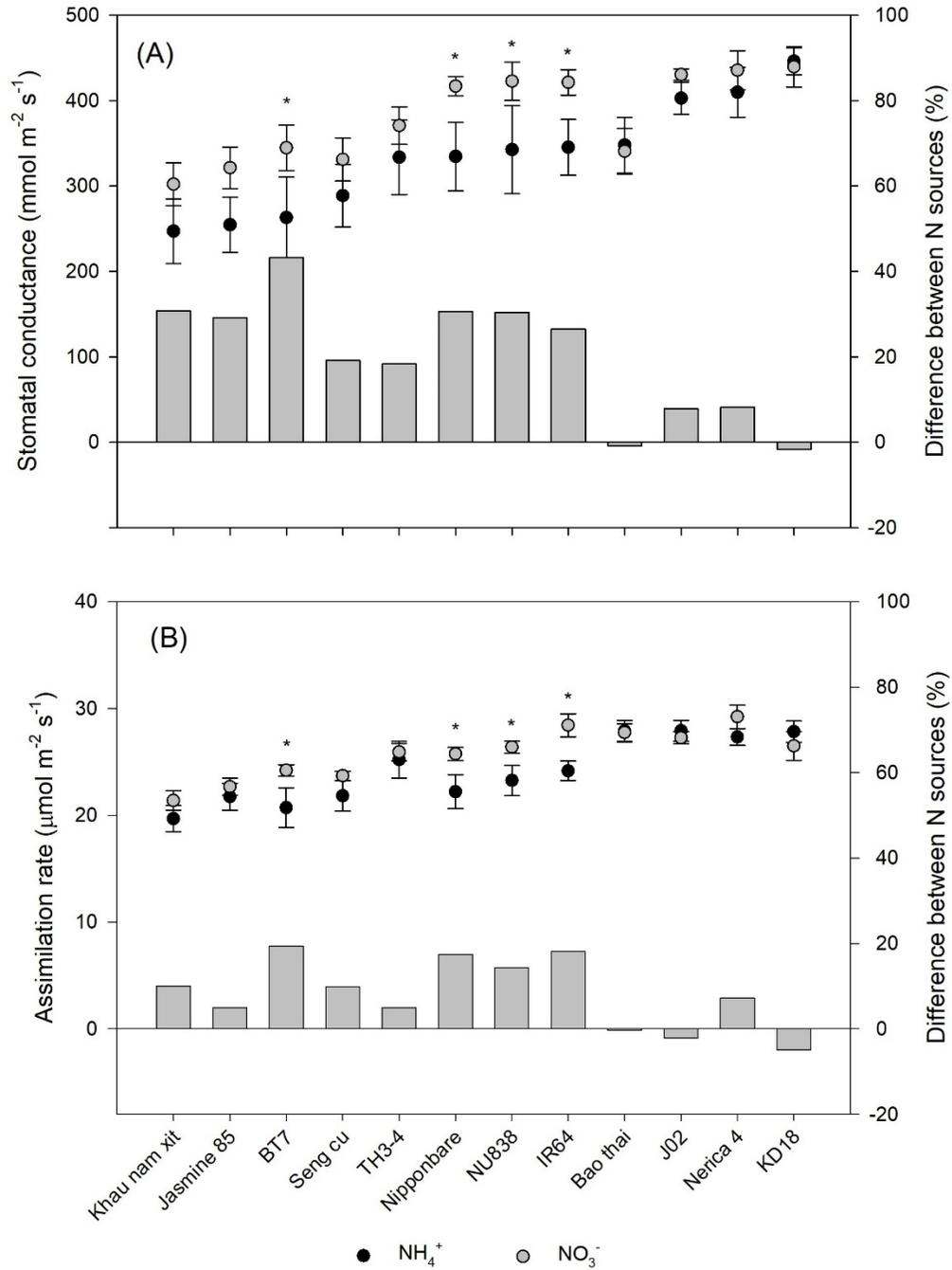


Figure 3.1: Stomatal conductance (A) and assimilation rate (B) of 12 rice varieties in response to nitrogen source (NH_4^+ or NO_3^-) at high VPD (“standard” greenhouse conditions; experiment 1). Bars show the difference (%) between NO_3^- and NH_4^+ treatments. Error bars indicate standard error of means (n = 4); * indicates significant differences between N-sources at $p < 0.05$.

There was genotypic variation in A , ranging from 19.7 to $29.2 \mu\text{mol m}^{-2} \text{s}^{-1}$ in Khau nam xit fed with NH_4^+ and Nerica 4 fed with NO_3^- , respectively (Fig. 3.1B). Significant differences in A between N forms were found in the same varieties, that already showed significant differences in g_s , IR64, BT7, NU838, and Nipponbare. The largest difference in A between plants fed with different N sources was observed in BT7, which showed a 19% higher A in NO_3^- than in NH_4^+ , followed by IR64 with 18%. In KD18, A was with $27.9 \mu\text{mol m}^{-2} \text{s}^{-1}$ slightly higher in NH_4^+ than in NO_3^- with $26.5 \mu\text{mol m}^{-2} \text{s}^{-1}$. A high positive correlation between g_s and A was found in all varieties (see appendix, Fig. 3.7).

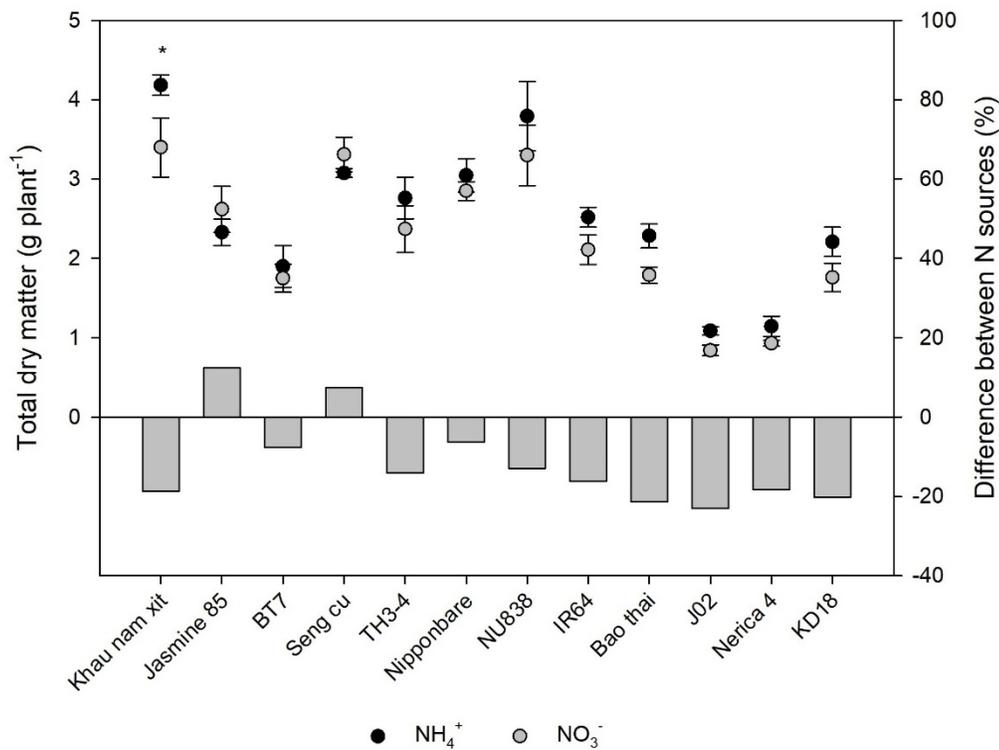


Figure 3.2: Total dry matter of 12 rice varieties in response to nitrogen source (NH_4^+ or NO_3^-) at high VPD (“standard” greenhouse conditions; experiment 1). Bars show the difference (%) between NO_3^- and NH_4^+ treatments. Error bars indicate standard error of means ($n=4$); * indicates significant differences between N sources at $p < 0.05$.

Total DM ranged from 0.84 to $4.19 \text{ g plant}^{-1}$ in J02 fed with NO_3^- and Khau nam xit fed with NH_4^+ , respectively (Fig. 3.2). Over all varieties, DM was on average $2.25 \text{ g plant}^{-1}$ significantly lower under NO_3^- nutrition than under NH_4^+ nutrition with $2.53 \text{ g plant}^{-1}$. However, looking at varieties individually, NO_3^- nutrition significantly reduced DM in Khau Nam xit alone.

Table 3.3: Stomatal conductance (g_s) and assimilation rate (A) of four rice varieties at low VPD and after one week exposure to high VPD, and the difference in g_s and A between low and high VPD (%) (Experiment 2). Values are means of 4 replications. Small letters indicate significant differences between N sources, while capital letters indicate significant differences between varieties ($p < 0.05$).

Rice variety	N source	Stomatal conductance ($\text{mmol m}^{-2} \text{s}^{-1}$)			Assimilation rate ($\mu\text{mol m}^{-2} \text{s}^{-1}$)		
		Low VPD	High VPD	(%)*	Low VPD	High VPD	(%)*
IR64	NH_4^+	749.0	B 329.5	b B -56.0	19.3	B 16.9	B -12.6
IR64	NO_3^-	786.5	B 435.0	a B -44.7	19.4	B 18.4	A -5.0
BT7	NH_4^+	700.5	B 273.1	B -61.0	19.2	B 13.4	C -29.9
BT7	NO_3^-	701.7	B 302.9	C -56.8	18.3	B 12.5	B -31.6
KD18	NH_4^+	1166.0	A 546.7	A -53.1	23.3	A 19.3	A -17.0
KD18	NO_3^-	1279.3	A 601.1	A -53.0	21.4	A 18.1	A -15.3
Jasmine 85	NH_4^+	911.4	B 482.7	A -47.0	19.5	B 17.2	a B -12.0
Jasmine 85	NO_3^-	1048.8	A 503.2	B -52.0	18.4	B 12.3	b B -33.0

* Difference between low and high VPD

Under low VPD in Experiment 2, N form did not influence g_s or A significantly but varietal differences in g_s and A were found (Tab. 3.3). One week after shifting the plants from low to high VPD, g_s was strongly decreased in all varieties and for both N sources (53% on average). At high VPD, NO_3^- nutrition significantly increased g_s in IR64, as compared to NH_4^+ -fed plants. At both VPD levels and for both N sources, KD18 showed the highest g_s , followed by Jasmine 85, IR64, and BT7. Shifting plants to high VPD also resulted in a strong decrease in A in all varieties and for both N sources (19% on average). Reductions of more than 30% in A were observed in BT7 under both N sources and in Jasmine 85 under NO_3^- nutrition, whereas a 5% reduction was observed in NO_3^- -fed plants of IR64. At high VPD, A only differed in plants fed with different N sources in Jasmine 85, where NO_3^- strongly reduced A relative to NH_4^+ .

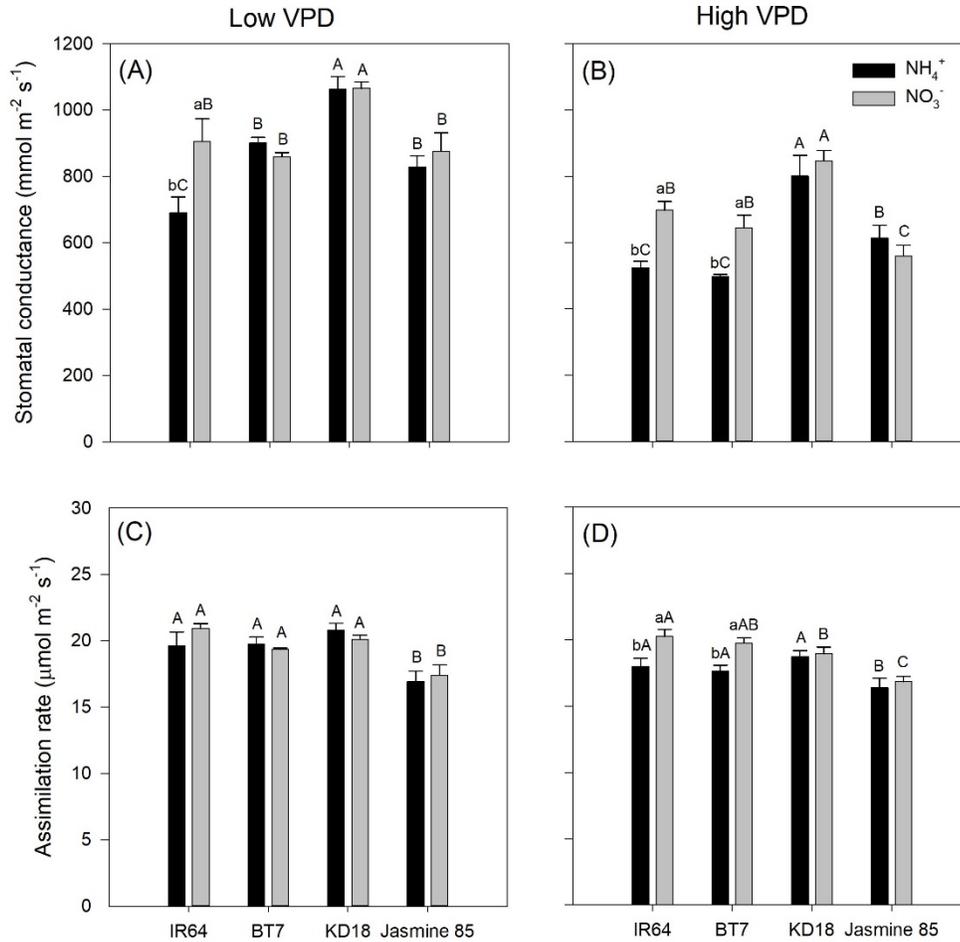


Figure 3.3: Stomatal conductance (A, B) and assimilation rate (C, D) of four rice varieties fed with different N sources (NH₄⁺ or NO₃⁻) at low and high VPD (Experiment 3). Columns and error bars show mean values and standard errors of 4 replications. Small letters indicate significant differences between N sources, while capital letters indicate significant differences between varieties, all at $p < 0.05$.

g_s and A of four rice varieties grown at low and high VPD are presented in Figure 3.3 (Experiment 3). At low VPD, N source only had an effect on IR64, where NO₃⁻ induced a higher g_s than NH₄⁺. In contrast, at high VPD, NO₃⁻ nutrition led to a significantly higher g_s and A in IR64 and BT7, whereas, no effect of N source was observed in KD18 and Jasmine 85. At both VPD levels and in both N sources, KD18 showed the highest g_s .

Comparing g_s and A between VPD levels, both parameters were lower under high VPD with a larger difference in g_s than in A. In comparison to low VPD, g_s and A were on average 26% and 5% lower at high VPD, respectively. The difference between VPD levels was larger in plants fed with NH₄⁺ than with NO₃⁻. While in NH₄⁺, g_s and A were 31% and 8% lower at

high VPD than at low VPD, they were 22% and 3% lower when fed with NO_3^- .

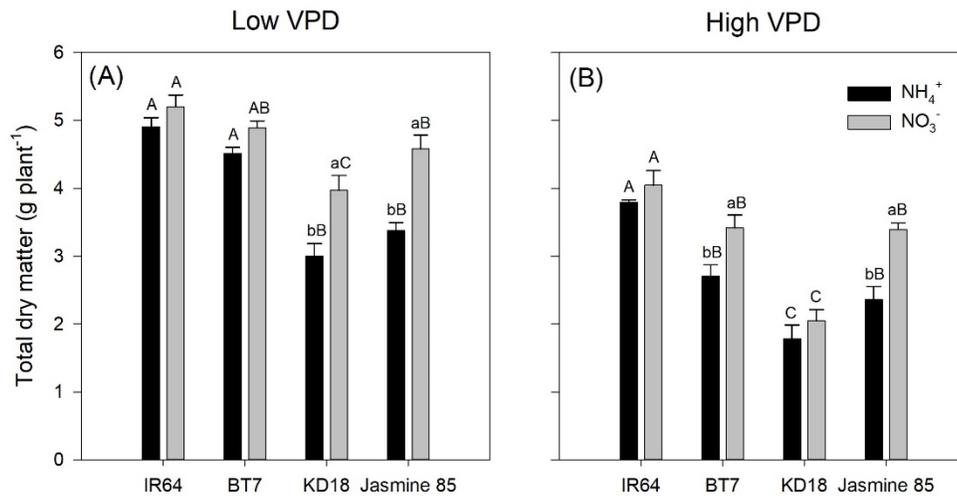


Figure 3.4: Total DM of four rice varieties fed with different N sources (NH_4^+ or NO_3^-) at low and high VPD (A, B) (Experiment 3). Columns and error bars show means and standard errors of 4 replications. Small letters indicate significant differences between N sources, while capital letters indicate significant differences between varieties, all at $p < 0.05$.

At low VPD, total DM of IR64 and BT7 did not differ between N sources, whereas NH_4^+ nutrition led to lower total DM in KD18 and Jasmine 85 relative to NO_3^- (Fig. 3.4A). At low VPD and for both N sources, the highest DM was found in IR64 and BT7 (4.9 and 5.2 g plant^{-1} in IR64, and 4.5 and 4.9 g plant^{-1} in BT7 under NH_4^+ and NO_3^- , respectively), followed by Jasmine 85 (3.8 and 4.6 g plant^{-1} under NH_4^+ and NO_3^- , respectively) and KD18 (3.0 and 4.0 g plant^{-1} under NH_4^+ and NO_3^- , respectively). At high VPD, total DM of IR64 and KD18 did not differ between N sources, while NH_4^+ led to a lower DM in BT7 and Jasmine 85 relative to NO_3^- (Fig. 3.4B). At high VPD and for both N sources, the highest DM was observed in IR64 (3.8 and 4.1 g plant^{-1} under NH_4^+ and NO_3^- , respectively), followed by BT7 (2.7 and 3.4 g plant^{-1} under NH_4^+ and NO_3^- , respectively), Jasmine 85 (2.4 and 3.4 g plant^{-1} under NH_4^+ and NO_3^- , respectively), and the lowest DM was found in KD18 (1.8 and 2.1 g plant^{-1} under NH_4^+ and NO_3^- , respectively).

At low VPD, a significant difference in root/shoot (R/S) ratio between plants fed with different N forms was observed in KD18 alone, where NO_3^- nutrition led to a higher R/S ratio (0.33 g/g) than NH_4^+ (0.29 g/g ; Fig. 3.5A). At high VPD, NO_3^- significantly increased R/S

ratio in all varieties but not in KD18 (Fig. 3.5B). The largest increase was found in BT7, where R/S ratio was 0.19 g/g in NH_4^+ and 0.27 g/g in NO_3^- . At both VPD levels and with both N sources, IR64 showed the highest R/S ratio. At high VPD, R/S ratio was positively correlated with g_s of IR64 and BT7, but not with g_s of KD18 and Jasmine 85 (see appendix, Fig. 3.8).

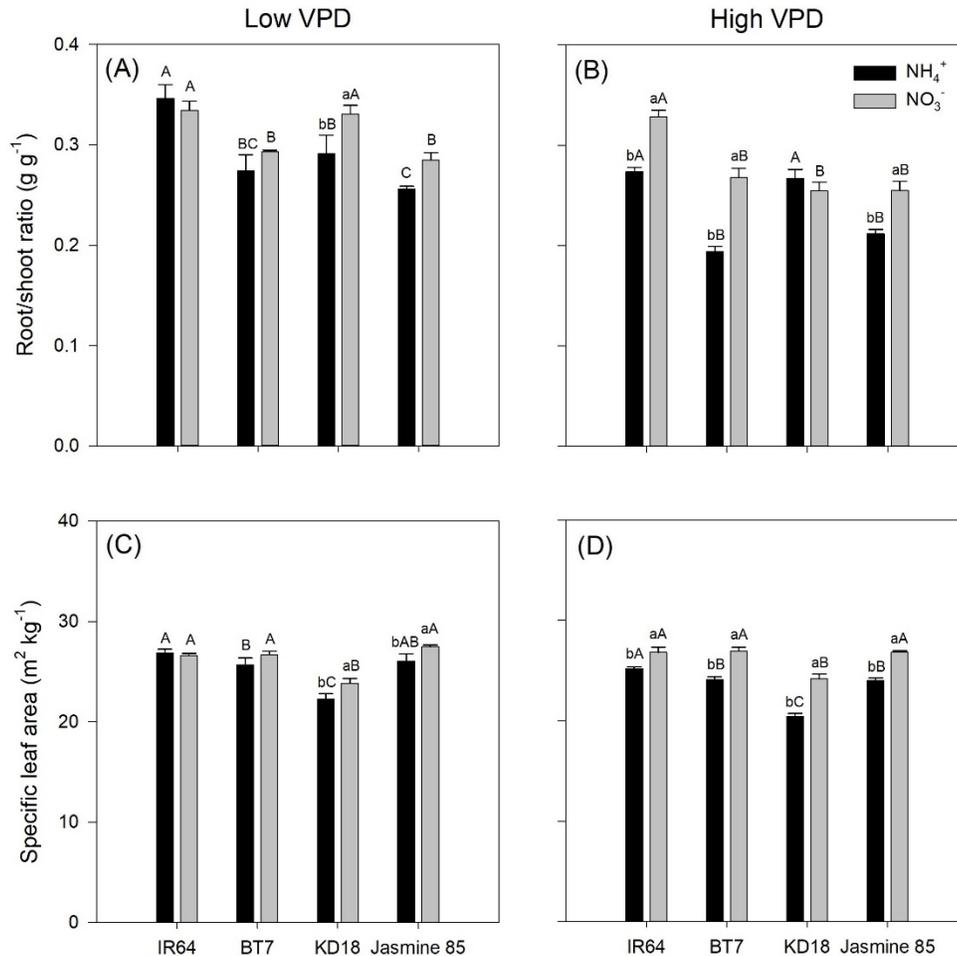


Figure 3.5: Root/shoot ratio (A, B) and specific leaf area (C, D) of four rice varieties fed with different N sources (NH_4^+ or NO_3^-) at low and high VPD (Experiment 3). Columns and error bars show means and standard errors of 4 replications. Small letters indicate significant differences between N sources, while capital letters indicate significant differences between varieties, all at p < 0.05.

NO_3^- nutrition led to a higher SLA in KD18 and Jasmine 85 at low VPD and in all varieties at high VPD relative to NH_4^+ (Fig. 3.5C, D). At both VPD levels and with both N sources, the lowest SLA was found in KD18.

At low VPD, NO_3^- -fed plants showed a significantly higher starch content in the leaves compared to NH_4^+ -fed plants, with an exception for Jasmine 85 (Fig. 3.6). At high VPD, NO_3^- nutrition led to significantly higher starch content in BT7 and Jasmine 85. With both N sources, BT7 showed the highest starch content in the leaves at low VPD, while at high VPD, the highest starch content was observed in IR64.

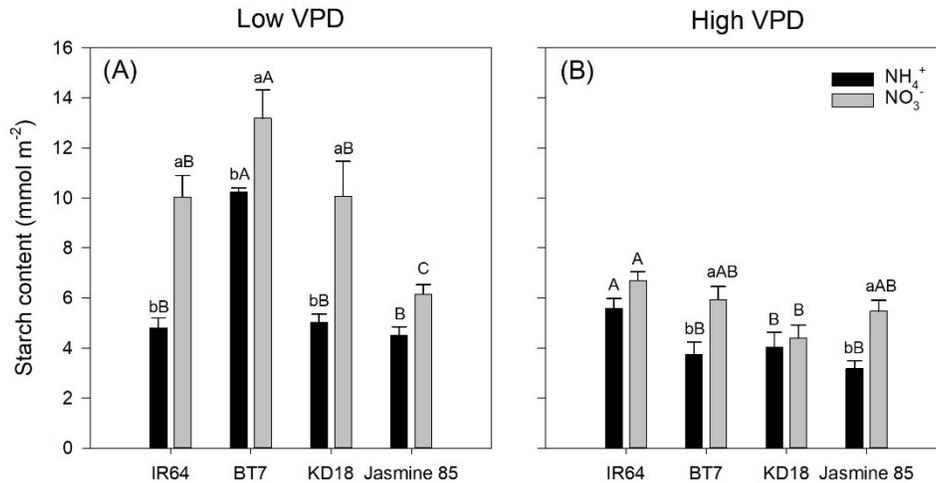


Figure 3.6: Starch content in leaves of four rice varieties fed with different N sources (NH_4^+ or NO_3^-) at low and high VPD (A, B) (Experiment 3). Mean values of 4 replications are presented. Small letters indicate significant differences between nitrogen sources, while capital letters indicate significant differences between varieties, all at $p < 0.05$.

3.4 Discussion

3.4.1 NO_3^- induced higher g_s and A relative to NH_4^+ at high VPD but not at low VPD

At low VPD, gas exchange parameters were hardly affected by N source. Among the four rice varieties (IR64, BT7, KD18, and Jasmine 85) at low VPD in experiments 2 and 3, only g_s of IR64 showed a significant response to N source in experiment 3, with g_s being higher in NO_3^- than in NH_4^+ . In previous studies, N source did not affect leaf gas exchange of rice under non-water stress conditions (S. Guo et al., 2008; Ji and Peng, 2005; Li et al., 2009; Tran et al., 2015; Zhou et al., 2011), whereas NH_4^+ significantly reduced photosynthesis in some other crops, for example, cucumber (Zhou et al., 2011), durum wheat (*Triticum durum*; Torralbo et al., 2019), and eggplant (*Solanum melongena* L.; Claussen and Lenz, 1995). Excessive accumulation of NH_4^+ in the cytosol is toxic, leading to tissue necrosis

(Marschner, 2011) but a substantial amount of N can be translocated as NH_4^+ from the root to the shoot (Schjoerring et al., 2002). In various species, NH_4^+ as exclusive N source led to toxicity symptoms (Britto and Kronzucker, 2002). Therefore, plants sensitive to NH_4^+ decrease g_s as a strategy to reduce NH_4^+ transport towards the leaves (Torralbo et al., 2019). However, lowland rice is considered NH_4^+ -tolerant (Zhou et al., 2011), since activities of N-assimilating enzymes, both in roots and shoots, are much higher than in other, more sensitive crops (e.g. tomato, maize; Magalhães and Huber, 1989). Most of the NH_4^+ taken up is assimilated in the root (Marschner, 2011; Tabuchi et al., 2007). However, NH_4^+ assimilation consumes carbon skeletons and a reduction in photosynthate concentration after NH_4^+ uptake has been observed in roots (Bowman and Paul, 1988) as well as in leaves (Raab and Terry, 1995). In our study, NH_4^+ -assimilating enzyme activities were not determined, however, starch concentrations in the leaves were lower in NH_4^+ -fed plants than in NO_3^- -fed plants at low VPD, while A did not differ between plants fed with the two N sources, suggesting translocation of carbohydrates to the roots probably for NH_4^+ assimilation and detoxification.

In contrast, different N sources led to significant differences in leaf gas exchange at high VPD under “standard” greenhouse conditions (experiment 1), with higher g_s and A in four out of 12 varieties, IR64, BT7, NU838, and Nipponbare fed with NO_3^- . The same was observed at high VPD in the growth chamber (Experiment 3) where higher g_s and A were found in IR64 and BT7 under NO_3^- nutrition relative to NH_4^+ . Since NH_4^+ and NO_3^- are taken up at different rates (Vu et al., 2020), supplying different N sources could lead to different N concentrations in the leaves and, thus, differences in chlorophyll concentrations. However, chlorophyll concentration was measured directly spectrophotometrically after ethanol extraction, but no differences between N treatments were found (see appendix, Fig. 3.9). Several previous studies demonstrated that the effect of N source on A is associated with g_s (S. Guo, Zhou, Shen, & Zhang, 2007; Torralbo et al., 2019; Zhou et al., 2011). In our study, A was closely correlated with g_s , which supports the hypothesis that g_s limits A in response to NH_4^+ nutrition. NO_3^- has been linked to g_s , as its uptake and accumulation in guard cells induces their depolarization and, eventually, stomatal opening (F.-Q. Guo et al., 2003). Since NO_3^- can be easily transported and stored in the leaves (Marschner, 2011), it can be assumed that NO_3^- concentration in the leaves was higher in NO_3^- relative to NH_4^+ treated plants, which consequently enhanced stomatal opening at high VPD maintaining high A. Since g_s

limits A more strongly at high transpirational demand (*Ohsumi et al., 2008*), we hypothesize that the advantageous effects of NO_3^- on g_s and A were only observed at high VPD.

Assimilation of N sources and water uptake and use seem to be linked, since in previous studies, leaf gas exchange of rice plants did not differ between N sources under well-water conditions, but NO_3^- nutrition led to reduced g_s , A, SPAD, and water consumption under PEG-induced water stress compared to NH_4^+ (*Ding et al., 2015; S. Guo et al., 2008*). Under water deficit, NH_4^+ improved the water relations of rice plants via maintaining water uptake (g plant^{-1} ; *S. Guo et al., 2008*), whereas NO_3^- increased the share of root aerenchyma, leading to decreased water uptake (*Yang et al., 2012*). In our experiments, a decrease of WUEi (A/g_s) under NO_3^- nutrition at high VPD (Tab. 3.4) shows high transpirational costs for CO_2 assimilation, which will be a disadvantage under conditions of soil water deficit. However, water supply was not a limiting factor in our hydroponic experimental setup.

Moreover, g_s can be seen as an important indicator of the plant's water status (*Parkash and Singh, 2020*), with plants being considered as water stressed, if g_s is reduced by 40% compared to well-watered conditions (*Perdomo et al., 2016*). Under this precondition, plants acclimated to high VPD did not suffer from severe water deficit in Experiment 3, since g_s was on average only 27% lower than that of plants at low VPD. *Zhong et al. (2018)* postulated that the larger stomatal aperture caused by NO_3^- is advantageous for the plant, provided that the leaf water content is not impaired by water deficit, which was observed in Experiment 3. In contrast, in Experiment 2, rice plants were transferred from low to high VPD, and after 1 week, g_s was still reduced by more than 50% on average, implying that the leaf water status was significantly affected and plants suffered from water stress. Accordingly, A was substantially decreased after transfer of the plants to high VPD in both N treatments, and in Jasmine 85, NO_3^- led to a significantly lower A relative to NH_4^+ , as observed in previous studies (*Ding et al., 2015; S. Guo et al., 2008*). Therefore, we hypothesize that NO_3^- nutrition can improve leaf gas exchange in specific rice varieties at high VPD, provided an adequate water supply and sufficient time for acclimation.

The uptake, reduction, and assimilation of NO_3^- consumes more energy than that of NH_4^+ (*Raven, 1985*). However, a surplus of NADPH generation during the light reaction is not completely consumed during the assimilation of CO_2 , and NO_3^- assimilation in the leaves

represents an additional NADPH sink, which is not available in NH_4^+ -fed plants (*S. Guo, Zhou, et al., 2007*). Therefore, NO_3^- nutrition could protect chloroplasts from photo-damage and maintain the stability of the photosynthetic apparatus, when light absorption exceeds the capacity for its utilization during photosynthesis (*Zhang et al., 2014*). Moreover, NADPH generation is driven by light intensity (*Hashida and Kawai-Yamada, 2019*), which could explain the higher A in NO_3^- -fed plants in our first experiment in greenhouse condition, where light intensity was higher than in the other experiments conducted in growth chambers.

Table 3.4: Intrinsic water-use efficiencies (WUEi) of four rice varieties at low and high VPD and the difference in WUEi between the two VPDs (%) in experiment 2 and 3. Data presented are mean values of 4 replications. Different small letters indicate significant differences between N sources, while different capital letters indicate significant differences between varieties.

		Intrinsic water-use efficiencies (WUEi) ($\mu\text{mol}/\text{mmol}$)					
		Experiment 2			Experiment 3		
Rice cultivar	N source	Low VPD	High VPD	(%)*	Low VPD	High VPD	(%)*
IR64	NH_4^+	0.026 A	0.051 a A	98.6	0.029 a A	0.034 a A	18.6
IR64	NO_3^-	0.025 A	0.042 b A	71.7	0.025 b A	0.028 b A	10.4
BT7	NH_4^+	0.027 A	0.049 a A	79.9	0.022 B	0.037 a A	69.8
BT7	NO_3^-	0.026 A	0.041 b A	58.6	0.023 A	0.031 b A	37.1
KD18	NH_4^+	0.020 B	0.035 a B	77.1	0.020 B	0.024 C	21.4
KD18	NO_3^-	0.017 B	0.030 b B	80.2	0.019 B	0.023 B	19.1
Jasmine 85	NH_4^+	0.021 B	0.036 a B	66.3	0.021 B	0.027 B	28.3
Jasmine 85	NO_3^-	0.018 B	0.025 b B	39.6	0.019 B	0.030 A	59.6

* Difference between low and high VPD

3.4.2 Varietal variation of leaf gas exchange in response to N source at high VPD

Rice varieties showed large differences in leaf gas exchange in response to N source at high VPD where IR64, BT7, NU838, and Nipponbare showed higher g_s and A in NO_3^- relative to

NH_4^+ nutrition, whereas no significant difference was found between N treatments for the other varieties. Since A was closely correlated with g_s across varieties, the difference in A between varieties may be attributed to differences in the response of g_s to N source at high VPD. After long term exposure to high VPD in Experiment 3, NO_3^- induced a higher R/S ratio in IR64 and BT7, with a high positive correlation between R/S ratio and g_s (see appendix, Fig. 3.8). Varietal differences in root characteristics in response to N source have been observed before. *Song et al.* (2011) found that the presence of NO_3^- improved root initiation and root growth in varieties with a high N-use efficiency only. Plant N content was not measured in our experiments, however, N was entirely depleted in the residual nutrition solution after 7 days and, thus, we assume that the entire N provided in the nutrient solution was taken up. Since in Experiment 3, IR64, BT7, and Jasmine 85 showed a higher biomass than KD18 across N forms, they probably had a higher N-use efficiency. The higher R/S ratio under NO_3^- nutrition in IR64 and BT7 at high VPD could explain the higher g_s , since a high R/S ratio supports water uptake, which is consistent with higher transpiration rates in NO_3^- -fed plants (see appendix, Fig. 3.10). Probably since morphological adaptations need some time to develop, differences in R/S ratio were not observed after short-term exposure to high VPD, but only at long-term.

Despite differences in g_s and A, no differences in R/S ratio between N treatments were found in the first experiment, showing that R/S ratio is not the only reason for differences in leaf gas exchange in response to N form. NO_3^- and amino acids serve as osmotic agents in plant tissues, which are involved in regulating the acclimation of photosynthesis in rice plants, however, varietal differences were observed in accumulation and assimilation of NO_3^- (*Fan et al.*, 2007; *Zhong et al.*, 2018). There were differences in uptake rate as well as assimilation rate of NO_3^- between rice varieties at high VPD, but not at low VPD (*Vu et al.*, 2020). Moreover, rice varieties also show a large variation in osmotic adjustment as well as dehydration tolerance (*Lilley and Ludlow*, 1996). Therefore, we hypothesize that the difference in g_s of rice varieties in response to N form under high VPD may be partly attributed to the varietal difference in osmotic adjustment caused by assimilation and accumulation of NO_3^- .

NO_3^- nutrition led to a higher SLA in KD18 and Jasmine 85 at low VPD, and in all rice varieties at high VPD in Experiment 3. Several authors have highlighted the advantages of a

high SLA for photosynthesis. *Evans and Poorter (2001)* demonstrated that a high SLA is important for maximum carbon gain per unit leaf mass, especially at low-light conditions (*Schmieder et al., 2021*). While thicker leaves have more structural material relative to metabolic components and increased internal shading, leading to lower light absorption (*Terashima and Hikosaka, 1995*), they also slow down intercellular gaseous diffusion, which probably limits CO₂ assimilation (*Parkhurst, 1994*). One reason for higher SLA in NO₃⁻-fed plants is that NO₃⁻ nutrition can increase cation uptake and leading to higher concentrations of osmolytes (i.e. NO₃⁻, cations), resulting in an increased leaf expansion (*Chaillou et al., 1986; Raab and Terry, 1995*).

IR64 and BT7 showed a higher WUE_i than KD18 and Jasmine 85 at both VPDs and in Experiments 2 and 3 (Tab. 3.4). *Gilbert et al. (2011)* demonstrated a wide varietal variation in WUE_i, which was attributed to the variation in *g_s* and the curvature of the relationship between A and *g_s*. Both, reducing *g_s* per amount of CO₂ assimilated or enhancing the assimilation rate at a given *g_s* can improve WUE_i (*Blankenagel et al., 2018*). In our study, IR64 and BT7 showed a high WUE_i, which is especially beneficial at high transpirational demand. Since both varieties also showed a higher A in NO₃⁻ treatments, we hypothesize that NO₃⁻ may increase leaf gas exchange in varieties with high WUE_i. As the number of varieties in our study was limited, further research would be needed to confirm this finding.

3.4.3 Growth response of different rice varieties to N-source at low and high VPD

In most cases, when A of NO₃⁻-fed plants exceeded A of NH₄⁺-fed plants, no difference in plant dry matter was found, showing that the advantage of increased carbon assimilation is not directly translated into growth. Since NO₃⁻ assimilation has a higher energy demand than the assimilation of NH₄⁺ (*Bloom et al., 1992; Raven, 1985; Salsac et al., 1987*), we assume that the higher carbon assimilation in NO₃⁻-grown plants mainly compensated the higher demand of assimilates for NO₃⁻ assimilation.

In Experiment 3, total DM was lower in NH₄⁺ than in NO₃⁻ treated plants in KD18 and Jasmine 85 at low VPD, and in BT7 and Jasmine 85 at high VPD. One reason for this was a large share of dead leaves of KD18 and Jasmine 85 in the NH₄⁺ treatment at both VPD levels, leading to a relatively low total dry matter (see appendix, Fig. 3.11). Dead leaves showed a striking orange coloration-like symptoms of leaf-oranging, which were probably caused by

NH_4^+ toxicity as described by *Liao et al.* (1994). Symptoms of NH_4^+ toxicity are attributed to a decline in cation uptake, acidification of the external medium, disturbance of intracellular pH or changes in the amino acid or organic acid profile of the plants (*Britto and Kronzucker, 2002*). In our experiment, the pH of the nutrient solution was adjusted daily; however, a large decline in pH was observed in the NH_4^+ nutrient solution within the day. Moreover, we assume that these varieties suffered from NH_4^+ toxicity even at a moderate concentration, since rice plants can accumulate substantial amounts of un-metabolized NH_4^+ in the cytoplasm and vacuole in the root even at low external NH_4^+ concentrations (*Wang et al., 1993*). Although rice is NH_4^+ tolerant, it can suffer from NH_4^+ toxicity due to excessive accumulation of NH_4^+ in the leaves (*Chen et al., 2013; Liao et al., 1994*), which has been associated with two factors. Firstly, a reduction in the activity of assimilating enzymes due to antagonistic effects between NH_4^+ and cations, particularly K^+ , and second, an uptake rate of NH_4^+ exceeding the assimilation capacity of the enzymes (*Liao et al., 1994*). Varieties with a high N-use efficiency showed a greater capacity to resist high NH_4^+ concentrations, whereas susceptibility of varieties with low N-use efficiency was related to a relative poor regulation of plasma-membrane influx of NH_4^+ under high NH_4^+ , accompanied by enhanced root respiration (*Chen et al., 2013*). Moreover, previous studies have shown that rice plants in flooded soils also take up substantial amounts of NO_3^- instead of NH_4^+ through nitrification in the rhizosphere caused by the release of oxygen from the roots (*Kirk, 2001; Kirk and Kronzucker, 2005*), but this might be marginal in our study as the experiment was conducted in nutrient solution. However, the mechanism of NH_4^+ uptake and the variation in NH_4^+ resistance of rice varieties at different N concentrations/contents and diurnal VPDs need to be elucidated.

3.5 Conclusion

The present work evaluated the effect of N sources on leaf gas exchange and growth of different rice varieties at low and high VPD. N sources significantly influenced leaf gas exchange of rice plants at high VPD with a higher A in NO_3^- than in NH_4^+ -fed plants, which was mainly attributed to higher g_s and supported by a higher R/S ratio. However, this effect varied among rice varieties and was not observed at low VPD or shortly after transferring plants from low to high VPD. With higher transpirational costs for photosynthesis in NO_3^- -

fed plants, we hypothesize that NO_3^- nutrition can improve leaf gas exchange of rice plants at high VPD in high WUE_i varieties, provided a sufficient water supply. Some varieties showed symptoms of NH_4^+ toxicity even at moderate concentrations, indicating genotypic diversity in transport and assimilation capacity of NH_4^+ among rice varieties. Since a high R/S ratio was not a unique factor improving g_s in NO_3^- supplied plants, further studies are needed to elucidate the role of NO_3^- uptake and assimilation in controlling g_s at high VPD.

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Appendix

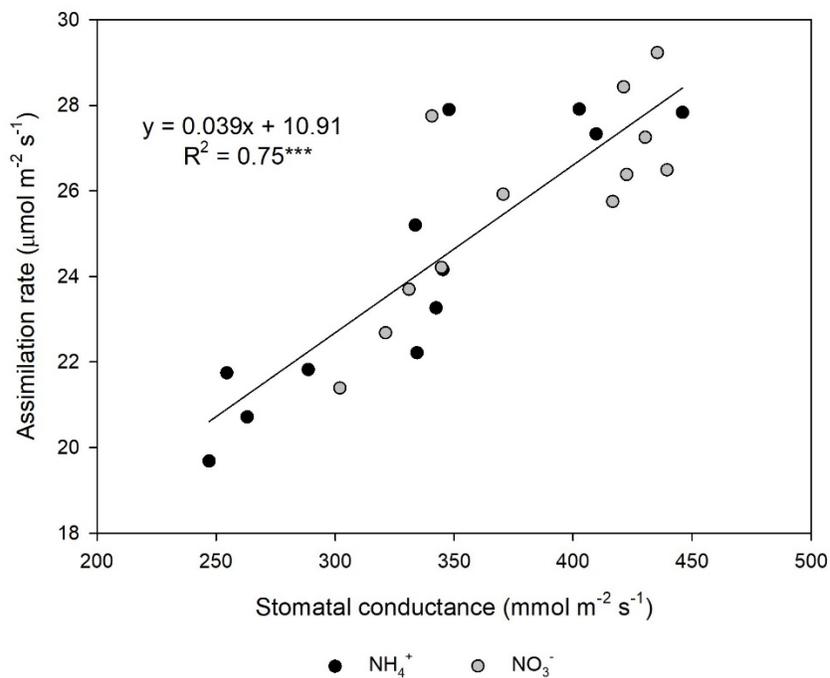


Figure 3.7: Linear regression between assimilation rate (A) and stomatal conductance (g_s) of 12 rice varieties at high VPD (“standard greenhouse”; Experiment 1). The regression was performed over 12 varieties with two nitrogen sources ($n = 24$). *** significant at $p \leq 0.001$.

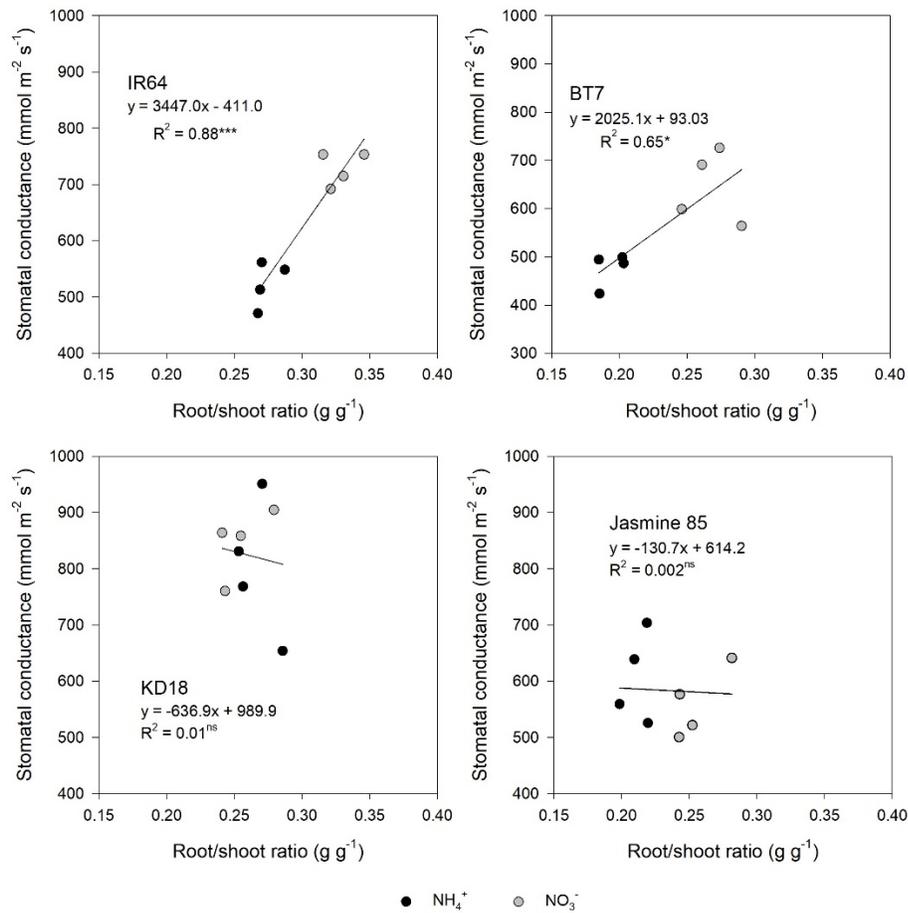


Figure 3.8: Linear regression between stomatal conductance (g_s) and root/shoot ratio of 4 rice varieties at high VPD (Experiment 3). The regression was performed over two nitrogen sources with 4 individual plants as replications ($n = 8$). ***, * significant at $p \leq 0.001$ and $p \leq 0.05$, respectively; ns not significant.

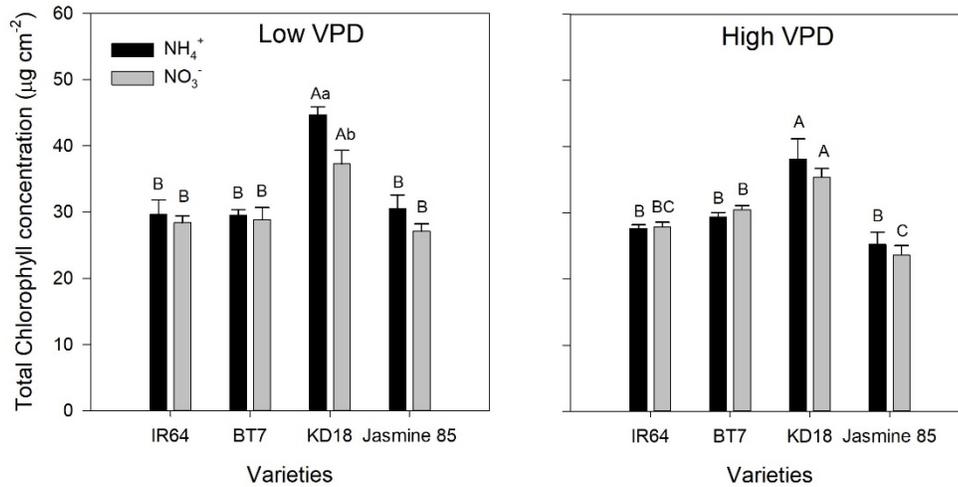


Figure 3.9: Chlorophyll concentration of four rice varieties fed with different N sources (NH₄⁺ or NO₃⁻) at low and high VPD (Experiment 3). Columns and error bars show means and standard errors of 4 replications. Small letters indicate significant differences between N sources, while capital letters indicate significant differences between varieties, all at $p < 0.05$.

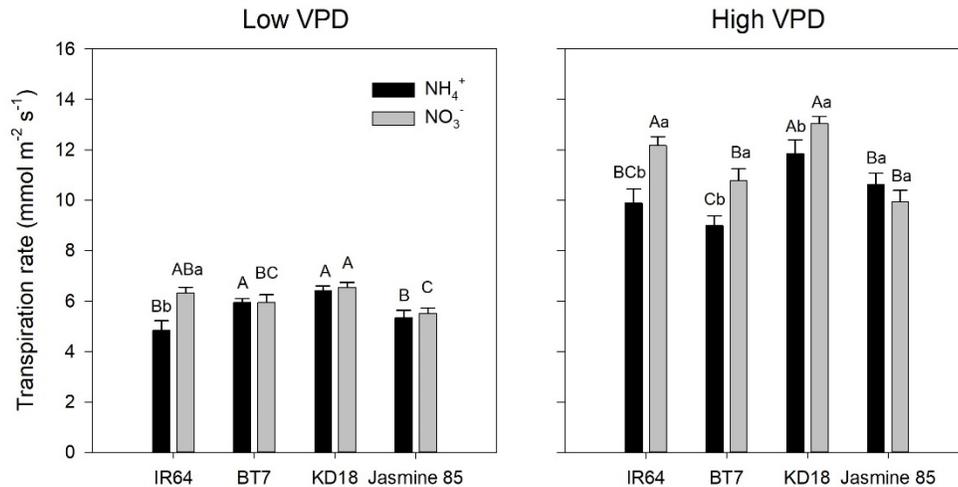


Figure 3.10: Transpiration rate of four rice varieties fed with different N sources (NH₄⁺ or NO₃⁻) at low and high VPD (Experiment 3). Columns and error bars show means and standard errors of 4 replications. Small letters indicate significant differences between N sources, while capital letters indicate significant differences between varieties, all at $p < 0.05$.

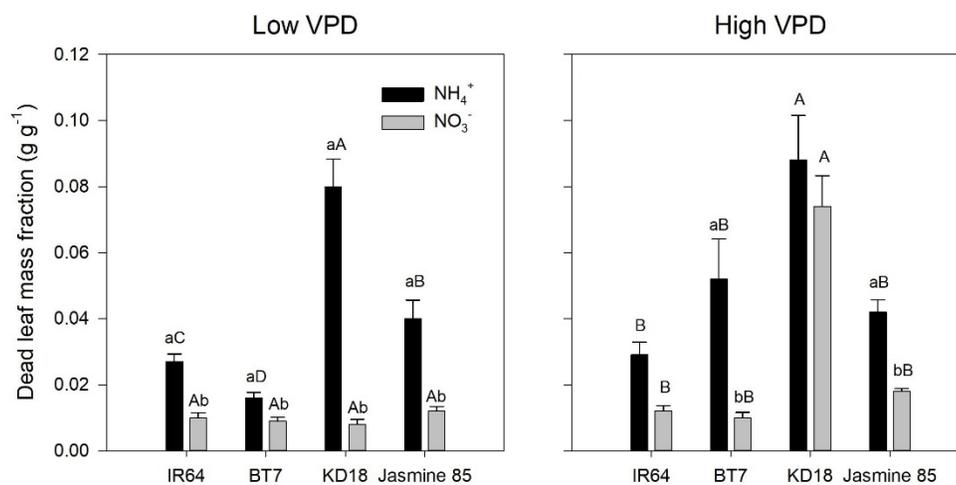


Figure 3.11: Dead leaf fraction of four rice varieties fed with different N sources (NH₄⁺ or NO₃⁻) at low and high VPD (Experiment 3). Mean values of 4 replications are presented. Small letters indicate significant differences between nitrogen sources, while capital letters indicate significant differences between varieties, all at $p < 0.05$.

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Chapter 4

Rice-Weed Competition in Response to Nitrogen Form and Vapor Pressure Deficit*

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Abstract

In lowland rice fields, implementation of water-saving irrigation practices results in increased availability of nitrate (NO_3^-) in the soil due to nitrification. The absence of the water layer also favors germination of upland weeds. Since plant species show a specific preference for either ammonium (NH_4^+) or NO_3^- as nitrogen (N) source, changes in both soil NO_3^- concentration and weed flora may affect the competition between rice and weeds. The study aimed to evaluate the effects of N source on growth, N uptake, and competition between rice and common upland and lowland weeds. Two rice varieties (NU838 and KD18) differing in growth characteristics and two weed species (*Echinochloa crus-galli* and *Solanum nigrum*) differing in their natural habitat were selected. Rice and weeds were grown hydroponically as monoculture or mixed culture at low or high vapor pressure deficit (VPD). N was supplied as 75%/25% or 25%/75% $\text{NH}_4^+/\text{NO}_3^-$. N uptake rates were measured in the first week, whereas dry matter (DM), N concentration in the plant, total N uptake, and the activities of nitrate reductase and glutamine synthetase in the fresh leaves were determined two weeks after the onset of treatments. Independent of N source, both rice varieties and *E. crus-galli* took up a larger share of NH_4^+ , whereas *S. nigrum* took up a larger share of NO_3^- . N uptake of rice and *E. crus-galli* was hardly affected by N source, whereas high NO_3^- led to significantly higher N uptake rates and total N uptake of *S. nigrum*. NU838 showed a higher competitiveness against weeds than KD18. N source did not affect DM of rice in monoculture, but strongly affected the growth of weeds, as high NO_3^- decreased the competitiveness of *E. crus-galli* against NU838 but increased the competitiveness of *S. nigrum* against NU838. High VPD did not affect DM but increased N uptake of *S. nigrum*, leading to increased competitiveness of the weed at high transpirational demand.

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Competitiveness for N uptake appears to be an important trait as the relative N concentration in mixed plant communities was correlated with the activity of N-assimilating enzymes and leaf growth, with a stronger response in rice than in weeds. Our results support the hypothesis that increased availability of NO_3^- in aerobic rice soils may be advantageous for the competitiveness of upland weeds, especially at high VPD, whereas, it may be disadvantageous for common lowland weeds.

Key words: Alternative wetting and drying, *Echinochloa crus-galli*, plant competitiveness, *Solanum nigrum*, water-saving irrigation, weeds in lowland rice.

4.1 Introduction

Weeds are considered an important biological constraint in crop production. They compete with crops for nutrients, water, and light, and also serve as hosts for pests and diseases, resulting in reduced crop growth and yield (Zimdahl, 2018). For rice, the global yield loss due to weeds has been estimated at 35%, much higher than that due to animal pests and fungal and bacterial pathogens (24 and 16%, respectively) (Oerke and Dehne, 2004). Due to weed encroachment, grain yield can decrease by 50 - 60% in transplanted rice and 70 - 80% in direct seeded rice (Dass et al., 2017), and even complete crop failure has been observed under certain conditions (Matloob et al., 2015).

In conventional rice systems, continuous flooding is considered an effective method of weed control. Flooding creates unfavorable conditions for weed seed germination and also suppresses the growth of weed seedlings during early growth stages due to altered soil chemistry, reduced oxygen levels, accumulation of carbon dioxide and toxic gasses in the soil (Smith and Fox, 1973; Zimdahl, 2018) or reduced fluctuation of soil temperature (Chauhan and Johnson, 2009; Pons, 1982). Previous studies have shown that increasing the duration and depth of flooding significantly suppresses the emergence and growth of many important weeds in lowland rice, such as *Echinochloa* spp. (Chauhan and Johnson, 2011; Estioko et al., 2014), *Leptochloa chinensis* (Benvenuti et al., 2004; Chauhan and Johnson, 2008), *Cyperus difformis*, *Cyperus iria*, and *Fimbristylis miliacea* (Begum et al., 2006; Chauhan and Johnson, 2009). However, maintaining a ponded water layer is becoming increasingly difficult due to increasing water scarcity (Rodenburg and Johnson, 2009) as a

result of global warming (*Dinar et al., 2019; Flörke et al., 2018; IPCC, 2018; Morriso et al., 2009*).

Water-saving irrigation technologies (e.g., alternate wetting and drying - AWD) are being promoted in various rice-growing areas to adapt to water scarcity, but the absence of the water layer favors weed germination, leading to increased weed pressure (*Begum et al., 2006; Rodenburg et al., 2011; Van der Hoek et al., 2001*). Many studies have shown that saturated soil conditions significantly increase the number of weed species, density, and biomass compared to continuous flooding (*Bhager et al., 1999; Mahajan et al., 2009*). This is especially true for sedges and grass species (*Bhuiyan et al., 2017; Hill et al., 2001*). Maintaining soil saturation at the early stage (pre-sowing to 2-3 leaf stage) in direct-seeded rice also resulted in the largest weed seed banks, especially in sedges, grasses, and some upland weeds (*Chen et al., 2017*). Therefore, implementing water-saving irrigation practices may cause a shift in the weed composition in lowland rice, altering the competitive relationship between rice and weeds (*Brim-DeForest et al., 2017; Kent and Johnson, 2001; Luo et al., 2017; Rodenburg et al., 2011*).

In addition, the water regime in the field also affects the transformation of nitrogen (N) forms in the soil due to changes in soil oxygen levels, which may affect the competition between rice and weeds in terms of N uptake. Ammonium (NH_4^+) is the predominant N form in flooded lowland, but aerobic conditions during dry periods in AWD favor nitrification, resulting in higher availability of nitrate (NO_3^-) in the soil (*Buresh et al., 2008; Chunmei et al., 2020; Dong et al., 2012*). Both N forms are taken up and metabolized by plants. However, *Yang et al. (2018)* argued that the N form of preference for uptake varies among plant communities, but is consistent for coexisting species within each community. Since rice plants preferably take up more NH_4^+ than NO_3^- (*Vu et al., 2020*), weeds in lowland rice might also show a similar trend. In contrast, upland weed species might preferentially take up more NO_3^- than NH_4^+ , such as redroot pigweed (*Amaranthus retroflexus* L.) (*Teyker et al., 1991*) and lamb's quarters (*Chenopodium album* L.) (*Kirkby, 1967*), as their growth, N uptake, and N assimilation were enhanced under NO_3^- compared to NH_4^+ nutrition.

Because lowland rice is grown in various production systems (e.g., direct-seeded or transplanted, monocropping or in rotation with upland crops) and under different water

managements (e.g., continuous flooding or water-saving irrigation), weed composition and seed banks in the soil are diverse. Many weed species that usually occur under upland conditions can become more harmful in lowland rice if water management creates favorable conditions for their germination (e.g., during dry periods in AWD). Moreover, rice-growing regions and seasons also vary significantly in terms of vapor pressure deficit (VPD), from less than 1.0 kPa in Southeast Asia (humid environment) to 4.0 kPa in Senegal (dry environment) (Julia and Dingkuhn, 2013). VPD describes the difference between the saturation vapor pressure and the actual vapor pressure at a given temperature. VPD has the potential to affect plant growth (Stuerz and Asch 2019), water uptake and N assimilation (Vu et al. 2020), and can alter physiological responses of plants via its effects on stomatal conductance and transpiration (Vu et al. 2021; Stuerz and Asch 2021), as plants close their stomata in response to high VPD to minimize water loss. Moreover, global VPD has increased strongly over the past 30 years and is predicted to increase continuously throughout the century (Yuan et al. 2019). The objective of this study was to evaluate the competitiveness of rice and weed species (lowland and upland weeds) in terms of nutrient uptake and biomass accumulation under different N forms and VPDs. We hypothesized that: (1) a higher share of NO_3^- will decrease N uptake of the lowland weed species but increase N uptake of the upland weed species, resulting in a decrease in total N uptake by rice competing with the upland species; (2) Growth of upland weeds is favored by NO_3^- while growth of lowland weeds is favored by NH_4^+ , thus a higher share of NO_3^- may increase the competitiveness of upland species; (3) Rice varieties and weeds show differences in growth and N uptake under varying VPD, which alters the competition between rice and weed.

4.2 Materials and methods

The studies were conducted in 2020 in the greenhouse of the Institute of Agricultural Sciences in the Tropics at the University of Hohenheim, Germany.

4.2.1 Plant materials

Two rice varieties (Nhi uu 838 - NU838 and Khang dan 18 - KD18) differing in growth characteristics were selected to study varietal variation in competition with weeds. NU838 is a hybrid variety, high-tillering, with high yield, and KD18 is an inbred variety with moderate tillering and moderate yield (Pham et al., 2009).

To simulate the altered weed flora under water-saving irrigation, two weed species (Barnyard grass - *E. crus-galli* and Black nightshade - *Solanum nigrum* L.) differing in their growth characteristics and common in different ecological environments were included in the study. *E. crus-galli* is one of the most common and detrimental weeds in lowland rice (Holm et al., 1991). Seeds of *E. crus-galli* were collected from a rice research farm at the Faculty of Agronomy, Vietnam National University of Agriculture (VNUA), in summer 2018 and subsequently stored in a refrigerator. *S. nigrum* is not a common weed in lowland rice, but we hypothesize that with the application of AWD, some weed species that are common in upland rice will spread on saturated soils in lowland rice. *S. nigrum* is a common weed in various crops in both temperate and tropical regions, especially on soils with a high-water content. The weed has a strong primary root system and is well adapted to fertile soils with high N and phosphorus content (Holm et al., 1991). Because of these characteristics, *S. nigrum* was selected as potential weed in lowland rice under AWD. Seeds of *S. nigrum* were obtained from Herbiseed (England).

4.2.2 Experimental design

The seeds of rice and weeds were germinated on filter paper for 4 days and then grown individually in sand irrigated with tap water for the next 4 days. 50% standard YOSHIDA nutrient solution (Yoshida et al., 1976) was supplied for the next four days and then seedlings were transplanted into plastic boxes (40 x 30 x 12 cm) containing 8.5 L of 100% standard YOSHIDA nutrient solution (at a density of 60 seedlings per box) for another 8 days.

Treatments started with transplanting seedlings of rice and weeds into 1 L pots containing modified nutrient solution with an N concentration of 2.86 mM, differing in the ratio of N forms (75%/25% or 25%/75% $\text{NH}_4^+/\text{NO}_3^-$). Other macro- and micronutrients were provided in equal amounts in both N source treatments, based on the composition of YOSHIDA nutrient solution. Each pot contained two plants with either rice/rice or weed/weed as monoculture or rice/weed in competition as mixed culture (Tab. 4.1). Treatments representing the competition between both rice varieties or both weed species were not included in the experiment. Plants were placed at a distance of 6 cm in each pot. The nutrient solution was renewed every 7 days, while the pH was adjusted daily to 5.8 ± 0.1 with 1N NaOH or HCl. To inhibit nitrification, 7 μM dicyandiamide ($\text{C}_2\text{H}_4\text{N}_4$) was added to both N treatments (Duan et al., 2006).

Pots were placed in two separate self-constructed VPD chambers (low and high VPD). The average daytime and nighttime VPD was 0.5 and 0.2 kPa in the low VPD chamber and 2.2 and 1.6 kPa in the high VPD chamber, respectively. Air temperature was 31 and 25°C on average at day and night in both VPD chambers. Day and night cycles were set to a 12 h photoperiod from 7 am to 7 pm and the photosynthetic photon flux density (PPFD) at canopy level was approx. 850 $\mu\text{mol m}^{-2} \text{s}^{-1}$.

Table 4.1: Planting treatments

Varieties/Species	Neighbor plants	NH ₄ ⁺ /NO ₃ ⁻ ratios
NU838	NU838	75/25
NU838	<i>E. crus-galli</i>	75/25
NU838	<i>S. nigrum</i>	75/25
NU838	NU838	25/75
NU838	<i>E. crus-galli</i>	25/75
NU838	<i>S. nigrum</i>	25/75
KD18	KD18	75/25
KD18	<i>E. crus-galli</i>	75/25
KD18	<i>S. nigrum</i>	75/25
KD18	KD18	25/75
KD18	<i>E. crus-galli</i>	25/75
KD18	<i>S. nigrum</i>	25/75
<i>E. crus-galli</i>	<i>E. crus-galli</i>	75/25
<i>E. crus-galli</i>	<i>E. crus-galli</i>	25/75
<i>S. nigrum</i>	<i>S. nigrum</i>	75/25
<i>S. nigrum</i>	<i>S. nigrum</i>	25/75

4.2.3 Nutrient uptake measurement

Nutrient uptake was determined on 7 consecutive days during the first week of treatment. For this, each day at the same time, nutrient solution was sampled and stored in the

refrigerator (4-6°C) after weighing the pots for water loss determination. Concentration of NH_4^+ and NO_3^- in the nutrient solution was determined using a continuous flow analysis with an Autoanalyzer (Technicon/Alliance Instruments, Germany). Daily plant fresh weight (FW) was interpolated from FW at day 1 and day 8 using an exponential function. The uptake rate of N was calculated in $\mu\text{mol g}^{-1} \text{FW h}^{-1}$ (Vu et al., 2020). To account for evaporative losses, 6 pots without plants were randomly placed in each VPD chamber and weighed daily.

4.2.4 Determination of enzyme activities

Two weeks after the onset of treatment and one day after renewing nutrient solution, samples of roots (approx. 0.2 g) and of the youngest fully developed leaf on the main stem (approx. 1 cm^2) were taken at midday for nitrate reductase (NR) and glutamine synthetase (GS) analysis. Samples were quickly frozen in liquid N and stored at -80°C (Hartmann and Asch, 2019). Activities of NR and GS enzymes were determined according to the methods described by Vu et al. (2020).

4.2.5 Dry matter accumulation and competitive index

Plants were harvested 2 weeks after onset of treatment. For dry matter (DM) determination, roots, stems, and leaves were separated and weighed after drying at 60°C for more than 48 hours.

Competition between rice and weed in the rice-weed treatment was assessed using the competitive index (CI) described by Aminpanah et al. (2013) as:

$$CI = \left(\frac{DM_R}{DM_{RM}} \right) / \left(\frac{DM_W}{DM_{WM}} \right)$$

With DM_R as dry matter (g) of the rice plant in competition with the weed; DM_{RM} as mean dry matter of all rice plants in competition with a weed; DM_W as dry matter of the weed in competition with rice; and DM_{WM} as mean dry matter of the weeds in competition with rice. Higher CI indicates superior competitiveness.

4.2.6 Total nitrogen uptake

Dry plant samples were finely ground and then analyzed at the Core Facility of the University of Hohenheim, where N concentration (%) in the DM was determined with an elemental analyser Vario MAX CNS (Co. Elementar).

$$\text{Total N uptake (mg plant}^{-1}\text{)} = (N_a * DM_a - N_b * DM_b) * 10$$

Where N_b and N_a is N concentration (%) in plant DM before and after the treatment period, respectively, while DM_b and DM_a is dry matter (g) of plants before and after the treatment period, respectively.

4.2.7 Statistical analysis

Data analysis was performed with SAS (Statistical Analysis System, version 9.4, USA) for each VPD separately, as VPD was not included as a factor due to the lack of degrees of freedom. ANOVA (Analysis of variance) was used to examine the effects of varieties/species, competitor plant, N form ratio and their interactions. For daily nitrogen uptake and CI, the effects of planting treatments, N-form ratio and their interaction were tested. Means were separated using LSD test (Least significant difference) at $p < 0.05$.

4.3 Results

4.3.1 Effect of nitrogen source and plant-competition on biomass accumulation of rice and weeds

DM of NU838 was 3.1 and 2.6 g plant⁻¹ on average at low and high VPD, respectively (Fig. 4.1). At low VPD, competition with *S. nigrum* at high NH_4^+ (75%/25% NH_4^+/NO_3^-) led to a significantly higher DM of NU838 in comparison to all other treatments. At high VPD, no effect of N source or weed competition was observed.

On average, DM of KD18 was 1.4 and 0.9 g plant⁻¹ at low and high VPD, respectively (Fig. 4.1). At low VPD, in competition with *E. crus-galli* at high NH_4^+ , DM of KD18 was significantly lower than in other treatments. At high VPD, no significant treatment effects were observed.

DM of *E. crus-galli* was 3.2 and 2.9 g plant⁻¹ on average at low and high VPD, respectively (Fig. 4.1). N source did not affect DM of *E. crus-galli* in monoculture, but in competition with rice, DM was significantly lower at high NO_3^- (25%/75% NH_4^+/NO_3^-) than at high NH_4^+ . However, in competition with NU838 at high VPD, N source did not result in significant differences. Compared with monoculture, DM of *E. crus-galli* was greatly reduced in competition with NU838 at high NO_3^- , but not at high NH_4^+ . In contrast, in competition with KD18, DM was significantly higher than in monoculture in both N treatments, except for high NO_3^- at high VPD.

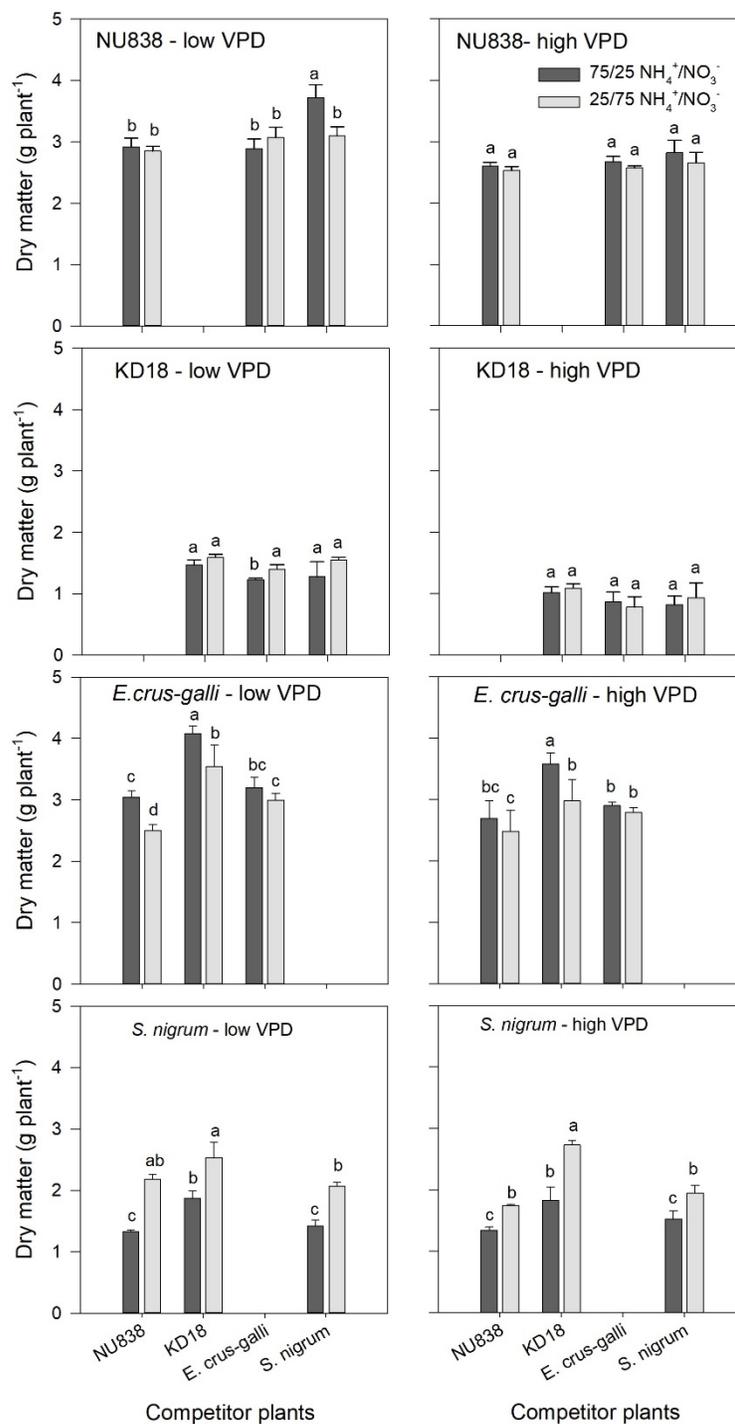


Figure 4.1: Total dry matter of two rice varieties (NU838 and KD18) and two weed species (*E. crus-galli* and *S. nigrum*) as affected by neighbor plant and N source at low and high VPD. Missing values = species was not part of the pairing. Error bars indicate standard error of means (n = 6 and 3 for monoculture and mixed culture, respectively). Different letters indicate significant differences between treatments.

Under both low and high VPD, DM of *S. nigrum* was on average 1.9 g plant⁻¹ (Fig. 4.1). Independent of neighboring plant and VPD, DM of *S. nigrum* was significantly higher at high NO₃⁻ than at high NH₄⁺. Compared with monoculture, DM of *S. nigrum* was significantly higher when grown with KD18, but did not differ when grown with NU838.

4.3.2 Effect of nitrogen source on weed competitive index of two rice varieties

NU838 showed a significantly higher CI than KD18 at both VPDs (Fig. 4.2). High NO₃⁻ increased CI of NU838 in competition with *E. crus-galli* at low VPD, but strongly decreased CI of NU838 in competition with *S. nigrum* at both VPDs. At low VPD, NU838 showed a significantly higher CI in competition with *S. nigrum* than in competition with *E. crus-galli* at high NH₄⁺, while the opposite was observed at high NO₃⁻. However, at high VPD, CI of NU838 was similar when grown with both weed species in both N sources. CI of KD18 did not differ with neighboring plant or N source.

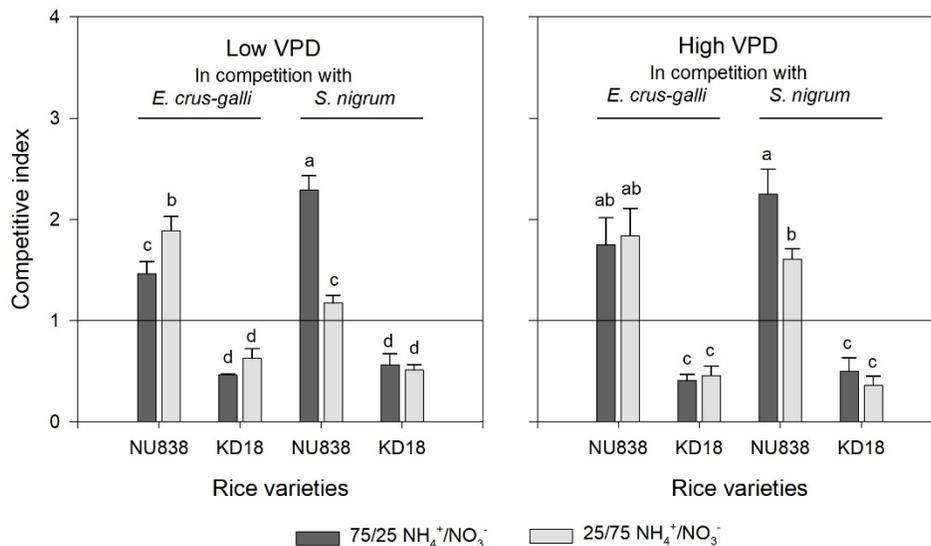


Figure 4.2: Competitive index of two rice varieties (NU838 and KD18) in competition with two weed species (*E. crus-galli* and *S. nigrum*) grown with different N sources at low and high VPD. Error bars indicate standard error of means (n = 3). Different letters indicate significant differences between treatments.

4.3.3 Effect of nitrogen source and plant-competition on nitrogen uptake of rice and weeds

Figure 4.3 shows the average N uptake rates of rice and weeds in monoculture from differently composed nutrient solutions during the first four days after renewing the nutrient

solution. As in competition treatments, N uptake rates of rice and weeds could not be distinguished, only uptake rates in monoculture are shown. At both VPDs, *E. crus-galli* had the highest N uptake rates, while the lowest uptake rates were observed in *S. nigrum*. N uptake rates of *E. crus-galli* and KD18 were not influenced by N source, whereas high NO_3^- led to significantly lower N uptake rates in NU838 and significantly higher N uptake rates in *S. nigrum*. Independent of N source, both rice varieties and *E. crus-galli* took up a larger share of NH_4^+ , whereas *S. nigrum* took up a larger share of NO_3^- .

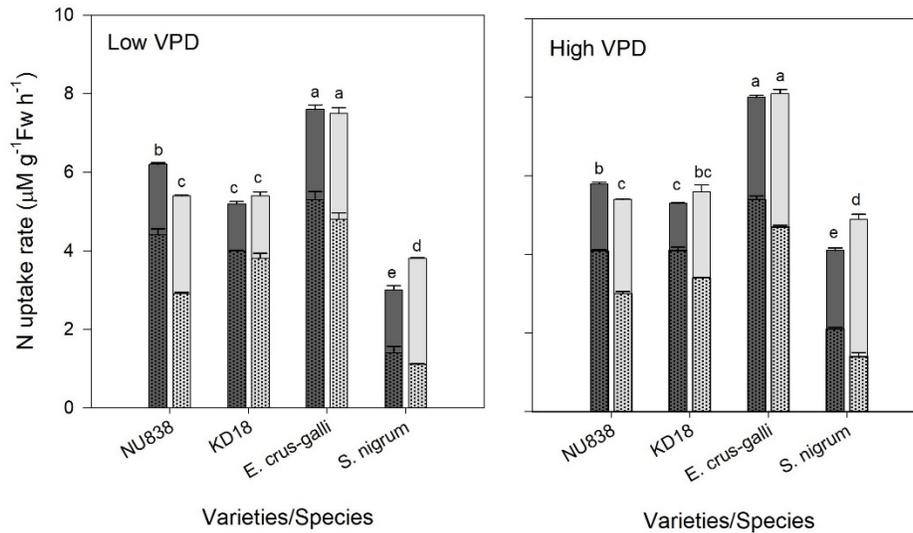


Figure 4.3: Ammonium and nitrate uptake rates of two rice varieties (NU838 and KD18) and two weed species (*E. crus-galli* and *S. nigrum*) in monoculture during the first four days after the onset of treatment. Dark and light colors indicate the treatments 75/25 $\text{NH}_4^+/\text{NO}_3^-$ and 25/75 $\text{NH}_4^+/\text{NO}_3^-$ nutrition, respectively. The patterned bar shows the NH_4^+ uptake rate and clear bar shows the NO_3^- uptake rate. Error bars show standard error ($n = 3$). Different letters indicate significant differences in total N uptake rate between treatments.

NU838 and *E. crus-galli* showed the highest total N uptake (70.8 and 66.5 mg plant^{-1} in NU838, and 68.3 and 70.3 mg plant^{-1} in *E. crus-galli* at low and high VPD, respectively), followed by *S. nigrum* (40.1 and 48.2 mg plant^{-1} at low and high VPD, respectively) and KD18 (43.7 and 30.7 mg plant^{-1} at low and high VPD, respectively) (Fig. 4.4).

Total N uptake of NU838 in competition with *S. nigrum* was higher at high NH_4^+ , but there was no significant effect of N source in KD18 and *E. crus-galli*. In contrast, total N uptake in *S. nigrum* was always higher at high NO_3^- than at high NH_4^+ for all plant combinations and at both VPDs.

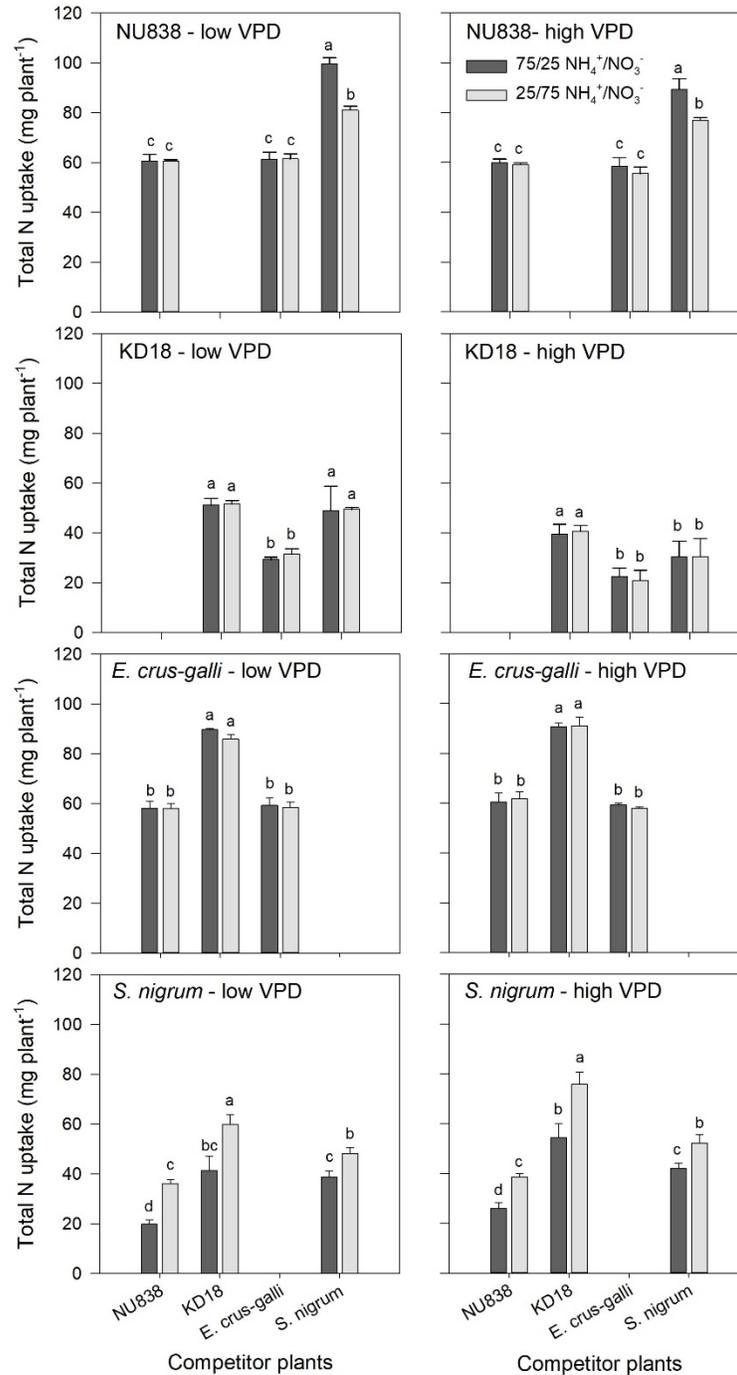


Figure 4.4: Total nitrogen uptake of two rice varieties (NU838 and KD18) and two weed species (*E. crus-galli* and *S. nigrum*) as affected by neighboring plants and N source at low and high VPD. Missing values = species was not part of the pairing. Error bars indicate standard error of means (n = 6 and 3 in monoculture and mixed culture, respectively). Different letters indicate significant differences between treatments.

In competition with *S. nigrum*, total N uptake of NU838 was increased by 57% at high NH_4^+ and by 32% at high NO_3^- in comparison to monoculture, while competition with *E. crus-galli* did not cause any significant difference in total N uptake of NU838. Total N uptake of KD18 was negatively affected by the presence of *E. crus-galli* at both VPDs and by the presence of *S. nigrum* at high VPD only. Compared with monoculture, competition with *E. crus-galli* reduced total N uptake of KD18 by 41 and 46% at low and high VPD on average across N treatments. At high VPD, competition with *S. nigrum* decreased total N uptake of KD18 by 24%.

Compared with monoculture, total N uptake of *E. crus-galli* did not differ in competition with NU838 but significantly increased in competition with KD18 at both VPDs. However, total N uptake of *S. nigrum* was significantly lower in competition with NU838 but significantly higher in competition with KD18, except at high NH_4^+ and low VPD.

4.3.4 Effect of nitrogen source and plant-competition on nitrogen assimilation of rice and weeds

In the leaves, both rice varieties showed significantly higher activities of N-assimilating enzymes than weeds, whereas the difference in enzyme activities between species was less pronounced in the roots (Tab. 4.2). Enzyme activities were significantly higher in KD18 in both roots and leaves than in NU838, except for GS in leaves at low VPD. *S. nigrum* had significantly higher activities of NR and GS than *E. crus-galli* in the roots, but a lower GS activity in the leaves.

Table 4.2: Nitrate reductase (NR) and glutamine synthetase (GS) activities in roots and leaves of two rice varieties (KD18 and NU838) and two weed species (*E. crus-galli* and *S. nigrum*) supplied with different N sources under low and high VPD. Small letters indicate significant differences between plant combinations within each variety/species. Capital letters indicates significant differences between N sources within each variety/species.

Plant name	Neighbor plants	NH ₄ ⁺ /NO ₃ ⁻	Low VPD				High VPD			
			Root NR (nM NO ₂ g ⁻¹ FW h ⁻¹)	Leaf NR (μM NO ₂ g ⁻¹ DW h ⁻¹)	Root GS (μM γ-GHA g ⁻¹ FW h ⁻¹)	Leaf GS (μM γ-GHA g ⁻¹ DW h ⁻¹)	Root NR (nM NO ₂ g ⁻¹ FW h ⁻¹)	Leaf NR (μM NO ₂ g ⁻¹ DW h ⁻¹)	Root GS (μM γ-GHA g ⁻¹ FW h ⁻¹)	Leaf GS (μM γ-GHA g ⁻¹ DW h ⁻¹)
NU838	NU838	75/25	80.7 a A	13.5 b B	14.0 a A	545.4 b A	81.7 a A	17.5 b B	16.3 a A	651.2 a A
NU838	<i>E. crus-galli</i>	75/25	65.8 a A	13.9 ab A	10.9 a A	654.1 ab A	82.9 a A	14.1 b A	21.2 a A	624.9 a A
NU838	<i>S. nigrum</i>	75/25	85.7 a A	24.2 a B	14.3 a A	737.2 a A	101.8 a A	29.6 a B	20.7 a A	713.7 a A
NU838	NU838	25/75	97.6 a A	27.2 b A	15.3 a A	616.8 a A	99.2 a A	35.6 b A	21.9 a A	609.7 b A
NU838	<i>E. crus-galli</i>	25/75	77.1 a A	16.1 c A	14.9 a A	641.5 a A	85.2 a A	21.8 c A	17.6 a A	754.4 a A
NU838	<i>S. nigrum</i>	25/75	98.3 a A	54.0 a A	13.8 a A	627.9 a A	111.4 a A	51.7 a A	21.5 a A	809.1 a A
Average			84.2	24.8	13.9	637.2	93.7	28.4	19.9	693.8
KD18	KD18	75/25	145.7 a A	38.8 a A	28.9 a A	638.2 a A	128.2 a B	38.3 b B	27.2 a A	763.0 b A
KD18	<i>E. crus-galli</i>	75/25	94.7 b A	11.2 b B	15.6 b A	457.6 b B	78.6 b A	20.0 c B	23.1 a A	626.2 b A
KD18	<i>S. nigrum</i>	75/25	127.3 ab A	35.0 a B	24.4 a A	747.8 a A	117.4 ab A	51.7 a A	26.4 a A	905.0 a A
KD18	KD18	25/75	178.0 a A	36.0 b A	22.9 a A	673.6 a A	204.7 a A	61.1 a A	33.5 a A	814.6 a A
KD18	<i>E. crus-galli</i>	25/75	89.4 b A	22.2 c A	17.5 a A	583.3 b A	117.3 b A	32.9 c A	26.9 a A	688.3 a A
KD18	<i>S. nigrum</i>	25/75	138.1 ab A	49.6 a A	21.0 a A	783.0 a A	108.4 b A	47.4 b A	30.1 a A	800.6 a A
Average			128.9	32.1	21.7	647.3	125.8	41.9	27.9	766.3
<i>E. crus-galli</i>	<i>E. crus-galli</i>	75/25	67.8 a A	3.3 a A	5.4 a A	588.4 a A	65.6 a A	3.8 a A	8.3 a A	566.3 a A
<i>E. crus-galli</i>	NU838	75/25	86.4 a A	3.0 a A	6.3 a A	566.2 a A	65.1 a A	4.4 a A	6.8 a A	572.4 a A
<i>E. crus-galli</i>	KD18	75/25	79.1 a A	3.7 a A	8.9 a A	570.6 a A	66.2 a A	4.2 a A	5.3 a A	608.9 a A
<i>E. crus-galli</i>	<i>E. crus-galli</i>	25/75	60.0 a A	3.0 a A	6.7 a A	560.7 a A	54.8 a A	3.3 a A	5.0 a A	589.9 a A
<i>E. crus-galli</i>	NU838	25/75	54.2 a A	4.2 a A	5.3 a A	597.4 a A	41.1 a A	5.9 a A	3.5 a A	599.5 a A
<i>E. crus-galli</i>	KD18	25/75	54.8 a A	4.4 a A	9.6 a A	603.6 a A	60.3 a A	6.7 a A	6.3 a A	639.5 a A
Average			67.0	3.6	7.0	581.1	58.9	4.7	5.9	596.1
<i>S. nigrum</i>	<i>S. nigrum</i>	75/25	158.1 c A	6.3 a A	26.2 a A	316.1 a A	141.7 ab A	5.5 a A	17.5 a A	386.4 ab A
<i>S. nigrum</i>	NU838	75/25	231.2 b A	4.3 a A	8.5 b B	199.9 a A	129.0 b A	5.0 a A	12.4 a A	247.7 b A
<i>S. nigrum</i>	KD18	75/25	337.5 a A	3.8 a A	22.9 a A	270.6 a A	186.3 a A	5.6 a A	16.3 a A	434.9 a A
<i>S. nigrum</i>	<i>S. nigrum</i>	25/75	89.4 a B	3.6 a A	8.4 a B	299.5 a A	81.5 a B	3.9 a A	7.8 a B	369.4 a A
<i>S. nigrum</i>	NU838	25/75	135.80 a B	2.5 a A	9.4 a B	210.2 a A	86.20 a A	3.0 a A	12.3 a A	341.3 a A
<i>S. nigrum</i>	KD18	25/75	102.7 a B	3.3 a A	11.9 a B	290.8 a A	100.8 a B	3.7 a A	9.2 a A	347.0 a A
Average			175.8	4.0	14.5	264.5	120.9	4.5	12.6	354.4

In NU838, no effect of weed competition or N source on enzyme activities was observed in the roots. However, competition with *E. crus-galli* resulted in significantly lower leaf NR at high NO_3^- , whereas competition with *S. nigrum* strongly increased activities of NR and GS in the leaves, except for GS at high NO_3^- at low VPD and high NH_4^+ at high VPD. Compared with high NH_4^+ , high NO_3^- led to significantly higher NR in the leaves of NU838, except in competition with *E. crus-galli*.

Independent of N source, competition with *E. crus-galli* led to significantly lower activities of N-assimilating enzymes in both roots and leaves of KD18, except for GS at high VPD. At low VPD, competition with *S. nigrum* resulted in significantly higher leaf NR in KD18 at high NO_3^- . At high VPD, competition with *S. nigrum* significantly reduced the activities of NR in both roots and leaves at high NO_3^- but increased the activities of NR and GS in leaves at high NH_4^+ . At low VPD, high NO_3^- resulted in significantly higher leaf NR in competition with both weed species and increased leaf GS in competition with *E. crus-galli*. At high VPD, high NO_3^- led to significantly higher NR in both roots and leaves in monoculture and leaf NR in competition with *E. crus-galli*.

The activities of N-assimilating enzymes in *E. crus-galli* were neither affected by N source nor by neighboring plant. At low VPD and high NH_4^+ , competition with NU838 led to significantly higher root NR but lower root GS in *S. nigrum*, whereas competition with KD18 led to higher root NR only. At low and high VPD, high NH_4^+ resulted in significantly higher activities of NR and GS in the roots of *S. nigrum* compared with high NO_3^- , except for root GS in competition with NU838.

To estimate the effect of competition between rice and weeds on N uptake, the activity of N-assimilating enzymes and on biomass production, relative plant N concentration, enzyme activities, and plant growth parameters were calculated as the ratio between plants in mixed culture and in monoculture, and a linear correlation was performed between the relative values (Fig. 4.5). A positive correlation was found between the relative N concentration and the relative activities of N-assimilating enzymes in the leaves, with a stronger correlation in rice than in weeds. The relative plant N concentration was also strongly correlated with the relative leaf area but not correlated with the relative root DM.

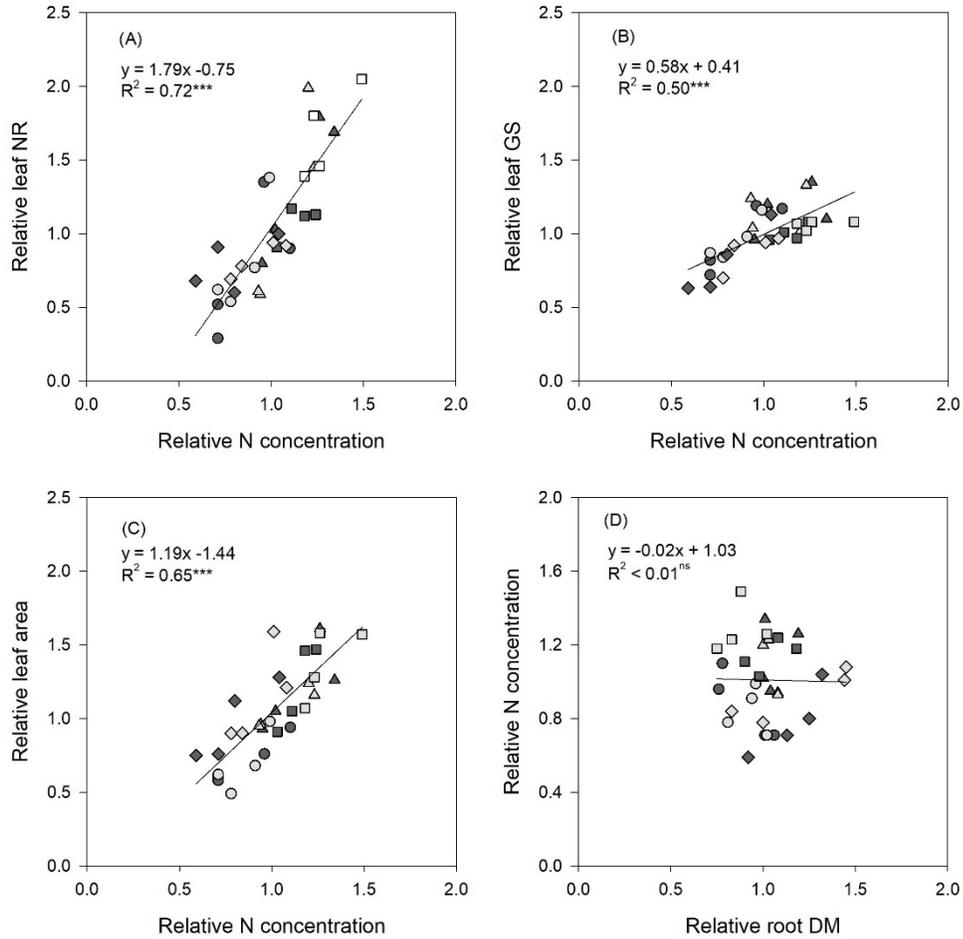


Figure 4.5: Linear correlation between N concentration, leaf N-assimilating enzymes and plant growth of plants in mixed culture relative to plants in monoculture. Dark grey is 75/25 $\text{NH}_4^+/\text{NO}_3^-$, light grey is 25/75 $\text{NH}_4^+/\text{NO}_3^-$. Triangles are NU838, circles are KD18, squares are *E. crus-galli*, and diamonds are *S. nigrum*. The correlation was performed over 4 varieties/species with 2 neighboring plants and 2 nitrogen sources at low and high VPD ($n = 32$).

4.4 Discussion

4.4.1 Effects of nitrogen source on uptake and assimilation of nitrogen in rice and weeds

Independent of N source, *E. crus-galli* showed the highest N uptake rate, whereas the lowest uptake rate was observed in *S. nigrum*. Nutrient uptake, transport, and accumulation are genetically determined and may differ both between and within plant species (Marschner, 2011). However, differences in N uptake rates between species could be partly attributed to root growth (Jiang et al., 2011), which we also found in our experiment, as root DM and N

uptake rate were significantly correlated (data not shown). In monoculture, the highest root DM was found in *E. crus-galli*, followed by NU838, KD18, and *S. nigrum*. For *S. nigrum* as an upland weed, root growth and nutrient uptake might have been inhibited due to insufficient oxygen supply, as plants were grown in nutrient solution without aeration. Rice and *E. crus-galli* are considered flood- and low-oxygen tolerant because of their continuous aerenchyma throughout the plant, whereas the development of aerenchyma is less pronounced in plant species normally found under upland conditions (Wagatsuma et al., 1990). Although the N uptake rate of *S. nigrum* was considerably lower than that of rice, its total N uptake reached on average 78 and 95% of rice at low and high VPD, respectively, demonstrating that *S. nigrum* can grow and take up a substantial amount of N under conditions of low oxygen.

In the current experiment, rice and *E. crus-galli* took up a higher share of NH_4^+ independent of N source, whereas the opposite was observed for *S. nigrum*. Plant communities differ in their preference for a specific N form due to adaptation to the availability of N form in the plant's native habitat (Wang and Macko, 2011; Yang et al., 2018). As *S. nigrum* showed a similar uptake preference for NO_3^- as other upland weeds (Chen and Chen, 2019; Kirkby, 1967; Teyker et al., 1991), increased availability of NO_3^- due to enhanced nitrification in the soil will facilitate N uptake by *S. nigrum*. In contrast, lowland rice is considered NH_4^+ tolerant (Zhou et al., 2011) and preferentially takes up NH_4^+ when NH_4^+ and NO_3^- are provided in equal concentrations in the nutrient solution (Vu et al., 2020; Yi et al., 2019). As the N form of preference is alike for coexisting species (Yang et al., 2018), we hypothesized that *E. crus-galli* preferentially takes up NH_4^+ , which has been confirmed in this study.

However, N source did not result in significant differences in total N uptake of rice and *E. crus-galli* in monoculture. Both rice varieties and *E. crus-galli* strongly increased the uptake of NO_3^- when NH_4^+ became less available in the nutrient solution (data not shown) and in NU838 and *E. crus-galli*, N in the nutrient solution was completely depleted at day 7, before the replacement of the nutrient solution. Therefore, N was limiting in both treatments for both species. In contrast, total N uptake of *S. nigrum* was significantly higher at high NO_3^- than at high NH_4^+ .

The N uptake rates of rice did not differ between both VPD conditions. However, the N uptake rate of the weeds was higher under high VPD with increases of 39 and 29% at high

NH_4^+ and high NO_3^- , respectively in *S. nigrum* and 5 and 9% at high NH_4^+ and high NO_3^- in *E. crus-galli*. In our previous study, N uptake of rice was hardly affected by VPD and was not correlated with water uptake (Vu et al., 2020), which was also observed in the current study. However, a significant correlation between N uptake and water uptake was found in *E. crus-galli* and *S. nigrum* (data not shown).

In rice and *E. crus-galli*, N source had no significant effect on GS activity, while their GS activity in the leaves was significantly higher than in *S. nigrum*. A potential reason is the predominant uptake of NH_4^+ of rice and *E. crus-galli* in both N treatments, as GS is one of the key enzymes in NH_4^+ assimilation (Marschner, 2011). Related to its high activity of GS, rice has been described as very efficient in NH_4^+ assimilation (Magalhães and Huber, 1989), which seems also the case for *E. crus-galli* according to our experiment.

In both rice varieties, high NO_3^- resulted in higher NR activity in the leaves, which was more pronounced at high than at low VPD, supporting the hypothesis that N assimilation of rice is affected by VPD (Vu et al., 2020). In contrast, high NH_4^+ resulted in significantly higher activities of NR and GS in roots of *S. nigrum*. Since NH_4^+ is mainly assimilated to amino acids in the root, the increased GS activity may be an adaptation of *S. nigrum* to the availability of NH_4^+ in the medium.

4.4.2 Effects of nitrogen source on biomass accumulation of rice and weeds

In our study, N sources did not affect DM of rice and *E. crus-galli* in monoculture. In nutrient solution, rice plants produced the largest DM in the mixture of both N forms (NH_4^+ and NO_3^-) (Duan et al., 2007; Guo et al., 2008), whereas there was no significant difference in DM between N forms alone (Guo et al., 2008; Zhou et al., 2011). According to Duan et al. (2006, 2007), the increase in rice DM under mixed N supply was attributed to increased N uptake related to the presence of NO_3^- . In the current experiment, NO_3^- was available as N source in both N treatments, while total N uptake did not differ.

Higher N uptake by *S. nigrum* at high NO_3^- than at high NH_4^+ , resulted in significantly greater DM. Many upland weed species are considered to be NH_4^+ -sensitive, with high NH_4^+ supply inhibiting plant growth (Chen and Chen, 2019; Kirkby, 1967; Teyker et al., 1991). However, the threshold for NH_4^+ toxicity varies greatly among species (Britto and Kronzucker, 2002). In our experiment, compared to high NO_3^- , high NH_4^+ caused a decrease of 58 and 41% in

root DM and R/S ratio on average, respectively, at low VPD, and of 52 and 35%, respectively, at high VPD, which are common symptoms of NH_4^+ toxicity (Bittsánszky et al., 2015; Britto and Kronzucker, 2002). Although an increase in GS activity in the roots of *S. nigrum* was observed at high NH_4^+ , the low activity of the enzyme observed in the leaves could impede for NH_4^+ assimilation. A considerable amount of NH_4^+ from the root may be translocated to the shoot (Schjoerring et al., 2002), leading to tissue necrosis with excessive accumulation of NH_4^+ in the cytosol (Marschner, 2011).

4.4.3 Effects of nitrogen source on competition between rice and weeds

Competition with *E. crus-galli* resulted in a significant decrease in N concentration, total N uptake, and DM in KD18, but did not affect NU838. The CI of NU838 against *E. crus-galli* was 3.1 and 4.1 times higher than that of KD18 at low and high VPD, respectively. The C4 plant *E. crus-galli* has higher growth and nutrient uptake rates than rice (Vu et al., 2013), and the presence of *E. crus-galli* significantly reduced the availability of nutrients to rice plants (Ulguim et al., 2020), resulting in growth and yield depressions in rice (Mennan et al., 2012; Perera et al., 1992; Ulguim et al., 2020; Zhu et al., 2008). However, rice showed wide genotypic variation in competitiveness against *E. crus-galli* (Aminpanah et al., 2013; Mennan et al., 2012). The most important traits related to competitiveness of rice against *E. crus-galli* were tillering, plant height, and early growth (Mennan et al., 2012), greater biomass production (Gealy et al., 2003), or early leaf area expansion rate (Lindquist and Kropff, 1996), whereas delayed crop establishment favored rapid growth of *E. crus-galli* (Ntanos and Koutroubas, 2000). As an advantage of hybrid varieties, NU838 has a greater growth rate, leaf area, and tiller number than KD18 (Pham et al., 2009).

Competition with *E. crus-galli* also resulted in significantly lower activities of N-assimilating enzymes and lower N concentrations in KD18 but not in NU838, suggesting poor competitiveness of KD18 with *E. crus-galli* in terms of N uptake. Perera et al. (1992) showed that inhibition of root growth of rice, resulting in lower resource uptake, was the major factor in the reduction of rice growth caused by *E. crus-galli*. In our experiment, competition with *E. crus-galli* did not result in lower root DM, but strongly reduced shoot DM and leaf area of KD18. The relative N concentration was strongly correlated with relative leaf enzyme activities and leaf area, but not with relative root growth and root enzyme activities,

indicating that maintaining growth and N assimilation in the aboveground part may improve plant competitiveness. Since nutrient uptake by roots requires energy that is derived from carbohydrates assimilated in the leaves (Marschner, 2011), increased leaf growth can ensure sufficient energy for nutrient uptake.

Compared with high NH_4^+ , high NO_3^- resulted in significantly lower DM of *E. crus-galli* in competition with rice. High NO_3^- induced significantly lower SPAD values in leaves of *E. crus-galli* in competition with rice one week after treatment initiation (data not shown). Since SPAD is closely correlated with N concentration per unit leaf area (Esfahani et al., 2008; Xiong et al., 2015), we hypothesize that leaf N status of *E. crus-galli* was negatively affected by rice competition at high NO_3^- in the first week. Further, N source did not affect the activities of N assimilating enzymes of *E. crus-galli* in all plant combinations or DM in monoculture. Therefore, the lower DM of *E. crus-galli* related to high NO_3^- might be rather an effect of the neighboring plant than of N assimilation itself. Since in most cases N source had no effect on DM and N uptake of both rice varieties in competition with *E. crus-galli*, the negative effect of high NO_3^- on *E. crus-galli* was not attributed to the higher biomass production of rice. NU838 and KD18 showed allelopathic effects against *E. crus-galli* (Tran et al. 2009), which could be an explanation for the reduction in growth of *E. crus-galli*. Moreover, *E. crus-galli* also has allelopathic potential when interacting with rice (Tran et al. 2018). However, allelochemicals of plants are diverse and are influenced by surrounding environmental factors including pH and nutrients (Tran et al. 2007, 2018). As the growth reduction was only observed at high NO_3^- , N form might affect the production and interaction of allelopathic compounds between rice and *E. crus-galli*.

In contrast, N concentration and total N uptake of NU838 was significantly increased in competition with *S. nigrum*, with a greater increase at high NH_4^+ . However, the significant increase in DM of NU838 was observed only at high NH_4^+ at low VPD. In KD18, competition with *S. nigrum* resulted in significantly lower N concentration at high NO_3^- and lower total N uptake at high VPD, but did not affect DM. The CI of NU838 against *S. nigrum* was significantly higher than that of KD18, but was significantly lower at high NO_3^- than that at high NH_4^+ . Moreover, the CI of NU838 was similar when grown with both weed species at high VPD, demonstrating a higher competitiveness of *S. nigrum* at high NO_3^- and high VPD, especially in terms of N uptake. The higher competitiveness of *S. nigrum* could result from

a higher growth rate at high NO_3^- or lower competitive pressure in terms of N uptake due to the difference in preference of N form for uptake between rice and the weed.

Rice and *E. crus-galli* showed a lower DM at high VPD than at low VPD, whereas DM of *S. nigrum* did not differ between the two VPD conditions, suggesting that *S. nigrum* is tolerant to high VPD. This could be due to the hair layer on leaves and stem of *S. nigrum* (Edmonds and Chweya, 1997), leading to less transpiration, which is beneficial in dry conditions (Wuenscher, 1970). In competition with NU838 and KD18, high VPD increased total N uptake of *S. nigrum* by 30 and 32%, respectively, at high NH_4^+ , and by 7 and 27%, respectively, at high NO_3^- . Therefore, our results suggest that upland weeds can compete with rice for nutrients in saturated soils with its enhanced nitrification under AWD, and could become a problem in rice production, especially at high VPD.

4.5 Conclusion

N source did not affect N uptake and growth of rice in monoculture, but significantly affected N uptake and growth of weeds, resulting in altered competition between rice and weeds. In the rice – *E. crus-galli* mixed culture, high NO_3^- did not reduce total N uptake but significantly declined growth of *E. crus-galli* compared to high NH_4^+ , leading to increased competitiveness of rice plants. However, N uptake and growth of *S. nigrum* were strongly favored by high NO_3^- , resulting in decreased competitiveness of rice compared to high NH_4^+ . Further, N uptake rate of *S. nigrum* was significantly increased at high VPD, leading to increased competitiveness of the weed. In both N treatments, NU838 outperformed KD18 in dry matter and competitiveness. Competitiveness in terms of N uptake appears to be an important trait in the competition between rice and weeds, as relative plant N concentration was correlated with relative leaf growth and N assimilation in the leaves, which was more pronounced in rice than in weeds. Our results suggest that increased nitrification in the soil is disadvantageous for rice in competition with upland weeds, especially in terms of N uptake at high VPD, while it may reduce the pressure of common lowland weeds.

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Chapter 5

General Discussion

Water management affects growth and grain yield of rice, which is partly related to root zone temperature (RZT), nitrification in the soil, and altered weed composition in the field. In water-saving irrigation systems (e.g., alternate wetting and drying – AWD), RZT is more dependent on air-temperature during drained periods due to the absence of a water layer acting as temperature buffer. Lower RZT reduces nutrient uptake by rice plants, which should be considered when implementing AWD during cold seasons. In addition, the altered RZT caused by both water management and global warming may lead to imbalanced nutrient uptake by rice plants and call for an adjusted fertilizer management. Nitrification under AWD increases the uptake and assimilation of nitrate (NO_3^-) by rice plants, which may improve leaf gas exchange and growth in some rice varieties at high vapor pressure deficit (VPD) due to its enhancing effects on stomatal conductance. However, appropriate water management must be applied to avoid drought stress and adverse effects on plant water status during drained periods. Along with nitrification, changes in weed flora under AWD alter the competition between rice and weed. Because NO_3^- affects the growth of weeds, rice might either benefit from increased nitrification in competition with lowland weeds or suffer in competition with upland weeds, especially at high VPD. Selecting varieties performing better under high NO_3^- may enhance the growth of rice and its competitiveness against weeds under water-saving irrigation systems.

5.1 Increasing root zone temperature in water-saving irrigation systems enhances nutrient uptake of rice plants

Global warming will increase RZT, particularly under water-saving irrigation measures (e.g., AWD) where RZT highly depends on air temperature during non-flooded periods (*Stuerz et al., 2014a*). However, the climate change related temperature increase was observed to be more pronounced during the night than during the day (*Davy et al., 2017; IPCC, 2018; Peng et al., 2004; Vose et al., 2005*). Root zone temperature (RZT) impacts root growth, nutrient, and water uptake and thus, regulates plant growth. Previous studies demonstrated that higher RZT significantly increased nutrient uptake in rice plants (*Setter and Greenway, 1988; Yang et al., 1993; Zia et al., 1994*). However, knowledge about the effects of increased RZT during

the day or night on nutrient and water uptake of rice plants was lacking, and therefore, it was studied in the current work at low and high VPD. RZT did not affect the water uptake rate of rice plants, whereas nutrient uptake increased with increasing RZT, both during the day and night. Nevertheless, the temperature response of nutrient uptake was more pronounced during the day than during the night. Furthermore, a close positive correlation between amino acid concentration in leaves at both day- and night-time and nitrogen (N) uptake rate during daytime was found. Therefore, rice plants under AWD may benefit from global warming in terms of nutrient uptake, with higher day temperature being more beneficial than higher night temperature.

Varietal differences in the response of nutrient uptake to RZT were observed. At low VPD, IR64 and NU838 showed a similar trend in nutrient uptake in response to RZT in our study. However, at high VPD, IR64 showed a greater increase in nutrient uptake per °C during the day than NU838, whereas NU838 showed a greater response than IR64 during the night. Differences in N uptake and assimilation between rice varieties were found in previous studies (*Chen et al.*, 2013; *Fan et al.*, 2007, 2005; *Subramanian and Sundaram*, 1985; *Zhong et al.*, 2018), however, not much is known about varietal differences in response to day and night RZT. As both varieties showed differences in the activities of N-assimilating enzymes at high VPD, we hypothesized that varietal differences in nutrient uptake in response to RZT might result from differences in nutrient assimilation, storage, and transport capacity. Therefore, rice varieties will benefit differently from an increase in RZT during the day or night at high VPD in terms of nutrient uptake.

Arai-Sanoh et al. (2010) found that the optimum soil temperature for root growth of rice is about 25°C, while *Sánchez et al.* (2014) reported that the optimum temperature for growth of rice plants is 27.6°C. However, in a study on the effects of day and night temperature on different rice varieties, *Yin et al.* (1996b) reported that the optimum temperature for the development of most rice varieties is 25 - 29°C at the night and about 2 - 4°C higher at the day. *Wassmann et al.* (2009) claimed that the growth rate of rice, depending on genotypes, linearly increases with increasing temperature in the range of 22 - 31°C. The optimum temperature not only differs between varieties, but also depends on development stage (*Sánchez et al.*, 2014; *Yoshida*, 1981). In our study on two rice varieties, a linear correlation between mean RZT and nutrient uptake rate was observed in the range of 19 - 29°C both

during the day and night, indicating that the optimum RZT for nutrient uptake is above 29°C. Since rice varieties showed wide variation in their optimum temperature for plant growth and development (Wassmann et al., 2009; Yin et al., 1996b, 1996a), the optimum RZT for nutrient uptake might differ among rice varieties. Moreover, temperature largely varies between rice-growing seasons and regions. Thus, plant breeding or selection of varieties with an optimum temperature fitting the actual temperature range can optimize nutrient uptake and growth of rice plants.

The increase in N uptake (NH_4^+ and NO_3^-) in response to temperature was larger than that for PO_4^{3-} and K^+ , which could result in unbalances in plant nutrition. The optimum use efficiency of nutrients in plants can be reached if they are taken up in balanced amounts (Janssen, 1998). Using the QUEFTS model (Quantitative Evaluation of the Fertility of Tropical Soils), Xu et al. (2015) reported that grain yield of rice increased linearly if nutrients were taken up in balanced amounts at a N:P:K ratio of 5.0:1.0:5.1 until the yield reached about 60 - 70% of the yield potential. Other studies also showed optimum ratios of nutrient uptake for yield in maize (Setiyono et al., 2010), wheat (Pathak et al., 2003), and radish (Zhang et al., 2019). Since the efficient use of a nutrient depends on its balanced supply and uptake with other nutrients (Aulakh and Malhi, 2005; Janssen, 1998; Yousaf et al., 2017), increasing temperature caused by both water management and global warming will call for an adjusted fertilizer management.

5.2 Enhanced nitrification may improve leaf gas exchange and growth of rice plants

Water-saving irrigation leads to increased oxygen levels and thus nitrification in the soil (Chunmei et al., 2020; Dong et al., 2012). Dong et al. (2012) showed that AWD resulted in a significantly higher potential nitrification activity than continuous flooding (CF), and NO_3^- concentration in the soil slightly increased in the early season and then remained constant. Chunmei et al. (2020) found a significantly higher nitrification in the soil under AWD, resulting in significantly decreased NH_4^+ concentrations but increased NO_3^- concentrations in the soil. In our studies, rice took up a higher share of NH_4^+ than NO_3^- when both forms of N were supplied, but NO_3^- uptake increased when NH_4^+ became less available. Further, rice plants under AWD showed a significantly higher NR activity in the leaves than under CF

(Chunmei et al., 2020), indicating that intensified nitrification under AWD stimulates the uptake and assimilation of NO_3^- .

Plants partially close their stomata at high transpirational demand to reduce water loss via the leaves, which results in decreased stomatal conductance and leaf gas exchange (Ohsumi et al., 2008; Pettigrew et al., 1990). This can constrain plant growth at high VPD. Since NO_3^- serves as osmoticum in the cells, accumulation of NO_3^- in guard cells may induce stomatal opening (Guo et al., 2003), which is beneficial for photosynthesis, provided that the leaf water content is not impaired by water deficit (Zhong et al., 2018). Studies on the impact of NO_3^- uptake and assimilation on leaf gas exchange should therefore consider plant water status. In the current study, NO_3^- -fed plants had a significantly higher stomatal conductance (g_s) and CO_2 assimilation rate (A) compared to NH_4^+ -fed plants at high VPD, but not at low VPD. However, the effects of NO_3^- on leaf gas exchange were only observed after two weeks, but not directly after moving the plants from low to high VPD. As a short-term effect of high VPD, g_s was reduced by more than 50% on average, implying that plants suffered from severe water deficit. In contrast, g_s of plants grown for two weeks at high VPD, was on average only 27% lower than that of plants at low VPD, demonstrating the adaptation capacity of rice plants to high transpirational demand. NO_3^- -fed plants also showed a higher root-to-shoot ratio compared to NH_4^+ -fed plants, which is advantageous in terms of water uptake and can help to maintain the plant water status at high VPD.

However, the effects of NO_3^- on leaf gas exchange of rice plants differed among rice varieties. In the current study, 4 out of 12 varieties showed a significantly higher leaf gas exchange with NO_3^- than with NH_4^+ as N source at high VPD. Previous studies argued that varietal differences in NO_3^- uptake, storage, and assimilation capacity can be found in rice (Chen et al., 2013; Fan et al., 2007, 2005; Zhong et al., 2018), as varieties differed in NO_3^- uptake kinetic, with a low-affinity NO_3^- transport system alone or both, low- and high-affinity NO_3^- transport systems (Fan et al., 2005). NO_3^- is preferentially stored in the vacuoles of epidermal cells in rice plants and the difference in remobilization of stored NO_3^- may be associated with differences in plant water content and N-use efficiency among rice varieties (Fan et al., 2007). Moreover, NO_3^- and amino acids serve as osmotic agents (Guo et al., 2003), which are involved in regulating stomata opening and the acclimation of photosynthesis in rice plants (Zhong et al., 2018). Therefore, we hypothesize that the difference in leaf gas exchange

of rice varieties in response to N source may be partly attributed to the varietal difference in osmotic adjustment caused by assimilation and accumulation of NO_3^- .

The increase in leaf gas exchange in NO_3^- -fed plants did not always result in higher dry matter (DM), which may be attributed to a greater energy demand for NO_3^- assimilation compared to NH_4^+ assimilation (Bloom et al., 1992; Raven, 1985; Salsac et al., 1987). The difference in DM between varieties in response to NO_3^- nutrition may result from differences in the preferential sites of N assimilation (root or shoot), which determines the energy expense related to the translocation of photosynthates (Subramanian and Sundaram, 1985). If NO_3^- is assimilated in the leaves, nicotinamide adenine dinucleotide hydrogen phosphate (NADPH) can be used that is generated during the light reaction (Guo et al., 2007; Zhang et al., 2014), which is energetically more efficient than sucrose synthesis and transport to the root for NO_3^- assimilation (Nunes-Nesi et al., 2010). Fan et al. (2005) demonstrated that some varieties performed best under NO_3^- nutrition, implying that these varieties use NO_3^- more efficiently than others. As rice varieties differ in their response to N source, it is hypothesized that some varieties can profit from the enhanced nitrification under AWD. In order to explore breeding options for varieties adapted to water-saving irrigation, this hypothesis should be validated in the field.

5.3 Promotion of nitrification alters competition between rice and weed

Weeds are a major constraint in crop production, including rice. In conventional lowland rice, the water layer significantly reduces weed seed germination and growth of weed seedling at the early stage (Begum et al., 2006; Bhager et al., 1999; Chauhan and Johnson, 2009; Estioko et al., 2014; Hill et al., 2001), resulting in lower weed pressure. However, this advantage is lost when switching to water-saving irrigation (Bhager et al., 1999; Rodenburg et al., 2011; Van der Hoek et al., 2001). Chen et al. (2017) also reported that maintaining soil saturation at early stage increased weed seedbanks in direct-seeded rice, including some upland weeds. Because rice is being cultivated in diverse systems, including rotation with upland crops, we hypothesize that weed seedbanks in paddy soil are diverse, including upland weeds. Many weed species have a long viability in the soil due to dormancy (Zimdahl, 2018) and their germination may be favored under moist soil conditions, increasing the concern and cost of weed control under AWD. A better understanding of the competitive relationship

between rice and weeds under AWD can help to reduce the dependency on herbicides and improve rice production profitability in the systems.

Echinochloa crus-galli is the most problematic weed in lowland rice worldwide because it is widespread and highly competitive, inducing significant suppression in plant growth and decrease in grain yield (Holm et al., 1991; Tran et al., 2018), while *Solanum nigrum* is a serious weed in many upland crops around the world (Edmonds and Chweya, 1997; Holm et al., 1991). In competition with rice in our study, *S. nigrum* was less competitive than *E. crus-galli*, possibly due to low oxygen levels inhibiting root growth of *S. nigrum* (Marschner, 2011). However, *S. nigrum* still took up a substantial amount of N, suggesting that it could be a significant competitor of rice in terms of nutrient uptake under AWD, where soil oxygen levels are improved (Chunmei et al., 2020). Furthermore, N form and VPD significantly affected the growth and competitiveness of both weed species. In competition with rice, high NO_3^- significantly decreased growth of *E. crus-galli* but increased growth of *S. nigrum*. Since NO_3^- assimilation may improve photosynthesis of C_4 plants (Cousins and Bloom, 2003; Marschner, 2011), but N source had no effect on N uptake and total DM of *E. crus-galli* in monoculture, it seems unlikely that the decrease in the growth of the weed is related to NO_3^- assimilation. As both rice and *E. crus-galli* exhibit allelopathic effects against each other (Tran et al., 2009, 2018), we hypothesize that there is an effect of N form on the allelopathic compound itself or in its production. Further research is needed to test this hypothesis. In contrast, *S. nigrum* is commonly found in upland soils (Holm et al., 1991), and therefore, enhanced N uptake, growth, and competitiveness at high NO_3^- was expected. In addition, its growth was not affected by high VPD, which could be related to the coating of hairs on the weed plant (Edmonds and Chweya, 1997), resulting in lower transpiration, which is beneficial in dry conditions (Wuenschel, 1970). Moreover, in contrast to rice, N uptake rate and water uptake rate of the weed were significantly correlated, which increases the competitiveness of the weed in terms of nutrient uptake at high VPD. Thus, increased availability of NO_3^- through nitrification in the soil under AWD is more beneficial for upland weeds than for lowland weeds.

The competitiveness of rice against weeds plays an important role in weed suppression. Highly competitive rice varieties have been characterized as vigorous seedlings, showing rapid growth and establishment, good tillering, strong roots, tallness, large leaf area, and

allelopathic effects (Dass et al., 2017). Mennan et al. (2012) studied the competitiveness of five rice varieties against *E. crus-galli* and found that tillering capacity, early growth, and plant height were the most important traits in terms of competitive strength. In terms of growth parameters and competitiveness, hybrid rice varieties are often superior to inbred rice varieties. Previous studies also demonstrated the advantage of hybrid varieties in competition with *Echinochloa- crus-galli* due to higher tillering capacity (Ni et al., 2004) and higher allelopathic effects (Tran et al., 2009). In our study, NU838, a hybrid variety showed significantly higher competitiveness against both weeds than KD18, an inbred variety. Further, N source had no effect on the growth of the two rice varieties in monoculture, but due to effects on weed growth, significant effects on the competition between rice and weeds were observed, with high NO_3^- increasing the competitiveness of NU838 when competing with *E. crus-galli* at low VPD but decreasing its competitiveness when competing with *S. nigrum*.

5.4 Outlook for improving rice growth under alternate wetting and drying

In comparison to CF, growth and yield of rice under water-saving irrigation slightly increased in the hot-wet season, but was reduced in the cold-dry season (Stuerz et al., 2014b). The decline in grain yield was attributed to a lower meristem temperature which affected leaf growth and leaf gas exchange, and thus limited carbohydrate gains (Stuerz et al., 2014a, 2014c). In the cold season, water temperature in paddy rice was higher than air temperature (Maruyama et al., 2017), indicating that soil temperature and RZT were lower without than with a ponded water layer, which was also observed by Stuerz et al., (2014a, 2014b). Since nutrient uptake is strongly correlated with RZT, lower RZT under AWD may lead to lower nutrient uptake in the cold season, which could partly explain the often observed decrease in growth and grain yield of rice plants. As RZT is more dependent on the air temperature during the drained periods, we hypothesize that implementation of AWD in warm seasons is more advantageous than in cold seasons. Further, as the combination of organic manure and mineral fertilizer significantly increased root growth and nutrient uptake of rice plants under AWD, leading to higher grain yield than CF (Yang et al., 2004), a balanced nutrient application could potentially minimize the effect of low RZT in AWD in the cold season.

As not only temperature but also VPD is predicted to continuously increase in the coming decades due to global warming (Yuan et al., 2019), rice under AWD could benefit from intensified nitrification related to positive effects on photosynthesis. Yang and Zhang (2010) revealed that under AWD with moderate soil drying, leaf photosynthesis rate did not reduce during drained periods but significantly increased after irrigation, whereas it was reduced under AWD with severe soil drying. Related to the increase in leaf photosynthesis and root growth, they argued that AWD not only saves irrigation water but also has the potential to increase grain yield, if the plant water status is not adversely affected during the drained periods (Yang and Zhang, 2010). Mote et al. (2016) also demonstrated that water content and leaf water potential of rice plants under AWD was significantly affected by irrigation regimes (ponded water level, depth and duration of receding water). Our work, therefore, suggests that the benefits of NO_3^- uptake related to intensified nitrification under AWD on photosynthesis and growth of rice plants can be fully exploited with finely coordinated water management.

Although upland weeds were less competitive than lowland weeds at low-oxygen levels, they still competed with rice in terms of nutrient uptake, resulting in lower availability of N for rice plants and inhibiting the growth of the slow-growing rice varieties, especially at high VPD. Moreover, the growth of the upland crop was favored by NO_3^- . Thus, preventing seeds of upland weeds from accumulating and germinating in the lowland will reduce competitive pressure for rice production under AWD. Although highly competitive rice varieties provide an advantage in competition with weeds, the change in soil N form also has significant effects on this relationship. Gealy et al. (2019) found that in competition with some rice varieties, DM of *E. crus-galli* was significantly lower under AWD compared to CF, but not in monoculture, which could be explained by the effect of increased NO_3^- concentration due to nitrification as observed in our study. Moreover, rice varieties showed differences in competitiveness against *E. crus-galli* under AWD but not under CF (Gealy et al., 2019). Thus, we hypothesize that rice varieties that perform better at high NO_3^- are more competitive against weeds. However, allelopathic effects can alter the competition between rice and weeds and further research is needed to address this issue. Knowledge on allelopathic effects between rice and upland weeds is lacking and the effects of changes in soil N source on the synthesis of allelopathic compounds should be investigated to enable future rice production to be resilient to weeds under water-saving irrigation.

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Chapter 6

Conclusions

This study aimed to investigate the effects of arising challenges in water-saving irrigation in the context of climate change, including increasing root zone temperature (RZT), increased soil nitrate (NO_3^-) concentration, and altered weed dynamics, on growth of lowland rice at varying VPDs.

Increasing RZT increased nutrient uptake in rice plants up to an optimum RZT above 29°C, while water uptake rate was not affected, indicating that rice plants may benefit from global warming in terms of nutrient uptake. Since nutrient uptake is independent of water uptake and VPD, the effect of global warming on nutrient uptake of rice is mainly attributed to higher RZT. However, the increase of nutrient uptake and assimilation with RZT was more pronounced during the day than during the night. Furthermore, as the increase in N uptake per °C was higher than that of PO_4^{3-} and K^+ , rising temperature will shift the ratio of nutrients taken up and may lead to an imbalance in plant nutrition, requiring an adjusted fertilizer management.

At high VPD, NO_3^- nutrition significantly increased photosynthesis in some rice varieties because of higher stomatal conductance and improved root growth. As VPD is predicted to continuously increase with global warming, promotion of nitrification in soil under AWD may be beneficial for rice photosynthesis, provided a sufficient water supply to maintain a high stomatal conductance. However, higher photosynthesis did not always result in higher biomass, probably due to the higher energy demand for NO_3^- assimilation. Since rice varieties showed different responses to N source, this trait should be considered in plant breeding for AWD. However, further studies are needed to clarify the role of NO_3^- uptake and assimilation in controlling stomatal conductance at high VPD.

NO_3^- significantly increased the growth and competitiveness of upland weeds but reduced that of lowland weeds, therefore affecting the competition between rice and weeds. Thus, nitrification under AWD could negatively affect the growth of rice in competition with upland weeds, but may reduce the pressure from lowland weeds. The hybrid rice variety was more competitive when grown with weeds than the inbred rice variety. Selecting varieties

performing better with NO_3^- may increase competitiveness against both upland and lowland weeds under AWD. However, our results indicate that N source might affect the synthesis of allelopathic compounds of rice and weeds, an aspect that needs to be investigated in further studies.

Affidavit

pursuant to Sec. 8(2) of the University of Hohenheim's doctoral degree regulations for Dr.sc.agr.

1. I hereby declare that I independently completed the doctoral thesis submitted on the topic
“Effects of temperature and vapor pressure deficit on genotypic responses to nitrogen nutrition and weed competition in lowland rice”
2. I only used the sources and aids documented and only made use of permissible assistance by third parties. In particular, I properly documented any contents which I used - either by directly quoting or paraphrasing - from other works.
3. I did not accept any assistance from a commercial doctoral agency or consulting firm.
4. I am aware of the meaning of this affidavit and the criminal penalties of an incorrect or incomplete affidavit.

I hereby confirm the correctness of the above declaration. I hereby affirm in lieu of oath that I have, to the best of my knowledge, declared nothing but the truth and have not omitted any information.

Stuttgart, July 22, 2021

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Journal Articles (Peer-review)

2021 - **Vu, Duy Hoang**, Sabine Stuerz, and Folkard Asch. "Rice-weed competition in response to nitrogen form and vapor pressure deficit." *Journal of Agronomy and Crop Science* (submitted).

2021 – **Vu, Duy Hoang**, Sabine Stuerz, Alejandro Pieters, and Folkard Asch. "Leaf gas-exchange of lowland rice in response to nitrogen source and vapor pressure deficit." *Journal of Plant Nutrition and Soil Science*, 1-13. <https://doi.org/10.1002/jpln.202100032>

2020 - **Vu, Duy Hoang**, Sabine Stuerz, and Folkard Asch. "Nutrient uptake and assimilation under varying day and night root zone temperatures in lowland rice." *Journal of Plant Nutrition and Soil Science* 183, 602-614. <https://doi.org/10.1002/jpln.201900522>

- 2020 - Stuerz, Sabine, Suchit P. Shrestha, Marc Schmierer, **Duy H. Vu**, Julia Hartmann, Abdoulaye Sow, Ando Razafindrazaka, Bayuh Belay Abera, Boshuwenda Andre Chuma, and Folkard Asch. “Climatic determinants of lowland rice development.” *Journal of Agronomy and Crop Science* 206 (4): 466–477. <https://doi.org/10.1111/jac.12419>
- 2018 - **Vu, Duy Hoang**, Thi Loan Nguyen, Thi Tam Bui, and Thi Thiem Tran. “Effects of fertilization ratios on the growth of pinto peanut (*Arachis pintoï*) under drought stress conditions.” *Vietnam Journal of Agricultural Sciences* 1 (4): 249–260. <https://doi.org/10.31817/vjas.2018.1.4.01>
- 2017 - **Vu, Duy Hoang**, and Duc Thang Vu. “Effect of transplanting dates and plant density on growth and grain yield of photoperiod sensitive rice var. Bao thai lun.” *Vietnam Journal of Agricultural Sciences* 15 (2): 137–145.
- 2015 - **Vu, Duy Hoang**, and Thi Thanh Binh Ha. “Effect of maize - soybean intercropping and hand weeding on weed control.” *Journal of Science and Development* 13 (3): 354–363.
- 2014 - **Vu, Duy Hoang**, Thi Thanh Binh Ha, and Trung Kien Nguyen. “Effect of nitrogen application on growth and development of barnyard grass (*Echinochloa crus-galli* (L.) Beauv) and rice (*Oryza sativa* L.)” *Journal of Crop Protection Science* 2: 9–14.
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- 2013 - **Vu, Duy Hoang**, Tat Canh Nguyen, Van Bien Nguyen, and Thi Hong Linh Nhu. “Effect of biochar and foliar fertilizer on growth and yield of tomato grown on sandy soil.” *Journal of Science and Development* 11 (5): 603–613.
- 2012 - Ha, Thi Thanh Binh, Xuan Mai Nguyen, Mai Thom Nguyen, Thi Phong Thu Thieu, **Duy Hoang Vu**, and Thi Phuong Lan Nguyen. “Influence of plant density and nitrogen fertilizer on growth and yield of maize on small cave in the mountain land, Dong Van, Ha Giang.” *Science and Technology Journal of Agriculture and Rural Development* 186: 43–48.
- 2011 - Ha, Thi Thanh Binh, Xuan Mai Nguyen, Thi Phong Thu Thieu, **Duy Hoang Vu**, Mai Thom Nguyen, and Thi Phuong Lan Nguyen. “Influence of plant spacing and nitrogen fertilizer on growth and yield of maize (*Zea Mays*) on sloping land Yen Minh, Ha Giang.” *Journal of Science and Development* 9 (6): 861–866.

Book

- 2016 - Ha Thi Thanh Binh, **Vu Duy Hoang**, Nguyen Tat Canh, and Chu Anh Tiep. *Weeds and their control*. Agricultural Academy Publishing House (in Vietnamese).

CONFERENCE ACTIVITY

Presentation

- 2020 - **Duy Hoang Vu**, Sabine Stuerz, and Forkard Asch. “*Leaf gas exchange of lowland rice in response to nitrogen source and vapor pressure deficit*”. Food and nutrition security and its resilience to global crisis (Tropentag). Virtual Conference. 9-11th September.
- 2019 - **Duy Hoang Vu**, Sabine Stuerz, and Forkard Asch. “*Nutrient and water uptake of rice in response to day and night root zone temperatures under different vapor pressure deficits*”. International research on food security, natural resource management and rural development: Filling gaps and removing traps for sustainable resources management (Tropentag). Universities of Kassel and Goettingen, Germany. 18-20th September.
- 2016 - **Duy Hoang Vu**, Sabine Stuerz, and Forkard Asch. “*Root and meristem temperature related to growth of rice under climate change*”. German Alumni: Agricultural injury and adapted strategy under climate change. An Giang University, Vietnam, 15-18th November
- 2016 - **Duy Hoang Vu** and Nguyen Thi Huong. “*Growth response of Brussel Sprout (*Brassica oleracea* var. *gemmifera*) to different fertilizer application levels*”. ISSAAS 2016 international Congress and General Meeting: National and Global Good Agricultural practice in Southeast Asia. Vietnam National University of Agriculture, 5-7th November.
- 2015 - **Duy Hoang Vu**, Sabine Stuerz, and Forkard Asch. “*Growth response of rice to different vapor pressure deficits and diurnal temperature patterns*”. International conference on management of land use systems for enhanced food security: conflicts, controversies and resolutions (Tropentag). Humboldt University in Berlin, Germany, 16-18th September.

GRANT AND FELLOWSHIPS

- 2017 - 2021 PhD-scholarship. DAAD (German Academic Exchange Service), Germany.
- 2013 - 2015 Master-scholarship. Ministry of Agriculture and Rural Development, Vietnam.

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