Studies on improving phosphorus use efficiency of potato: effects on plant growth, physiology, and quality of tubers

Dissertation

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Leangsrun Chea

Born on 07 June 1992 in Cambodia

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Referees:

1. Prof. Dr. Elke Pawelzik
2. Prof. Dr. Klaus Dittert
3. Prof. Dr. Michaela Dippold

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List of abbreviations

3PGA	: 3-phosphoglycerate
ABTS	: 2,2’-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid
Acetyl-CoA	: Acetyl Coenzyme A
ANOVA	: Analysis of variance
ATP	: Adenosine 5'-triphosphate
DAE	: Days after emergence
DAT	: Days after transplanting in hydroponic system
DM	: Dry matter
DPPH	: 2,2-diphenyl-1-picrylhydrazyl
DSMZ	: German Collection of Microorganisms and Cell Cultures GmbH
FM	: Fresh matter
GABA	: γ-aminobutyrate
HSD	: Honestly signicant difference
IAA	: Indole-3-acetic acid
NMR	: Nuclear magnetic resonance
P	: Phosphorus
PCoA	: Principal coordinates analysis
PEP	: Phosphoenolpyruvate
PGPR	: Plant growth-promoting rhizobacteria
P_i	: Inorganic phosphate
PUE	: Phosphorus use efficiency
PUpE	: Phosphorus uptake efficiency
PUtE	: Phosphorus utilization efficiency
TAA	: Total free amino acids
TAC	: Total anthocyanin concentration
TCA	: Tricarboxylic acid
TEAC	: Trolox equivalent antioxidant capacity
TFC	: Total flavonoid concentration
TPC	: Total phenolic concentration

Chapter 1. General introduction

1.1. Background of the study

1.1.1. Importance of phosphorus management in potato production

Potato is a staple food crop and vegetable providing high energy and high nutritional values, which contributes to food security worldwide (Burgos et al., 2020). The world potato production has increased substantially in the last decades, and it attained almost 400 million tons in 2019. However, in Europe potato production has continuously declined, and it accounts for 29% of global production. Within Europe, the highest potato production is seen in Germany, achieving 10 million tons in 2019 (FAO, 2021). Compared to cereals, potato produces more dry matter and protein per unit of production area and time (Storey, 2007; Lutaladio and Castaldi, 2009). The tubers of potato are rich in carbohydrates (about 75% of total dry matter), essential minerals (mainly potassium [K], phosphorus [P], calcium [Ca], magnesium [Mg], and sodium [Na]), and antioxidant compounds (Storey, 2007). The major antioxidants are hydrophilic antioxidants such as polyphenols (1.2-4.4 mg g^{-1} fresh weight) and ascorbic acid (80-540 µg g^{-1} fresh weight) (Andre et al., 2007; Storey, 2007). These antioxidants are thought to play important roles in human nutrition by preventing chronic and degenerative disease (Akyol et al., 2016).

Nutrient management is an important factor determining for potato yield and tuber quality (Westermann, 2005; Koch et al., 2019). Among the essential nutrients, potato demands a relatively high P in the soils (Koch et al., 2019), which is four times higher than that of cereals to achieve 95% of its potential yield (Table 1.1; Nawara et al., 2017). The high requirement of potato is caused by its root inefficiency in P uptake at low soil P concentration (Dechassa et al., 2003). P plays indispensable roles in energy transfer and photosynthesis necessary for metabolic processes during plant growth and development (Hawkesford et al., 2012). A concentration of 3-5 mg P g^{-1} dry matter, in average, is required for optimum plant growth (Hawkesford et al., 2012). In plants, P exists as inorganic orthophosphate forms (P_i) and organic phosphate esters (Veneklaas et al., 2012). A large amount of P is required for cell division and growth in meristematic tissue, which is important for root growth (Weil and Brady, 2016). Furthermore, P is a major constituent of adenosine triphosphate (ATP) for consumption in Calvin cycle in order to convert assimilated CO_2 into carbohydrates in the photosynthesis reaction (Carstensen et al., 2018). Sucrose transports to various parts of plant and starch synthesis are also ATP-dependent processes (Taiz and Zeiger, 2010; Hawkesford et al., 2012). In addition to the importance of P in plant growth and development, adequate P supply is also necessary to achieve higher crop yield and crop quality. Several studies—such as Dechassa et al. (2003), Rosen and Bierman (2008), and Fernandes et al. (2015)—reported the significant increase of potato yield in response to increasing P applications. If other nutrients are available at optimum levels, sufficient P supply results in cation-anion balance, and subsequently, increases nutrient uptake and translocations among various parts of plant (Föhse et al., 1991). The increased allocation of nutrients—such as nitrogen (N), sulfur (S), P, K, and Mg—to tubers of

potato is the major determinant for the production of dry matter, starch, protein, vitamins, and antioxidants (Naumann et al., 2020). For instance, Leonel et al. (2017) showed higher dry matter, starch, and protein, but lower total sugar concentrations in tuber in response to increasing P application. However, Xing et al. (2020) reported a non-significant correlation between P availability in the soils and soluble sugar concentration in potato tubers. Sugar accumulation in the tubers may be cultivar-specific because potato cultivars intended for starch production tend to have less soluble sugars due to high conversion rate from sugars into starch (Santos et al., 2019). These results indicate the importance of sufficient P supply and its interaction with cultivar effect in determining potato productivity and tuber quality. However, a sufficient P level in the soils cannot always be maintained; therefore, plants need to react and develop strategies in order to cope with low and, eventually, excess P availability.

Table 1.1. Average of available P in the soils to achieve 95% relative yield and roots traits of different crops

Crop	Olsen P (mg kg^{-1} soil)	Root length (10^3 cm)	% roots in the top 20 cm soil
Wheat	18	86	53
Potato	76	21	67
Sugar beet	23	52	52
Maize	18	50	45

Adapted from Nawara et al. (2017) and Yamaguchi and Tanaka (1990)

1.1.2. Morphological and physiological responses of plants to varying P availability

In the soils, total P concentration ranges between 200 and 800 mg kg^{-1}, depending on soil types and weathering processes (Tiessen, 2008). Majority of soil P is present in organic forms, and it is not available for many crop plants to uptake (Mengel et al., 2001). Although soils in Europe are rich in plant-available P (Tóth et al., 2014), it is not always sufficient to fulfill plant requirement—especially for potato (Bucher and Kossmann, 2007). P fertilizers are applied to increase soil P and crop yield, but up to 80% of the applied P turns into insoluble complex and immobile forms (Vance et al., 2003). These plant-unavailable forms are prone to surface runoff, which contributes to eutrophication (MacDonald et al., 2011) and subsequently, increases methane emission (Beaulieu et al., 2019). Meanwhile, there is an increasing concern over the scarcity of P resource, and it is believed to reach the peak of supply before 2090 (Cordell and White, 2014). Since potato is extremely sensitive to low P availability (Hopkins and Hansen, 2019), it remains a challenge to maintain high yield and quality while reducing P application in order to minimize P loss into the environment. Therefore, information on plant responses under varying P availability is helpful for developing strategies for improved PUE in potato production.

To ensure growth under limited P availability, plants develop adaptation mechanisms from plant to cell level. At plant level, plants display a variety of symptoms through reduced biomass, early leaf senescence, and ultimately, decreased crop yield (Mengel et al., 2001; Wang et al., 2015). The reduction of biomass under P deficiency results from inhibited leaf expansion and less leaf number (Fredeen et al., 1989). Shoot growth is

strongly inhibited compared to roots, resulted in increased root-to-shoot ratio. This is caused by increase in nutrient and carbohydrate partitioning to roots in order to improve root morphology enhancing P uptake under limited P availability (Vance et al., 2003; Wissuwa et al., 2005). These responses are signaled by sugar and shoot P status under P starvation (Hawkesford et al., 2012). Additionally, limited P availability also induces nutrient imbalance, which influences the uptake of other nutrients and finally, affects plant nutritional status (Fernandes and Soratto, 2012). Beside the morphological and nutritional alternations, P deficiency also suppresses photosynthesis, but it enhances the formation of plant secondary metabolites (Müller et al., 2015; Wang et al., 2015). The modulation of leaf anthocyanins, flavonoids, amino acids, and plant metabolites under P deprivation are necessary for leaf photoprotection and to enhance internal PUE (Vance et al., 2003; Gunes and Inal, 2009; Nguyen et al., 2019). However, when P fertilizers are excessively applied to the soils, P may become toxic if it is accumulated by plants at high concentrations (Silber et al., 2002). P toxicity was reported for the first time in wheat when leaf P concentration exceeded 10 mg g^{-1} of dry matter (Bhatti and Loneragan, 1970). Taiz and Zeiger (2010) also defined toxic nutrient condition when growth or yield declines as the nutrient concentration of plant tissue increases beyond the sufficient levels. In addition to growth reduction, high P availability conditions also induces iron (Fe) and zinc (Zn) deficiency (Barben et al., 2010; Fernandes and Soratto, 2012). The adaptation of plants under low and high P availability ultimately define plant P efficiency under specific P supply (Deng et al., 2018; Irfan et al., 2020).

1.1.3. Benefits of plant growth-promoting rhizobacteria in P deficiency amelioration and P use efficiency improvement

Plant growth-promoting rhizobacteria (PGPR) was firstly defined by Kloepper et al. (1980) as the bacteria that inhabit the rhizosphere, improve plant heath, and may also enhance plant growth. The colonization of these bacteria occurs on the root surface as rhizosphere bacteria, and sometimes they are able to colonize root inner tissues as endophytic bacteria (Barea et al., 2005). There are wide ranges of direct and indirect effects of PGPR to enhance plant health and growth such as defensive mechanisms against pests, free atmospheric nitrogen fixation, phosphate solubilization, soil nutrient availability improvement, and root growth and nutrient uptake enhancement (Solano et al., 2008). Root growth modifications induced by PGPR is achieved by the production of phytohormone and secondary metabolites that modulate hormonal pathway of plants involved in root system development (Vacheron et al., 2013). Root morphological alterations by PGPR is seen as potential benefits for plants to enhance P uptake under the deficiency conditions (Etesami and Adl, 2020). Naqqash et al. (2016) reported the production of indole-3-acetic acid (IAA) of diverse PGPR strains—including *Azospirillum sp.* and *Pseudomonas sp.*—and the inoculation of these PGPR resulted in enhanced plant biomass of potato. Furthermore, Shakeel et al. (2015) showed that inoculation of plant growth-promoting *Bacillus sp.* improved not only growth and yield, but it also promoted nutrient translocation in rice. Because of the limited and shallow root systems of potato, the inoculation of PGPR on potato roots may also provide benefits in improving root morphology and nutrient uptake, which ultimately reduces P fertilizer application and improves PUE.

1.2. Research gaps

P is important for early shoot and root development of potato, and it contributes the most to increasing tuber yield (White et al., 2018). There are studies to assess the effect of P application on plant morphological and physiological responses of potato under different conditions such as hydroponic system (Barben et al., 2010; Fernandes and Soratto, 2012; Fernandes and Soratto, 2016), pot conditions (Dechassa et al., 2003; Ekelöf et al., 2012; Wacker-Fester et al., 2019), and field trials (Rosen and Bierman, 2008; Sandaña, 2016; Wacker-Fester et al., 2019). However, the results differed in accordance to growing conditions. Consequently, the threshold levels of applied P for deficient-, optimal-, and toxic-P conditions were defined differently. Under field conditions, the effect of applied P can be inhibited by either soil organic P mobilization to balance plant-available P, or the immobilization and complexation of this mineral with metallic ions that reduce its availability for plants to uptake (Vance et al., 2003). For instance, Wacker-Fester et al. (2019) reported non-significant differences in potato yield between P fertilized and non-fertilized treatments in a long-term field trial. To overcome this challenge, hydroponic system and pot conditions by using low initial P concentration in the soils are promising tools for assessing plant responses under varying P application (Baron et al., 2018; Vogel et al., 2018). However, the results from previous hydroponic studies also varied, which could be mainly caused by P requirement of different cultivars. Sandaña (2016) and Wacker-Fester et al. (2019) also reported genotypic difference in plant biomass and total P content of potato cultivars in response to low and high P supply. Furthermore, significant variations among cultivars in PUE were observed in other crops, such as in rice (Irfan et al., 2020), sunflower (Gunes and Inal, 2009), and wheat (Ozturk et al., 2005). These results indicate opportunities to explore cultivars with higher PUE in order to reduce P fertilizer application. Therefore, it is necessary to evaluate further potato cultivars to characterize their P efficiency mechanisms under various P availability.

The assessments of applied P on potato have been mainly focused on biomass partitioning (Barben et al., 2010; Fernandes and Soratto, 2012; Wacker-Fester et al., 2019), mineral allocation (Fernandes and Soratto, 2012), root morphology (Fernandes et al., 2014; Wacker-Fester et al., 2019), tuber yield (Dechassa et al., 2003; Sandaña, 2016), and PUE (Sandaña, 2016; Wacker-Fester et al., 2019). The characterization of these parameters was mainly based on experiments under P-deficient conditions, with exception of the studies of Barben et al. (2010) and Fernandes and Soratto (2012), who also reported plant biomass responses under P toxicity. In Arabidopsis, Stewart et al. (2001) showed a four-time increase in leaf flavonoids under P deficiency while Gunes and Inal (2009) also reported a relative accumulation of leaf anthocyanin in sunflower cultivars under P starvation. These secondary metabolites serve to mediate oxidative stress for plants under P deficiency, which is signaled by proline (Signorelli et al., 2014; Aleksza et al., 2017). Moreover, Nguyen et al. (2019) found a significant increase in organic acids, but a decrease in sugars in roots of wheat under low P supply. This finding indicates that P deficiency also alters metabolic pathways of plants. However, there is a lack of research in regard to the biochemical adaptations and metabolite profiling for potato under P deficiency. Along with the findings of Wissuwa et al. (2005) on the importance of P allocation to roots in modifying root

morphology, Nadira et al. (2016) also reported the roles of root phytohormones in regulating root growth in response to P deficiency in barley. However, there is no study investigating the factors that contribute to root morphology and P uptake under P availability of potato. Furthermore, little is known about the physiological and metabolic responses of potato under P toxicity, beside the reports on Fe, Zn, and manganese (Mn) deficiency under high P supply (Barben et al., 2010; Fernandes and Soratto, 2012). Although genes associated with P uptake and translocation—such as family *StPHT1* and *StPHT2*—were already identified and their regulations under P deficiency were assessed in potato (Liu et al., 2017), the responses of these genes in different potato cultivars and under excess P supply have not been documented. Therefore, characterizing morphological, physiological, and biochemical responses of potato under low and high P availability is essential to fill these knowledge gaps and understand how plants react and use P when it is either deficient or available in abundance.

There have been growing interest to explore the potential benefits of PGPR in increasing plant tolerance under P deficiency. The effects of PGPR have been also tested on different crop plants in both soil and hydroponic conditions, as reviewed by Zaidi et al. (2015) and in reports of Naqqash et al. (2016) and Sheridan et al. (2017). These results suggest the positive effect of PGPR inoculation to improve plant growth not only in soil medium, but also in nutrient solution. The assessment of growth-promoting effects of PGPR in hydroponic system has advantages by minimizing the interference of well-structured and regulated community of microorganisms in the rhizosphere that usually occurs in the soils (Backer et al., 2018). However, it still requires a deeper look at the plant-PGPR interaction under P deficiency through assessing root-associated bacterial community and their effects on root growth and P uptake, in which the studies of Naqqash et al. (2016) and Sheridan et al. (2017) did not report. Moreover, although a diverse set of PGPR instead of a single strain was suggested to benefit plant growth (Menéndez and Paço, 2020), it is necessary to elucidate the plant growth-promoting effects of the individual strain in order to assess its efficiency and possible mechanisms to ameliorate P deficiency condition.

The quality of potato tubers is largely influenced by P availability (Naumann et al., 2020). In contrast to the increasing attempts in determining plant morpho-physiological and biochemical responses of potato under P deficiency, the impact of limited P availability on quality of potato tubers is less documented. There are only a few reports on tuber yield, dry matter, starch, protein, and sugars under P deficiency (Öztürk et al., 2010; Fernandes et al., 2015; Leonel et al., 2017). Plant growth limitation under P starvation implies a shortage of P and carbohydrate for translocation, which consequently, influence mineral concentration and quality parameters of the tubers. These effects are also associated with the physiological and metabolic response of the whole plant to translocate the nutrients and carbohydrates for tuber yield and quality formation (Koch et al., 2019). However, to what extent that P deficiency alters quality traits of potato is less reported. There is also no study that links physiological adaptations and tuber quality under P deficiency. Wang and Frei (2011) showed in their review an increase of micronutrients, protein, and antioxidant capacity in potato tubers and grains of various crops as a result of nutrient uptake alteration and the modulation of key enzymes under abiotic

stress. Taking these results together with the report of Stewart et al. (2001) on the accumulation of total flavonoids in leaves, and Nguyen et al. (2019) on the increased amino acids in leaves and roots under P deficiency, stress induced by limited P availability may also stimulate minerals, protein concentration, and antioxidant capacity in tubers. However, this speculation requires empirical studies for elucidation and to explain the link between plant adaptation and tuber quality under limited P supply.

1.3. Objectives and research questions of the dissertation

The goal of this dissertation is to assess P efficiency mechanisms for potato as influenced by P availability, cultivar, and PGPR inoculation. Furthermore, the implications of P deficiency on tuber quality are also revealed. To achieve the overall goal, pot and hydroponic experiments were conducted under greenhouse and outdoor conditions with specific objectives and research questions in accordance with the chapters as outlined below:

- **Objective 1**: To characterize plant morphology, mineral allocation, and metabolite accumulation of potato under various P levels (**Chapter 2**)
    - **Research question 1**: To what extent P deficiency and toxicity modulate plant biomass allocation, uptake of minerals, and metabolite compounds?
- **Objective 2**: To assess the benefits of PGPR inoculation in P deficiency amelioration (**Chapter 2 and 3**)
    - **Research question 2**: How effective are PGPR co-inoculation and single strain addition in enhancing P uptake of potato under low P supply?
- **Objective 3**: To assess the morphological and physiological adaptation responses of different cultivars under low and high P supply (**Chapter 3 and 4**)
    - **Research question 3**: What are the possible factors determining plant P efficiency and root morphology under low and high P supply?
- **Objective 4**: To determine the effect of low P supply on yield and quality of tubers (**Chapter 4**)
    - **Research question 4**: How does limited P availability influence quality-relevant tuber compounds and antioxidant capacity of different cultivars?

1.4. Overview of the dissertation

In response to the above objectives and research questions, this dissertation presents the results of three studies corresponding to the following chapters.

- **Study 1** (Chapter 2): "**Morphological and metabolite responses of potato under various phosphorus levels and their amelioration by plant growth-promoting rhizobacteria**" was published in the *International Journal of Molecular Sciences*. Potato (cv. Milva) was grown under varying P levels (0-40 mg L^{-1}) in nutrient solution. Additionally, a diverse set of PGPR strains including *Variovorax paradoxus* DSM 30034 (NBRC 15149), *Azoarcus sp.* DSM 9506, *Azospirillum sp.* DSM 1842, *Bacillus subtilis* DSM 21393, and *Pseudomonas putida* DSM 6125 (KT2440) were co-inoculated mainly at low P levels (0-2 mg L^{-1}). The

results addressed the first objective by characterizing the influence of P deficiency and toxicity on plant growth, uptake of minerals, and plant metabolites. This chapter also fulfilled part of the second objective by revealing the effect of diverse PGPR strains inoculation on root growth and P uptake under low P levels. The findings in this study provide a deeper understanding of P deficiency and toxicity responses in potato in regard to nutrient interaction and metabolite changes, which are closely related to tolerance mechanisms under both P deficiency and toxicity. It also suggests the potential of PGPR co-inoculation to increase potato plant tolerance under P deficiency.

- **Study 2** (Chapter 3)**: "Effect of phosphorus availability and plant growth-promoting *Bacillus subtilis* on phosphorus efficiency of two potato cultivars"** is currently under review in the journal *Physiologia Plantarum.* The Study 1 reported the sensitivity of potato not only under limited P availability, but also under high P supply through plant biomass reduction. The positive effect of PGPR co-inoculation also provided the opportunity to further elucidate the plant growth-promoting effect of individual strain. Therefore, Study 2 was conducted to characterize two cultivars—a table cultivar (cv. Milva) and a starch cultivar (cv. Lady Claire)—under various P levels (0.5-30 mg L^{-1}) in nutrient solution. Similar to Study 1, plant growth-promoting *Bacillus subtilis* DSM 21393 was inoculated mainly under low P levels (0.5 and 2 mg L^{-1}) and compared with plants without the inoculants. This study was aimed to complement the second objective and part of the third objective of this dissertation by elucidating the adaptation response of two cultivars at plant and root level under various P availability. The results showed the tolerance of Milva and sensitivity of Lady Claire under low P levels. Nevertheless, Lady Claire was efficient in P uptake under high P supply. The possible factors determining PUE and root morphology were also revealed. Because Lady Claire lacked adaptation strategies under P deficiency, it was relatively more responsive than Milva in regard to the symbiotic interaction with *B. subtilis* to increase P uptake

- **Study 3** (Chapter 4): "**Cultivar-dependent responses in plant growth, leaf physiology, phosphorus use efficiency, and tuber quality for potato under limited phosphorus availability conditions**" was published in the journal *Frontiers in Plant Science*. The study used six potato cultivars consisted of four table cultivars (Agria, Lilly, Milva, and Sieglinde) and two starch cultivars (Lady Claire and Verdi). These cultivars were assessed in pot conditions with the application of P_{low}= 0.02 g P kg^{-1}, P_{med}= 0.2 g P kg^{-1}, and P_{high}= 1.2 g P kg^{-1}. The study was conducted with the aim to fulfill the third and fourth objectives in this dissertation. The results revealed a significant variation of cultivars in response to P availability. We were also able to identify P-efficient and P-inefficient cultivars based on their tuber yield formation under P deficiency. Additionally, leaf physiological adaptation and PUE of these cultivars under P starvation were explained. The study also elucidated the impact of P deficiency on tuber quality of P-efficient cultivars.

In addition to the studies reported in Chapters 2 to 4, Chapter 5 compiles the key findings of the three studies and discuss the mechanisms underlying P efficiency of potato and its impact on tuber quality as well as the outlooks for future research. Furthermore, Chapter 6 also provides the conclusions that can be drawn from the studies.

Chapter 2. Morphological and metabolite responses of potato under various phosphorus levels and their amelioration by plant growth-promoting rhizobacteria

Leangsrun Chea[1], Birgit Pfeiffer[2], Dominik Schneider[2], Rolf Daniel[2], Elke Pawelzik[1], and Marcel Naumann[1]

[1]Division Quality of Plant Products, Department of Crop Sciences, University of Göttingen, Carl-Sprengel-Weg 1, 37075 Göttingen, Germany

[2]University of Göttingen, Institute of Microbiology and Genetics, Department of Genomic and Applied Microbiology, Grisebachstraße 8, 37077 Göttingen, Germany

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Author contribution

Leangsrun Chea	: performed the experiment, analyzed data, drafted the manuscript, and revised the manuscript based on suggestions from other co-authors
Birgit Pfeiffer	: conducted DNA isolation, 16S rRNA gene amplification, analyzed the data, and revised the manuscript
Dominik Schneider	: contributed to 16S rRNA gene data processing and revised the manuscript
Rolf Daniel	: conducted amplicon-based analysis and revised the manuscript
Elke Pawelzik	: supervised and revised the manuscript
Marcel Naumann	: supervised, designed the experiment, and revised the manuscript

Abstract

Low phosphorus (P) availability is a major limiting factor in potato. P fertilizer is applied to enhance P in the soils; however, it may become toxic when plants accumulate at high concentrations. Therefore, it is necessary to gain more knowledge of the morphological and biochemical processes associated with P deficiency and toxicity in potato, as well as to explore an alternative approach to ameliorate the P deficiency condition. A comprehensive study was conducted (I) to assess plant morphology, mineral allocation, and metabolites of potato in response to P deficiency and toxicity; and (II) to evaluate the potency of plant growth-promoting rhizobacteria (PGPR) in improving plant biomass, P uptake, and metabolites at low P levels. The results revealed a reduction in plant height and biomass by 60-80% under P deficiency compared to P optimum. P deficiency and toxicity conditions also altered the mineral concentration and allocation in different parts of the plant due to nutrient imbalance. The stress induced by both P deficiency and toxicity was evident from an accumulation of proline and total free amino acids in young leaves and roots. Furthermore, root metabolite analyses revealed that P deficiency reduced sugars by 50-80% and organic acids by 20-90%, but increased amino acids by 1.5-14.8 times. However, the effect of P toxicity on metabolic changes in roots was less pronounced. Under P deficiency, PGPR significantly improved the root and shoot biomass, total root length, and root surface area by 32-45%. This finding suggests the benefit of PGPR inoculation to increase potato plant tolerance under P deficiency.

Keywords: plant biomass; metabolite profiling; mineral nutrients; phosphorus deficiency; phosphorus toxicity; potato; plant growth-promoting rhizobacteria; secondary metabolites

2.1. Introduction

Phosphorus (P) is an essential element for plant growth and metabolism (Hawkesford et al., 2012). Although P in natural soils is abundant, its availability for plants is very limited (Vance et al., 2003). Since the 1950s, inorganic P fertilizer use has rapidly increased to enhance P availability in the soils in order to increase crop yields (MacDonald et al., 2011). Excessive P application often results in low P use efficiency because the majority of applied P becomes insoluble complexes and is lost through surface run-off, causing eutrophication (Vance et al., 2003). Meanwhile, almost 30% of global arable land is P deficient due to less accessibility to P fertilizers in the regions (MacDonald et al., 2011). Therefore, it is necessary to optimize P fertilization practices in order to improve P efficiency.

Compared to other crops, potato has a relatively high P demand. If all other nutrients are available in sufficient amount, potato needs a soil P concentration of about 76 mg kg^{-1} of Olsen P, which is four times higher than the demand of cereals to achieve 95% of its yield potential (Nawara et al., 2017). This high requirement is caused by the shallow root system and inefficiency in P uptake at low soil P concentration (Dechassa et al., 2003), making P a major limiting factor in potato production. P is important for early root and shoot development of the plant, and it also contributes most to increasing tuber yield (White et al., 2018). Continuous supply of inorganic P may result in high P accumulation in the soil, and it subsequently creates P toxicity causing plant biomass reduction (Barben et al., 2010). However, assessing responses of potato to low and high P supply is difficult in field conditions. In soils effects of P application can be inhibited by either soil organic P mobilization to balance available P concentration, or complexation of P and metallic ions that reduce availability of applied P (Vance et al., 2003). The hydroponic system is a more suitable tool to assess the plant growth and metabolite responses under P deficiency and toxicity (Baron et al., 2018). Even if the hydroponic condition does not always reflect the growth conditions of plants in the field, it is widely used to assess the mechanisms underlying plant responses under varying P availability. This is because the interference by organic P solubility and plant–plant interaction, which often occurs in the fields, is omitted or minimized (Wang et al., 2015; Baron et al., 2018).

To ensure growth, plants require adaptation mechanisms from plant to cell level to cope with P deficiency and toxicity conditions. Root growth is improved under P deficiency compared to shoot growth, in order to enable the root system to exploit limited available P (Vance et al., 2003; Wissuwa et al., 2005). Deficient and excess-P application also induce nutrient imbalance, which affects the uptake of other nutrients by the plant and influences crop nutritional status (Fernandes and Soratto, 2012). Apart from these morphological and nutritional interferences, P deficiency also regulates secondary metabolites such as accumulation of anthocyanin, flavonoids, and amino acids in the leaves. Stewart et al. (2001) reported that flavonoid concentration of Arabidopsis and tomato was four and two times higher when P availability was reduced from 6.3 mM to 0 mM, respectively. Plant stress induced by either P deficiency or toxicity leads also to the stimulation of proline, which helps prevent the plant cellular structures from oxidative damage and triggers the accumulation of the abovementioned secondary metabolites (Signorelli et al., 2014; Aleksza et al., 2017).

Moreover, modifications of plant metabolic pathways can occur under P deficiency to enhance internal P use (Vance et al., 2003). Nguyen et al. (2019) found a significant increase in organic acids, but a decrease in sugars and phosphorylated sugars in roots of wheat cultivars under low P supply. However, there is a lack of research regarding the biochemical adaptation of potato under P deficiency. There is also no information available on the P toxicity of potato that elucidates its physiological and metabolic responses, except some reports showing biomass reduction and micronutrient (i.e. iron [Fe], zinc [Zn], and manganese [Mn]) deficiency under excess P conditions (Barben et al., 2010; Fernandes and Soratto, 2012). Therefore, understanding and characterizing potato response to P deficiency and toxicity is necessary to improve the plant's tolerance to these conditions.

Furthermore, there has been a growing interest in exploring the benefit of plant growth-promoting rhizobacteria (PGPR) in increasing P efficiency. Many PGPR can produce secondary metabolites and phytohormones that can stimulate the hormonal pathways of plants involved in root development (Vacheron et al., 2013). These positive effects of PGPR have been reported in pea (Jiang et al., 2012) and other vegetable crops, including potato (Andreote et al., 2009; Naqqash et al., 2016). Menéndez and Paço (2020) demonstrated the benefit of using a diverse set of PGPR instead of single-strain inoculant on plants; however, it requires a deeper look at plant-PGPR interaction. To the best of our knowledge, little studies have been conducted to understand the interactive effect of PGPR and P on root growth and P uptake of potato.

In the present study, a range of P fertilization levels (0-40 mg L^{-1}) were tested on potato plants in a hydroponic condition. Five bacterial strains including *Variovorax paradoxus* DSM 30034 (NBRC 15149), *Azoarcus sp*. DSM 9506, *Azospirillum sp*. DSM 1842, *Bacillus subtilis* DSM 21393, and *Pseudomonas putida* DSM 6125 (KT2440) were co-inoculated mainly at low P levels (0-2 mg L^{-1}). These strains were selected based on literature research of their growth-promoting effects on various crop plants including vegetables, field crops, and paddy rice in which the submerged conditions of the latest crop are similar to the hydroponic condition. The objectives of the present study were (I) to assess plant morphology, mineral allocation, and metabolite compounds of potato in response to different P applications and (II) to evaluate how PGPR affects plant biomass, P uptake, and metabolites at low P levels in nutrient solution. Consequently, the present study shows the first on metabolite profiling of potato root under P deficiency and toxicity conditions. The potential application of PGPR for potato root growth enhancement is also elucidated.

2.2. Materials and methods

2.2.1. Experimental setup and plant cultivation

The hydroponic experiment was conducted under greenhouse conditions. External light was supplied using sodium vapor lamps with photosynthetic photon flux density (PPFD) of 400 µmol m^{-2} s^{-1} at plant level from 6 am to 10 pm. The average temperature during the growing period was 19.3 ±5.0°C, and the average relative humidity was 42.8±9.9%. The experiment was arranged in a randomized complete block design under different P levels and PGPR inoculation scenarios with four replications. The P levels were 0, 1, 2, 5, 12, 30, and 40 mg P L^{-1} with the use of $Ca(H_2PO_4)_2*H_2O$, and these P treatments are referred as P0, P1, P2, P5, P12,

P30, and P40, respectively, in the entire manuscript. To assess the effects of PGPR, the low P levels (P0, P1, and P2) were treated with and without PGPR inoculation (Figure 2.1A).

Seedlings of the potato cultivar "Milva" (Europlant Pflanzenzucht, Lüneburg, Germany) were raised in a pot (one seedling per pot) filled with 3 kg of quartz sand, which received 8 mg P kg^{-1}. Other nutrients were applied at optimum (Supplemtary Table 2.1). Four weeks later, the healthy and uniformly grown seedlings were removed from quartz sand and transplanted in 6 L pots (one plant per pot), which contained nutrient solution with different P concentrations. Other nutrients were applied at sufficient amounts (Supplementary Table 2.2). The nutrient solution had a pH of 5.5-6.5. During the first two days after seedling transplanting in nutrient solution (DAT), the nutrients were supplied at 20% of the full concentration, and it was increased it to 50%, 70%, and 100% every two days. The nutrient solution was constantly aerated (Figure 2.1B). The nutrient solution was renewed once per week until the end of the experiment.

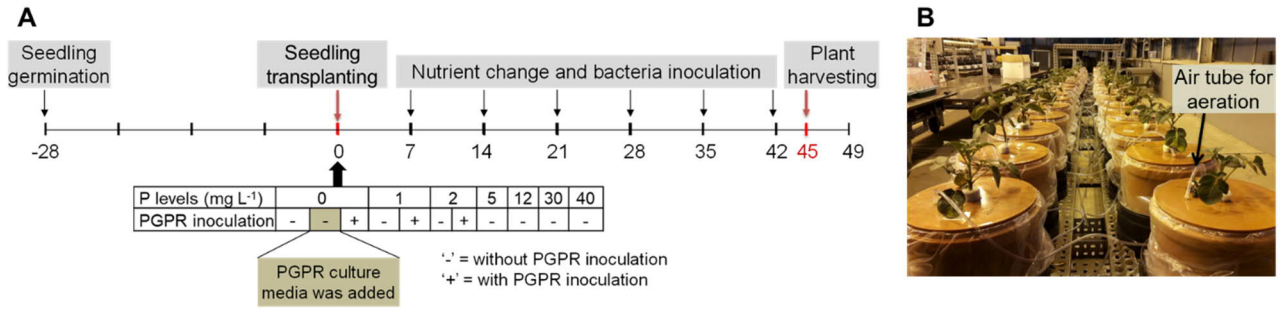


Figure 2.1. (A) Experimental design and timeline and **(B)** growing condition and pot arrangement in the greenhouse

2.2.2. Bacteria culture and inoculation

Five taxonomically diverse PGPR including *Variovorax paradoxus* DSM 30034 (NBRC 15149), *Azoarcus sp*. DSM 9506, *Azospirillum sp.* DSM 1842, *Bacillus subtilis* DSM 21393, and *Pseudomonas putida* DSM 6125 (KT2440) were obtained from German Collection of Microorganism and Cell Culture (DSMZ, Braunschweig, Germany). The biome of each bacterium is listed in Table 2.1. The bacteria cultivation procedures are depicted in Supplementary Figure 2.1. For inoculation, a 50 mL mixture of the five bacteria cultures was prepared by adjusting the density of each strain based on optical density measurement at 600 nm (OD_{600}). The mixture, which contained 2.8-10 x 10^9 colony-forming units (CFU) per ml of each strain, was then used to inoculate the nutrient solution at 7 DAT. A control treatment under P0 was also established by adding sterile media (Figure 2.1A, highlighted in ochre). Both bacteria mixture and sterile media were renewed once a week after the exchange of nutrient solution.

Table 2.1. List of the bacteria strains, biome, and their growth-promoting effects

Bacterium	DSMZ[#] code	Biome	Plant growth-promoting effects
Variovorax paradoxus	DSM 30034	Soil	Rhizosphere and endophere bacterium of potato (Han et al., 2011; Han et al., 2013) Producing indole-3-acetic acid (IAA); improving plant biomass and P uptake of pea (Jiang et al., 2012)
Azoarcus sp.	DSM 9506	Laboratory aquifer column	Endophere bacterium, producing IAA, fixing N_2, and enhancing P uptake of rice (Fernández et al., 2014)
Azospirillum sp.	DSM 1842	Maize roots	Endosphere bacterium and IAA synthesis in rice (Kaneko et al., 2010) Producing IAA and promoting plant biomass of potato (Naqqash et al., 2016)
Bacillus subtilis	DSM 21393	Potato tubers	Endophere bacterium of maize (Gond et al., 2015) Producing IAA; improving root length and plant biomass of potato (Pathak et al., 2019)
Pseudomonas putida	DSM 6125	Unknown	Endosphere bacterium of potato (Andreote et al., 2009) Producing IAA, improving root growth of canola (Patten and Glick, 2002)

[#]DSMZ= German Collection of Microorganism and Cell Culture (Braunschweig, Germany)

2.2.3. Quantification of bacteria-derived indole-3-acetic acid

Each strain culture was diluted to 0.4 at OD_{600}. A total of 50 mL of the diluted culture was centrifuged at 2,700 *g* for 15 min, to collect the supernatant for colorimetric quantification of indole-3-acetic acid (IAA) based on Sarwar and Kremer (1995). Then, 150 µL of culture supernatant was added to 100 µL of Salkowski reagent (34.3% of $HClO_4$ and 10 mM of $FeCl_3$). Fresh, sterile media for each bacterium were used as blanks. The mixture was incubated for 30 min at room temperature and read in a plate reader (Biotek, Winooski, United States) at 530 nm absorbance.

2.2.4. Plant growth measurements, harvest, and sample processing

Plant height was measured at 11, 18, 28, and 45 DAT. At 18 and 45 DAT, the number of leaves was also counted. Whole plants were harvested at 45 DAT and separated into shoots (aerial parts of plants) and roots. The roots were washed in distilled water three times and dried with tissue paper. The shoots were further separated into young leaves, old leaves, and main stem for sample preparation and analyses. Young leaves were defined as petioles at the third and fourth position from the top, and old leaves as petioles at the first and second position from the bottom with less than 30% chlorosis and necrosis. Side shoots and remaining leaves were not included in sample preparation, but they were included for shoot biomass calculation. All samples were then oven-dried at 60°C for 72 hours. A part of the root samples was immediately frozen in liquid nitrogen and afterward freeze-dried for four days using an EPSILON 2-40 freeze dryer (Christ, Osterode am Harz, Germany). After that, all samples were milled (DFH 48 Culatti, Kinematica, Malters, Switzerland) to obtain a fine power (<0.5 mm).

To quantify the effect of PGPR on root growth, one-third of the fresh root sample of PGPR-treated and non-treated plants of the same P level was scanned for total root length and root surface area. Each sample was split into subsamples of 5-10 root segments. To minimize the overlapping, root segments of each subsample were separated and placed in a transparent water bath (20 x 24 x 2 cm) containing 1 cm of water. A digital image of each subsample was acquired using an EPSON Perfection V800 Photo scanner (Epson, Nagano, Japan) and analyzed using WinRHIZO image analysis software (Regent Instruments, Quebec, Canada).

2.2.5. Mineral analyses

Based on the method described by Koch et al. (2019), the concentrations of P, potassium (K), calcium (Ca), magnesium (Mg), sulfur (S), Fe, Mn, and Zn were determined in young leaves, old leaves, stem, and roots. About 3.5-4 mg of each sample was also weighed in a 5 x 9 mm tin capsule (IVA Analysentechnik, Meerbusch, Germany) and analyzed for nitrogen (N) and carbon (C) concentration against acetanilide standard using dry combustion method in a Vario EL analyzer (Elementar, Langenselbold, Germany).

2.2.6. Chlorophyll, free proline, and total free amino acid analyses

A total of 20 mg of each oven-dried young leaf, old leaf, and root samples was mixed with 250 µL of 80% ethanol for 30 min at 95°C. It was then centrifuged at 10,600 *g* for 10 min to collect the supernatant. The procedure was repeated again with 150 µL of 80% ethanol and then three times more with 250 µL of 50% ethanol. The supernatants from all steps were combined. For analyses, the chlorophyll concentration of young leaves was photometrically determined based on Koch et al. (2019). Free proline concentration was measured according to Bates et al. (1973). Total free amino acid concentration (TAA) was determined based on the reaction of amino acids with fluorescamine. A total of 2 µL of the extract or glutamic acid standard was mixed with 15 µL of sodium borate buffer (0.1M; pH 8.0), 90 µL of fluorescamine (0.1% in acetonitrile), and 10 µL of water. After incubation for 5 min, the fluorescence was measured in a plate reader at 360/40 nm excitation and 460/40 nm emission.

2.2.7. Secondary metabolite analyses

A total of 50 mg of oven-dried young leaf, old leaf, and root sample was extracted twice with 1 mL of 60% methanol containing 1% HCl. The supernatant was collected and filled up to 2 mL with the acidified methanol. For measurements, total anthocyanins (TAC) in young leaves were determined spectrophotometrically, following Hada et al. (2003), by using Cyanidin-3-glucoside as standard. The $AlCl_3$ colorimetric method of Chandra et al. (2014) was adapted to determine total flavonoid concentration (TFC) in the plant material by using Quercetin-3-glucoside standard in a plate reader. Total phenolic concentration (TPC) was determined using the Folin–Ciocalteu assay. A total of 300 µL of the extract was mixed with 1 mL of 0.5 M NaOH, 2.6 mL of water, and 100 µL of the Folin-Ciocalteu reagent, and the mixture was incubated for 15 min at 37°C. After cooling, the TPC was measured in a UV-Vis spectrophotometer (Hewlett Packard, Boeblingen, Germany) at 736 nm absorbance. Gallic acid was used as standard.

2.2.8. Root metabolite profiling

The metabolite extraction and analytical procedures were conducted at Lifespin (Regensburg, Germany). Metabolites in roots were extracted by homogenizing 100 mg of freeze-dried samples with 1.5 mL of 1 M phosphate buffer (pH 6.8) containing 5% D2O and 0.01% NaN3. The mixture was incubated for 20 min at 85°C and cooled down for 40 min to room temperature. The supernatant was collected after 10 min centrifugation at 10,600 *g*. Then, 630 µL of the aliquot was suspended with 70 µL of additive solution (Lifespin, Regensburg, Germany) containing internal reference standards. Afterward, 600 µL of the mixture was filled in a 5 mm NMR-tube and analyzed by nuclear magnetic resonance (NMR) spectroscopy using a Bruker AVANCE III HD 600 MHz spectrometer (1D 1H noesygppr NMR spectrum, NS=32, T=298K). The obtained spectra were analyzed with Lifespin's proprietary profiling software (V1.2.3, customized for potato root extracts). The metabolite was identified by comparison with Lifespin's database and subsequently quantified against pyrazine (8.66 ppm, 4 protons). The concentration of each metabolite was expressed as mg per g dry matter (mg g^{-1}).

2.2.9. Root-associated bacterial community

a. DNA isolation and 16S rRNA gene amplification

To analyze the root-associated bacterial community, DNA was extracted from 500 mg freeze-dried root samples using the DNeasy PowerSoil Kit (Qiagen, Hilden, Germany) as recommended by the manufacturer. The obtained DNA was used for amplification of the V3-V4 region of the 16S rRNA gene. The bacterial primer pair S-D-Bact-0341-b-S-17 and S-D-Bact-0785-a-A-21 targeting the V3–V4 region was used as described by Klindworth et al. (2013) with addition of adapters for Illumina MiSeq sequencing. The PCR reaction mixture contained 5-fold Phusion GC buffer, 200 µM of each of the four deoxynucleoside triphosphates, 5% DMSO, 0.4 µM of each primer, 1 U of Phusion HF DNA polymerase (Fisher Scientific, Osterode am Harz, Germany), and 25 ng of DNA as template. The cycling scheme used for DNA amplification was as follows: initial denaturation at 98°C for 5 min and 25 cycles of denaturation at 98°C for 45 s, annealing at 60°C for 30 s and extension at 72°C for 30 s, followed by a final extension at 72°C for 10 min. PCR reactions were performed in triplicate for each sample. The resulting PCR products were pooled equimolar and were purified using the GeneRead Size Selection kit (Qiagen, Hilde, Germany) as recommended by the manufacturer. The PCR products were quantified using the Quant-iT dsDNA HS assay kit and a Qubit fluorometer, as described by the manufacturer (Invitrogen, Osterode am Harz, Germany). Indexing of the PCR products was performed by the Göttingen Genomics Lab (Göttingen, Germany) using the Nextera XT Index kit as recommended by the supplier (Illumina, San Diego, United States). Sequencing of 16S rRNA genes was performed using the dual index paired-end approach (2 × 300 bp) with v3 chemistry for the Illumina MiSeq platform as recommended by the manufacturer (Illumina, San Diego, United States).

b. Sequence data processing and analyses

Adapter removal and quality filtering of raw paired-end sequences was done using fastp v0.19.6 (Chen et al., 2018), with base correction in overlapping regions, a Phred quality score of 20, size filtering of sequences longer 50 bp and per read trimming by quality with sliding windows of 4 and soft-clipping. The quality-filtered paired-end reads were merged by PEAR v0.9.11 with default parameters (Zhang et al., 2014). Primer removal was conducted using cutadapt v1.18 (Martin, 2011). Subsequently, dereplication, denoising, as well as chimera detection and removal (*de novo* followed by reference based) was performed with VSEARCH v2.13.0 (Rognes et al., 2016). Finally, the raw reads were mapped to amplicon sequence variants (ASVs). The ASVs were taxonomically classified with BLAST+ v2.7.1 against the SILVA 132 SSU reference database (Quast et al., 2013). Subsequently, extrinsic domain ASVs and chloroplasts were removed from the dataset. Sample comparisons were performed at the same surveying effort of 8,333 sequences per sample. Statistical analyses were done using the ASV table in R version 3.5.3 (R Core Team, 2019). Species richness, alpha diversity estimates, and rarefaction curves were determined using ampvis2 v2.4.7 (Andersen et al., 2018). To analyze the root-associated microbial communities in the differently treated plants, PCoA based on Bray–Curtis distance measures was performed without data transformation (Bray and Curtis, 1957). The correlation of environmental parameters to the bacterial community composition was performed using the envfit function of the vegan package version 2.5-4 (Oksanen et al., 2019) and projected into the ordination with arrows with a p-value cutoff of ≤ 0.05.

2.2.10. Statistical analysis

Data of plant growth, mineral concentration, secondary metabolites, and metabolite profiles were subjected to an analysis of variance (ANOVA). Tukey's HSD test at $p \leq 0.05$ was conducted for pairwise comparison of the treatments when there were significant differences of ANOVA. Contrast analyses were performed to answer relevant questions regarding different effects including the effect of P, the effect of PGPR at low P levels (P0-P2), and the effect of bacteria by excluding culture media (P0+PGPR vs. P0+M). These analyses were performed with Statistix 8.0 (Analytical Software, Tallahassee, United States).

2.3. Results

2.3.1. Effect of P application on plant morphology and leaf chlorophyll concentration

The important plant morphological traits and leaf chlorophyll were assessed in response to varying P availability in nutrient solution. Plants under P0 showed deficiency symptoms through stagnated height development and significant reduction in leaf number during the growing period (Figures 2.2A-C). With regard to increasing P application, root and shoot biomass significantly increased up to P5. Root and shoot biomass of P5 plants were 12- and 36-fold higher, respectively, than that of P0, but they were not significantly different from P1 and P2. Increasing P supply higher than P5 resulted in a significant decrease in root biomass by 44-53%, as well as in shoot biomass by 14-30%, under both P30 and P40 compared to P5 (Figure 2.2D). Therefore, shoot growth was severely impaired under P limitation (P0). Excess P application (P30 and P40) strongly

inhibited root biomass, implying P toxicity conditions for plants. These results also suggest that P5 may be the optimum P application for potato plant growth in this study. However, leaf chlorophyll concentration increased by 17-20% under both P limitation and excess compared to P5, even though it was not significant (Figure 2.2E).

2.3.2. Effect of P application on nutrient concentration in different parts of plant

Young leaves, old leaves, stem, and roots of potato were harvested 45 days after the onset of P treatments for quantifying nutrient concentrations. Tissue P analyses revealed that P concentration in all parts of plant increased at different magnitudes in response to increasing P levels in the nutrient solution up to P30 and, subsequently, decreased at P40 by 20%, 17%, 9%, and 46% in young leaves, old leaves, stem, and roots, respectively, compared to those at P30 (Figure 2.3A).

Furthermore, Figures 2.3B-I and Supplementary Figure 2.1 show that varying P application to the nutrient solution also significantly induced different concentrations and distribution patterns of macro- and micro-nutrients among plant parts. Plants under P0 exhibited 23-82% higher in N, N-to-P ratio, Mg, Fe, Mn, and Zn concentration, but 33–60% lower in the C-to-N ratio in all studied parts compared to plants under optimum P (P5). In response to increasing P availability, Mg, Ca, Fe, and Mn concentrations in young leaves, old leaves, and stem decreased at P40 by 40-63% compared to those at P5 (Figures 2.3E-H).

2.3.3. Effect of P application on plant secondary metabolites and total free amino acids

In order to confirm P deficiency and toxicity conditions in the potato plant, secondary metabolites and amino acids such as TPC, TFC, TAA, and proline concentration in young leaves, old leaves, and roots, and TAC in young leaves were quantified. Figures 2.4 shows that TFC, TAC, TAA, and proline concentrations in young leaves and roots of plants at P0 were 60–200% higher than in those under P5. However, TAC in young leaves decreased in response to increasing P application until P5, followed by a subsequent increase at P40 by 25% compared to those at P5. Increasing P supply to the nutrient solution also led to decreasing of TAA and proline in all parts of plant, but they recovered in young leaves and roots under P30 with a subsequent increase under P40. This recovery was more evident in roots, in which the TAA and proline concentrations increased by 1.5 and 2 times with the application of P30 and P40, respectively, compared to P5. The high accumulation of these secondary metabolites, especially TAA and proline, at P0, P30, and P40 indicate P deficiency- and toxicity-induced stress conditions in plants. There was no clear indication for P deficiency at P1 and P2, and P toxicity at P12.

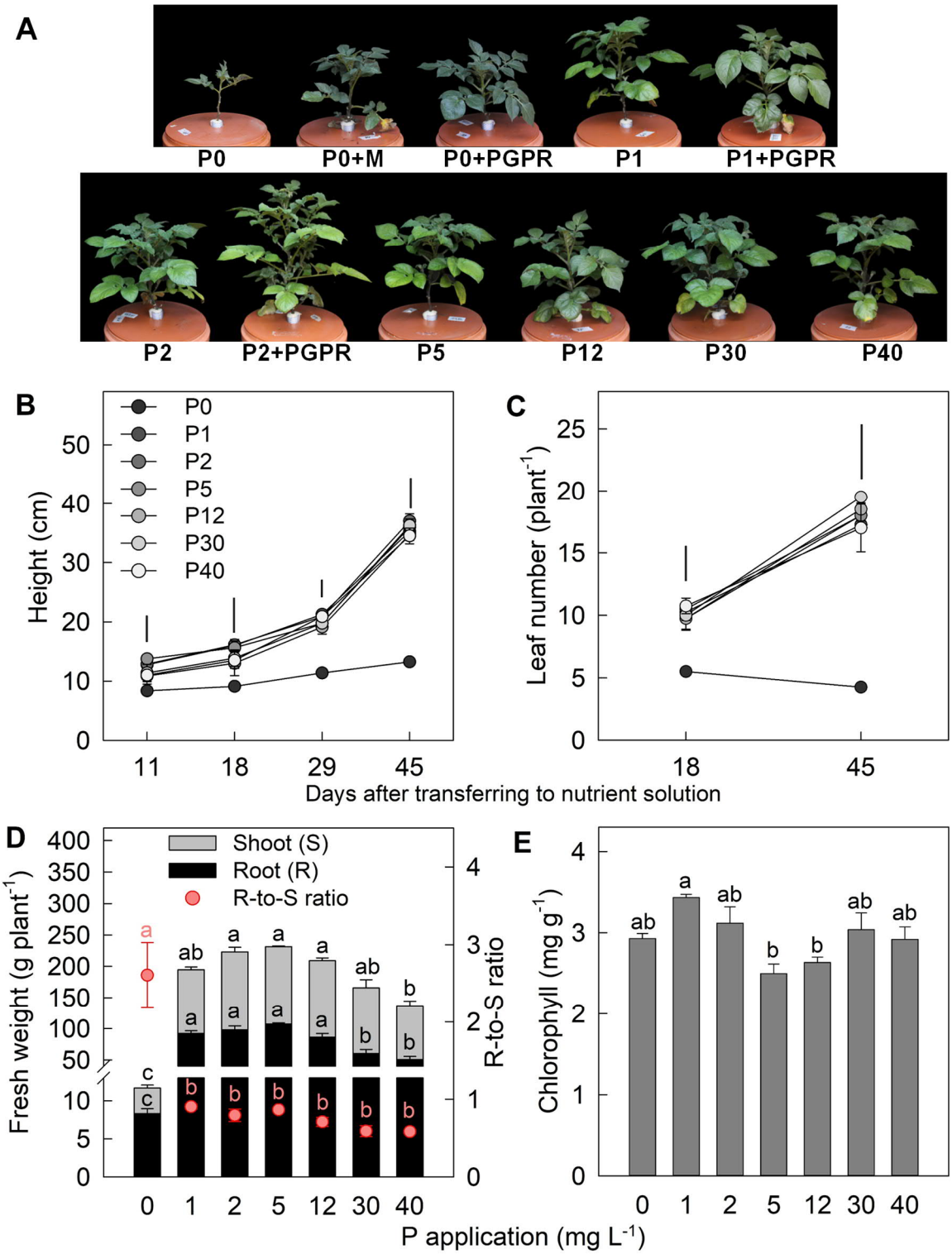


Figure 2.2. (**A**) Plant shoot phenotype at 30 days after seedling transplanting (DAT) as affected by different P levels and PGPR incolucation and (**B-E**) the influence of P application (0-40 mg L^{-1}) on morphology and leaf chlorophyll concentration of potato plants. Error bar represents standard error of means. Vertical bars in (**B**) and (**C**) represent critical value for comparisons of plant height and leaf number among P treatments in each measurement date by Tukey's HSD test at $p \leq 0.05$. Different letters in (**D**) and (**E**) of the same parameter indicate significant difference by Tukey's HSD test at $p \leq 0.05$.

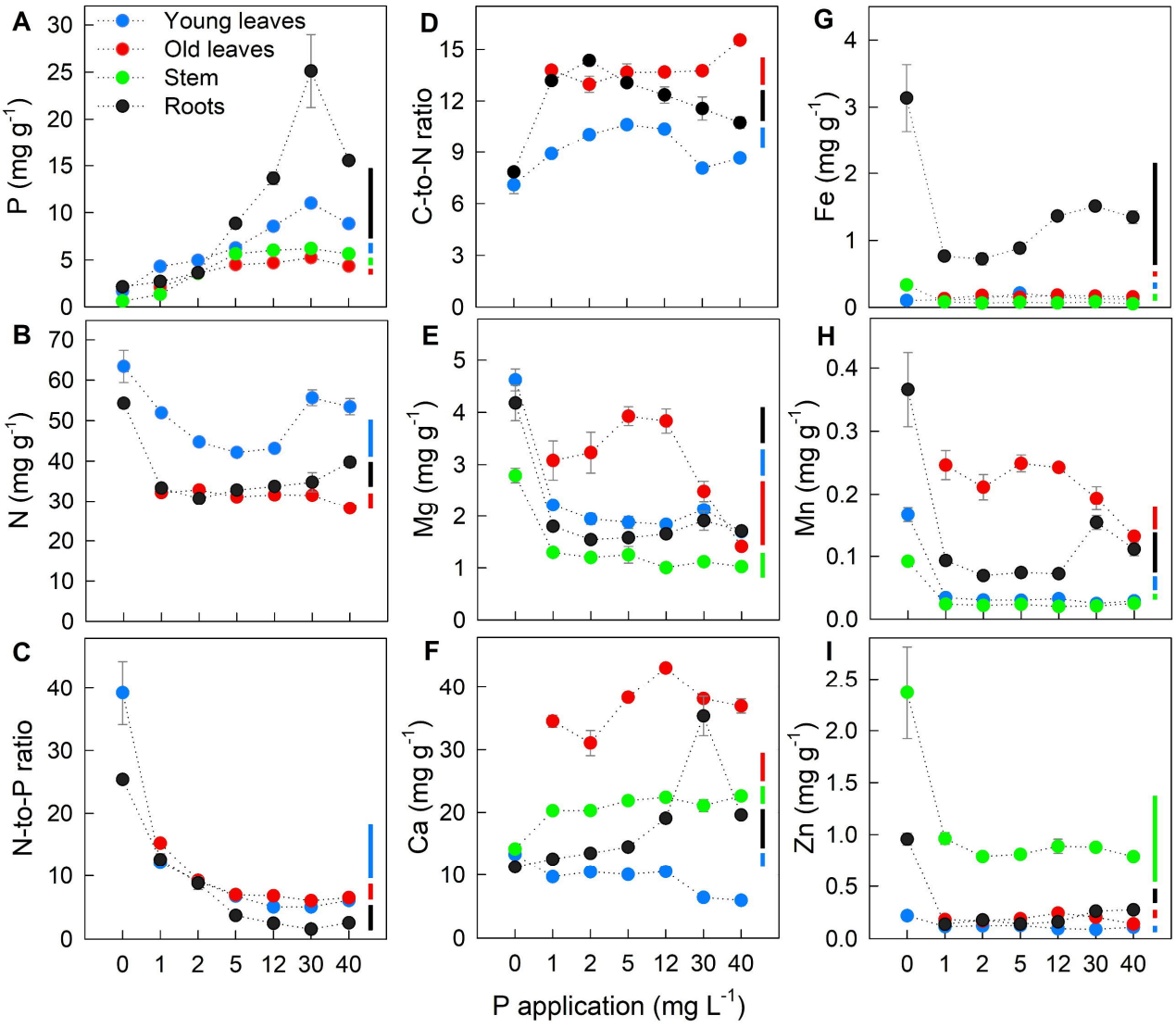


Figure 2.3. (**A-I**) Effect of P application on mineral concentration and mineral ratios in young leaves, old leaves, stem, and roots. For all measured traits, P0 data for old leaves are missing due to insufficient sample material for analyses. Error bars indicate standard error of means (n=4). Vertical bars represent critical value for comparisons among P treatments in each part of plant by Tukey's HSD test at $p \leq 0.05$.

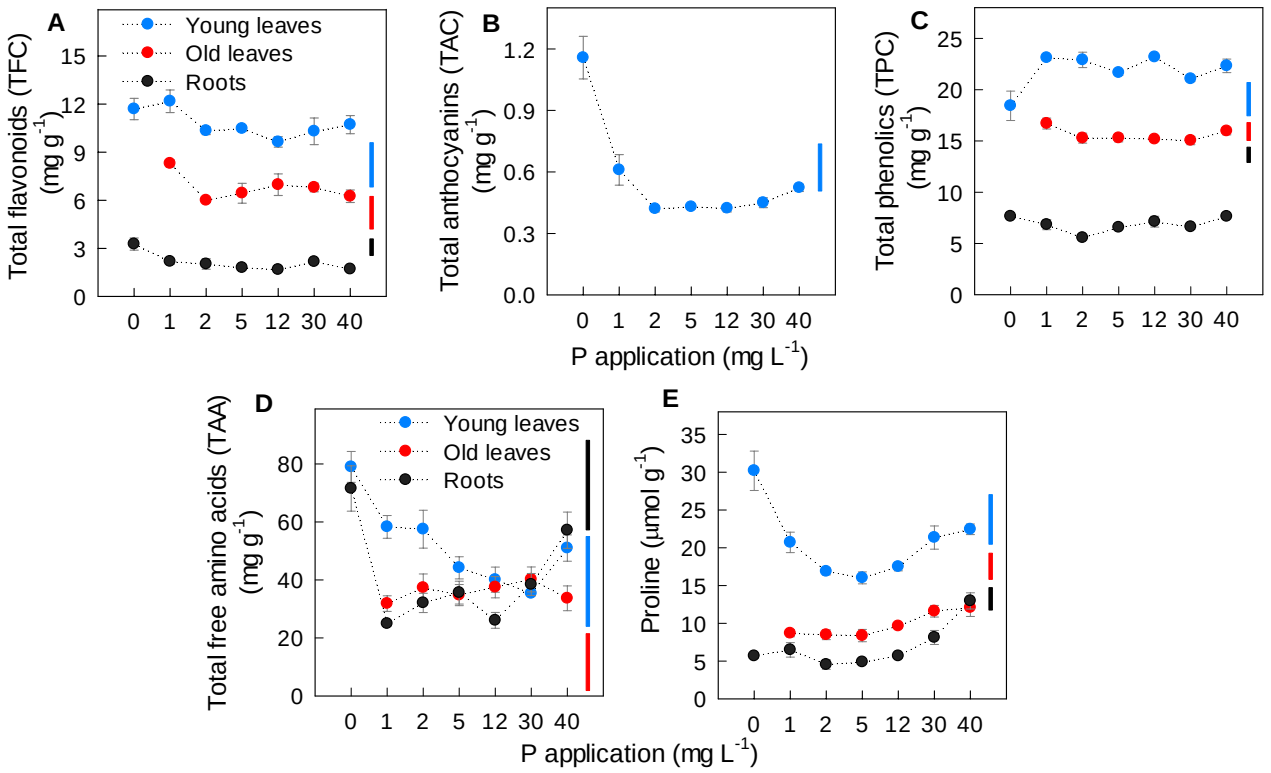


Figure 2.4. Effect of P application on the concentration of secondary metabolites, total free amino acids, and proline in young leaves, old leaves, and roots. For all measured traits, P0 data of old leaves are missing due to insufficient sample material for analyses. Error bars indicate standard error of means (n=4). Vertical bars represent critical value for comparisons among P treatments in each part of plant by Tukey's HSD test at $p \leq 0.05$.

2.3.4. Root metabolite profiling under P deficiency and toxicity conditions

Since there was a relatively high accumulation of TAA at P deficiency (P0) and P toxicity (P30 and P40) compared with P optimum (P5), a further investigation was conducted on the metabolic changes in roots under deficient- and toxic-P conditions in comparison with P optimum by using nuclear magnetic resonance (NMR) spectroscopy. A total of 100 metabolites were detected in roots, of which 37 were above the detection limit of measurements. These metabolites include amino acids, sugars, organic acids, and other organic compounds (Supplementary Table 2.3).

Figure 2.5 shows the pathway and fold changes of metabolites in root tissue under P deficiency and toxicity compared with the optimal P condition. P deficiency significantly reduced the concentration of sugars including sucrose, fructose, mannitol, and mannose by 50-80%. Organic acids generally decreased under P deficiency by 20-90%. These acids included glycerate, pyruvate, formate, acetate, α-ketoglutarate, succinate, fumarate, and malate. In contrast, amino acids significantly increased by 1.5-14.8 times under P deficiency. Of these amino acids, asparagine presented the highest fold change (14.8).

The effects of P toxicity were more evident in organic acids and their precursors than in sugars and amino acids (Figure 2.5). Toxic P conditions, either at P30 or P40, significantly decreased glycerine, methanol, acetate, acetaldehyde, succinate, fumarate, and malate by 20-80%. The significant decrease in sugars (by 50-60%) under P toxicity was found only in fructose, glucose, and mannose. Similar to P deficiency, P toxicity condition also caused a 1.8- to 4.3-time increase in amino acids such as alanine, valine, and γ-Amino-butyrate (GABA). 1,3-Butanediol also increased 2.5-2.7 times under P toxicity. Therefore, the conditions of both P deficiency or P toxicity significantly reduced sugar and organic acid levels, but increased amino acids concentrations in the roots.

2.3.5. Effect of PGPR inoculation on plant growth and secondary metabolites under low P supply

To test the hypothesis that PGPR inoculation improves plant growth under low P supply, a mixture of five potential plant growth-promoting strains was co-inoculated in the nutrient solution with the application of P0, P1, and P2. Plants with and without PGPR inoculation were compared to reveal the effect of PGPR under specific P treatment. In addition, a control with added sterile media was conducted under P0. The inoculants and media were renewed once a week. At 1 and 7 days after inoculation, nutrient solution was plated on strain-specific nutrient agar. After a 24-h incubation of plates in room temperature, there was growth of bacteria in which the colonies matched in appearance to the original strain. This observation indicates the viability of the inoculants in nutrient solution at both 1 and 7 days after inoculation. Furthermore, *in-vitro* determination of IAA indicated that each bacterial strain was able to produce IAA at different levels ranging from 0.2 mg L^{-1} (*Variovorax paradoxus* DSM 30034) to 7.15 mg L^{-1} (*Pseudomonas putida* DSM 6125) (Figure 2.6). Table 2.2 shows that the effect of PGPR was observed only on plants under P0 for plant height, leaf number, root biomass, shoot biomass, and total biomass. By considering the media effect under P0, the results revealed that PGPR significantly increased root, shoot, and total biomass by 45%, 33%, and 42%, respectively. Total root length and root surface area of PGPR-inoculated plants were also 44% and 32%, respectively, higher than media-treated plants (Table 2.2). However, PGPR addition reduced P concentration in roots of P0 plants by 17% and young leaves of P1 plants by 19%. Nevertheless, PGPR inoculation tended to increase root P uptake by 28% although there was no effect on root specific P uptake compared with sterile media addition under P0. TAA decreased by 40% in young leaves and increased by 78% in roots by PGPR under P0. PGPR also increased root TAA under P1 by 3-fold (Table 2.3). These results were similar to our pre-experiment (results not shown), and they indicate an improvement in plant growth by PGPR inoculation under P0 with an addition of culture media; however, PGPR also altered amino acid biosynthesis in plant roots at both P0 and P1 levels.

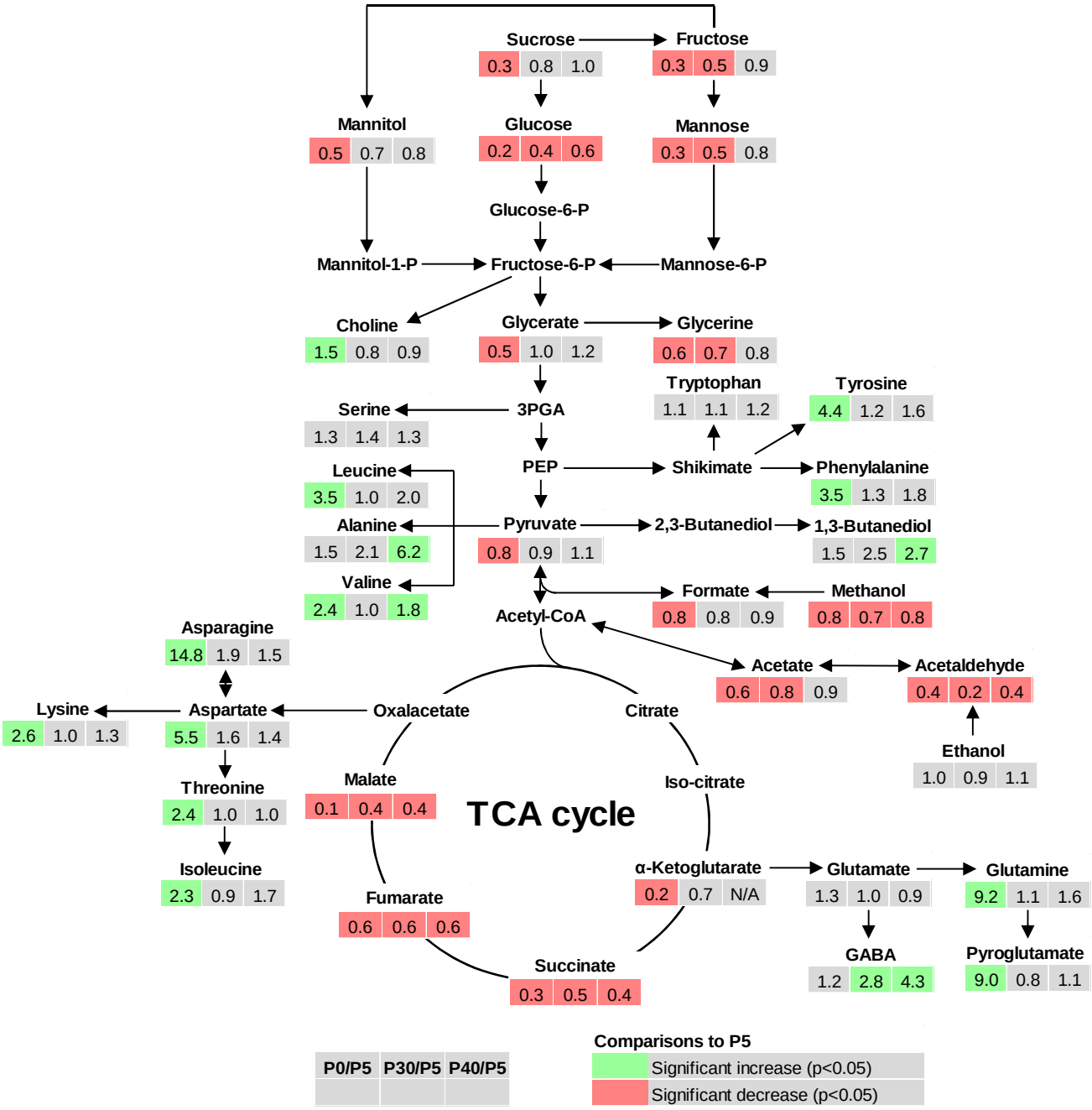


Figure 2.5. Effect of P deficiency and toxicity on metabolite concentration in roots of potato plants. The relative ratios were calculated by the division of metabolite concentration under P0, P30, and P40 with those under P5 as P sufficient treatment (n=4). Light green and red present the significant increase and decrease ($p \leq 0.05$), respectively. N/A=comparison not possible due to incomplete data; 3PGA=3-phosphoglycerate; PEP=Phosphoenolpyruvate; Acetyl-CoA= Acetyl Coenzyme A; TCA= tricarboxylic acid; GABA= γ-aminobutyrate

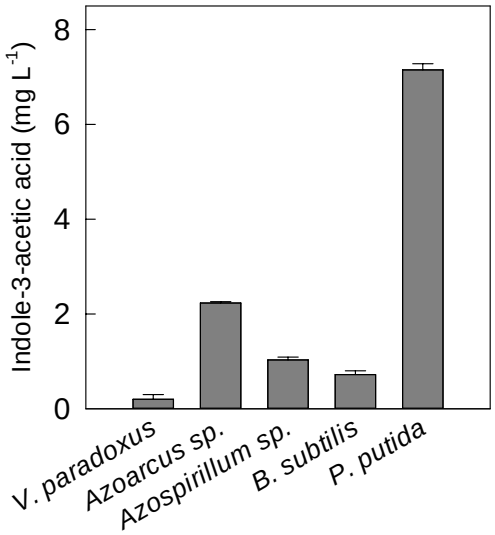


Figure 2.6. Indole-3-acetic acid (IAA) concentration produced by each bacteria strain in its respectively culture at OD_{600}=0.4

Table 2.2. Effect of PGPR on shoot and root growth under low P application

P level (mg L^{-1})	PGPR	Plant height (cm)	Leaf Number (plant^{-1})	Root biomass (g plant^{-1})	Shoot biomass (g plant^{-1})	R-to-S ratio	Total root length (10^4 cm)	Root surface area (10^3 cm^2)
0	-PGPR	13.3±0.5^b	4.3±0.3^b	8.3±0.7^c	3.4±0.4^c	2.6±0.4	-	-
	+M	20.5±0.9^a	14.3±1.4^a	52.0±3.3^b	26.3±0.7^b	2.0±0.1	4.7±0.0^b	2.8±0.0^b
	+PGPR	20.6±0.9^a	15.3±0.5^a	76.1±2.6^a	35.1±2.2^a	2.2±0.2	6.8±1.0^a	3.7±0.4^a
1	-PGPR	35.3±1.1	17.3±0.3	92.3±4.3	102.2±4.7	0.9±0.0	6.6±0.0	3.6±0.0
	+PGPR	32.8±2.1	17.3±1.3	79.9±6.8	103.9±7.8	0.8±0.0	6.2±0.5	3.5±0.2
2	-PGPR	37.0±1.2	18.0±1.0	97.9±6.3	125.2±7.4	0.8±0.1	7.6±0.5	4.3±0.3
	+PGPR	38.0±1.6	17.0±0.9	85.7±7.0	115.6±10.8	0.8±0.1	6.4±0.2	3.4±0.1

Mean ±SE with different letters in the same column and same P level are significantly different at p≤0.05 by Tukey's HSD test. No indication means non-significant difference. M=sterile culture media

Table 2.3 Effect of PGPR on P concentration, root P uptake, specific P uptake, total free amino acids (TAA), and proline concentrations in young leaves and roots under low P levels

P level	PGPR	P concentration (mg g^{-1})		Root P Uptake	Specific P uptake	TAA (mg g^{-1})		Proline (µmol g^{-1})	
(mg L^{-1})		Leaves	Roots	(mg $plant^{-1}$)	(µg P cm^{-2})	Leaves	Roots	Leaves	Roots
0	-PGPR	1.7±0.2^{b}	2.1±0.1^{a}	1.1±0.1^{b}	-	79.0±8.3^{a}	71.6±7.8^{a}	30.2±2.6	5.7±0.3
	+M	2.4±0.1^{a}	2.3±0.1^{a}	7.2±0.4^{a}	2.6±0.2	90.1±3.9^{a}	37.6±4.1^{b}	23.1±1.1	6.3±0.8
	+PGPR	2.3±0.1^{a}	1.9±0.1^{b}	9.2±0.9^{a}	2.7±0.6	54.0±8.7^{b}	67.0±9.3^{a}	23.2±1.8	8.0±0.5
1	-PGPR	4.3±0.1^{a}	2.7±0.2	15.6±1.3	3.9±0.3	58.3±3.9	24.5±0.7^{b}	20.7±1.4	6.5±1.0
	+PGPR	3.5±0.1^{b}	2.5±0.2	12.4±1.0	3.6±0.3	58.2±3.3	82.4±2.3^{a}	20.5±1.1	6.5±0.0
2	-PGPR	4.9±0.3	3.6±0.4	22.7±4.1	4.4±0.5	57.5±12.5	32.1±3.3	16.9±0.4	4.5±0.6
	+PGPR	4.6±0.2	3.8±0.2	20.3±1.8	5.5±0.6	42.5±4.4	45.1±7.7	19.7±0.6	5.1±0.3

Mean ±SE with different letters in the same column and same P level are significantly different at p≤0.05 by Tukey's HSD test. No indication means non-significant difference. M=sterile culture media

2.3.6. Root-associated bacterial community under low P supply

In order to gain a better understanding of how PGPR co-inoculation interacted with plants, the root-associated bacterial community was also determined by amplicon-based 16S rRNA gene analysis. Overall, 1,092,304 raw reads were produced, of which 820,818 remained after quality filtering, and chimera removal. Within the obtained dataset, the 25 most abundant root-associated bacterial orders are shown in Supplementary Figure 2.3. Furthermore, it was possible to classify the reads partially at genus or species level. Thus, three of the five inoculated PGPR strains on the species level and four of them at the genus level were identified within the dataset, as shown in Figure 2.7A. In addition, Faith's phylogenetic diversity (PD) was lowest in the P0 supplemented with sterile media (41.3), and highest in P1 and P2 without PGPR addition (45.5 and 45.7), while the treatments with PGPR addition had a similar PD (44.3, 44.9, and 44.5). The principal coordinates analysis (PCoA) revealed a significant correlation of the bacterial community composition with plant height, root and shoot biomass, as well as the root-to-shoot ratio (Figure 2.7B). The bacterial communities of P treatments without PGPR addition cluster together and separately from those of the P treatments with PGPR as well as sterile media addition. This indicates differences in bacterial community composition between PGPR treated and untreated roots, independent from the impact of P fertilization on the community (Supplementary Figure 2.3).

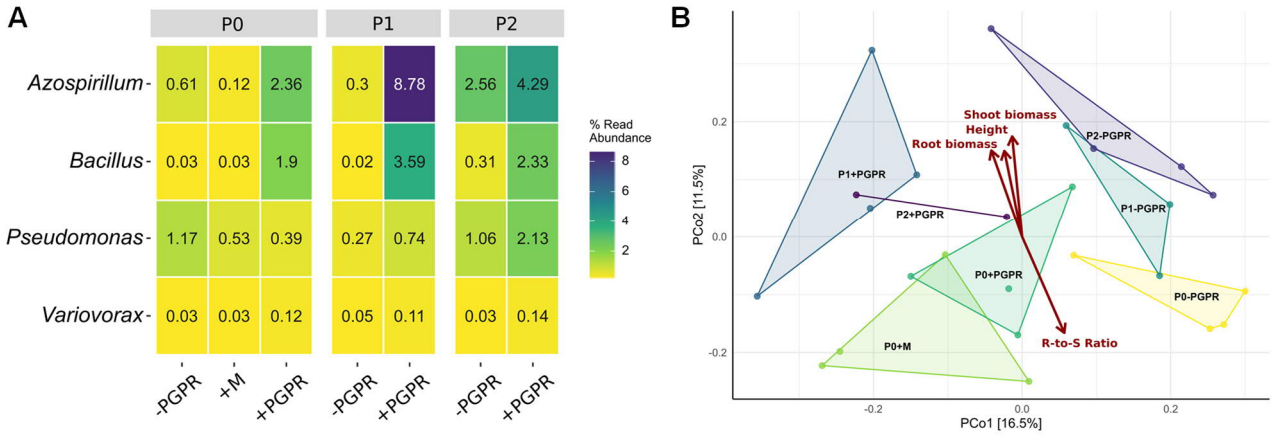


Figure 2.7. (**A**) Bacterial community composition at genus level detected in and on the root tissue of PGPR-inoculated and non-inoculated plants at the application of P0, P1, and P2, and (**B**) their correlation with plant growth parameters. The correlation between bacterial community composition and plant growth was performed with principal coordinates analysis (PCoA) using Bray-Curtis distance units. The correlations are significant at p= 0.009, p= 0.003, p= 0.001, and p= 0.001 for plant height, root biomass, shoot biomass, and root-to-shoot ratio, respectively. The analysis was based on four biological replications (n= 4), except for those under P2+PGPR with n= 2 and P1-PGPR with n= 3 due to insufficient sample material.

2.4. Discussion

2.4.1. P deficiency and toxicity conditions in potato

Understanding the morphophysiological and metabolic responses to P deficiency and toxicity conditions is important to understand how potato plants use P when it is either in deficit or in high abundance. Characterization of plant responses to excess P supply in potato has not been elucidated yet. Results of the present study show P deficiency at P0 and P toxicity at P30 and P40, and P optimum at P5. Huett et al. (1997) also reported P deficiency and adequate P supply if the P concentration in leaf tissue is below 2.3 mg g^{-1} and between 3.0-6.0 mg g^{-1}, respectively. Therefore, potato plants grown under P1 and P2 were also within the range of sufficient P supply. The survival, biomass production, and P accumulation of plants under the condition without P (P0) in the present study was due to the carry-over effect of P during seedling germination. In contrast, Fernandes and Soratto (2012) and Barben et al. (2010) observed P deficiency symptoms at 1 mg P L^{-1} in nutrient solution. This difference may be due to the use of different cultivars. Nevertheless, the definitions of P optimum (5 mg L^{-1}) and P toxicity (≥30 mg L^{-1}) are comparable with that of Fernandes and Soratto (2012) and Barben et al. (2010). This result is also in agreement with the result of Bhatti and Loneragan (1970), who reported P toxicity in wheat for the first time when leaf P concentration exceeded 10 mg g^{-1} of dry matter.

2.4.2. Effect of P deficiency and toxicity on plant growth and nutrient assimilation

The decreased leaf number under P deficiency implied that leaf initiation rates and shoot apical meristem activity were inhibited, which subsequently, reduced cell expansion and individual leaf size (Chiera et al., 2002). Although plant height and leaf number under P toxicity were not affected, leaf expansion could be restricted, causing reduced shoot biomass. Therefore, less expanded leaves of plants under P deficiency and toxicity resulted in a relative increase of the chlorophyll concentration. Similarly reported by Mengel et al. (2001), the leaves of P-deficient plants eventually become thick and appear to be bluish green. Under P deficiency, impaired shoot growth could be caused by the preferential assimilate distribution to roots for improved P uptake, causing a high root-to-shoot ratio (Wang et al., 2015). In this situation, plants require a P conservation strategy through the mobilization of P from old leaves to young leaves and roots (Schachtman et al., 1998). Consequently, old leaves are shed off (Figure 2.2A). In contrast to P deficiency, root biomass is reduced at a greater magnitude than shoot biomass under P toxicity (Figure 2.2D). The reduction of root growth under P toxicity could be due to less availability of P in the roots. The reduced P could be linked to a reduction in phosphate uptake activities under excess P application to prevent the accumulation of toxic P concentration in the roots while the phosphate mobilization from roots to shoots still occurs (Schachtman et al., 1998; Shukla et al., 2017). Consequently, P concentration in young leaves, stem, and old leaves is less affected. A further experiment may be required to elucidate P transporter genes in various parts of plant.

The pH range 5.5–6.5 of nutrient solution in the present study is optimal for the availability of nutrients (Kane et al., 2006); therefore, the uptake of other minerals by plants is solely affected by P availability in the nutrient solution. In line with the previous report by Pang et al. (2011) on legumes, we found an increased N concentration in leaves under P0, followed by a substantial decrease until P12, but recovery at increased P application to P30 and P40 (Figure 2.3B). This could explain the results with respect to the relative increase of leaf chlorophyll (Figure 1E), which is approximately proportional to leaf N under P limiting condition (Shi et al., 2019). The accumulation of N under P deficiency and toxicity could be an adaptive response to meet the N demand for protein synthesis under stress conditions (Misson et al., 2005). The variations in tissue N concentrations under varying P supply also, subsequently, influenced the C-to-N ratio (Figure 2.3D), which indicates stolon elongation and tuber yield formation (Zheng et al., 2018). The high concentrations of Mg, Fe, Mn, and Zn under P deficiency (Figures 2.3E, G-I) imply that the uptake and utilization of these minerals were altered. Owing to the roles of Mg in photosynthesis and assimilate transport (Koch et al., 2019a), plants need a huge amount of this element for P and carbohydrate translocation under P deprivation. The increased uptake of metallic ions such as Fe and Zn under P deficiency could be a plant strategy to decrease their amounts in the rhizosphere in order to reduce the complex formation between phosphate and these ions (Hirsch et al., 2006). Another explanation for elevated Fe and Zn in roots could be due to the increased availability of these minerals in the absence of P in the nutrient solution. In Arabidopsis, Misson et al. (2005) showed a suppression of iron transporter *IRT1* in roots under P deficiency which is a mechanism in maintenance of Fe homeostasis. Moreover, excess P supply also resulted in the accumulation of Fe in roots (Figure 2.3G), which could react

with hydrogen peroxide in plants to produce hydroxyl radicals, leading to the death of meristematic cells and causing root biomass reduction (Shukla et al., 2017). It has been shown that an excess supply of P reduces Zn and Ca availability near the root surface, preventing its uptake and translocation to the shoots (Hawkesford et al., 2012). In the present study a similar tendency was observed, but leaf Zn concentration was still in a sufficient range (>0.015 mg g^{-1}; Huett et al. (1997)). Therefore, P-induced Zn deficiency does not account for P toxicity.

2.4.3. Effect of P deficiency and toxicity on plant secondary metabolites and root metabolite profiling

Plants use various adaptation strategies in response to stress induced by P deficiency and toxicity. Under P deficiency, the accumulation of secondary metabolites and TAA in potato plants ranged from 60-200% higher than those under optimal P conditions (Figures 2.4). The previously reported accumulation of TFC in tomato and Arabidopsis (Stewart et al., 2001), leaf TAC in sunflower (Gunes and Inal, 2009), and TAA in barley (Criado et al., 2017) under P deficiency are also within this range. However, Aleksza et al. (2017) found 3.5-fold increase of proline in Arabidopsis without P application. The variation of the results may be caused by different plant species and experimental conditions. Moreover, there was also an accumulation of these compounds at different magnitudes under P toxicity. The analyses were conducted using oven-dried (60°C) samples, but this sample-processing method did not significantly influence the results based on a pre-test by comparing oven- and freeze-dried samples (Supplementary Table 2.2), which was also in agreement with Volf et al. (2014). Fini et al. (2011) reported a disruption of photosynthetic apparatus under stress that could be induced by P deficiency or toxicity, leading to increased sensitivity of plant to high light. Therefore, the upregulation of TFC and TAC in leaves occurs as a protection mechanism against light-induced oxidative damage under P deficiency and toxicity stress (Fini et al., 2011). The increase of leaf TAC under P toxicity was also reported in Arabidopsis (Shukla et al., 2017). Proline also acts as a stress indicator, leading to further increase of stress-related metabolites. In Arabidopsis, the accumulation of proline under P starvation is mediated by stress-induced abscisic acid, and it is also caused by the upregulation of *P5CS1* and *PDH2* genes, which control proline metabolism (Aleksza et al., 2017). The mechanism underlying proline accumulation under P toxicity has not been explained yet; however, proline in roots probably acts to scavenge hydroxyl radicals (Signorelli et al., 2015), which are produced under elevated Fe conditions (Figure 2.3G).

In addition to the secondary metabolite quantification, a comprehensive metabolite profiling of roots provided us valuable information about how primary and secondary metabolites change in response to P deficiency and toxicity (Figure 2.5). Sugars decreased in roots under P deficiency at a greater scale than those under P toxicity. Under P deficiency, low cytosolic P results in a reduced phosphate release from the Calvin cycle for sucrose synthesis (Wissuwa et al., 2005). Owing to high leaf and root Mg, carbohydrate transport from leaves to roots may not be inhibited under P deficiency (Tränkner et al., 2018; Koch et al., 2019a). Therefore, the reduction of leaf sucrose leads to a decrease in reducing sugars and their concentrations in roots. A strong reduction in glucose under P deficiency results in a decrease of pyruvate. However, high cytosolic P

under P toxicity alters sugar metabolism due to over-expression of hexokinase activity under elevated ATP levels (Silber et al., 2002). Consequently, hexose sugars (glucose, fructose, and mannose) may be rapidly converted to their phosphorylated forms, resulting in a low hexose sugar concentration in roots under P toxicity condition. If this hypothesis is true, improving phosphorylated sugars in roots is important for the biosynthesis of essential molecules such as amino acids (Nguyen et al., 2019).

The levels of aromatic amino acids (tyrosine and phenylalanine) were enhanced under P deficiency. P toxicity condition also tended to increase the concentration of these aromatic amino acids (Figure 2.5). The increase in the amount of phenylalanine and tyrosine in roots was consistent with the accumulation of TFC in roots under both P deficiency and toxicity. This is probably due to the enhanced phenylalanine/tyrosine ammonia lyase activity under stress conditions to release nitrogen for phenylalanine and tyrosine metabolism, which contribute to protein synthesis. Meanwhile, the carbon products are transferred into flavonoid biosynthesis pathway via 4-coumaroyl-Coenzyme A (Stewart et al., 2001). The high activity of phenylalanine ammonia lyase was also reported on maize roots under P deficiency (Li et al., 2007).

The P deficiency and toxicity conditions also reduced the production of most of the organic acids (i.e. succinate, fumarate, and malate) in the TCA cycle. The reduction in α-ketoglutarate was observed only under P deficiency. This is similar to the results of Huang et al. (2008) on barley roots under deficit P supply. However, Müller et al. (2015) reported an enhanced organic acid concentration in lupin roots, whereas Nguyen et al. (2019) found no change in organic acid concentrations in maize roots under P deficiency. These differences could be caused by plant species, cultivation systems (pot vs. hydroponic condition), and harvesting stage. The shortage of carbohydrates under P deficiency could be responsible for the reduced levels of organic acids in roots. For instance, fumarate represents a great percentage of fixed carbon; therefore, photosynthesis impairment induced by P deficiency results in low carbon fixation in leaves and its translocation into the roots, causing low fumarate in roots (Chia et al., 2000; Misson et al., 2005). Moreover, organic acids might have been secreted into the nutrient solution in response to P starvation (Wang et al., 2015); thus, their concentrations in roots remain low. The mechanisms for the decrease in the levels of organic acids under P toxicity are not clear; however, it could also be related to the shortage of carbohydrate. Another explanation for the low organic acid concentrations in roots could be their high metabolism under stress conditions induced by both P deficiency and toxicity. For instance, α-ketoglutarate is the precursor for γ-aminobutyrate (GABA), which is produced for plant abiotic stress protection (Singh and Roychoudhury, 2020). Moreover, GABA can also be formed from proline by nonenzymatic pathways (Signorelli et al., 2015), explaining the consistently high proline and GABA concentrations under both P deficiency and toxicity.

In line with the increased levels of amino acids in barley and lupin under P starvation (Huang et al., 2008; Müller et al., 2015), P deficiency in the present study enhanced the concentrations of many amino acids in the roots. Of all the measured amino acids, aspartate plays an important role in the biosynthesis of other amino acids such as asparagine, threonine, lysine, and isoleucine (Nguyen et al., 2019). Therefore, the accumulation

of aspartate results in increased concentration of other amino acids in roots. Genes responsible for aspartate family pathways are stimulated under stress conditions due to carbohydrate deprivation (Galili, 2011). Moreover, the upregulation of glutamine in P-deficient roots can be an adaptive response related to N concentration for protein synthesis because glutamine plays an indispensable role as amino donor in the biosynthesis of amino acids and N-containing compounds (Misson et al., 2005). However, P toxicity increased the level of amino acids to a lesser extent, which is evident only for alanine and valine. Their accumulation is a protective mechanism of plants in response to stress induced by P toxicity. For instance, alanine is a well-known stress-responsive amino acid, and its metabolism is activated under various stress conditions (Galili, 2011). Therefore, higher amino acid concentrations in the roots may lead to potato plants being more tolerant of P deficiency and toxicity.

2.5.4. Plant growth promotion by PGPR under low P supply

The co-inoculation of five PGPR strains improved plant biomass, total root length, root surface area, and plant secondary metabolites under P0 (Tables 2.2 and 2.3). The *in-vitro* determination of IAA in the bacterial cultures revealed a substantial IAA production by each strain. Even though phytohormones in the roots were not quantified in this study, bacterially derived IAA may also contribute to root growth and, subsequently, shoot biomass enhancement. There have been reports of the production of IAA and other phytohormones by *Variovorax paradoxus* (Jiang et al., 2012), *Azoarcus sp.* (Fernández et al., 2014), *Azospirillum sp.* (Kaneko et al., 2010), *Bacillus subtilis* (Pathak et al., 2019), and *Pseudomonas putida* (Patten and Glick, 2002). Most of these reports have shown the role of IAA in root system development. Present in a low concentration, IAA can stimulate primary root growth, whereas high IAA concentrations enhance root hairs and lateral roots (Vacheron et al., 2013). Consequently, total root length and root surface area are improved to exploit limited amount of P in the nutrient solution. Consequently, root P uptake was enhanced. These results also suggest a further study on root phytohormone determination, which could be influenced by low P availability and PGPR inoculation. In the present study, the addition of the bacterial culture media increased P concentration of the nutrient solution by 0.5 mg P L^{-1}. This low amount of P can be beneficial for plants through improved P uptake by root length elongation of PGPR-inoculated plants. The further addition of P might have a minor or even no impact, as PGPR also need P for their growth, survival, and functioning (Kisand et al., 2001). However, the low root P concentration of PGPR-treated plants may be due to the dilution effect. The TAA in roots was also altered by PGPR. Mhlongo et al. (2020) reported the regulation of aromatic amino acids in roots of tomato following *Pseudomonas sp.* inoculation. These amino acids are important for secondary metabolite biosynthesis in connection with cell wall lignification and chemical defense against pathogens. Nevertheless, an amino acid such as tryptophan is the precursor of IAA, which is a plant defense metabolite and also facilitates plant root elongation (Weston et al., 2012; Vacheron et al., 2013). The minor response of PGPR under P1 and P2 could be caused by less reliance of plants on PGPR to improve root growth and nutrient uptake. Based on the results described above, P1 and P2 might be already sufficient for plant growth and development. Plant-microbial

symbiosis is a carbon-expensive process; therefore, plants try to make cost-benefit analyses for the efficient use of their carbohydrates (Smith and Read, 2008).

Additionally, determining the root-associated bacterial community composition provides further insight into PGPR–plant interactions. The results show the identification of three of the added species in the derived dataset on species level and four of them at the genus as well as at the family level, indicating an attachment to—or even a penetration of—the roots, as well as a survival of the added PGPR. The absence of *Azoarcus* could be explained by a missing root association and/or competition between all PGPR. Furthermore, PD suggests a stabilization of the bacterial community and diversity by PGPR addition. The PD for treatments with PGPR addition is stable across the different P levels, suggesting that the influence of PGPR is greater than that of the P addition. Compared to P0 without PGPR inoculation, the PD is increased under P1 and P2 and decreased under P0+M treatment. The bacterial community composition is affected by PGPR and P addition, which is also illustrated by the topology of the PCoA. Overall, the PCoA revealed a significant correlation of the bacterial community composition with plant height, root and shoot biomass, and the root-to-shoot ratio, indicating an effect of the media addition, but also a stronger and more pronounced effect of the PGPR addition on the bacterial community. A positive effect of PGPR addition on plant development and the composition of rhizosphere bacterial communities has also been shown by e.g. Chen et al. (2020) and Pereira et al. (2020). Nevertheless, the root-associated microbial community composition was also affected by the P addition, which has also been shown for the rhizosphere inhabiting microbial community of blueberries (Pantigoso et al., 2018).

2.5. Conclusion

The results of the present study contribute to a deeper understanding of P deficiency- and toxicity-adaptive responses in potato plants. In addition to the previous reports on P deficiency in potato plants, further insights into nutrient interactions and metabolite changes were elucidated, which are closely related to tolerance mechanisms under both P deficiency and toxicity. The morphological and biochemical changes under P deficiency and toxicity are summarized in Figure 2.8. There was a stronger switch from primary to secondary metabolism under P deficiency compared to those under P toxicity. Moreover, PGPR could increase plant tolerance under P deficiency through their contribution to root growth and plant biomass improvement (Figure 2.9). The root-associated microbial communities showed an impact of the PGPR addition on the community composition; they also revealed a significant correlation with plant height and root as well as shoot biomass. Although the potato plant responses to P deficiency and toxicity condition was characterized, further studies may be required to test the specificity of these responses by other cultivars. The positive effects of PGPR co-inoculation also provide an outlook to assess the effect of individual strains on plant biomass, P uptake, and secondary metabolites of potato.

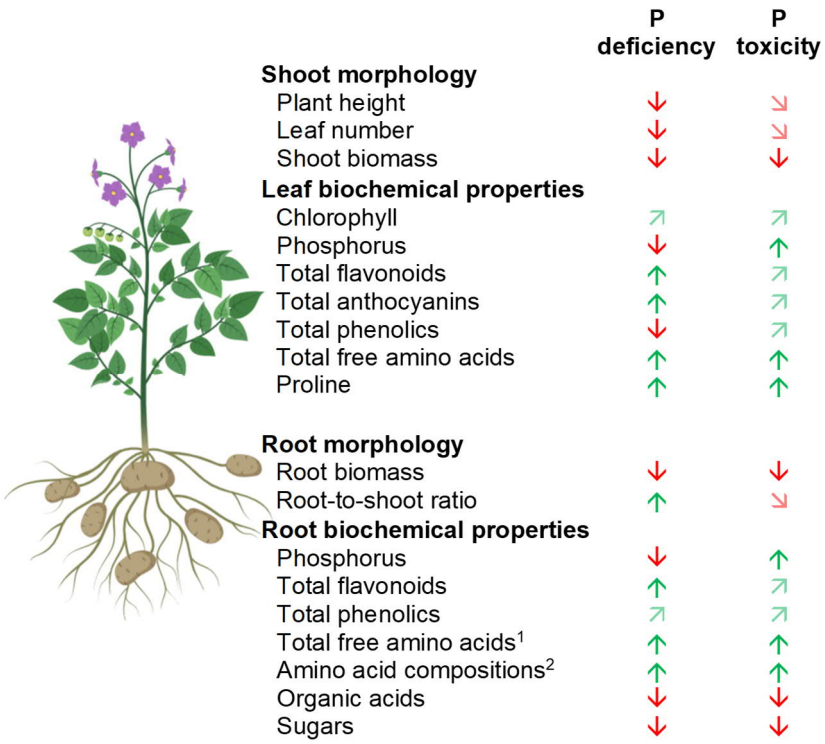


Figure 2.8. Summary of the morphological and biochemical responses of the potato plants to deficient- and toxic-P conditions. Green and red arrows indicate significant increase and decrease ($p \leq 0.05$) in concentration, respectively, compared to optimum P condition. Light green and light red diagonal arrows indicate a tendency of increase and decrease, respectively. [1]based on colorimetric determination; [2]based on nuclear magnetic resonance (NMR) spectroscopy determination. Figure was created using BioRender (https://biorender.com/) as part of Academic License.

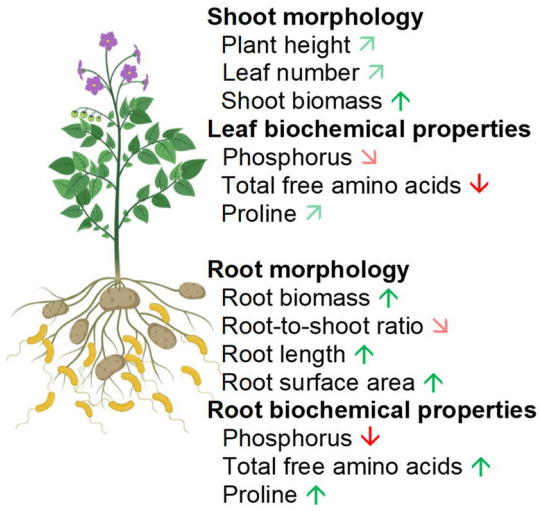


Figure 2.9. Summary of the morphological and biochemical responses of the potato plants to PGPR inoculation under P deficiency. Green and red arrows indicate significant increase and decrease ($p \leq 0.05$) in concentration, respectively, compared to optimum P condition. Light green and light red diagonal arrows indicate a tendency of increase and decrease, respectively. Figure was created using BioRender (https://biorender.com/) as part of Academic License.

Supplementary materials

Supplementary Table 2.1. Concentration of nutrients applied in quartz sand for potato seedling germination

Nutrients	Concentration (mg kg^{-1} soil)	Nutrient sources
P	8	$Ca(H_2PO_4)_2*H_2O$
N	300	$Ca(NO_3)_2*4H_2O$
K	330	K_2SO_4
Ca	1300	$CaCO_3$, $Ca(H_2PO_4)_2*H_2O$, $Ca(NO_3)_2*4H_2O$
S	250	K_2SO_4, $MgSO_4*7H_2O$, $CuSO_4*5H_2O$, $ZnSO_4*7H_2O$, $MnSO_4*H_2O$
Mg	100	$MgSO_4*7H_2O$
Cu	0.002	$CuSO_4*5H_2O$
EDTA-Fe	0.003	$C_{10}H_{12}FeN_2NaO_8*3H_2O$
Zn	0.002	$ZnSO_4*7H_2O$
B	0.0006	H_3BO_3
Mo	0.002	$Na_2MoO_4*2H_2O$
Mn	0.006	$MnSO_4*H_2O$

Supplementary Table 2.2. Concentration of nutrients applied in the hydroponic system

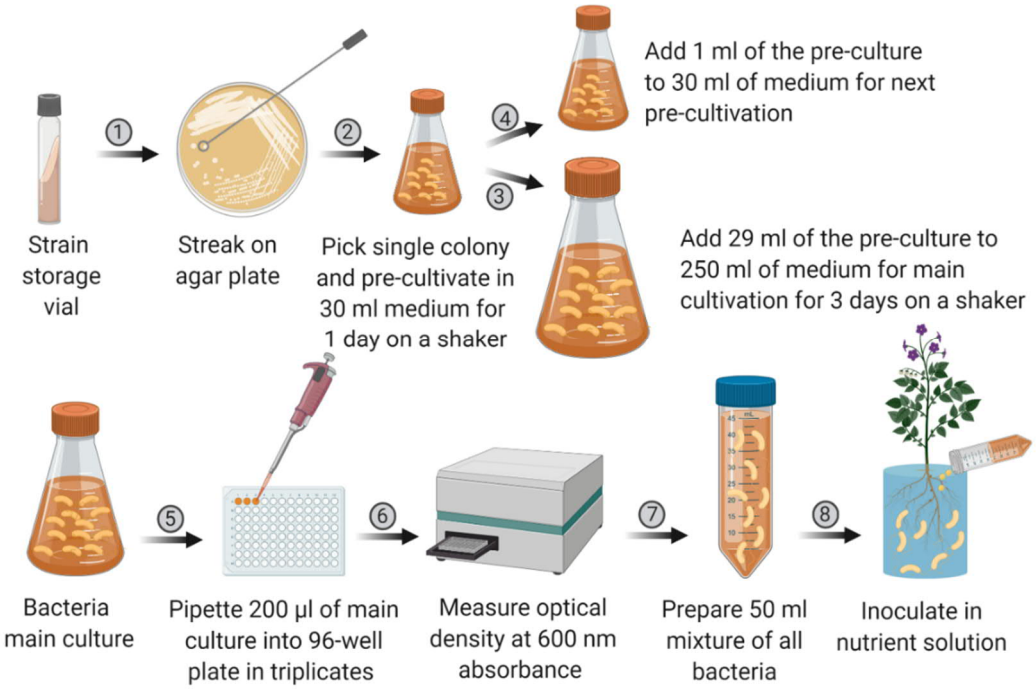
Nutrient	Concentration (mg L^{-1})	Nutrient sources
P	0-40	$Ca(H_2PO_4)_2*H_2O$
N	56.04	NH_4NO_3, $Ca(NO_3)_2*4H_2O$
K	104.25	K_2SO_4
Ca	69.05	$Ca(NO_3)_2*4H_2O$, $CaCl_2*2H_2O$
S	45.01	K_2SO_4, $MgSO_4*7H_2O$, $CuSO_4*5H_2O$, $ZnSO_4*7H_2O$, $MnSO_4*$
Mg	1.62	$MgSO_4*7H_2O$
Cu	0.02	$CuSO_4*5H_2O$
EDTA-Fe	5.58	$C_{10}H_{12}FeN_2NaO_8*3H_2O$
Zn	0.09	$ZnSO_4*7H_2O$
B	0.14	H_3BO_3
Mo	0.13	$H_{24}Mo_7N_6O_{24}*4H_2O$
Mn	0.07	$MnSO_4*H_2O$
Na	2.32	$C_{10}H_{12}FeN_2NaO_8*3H_2O$
Cl	2.83	$CaCl_2*2H_2O$

Supplementary Table 2.3. Metabolite concentration (mean±SE) in roots of potato plants under different P application

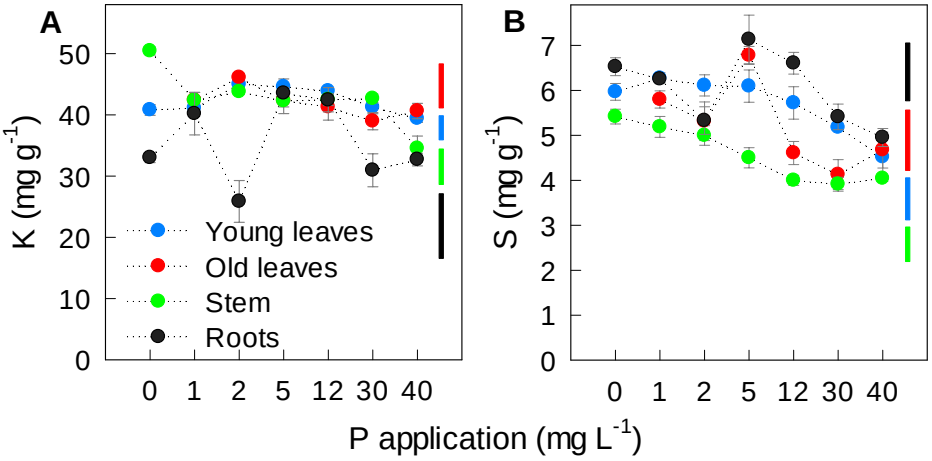
Metabolite	**P application in nutrient solution**			
(mg g^{-1})	**P0**	**P5**	**P30**	**P40**
Amino acids				
Alanine	0.68±0.06	0.47±0.06	0.99±0.51	2.86±0.79
Asparagine	46.58±23.31	3.14±0.38	6.03±1.74	4.61±0.61
Aspartate	6.10±1.80	1.12±0.14	1.83±0.23	1.61±0.13
Glutamate	2.05±0.36	1.56±0.21	1.50±0.41	1.46±0.09
Glutamine	24.23±8.76	2.63±0.24	2.89±0.97	4.15±0.82
Isoleucine	1.01±0.20	0.44±0.03	0.39±0.05	0.73±0.20
Leucine	0.88±0.27	0.25±0.02	0.26±0.01	0.51±0.15
Lysine	0.39±0.00	0.15±0.02	0.15±0.01	0.20±0.05
Phenylalanine	0.54±0.14	0.15±0.01	0.20±0.02	0.27±0.04
Pyroglutamate	45.87±26.53	5.09±1.43	4.19±1.19	5.55±1.31
Serine	1.23±0.04	0.95±0.36	1.29±0.32	1.23±0.41
Threonine	1.03±0.21	0.43±0.07	0.44±0.06	0.43±0.10
Tryptophan	0.16±0.03	0.15±0.01	0.16±0.01	0.18±0.01
Tyrosine	0.53±0.27	0.12±0.01	0.15±0.02	0.20±0.05
Valine	1.28±0.22	0.53±0.02	0.55±0.09	0.93±0.16
γ-amino-butyrate (GABA)	1.85±0.26	1.60±0.12	4.41±1.45	6.95±0.54
Sugars				
Fructose	6.87±2.43	25.75±0.56	12.85±3.96	23.93±3.29
Glucose	10.20±3.01	49.85±3.38	17.83±7.06	32.13±7.62
Mannitol	0.59±0.11	1.20±0.14	0.89±0.32	0.93±0.18
Mannose	0.59±0.00	1.79±0.10	0.95±0.31	1.45±0.26
Myo-inositol	0.81±0.40	1.16±0.26	1.03±0.42	1.30±0.41
Sucrose	11.90±1.72	40.85±2.50	34.68±2.98	39.80±2.64
Organic acids				
α-Ketoglutarate	0.13±0.07	0.79±0.30	0.53±0.06	-
Acetate	1.53±0.12	2.66±0.11	2.04±0.24	2.49±0.16
Formate	0.66±0.02	0.85±0.04	0.71±0.09	0.73±0.03
Fumarate	0.60±0.02	1.00±0.11	0.55±0.05	0.62±0.03
Glycerate	0.16±0.03	0.36±0.02	0.35±0.05	0.42±0.09
Malate	4.33±1.16	34.60±2.06	14.90±3.75	13.23±1.86
Pyruvate	0.04±0.00	0.05±0.00	0.05±0.00	0.05±0.01
Succinate	1.04±0.36	3.38±0.62	1.59±0.23	1.48±0.14
Other organic compounds				
1,3-Butanediol	0.06±0.01	0.04±0.01	0.10±0.04	0.11±0.01
Acetaldehyde	0.02±0.01	0.05±0.01	0.01±0.00	0.02±0.00
Acetone	0.02±0.00	0.01±0.00	0.02±0.00	0.02±0.00
Ethanol	0.02±0.01	0.03±0.00	0.02±0.00	0.03±0.00
Glycerine	1.98±0.38	3.55±0.19	2.33±0.31	2.99±0.25
Methanol	3.72±0.09	4.70±0.32	3.11±0.38	3.56±0.29
Choline	3.63±0.69	2.45±0.15	1.84±0.10	2.13±0.16

Supplementary Table 2.4. A comparison between oven-dried and freeze-dried leaf samples on total phenolic concentration (mg g^{-1} DM)

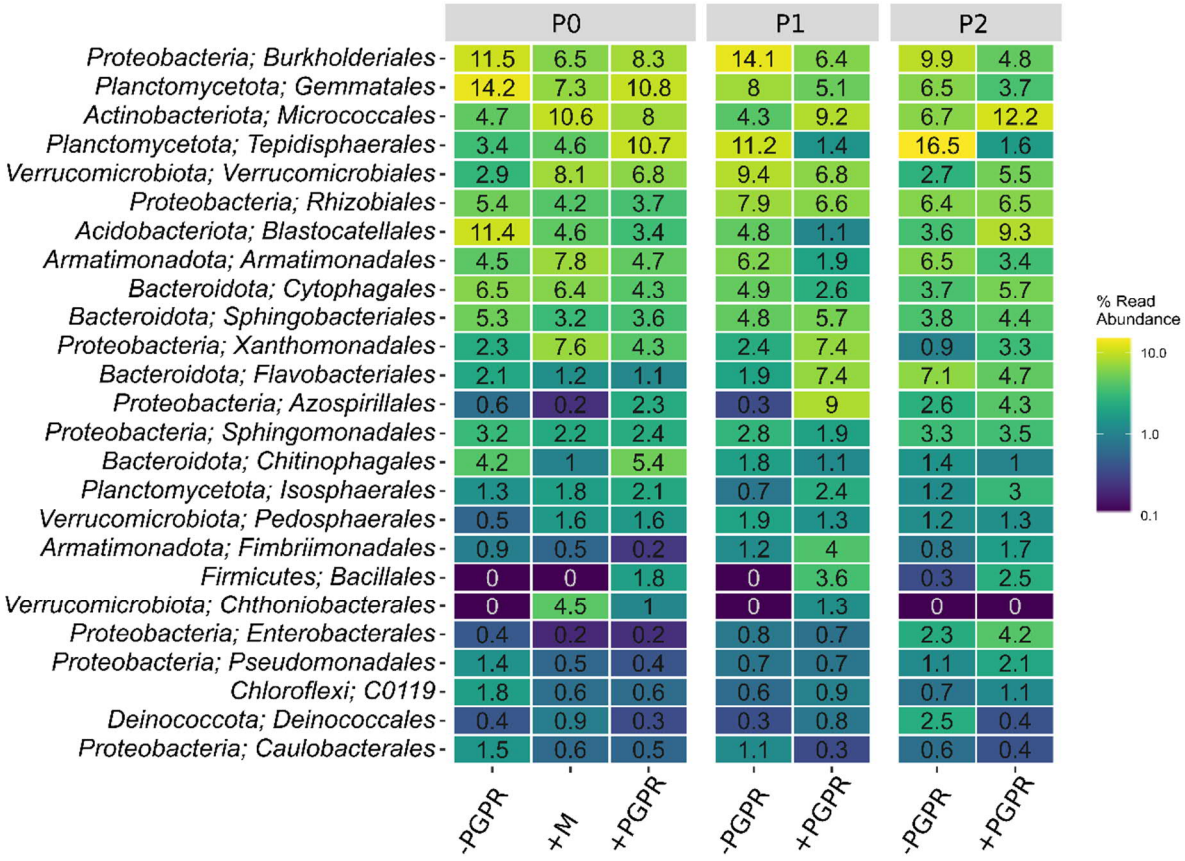
Sample	60°C oven-drying	Freeze-drying
1	16.00	16.36
2	10.89	12.79
3	12.17	13.21
4	14.73	14.66
5	11.24	13.78



Supplementary Figure 2.1. Schematic representation of bacteria cultivation and inoculation procedures. Nutrient broth for each bacteria cultivation was in accordance to supplier's recommendation. Figure was created using BioRender (https://biorender.com/) as part of Academic License.



Supplementary Figure 2.2. Effect of P application on concentrations of K and S in young leaves, old leaves, stem, and roots**.** For all measured traits, P0 data of old leaves are missing due to insufficient sample material for analyses. Error bars indicate standard error of means (n=4). Vertical bars represent critical value for comparisons among P treatments in each plant part by Tukey's HSD test at p≤0.05.



Supplementary Figure 2.3. Heatmap depicting the 25 most abundant root-associated bacterial orders occurring in the data set. The shown mean values are based on the calculation of 4 biological replicates (n=4), except for those under P2+PGPR with n=2 and P1-PGPR with n=3 due to insufficient sample material.

Chapter 3. Effect of phosphorus availability and plant growth-promoting *Bacillus subtilis* on phosphorus efficiency of two potato cultivars

Leangsrun Chea[1], Mohammad Alhussein[2], Petr Karlovsky[2], Elke Pawelzik[1], Marcel Naumann[1*]

[1]Division Quality of Plant Products, Department of Crop Sciences, University of Goettingen, Carl-Sprengel-Weg 1, 37075 Goettingen, Germany

[2]Division of Molecular Phytopathology and Mycotoxin Research, Department of Crop Sciences, University of Goettingen, Grisebachstraße 6, 37077 Goettingen, Germany

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Author contribution

Leangsrun Chea : performed the experiment, analyzed the data, drafted the manuscript, and revised the manuscript based on suggestions from other co-authors.

Mohammad Alhussein : contributed the phytohormone analyses and revised the manuscript.

Petr Karlovsky : contributed the phytohormone analyses and revised the manuscript.

Elke Pawelzik : supervised and revised the manuscript

Marcel Naumann : supervised, conducted RT-qPCR and root surface fluorescent labeling, and revised the manuscript.

Abstract

Plants develop different strategies in response to low and high phosphorus (P) supply. Potato has a low P use efficiency (PUE) compared to other crops, which is caused by its limited root system. The present study aimed to assess plant morphological and physiological adaptation responses that characterize root system modifications and PUE in potato under various P availability. Furthermore, the effect of plant growth-promoting *Bacillus subtilis* on P deficiency amelioration was investigated. Two potato cultivars—a table potato (cv. Milva) and a starch potato (cv. Lady Claire)—were grown under varying P levels (0.5, 2, 5, and 30 mg L^{-1}). Additionally, plants under 0.5 and 2 mg P L^{-1} were treated with and without *Bacillus subtilis* in the nutrient solution. The results revealed the capability of Milva to allocate biomass, P, and sugars to roots under low P supply, causing high P uptake and PUE. In contrast, Lady Claire was not efficient in P uptake under low P levels, but this cultivar was efficient in P uptake under high P availability. Total P uptake of both cultivars was influenced by modifications of root morphology, which was controlled by the P, sugar, and indole-3-acetic acid concentration in roots. The *StPHT1;1* and *StPHT2;1* genes were also important for P translocation to shoots, especially in Lady Claire. Furthermore, *B. subtilis* tend to mitigate P deficiency in Lady Claire more efficiently than in Milva. This information could be helpful to develop strategies to improve PUE in potato under specific P conditions.

Keywords: plant growth-promoting *Bacillus subtilis*, potato, phosphorus, phosphorus use efficiency, root system

3.1. Introduction

Potato is a staple food crop and vegetable providing high energy and nutritional values that contribute to food security worldwide (Burgos et al., 2020). However, potato has a low phosphorus (P) use efficiency (PUE) compared to other crops, making P an important factor determining the production. Inorganic P (P_i) in many soils is present at a low concentration; thus, its availability for plant uptake is limited (Bucher and Kossmann, 2007). P fertilizers are applied to increase soil P concentration, but high P application often contributes to surface water pollution (Sandaña, 2016; Wacker-Fester et al., 2019) and methane emission (Beaulieu et al., 2019). This situation poses a challenge to the improvement of potato production, while minimizing environmental problems. Therefore, it is necessary to deepen our understanding of P efficiency in potato in order to optimize P supply.

P is important for root and shoot development in potato (White et al., 2018). Our recent study found a reduction in potato biomass by >40% and sugar concentration in roots by >20% under very low (0 mg P L^{-1}) and, surprisingly, high P (≥30 mg P L^{-1}) supply in the nutrient solution due to toxic-P conditions (Chea et al., 2021b). This finding confirmed previous reports with regards to the sensitivity of potato to limited P availability (Dechassa et al., 2003; Fernandes et al., 2014; Wacker-Fester et al., 2019), and it showed the susceptibility of potato to high P supply. Therefore, a further investigation on sugar translocation to various parts of plant is necessary to explain the shortage of sugars in roots, especially under P deficiency. In response to these extreme P conditions, plants display a variety of adaption mechanisms, which are dependent on the cultivar and finally drive plant P efficiency under specific P supply (Deng et al., 2018; Irfan et al., 2020). PUE is divided into P uptake efficiency (PUpE)—the capacity of cultivars to exploit the available P—and P utilization efficiency (PUtE)—the capacity of cultivars to produce biomass from the absorbed P (Irfan et al., 2020). Improved root morphology—such as total root length, root surface area, and root volume—is necessary to increase PUpE under P starvation (Heuer et al., 2017). Therefore, root growth is enhanced under P deficiency compared to shoot growth, causing an increased root-to-shoot ratio (Chea et al., 2021b). In addition to the modification of root morphology enhancing P uptake, Mori et al. (2016) suggested a second adaptation strategy consisting of increased root efficiency under P deficiency. Root efficiency is the capacity of roots to uptake P per unit of root surface area. Moreover, specific P uptake—P uptake per unit of root length—is also an important trait for plants grown under P limitation (Marschner et al., 2007). Modifications of root morphology as well as root uptake capacity—including specific P uptake and root efficiency—imply that, under P deficiency, roots play an essential role as a source organ to supply P for various plant parts, and they simultaneously act as a strong sink organ for P. In rice, enhanced root growth under P deficiency was strongly determined by greater P rather than carbohydrate allocation to roots (Wissuwa et al., 2005). Meanwhile, there is also increasing evidence suggesting the involvement of phytohormones in root morphology modification under P deficiency (Niu et al., 2013; Nadira et al., 2016). For instance, indole-3-acetic acid (IAA) serves as a signaling molecule in the growth of lateral roots and root hair (Pérez-Torres et al., 2008). Furthermore, at the molecular level, a variety of P transporter genes are also regulated to enhance P uptake by roots. Liu et al.

(2017) found an increase in the expression of *PHT1* and *PHT2* gene families in potato roots and leaves in response to P deficiency. This finding indicates the importance of these two gene families in enhancing P uptake by roots and translocation to shoots. The translocation of absorbed P into shoots is necessary to improve PUtE under P starvation. However, besides the abovementioned adaption mechanism of plants to P deficiency, there is a lack of knowledge on potato in regard to plant morphological, physiological, and molecular modifications under low and high P availability. There have only been reports by Chea et al. (2021b) on mineral homeostasis and metabolites in potato and Shukla et al. (2017) on plant growth and development in Arabidopsis in response to high P supply. Therefore, it is necessary to further characterize the mechanisms of P efficiency in potato cultivars under low and high P supply.

Furthermore, our recent study also showed the effectiveness of plant growth-promoting rhizobacteria (PGPR) co-inoculation on root growth stimulation, which increased P uptake and plant biomass of potato—compared to non-inoculated plants—under P deficiency (Chea et al., 2021b). Although a diverse set of PGPR was suggested to benefit plant growth rather than a single strain (Menéndez and Paço, 2020), it is necessary to elucidate the plant growth-promoting effects of individual strains. The improvement of root morphology may also result from PGPR-induced production of hormones such as IAA and abscisic acid (ABA) in the roots (Vacheron et al., 2013). Furthermore, some PGPR are capable of producing phytohormones themselves—such as IAA (Chea et al., 2021b). However, besides the growth-promoting effect improving P uptake, plant-PGPR symbiosis is an energy-expensive process, which also requires P and photoassimilates from plants (Kisand et al., 2001; Smith and Read, 2008). In maize, Molina-Romero et al. (2021) also reported the dependence of the growth-promoting effect of PGPR on the cultivar and P availability. Therefore, the symbiosis between plants and PGPR is largely influenced by the dependence of cultivars on the benefits from the association with bacteria.

In the present study, a table potato—cv. Milva—and a starch potato—cv. Lady Claire—were evaluated for their adaptation responses under various P levels (0.5–30 mg L^{-1}) in a nutrient solution. Plant growth-promoting *Bacillus subtilis* was inoculated mainly under low P levels (0.5 and 2 mg L^{-1}) and compared with plants without the inoculant. The selection of this strain was based on its relatively high abundance, i.e. 1.9-3.6% of the total bacterial community compositions at the genus level in association with roots across low P levels, according to our previous study (Chea et al., 2021b). The present study intended to (I) characterize plant morphology and molecular adaptations of potato cultivars in response to various P levels, (II) investigate the factors controlling root morphology and PUE of the cultivars, and (III) evaluate the effect of *B. subtilis* on root system modifications and plant P status. Differences in P efficiency between the two contrasting potato cultivars and their responses to *B. subtilis* are revealed.

3.2. Materials and methods

3.2.1. Plant materials and reagents

Potato cultivars Milva and Lady Claire were received from Europlant Pflanzenzucht (Lüneburg, Germany) and Meijer Potato (Rilland, the Netherlands), respectively. The selection of these cultivars was based on their different responses to P availability in our earlier study (Chea et al., 2021a). For germination, a single bud of 1-2 cm was taken from each potato seed using a ball shaper and planted at a 2 cm depth in a 2-L pot filled with quartz sand containing nutrients, as described in Chea et al. (2021b). Four weeks after planting, the uniformly grown seedlings were removed from quartz sand and washed in distilled water before transferring to a hydroponic system.

Acetonitrile, HNO_3, H_2O_2, $K_4[Fe(CN)_6]*3H_2O$, $ZnSO_4*7H_2O$, and the reagents used for the nutrient solution preparation were obtained from Carl Roth (Karlsruhe, Germany). Abscisic acid and indole-3-acetic acid were purchased from Sigma-Aldrich (Steinheim, Germany). Jasmonic acid, jasmonic acid-d5, *trans*-zeatin, and *trans*-zeatin-d5 were purchased from Cayman Chemical (Michigan, United States). Indoleacetic acid-d4 and *cis,trans*-abscisic acid-d6 were purchased from Toronto Research Chemical (Toronto, Canada). LC-MS grade 2-propanol and methanol were purchased from Th. Geyer (Hoexter, Germany). Formic acid was purchased from Carlo Erba Reagents (Val-de-Reuil, France). Water was purified using an Arium pro ultrapure water system (Sartorius, Goettingen, Germany).

3.2.2. Experimental setup, plant cultivation, and *B. subtilis* inoculation

The experiment was arranged in a factorial randomized complete block design with five replications under greenhouse conditions. External light of 400 µmol m^{-2} s^{-1} photosynthetic photon flux density was supplied at a 16h/8h day/night regime. The average temperature was 21.0±1.5°C. Potato seedlings were transferred into a 6-L pot (one plant per pot) containing nutrient solution with different P concentrations of 0.5, 2, 5, and 30 mg L^{-1} by using KH_2PO_4. These P treatments are referred as P0.5, P2, P5, and P30, respectively, hereafter. Other nutrients were supplied at the optimum levels following Chea et al. (2021b). The K concentration in each pot was balanced by using K_2SO_4. The pH of the nutrient solution was in the range of 5.5-6.5. The experiment was initiated by supplying nutrients at 25% of the full concentration, which was then increased to 50%, 75%, and 100%, every two days subsequently. Afterwards, the nutrient solution was renewed once per week. Between the nutrient solution renewals, distilled water was added as needed to each pot to maintain the volume. Aeration was maintained by using polyethylene tubes dipped into the nutrient solution of each pot.

A strain of *B. subtilis* DSM 21393 was derived from the German Collection of Microorganism and Cell Culture (DSMZ, Braunschweig, Germany). In order to assess the growth-promoting effects of *B. subtilis*, each of P0.5 and P2 was treated with and without *B. subtilis* in the nutrient solution. Before inoculation, the bacterial strain was plated and cultivated in nutrient broth according to the procedures described in Chea et al. (2021b). A total of 50 mL of *B. subtilis* culture (~2.8 x 10^9 colony-forming units mL^{-1}) was then centrifuged at 2,660 *g* for 15 min. The supernatant was discarded, and the bacterial cells were homogenized and suspended in nutrient

solution of the plants at eight days after transplanting (DAT) the seedlings a hydroponic system. Afterwards, bacterial cells were inoculated once per week after renewal of the nutrient solution for the plants.

3.2.3. Plant harvesting and sample preparation

At 25 DAT, a young fully developed leaves at fourth position from the top was sampled to determine leaf nutritional status. At 42 DAT, the whole plants were harvested, and they were separated into young leaves (fourth position from the top), old leaves (first position from the bottom), main stem, remaining shoots (including side-shoots and stolon), and roots. A sub-sample of young leaves and roots were freshly ground with liquid nitrogen and stored at -20°C. Another sub-sample of roots was stored at -20°C for root scanning. The remaining sub-samples of each plant part were freeze-dried in an EPSILON 2-40 freeze dryer (Christ, Osterode am Harz, Germany) for four days. Shoot and root dry matter (DM) of each plant was then calculated. Shoot DM was the sum of DM of all shoot parts—including young leaves at 25 DAT and young leaves, old leaves, stem, and remaining shoots at 42 DAT. The samples were then milled using a hammer mill (DFH 48 Culatti, Kinematica, Malters, Switzerland) with a 0.5 mm sieve.

3.2.4. P and sugar analyses in plant tissue

P concentration in young leaves, old leaves, stem, remaining shoots, and roots was determined by extracting 100 mg of freeze-dried sample with 4 mL of 60% HNO_3 and 2 mL of 30% H_2O_2 according to the method described by Koch et al. (2019a) and analyzed in an inductively coupled plasma optical emission spectrometry (ICP-OES) (Varian, Palo Alto, United States). P content in shoots was calculated as a sum of total P in young leaves at both sampling dates, stem, old leaves, and remaining shoots. P content of each shoot part was calculated by multiplying the P concentration by its respective DM. P content in roots was also obtained through a multiplication of P concentration in roots with its DM. The combination of P content in shoots and roots resulted in total P uptake. The calculation of PUpE, PUtE, and PUE was adapted from the methods of Wacker-Fester et al. (2019) and Sandaña (2016) as follows:

$$\text{PUpE} = \frac{\text{Total P uptake (mg plant}^{-1})}{\text{Total applied P (mg pot}^{-1})}$$

$$\text{PUtE} = \frac{\text{Shoot DM (g plant}^{-1})}{\text{Total P uptake (mg plant}^{-1})}$$

$$\text{PUE} = \text{PUpE} \times \text{PUtE}$$

To measure the soluble sugar concentration in young leaves, old leaves, stem, and roots, 50 mg of freeze-dried sample was extracted with 700 µL of 80% acetonitrile. Then, 50 µL of 3.6% $K_4[Fe(CN)_6]*3H_2O$ and 50 µL of 7.2% $ZnSO_4*7H_2O$ were subsequently added to precipitate proteins, followed by 30 min centrifugation at 15,000 *g*. The supernatant was collected and stored at -20°C. For sugar quantification, the extract was thawed and centrifuged for 30 min at 15,000 *g*. The supernatant was then filtered through a 0.45 µm membrane with a 13 mm syringe (VWR, Darmstadt, Germany). Finally, 20 µL of the filtered extract was used for the

quantification of sucrose, fructose, and glucose by high-performance liquid chromatography (HPLC) (Jasco, Pfungstadt, Germany). As the eluent, 80% acetonitrile was used through a 5 µm LiChrospher 100 NH2 column (Merck, Darmstadt, Germany) at 22°C and 1 mL min^{-1} flow rate. The sugar content in shoots and roots was then calculated.

3.2.5. Root scanning and surface fluorescent labeling

Root samples stored at -20°C were thawed and scanned to determine total root length, root surface area, and root volume according to the procedures described in Chea et al. (2021b). Specific root length was determined as root length per root DM. Specific P uptake and root efficiency were calculated as total P uptake per unit of root length and root surface area, respectively.

To assess the presence of root-associated *B. subtilis*, root sample of *B. subtilis* inoculated and non-inoculated plants were labeled by using Cellbrite Fix Membrane Stains (Biotum, Hayward, United States) according to the manufacturer's protocol. Briefly, the roots were washed with phosphate-buffered saline. They were then suspended in a 10-fold diluted Cellbrite Fix Membrane Dye and incubated for 15 min at room temperature. Afterwards, the labeled roots were washed twice with phosphate-buffered saline and mounted on glass slide for imaging with an LSM 780 confocal laser-scanning microscope (Zeiss, Oberkochen, Germany). The Cellbrite Fix Membrane Dye was excited at 488 nm by using an argon laser and emission filtering was achieved using a 493 nm to 630-nm bandpass filter. Image processing was performed with ZEN 2013 software (Zeiss, Oberkochen, Germany).

3.2.6. Quantitative polymerase chain reaction (PCR) analyses of P transporters

Young leaf and root samples at 42 DAT were ground with liquid nitrogen. Approximately 100 mg of plant material from each sample was used for RNA extraction, which was performed according to the manufacturer's instructions using the innuPREP Plant RNA Kit (Analytik Jena, Jena, Germany). Afterwards, 750 µg of total RNA was used to perform cDNA synthesis by using the iScript™ cDNA Synthesis Kit (Bio-Rad, Hercules, United States) in accordance with the manufacturer's instructions. Quantitative PCR (qPCR) was performed with the SsoAdvanced Universal SYBR Green Supermix (Bio-Rad, Hercules, United States) according to the manufacturer's instructions, using a protocol previously published by Koch et al. (2019a). The primers used in this study for the P transporter genes (*StPHT1;1*, *StPHT1;7*, and *StPHT2;1*) and housekeeping gene (*StUBIQUITIN*) were taken from the studies of Liu et al. (2017) and Koch et al. (2019a), respectively.

3.2.7. Quantification of phytohormones in roots

a. Phytohormone extraction

Phytohormones were extracted from the frozen ground roots using a slightly modified protocol of Müller and Munné-Bosch (2011). Briefly, 2 g of ground roots were suspended in 5 mL of cold extraction solution (methanol:isopropanol, 20:80) with 0.1% formic acid [v/v] and sonicated for 10 min at 5-8°C. The suspension

was shaken at 4-5°C and 390 rpm for 2 h, followed by a centrifugation at 15,800 *g* for 10 minutes. An aliquot of 1 mL was transferred from each sample into an HPLC amber glass vial and analyzed immediately.

b. HPLC-MS/MS

The analysis was carried out using HPLC system 1290 Infinity II (Agilent Technologies, Waldbronn, Germany) and the Agilent 6460 triple quadrupole detector (Agilent Technologies, Waldbronn, Germany). Chromatographic separation was performed using Zorbax Eclipse Plus C18 column, 50 x 2.1 mm with 1.8 µm particle size (Agilent Technologies, Waldbronn, Germany). The column was kept at 40°C, and the injection volume was 5 µL. Solvent A was water with 0.1% formic acid [v/v], and solvent B was methanol with 0.1% formic acid [v/v]. The gradient was as follows: 0 to 0.2 min, 5% B; 0.2 to 6 min, 5% to 75% B; 6 to 6.50 min, 75% to 98% B; 6.50 to 8.50 min, 98% B; 8.50 to 9 min, 98% to 5% B; 9 to 12 min, 5%. The eluent was ionized using an electrospray ionization (ESI) source with the following parameters: nebulizer pressure, 60 psi; capillary voltage, 4000 V; nitrogen temperature, 350°C; gas flow, 13 L min^{-1}. Phytohormones were analyzed in multiple reaction monitoring (MRM) mode. The acquisition parameters are described in Supplementary Table 3.1. The calibration curve consisted of 12 concentrations from 0.48 to 1,000 µg L^{-1}. Blank samples were analyzed after every seventh sample, and a quality control standard (250 µg L^{-1}) was analyzed after every 15^{th} samples. The limit of detection (LOD) and limit of quantification (LOQ) were calculated based on the standard deviation of the blank (Wenzl et al., 2016). The overall process efficiency was estimated as described by Matuszewski et al. (2003) using the equation:

$$PE\ (\%) = C/A \times 100$$

C: peak areas for the isotopically labeled standards spiked before extraction

A: peak areas for isotopically labeled standards in pure solvents

3.2.8. Statistical analysis

The data obtained from measurements were analyzed separately between the effect of varying P applications and *B. subtilis* inoculation at low P levels. Three-way analysis of variance (ANOVA) was used to determine the significant effects of P levels, cultivars, and the interactions. Furthermore, the interactions were sliced to compare P treatments for each cultivar as well as comparison among cultivars at the same P levels. The pairwise comparisons were performed using Tukey's HSD test at $p < 0.05$. To determine the effect of *B. subtilis* inoculation, plants with and without the inoculant were compared using a paired t-test at $p < 0.05$ within each P treatment and cultivar. The correlations among observed traits were evaluated by a Pearson's correlation. These analyses were conducted following the methods of Gomez and Gomez (1984) by using the Statistix 8.0 software (Analytical Software, Tallahassee, United States). Graphical presentations were prepared in SigmaPlot 12.5 (Systat Software, San Jose, United States).

3.3. Results

3.3.1. Effect of P on plant biomass partitioning and P status

Whole plant phenotyping was documented at 25 DAT (Figure 3.1A). In order to assess plant biomass partitioning in response to different P levels (0.5, 2, 5, and 30 mg L^{-1}), shoot and root DM were assessed at 42 DAT. Shoot DM, root DM, and root-to-shoot ratio of both cultivars were significantly affected by P level (Figure 3.1B-D). There was also a significant interaction between P level and cultivar in root DM. Shoot and root DM of Lady Claire and Milva increased in response to increasing P supply at different magnitudes, but there was no significant increase in shoot DM of Milva above P5 and root DM of Lady Claire above P2. The root-to-shoot ratio of both cultivars gradually decreased at higher P availability, but it tended to increase in Milva at P30 by 26% compared with P5. A comparison between cultivars at the same P level shows that Lady Claire had significantly greater shoot DM than Milva under P30. However, Milva had greater root DM and root-to-shoot ratio than Lady Claire under all P treatments.

Furthermore, P concentration in various plant tissues and sampling dates—young leaves at 25 DAT as well as young leaves, old leaves, stem, roots at 42 DAT, and total P content in shoots and roots—were significantly influenced by P availability, cultivar, and their interaction (Table 3.1). For both cultivars, the P concentration in young leaves decreased from 25 DAT to 42 DAT by 10-30% across all P levels, except in Milva under P30. At harvest (42 DAT), P concentration in different parts of the plant and P content in shoots and roots generally increased in response to increasing P supply. Interestingly, the P concentration in roots under P0.5 was 25-30% higher than under P2. Further increases of P supply to P30 resulted in enhanced P concentration in roots up to 30.18 mg g^{-1} in Lady Claire and 16.50 mg g^{-1} in Milva. A comparison between cultivars reveals that Milva displayed a higher P concentration in young and old leaves as well as P content in shoots and roots than Lady Claire under P0.5 and P2, although it was not significant. Under P30, Lady Claire had a greater P concentration in old leaves and roots as well as P content in shoots and roots, but the P concentration in young leaves and stem was lower than in Milva.

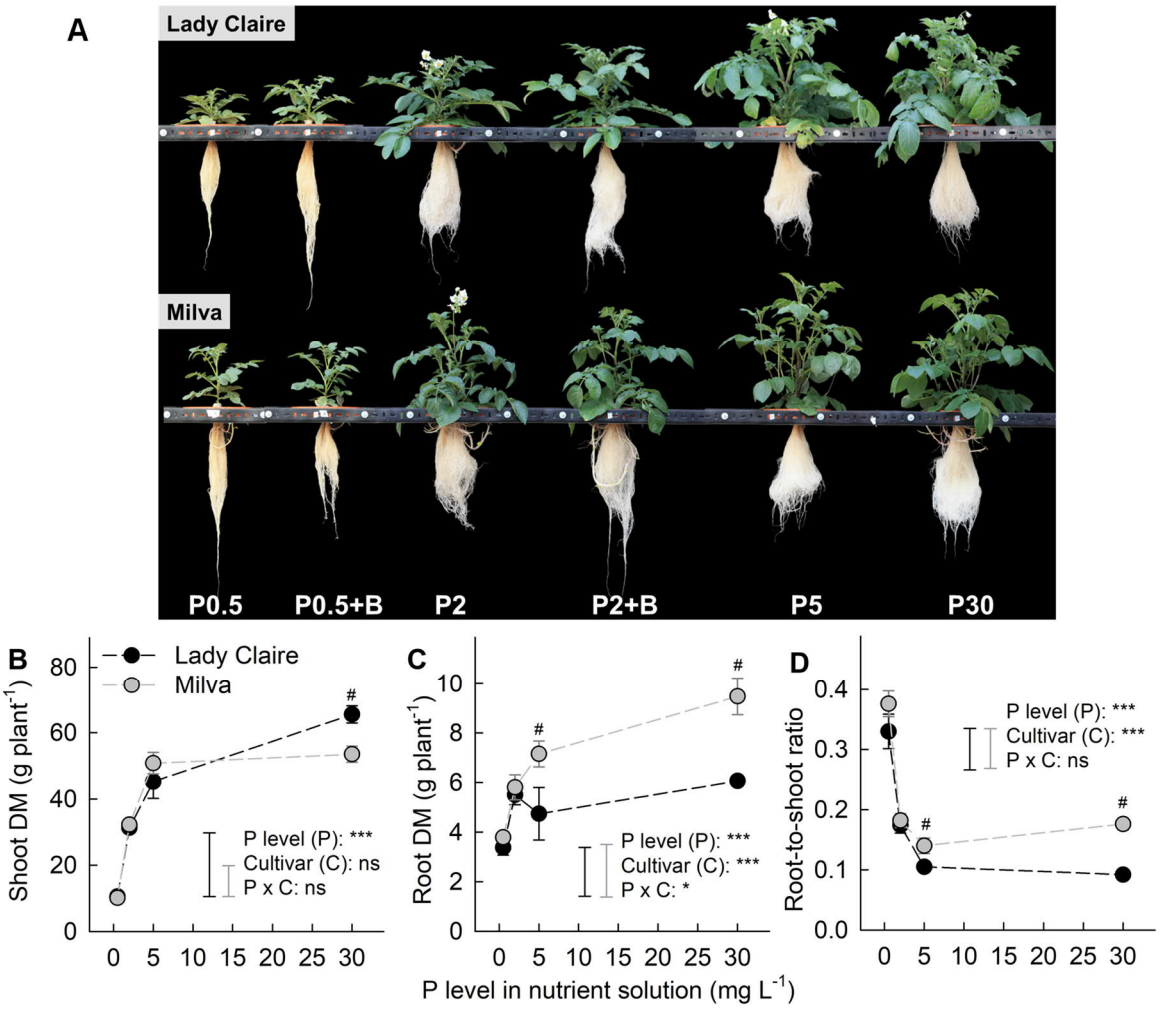


Figure 3.1. (**A**) Plant phenotypes of the potato cultivars Lady Claire and Milva as affected by P availability and *Bacillus subtilis* inoculation and the effect of varying P levels (0.5, 2, 5, and 30 mg L^{-1}) on the (**B**) shoot DM, (**C**) root DM, and (**D**) root-to-shoot ratio of the cultivars. The values are mean ± SE (n=5). ns, *, and *** indicate non-significant and significant differences at $p<0.05$ and $p<0.001$, respectively. Black and grey vertical bars represent critical value for comparisons among P treatments of each cultivar by Tukey's HSD test at $p<0.05$. # indicates a significant difference between cultivars at the same each P level while no indication means non-significant difference. B = *Bacillus subtilis*, DM = dry matter.

Table 3.1. P concentration in different parts of plant and P content in shoots and roots

Cultivar	P level	P concentration (mg g^{-1} DM)					P content (mg plant^{-1})	
		Young leaves	Young leaves	Old leaves	Stem	Roots	Shoots	Roots
	(mg L^{-1})	25 DAT	42 DAT	42 DAT	42 DAT	42 DAT		
Lady Claire	0.5	1.46±0.07 ^{c}	1.17±0.05^{c}	1.06±0.05^{c}	1.05±0.06^{b}	2.17±0.09^{b}	12.77±1.24^{d}	7.28±0.57^{b}
	2	1.32±0.07^{c}	1.19±0.02^{c}	1.07±0.04^{c}	1.01±0.06^{b}	1.74±0.04^{b}	32.80±3.04^{c}	9.55±0.65^{b}
	5	2.23±0.16^{b}	1.95±0.14^{b}	2.65±0.23$^{b\#}$	1.42±0.15^{b}	2.71±0.24^{b}	65.54±9.26^{b}	8.60±3.58^{b}
	30	8.41±0.23$^{a\#}$	5.81±0.20^{a}	7.08±0.15$^{a\#}$	4.93±0.24$^{a\#}$	30.18±0.87$^{a\#}$	367.94±9.72^{a}	182.85±5.27^{a}
Milva	0.5	1.93±0.07^{c}	1.70±0.15^{c}	1.46±0.08^{b}	0.97±0.03^{c}	2.19±0.10^{b}	14.16±0.65^{d}	7.90±0.28^{c}
	2	2.19±0.04^{c}	1.78±0.05^{c}	1.09±0.07^{b}	0.92±0.02^{c}	1.67±0.07^{c}	51.81±3.57^{c}	9.56±0.50^{c}
	5	2.96±0.16^{b}	2.43±0.12^{b}	1.39±0.07$^{b\#}$	1.53±0.04^{b}	2.62±0.06^{b}	112.27±2.45^{b}	18.76±1.48^{b}
	30	6.02±0.29$^{a\#}$	6.47±0.15^{a}	4.84±0.28$^{a\#}$	6.23±0.09$^{a\#}$	16.50±0.21$^{a\#}$	309.98±9.73^{a}	156.75±9.27^{a}
P level (P)		***	***	***	***	***	***	***
Cultivar (C)		ns	***	***	***	***	ns	ns
P x C		***	ns	***	***	***	*	ns

ns, *, and *** indicate non-significant and significant differences at $p<0.05$ and $p<0.001$, respectively, by ANOVA. Mean values ± SE (n=5) with different letters in the same column indicate significant differences between P levels of each cultivar. $^{\#}$ indicates a significant difference between cultivars at the same P level while no indication means a non-significant difference. Shoot P content is the combination of P content in all shoot parts including young leaves at 25 DAT.

3.3.2. Quantitative PCR determination of P transporters

The analyses via qPCR revealed variation in the expression levels of *StPHT1;1* and *StPHT1;7* in leaves and roots as well as *StPHT2;1* in leaves (Figure 3.2). The relative expression levels of each gene in leaves and roots were based on the fold changes compared to the expression levels of a mixed cDNA sample, which included all cDNA samples used in this study for leaves and roots, respectively. Furthermore, by comparing the expression levels with the same mixed cDNA (either in leaves or roots), relative expression levels of *StPHT1;1* and *StPHT1;7* in roots were higher than in leaves (data not shown). Although the effects of P level, cultivar, and the interaction were not significant, the transcript levels of *StPHT1;1*, *StPHT1;7,* and *StPHT2;1* in leaves tended to be higher in Lady Claire than in Milva. In roots, the expression levels of *StPHT1;1* and *StPHT1;7* were stable in Lady Claire; however, *StPHT1;7* tended to decrease in response to increasing P levels.

3.3.3. PUpE, PUtE, and PUE of potato cultivars under varying P levels

In both cultivars, PUpE, PUtE, and PUE were significantly affected by P levels (Figure 3.3 A-C). Among P treatments, P0.5 obtained the highest PUpE and PUE. Increasing P availability decreased PUpE and PUE. In contrast, the highest PUtE was observed under P5. Lower or higher P availability resulted in a significant reduction of PUtE in both cultivars. PUpE and PUE were higher in Milva than in Lady Claire under P0.5, P2, and P5, but these P efficiency indices were lower in Milva under P30.

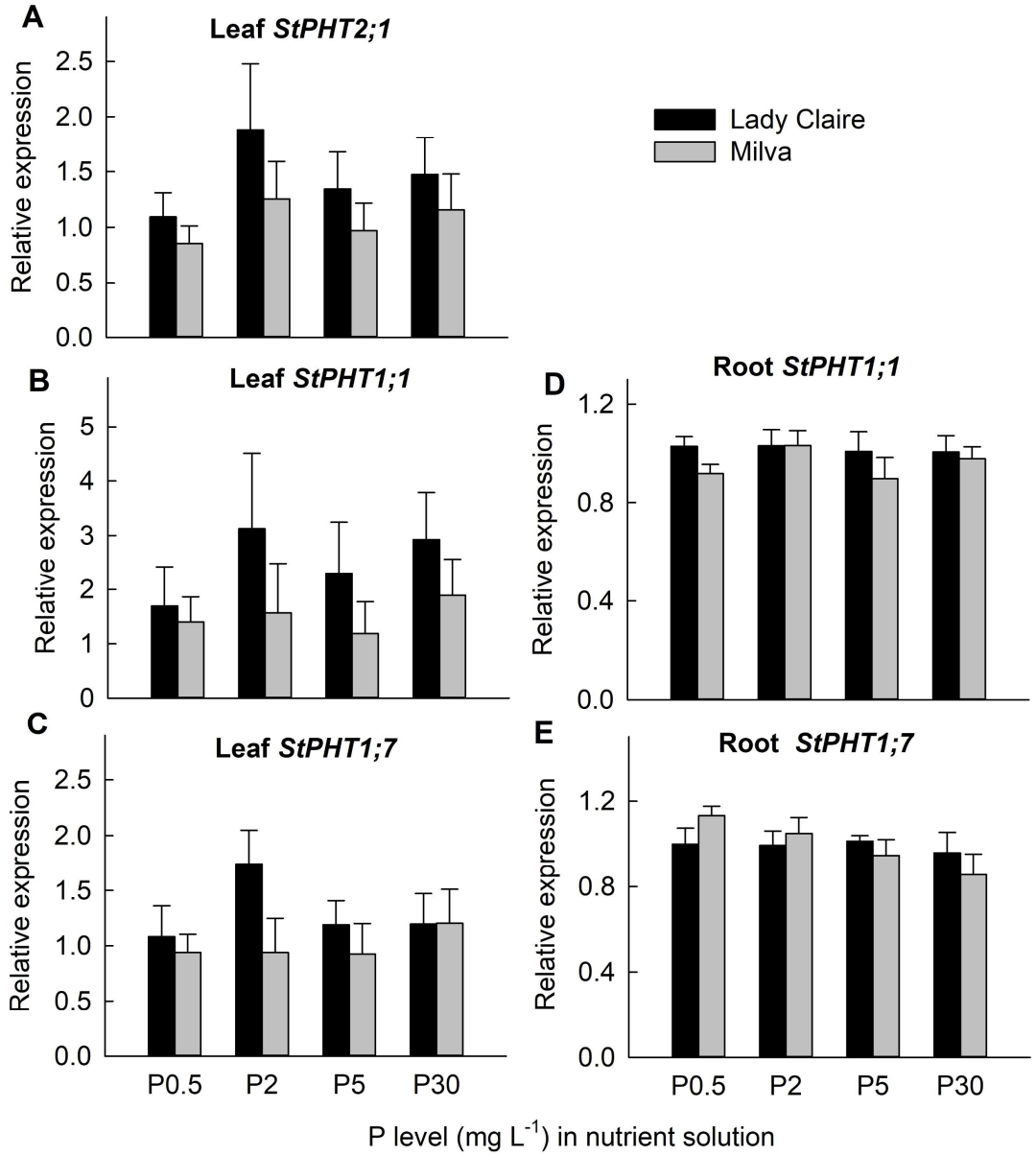


Figure 3.2. Relative expression of P uptake and translocation gene in young leaves and roots at 42 DAT as influenced by varying P levels. The transcription in leaves and roots is presented as relative values to a mix of all cDNA from all treatments in leaves and roots, respectively. The effects of P level (P), cultivar (C), and the interaction (P x C) are not significant by ANOVA. The cultivars are also not significantly different at the same P level.

3.3.4. Root morphology and phytohormones in roots under varying P levels

Since root DM was strongly influenced by cultivar and P level, a proportion of fresh root samples was scanned and calculated root system-associated parameters. Total root length, root surface area, root volume, specific P uptake, and root efficiency were also significantly affected by P level, cultivar, and their interaction (Figure 3.3 D-I). Although, root length density differed among the cultivars, it was less affected by P level. Total root length, root surface area, and root volume increased as P supply increased up to P2 in Lady Claire and P5 in Milva, while there was no significant increment above this P level in each cultivar. Moreover,

specific P uptake and root efficiency increased under increasing P levels and achieved their maximum values under P30. At the same P level, Milva exhibited greater total root length, root surface area, and root volume than Lady Claire, mainly under P0.5, P5, and P30. Nevertheless, Lady Claire also tended to reveal higher root length density than Milva under P0.5. Furthermore, specific P uptake and root efficiency of Lady Claire under P30 were 75-89% higher than those of Milva. Total root length and root surface area significantly correlated with total P uptake in Milva, but there were no significant associations in Lady Claire. In both cultivars, specific P uptake and root efficiency positively correlated with total P uptake (Supplementary Figure 3.1).

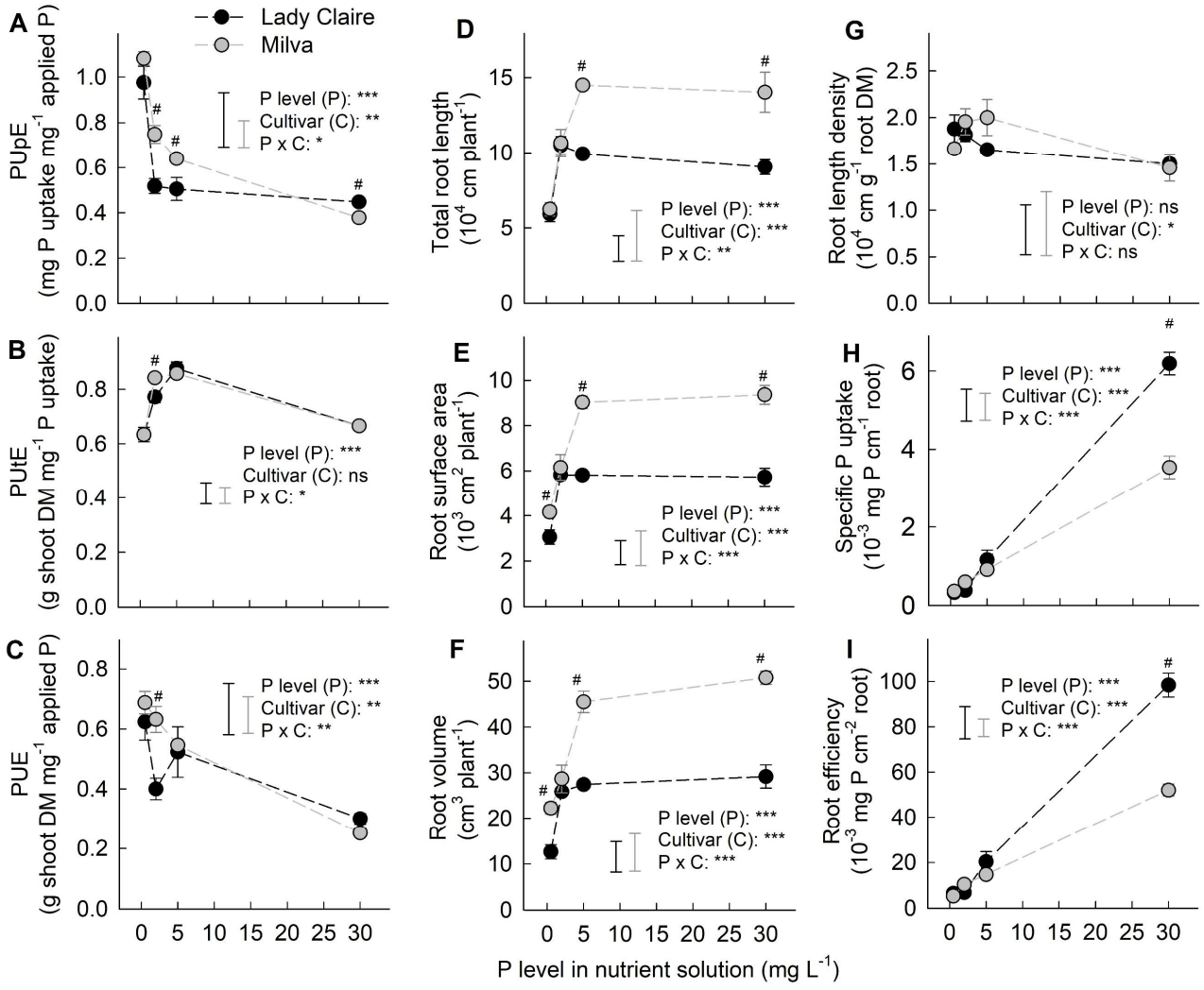


Figure 3.3. Effect of varying P levels (0.5, 2, 5, and 30 mg L^{-1}) on (**A**) P uptake efficiency (PUpE), (**B**) P utilization efficiency (PUtE), (**C**) P use efficiency (PUE), (**D-G**) root morphology, and (**H, I**) root P uptake capacity in the cultivars Lady Claire and Milva. The values are mean ± SE (n=5). PUpE = total plant P uptake/total applied P, PUtE = shoot dry matter/plant P uptake, and PUE = PUpE x PUtE. ns, *, and *** indicate non-significant and significant differences at $p<0.05$ and $p<0.001$, respectively. Black and grey vertical bars represent critical value for comparisons among P treatments of each cultivar by Tukey's HSD test at $p<0.05$. # indicates a significant difference between cultivars at the same each P level while no indication means a non-significant difference.

The analyses of phytohormone in roots revealed the presence of trans-zeatin, IAA, ABA, and JA. However, the concentration of trans-zeatin, ABA, and JA in many samples was below the LOD and LOQ. The effects of P levels, cultivars, and their interaction on root IAA concentration were not significant. Nevertheless, IAA concentration in roots of Milva was relatively higher than in Lady Claire across P treatments (Figure 3.4). In Milva, the highest IAA was observed under P2 which was 2-3 times higher than P0.5, P5, and P30. In contrast, in Lady Claire P2 had the lowest IAA concentration, which was about 50% lower than P0.5 and P30.

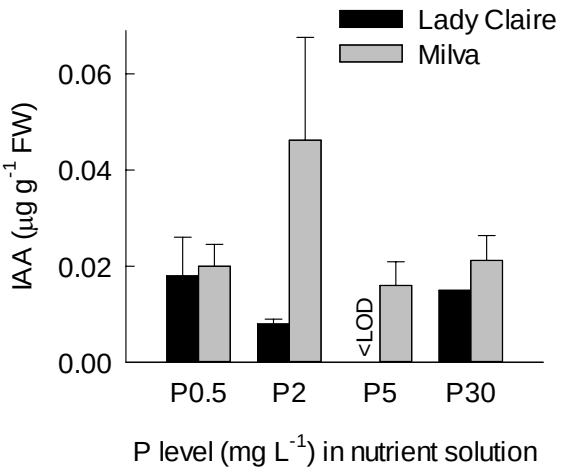


Figure 3.4. Indole-3-acetic acid (IAA) concentration in roots under different P levels in the cultivars Lady Claire and Milva. The values are means ± SE (n=5). The effects of P level (P), cultivar (C), and the interaction (P x C) are not significant by ANOVA. The cultivars are also not significantly different at the same P level. LOD = limit of detection.

3.3.5. Effect of P on sugar concentration in different parts of plant

The analyses of sugar concentration in all parts of plants at all sampling dates under various P levels showed a significant increase in sugar concentration and content of both cultivars in response to increasing P supply (Table 3.2). In young leaves, the sugar concentration increased from 25 DAT to 45 DAT in all P treatments of both cultivars, except Milva under P0.5, in which the sugar concentration was reduced by 17%. At 42 DAT, Milva contained less sugar in young leaves and old leaves, but higher sugar concentration in stem and roots than in Lady Claire under P0.5, P2, and P5. However, sugar concentration in young leaves, old leaves, and roots of Lady Claire were higher than in Milva under P30. Furthermore, the correlation analyses showed overall positive correlations between root sugar concentration with total root length, root surface area, specific P uptake, and root efficiency, although these relationships differed between cultivars (Supplementary Figure 3.2).

Table 3.2. Influences of P supply on sugar partitioning into various parts of plant

Cultivar	P level (mg L^{-1})	Sugar concentration (mg g^{-1} DM)					Sugar content (g $plant^{-1}$)	
		Young leaves 25 DAT	Young leaves 42 DAT	Old leaves 42 DAT	Stem 42 DAT	Roots 42 DAT	Shoots	Roots
Lady Claire	0.5	$12.39\pm0.57^{c\#}$	14.94 ± 1.52^{d}	0.74 ± 0.06^{b}	$22.21\pm1.79^{c\#}$	$11.53\pm1.10^{b\#}$	0.22 ± 0.04^{d}	$0.03\pm0.01^{d\#}$
	2	$13.48\pm1.55^{c\#}$	26.04 ± 4.85^{c}	$1.54\pm0.21^{ab\#}$	$23.11\pm2.47^{c\#}$	$15.82\pm1.24^{b\#}$	0.59 ± 0.02^{c}	$0.08\pm0.01^{c\#}$
	5	$35.72\pm5.69^{b\#}$	57.20 ± 9.94^{b}	1.31 ± 0.13^{a}	$37.43\pm4.16^{b\#}$	31.75 ± 3.23^{a}	1.81 ± 0.32^{b}	$0.18\pm0.01^{b\#}$
	30	$55.98\pm8.53^{a\#}$	$84.82\pm6.78^{a\#}$	1.69 ± 0.10^{a}	$60.99\pm1.84^{a\#}$	$39.26\pm3.50^{a\#}$	2.93 ± 0.36^{a}	0.24 ± 0.02^{a}
Milva	0.5	$16.93\pm1.19^{b\#}$	14.04 ± 0.69^{d}	0.80 ± 0.04^{b}	$29.22\pm0.96^{d\#}$	$14.01\pm0.84^{d\#}$	0.14 ± 0.01^{c}	$0.05\pm0.01^{c\#}$
	2	$18.29\pm0.34^{b\#}$	22.98 ± 1.00^{c}	$0.92\pm0.07^{ab\#}$	$40.99\pm1.41^{c\#}$	$20.14\pm1.51^{c\#}$	0.67 ± 0.07^{b}	$0.12\pm0.01^{b\#}$
	5	$26.94\pm0.87^{a\#}$	50.04 ± 4.92^{b}	1.23 ± 0.13^{ab}	$72.00\pm3.45^{a\#}$	35.91 ± 2.36^{a}	2.01 ± 0.16^{a}	$0.26\pm0.02^{a\#}$
	30	$29.69\pm1.21^{a\#}$	$73.28\pm2.65^{a\#}$	1.39 ± 0.20^{a}	$68.75\pm2.29^{b\#}$	$26.98\pm1.03^{b\#}$	2.34 ± 0.23^{a}	0.26 ± 0.02^{a}
P level (P)		***	***	***	***	***	***	***
Cultivar (C)		*	ns	*	***	*	ns	**
P x C		**	ns	*	***	***	ns	ns

ns, *, * and *** indicate non-significant and significant differences at $p<0.05$, $p<0.01$, and $p<0.001$, respectively, by ANOVA. Mean values ± SE (n=5) with different letters in the same column indicate a significant difference between P levels of each cultivar. # indicates a significant difference between cultivars at the same P level while no indication means a non-significant difference.

3.3.6. Effect of *B. subtilis* on the PUE of potato cultivars under low P levels

Surface fluorescent labeling with Cellbrite Fix Membrane Stains on root segments with and without *B. subtilis* addition showed a greater colonization of bacterial colonies on the root surface under *B. subtilis* inoculation than in the non-inoculated treatment (Figure 3.5 and Supplementary Figure 3.3). This observation indicates the attachment of *B. subtilis* on the root surface—and even inside the roots—of both cultivars.

In order to assess the plant growth-promoting effects of *B. subtilis* inoculation, plants with and without the inoculants were compared. In Lady Claire, *B. subtilis* tended to enhance the P content in shoots by 13-25% and in roots by 4-13% at both P0.5 and P2. Moreover, the addition of inoculant stimulated the relative expression levels of *StPHT1;1* and *StPHT2;1* in leaves of Lady Claire by two-fold under P0.5. However, in Milva, the effect of *B. subtilis* on the P content in shoots and roots was lower, but the inoculant also provoked *StPHT1;1* in leaves by three-fold under P0.5 (Table 3.3).

Furthermore, *B. subtilis* tended to increase PUpE, PUE, total root length, and root surface area under P0.5 and root efficiency under P2 in Lady Claire by 19-31%. However, the inoculant had less effects on PUpE, PUE, and root traits in Milva. Nevertheless, the IAA concentration in roots of *B. subtilis*-inoculated P0.5 of both cultivars was 65-90% higher than that without the inoculant. Furthermore, the sugar content in roots was 20-33% higher under *B. subtilis* inoculated than non-inoculated plants in Lady Claire under both P levels and in Milva under P0.5 (Figure 3.6).

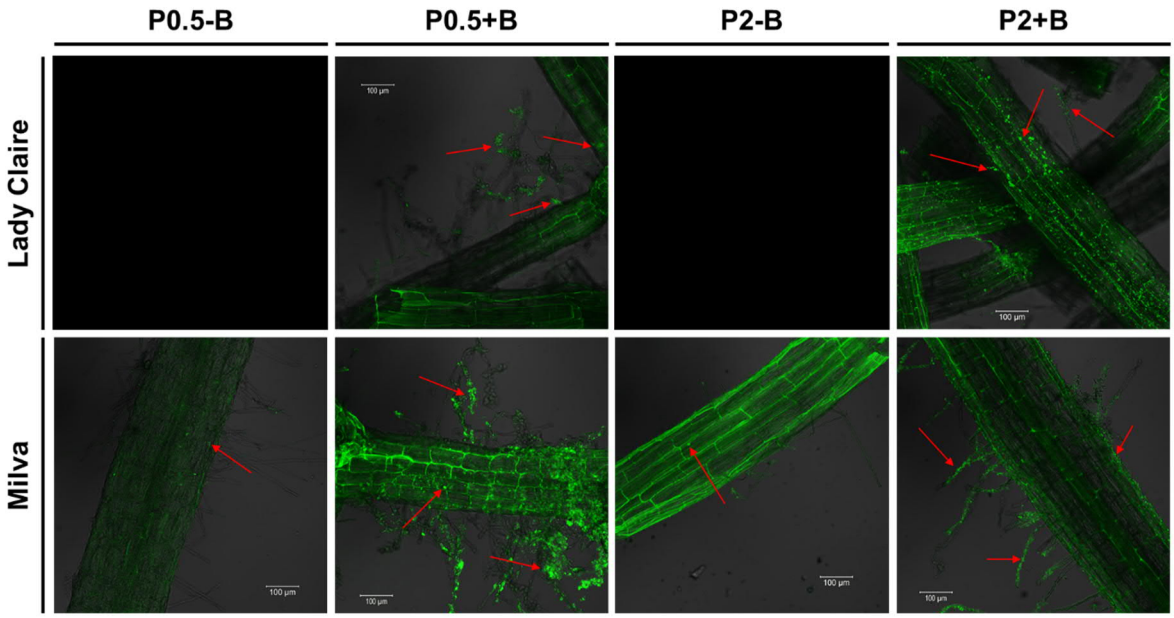


Figure 3.5. Microscopic images of roots with and without *Bacillus subtilis* inoculation by using Cellbrite Fix Membrane Stains. The images of Lady Claire under P0.5 and P2 are not available. Arrows indicate the bacterial colonies attached to the roots. B = *Bacillus subtilis.*

Table 3.3. Effect of *Bacillus subtilis* on P content in shoots and roots and P transport genes in leaves

Cultivar	**P level (mg L^{-1})**	***B. subtilis* inoculation**	**P content (mg $plant^{-1}$)**		**P transport genes in leaves (relative expression)**		
			Shoots	**Roots**	***StPHT1;1***	***StPHT1;7***	***StPHT2;1***
Lady Claire	0.5	-B	12.77±1.24	7.28±0.57	1.69±0.73	1.08±0.28	1.09±0.22
		+B	15.86±1.92	8.24±0.76	3.68±1.29	1.52±0.38	1.96±0.38
	2	-B	32.80±3.04	9.55±0.65	3.12±1.38	1.74±0.30	1.88±0.60
		+B	36.93±1.98	9.87±0.45	2.55±1.17	1.27±0.27	1.39±0.44
Milva	0.5	-B	14.16±0.65	7.90±0.28	1.40±0.47[b]	0.94±0.17	0.85±0.16
		+B	15.08±0.96	8.25±0.43	4.20±1.08[a]	1.56±0.17	1.83±0.41
	2	-B	51.81±3.57	9.56±0.50	1.56±0.92	0.94±0.31	1.25±0.34
		+B	51.81±3.15	9.75±0.44	0.57±0.24	0.86±0.17	0.60±0.21

Transcription levels are presented as relative values to a mix of cDNA from all treatments. Means ± SE (n=5) followed by different lowercase letters in the same column are significantly different between with and without *Bacillus subtilis* inoculation at the same P level and cultivar. B = *Bacillus subtilis*, no indication = not significantly different.

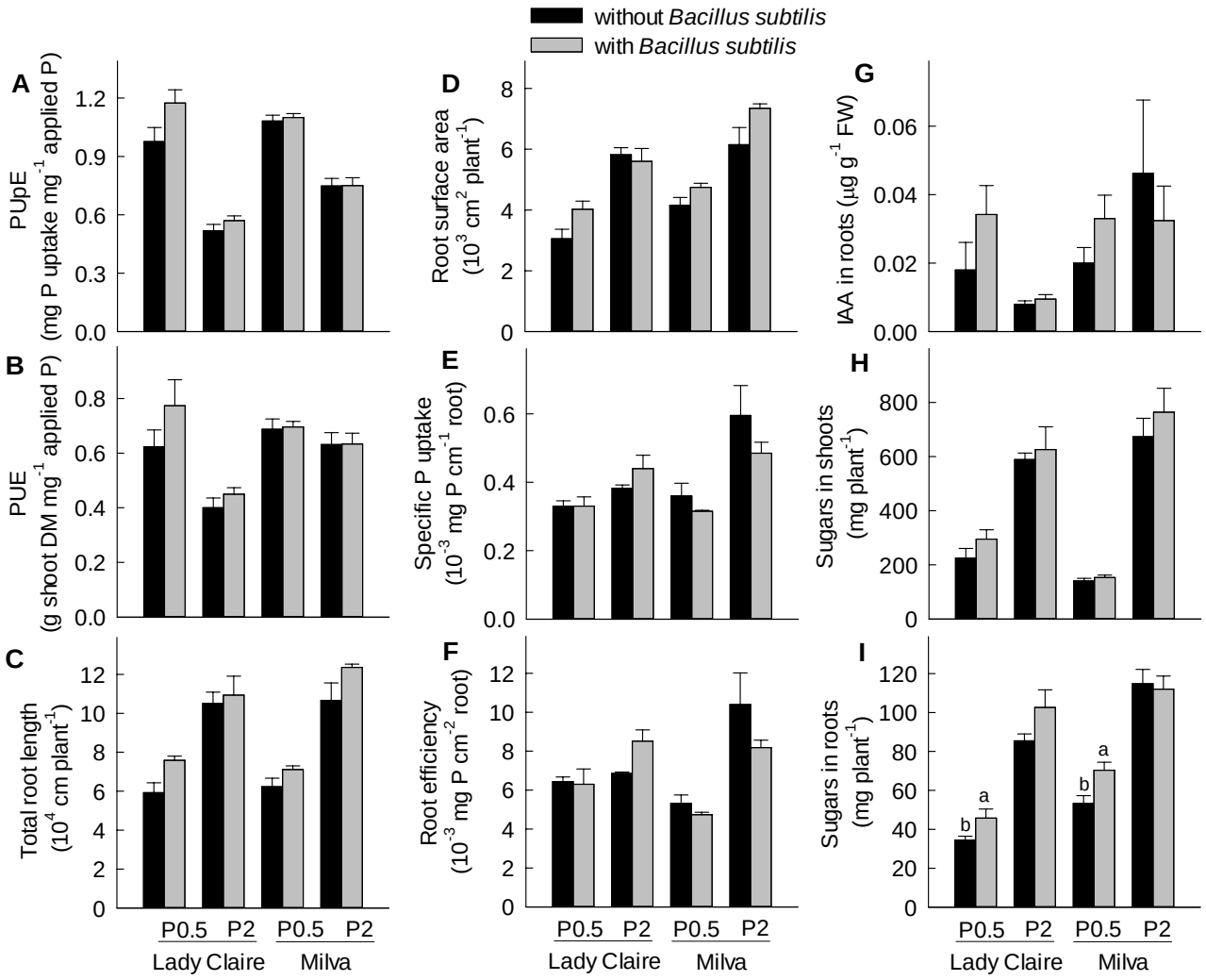


Figure 3.6. Effect of *Bacillus subtilis* inoculation on (**A**) P uptake efficiency (PUpE), (**B**) P use efficiency (PUE), (**C-F**) root characteristics, (**G**) indole-3-acetic acid (IAA) in roots, and (**H-I**) sugar content in shoots and roots. Mean ± SE (n=5) with different lowercase letters are significantly different at the same P level and cultivar. No indication = not significantly different.

3.4. Discussion

Our recent study attempted to characterize plant morphology, mineral nutrients, and metabolites of potato under low and high P availability. Furthermore, co-inoculation of PGPR ameliorated P deficiency through root growth enhancement (Chea et al., 2021b). We were also able to identify potato cultivars Lady Claire and Milva as P-inefficient and P-efficient cultivars, respectively, under P deficiency (Chea et al., 2021a). In addition to confirming these findings, the present study provided further information on root morphology, physiology, and molecular responses, which regulate P efficiency of Lady Claire and Milva in response to various P levels and *B. subtilis* inoculation.

3.4.1. Responses of plant DM, P uptake, root morphology, and sugar allocation to varying P availability

The P concentration in young leaves of both cultivars under P0.5 and P2 corresponded to a deficient and suboptimal P supply, respectively, according to Huett et al. (1997). The plants under these P levels displayed reduced shoot growth and an increased root-to-shoot ratio compared with sufficient (P5) and high (P30) P supply, which confirmed our recent report (Chea et al., 2021b). The differences in shoot and root DM between potato cultivars and P treatments were previously reported (Fernandes et al., 2014). In the present study, the differences between cultivars in root DM were greater than in shoot DM across P treatments (Figure 3.1). Therefore, root DM could serve as a measure of the efficiency of potato cultivars under varying P availability. Under low and high P supply, Milva produced greater root DM than Lady Claire, which resulted in an enhanced root-to-shoot ratio. Owing to the limited and shallow root system of potato, improved root growth is important in plants to increase P uptake under limited P supply (Fernandes et al., 2014). Under high P availability, enhanced root growth also serves as a storage depot for excess P, preventing toxic effects (Shane et al., 2004).

In addition to the modification of dry matter partitioning in response to varying P supply, translocation of absorbed P to various parts of plant may also account for difference in P efficiency between cultivars (Lambers et al., 2010; Irfan et al., 2020). A higher root P concentration in both cultivars under P0.5 compared to P2 indicates improved P allocation to roots under low P availability. Although internal plant P is very limited under P deficiency, additional P allocation to roots could stimulate root growth and further P uptake (Wissuwa et al., 2005). Both cultivars had similar P concentration in roots under P0.5, whereas the P concentration in young leaves of Milva (both 25 DAT and 42 DAT) was higher than in Lady Claire. This implies that, in spite of internal P allocation to roots, Milva also translocated the sparingly available P to young leaves, thereby enhancing shoot growth. Furthermore, in old leaves Milva had similar P concentrations between the P0.5 and P5. P0.5 condition, which probably did not induce such a severe P deficiency in Milva that it required P mobilization from old leaves. However, the P concentration in roots of Lady Claire was almost two-fold higher than in Milva under P30. Therefore, the roots of Lady Claire were able to store more P without affecting plant growth under high P supply. Consequently, the P content in the shoots and roots of Lady Claire was greater than that in Milva. This indicates the efficiency of Lady Claire in P uptake, which may lead to a reduction in P loss to the environment under high P supply.

In the rhizosphere, P uptake is largely controlled by proton-coupled P symporters of the PHT1 family (Młodzińska and Zboińska, 2016). In the present study, *StPHT1;1* and *StPHT1;7* were found to be expressed in roots of potato in response to various P levels, although there were no significant differences. *StPHT1;1* and *StPHT1;7* transcripts were also found in leaves, suggesting that these genes are involved not only in root P uptake but also in P translocation to shoots. Similar to the report by Liu et al. (2017), *StPHT2;1* in leaves was upregulated under low P supply, especially under P2, which was about 30% higher than under sufficient (P5) conditions, though it was not significant. In Arabidopsis, *AtPHT2;1* plays an important role in mediating P_i translocation from cytosol to chloroplast (Guo et al., 2013). The less significant effects of P availability on

transcription levels of P transporter genes reported in the present study may be caused by sampling time. P in the nutrient solution was rapidly depleted, especially at low P levels, within 24 hours after nutrient renewal (Supplementary Figure 3.4); however, leaves and root samples were collected 42 days after the onset of P treatments and 7 days after a nutrient change. In barley, Ren et al. (2018) also reported a decreased expression level of genes associated with P uptake and translocation under P deficiency after resupplying the nutrients. The relatively reduced expression level of *StPHT1;7* in roots of Milva under P30 supported the P concentration results, implying minimized P uptake activity under high P supply to avoid toxic P conditions in roots. Nevertheless, the translocation of P to young leaves still occurred, indicated by relatively constant expression levels of *StPHT1;1* (Figure 3.2B). Modulation of *AtPHT1;7* in P uptake was also reported in Arabidopsis, which is homologous to *StPHT1;7* (Cao et al., 2020). Similarly, Ayadi et al. (2015) showed the important role of *AtPHT1;1* in phosphate translocation from roots to leaves under high P availability in Arabidopsis. The expression levels of *StPHT2;1* and *StPHT1;1* in leaves of Lady Claire were generally higher than in Milva (Figure 3.2A,B). Therefore, stimulation of *StPHT2;1* and *StPHT1;1* suggests the importance of these genes in P translocation to the young leaves of Lady Claire.

Under low P availability, greater root DM and P uptake of Milva resulted in enhanced PUpE and PUE compared with Lady Claire. Since potato tubers were not available at early harvest (42 DAT) under hydroponic conditions, the determination of PUE and PUtE was based on the amount of shoot DM produced per unit of supplied P and absorbed P, respectively. PUpE values under P0.5 (>1 mg P uptake per mg of applied P) have to be treated with caution because of the carry-over effect of applied P in seedling germination. All seedlings received 8 mg P kg^{-1} soil when they were raised in quartz sand prior to the transfer into the nutrient solution. Therefore, the seedlings already accumulated a certain amount of P before the onset of P treatments. PUpE is important in supplying P to maintain plant growth under P deficiency. The high PUtE of both cultivars under P2 and P5 indicate the relative importance in the improvement of PUE under sub-optimal and optimal P conditions, as similarly observed by Wacker-Fester et al. (2019) and Sandaña (2016). Dissanayaka et al. (2021) also showed the impaired PUtE under P deficiency, which may be caused by inefficient energy metabolism for P translocation to shoots, causing low shoot DM. Under high P supply, plants tend to take up more P than they actually need—especially in nutrient solution—until the root P uptake is reduced. The excess P is sequestered in vacuoles to prevent P toxicity in the cytoplasm, and it also serves as P storage pool in plants (Shane et al., 2004; Liu et al., 2015). Consequently, utilization efficiency of absorbed P in shoot biomass production is reduced. Nevertheless, the PUtE of Milva was significantly higher than of Lady Claire under less severe P deficiency (P2) in the present study. This finding indicates the efficiency of Milva in P uptake and P utilization under low P supply. Lady Claire had a relatively high root P uptake capacity and shoot DM under high P supply, but its PUtE was comparable to Milva because the large amount of absorbed P was stored in the roots.

Improved plant PUpE is influenced by the modification of root morphology (Fernandes et al., 2014; Heuer et al., 2017). Under low P supply, total root length, root surface area, specific P uptake, and root efficiency of

Milva were greater than of Lady Claire, which, subsequently, resulted in enhanced P uptake (Figure 3.3D-I, Supplementary Figure 3.1). Mori et al. (2016) also confirmed that large root surface area is important to enhance P uptake under limited P availability. In Lady Claire, improved total root length and root surface area may enhance P uptake only under low P availability (Supplementary Figure 3.1). However, under high P supply, Lady Claire tended to adapt another strategy by conserving root elongation but stimulating specific P uptake and root efficiency, as similarly observed by Mori et al. (2016) under sub-optimal P conditions. These results further emphasize the efficiency of Milva and Lady Claire in P uptake under low and high P supply, respectively. Although root length density is also an important trait in P uptake (Campos et al., 2018), this parameter was less affected by P treatments and it was negatively associated with plant P uptake in the present study (Table 3.1 and Figure 3.3G). Wacker-Fester et al. (2019) also reported similar findings in their study on the increase in root length density, which is likely a symptom of plants under P deficiency.

Modification of root morphology, especially under P deficiency, may be mediated by phytohormones, which was reported in barley (Nadira et al., 2016) and tomato (de Groot et al., 2003). In the present study, although a set of different phytohormones (IAA, trans-zeatin, ABA, and JA) were quantified in roots; three of them were present in very low concentrations in many treatments, i.e. below the LOD or LOQ. Nevertheless, the ranges of IAA in all P treatments of both cultivars were similar to those observed in the roots of barley (Nadira et al., 2016). Hammond et al. (2004) also reported evidence that IAA is involved in lateral root development, root hair elongation, and root density modulation under optimal P conditions. Therefore, the relatively higher IAA concentration in roots of Milva than Lady Claire may also explain the role of IAA in stimulating the root morphology of Milva under sufficient P supply (P5) as well as sub-optional P conditions (P2). Moreover, Rietz et al. (2010) proposed the role of IAA to regulate root responses in *Arabidopsis* through its interaction with patatin-related phospholipase. Even though P treatment had no significant effect on root IAA concentration in both cultivars, the variability of root IAA among P treatments tended to be higher than in Milva. This result indicates IAA responsiveness in roots of Milva under varying P levels. In *Arabidopsis*, López-Bucio et al. (2002) reported the importance of plant response in root IAA in modifying root architecture under different P availability. In potato, our result provides firsthand evidence of the presence of IAA in roots, which requires further investigations to understand the mechanisms underlying the role of IAA in root morphological alterations under varying P supply.

A major role of roots is to take up and distribute P to other parts of the plant; however, roots also require substantial P and photoassimilates—in addition to the abovementioned phytohormone alterations—to modify their morphology, especially in response to P starvation (Wissuwa et al., 2005). P limitation can reduce P availability in the chloroplast, which further leads to a reduction in carbon assimilation (Lemoine et al., 2013). Nevertheless, plants need to efficiently translocate available sugars to roots to modify the root system while coping with P limitation (Hammond and White, 2008). Similarly, Lemoine et al. (2013) and Hermans et al. (2006) reported increased sucrose translocation into the phloem under P deficiency, which is ready for the long-distance transport to roots. In the present study, the sugar concentration in young leaves of Milva under

low P supply was greater than that in Lady Claire at early growth (25 DAT), but it was reduced at the later growth stage (42 DAT). Meanwhile, there was a relative accumulation of sugar in stem and roots of Milva under this condition (Table 3.2). These results suggest an improved phloem loading of Milva, which has potential for sugar translocation from photosynthetically active tissue to roots. However, the sugar translocation to roots might be reduced in Lady Claire under P deficiency, resulted in a relatively high sugar concentration in young leaves as well as sugar content in shoots. Nevertheless, a high root sugar concentration was observed under high P supply in Lady Claire, which may link to enhanced specific P uptake and root efficiency. Furthermore, the positive overall correlations between root sugar concentration and root traits (Supplementary Figure 3.2) suggest the importance of sugar allocation—in addition to P—to roots in enhancing root morphology and P uptake capacity in response to both low and high P availability in potato. Therefore, although sugar translocation from leaves to roots is not limited under P deficiency as reported by Wissuwa et al. (2005), additional sugar partitioning to roots by P-efficient cultivars modulates root growth, and ultimately, stimulates total P uptake.

3.4.2. Cultivar-dependent effect of plant growth-promoting *B. subtilis* on root growth improvement under P deficiency

B. subtilis inoculation improved plant P content, PUE, and root morphology under P0.5 at greater magnitudes than under P2 of both cultivars even though the respective increments were not significantly different (Table 3.3 and Figure 3.6). Although the viability of *B. subtilis* in the nutrient solution was not determined in this study, we were able to detect the occurrence of this strain on the surface and in root intercellular spaces (Figure 3.5 and Supplementary Figure 3.3). This observation was in agreement with Beauregard et al. (2013) who also found attachment of *B. subtilis* to the roots of Arabidopsis after 24 h of inoculation. Furthermore, our recent report showed the present of this strain at the genus level in association with potato roots. We were also able to detect bacterial colony growth after plating the nutrient solution on a strain-specific agar and the appearance of the colonies matched that of the original strain (Chea et al., 2021b). These observations imply the survival of the inoculant in nutrient solution and their attachment to the roots of potato. Similarly, Eckshtain-Levi et al. (2020) also reported the colonization of *B. subtilis* on Arabidopsis roots under hydroponic conditions.

The increase in P content in shoots and roots under *B. subtilis* treatment was more noticeable in Lady Claire than in Milva. This indicates a greater benefit of Lady Claire in the symbiosis with plant growth-promoting *B. subtilis* under very low P levels with regard to P uptake. Furthermore, P translocation genes (*StPHT1;1*, *StPHT1;7*, and *StPHT2;1*) in leaves were upregulated in *B. subtilis*-treated plants compared with non-inoculated treatments. Therefore, *B. subtilis* provided benefits to both total P uptake as well as P translocation to young leaves. The increase in P uptake modulated by *B. subtilis* resulted in enhanced PUpE and PUE, especially under P0.5, which may be caused by improved root morphology, including total root length and root surface area (Figure 3.6A-D). The increased total root length and root surface area under *B. subtilis* inoculation in the present study were up to 50% of the increase observed in our recent report by using

five diverse PGPR co-inoculations (Chea et al., 2021b). This evidence suggests the efficiency of *B. subtilis* inoculation as a single strain in promoting root growth. The greater responsiveness in total P uptake as well as total root length and root surface area of Lady Claire compared to Milva could be explained by the inefficiency of Lady Claire under low P supply; therefore, the benefit from the symbiosis was higher in Lady Claire than in Milva to ameliorate P-deficient conditions. In contrast, Milva was more tolerant under P deficiency; therefore, the growth of this cultivar was less reliant on the benefits that *B. subtilis* could provide to improve root growth and P uptake. Nevertheless, IAA and sugar content in the roots of both cultivars were improved at similar levels under *B. subtilis* inoculation compared with non-inoculated plants (Figure 3.6G,I). In our earlier report, *B. subtilis* was able to produce a substantial amount of IAA in the nutrient broth (Chea et al., 2021b). In addition to this finding, the present study further shows that *B. subtilis* also stimulated IAA production in roots, which resulted in enhanced root morphology, PUpE, and PUE. The increased IAA in roots could be caused by the interference of *B. subtilis* in hormonal pathways through the stimulation of genes associated with auxin production and the direct IAA excretion by the bacteria after being attached to—or even penetrating into—the roots. Furthermore, *B. subtilis* may also produce IAA in nutrient solution, which could benefit plant root uptake (Strader and Bartel, 2011). Similarly, Pathak et al. (2019) also reported the production of IAA by *B. subtilis*, which further enhanced vertical root length of potato cultivars by 10-14%. IAA can modulate primary root growth and root hair formation, resulting in increased total root length and root surface area (Vacheron et al., 2013). In maize, a marginal addition of IAA in nutrient solution (100 µmol IAA L^{-1}) enhanced root surface area by 13% under P deficiency (Wittenmayer and Merbach, 2005). Consequently, root morphology was enhanced to exploit limited P availability under P deficiency. Furthermore, the present study shows the benefit of *B. subtilis* to increase P uptake by roots, such as specific P uptake and root efficiency, of Lady Claire under P2, although the root length and root surface area were not improved (Figure 3.6E,F). This suggests the potential modulation of *B. subtilis* to improve root morphology and PUE under low P availability and root efficiency under less severe P conditions for a P-inefficient cultivar. The P-efficient cultivar Milva was tolerant under P deficiency, and plant growth was less reliant on plant growth-promoting *B. subtilis*.

3.5. Conclusion

The present study shows considerable variation between potato cultivars in response to P availability. The results provide evidence for genotypic differences among two potato cultivars in terms of P efficiency. Milva was efficient under low P supply through its increased biomass, P, and sugar allocation to roots, which resulted in enhanced PUpE as well as enhanced PUE. Furthermore, this cultivar was also IAA responsive, which may help enhance root morphology across P treatments. In contrast, Lady Claire lacked the traits associated with P and sugar allocation to roots for enhanced root growth; thus, this cultivar was less efficient under limited P availability. Nevertheless, Lady Claire was able to increase specific P uptake and root efficiency under high P availability, which may be beneficial to the environment due to reduced P loss. Furthermore, under P starvation, Lady Claire was relatively responsive to *B. subtilis*, which improved P translocation to shoots, PUpE, PUE, and root morphology at a greater magnitude than in Milva. Additionally, *B. subtilis* inoculation

was able to improve specific P uptake and root efficiency of Lady Claire under less severe P deficiency. Based on the different responses of these two cultivars, a mechanism underlying the responses of potato to varying P levels and *B. subtilis* inoculation is suggested in Figure 3.7. This information could be helpful to develop strategies identify traits for future breeding programs aiming to improve PUE in potato under specific P conditions. Additional studies are required to understand the mechanisms underlying the role of IAA in root morphology modification and molecular responses in sugar translocation into various plant parts under P availability.

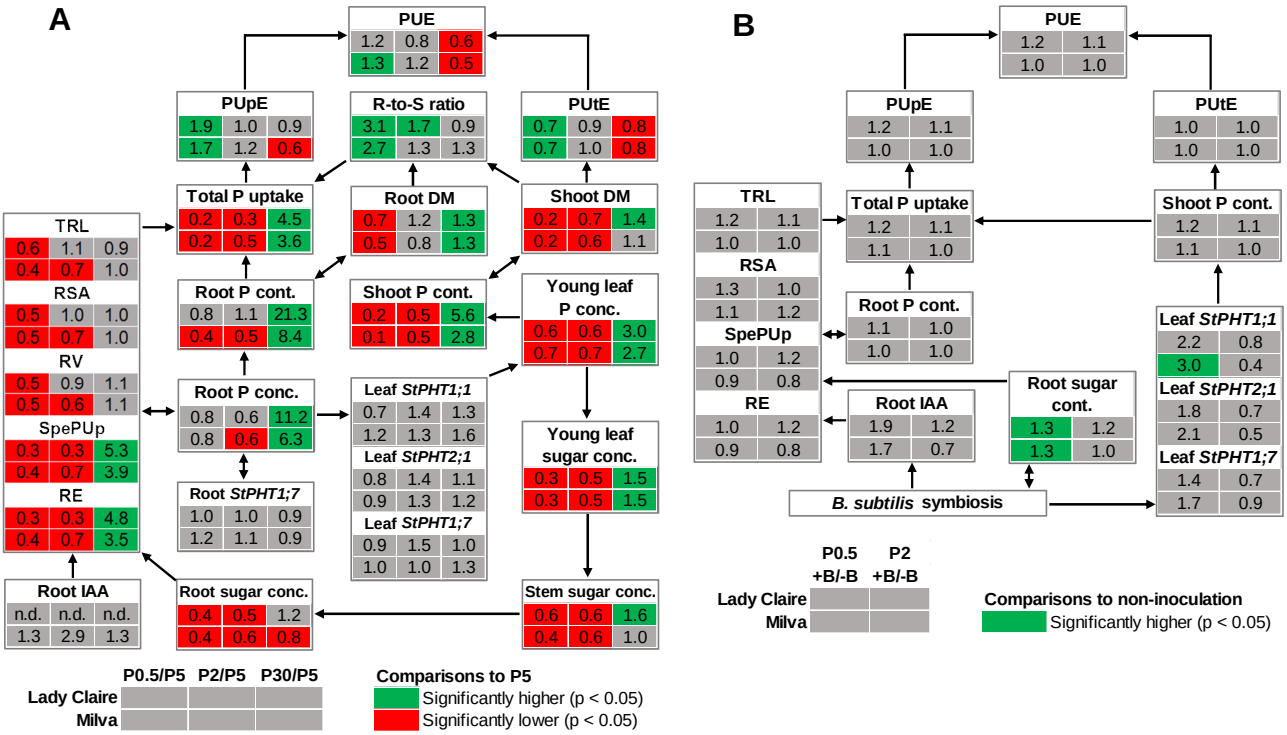
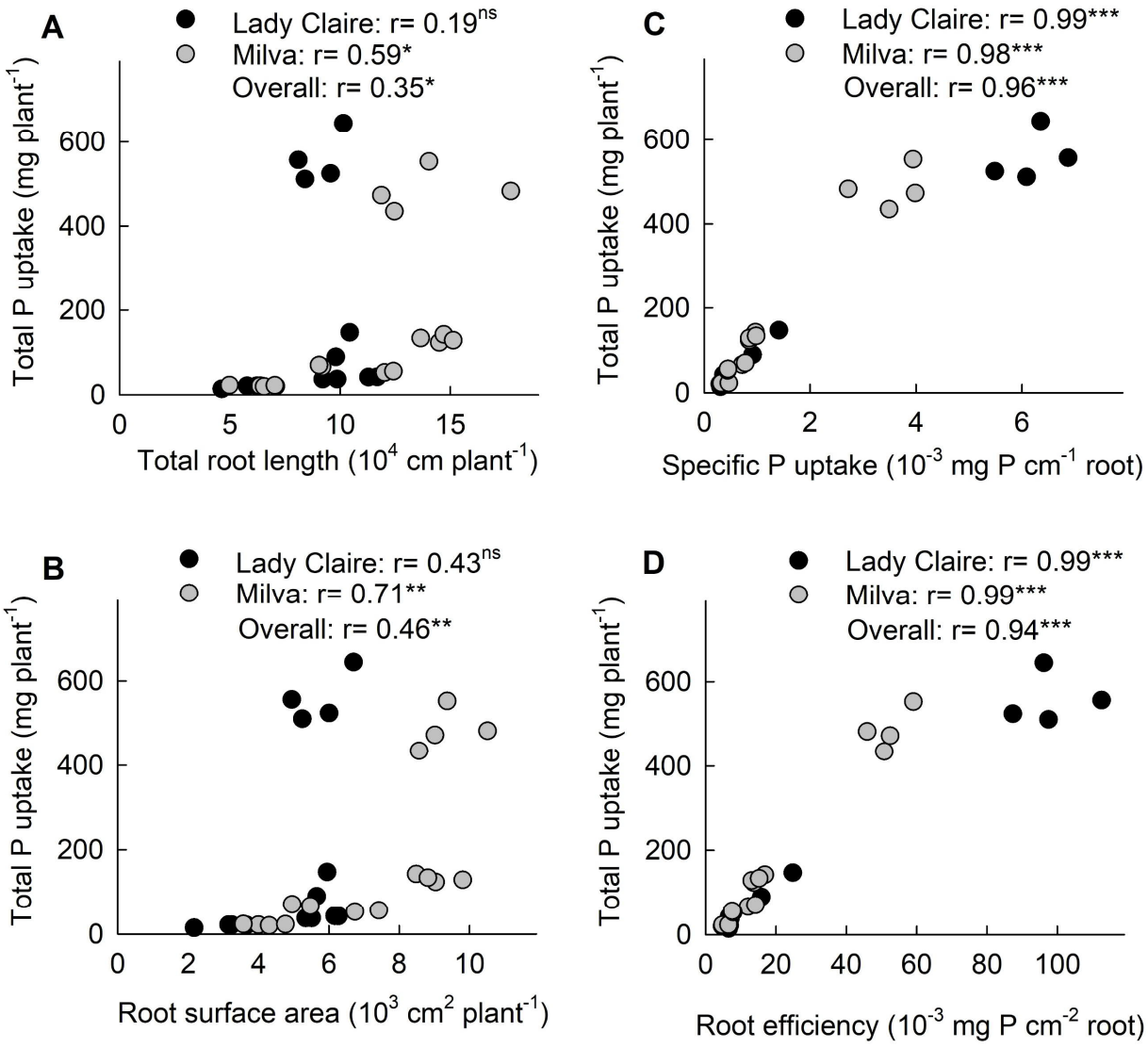


Figure 7. (**A**) Schematic representation underlying possible P efficiency mechanisms in potato as influenced by P availability and (**B**) the possible effects of *B. subtilis* on P deficiency amelioration. The relative values in (**A**) were calculated by the division of values of each trait at P0.5, P2, and P30 with those at P5 in the same cultivar while the values in (**B**) were determined by the division between *B. subtilis* inoculated and non-inoculated treatment in the same P level and cultivar. Green and red indicate the significant higher and lower ($p < 0.05$) values compared to P5, respectively. Arrows with one direction show possible determining factor of a trait and arrows with double direction show reversible causality. PUE = P use efficiency, PUpE = P uptake efficiency, PUtE = P utilization efficiency, R-to-S ratio = root-to-shoot ratio, DM = dry matter, TRL = total root length, RSA = root surface area, SpePUp = specific P uptake, RE = root efficiency, IAA = indole-3-acetic acid, conc. = concentration, cont. = content, n.d. = not determined because of no value was available under P5 for the calculation.

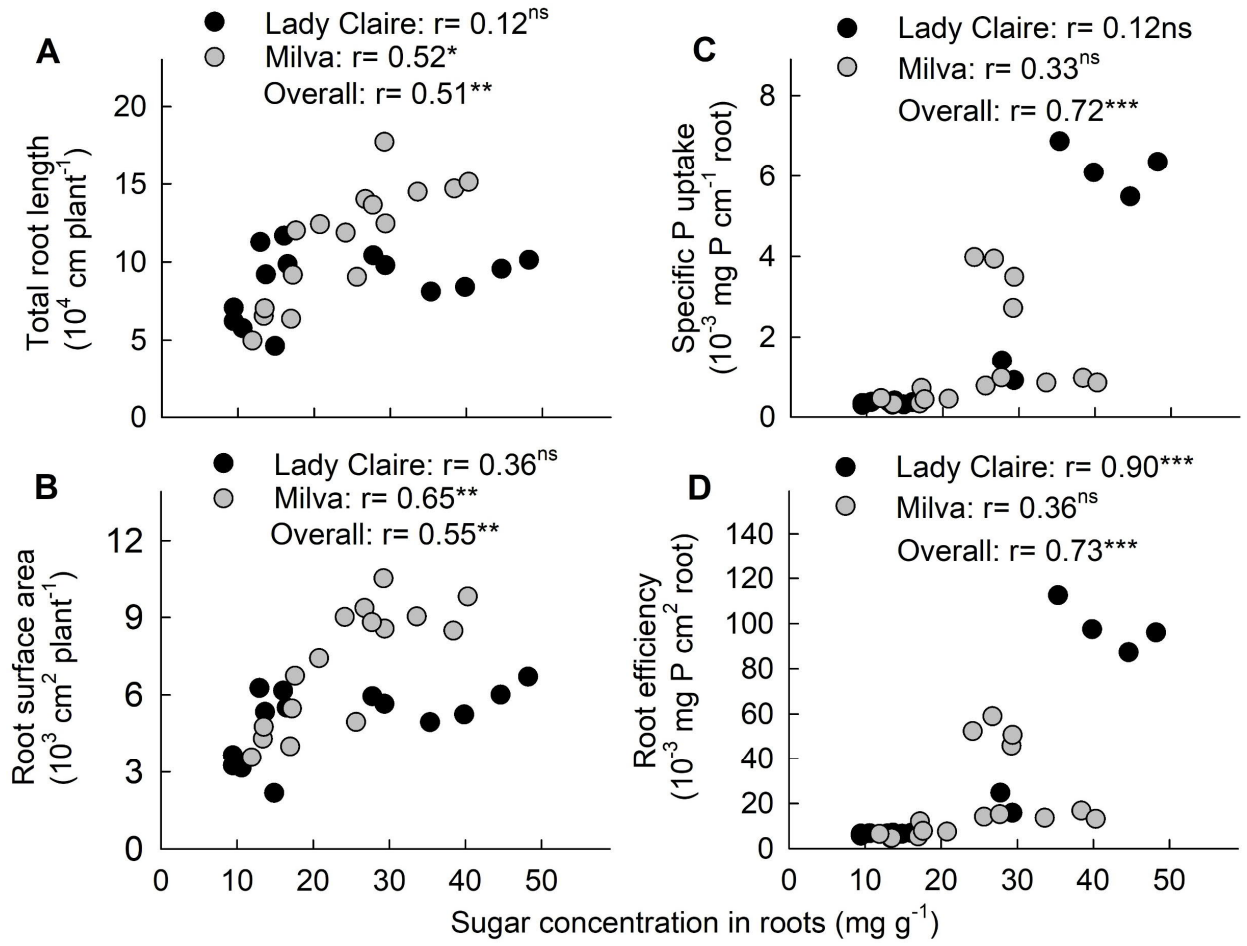
Supplementary materials

Supplementary Table 3.1. Acquisition parameters for phytohormones analysis.

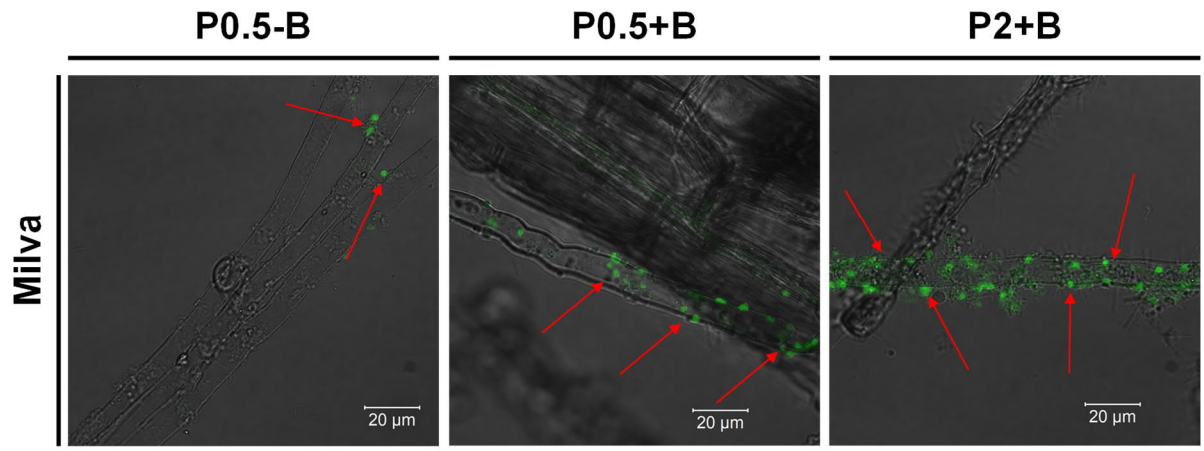
Compound	RT [min]	Polarity	Parent Ion [m/z]	Fragmentor V	Collision Energy V	Product Ion [m/z]
Trans-zeatin	1.93	+	220.1	100	15	136.1
					9	202.1
					22	148.1
Trans-zeatin-d5	1.92	+	225.1	105	16	137.1
					10	207.2
Indoleacetic acid (IAA)	3.88	+	176.1	84	14	130
					38	103.1
					40	77.2
Indoleacetic acid-d4	3.87	+	180.1	84	15	133.2
					38	106.1
					45	79.2
Abscisic acid (ABA)	4.68	-	263.1	85	4	153.1
					5	219.1
					12	204.1
Abscisic acid-d6	4.67	-	269.1	88	4	159.1
					8	225.1
Jasmonic acid (JA)	5.20	+	211.2	85	8	133.1
					8	151.1
					5	193
Jasmonic acid-d5	5.19	+	216.1	85	8	135.1
					9	153.2
					7	198.2



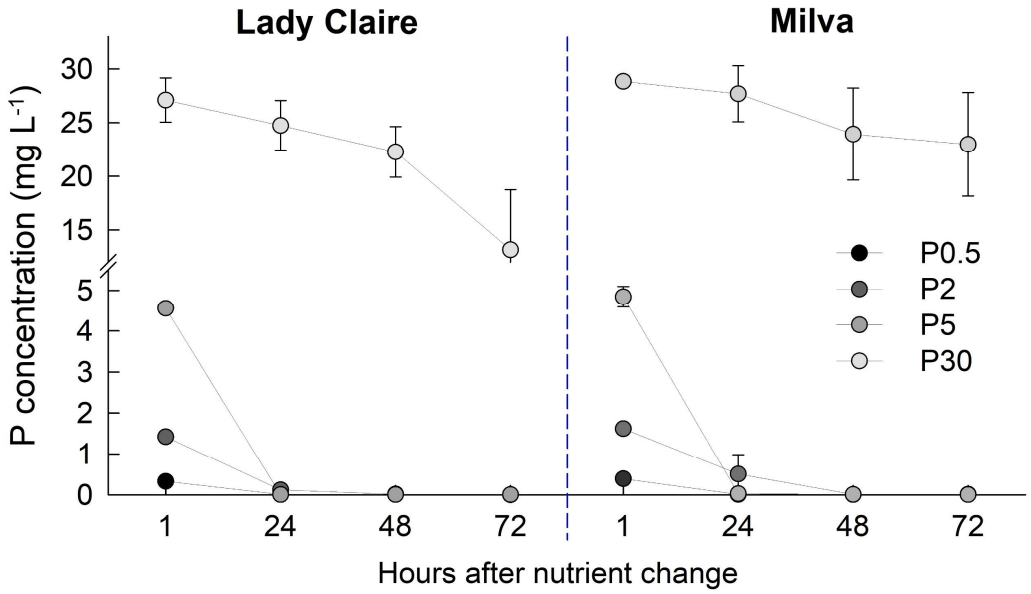
Supplementary Figure 3.1. Correlation between root morphological traits and total P uptake. ns, *, and *** indicate non-significant and significant difference at $p<0.05$ and $p<0.001$, respectively, by Pearson correlation.



Supplementary Figure 3.2. Correlation between root sugar concentration and root morphological traits. ns, *, and *** indicate non-significant and significant difference at $p<0.05$ and $p<0.001$, respectively, by Pearson correlation.



Supplementary Figure 3.3. Microscopic images of roots of cultivar Milva with and without *Bacillus subtilis* inoculation by using Cellbrite Fix Membrane Stains. Arrows indicate the bacterial colonies attached to the roots. B = *Bacillus subtilis.*



Supplementary Figure 3.4. P concentration in each pot after nutrient renewal

Chapter 4. Cultivar-dependent responses in plant growth, leaf physiology, phosphorus use efficiency, and tuber quality of potato under limited phosphorus availability conditions

Leangsrun Chea[1], Ana Meijide[2], Catharina Meinen[2], Elke Pawelzik[1], Marcel Naumann[1]

[1]Division of Quality of Plant Products, Department of Crop Sciences, University of Goettingen, Carl-Sprengel-Weg 1, 37075 Goettingen, Germany

[2]Division of Agronomy, Department of Crop Sciences, University of Goettingen, Von-Siebold-Straße 8, 37075 Goettingen, Germany

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Author contribution

Leangsrun Chea : performed the experiment, analyzed data, drafted the manuscript, and revised the manuscript based on suggestions from other co-authors

Ana Meijide : contributed in photosynthetic data analyses and revised the manuscript

Catharina Meinen : contributed in photosynthetic measurements and revised the manuscript

Elke Pawelzik : supervised and revised the manuscript

Marcel Naumann : supervised, designed the experiment, and revised the manuscript

Abstract

Limited availability of phosphorus (P) in soils causes a major constraint in the productivity of potato, which requires increased knowledge on plant adaptation responses under this condition. In the present study, six potato cultivars—Agria, Lady Claire, Milva, Lilly, Sieglinde, and Verdi—were assessed for their responses on plant growth, leaf physiology, P use efficiency (PUE), and tuber quality under three P levels (P_{low}, P_{med}, and P_{high}). The results reveal a significant variation of cultivars in response to different P availability. P-efficient cultivars—Agria, Milva, and Lilly—possessed substantial plant biomass, tuber yield, and high P uptake efficiency (PUpE) under low P supply. The P-inefficient cultivars—Lady Claire, Sieglinde, and Verdi—lacked the ability to produce tubers under P deprivation, as well as the ability for efficient P uptake under low P level, but they were efficient in P uptake under high soil P. Improved PUpE is important for plant tolerance under limited P availability, which results in efficient use of the applied P. At the leaf level, increased accumulations of nitrate, sulfate, sucrose, and proline are necessary for a plant to acclimate to P deficiency-induced stress and to mobilize leaf inorganic phosphate to increase internal PUE and photosynthesis. The reduction of plant biomass and tuber yield under P deficiency could be caused by the reduced CO_2 assimilation. Furthermore, P deficiency significantly reduced tuber yield, dry matter, and starch concentration in Agria, Milva, and Lilly. Nevertheless, contents of tuber protein, sugars, and minerals, as well as antioxidant capacity, were enhanced under this condition in these cultivars. These results highlight the important traits contributing to potato plant tolerance under P deficiency and indicate an opportunity to improve P efficiency and tuber quality of potato under the deficient conditions by using more efficient cultivars. Future research on evaluating molecular mechanisms related to P and sucrose translocation, as well as minimizing tuber yield reduction under limited P availability is necessary.

Keywords: ATP, cultivars, phosphorus deficiency, phosphorus efficiency, leaf, potato, sugars, tuber quality

4.1. Introduction

Potato demands high phosphorus (P) availability in soils because its roots are relatively inefficient in P uptake under low soil P concentration (Sandaña, 2016; Wacker-Fester et al., 2019). P fertilizers have been applied to increase soil P and tuber yield. However, up to 80% of the applied P turns into insoluble complex and organically bound forms, which are not readily available for plants (Shi et al., 2019). The unavailable P tends to remain in the upper soil layers, and has a tendency to flow towards surface water, which causes eutrophication (King et al., 2015). Considering the environmental concerns along with a decrease in globally reserved P resources (Cordell and White, 2014), improved agronomic practices which reduce P fertilizer input are necessary to enhance P efficiency for potato production.

For potato plants, phosphorus use efficiency (PUE) can be defined as the capability of plants to produce biomass or tuber yield per unit of applied P (Veneklaas et al., 2012). Under low P availability, PUE is strongly influenced by P uptake efficiency (PUpE)—the amount of total plant P uptake per unit of applied P (Sandaña, 2016). Plants respond to limited P availability through morphological and physiological adjustments to acclimate to deficient-P conditions. Our previous work reported an increased root biomass compared to the shoots of potato (cv. Milva) under P deficiency, which indicates a preferential photoassimilate allocation to roots to enhance P uptake (Chea et al., 2021b). However, sugar concentrations in roots were reduced by 50–80% under this condition. Based on these results, additional investigations on leaf biochemical alterations under P deficiency are required to explain the shortage of photoassimilates for translocation. Owing the indispensable role of P in energy transfer, a marginal P deficiency could reduce the adenosine triphosphate (ATP) synthesis for consumption in the Calvin cycle causing a reduction in total photosynthetic rates (Carstensen et al., 2018; Dixon et al., 2020). Electron transport and ATP synthesis mediation in the Calvin cycle is important to increase photosynthesis (Simkin et al., 2019). Furthermore, a large amount of inorganic P (P_i) in leaf tissues has to be generated through internal P recycling to maintain its concentration in stroma for carbon fixation (Wissuwa et al., 2005). Therefore, efficient use of plant internal P for photosynthesis is essential to ensure sufficient photoassimilates for shoot growth and translocation. However, Wissuwa et al. (2005) and Fredeen et al. (1989) showed an accumulation of soluble sugars under P deficiency, which suggested that the utilization of photoassimilates for plant growth was restricted. In maize, Plénet et al. (2000) reported a direct effect of P deficiency on growth rather than on leaf photosynthesis. Little is known about potato with respect to photosynthesis and leaf biochemical alteration induced by P deficiency. Additionally, the stress caused by P deficiency provokes the accumulation of osmolytes, such as proline, for stress detoxification (Hayat et al., 2012) and also greater uptake of essential minerals, such as nitrogen (N) and sulfur (S) (Chea et al., 2021b). These adaptation mechanisms are necessary for plant viability under P deficiency. Therefore, potato cultivars with enhanced PUE and photosynthetic capacity, under limited P availability, could be an alternative strategy to overcome situations of P deficiency.

The P availability in soils largely influences potato tuber yield and quality (Naumann et al., 2020). However, besides the above mentioned morphological and biochemical responses, the impact of limited P

availability on potato tuber quality is less documented, except for a few reports on tuber yield, dry matter, starch, protein, and sugars (Öztürk et al., 2010; Fernandes et al., 2015; Leonel et al., 2017). Wang and Frei (2011) showed an increased concentration of micronutrients, protein, and antioxidant capacity in potato tubers and grains of various crops as a result of nutrient uptake alteration and the modulation of key enzymes under abiotic stress. Moreover, our recent discovery showed an increase in leaf minerals and antioxidant compounds, such as total flavonoids and total phenolics, in response to P deficiency (Chea et al., 2021b); thus, we can hypothesize that P deficiency may also stimulate the uptake of minerals and the antioxidant capacity in tubers.

In the present study, we sought to assess (I) the impact of P deficiency on plant growth, PUE, photosynthetic characteristics, and leaf biochemical properties and (II) the effects of P deficiency on quality of potato tubers. The goals of our study are to identify P-efficient cultivars and to provide further insights into tolerant mechanisms of potato under P deficiency. We also provide the first report on the implications of P deficiency on ascorbic acid and antioxidant capacity of potato tubers.

4.2. Materials and Methods

4.2.1. Plant materials

Six potato cultivars were used, which consisted of four table potatoes—Agria, Milva, Lilly, and Sieglinde—and two starch potatoes—Lady Claire and Verdi. Lady Claire was obtained from Meijer Potato (Rilland, the Netherlands) while Agria, Lilly, and Sieglinde were obtained from Kartoffel Mueller (Nersingen, Germany). Milva and Verdi were obtained from Europlant Pflanzenzucht (Lüneburg, Germany). These cultivars were selected based on their popularity for production in the respected regions, and their differences in morphological and yield characteristics. Additionally, Sieglinde is known for its limited biomass and tuber yield production and it is a sensitive cultivar to nutrient deficiency (Mauromicale et al., 2006). The description of each cultivar is shown in Supplementary Table 4.1.

4.2.2. Experimental setup and crop management

The experiment was performed under outdoor conditions by using six cultivars and three P levels (0.02, 0.2, and 1.2 g kg^{-1} soil; designated as P_{low}, P_{med}, and P_{high} hereafter) with four replications. The average temperature during this period (June–September 2019) was 19.0±6.5°C. Diurnal photosynthetic active radiation, temperature, relative humidity, and daily precipitation are shown in Supplementary Figure 4.1. The sandy soil used in the experiment had a pH of 4.8 and extractable calcium acetate lactate P (CAL-P) of 0.06 g kg^{-1} soil. To the lower P concentration, the soil was mixed with equal amounts of medium-sized quartz sand, so that all treatments had the same initial soil P. Afterward, the different P treatments were induced by applying $Ca(H_2PO_4)_2$ basally as powder. The application of other nutrients is shown in Supplementary Table 4.2. Soil Ca concentration was balanced by addition of $CaCO_3$. After all the nutrients were applied, soil pH was in the range of 5.5 to 7.0. Then, the soil mixture, with a bulk density of 1.1 kg dm^{-3}, was filled in 6 L pots (Mitscherlich, STOMA, Germany). Afterwards, a single germ bud of 1-2 cm was taken from each potato seed by using a ball shaper and was planted in each pot at a depth of 5 cm. These procedures were adapted from

Koch et al. (2019a) and Wacker-Fester et al. (2019). All seedlings were germinated within ten days after the plantation. Water was supplied regularly to maintain optimum soil moisture based on visual observation.

4.2.3. Plant growth assessment, leaf gas exchange measurements, and sample preparation

The plant height development was monitored during the experiment. Leaf gas exchange measurements were conducted on the terminal leaf of a fully developed leaflet at the fourth position from the top by using a portable photosynthesis system (LI-6800, LI-COR Biosciences, Lincoln, United States) after 35, 53, and 70 days of the emergence (DAE). Prior to the measurements, each plant was adapted for one hour in constant environmental conditions (temperature = 20˚C, relative humidity = 60%, and photosynthetic photon flux density [PPFD] = 400 µmol m^{-2} s^{-1} at plant level) in a climate chamber. For the measurement, the CO_2 concentration, relative humidity, and leaf temperature inside the cuvette (4 cm^2) were adjusted to 400 ppm, 50%, and 25˚C, respectively. Light response curves were generated by starting with a PPFD of 1400 µmol m^{-2} s^{-1} in 13 steps (180 seconds per step) to zero. Based on the light response curve measurements, the CO_2 assimilation rate, stomatal conductance, and intercellular CO_2 concentration measured at a PPFD of 400 µmol m^{-2} s^{-1} were compared for different cultivars and P treatments at different stages of development.

The whole leaflet at the fourth position from the top was taken 53 DAE after leaf gas exchange measurements for mineral and biochemical analyses. A part of the sample was freshly ground with liquid nitrogen and was stored at -20˚C. The plants were mature and the tubers were fully developed at 87 DAE; therefore, the whole plants were harvested, and were partitioned into shoots, roots, and tubers. A part of fresh tuber was used for ascorbic acid determination, immediately after cutting, as described below. The sub-samples of each plant part were freeze-dried in a freeze dryer (EPSILON 2-40, Christ, Osterode am Harz, Germany) for four days. The biomass of the plant was then calculated as a combination of total shoots (including sampled young leaves at 53 DAE) and root dry matter (DM). Dry samples were grinded in a hammer mill (DFH 48 Culatti, Kinematica, Malters, Switzerland) with a 0.5 mm sieve.

4.2.4. Analyses of minerals and ions in plant tissues and residual P in soils

Minerals in leaves, shoots, roots, and tubers were analyzed by extracting 100 mg of freeze-dried sample—according to Koch et al. (2019)—to determine the concentration of P, S, potassium (K), calcium (Ca), magnesium (Mg), manganese (Mn), iron (Fe), and zinc (Zn). These mineral concentrations were determined by an inductively coupled plasma optical emission spectrometry (ICP-OES) (Varian, Palo Alto, United States). Shoot, root, and tuber P contents were calculated by multiplying P concentration of the respective plant part with its dry matter content. Total P uptake of each plant was calculated as the combination of shoot, root, and tuber P content. Afterwards, PUpE and PUE were calculated based on Sandaña (2016) as follow:

$$\text{PUpE (mg P uptake mg}^{-1}\text{ applied P)} = \frac{\text{Total P uptake (mg plant}^{-1}\text{)}}{\text{Applied P (mg pot}^{-1}\text{)}}$$

$$\text{PUE (g tuber DM mg}^{-1}\text{ applied P)} = \frac{\text{Tuber DM (g plant}^{-1}\text{)}}{\text{Applied P (mg pot}^{-1}\text{)}}$$

The applied P of each treatment was determined by multiplication of the applied P concentration with the soil dry weight per pot. Carbon (C) and N concentration in leaves and tubers were analyzed from 0.75 g of the freeze-dried sample by using dry combustion method in a Vario EL analyzer (Elementar, Langenselbold, Germany). Phosphate (PO_4^{3-}), nitrate (NO_3^-), and sulfate (SO_4^{2-}) in leaves and tubers were determined by extracting 20 mg of freeze-dried sample with 1 mL of 0.1 M HCl and these extracts were analyzed in an Ion Chromatography system (ECO IC, Metrohm, Herisau, Switzerland) in accordance with the procedures described by Koch et al. (2020). The concentration of each ion was expressed as mg of the respective mineral per unit of sample DM. After the harvest, available P in the soil was extracted following the Olsen method by using bicarbonate solution (Hartmann et al., 2019) and was determined in accordance with molybdenum blue procedures (Murphy and Riley, 1962) in a UV-Vis spectrophotometer (HP 8453, Hewlett Packard, Böblingen, Germany) at 882 nm absorbance.

4.2.5. Analyses of chlorophyll, free proline, ATP, and protein in leaves

Leaf chlorophyll and proline were extracted by homogenizing 20 mg of freshly ground sample with 250 µL of 80% ethanol at 95°C. The mixture was then centrifuged at 10,600 *g* for 10 min to collect the supernatant. The procedures were sequentially repeated twice with 150 µL of 80% ethanol and 150 µL of 50% ethanol. The supernatants from each step were pooled and measured for leaf chlorophyll and free proline concentration according to Koch et al. (2019a) and Chea et al. (2021b), respectively.

For ATP and protein measurements, 100 mg of fresh leaf sample were extracted with 1 mL of cold 5% trichloroacetic acid for 5 min, and the mixture was centrifuged for 10 min at 13,000 *g* at 4°C to collect supernatant. The ATP concentration in the supernatant was determined by using ATPLite assay kit (PerkinElmer, Waltham, United States) in accordance with the introductions of the manufacturer. The pellet from the sample extract was resuspended with 400 µL of 0.1 M NaOH for 30 min at 95°C. After the centrifugation for 10 min at 10,000 *g* protein concentration was determined by using Bradford protein kit (Merck, Darmstadt, Germany) based on modified methods of Zor and Selinger (1996). Bovine serum albumin was used as the standard.

4.2.6. Sugar analyses in leaves and tubers

The soluble sugars in leaves were extracted by homogenizing 50 mg freeze-dried sample with 700 µL of 80% acetonitrile on a shaker at 420 rpm for 3 h. Then, 50 µL of 3.6% $K_4[Fe(CN)_6]*3H_2O$ and 50 µL of 7.2% $ZnSO_4*7H_2O$ were, subsequently, added to precipitate proteins, followed by 30 min of centrifugation at 15,000 *g* to collect the supernatant and it was stored at -20°C. For tubers, 0.75 g of each freeze-dried sample was extracted with 3 mL of distilled water by shaking for 1 h at 420 rpm. The protein precipitation was executed by sequentially adding 0.5 mL of 3.6% $K_4[Fe(CN)_6]*3H_2O$ and 0.5 mL of 7.2% $ZnSO_4*7H_2O$ in the mixture, which was then subjected to 20 min of centrifugation at 2,600 *g* to collect the supernatant. The extraction procedures were repeated twice without protein precipitation. The supernatants from each step were pooled and filled up to 10 mL with distilled water and were stored at -20°C. For measurement, the extract was thawed

and centrifuged for 30 min at 15,000 *g*. The supernatant was then filtered through a 0.45 µm membrane with the help of a 13 mm syringe (VWR, Darmstadt, Germany). Finally, 20 µL of the filtered extract was used for the quantification of soluble sugars (sucrose, glucose, and fructose) by high-performance liquid chromatography (Jasco, Pfungstadt, Germany). As eluent, 80% acetonitrile was used through a 5 µm column (LiChrospher 100 NH2, Merck, Darmstadt, Germany) at 22°C and with a flow rate of 1 mL min^{-1}.

4.2.7. Tuber quality analyses

The starch concentration of tubers was determined by polarimetric method according to the procedures of Koch et al. (2019a). Tuber N concentration was converted to crude protein concentration with a factor of 6.25 (AOAC, 2005). Ascorbic acid of potato tuber was analyzed based on a 2,6-Dichlorophenolindophenol (DIP) titrimetric method described in Sonntag et al. (2020).

To determine total phenolics (TPC), total flavonoids (TFC), and antioxidant capacity, 100 mg of freeze-dried tuber sample were extracted twice with 1 mL of 99.9% methanol. The supernatants were combined and filled up to 2 mL with methanol. TPC and TFC of the extract were analyzed according to Chea et al. (2021b). Antioxidant capacity was determined based on 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay and Trolox equivalent antioxidant capacity (TEAC) assay—based on Kaur et al. (2013)—with slight modifications. For DPPH assay, 20 µL of the extract was suspended with 180 µL of 0.2 mM DPPH. After incubating for 30 min in darkness, the mixture was read in a plate reader (Synergy HTX, Biotek, Winooski, United States) at 515 nm absorbance. TEAC assay was based on the ability of antioxidants to scavenge 2,2'-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid (ABTS) radical cation. For measurement, 10 µL of the extract was mixed with 150 µL of ABTS working solution, containing 0.15 mM of ABTS and 0.5 mM of $K_2S_2O_8$. The mixture was incubated at room temperature for 10 min in darkness, and the absorbance was read in a plate reader at 734 nm. In both the assays, 99.9% of methanol was used as a negative control and radical scavenging capacity was determined based on the difference of the negative control and sample extract absorbance. The antioxidant capacity was calculated against Trolox standard calibration curve and expressed as µmol Trolox equivalent (TE) g^{-1} DM.

4.2.8. Statistical analysis

Data of plant growth, mineral concentration, leaf biochemical properties, P efficiency, and tuber quality parameters were subjected to two-way analysis of variance (ANOVA). Tukey's HSD test at $p < 0.05$ was performed for pairwise comparisons among the P treatments when there were significant differences in ANOVA. The association among the observed traits were assessed by using a Pearson's correlation. These analyses were conducted following the methods of Gomez and Gomez (1984) by using the Statistix 8.0 software (Analytical Software, Tallahassee, United States). Graphical presentations were prepared in Sigmaplot 12.5 (Systat Software, San Jose, United States).

4.3. Results

4.3.1. Plant growth and tuber yield

The P applications had a significant effect on plant height, plant biomass, and tuber yield for all cultivars (Figure 4.1). The effects of cultivars and the interaction between cultivars and P levels were also significant (Supplementary Table 4.3). Plant height of each cultivar evolved at different rates during the growing period depending on the P applications (Figure 4.1A,B). Plant biomass of Lady Claire, Lilly, Sieglinde, and Verdi was strongly inhibited under P_{low} treatment (0.40-1.13 g plant^{-1}), and it increased by 24- to 85-fold under higher P availability. Agria and Milva had relatively greater plant biomass (6.16 and 9.70 g plant^{-1}, respectively) than other cultivars under P_{low}, and it also increased in response to higher P levels (Figure 4.1C). However, under P_{low}, Lady Claire, Sieglinde, and Verdi were not able to produce tubers, while tuber yield of Agria, Lilly, and Milva ranged from 45.90-79.42 g plant^{-1} (Figure 4.1D). The variations in mean values of plant height, plant biomass, and tuber yield among cultivars and P treatments are shown in Supplementary Table 4.4.

4.3.2. Tissue P concentration, PUE, and residual soil P

Mineral analyses of leaves at 53 DAE revealed low P concentrations under P_{low}, which ranged from 0.82 mg g^{-1} (Sieglinde) to 1.19 mg g^{-1} (Agria) (Figure 4.2A, Supplementary Table 4.4). The application of P_{med} and P_{high} increased leaf P by 50–83% and 3- to 5-fold, respectively, compared to P_{low}. We also observed a reduction of leaf phosphate under P_{low} (Figure 4.2B), but it was at a lower magnitude than that of leaf P. The P_{low} treatment reduced shoot P concentrations, but there was no significant difference between P_{low} and P_{med} in root and tuber P concentration (Figure 4.2C-E). Low P availability in soil suppressed total P uptake, but it enhanced PUpE of Agria and Milva by 44% (Figure 4.2F,G). In contrast, PUpE of Lady Claire, Sieglinde, and Verdi was hampered under low soil P. Furthermore, PUE remained highest for Agria among the cultivars under P_{low} conditions (Figure 4.2H). We were unable to determine the PUE of Lady Claire, Sieglinde, and Verdi under P_{low} due to the absence of tubers; thus, we assumed a very low PUE for these cultivars under low P availability. The analysis of soil P after harvest, of which the Olsen extraction method was used, revealed that soil P concentration under P_{high} conditions was 14-16 times and 7-9 times higher than P_{low} and P_{med} conditions, respectively (Figure 4.2I).

Furthermore, Pearson's correlations of traits associated with plant P concentration and PUE show positive correlations among leaf P, shoot P, plant biomass, total P uptake, and applied P levels. However, these parameters negatively correlated with PUE. PUE was positively associated with PUpE (Figure 4.3).

4.3.3. Leaf minerals and ions

In response to the P_{low} treatment, leaf essential minerals and ions such as N, nitrate, S, and sulfate significantly increased compared to higher P availability (Table 4.1). Furthermore, there were significant correlations between leaf N and S with their respective ionic forms (Supplementary Figure 4.2). The impact of P deficiency on other leaf macro and micronutrients are shown in Supplementary Table 4.5.

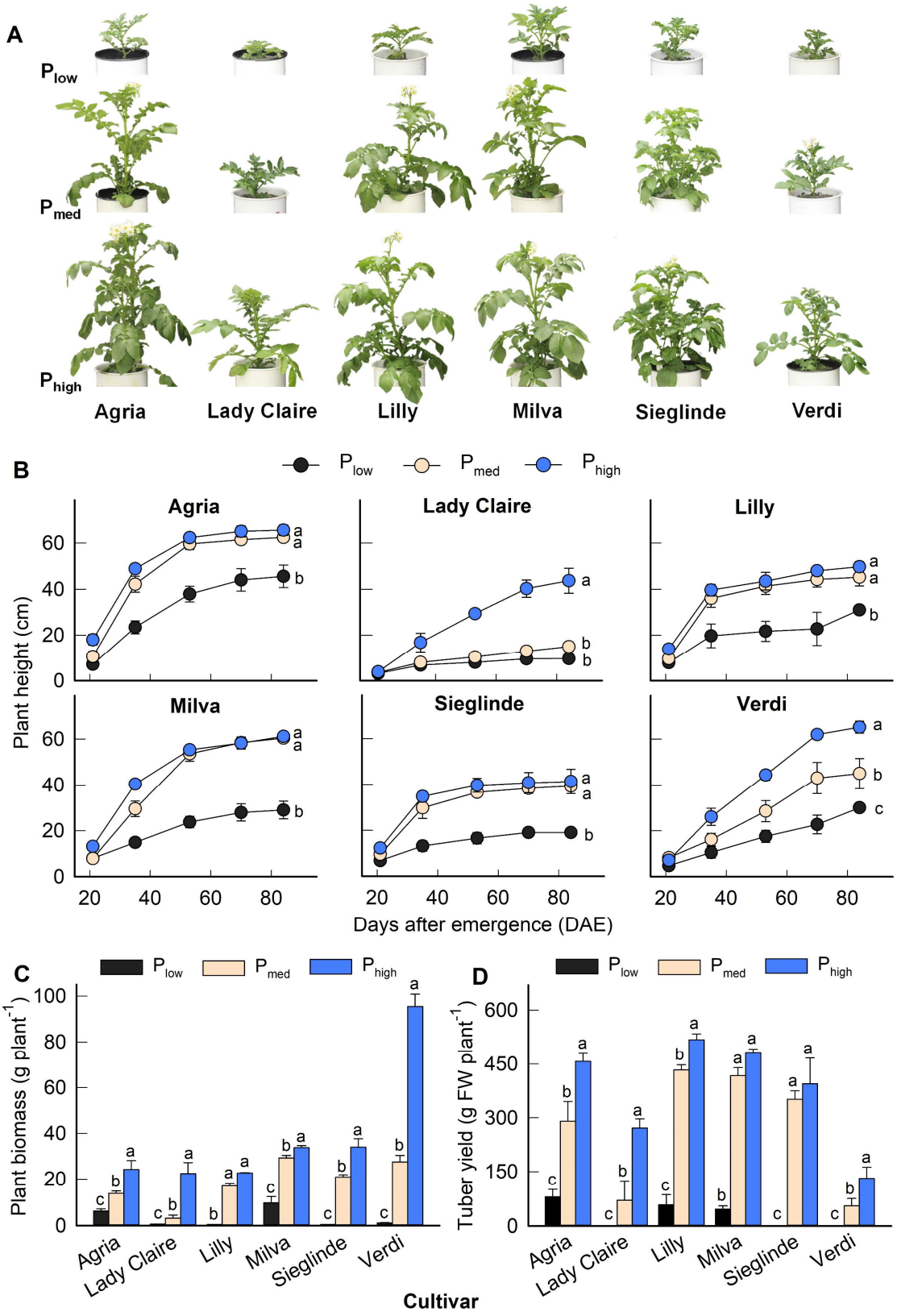


Figure 4.1. **(A)** Shoot phenotype 40 days after emergence (DAE), **(B)** plant height development, **(C)** plant biomass, and **(D)** tuber yield of potato cultivars with the application of P_{low}, P_{med}, and P_{high}. Lowercase letters indicate significant difference between P treatments of each cultivar by Tukey's HSD test at $p < 0.05$. Error bar represents standard error of means (n = 4). FW = fresh weight.

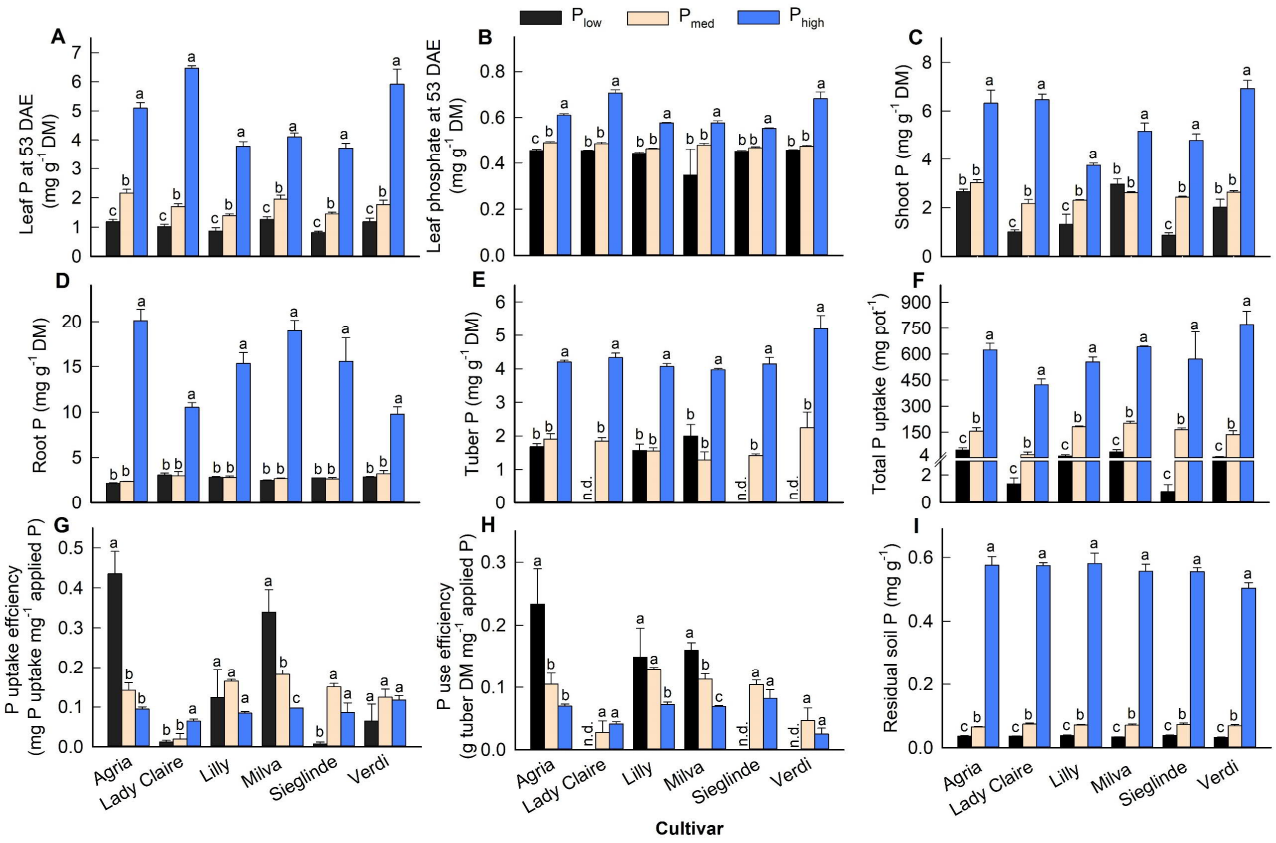


Figure 4.2. (**A-E**) Phosphorus (P) concentration in different parts of the plant, (**F**) total P uptake, (**G**) P uptake efficiency (PUpE), (**H**) P use efficiency (PUE), and (**I**) residual soil P concentration of potato cultivars with the application of P_{low}, P_{med}, and P_{high}. PUpE = amount of total P uptake per unit of applied P, PUE = tuber dry matter production per unit of P uptake. Phosphate concentration (**B**) is expressed as mg of mineral (P) per unit of DM. Different lowercase letters indicate significant difference between P treatments of each cultivar by Tukey's HSD test at $p < 0.05$. Error bar represents standard error of means (n = 4). DM = dry matter, n.d. = not determined because of no tuber production.

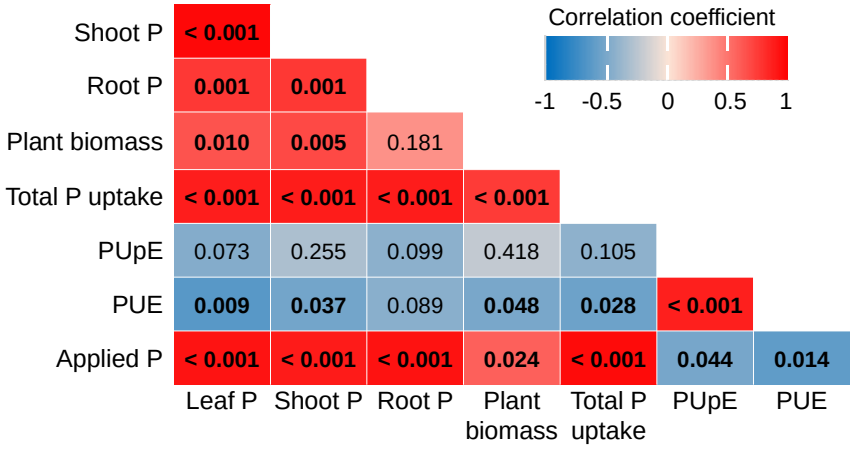


Figure 4.3. Correlation between plant P concentration, biomass, total P uptake, P uptake efficiency (PUpE), P use efficiency (PUE), and P applications (applied P). Color gradients represent Pearson's correlation coefficient and values in bold of each cell indicate p-values which are significant at $p < 0.05$.

Table 4.1. Leaf concentration (mg g^{-1} DM) of nitrogen (N), nitrate, sulfur (S), and sulfate of potato cultivar with the application of P_{low}, P_{med}, and P_{high}

	Cultivar					
	Agria	**Lady Claire**	**Lilly**	**Milva**	**Sieglinde**	**Verdi**
N						
P_{low}	57.23±1.00 ^{a}	56.87±0.91 ^{a}	54.66±1.24 ^{a}	56.25±0.82 ^{b}	58.12±3.87 ^{a}	57.47±4.85 ^{a}
P_{med}	52.40±1.61 ^{ab}	45.83±0.60 ^{b}	45.90±2.02 ^{a}	53.83±1.50 ^{b}	51.55±5.04 ^{a}	51.26±3.25 ^{a}
P_{high}	47.27±1.83 ^{b}	62.77±2.51 ^{a}	42.89±3.74 ^{a}	45.62±2.56 ^{a}	42.33±0.85 ^{a}	55.57±1.98 ^{a}
Nitrate						
P_{low}	10.46±0.52 ^{a}	13.52±1.16 ^{a}	16.33±0.75 ^{a}	7.31±2.45 ^{a}	12.50±1.20 ^{a}	14.31±1.65 ^{a}
P_{med}	6.06±0.51 ^{b}	14.67±0.53 ^{a}	8.16±0.30 ^{b}	5.92±0.28 ^{b}	6.74±0.90 ^{b}	9.14±1.71 ^{b}
P_{high}	4.32±0.19 ^{b}	9.97±0.47 ^{b}	6.81±0.20 ^{c}	4.65±0.25 ^{c}	3.44±0.12 ^{c}	6.42±0.39 ^{c}
S						
P_{low}	7.53±0.73 ^{a}	6.96±0.23 ^{a}	12.13±0.75 ^{a}	11.93±0.99 ^{a}	8.25±0.24 ^{a}	5.21±0.39 ^{a}
P_{med}	4.92±0.20 ^{b}	7.50±1.76 ^{a}	5.25±0.26 ^{b}	5.59±0.74 ^{b}	4.62±0.24 ^{b}	3.72±0.40 ^{ab}
P_{high}	3.98±0.27 ^{c}	3.85±0.10 ^{b}	3.63±0.46 ^{c}	3.87±0.19 ^{c}	3.05±0.13 ^{c}	3.56±0.14 ^{b}
Sulfate						
P_{low}	5.88±0.44 ^{a}	5.86±0.20 ^{a}	8.89±0.12 ^{a}	6.92±2.36 ^{a}	6.54±0.35 ^{a}	4.77±0.31 ^{a}
P_{med}	4.19±0.06 ^{b}	6.23±1.15 ^{a}	4.70±0.19 ^{b}	4.42±0.16 ^{b}	4.00±0.08 ^{b}	3.72±0.08 ^{b}
P_{high}	3.80±0.12 ^{b}	3.70±0.06 ^{b}	3.76±0.08 ^{c}	3.87±0.11 ^{b}	3.54±0.05 ^{b}	3.60±0.05 ^{b}

Nitrate and sulfate concentrations are expressed as mg of respective mineral per unit of dry matter (DM). Mean values ± SE (n = 4) with different lowercase letters indicate significant difference between P treatments of each cultivar by Tukey's HSD test at $p < 0.05$.

4.3.4. Leaf gas exchange and leaf biochemical characteristics

CO_2 assimilation rate and stomatal conductance were significantly affected by the cultivars and P levels on all measurement dates (Figure 4.4A,B and Supplementary Table 4.3). Generally, both parameters were reduced by 13-90% on all measurement dates under P_{low} compared to P_{med}. Across the measurement dates, CO_2 assimilation and stomatal conductance of Agria and Milva were relatively higher than other cultivars under P_{low}. We observed an increased trend in CO_2 assimilation and stomatal conductance of Lady Claire, Sieglinde, and Verdi under P_{high} compared with P_{med}, but they were either stable or reduced by 16-45% for Agria, Lilly, and Milva. The influence of P applications on intercellular CO_2 concentration was to a variable extent in regards to cultivars and measurement dates (Figure 4.4C). For Lady Claire, Lilly, Sieglinde, and Verdi, the intercellular CO_2 concentration was enhanced under P_{low} on at least one of the measurement dates.

Furthermore, the analyses of leaves sampled at 53 DAE show that leaf ATP ranged from 3.02 nmol g^{-1} (Milva) to 22.87 nmol g^{-1} (Sieglinde) under P_{low}, and it was enhanced by 2-12 times under P_{high} (Figure 4.5A, Supplementary Table 4.6). Leaf protein and chlorophyll concentrations of each cultivar were less affected by P applications (Figure 4.5B,C). Nevertheless, leaf proline under P_{low} ranged from 5.10 µmol g^{-1} (Agria) to 9.80 µmol g^{-1} (Sieglinde). At higher P application (P_{high}), leaf proline was reduced by 14-63% (Figure 4.5D, Supplementary Table 4.6). Even though P availability in the soil had less influence on leaf sucrose in all

cultivars, except for Lady Claire and Milva, reducing sugars (fructose and glucose) were significantly increased in response to higher P supply (Figure 4.5E,F). Additionally, total soluble sugars positively correlated with ATP concentration and CO_2 assimilation although correlation coefficient between the total soluble sugars and CO_2 assimilation was moderate (Figure 4.6).

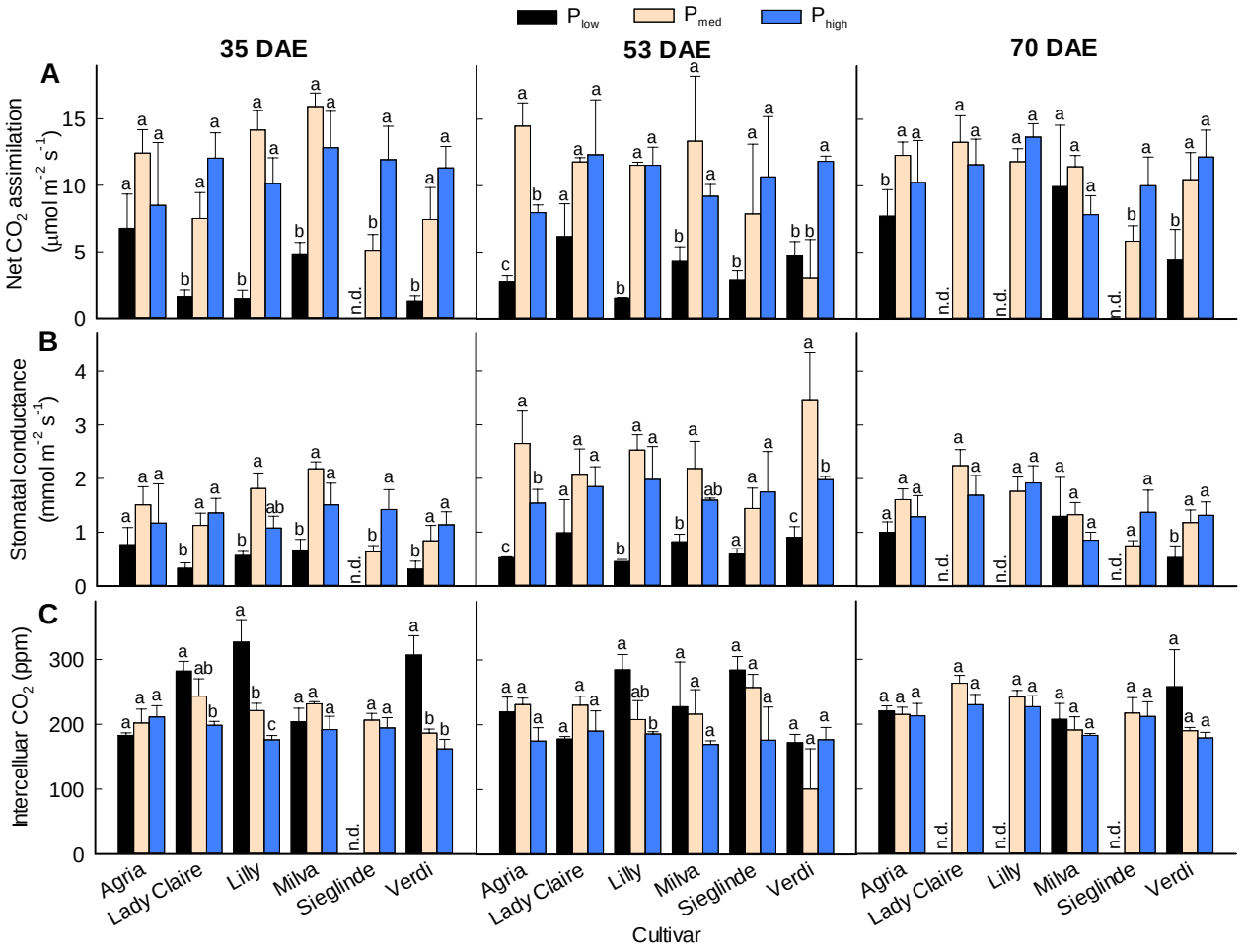


Figure 4.4. (**A**) CO_2 assimilation, (**B**) stomatal conductance, and (**C**) intercellular CO_2 concentration of potato cultivars with the application of P_{low}, P_{med}, and P_{high}. Different lowercase letters indicate significant difference between P treatments of each cultivar by Tukey's HSD test at $p < 0.05$. Error bar represents standard error of means (n = 2-3), n.d. = not determined because of too small leaf area to fit with the cuvette.

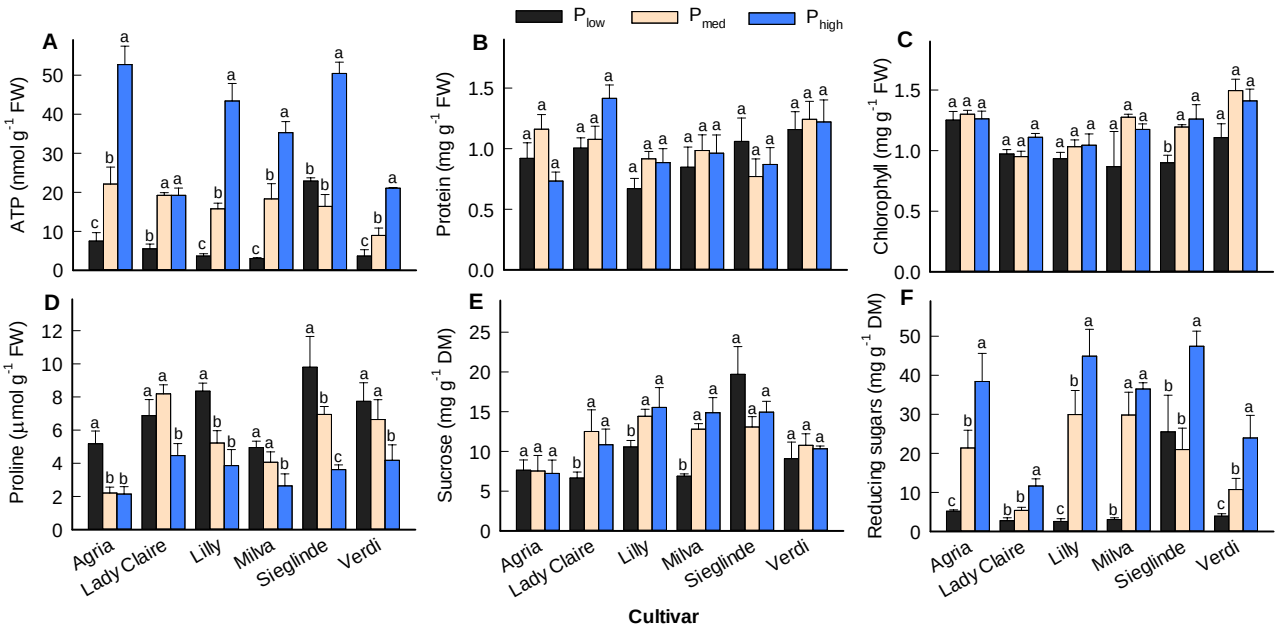


Figure 4.5. (**A-F**) Leaf biochemical characteristics of potato cultivars 53 DAE with the application of P_{low}, P_{med}, and P_{high}. Different lowercase letters indicate significant difference between P treatments of each cultivar by Tukey's HSD test at $p < 0.05$. Error bar represents standard error of means (n = 4). FW = fresh weight, DM = dry matter, ATP = adenosine triphosphate.

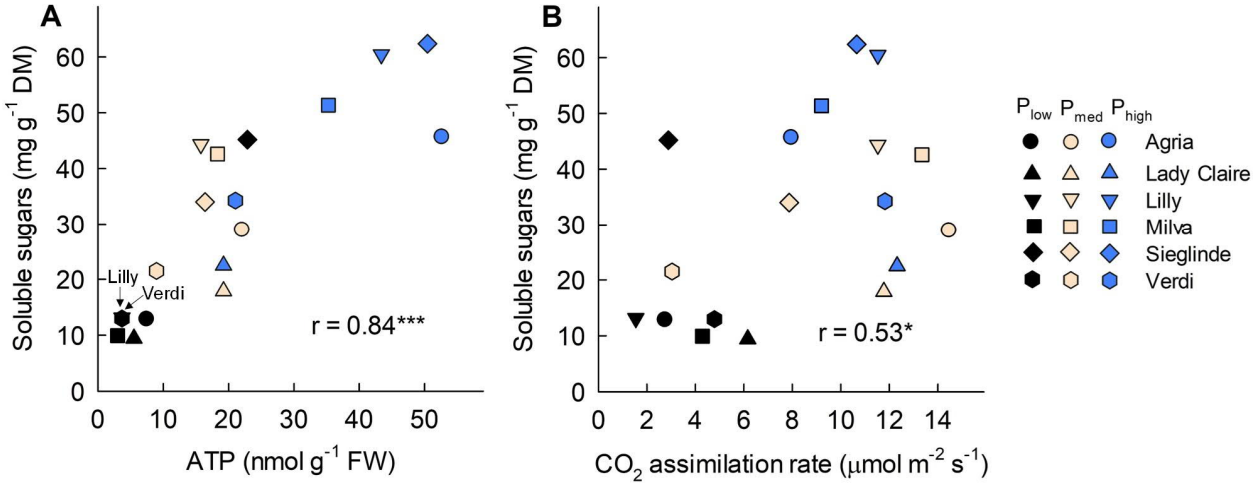


Figure 4.6. Correlation among leaf soluble sugars with (**A**) adenosine triphosphate (ATP) concentration and (**B**) CO_2 assimilation rate measured at 53 DAE. FW = fresh weight, DM = dry matter, * and *** indicate significant correlation at $p < 0.05$ and $p < 0.001$, respectively.

4.3.5. Tuber quality

Since Lady Claire, Sieglinde, and Verdi were unable to produce tubers under P_{low} condition, the results are demonstrated only for Agria, Lilly, and Milva to assess the impact of P_{low} on tuber quality characteristics. The significant effects of cultivars, P levels, and their interactions were observed in many quality parameters (Supplementary Table 4.7). Figure 4.7 shows that P_{low} reduced tuber DM by 22-32%, starch content by 14-23%, and ascorbic acid by 10-25% compared to both P_{med} and P_{high}. Nevertheless, protein in the tuber was increased

under P_{low} by 27-64% and 71-85% compared to P_{med} and P_{high}, respectively. Tuber soluble sugars of Agria and Milva under P_{low} were higher than those under higher P levels; however, the soluble sugars of Lilly were not significantly different between the P treatments. There was no significant difference in TPC and TFC among P applications of Agria, but increasing P supply resulted in decreasing TPC and TFC for Lilly and Milva. Furthermore, antioxidant capacities (DPPH and TEAC) of P_{low} were also 11-57% higher than P_{med} and P_{high}. Besides these tuber quality characteristics, P applications also affected mineral and ion concentrations to variable extents (Table 4.2). Although, P_{low} reduced tuber C and PO_4^{3-} concentration by 2-40%, it increased the concentrations of other minerals and ions (K, Ca, Mg, S, Cu, Fe, Mn, Zn, NO_3^-, and SO_4^{3-}) by 20-85% compared to P_{high}.

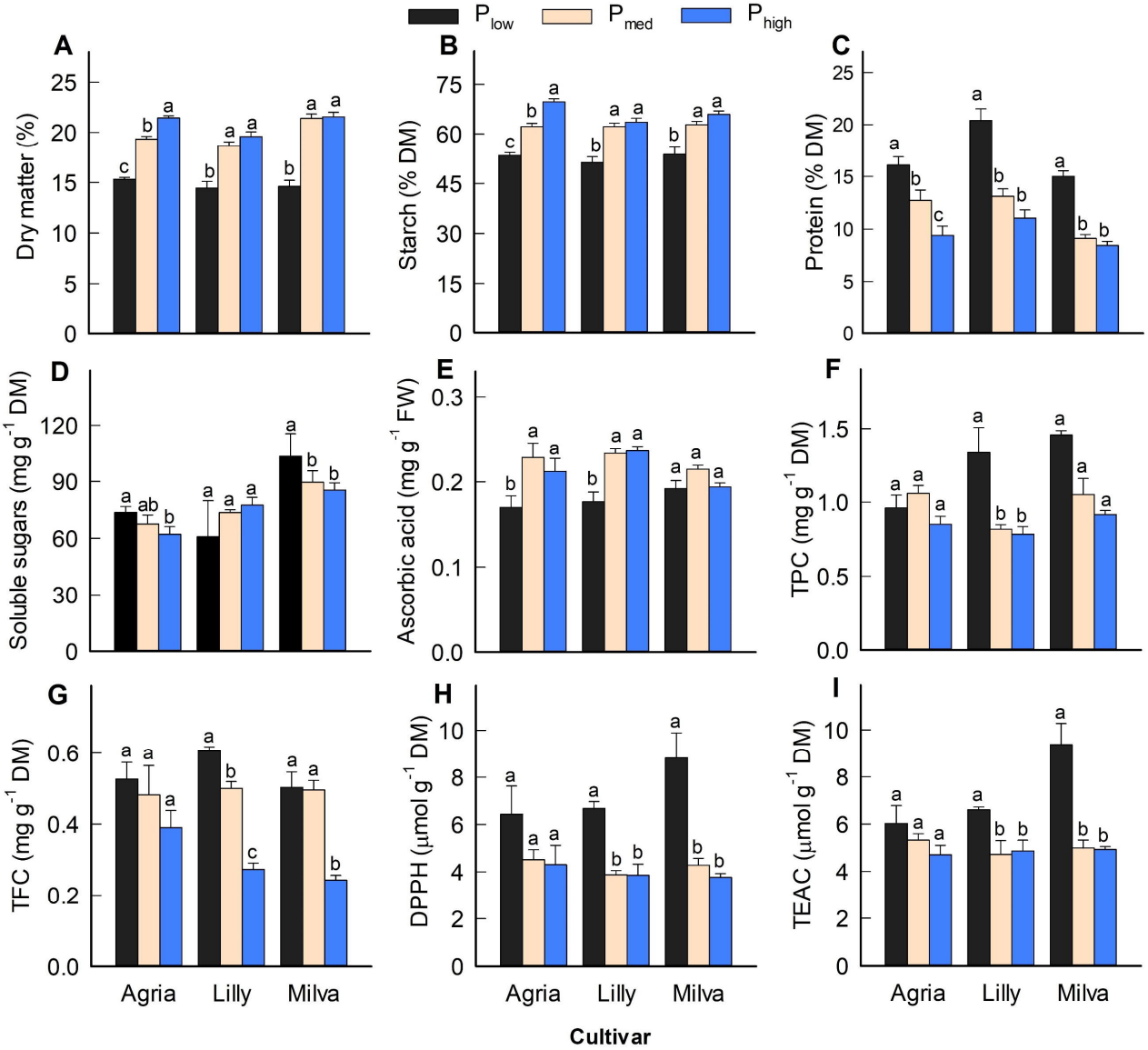


Figure 4.7. Tuber quality characteristics of potato cultivars under P_{low}, P_{med}, and P_{high} application. Different lowercase letters indicate significant difference between P treatments of each cultivar by Tukey's HSD test at $p < 0.05$. DM = dry matter, FW = fresh weight, TPC = total phenolic concentration, TFC = total flavonoid concentration, DPPH = antioxidant capacity by 2,2-diphenyl-1-picrylhydrazyl assay, TEAC = Trolox equivalent antioxidant capacity.

Table 4.2. Tuber mineral and ion concentration of potato cultivars with the application of P_{low}, P_{med}, and P_{high}

	Cultivar		
	Agria	**Lilly**	**Milva**
Phosphate (mg g^{-1} DM)			
P_{low}	0.98±0.06^{b}	0.71±0.09 ^{b}	1.22±0.14^{b}
P_{med}	0.91±0.08^{b}	0.66±0.06 ^{b}	0.61±0.10^{b}
P_{high}	1.86±0.07^{a}	1.86±0.07 ^{a}	2.36±0.10^{a}
Nitrate (mg g^{-1} DM)			
P_{low}	6.49±0.47^{a}	10.77±0.95 ^{a}	10.66±1.17^{a}
P_{med}	0.66±0.61^{b}	3.03±0.60 ^{b}	1.34±0.83^{b}
P_{high}	0.74±0.53^{b}	1.58±0.67 ^{b}	0.88±0.83^{b}
C (mg g^{-1} DM)			
P_{low}	408.31±2.02^{b}	399.20±5.15 ^{b}	403.51±1.53^{b}
P_{med}	417.99±2.61^{a}	419.77±3.25 ^{a}	418.34±1.08^{a}
P_{high}	419.52±2.26^{a}	412.17±3.64 ^{ab}	417.23±1.08^{a}
K (mg g^{-1} DM)			
P_{low}	31.73±0.39^{a}	32.27±1.06 ^{a}	32.87±0.95^{a}
P_{med}	16.91±0.51^{b}	18.43±0.67 ^{b}	16.63±0.67^{b}
P_{high}	15.22±0.44^{c}	16.03±0.75 ^{b}	15.07±0.67^{b}
Ca (mg g^{-1} DM)			
P_{low}	2.18±0.14^{a}	2.83±0.29 ^{a}	4.56±0.58^{a}
P_{med}	0.55±0.18^{b}	1.00±0.18 ^{b}	0.74±0.41^{b}
P_{high}	0.72±0.15^{b}	0.85±0.20 ^{b}	0.79±0.41^{b}
Mg (mg g^{-1} DM)			
P_{low}	1.71±0.06^{a}	1.53±0.08 ^{a}	1.76±0.14^{a}
P_{med}	1.25±0.08^{b}	1.29±0.05 ^{b}	0.94±0.10^{b}
P_{high}	1.06±0.07^{b}	1.18±0.06 ^{b}	0.89±0.10^{b}
S (mg g^{-1} DM)			
P_{low}	3.12±0.04^{a}	3.66±0.19 ^{a}	4.23±0.51^{a}
P_{med}	2.37±0.06^{b}	2.72±0.12 ^{b}	2.22±0.36^{b}
P_{high}	1.74±0.05^{c}	2.11±0.13 ^{c}	1.87±0.36^{b}
Sulfate (mg g^{-1} DM)			
P_{low}	2.66±0.10^{a}	3.63±0.68 ^{a}	3.44±0.58^{a}
P_{med}	2.81±0.22^{a}	2.70±0.19 ^{a}	1.94±0.10^{b}
P_{high}	1.63±0.06^{b}	1.69±0.05 ^{b}	1.53±0.08^{b}
Fe (µg g^{-1} DM)			
P_{low}	48.70±5.11^{a}	46.70±9.46 ^{a}	77.50±9.80^{a}
P_{med}	35.60±6.60^{ab}	31.00±5.98 ^{a}	37.50±7.60^{b}
P_{high}	22.50±5.72^{b}	52.50±6.69 ^{a}	62.50±7.60^{ab}
Mn (µg g^{-1} DM)			
P_{low}	20.40±1.02^{a}	27.70±2.11 ^{a}	22.40±3.51^{a}
P_{med}	9.30±1.31^{b}	12.30±1.33 ^{b}	7.00±2.48^{b}
P_{high}	9.50±1.14^{b}	12.50±1.48 ^{b}	10.80±2.48^{ab}
Zn (µg g^{-1} DM)			
P_{low}	47.10±1.47^{a}	65.00±3.65 ^{a}	55.00±6.45^{a}
P_{med}	38.10±1.90^{b}	45.00±2.31 ^{b}	30.00±4.56^{b}
P_{high}	32.50±1.65^{b}	35.00±2.58 ^{c}	40.00±4.56^{ab}

Phosphate, nitrate, and sulfate concentrations are expressed as mg of respective mineral per unit of dry matter (DM). Mean values ± SE (n = 4) with different lowercase letters indicate significant difference between P treatments of each cultivar by Tukey's HSD test at $p < 0.05$.

4.4. Discussion

A pot experiment was conducted to elucidate the responses of six potato cultivars to soil P levels (P_{low}, P_{med}, and P_{high}). In pot conditions, the availability of applied P for the plants is less interfered by P immobilization and buffering capacity of the soils, which usually occur in field conditions. The results provide further understanding of the impacts of P deficiency and its tolerance mechanisms of potato cultivars from plant to leaf levels.

4.4.1. Different responses of plant growth and PUE to P availability

The greatest plant height, plant biomass, and tuber yield were seen under P_{high}, which indicates that in our experiments there was sufficient P available in soils with P application at 1.2 g kg^{-1}. All cultivars exhibited deficiency under P_{low} conditions through stagnated height development, reduced plant biomass, and less or no tuber yield formation (Figure 4.1). The reduction of plant biomass under P deficiency was at a greater magnitude than that reported in pot studies by Lee et al. (2013) and Wacker-Fester et al. (2019), which suggested that P deficiency condition in our pot study was severe. The variation in plant biomass and tuber yield of potato cultivars was also documented (Lee et al., 2013; Soratto et al., 2015; Wacker-Fester et al., 2019), which allowed us to identify P-efficient cultivars. In potato, tuber yield is important for classifying P responsiveness of cultivars (Soratto et al., 2015); thus, it is an excellent parameter that indicates the tolerance of cultivars to P deficiency. In the present study, P-efficient cultivars—Agria, Milva, and Lilly—produced substantial tuber yield, but the P-inefficiency cultivars—Lady Claire, Sieglinde, and Verdi—were unable to produce tubers under P deficiency. Furthermore, Lady Claire, Sieglinde, and Verdi maintained substantial tissue P concentration, plant biomass, and they had relatively high P uptake and PUE under P-deficient condition (Figures 4.1 and 4.2A-H). The ranges of P uptake and PUE were similar to Sandaña (2016) in field conditions. In contrast, although Lady Claire, Sieglinde, and Verdi had similar plant P concentration compared to other cultivars, they were characterized by very low plant biomass, low PUpE, and low PUE under P deficiency. This indicates that these cultivars conserved the sparingly available P in the plant for viability, and they might lack traits associated with enhanced P uptake and allocation for growth and tuber formation. The separation of cultivars for their efficiency under limited P availability was also confirmed based on the principal component analysis of traits associated with P efficiency, as shown in Supplementary Figure 4.3. Although, residual P availability in soils under P deficiency was similar among the cultivars, the enhanced P uptake of P-efficient cultivars may have been caused by improved root traits, which could modify the amount of P available for plants (Lee et al., 2013). Therefore, improved PUpE is important to enhance PUE under limited P supply (Wang et al., 2010). The increased PUpE and PUE under high P availability of Lady Claire, Sieglinde, and Verdi suggests that these cultivars are efficient in P uptake when soil P availability is not a limiting factor. Increased P availability in soil resulted in high plant P concentration, P uptake, and plant biomass (Figure 4.3), but it cannot give assurance for enhanced PUE if the PUpE is low. Consequently, high residual soil P, after harvest, implies an inefficient use of P fertilizer, which is a potential risk for the environmental under field conditions (Heuer et al., 2017).

4.4.2. Importance of leaf minerals and ions in mitigating P deficiency

In plants, P exists as inorganic orthophosphate forms (PO_4^{3-} or P_i) and organic phosphate esters (Veneklaas et al., 2012). Our results reveal a lesser reduction of leaf P_i compared to leaf P under P deficiency which could be caused by an increase of P_i fraction under this condition. Under P deficiency, plants sense cytosolic P_i shortage through recycling the entire P in the vacuole, to increase P_i concentration for efflux into cytosol and chloroplast (Shen et al., 2011; Long et al., 2019). Therefore, P translocation to active photosynthetic tissue and internal P recycling could be adaptations of potato cultivars under stress induced by P deprivation. Moreover, leaf nitrate also rose under P deficiency. The mechanisms underlying nitrate accumulation have not been thoroughly understood; however, it could be caused by nitric oxide generation due to oxidative stress acclimation under P deficiency (Fu et al., 2018). High nitric oxide production was also reported on soybean leaves (Ramos-Artuso et al., 2019) and Arabidopsis (Royo et al., 2015) under P deprivation. In the complex nitrate cycle in plants, nitrate is required for nitric oxide biosynthesis and the turnover of nitric oxide also produces nitrate. This complete cycle is regulated by nitrate reductase (Astier et al., 2018). Furthermore, nitrate assimilation is an energetically costly process (Nunes-Nesi et al., 2010), which could be inhibited under P deficiency resulting in an accumulation of nitrate in leaves. Additionally, the uptake and allocation of other N forms such as ammonium and amides may also be altered under P deficiency, as implied by the relationship between leaf N and nitrate in Supplementary Figure 4.2. Furthermore, leaf sulfate concentration may also increase to fulfill the S-demand of sulfolipid generation for replacing phospholipid under P deficiency and to balance leaf anion-to-cation ratio under low phosphate ions (Misson et al., 2005; Rouached, 2011).

4.4.3. Leaf photosynthesis and biochemical adaption in response to P deficiency

Besides the alterations in leaf minerals and ions, P deficiency reduced stomatal conductance and CO_2 assimilation rate at different magnitudes (Figure 4.4A,B). The reduction of photosynthesis under P deficiency was also observed in barley (Carstensen et al., 2018) and soybean (Singh and Reddy, 2015). P deficiency suppresses photosynthesis rate through the disruption on electron transport and reduction in ATP and NADPH synthesis (Carstensen et al., 2018). In the present study, although fast photosynthesis measurements were conducted at 180 seconds at each radiation level, it was sufficient to obtain steady-state results because the PPFD inside the cuvette for the data used in this study and at ambient conditions before the measurements were the same. Our results further reveal that at least two of the three P-efficient cultivars (viz. Agria and Milva) had relatively high CO_2 assimilation rates and stomatal conductance—especially at 35 and 70 DAE—, which explained the substantial shoot biomass production and tuber yield of these cultivars under P starvation. The minimal disruption on photosynthesis of these cultivars under P deficiency could be caused by improved P allocation to leaves to increase the leaf area. In soybean, Chaudhary et al. (2008) also showed the importance of P allocation to shoots to increase PUE and leaf area under P deprivation. However, the reduction of CO_2 assimilation in P-efficient cultivars under high P supply might be linked to their high leaf area (Figure 4.1A). Leaves of the plants expand in response to high P supply, causing high leaf area (Shi et al., 2019), and the leaves, eventually, become thinner. Therefore, photosynthetic machinery per unit of leaf area may be reduced.

Nevertheless, increased leaf area under high P supply could compensate the reduction in photosynthesis because of a greater light interception.

Although leaf photosynthesis was reduced by P deficiency, leaf protein, and chlorophyll were less affected (Figure 4.5B,C). In leaves, the majority of N is present in chlorophyll and in proteins of the thylakoids (Perchlik and Tegeder, 2018); therefore, in the present study the accumulation of leaf N may contribute to maintaining protein and chlorophyll in leaves under P deficiency. A similar observation in maize indicated that leaves of P-deficient plants are less associated with the reduction of chlorophyll concentration, because these leaves eventually become thicker, and appear to be bluish green (Plénet et al., 2000). However, our results reveal that P deficiency also modulates leaf ATP, sucrose, reducing sugars, and proline at different levels depending on the cultivars. Since P applications did not significantly affect leaf protein concentration (Figure 4.5B,C), the effects of P levels on ATP and proline could be compared and discussed on the basis per leaf fresh weight. At the leaf level, P plays an essential role as a substrate for ATP synthesis in the chloroplast (Carstensen et al., 2018). Even though, chloroplast P transporter (*AtPHT4;1*) is proposed to mediate chloroplast Pi for ATP synthesis activities under limited P supply in Arabidopsis (Karlsson et al., 2015), in our study sufficient chloroplast P_i may have not been maintained under severe P deficiency, which ultimately results in a reduction of ATP. ATP limitation under P deficiency hampers the use of NADPH in the Calvin cycle (Carstensen et al., 2018); thus, the CO_2 assimilation is reduced for sugar production.

In our study, P deficiency also influenced sugar metabolism. There was no significant reduction in leaf sucrose concentration in many cultivars under P deficiency (Figure 4.5E). This could be due to a high cleavage of sucrose to fructose and glucose under high P availability, but under P deficiency the conversion of sucrose was inhibited. This resulted in a huge increase of these reducing sugars in response to increasing P applications, especially for Agria, Lilly, and Milva (Figure 4.5F). The conversion of sucrose to reducing sugars is regulated by enzymes such as invertase, sucrose phosphate synthase, and fructose 1,6-bisphosphatase, which are enhanced under sufficient P supply (García-Caparrós et al., 2021). The less or no reduction of sucrose in the leaves under P deficiency could also be caused by either a limited sugar transport or sugar conservation response of plants. Under P deficiency, plants maintain large amounts of compatible non-toxic solutes such as sucrose and proline for cellular osmotic adjustment, stabilizing cell structure, and scavenging free radicals (Ashraf and Foolad, 2007; Hayat et al., 2012). In Arabidopsis, high sucrose level is important to induce P starvation-responsive genes for mobilizing plant internal P (Lei et al., 2011). Under stress conditions, induced by P deficiency, proline is synthesized from glutamate in the cytosol; nevertheless, depending upon the recovery from stress, proline is rapidly oxidized into glutamate, while ATP is also generated during this oxidation process to maintain leaf viability (Launay et al., 2019). Among the cultivars, we found that Sieglinde had relatively high concentrations of ATP, proline, sucrose, and total reducing sugar in young leaves compared to other cultivars under P deficiency conditions. These results indicate a high stress intensity of this cultivar under P deficiency, which may have inhibited sugar translocation. It also recommends further investigations on sucrose transporters that may explain molecular mechanisms underlying relatively high sucrose of these

cultivars under low P availability. In our study, there was a positive correlation between concentration of leaf total soluble sugars with ATP concentration (Figure 4.6A). The correlation between total soluble sugars and CO_2 assimilation was also positively significant (Figure 4.6B), but it was at less extent which suggest that improved CO_2 assimilation is important for sugar production, provided that there is sufficient amount of ATP in the reaction to convert the assimilated CO_2 into sugars.

4.4.4. Impacts of P deficiency on tuber quality

We present the implication of P deficiency on tuber quality of potato in addition to plant agronomic and biochemical characteristics as outlined above. P is involved in a number of key enzymes that regulate starch synthesis, and it is also a key element of starch composition (Nielsen et al., 1994; Naumann et al., 2020). Although we did not observe a significant difference in tuber P concentration between P_{low} and P_{med}, a reduction of P application resulted in significant decrease in tuber dry matter and starch concentration (Figure 4.7A,B). This suggests a compromise between improving PUE and these quality parameters. The tuber DM of all cultivars under P deficiency was also below the range (16–18%) of many potatoes available in the markets (Storey, 2007), which may have a significant impact on the market acceptability. Nevertheless, protein concentration was enhanced under low P availability (Figure 4.7C), which could be caused by increased N uptake as indicated by high leaf N concentration (Table 4.1). These results are similar to those reported by Leonel et al. (2017). However, Fernandes et al. (2015) and Öztürk et al. (2010) did not observe significant differences in tuber DM, starch, and protein concentrations between low and high soil P availability. The inconsistency of these results might be due to cultivar differences in DM and starch concentration. Furthermore, the P deficiency condition reported in those studies might be less severe—compared to our study—and therefore did not alter the DM, starch, and protein concentrations in the tubers. Sugar concentration of the tubers is also important for fresh market (Storey, 2007). In our study, P deficiency enhanced sugar concentration in tubers for Agria and Milva, but it had no significant effect in Lilly (Figure 4.7D). This indicates that under P efficiency, the limited carbohydrates allocated into tubers might be converted into sugars rather than starch. Similar to our findings, Xing et al. (2020) reported a non-significant relationship between soil P availability and total soluble sugars in tubers, because some potato tubers grown under low soil P also contain relatively high sugar concentration.

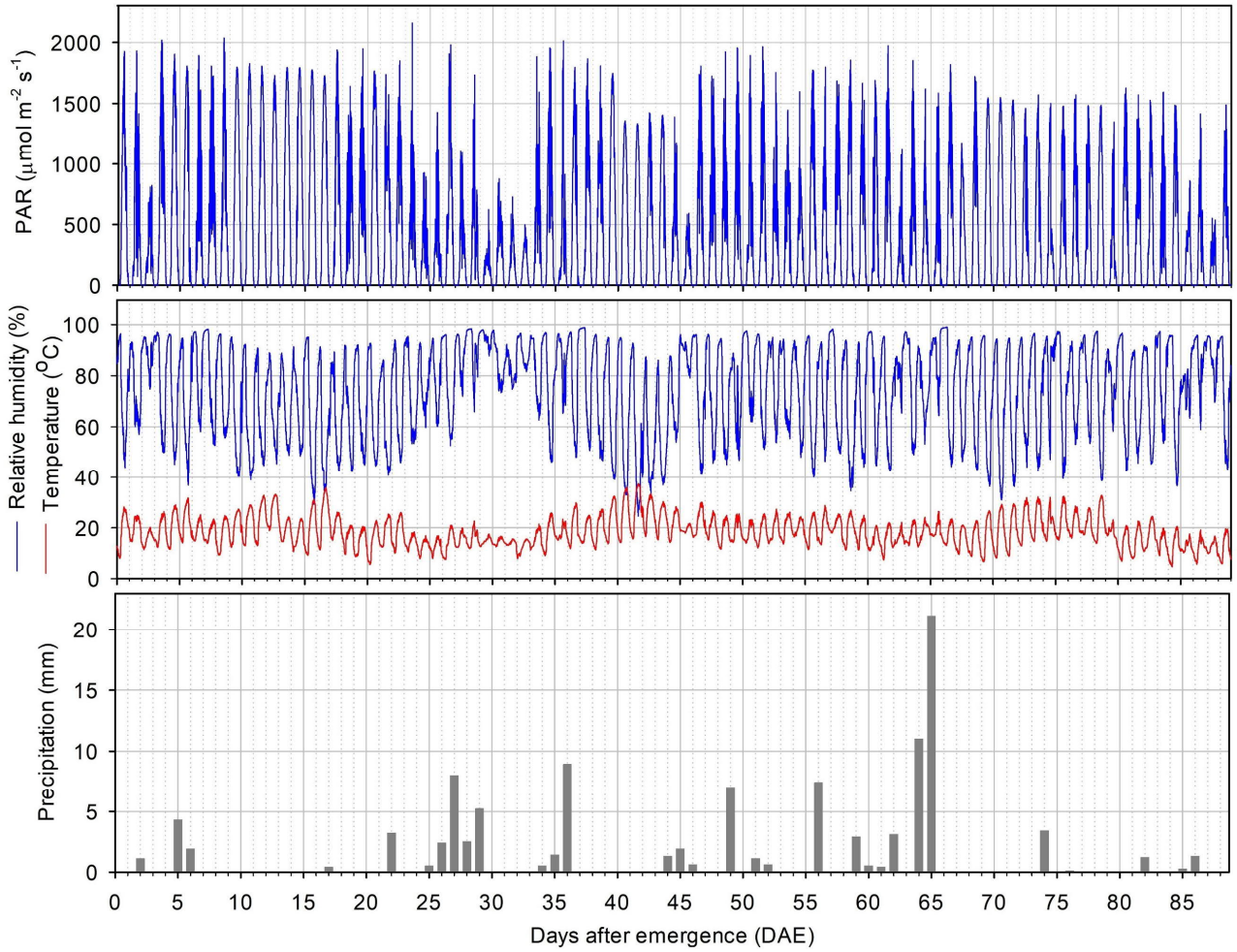
The analyses of ascorbic acid, antioxidant capacity, and minerals in the tubers revealed a reduction of ascorbic acid in all cultivars except in Milva, but an increase in TPC, TFC, DPPH, TEAC, minerals, and ions under P deficiency (Figure 4.7E-I, Table 4.2). The concentration of ascorbic acid under different P treatments was within the ranges (0.1-0.25 mg g^{-1} FW) of potato in the markets (Storey, 2007), which implies that the reduction of ascorbic acid under P deficiency could be neglected. Ascorbic acid accounts for about 13% of the total antioxidant capacity of tuber (Storey, 2007); therefore, about 20% reduction of ascorbic acid under P deficiency could be compensated by an increase of other antioxidants such as, TPC and TFC, while the total antioxidant capacity increased significantly under this condition. Although the tuber sample extraction for antioxidant capacity measurement was conducted using methanol, which aimed for hydrophilic antioxidants

(Kaur et al., 2013), these antioxidants contribute the most to the total antioxidant capacity of potato tubers (Andre et al., 2007). The accumulation of these antioxidants is caused by oxidative stress induced by P deficiency, which provokes numerous plant response reactions for the antioxidant systems (Wang and Frei, 2011). The increased concentration of minerals in the leaves also contributed to improve the concentration of these minerals and ions in tubers. However, nitrate concentration in tubers under P deficiency exceeded the general range (<200 mg kg^{-1} FW) for potato, based on the classification of vegetables, according to nitrate concentration published by Santamaria (2006). High nitrate concentration could be caused by a higher proportion of tuber skin when the size of the tubers is small under P deficiency (data not shown). Increased concentrations of protein, minerals, and ions—except P and phosphate—under P deficiency could also be a result of tuber yield reduction; thus, the concentration is less diluted by the shortage of carbohydrates (Wang and Frei, 2011). The increased concentrations of tuber phytochemicals and minerals are valuable to promote health and physical well-being of consumers (Andre et al., 2007; Wang and Frei, 2011).

4.5. Conclusion

There is a significant variation in plant responses to P deficiency that exists among the tested potato cultivars. We could identify the P-efficient cultivars—Agria, Milva, and Lilly—possessing substantial plant biomass, tuber yield, and high PUpE under low P supply and they may be suitable for production in limited P condition. The P-inefficient cultivars—Lady Claire, Sieglinde, and Verdi—lacked PUpE and the ability to produce tubers under P deficiency. Nevertheless, these cultivars may be efficient in P uptake at high P availability in the soils, which lead to a reduction of P loss in the environment. In response to low supply of P, potato plants attempted to maintain essential ions (such as phosphate, nitrate, and sulfate) and compatible solutes (such as proline and sucrose) to improve internal PUE, and acclimate stress, induced by P deficiency. Leaf photosynthesis also decreased under P deficiency, which was associated with ATP reduction and further resulted in reduced plant biomass and tuber yield. Furthermore, even though P deficiency significantly reduced tuber dry matter, concentrations of sugars and minerals, and the antioxidant capacity were enhanced for P-efficient cultivars, which can contribute to better nutritional properties of potato tubers. These results indicate a possibility to improve PUE and tuber quality of potato under P-deficient conditions by using P-efficient cultivars. Therefore, a future focus on evaluating molecular mechanisms related to P and sucrose transporters will increase knowledge of internal PUE under limited P availability.

Supplementary materials



Supplementary Figure 4.1. Diurnal photosynthetic active radiation (PAR), temperature, relative humidity, and daily precipitation during the experiment (17 June to 12 September 2019). (Data source: Experimental Botanical Garden, University of Goettingen)

Supplementary Table 4.1. Description of potato cultivars

Cultivars	Maturity	Tuber skin color	Tuber dry matter	Destination
Agria	Medium	Yellow	Medium	Ware potato, convenience products, french fries, flakes, crisps
Lady Claire	Medium-late	White	High	French fries, crisps
Lilly	Early-medium	Yellow	Medium	Ware potato convenience products
Milva	Medium	Yellow	Medium	Ware potato, convenience products
Sieglinde	Early	Yellow	Medium	Ware potato, convenience products
Verdi	Medium	Yellow	High	Crisps

The further information of each cultivar can be found in the variety catalogues below:

Lilly: https://www.solana.de/kartoffelsorten-detail_en/items/lilly.html?pdf=create&id=24

Agria and Milva: https://www.europlant.biz/fileadmin/user_upload/Brosch%C3%BCren/Europlant_A6_Varieties_E_2018-19.pdf

Lady Claire: https://www.meijerpotato.co.uk/our-varieties/crisping/lady-claire/

Sieglinde: http://heritagepotato.ca/heritage-potatoes/profiles/sieglinde/; https://www.europotato.org/varieties/view/Sieglinde-E

Verdi: https://www.solana.de/kartoffelsorten-detail_en/items/verdi.html

Supplementary Table 4.2. Concentration of nutrients applied in the soil

Nutrient	Concentration (mg kg^{-1} soil)	Nutrient source
N[1]	300; 500	$Ca(NO_3)_2$* $4H_2O$
K	330	K_2SO_4
Ca	1300	$CaCO_3$, $Ca(NO_3)_2$*$4H_2O$
S	250	K_2SO_4, $MgSO_4$*$7H_2O$, $CuSO_4$*$5H_2O$, $ZnSO_4$*$7H_2O$, $MnSO_4$*H_2O
Mg	100	$MgSO_4$*$7H_2O$
Cu	0.002	$CuSO_4$*$5H_2O$
EDTA-Fe	0.003	$C_{10}H_{12}FeN_2NaO_8$*$3H_2O$
Zn	0.002	$ZnSO_4$*$7H_2O$
B	0.0006	H_3BO_3
Mo	0.002	Na_2MoO_4*$2H_2O$
Mn	0.006	$MnSO_4$*H_2O

[1]N was applied at 300 mg kg^{-1} for Agria, Lilly, Milva, and Sieglinde and at 500 mg kg^{-1} N for Lady Claire and Verdi according to the fertilizer recommendations by seed suppliers. N was added in splits, 75% basally and 25% top-dressing at 50 DAE. These nutrients were dissolved in water and applied to the soils. After all nutrients were added, soil pH of P_{low}, P_{med}, and P_{high} was in range of 6.8–7.0, 6.3–6.6, and 5.5–5.8, respectively.

Supplementary Table 4.3. Analysis of variance (ANOVA) of plant growth, P use efficiency, leaf minerals, and leaf biochemical characteristics of six potato cultivars under P_{low}, P_{med}, and P_{high}

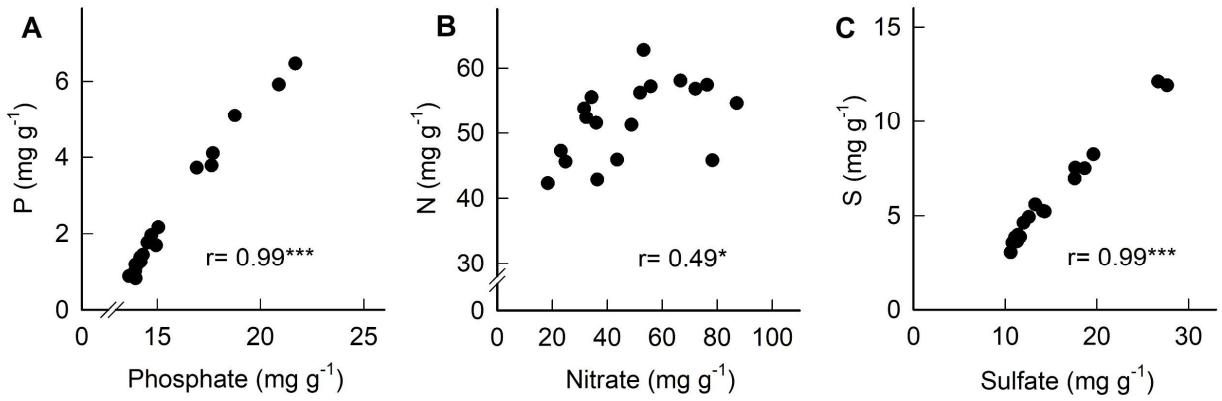
Parameters	ANOVA		
	Cultivar (C)	P level (P)	C x P
Plant height	***	***	***
Plant biomass	***	***	***
Tuber yield	***	***	***
P concentration			
Leaf at 53DAE	***	***	***
Shoot	***	***	***
Root	***	***	***
Tuber	***	***	***
Total P uptake	***	***	*
P uptake efficiency (PUpE)	***	*	***
P use efficiency (PUE)	***	*	***
Leaf photosynthesis			
CO_2 assimilation 35 DAE	***	***	***
CO_2 assimilation 53 DAE	***	***	***
CO_2 assimilation 70 DAE	*	*	**
Stomatal conductance 35 DAE	*	***	**
Stomatal conductance 53 DAE	**	***	***
Stomatal conductance 70 DAE	ns	ns	***
Intercellular CO_2 35 DAE	**	*	**
Intercellular CO_2 53 DAE	*	ns	**
Intercellular CO_2 70 DAE	ns	ns	*
Leaf mineral concentration			
N	***	***	***
C	**	***	ns
K	**	***	***
S	***	***	***
Ca	***	***	**
Mg	***	***	***
Mn	***	***	***
Fe	ns	ns	ns
Zn	ns	****	ns
Leaf ions			
Phosphate	***	***	***
Nitrate	***	***	***
Sulfate	***	***	***
Leaf biochemical properties			
ATP	***	***	***
Protein	**	ns	ns
Chlorophyll	***	ns	ns
Proline	***	***	ns
Sucrose	***	***	*
Reducing sugars	***	***	***

ns, *, **, and *** indicate non-significance and significance at $p<0.05$, $p<0.01$, and $p<0.001$, respectively.

Supplementary Table 4.4. Means±SE of plant morphology, P concentration, and P use efficiency

	Cultivar					
	Agria	**Lady Claire**	**Lilly**	**Milva**	**Sieglinde**	**Verdi**
Plant height (cm)						
P_{low}	45.60±4.87	9.50±0.50	31.00±0.00	29.00±3.85	19.00±1.00	30.00±1.20
P_{med}	62.50±1.44	15.00±2.00	45.20±3.76	60.50±1.71	39.25±1.93	45.00±6.61
P_{high}	65.63±2.30	43.67±5.46	49.75±2.21	61.25±0.85	38.33±5.36	65.25±2.63
Plant biomass (g plant^{-1})						
P_{low}	6.16±0.95	0.59±0.04	0.41±0.03	9.70±2.73	0.40±0.06	1.14±0.17
P_{med}	13.71±1.09	3.12±1.37	16.99±1.19	29.26±1.18	20.93±0.93	27.54±2.87
P_{high}	164.88±17.95	169.46±30.75	77.60±12.63	184.80±14.17	164.78±16.49	690.47±32.16
Tuber yield (g plant^{-1})						
P_{low}	79.42±20.60	-	57.90±27.60	45.90±8.80	-	-
P_{med}	290.37±54.72	70.35±53.15	433.42±14.34	417.25±22.59	351.68±23.15	54.90±19.86
P_{high}	457.18±22.36	271.45±25.64	518.50±15.86	481.00±10.80	394.52±72.08	130.93±31.28
Leaf P (mg g^{-1} DM)						
P_{low}	1.19±0.07	1.01±0.08	0.87±0.12	1.26±0.09	0.82±0.05	1.18±0.12
P_{med}	2.17±0.12	1.70±0.10	1.39±0.06	1.96±0.13	1.45±0.06	1.77±0.16
P_{high}	5.10±0.19	6.46±0.09	3.78±0.16	4.10±0.14	3.72±0.16	5.92±0.52
Leaf Phosphate (mg g^{-1} DM)						
P_{low}	0.46±0.01	0.46±0.00	0.44±0.01	0.35±0.12	0.45±0.00	0.46±0.00
P_{med}	0.49±0.01	0.49±0.01	0.46±0.00	0.48±0.01	0.47±0.00	0.48±0.01
P_{high}	0.61±0.01	0.71±0.01	0.58±0.00	0.58±0.01	0.55±0.00	0.68±0.03
Shoot P (mg g^{-1} DM)						
P_{low}	2.66±0.10	1.01±0.08	1.32±0.41	2.97±0.21	0.87±0.10	2.02±0.33
P_{med}	3.03±0.12	2.17±0.17	2.30±0.03	2.62±0.03	2.43±0.04	2.63±0.07
P_{high}	6.32±0.54	6.47±0.23	3.74±0.10	5.16±0.34	4.78±0.27	6.93±0.34
Root P (mg g^{-1} DM)						
P_{low}	2.19±0.06	3.09±0.22	2.85±0.07	2.50±0.07	2.79±0.00	2.86±0.06
P_{med}	2.37±0.04	3.00±0.50	2.83±0.14	2.68±0.07	2.67±0.14	3.22±0.37
P_{high}	20.10±1.28	10.53±0.53	15.36±1.20	18.98±1.16	15.58±2.60	9.79±0.82
Tuber P (mg g^{-1} DM)						
P_{low}	1.68±0.09	-	1.57±0.19	2.00±0.34	-	-
P_{med}	1.91±0.16	1.85±0.10	1.55±0.09	1.28±0.24	1.42±0.05	2.24±0.47
P_{high}	4.21±0.05	4.33±0.14	4.07±0.09	3.97±0.03	4.15±0.19	5.20±0.40
Total P uptake (mg pot^{-1})						
P_{low}	15.46±2.01	0.97±0.09	0.69±0.04	28.52±7.85	0.32±0.06	2.37±0.45
P_{med}	40.71±2.75	7.46±3.47	39.16±2.46	76.45±2.47	50.87±2.26	75.54±7.97
P_{high}	24.22±3.87	22.41±4.86	22.67±0.19	33.79±0.88	34.00±3.67	95.46±5.33
P uptake efficiency (mg P uptake mg^{-1} applied P)						
P_{low}	0.44±0.06	0.01±0.00	0.12±0.07	0.34±0.06	0.01±0.00	0.06±0.04
P_{med}	0.14±0.02	0.02±0.01	0.17±0.00	0.18±0.01	0.15±0.01	0.12±0.02
P_{high}	0.09±0.01	0.06±0.01	0.08±0.00	0.10±0.00	0.09±0.02	0.12±0.01
P use efficiency (g tuber DM mg^{-1} applied P)						
P_{low}	0.23±0.06	-	0.15±0.05	0.16±0.01	-	-
P_{med}	0.11±0.02	0.03±0.02	0.13±0.00	0.11±0.01	0.10±0.01	0.05±0.02
P_{high}	0.07±0.00	0.04±0.00	0.07±0.00	0.07±0.00	0.08±0.01	0.03±0.01
Residual soil P (mg g^{-1})						
P_{low}	0.04±0.00	0.04±0.00	0.04±0.00	0.03±0.00	0.04±0.00	0.03±0.00
P_{med}	0.07±0.00	0.07±0.00	0.07±0.00	0.07±0.00	0.07±0.01	0.07±0.00
P_{high}	0.58±0.03	0.58±0.01	0.58±0.03	0.56±0.02	0.56±0.01	0.50±0.02

Phosphate concentrations is expressed as mg of the mineral (P) per unit of DM.



Supplementary Figure 4.2. Correlations between minerals and ions in young leaves at 53 DAE

Supplementary Table 4.5. Leaf mineral concentration (mg g^{-1} DM) of potato cultivars at 53 DAE under P_{low}, P_{med}, and P_{high} applications

	Cultivar					
	Agria	**Lady Claire**	**Lilly**	**Milva**	**Sieglinde**	**Verdi**
C						
P_{low}	384.04±3.61^{b}	355.05±5.79^{a}	342.96±9.38^{b}	381.19±0.87^{a}	354.00±3.48^{b}	368.03±9.12^{a}
P_{med}	417.17±2.77^{a}	388.57±9.45^{a}	404.71±9.09^{a}	403.27±9.10^{a}	427.10±9.40^{a}	391.91±9.05^{b}
P_{high}	430.07±1.70^{a}	381.38±5.19^{a}	405.25±4.12^{a}	410.19±9.91^{a}	426.46±3.66^{a}	405.14±6.90^{b}
K						
P_{low}	46.73±8.79^{a}	51.12±2.52^{a}	50.76±7.27^{a}	37.53±9.35^{a}	54.93±3.62^{a}	46.13±7.88^{a}
P_{med}	30.64±7.59^{b}	48.61±7.99^{a}	44.84±4.05^{a}	42.68±6.42^{a}	37.01±8.12^{b}	34.12±7.22^{a}
P_{high}	35.64±3.23^{b}	31.18±4.16^{b}	50.28±7.21^{a}	30.90±6.22^{a}	37.49±7.64^{b}	39.82±9.61^{a}
Ca						
P_{low}	23.71±2.24^{a}	42.31±2.82^{a}	34.15±6.14^{a}	21.30±0.51^{a}	28.68±3.33^{a}	36.45±3.09^{a}
P_{med}	17.31±2.52^{a}	43.99±3.39^{a}	25.65±2.06^{a}	20.08±0.89^{a}	22.38±1.27^{a}	22.39±3.20^{b}
P_{high}	20.90±2.74^{a}	26.13±2.46^{b}	28.72±3.70^{a}	20.45±1.40^{a}	20.82±1.87^{a}	18.88±1.15^{b}
Mg						
P_{low}	5.75±0.34^{b}	5.48±0.27^{a}	3.71±0.12^{a}	6.19±0.33^{a}	5.57±0.21^{b}	5.58±0.30^{b}
P_{med}	6.04±0.39^{a}	7.01±0.67^{a}	4.64±0.62^{a}	6.50±1.15^{a}	5.51±0.68^{b}	6.06±0.74^{ab}
P_{high}	6.66±1.48^{a}	6.25±0.69^{a}	4.84±0.35^{a}	6.84±0.93^{a}	7.17±0.95^{a}	6.56±1.28^{a}
Mn						
P_{low}	0.26±0.02^{a}	0.17±0.01^{b}	0.52±0.05^{a}	0.38±0.01^{a}	0.37±0.04^{a}	0.23±0.06^{a}
P_{med}	0.21±0.01^{a}	0.24±0.03^{ab}	0.30±0.03^{b}	0.18±0.01^{b}	0.29±0.03^{a}	0.19±0.04^{a}
P_{high}	0.26±0.04^{a}	0.36±0.04^{a}	0.38±0.03^{ab}	0.27±0.02^{b}	0.31±0.04^{a}	0.21±0.02^{a}
Fe						
P_{low}	0.15±0.01^{a}	0.14±0.02^{a}	0.17±0.03^{a}	0.18±0.06^{a}	0.19±0.01^{a}	0.13±0.01^{a}
P_{med}	0.17±0.02^{a}	0.16±0.02^{a}	0.16±0.01^{a}	0.15±0.01^{a}	0.14±0.01^{b}	0.14±0.03^{a}
P_{high}	0.17±0.00^{a}	0.13±0.01^{a}	0.17±0.02^{a}	0.16±0.01^{a}	0.14±0.00^{b}	0.12±0.01^{a}
Zn						
P_{low}	0.20±0.02^{a}	0.32±0.07^{a}	0.30±0.03^{a}	0.26±0.01^{a}	0.37±0.09^{a}	0.30±0.03^{a}
P_{med}	0.14±0.02^{b}	0.26±0.06^{b}	0.14±0.02^{b}	0.15±0.01^{b}	0.10±0.01^{b}	0.18±0.02^{ab}
P_{high}	0.14±0.01^{b}	0.16±0.02^{c}	0.15±0.01^{b}	0.16±0.01^{b}	0.13±0.00^{b}	0.13±0.02^{b}

Mean values ± SE (n=4) with different lowercase letters indicate significant difference between P treatments of each cultivar by Tukey's HSD test at $p<0.05$.

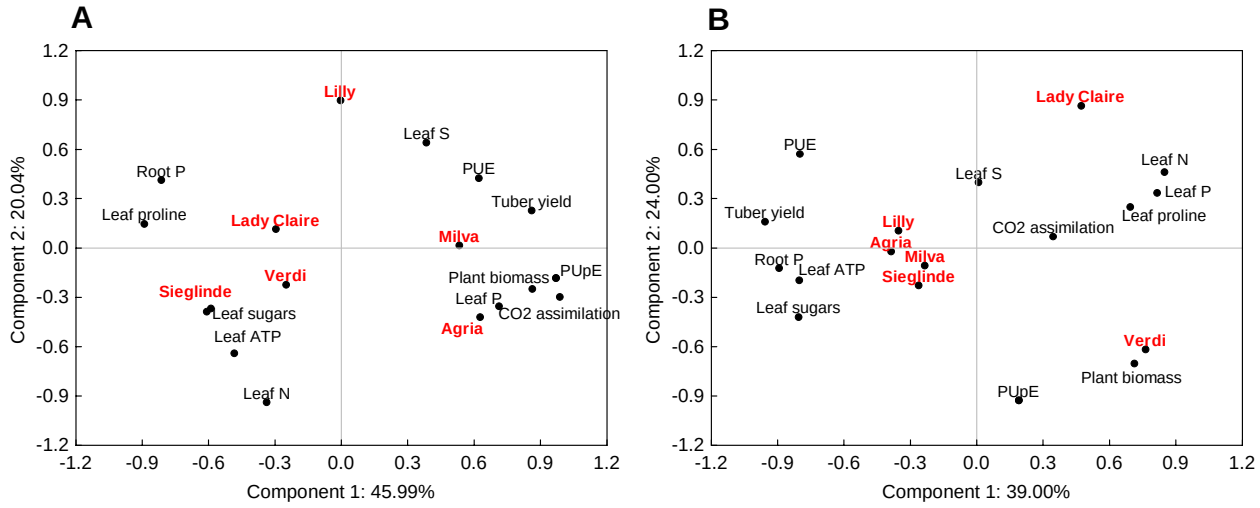
Supplementary Table 4.6. Means ±SE of leaf photosynthesis and biochemical properties

	Cultivar					
	Agria	**Lady Claire**	**Lilly**	**Milva**	**Sieglinde**	**Verdi**
CO_2 assimilation rate at 35 DAE (µmol m^{-2} s^{-1})						
P_{low}	6.75±2.57	1.62±0.50	1.47±0.64	4.84±0.85	-	1.29±0.40
P_{med}	12.41±1.77	7.50±1.95	14.15±1.46	15.92±1.02	5.12±1.18	7.42±2.41
P_{high}	8.49±4.72	12.03±1.91	10.13±1.93	12.82±2.73	11.92±2.52	11.30±1.61
CO_2 assimilation rate at 53 DAE (µmol m^{-2} s^{-1})						
P_{low}	2.76±0.46	6.16±2.47	1.54±0.04	4.29±1.11	2.89±0.70	4.79±1.00
P_{med}	14.47±1.75	11.77±0.33	11.53±0.23	13.35±4.87	7.87±5.24	3.04±2.91
P_{high}	7.97±0.58	12.32±4.13	11.53±1.35	9.20±0.88	10.66±4.53	11.82±0.39
CO_2 assimilation rate at 70 DAE (µmol m^{-2} s^{-1})						
P_{low}	7.71±1.99	-	-	9.94±4.61	-	4.40±2.32
P_{med}	12.28±1.00	13.27±2.00	11.80±0.98	11.42±0.85	5.83±1.16	10.46±2.03
P_{high}	10.24±3.15	11.57±1.93	13.66±1.01	7.83±1.43	10.00±2.16	12.16±2.02
Stomatal conductance at 35 DAE (mmol m^{-2} s^{-1})						
P_{low}	0.77±0.32	0.33±0.10	0.57±0.08	0.65±0.22	0.00±0.00	0.31±0.15
P_{med}	1.51±0.34	1.13±0.23	1.81±0.29	2.18±0.13	0.63±0.12	0.84±0.29
P_{high}	1.17±0.73	1.36±0.27	1.08±0.22	1.51±0.40	1.42±0.37	1.14±0.24
Stomatal conductance at 53 DAE (mmol m^{-2} s^{-1})						
P_{low}	0.53±0.02	0.99±0.62	0.45±0.04	0.82±0.14	0.59±0.10	0.90±0.20
P_{med}	2.65±0.60	2.08±0.47	2.53±0.29	2.18±0.51	1.44±0.38	3.46±2.88
P_{high}	1.54±0.25	1.85±0.37	1.98±0.61	1.60±0.04	1.75±0.76	1.98±0.06
Stomatal conductance at 53 DAE (mmol m^{-2} s^{-1})						
P_{low}	1.00±0.19	0.00±0.00	0.00±0.00	1.30±0.72	0.00±0.00	0.53±0.22
P_{med}	1.61±0.20	2.24±0.30	1.76±0.27	1.33±0.23	0.75±0.10	1.18±0.24
P_{high}	1.29±0.39	1.69±0.37	1.92±0.32	0.86±0.15	1.37±0.41	1.32±0.25
Intercelluar CO_2 at 35 DAE (ppm)						
P_{low}	182.83±3.84	282.07±15.00	326.70±34.73	204.10±20.66	-	306.76±30.00
P_{med}	202.02±21.37	243.46±26.79	220.94±11.37	231.55±3.30	206.34±10.49	186.24±6.38
P_{high}	211.24±17.47	198.39±6.14	175.93±6.72	191.71±20.63	194.24±15.86	161.98±14.86
Intercelluar CO_2 at 53 DAE (ppm)						
P_{low}	219.69±23.07	178.03±3.71	284.75±23.66	227.80±69.18	284.33±21.00	172.23±13.00
P_{med}	231.11±9.93	230.19±13.90	208.05±29.12	216.28±37.90	257.55±20.28	100.84±62.10
P_{high}	174.77±20.69	190.22±31.21	185.79±3.37	169.62±5.66	176.18±51.06	177.03±18.90
Intercelluar CO_2 at 70 DAE (ppm)						
P_{low}	220.38±7.90	-	-	207.46±24.55	-	257.77±57.03
P_{med}	215.05±11.00	263.23±12.00	241.96±10.05	190.91±20.31	217.06±23.82	189.83±5.13
P_{high}	212.76±19.12	230.02±15.75	226.86±16.94	182.70±2.86	211.89±22.56	178.63±8.75
ATP (nmol g^{-1} FW)						
P_{low}	10.56±3.74	5.52±1.23	3.68±0.58	2.25±0.80	18.67±4.28	7.45±4.12
P_{med}	22.11±4.34	13.41±5.85	13.85±2.40	24.50±7.32	16.40±3.02	13.58±4.98
P_{high}	45.93±8.26	25.43±6.48	35.25±9.29	31.27±4.90	44.58±6.53	25.38±4.34
Protein (mg g^{-1} FW)						
P_{low}	0.92±0.13	2.66±1.65	0.67±0.09	0.85±0.17	1.06±0.19	1.16±0.15
P_{med}	1.16±0.12	1.08±0.11	0.92±0.06	0.99±0.13	0.77±0.15	1.24±0.15
P_{high}	0.73±0.07	1.42±0.11	0.89±0.11	0.96±0.15	0.87±0.14	1.22±0.18
Chlorophyll (mg g^{-1} FW)						
P_{low}	1.25±0.07	0.97±0.04	0.93±0.05	0.87±0.29	0.90±0.06	1.11±0.11
P_{med}	1.30±0.03	0.95±0.05	1.03±0.06	1.28±0.02	1.20±0.02	1.50±0.09
P_{high}	1.26±0.06	1.11±0.03	1.05±0.09	1.18±0.05	1.26±0.12	1.41±0.10
Proline (µmol g^{-1} FW)						
P_{low}	5.18±0.77	6.88±0.97	8.35±0.49	4.95±0.38	9.80±1.85	7.74±1.12
P_{med}	2.20±0.36	8.19±0.54	5.22±0.74	4.07±0.62	6.95±0.48	6.64±1.20
P_{high}	2.15±0.45	4.46±0.72	3.86±0.97	2.64±0.72	3.62±0.29	4.18±0.93
Sucrose (mg g^{-1} DM)						
P_{low}	7.64±1.29	6.65±0.76	10.57±0.80	6.88±0.29	19.71±3.49	9.09±2.09
P_{med}	7.53±1.95	12.51±2.72	14.42±0.89	12.80±0.70	13.07±1.30	10.77±1.45
P_{high}	7.23±1.67	10.84±1.97	15.54±2.49	14.88±1.90	14.94±1.37	10.34±0.34
Reducing sugar (mg g^{-1} DM)						
P_{low}	5.16±0.38	2.77±0.72	2.55±0.71	3.05±0.45	25.50±9.39	3.92±0.63
P_{med}	21.37±4.56	5.39±0.81	29.93±6.16	29.80±5.86	20.95±5.49	10.73±2.85
P_{high}	38.41±7.20	11.65±1.81	44.89±6.88	36.48±1.60	47.41±3.86	23.93±5.80

Supplementary Table 4.7. Analysis of variance (ANOVA) of tuber quality of three potato cultivars with the application of P_{low}, P_{med}, and P_{high}

	ANOVA		
	Cultivar (C)	**P level (P)**	**C x P**
Dry matter	***	***	*
Starch	*	***	ns
Protein	***	***	ns
Soluble sugars	***	ns	ns
Ascorbic acid	ns	**	ns
TPC	*	***	**
TFC	ns	***	ns
DPPH	ns	***	ns
TEAC	*	***	*
Tuber minerals			
C	ns	***	ns
K	ns	***	ns
S	*	***	ns
Ca	**	***	**
Mg	*	***	**
Mn	**	***	ns
Fe	**	ns	ns
Zn	**	***	**
Tuber ions			
Phosphate	***	**	***
Nitrate	***	**	*
Sulfate	***	ns	**

ns, *, **, and *** indicate non-significance and significance at $p<0.05$, $p<0.01$, and $p<0.001$, respectively.



Supplementary Figure 4.3. Principal component analysis of traits associated with P efficiency of cultivars under (**A**) P_{low} and (**B**) P_{high} conditions. The leaf parameters such as P, S, N, proline, sugars, ATP, and CO2 assimilation rate were taken at 53 DAE. Root P, plant biomass, tuber yield, PUpE, and PUE were measured on plants at 87 DAE. PUpE= P uptake efficiency, PUE= P use efficiency.

Supplementary Table 4.8. Means±SE of quality parameters of tubers

	Cultivar		
	Agria	**Lilly**	**Miva**
Dry matter (%)			
P_{low}	15.30±0.20	14.43±0.67	14.59±0.63
P_{med}	19.33±0.26	18.62±0.43	21.39±0.44
P_{high}	21.43±0.22	19.58±0.48	21.55±0.44
Starch (% DM)			
P_{low}	53.41±0.83	51.26±1.71	53.77±2.10
P_{med}	62.16±1.07	62.22±1.08	62.75±1.05
P_{high}	69.70±0.92	63.56±1.21	65.91±1.05
Protein (% DM)			
P_{low}	16.11±0.77	20.39±1.13	15.01±0.54
P_{med}	12.72±0.99	13.10±0.71	9.10±0.38
P_{high}	9.41±0.86	11.03±0.80	8.44±0.38
Soluble sugars (mg g^{-1} DM)			
P_{low}	73.69±3.08	60.81±19.08	103.28±12.32
P_{med}	67.50±4.84	73.58±1.49	89.56±6.17
P_{high}	62.15±4.00	77.50±4.09	85.46±3.87
Ascorbic acid (mg g^{-1} FW)			
P_{low}	0.17±0.01	0.18±0.01	0.19±0.01
P_{med}	0.23±0.02	0.23±0.01	0.21±0.00
P_{high}	0.21±0.02	0.24±0.01	0.19±0.00
TPC (mg g^{-1} DM)			
P_{low}	0.96±0.09	1.34±0.17	1.46±0.03
P_{med}	1.06±0.05	0.82±0.03	1.05±0.11
P_{high}	0.85±0.05	0.78±0.05	0.92±0.03
TFC (mg g^{-1} DM)			
P_{low}	0.53±0.05	0.61±0.01	0.50±0.04
P_{med}	0.48±0.08	0.50±0.02	0.50±0.03
P_{high}	0.39±0.05	0.27±0.02	0.24±0.01
DPPH (µmol g^{-1} DM)			
P_{low}	6.43±1.19	6.67±0.30	8.82±1.07
P_{med}	4.51±0.42	3.88±0.18	4.28±0.29
P_{high}	4.31±0.81	3.86±0.47	3.77±0.16
TEAC (µmol g^{-1} DM)			
P_{low}	6.03±0.75	6.61±0.12	9.38±0.90
P_{med}	5.32±0.28	4.73±0.58	4.99±0.34
P_{high}	4.71±0.39	4.87±0.46	4.93±0.13

Chapter 5. General discussion

5.1. Background and research questions

Low phosphorus (P) availability in the soils is a major limiting factor for potato, which is a crop that demands high P (Koch et al., 2019b). Increasing P application often results in low P use efficiency (PUE) and contributes to eutrophication (Vance et al., 2003; MacDonald et al., 2011). Along with the concern over the scarcity of P resource (Cordell and White, 2014), improving PUE of potato could potentially reduce P fertilizer application and minimize surface water pollution. Several studies were conducted to assess potato responses under low P availability (Dechassa et al., 2003; Fernandes and Soratto, 2012; Wacker-Fester et al., 2019), but the obtained information was little in regard to plant biomass allocation, P uptake, and PUE. A comprehensive study from morphological to molecular level of plants is required to elucidate P efficiency mechanisms of potato under varying P availability—especially under limited P supply. A number of studies have also attempted to demonstrate the benefits of plant growth-promoting rhizobacteria (PGPR), which improve plant growth and nutrient uptake in many crops (Vacheron et al., 2013; Zaidi et al., 2015) as well as in potato (Naqqash et al., 2016; Sheridan et al., 2017), but the effect of PGPR on P uptake and PUE in potato under P deficiency has not been reported. There is also limited information on the effect of P deficiency on tuber quality. However, studies on the effect of P deficiency on plant physiology and tuber quality have been conducted separately. Consequently, information on the possible interactions between plant adaptation responses and tuber quality under P deficiency is limited.

The main objective of this dissertation was to characterize plant growth, physiology, and tuber quality of potato as influenced by P availability, cultivar, and PGPR. The information provides further insights on cultivar adaptation responses to varying P availability and PGPR inoculation. To achieve the overall goal of this dissertation, the following research questions were developed:

1. To what extent P deficiency and toxicity modulate plant biomass allocation, uptake of minerals, and metabolite compounds?
2. How effective are PGPR co-inoculation and single strain addition in enhancing P uptake under low P supply?
3. What are the possible factors determining plant PUE and root morphology under low and high P supply?
4. How does limited P availability influence tuber yield, quality-relevant tuber compounds and antioxidant capacity of different cultivars?

The experiments were conducted under hydroponic and pot conditions to minimize the interference of P immobilization and buffering capacity of the soils as well as the well-established microbial community, which usually occur in field conditions.

5.2. Key findings

The present study has contributed new knowledge on the response of potato to P availability and PGPR inoculation as below:

- Potato was sensitive not only to low P availability, but also to high P supply. Both P conditions reduced plant biomass and P concentration as well as altered the concentrations of other mineral nutrients and metabolite compounds in various parts of the plant, especially in young leaves and roots (**Chapter 2**).
- Based on the capability of plants in P uptake and tuber setting, P-efficient and P-inefficient cultivars under P deficiency were identified (**Chapter 3 and 4**).
- Although P utilization efficiency (PUtE) was impaired under P deficiency, plants attempted to accumulate osmolytes and recycle internal P in leaves, which was necessary for maintaining photoassimilate production to ensure plant viability and translocation of assimilates to roots. At higher P availability, both P uptake efficiency (PUpE) and PUtE contributed similarly to PUE (**Chapter 3 and 4**).
- Total P uptake of potato cultivars was influenced by modifications of root morphology, which was controlled by the concentrations of P, sugar, and indole-3-acetic acid (IAA) in roots (**Chapter 3**).
- Under P deficiency, the inoculation of PGPR—as mixture as well as individual strain—enhanced root morphology and, consequently, increased PUE. However, the increments were more pronounced for P-inefficient than for P-efficient cultivars (**Chapter 2 and 3**).
- Limited P availability substantially reduced tuber yield of potato; nevertheless, phytochemical compounds in tubers were higher in P-efficient cultivars under this condition (**Chapter 4**).

5.3. General discussion

P is involved in many processes of plant growth and metabolism (Hawkesford et al., 2012). Therefore, plants develop different strategies by modifying morphological and physiological traits in responses to low and high P availability. Characterization of these responses help to identify important traits associated to PUE, which can be distinguished into two main important mechanisms—PUpE and PUtE. PUpE is defined as the capacity of a plant to absorb the applied P and it is strongly influenced by root characteristics (Veneklaas et al., 2012). PUtE is the ability of plants to produce biomass or yield from the absorbed P and it is also referred as internal PUE, which is related to plant physiological and biochemical adaptation (van de Wiel et al., 2016).

5.3.1. Plant morphological adaptation strategies in modifying root system and P uptake under varying P availability

Improved P uptake is important to ensure plant growth under P deficiency and to minimize P loss to the environment under high P supply. Under P deficiency, plant biomass was significantly reduced in all studies (Chapter 2-4), whereas root growth was less affected causing high root-to-shoot ratio (Figures 2.2D and 3.1A-D). This could be explained by preferential biomass allocation to the roots to improve root system enhancing P uptake and PUpE (Wang et al., 2015). Modification of root morphology—such as total root length, root surface area, and root volume—is an energy-expensive process, and it is associated with the physiological

status of plants such as P and sugar in roots (Supplementary Figure 2.2). However, P and sugar concentrations in roots were significantly reduced under P starvation (Figures 2.3A, 2.5, and Tables 3.1, 3.2), which indicate a shortage of photoassimilates for translocation to the roots. This assumption was confirmed in Chapter 4 showing low net CO_2 assimilation rate, stomata conductance, and ATP concentration in young leaves under low P supply (Figures 4.4A, B and 4.5A). This results are in contrast to the observations of Wissuwa et al. (2005) in rice, who assumed that limiting photosynthetic source is less important under P deficiency because plants are able to produce enough assimilates under low P availability. The differences between these observations could be explained by the higher plant biomass resulting in stronger sink (yield) demand of potato than rice (Xu et al., 2019). Interestingly, the P-efficient cultivars (Agria, Lilly, and Milva) had substantial net CO_2 assimilation rate and/or stomata conductance (Figure 4.4A,B), which suggest that these cultivars attempted to maintain photosynthetic activities under P deficiency to produce sugars necessary for shoot growth and for their translocation to various parts of plant. As a result, these cultivars had relatively high total P uptake and the PUpE was also enhanced under P starvation (Figure 4.2G). However, the P-inefficient cultivars (Lady Claire, Sieglinde, and Verdi) were not able to produce tubers and had relatively low CO_2 assimilation rate and PUpE under P deficiency. This finding implies that Lady Claire, Sieglinde, and Verdi were unable to translocate sugar to roots although Verdi, for example, had high sugar concentration in young leaves at limited P availability. A comprehensive study to compare P-efficient (Milva) and P-inefficient (Lady Claire) cultivars showed that Milva had relatively high P and sugar concentrations in young leaves as well as in roots, which resulted in improved total root length, root surface area, and root volumes compared to Lady Claire under P deficiency (Chapter 3). Wissuwa et al. (2005) also reported that a tolerant cultivar under P deficiency preferentially distribute more P to roots to stimulate root growth and, ultimately, P uptake. These results suggest the increasing importance of a large root system under decreasing P availability. Similar to the observation of Mori et al. (2016), under less severe P deficiency, plants adapt another strategy by enhancing specific P uptake and root efficiency. Improved root efficiency is associated with the P uptake process at the root surface, which may not be efficient under severe P deficiency (Fernandes et al., 2014). In addition to this finding, our results show the importance of root morphology and root efficiency under high P availability to improve P uptake (Figures 3.3H, I and Supplementary Figure 3.1). Increased root biomass under high P supply could also serve as storage organ of excess P in plants (Shane et al., 2004). In the present study, Milva had a higher root biomass under high P supply, but the P concentration and P contents in roots were lower than in Lady Claire (Figure 3.1C and Table 3.2). The less accumulation of P in roots of Milva may be caused by reduced *StPHT1;7* transcription in roots (Figure 3.2E) to avoid toxic P condition. Modulation of *AtPHT1;7* in P uptake was also reported in Arabidopsis, which is homologous to *StPHT1;7* (Cao et al., 2020). This could indicate the sensitivity of Milva to high P supply, which was also evident when P supply was higher than 30 mg P L^{-1} resulting in biomass reduction (Chapter 2). Although root biomass, total root length, and root surface area of Lady Claire was lower than of Milva under high P, Lady Claire had higher P uptake, which was associated with specific P uptake and root efficiency under this condition. Therefore, improved total root length, root surface area, and root volume are important parameters enhancing P uptake under P deficiency.

Under less severe P deficiency and high P availability, specific P uptake and root efficiency play a significant role in controlling P uptake. Modification of these root characteristics and P uptake are associated with physiological responses of plants.

Despite modification of root morphology, plants also attempt to increase P availability in the rhizosphere under P deficiency (Wang et al., 2015). Our results show the accumulation of metallic ions—such as Fe and Zn—in various part of plants and less organic acids in the roots under P deficiency (Figures 2.3G,I and 2.5). The increased uptake of Fe and Zn could be a plant strategy to decrease their amounts in the rhizosphere and to minimize the formation of complexes between phosphate and the ionic form of these minerals (Hirsch et al., 2006). Although shortage of carbohydrates is mainly responsible for the decreased amount of organic acids in roots under P deficiency (Chia et al., 2000; Misson et al., 2005), the excretion of organic acids to the rhizosphere may also contribute to the low concentrations in roots. Wang et al. (2015) also reported an increased organic acid exudation from potato roots under P starvation. Organic acid secretion into the rhizosphere could help to mobilize P from oxides and hydroxides of Al and Fe as well as Ca-phosphate complex (Neumann and Römheld, 2007; Wang et al., 2015). These physiological adaptations are helpful to increase P availability for root uptake.

5.3.2. Plant physiological adaptation in improving PUtE and ensuring plant growth under varying P availability

Our results reveal an impaired PUtE under P deficiency, independently of cultivar differences (Figure 3.3B). Low shoot DM under P deficiency was mainly responsible for reduced PUtE. Dissanayaka et al. (2021) also showed in their review article under P deficiency conditions a reduced PUtE, which may be caused by inefficient energy metabolism for P translocation to shoots. The high PUtE of both cultivars—Lady Claire and Milva—under 2 mg L^{-1} and 5 mg L^{-1} of P supply indicates the relative importance of PUtE to improve PUE under sub-optimal and optimal P conditions, which was also observed also by Wacker-Fester et al. (2019) and Sandaña (2016). Nevertheless, PUtE of Milva was significantly higher than of Lady Claire under less severe P deficiency in the present study. This finding indicates the efficiency of Milva in P utilization under low P supply. However, the assessment of PUtE is merely based on the level of the shoot growth. In response to low P amounts in shoots, plants have also developed different mechanisms—for instance, recycling internal P, modifying uptake of other minerals, and modulating metabolic pathways—to enhance internal PUE and buffer the stress induced by P deficiency.

In plants, P exists in forms of inorganic orthophosphates (PO_4^{3-} or P_i) and organic phosphate esters (Veneklaas et al., 2012). We found a lesser reduction of leaf P_i compared to leaf P under P deficiency (Figure 4.2 A, B). This could be caused by increased P_i fraction under this condition. Under P starvation, plants detect cytosolic P_i shortage through recycling the entire P in the vacuole, to increase P_i concentration for efflux into cytosol and chloroplast (Shen et al., 2011; Long et al., 2019). Therefore, P translocation to active photosynthetic tissue and internal P recycling could be an adaptation of potato cultivars to ensure

photosynthesis under stress induced by P deprivation. Similarly observed by Lei et al. (2011), plants also attempted to maintain sucrose level in young leaves (Figure 4.5E), which is important to induce genes associated with mobilizing plant internal P. Since P and P_i were limited under P deficiency, plants increased the uptake of S to fulfill the S-demand of sulfolipid generation for replacing phospholipid and to balance leaf anion-to-cation ratio under low phosphate conditions (Misson et al., 2005; Rouached, 2011). As a results, S and sulfate concentrations in young leaves were increased (Table 4.1).

Under P deficiency, there was also an accumulation of secondary metabolites and amino acids in young leaves and roots (Chapter 2). This accumulation was in similar ranges as reported previously for the accumulation of total flavonoids (TFC) in tomato and Arabidopsis (Stewart et al., 2001), of leaf anthocyanins (TAC) in sunflower (Gunes and Inal, 2009), and total free amino acids (TAA) in barley (Criado et al., 2017), as well as of amino acids in barley and lupin (Huang et al., 2008; Müller et al., 2015). Furthermore, under P toxicity these compounds were also accumulated, but to a lesser extent compared to those under P deficiency. The upregulation of TFC and TAC in leaves act as protection mechanism against light-induced oxidative damage under deficient- and toxic-P conditions (Fini et al., 2011). Furthermore, amino acids serve as compatible osmolytes involves in pH regulation as well as detoxification of reactive oxygen species (ROS) and acts as a nitrogen and carbon reserve for the synthesis of specific enzymes (Ali et al., 2019). For instance, proline was produced in response to stress induced by P deficiency (Figures 2.4E and 4.5D). Proline is synthesized from glutamate in the cytosol; nevertheless, depending upon the recovery from stress, proline is rapidly oxidized again into glutamate, while ATP is generated during this oxidation process to maintain leaf viability (Launay et al., 2019). The increased TFC may also be controlled by aromatic amino acids biosynthesis (Ali et al., 2019). Especially in roots, we observed an increased amount of phenylalanine and tyrosine under P deficiency and P toxicity. This is probably related to the enhanced phenylalanine/tyrosine ammonia lyase activities under stress conditions, which release nitrogen for phenylalanine and tyrosine metabolism and contribute to protein synthesis. Meanwhile, the carbon products are transferred into flavonoid biosynthesis pathway via 4-coumaroyl-Coenzyme A (Stewart et al., 2001). Therefore, the higher secondary metabolites and amino acids in leaves and roots may lead to potato plants being more tolerant under deficient- and toxic-P conditions. These adaptation responses are necessary to ensure plant viability and internal PUE, regardless of the increased plant biomass, especially under P deficiency.

5.3.3. Benefit of PGPR in P deficiency amelioration

Under hydroponic conditions, where the described experiments were conducted, the addition of PGPR mixture and the *B. subtilis* strain increased plant biomass and root growth, which finally improved P uptake, PUpE, and PUE. The increased P uptake under P deficiency could be caused by improved root growth and the ability of the PGPR to take up the very low P concentration in the nutrient solution, which could not be utilised by the plant roots. In agreement with Menéndez and Paço (2020), the diverse PGPR strain co-inoculation was more beneficial on plant growth than single strain inoculation. The co-inoculation of five diverse PGPR strains increased total root length and root surface area up to 2-fold compared to those under *B. subtilis* addition (Table

2.2 and Figure 3.6C,D). Nevertheless, this result also suggests the efficiency of *B. subtilis* inoculation as single strain in promoting root growth. Plant-PGPR symbiosis is a carbon-expensive process for the plants (Smith and Read, 2008); therefore, they try to maximize their use of the limited carbohydrate, especially under limited P condition. Based on the two studies (Chapters 2 and 3), the increments in plant biomass, root morphology, P uptake, and PUE by PGPR inoculation were more pronouncing under P deficiency condition (0 and 0.5 mg P L^{-1}) than under higher P availability (1 and 2 mg P L^{-1}). This indicates the less reliance of plants on PGPR to improve growth and nutrient uptake. Considering cultivar difference, the results in Chapter 3 show that Lady Claire had greater responsiveness in total P uptake as well as total root length and root surface area to *B. subtilis* than Milva. This could be explained by the P inefficiency of Lady Claire under low P supply; therefore, the benefit of establishing a symbiosis was higher in Lady Claire than in Milva to ameliorate P-deficient condition. In contrast, Milva was more tolerant to P deficiency; therefore, the growth of this cultivar was less dependent on the benefits that *B. subtilis* could provide to improve root growth and P uptake.

The mechanism underlying PGPR-modulated P uptake and PUpE under low P supply could be elucidated through enhanced total root length, root surface area, specific P uptake, and root efficiency. In Chapter 3, IAA concentration in the roots under *B. subtilis* was higher than in non-inoculated plants (Figure 3.6G). At a low concentration, IAA can stimulate primary root growth, while high IAA concentrations enhance root hairs and lateral root growth (Vacheron et al., 2013). Consequently, total root length and root surface area are improved to scavenge the limited amount of P in the nutrient solution. Consequently, root P uptake was enhanced. Besides the modification in root system to increase P uptake, PGPR—especially *B. subtilis*—also regulated P distribution to shoots through enhanced genes (*StPHT1;1*, *StPHT1;7*, and *StPHT2;1*) responsible for P translocation in leaves (Table 3.3), which help to increase PUtE. Additionally, PGPR improved plant tolerance under P deficiency by increasing amino acid concentrations (Chapter 2). Mhlongo et al. (2020) reported the regulation of aromatic amino acids in tomato roots following *Pseudomonas sp.* inoculation. These amino acids are important for secondary metabolite biosynthesis in connection with cell wall integrity. Nevertheless, an amino acid such as tryptophan is the precursor of IAA, which facilitates plant root elongation (Weston et al., 2012; Vacheron et al., 2013). Therefore, beside improved P uptake and translocation, PGPR also interfere with metabolite biosynthesis to increase plant tolerance under limited P availability.

5.3.4. Relationship between plant physiological adaptations and tuber quality under P deficiency

Impaired CO_2 assimilation under P deficiency (Chapter 4) implies a shortage of carbohydrate for translocation to tubers (Lemoine et al., 2013). Therefore, differences in tuber formation provide an opportunity to identify potato cultivars for their efficiency in using limited available carbohydrates under P starvation. P-inefficient cultivars (Lady Claire, Sieglinde, and Verdi) were not able to produce tubers under low P supply. These cultivars were presumably able to conserve the sparingly available P and carbohydrates in the plant for viability, but they were unable to provide P and carbohydrates for tuber formation. Interestingly, the P-efficient cultivars were also able to maintain substantial CO_2 assimilation and set tubers under P deficiency. However, yield, dry matter, and starch contents of the tubers were significantly reduced. Nevertheless, we found

increased concentrations of sugars and secondary metabolites, and higher antioxidant capacity in tubers under P deficiency. Since the three P-efficient cultivars are table potatoes, the limited carbohydrate allocated to tubers might be converted to sugars rather than into starch, causing increased sugar but reduced starch content under P deficiency (Beckles and Thitisaksakul, 2011). The increased concentrations of total flavonoids and total phenolics in tubers (Figure 4.7F,G) also indicate that P deficiency stimulated the secondary metabolites not only in leaves, but also in tubers, which contributed to enhanced antioxidant capacity. The accumulation of these antioxidants is caused by oxidative stress induced by P deficiency, which provokes numerous plant response reactions for the antioxidant systems (Wang and Frei, 2011). Furthermore, stress induced by P deficiency increased protein and mineral concentrations in tubers (Figure 4.7C and Table 4.2). These findings complement the literature review of Wang and Frei (2011) by giving further insights toward increased knowledge on tuber quality of potato under deficient-P stress conditions. The increased concentrations of phytochemicals and minerals in the tuber are a valuable contribution to promote human health and physical well-being (Andre et al., 2007; Wang and Frei, 2011). These results also point out the possibility of reducing P supply to increase PUE and improve the quality of tubers. However, it remains a challenge to simultaneously improve PUE and increase dry matter and starch contents in tubers. Therefore, there is still a need to explore additional agronomic practices to minimize the reduction in yield, dry matter, and starch content of potato tubers when P availability is limited.

5.4. Future research directions

The results presented this dissertation provide opportunities for future studies as suggested below:

- **Assessing molecular mechanisms underlying sugar translocation and metabolism.** Sugar allocation to roots is important to modify root system; nevertheless, substantial amount of sugars (i.e. sucrose) has to be maintained in leaves to ensure growth and induce genes associated with mobilizing of plant internal P. Therefore, genes associated with sugar translocation from photosynthetic active tissues to roots should be assessed in order to reveal their responses in P-efficient and P-inefficient cultivars under deficient- and toxic-P conditions. Furthermore, it is potential to assess the phosphorylated sugars and their roles under P deficiency and toxicity.

- **Exploring the potential roles of amino acids under stress induced by P deficiency and toxicity.** Plants attempted to accumulate the amount of amino acids to increase their tolerance under stress induced by P deficiency and toxicity. Nevertheless, the functions of specific amino acids under P deficiency and toxicity should be empirically elucidated through the application of amino acids. Moreover, amino acid synthesis is an energy consuming process, which demands substantial amount of the limited carbohydrates under P stress. Therefore, an application of exogenous amino acids could provide options to ameliorate P stress in potato plants.

- **Evaluating P transporter genes under varying P availability**. We investigated the transcription levels of important P-transporter genes (*StPHT1;1*, *StPHT1;7*, and *StPHT2;1*) in leaves and roots; however, the obtained information was rather limited. This might be due to the timing of leaf and root sampling. Therefore,

the times from onset of P treatments to plant tissue sampling should be shortened, i.e. after 24 hours of the onset of treatments or nutrient exchange because P in nutrient solution is quickly taken up by plants within 24 hours under low P availability. Furthermore, more P-transporter genes could be studied and their responses in tubers and stems, besides leaves and rotos, should be investigated.

- **Assessing the efficiency and compatibility of PGPR strains.** We were able show positive effects of five diverse PGPR stains improving plant growth, root system, and P uptake. Furthermore, inoculation of *B. subtilis* as single strain incremented root morphology up to 50% compared with those under PGPR mixture co-inoculation. Therefore, it is necessary to elucidate the efficiency of individual strains in comparison to the mixture as well as their compatibility when they are co-inoculated.

- **Investing the factors associated with PGPR-mediated IAA in roots.** Inoculation of *B. subtilis* increased IAA concentration in roots. Furthermore, *in-vitro* determination of IAA indicates that the five bacterial strains were able to produce IAA at different levels. Therefore, separated experiments should be conducted to reveal (i) how PGPR directly interferes phytohormone pathway and (ii) to what extent plant roots are able to uptake IAA produced by PGPR at very low concentration in the nutrient solution. Additionally, based on phytohormone extraction in root samples glycoalkaloids were present at a great extent. Identification and determination of glycoalkaloids may help to explain their roles in improving plant tolerance under stress induced by P deficiency and the interference by PGPR.

- **Exploring agronomic practices to minimize yield reduction and increase tuber quality under P deficiency**. Reduced P supply potentially increased PUE and tuber quality; however, tuber yield was substantially decreased. We also showed the influence of PGPR in increasing plant growth under P deficiency; therefore, inoculation of PGPR may have the potential to minimize yield reductions and improve tuber quality. However, this assumption needs to be verified by empirical studies.

- **Long-term investigation on the effect of P availability and PGPR inoculation on potato under field conditions.** The experiments presented in this dissertation showed the potato responses under P availability and PGPR inoculation under hydroponic and pot conditions. Studies in field conditions are also important to reveal a long-term effect of P supply and PGPR inoculation and how other factors—such as organic P pool and existing soil microbiome—interfere these interventions.

Chapter 6. Conclusion and summary

6.1. Conclusion

The studies presented in this dissertation showed the sensitivity of potato under low and high P availability. Both P conditions inhibited plant biomass, interfered the uptake of minerals, increased secondary metabolite and amino acid concentration in leaves and roots, and decreased organic acids in roots. Under P deficiency, PUE was strongly influenced by PUpE through improved root system, which was controlled by physiological status of plants to allocate sugar and P to roots. In addition to the importance of root morphology, enhanced root efficiency was also necessary to increase P uptake at sub-optimal and high P supply. Although PUtE was impaired under P deficiency—caused by inefficient energy metabolism in P translocation—plants developed other strategies by recycle internal P and maintain osmolytes in leaves. These adaptations were necessary to maintain photosynthesis under limited P availability. There was also a high variation of cultivars in response to low and high P availability. We were able to identify P-efficient (Agria, Lilly, and Milva) and P-inefficient (Lady Claire, Sieglinde, and Verdi) cultivars under limited P supply. Nevertheless, Lady Claire and Verdi may be efficient in P uptake under high P availability, caused by their high biomass and root efficiency. The studies also revealed the positive effect of PGPR inoculation—by using a mixture of PGPR stains as well as *Bacillus subtilis* single strain—in improving root morphology, P uptake, and PUE under P deficiency in hydroponic conditions. The effect was more pronouncing in P-inefficient than P-efficient cultivars. Additionally, the increments in plant growth and PUE by PGPR was observed under severe P deficiency condition while the effect was less detectable at sub-optimal P or higher P availability. Based on our study, reducing P supply increased PUE and quality-relevant tuber compounds of potato. However, tuber yield, dry matter and starch contents were strongly reduced under P deficiency, which was driven by limited carbohydrate. therefore, there is opportunity to simultaneously increase PUE and tuber quality of potato by reducing P supply along with the use of P-efficient cultivars. The adaptation responses of potato under P availability and PGPR inoculation reported in this study are helpful to identify important traits and strategies to improve PUE and tuber quality in potato.

6.2. Summary

Plants develop different strategies in response to low and high P supply. Potato has a low P use efficiency (PUE) compared to other crops, which is caused by its limited root system. Therefore, it is necessary to gain more knowledge of the morphological and physiological processes associated with P deficiency and toxicity in potato, as well as to explore an alternative approach to ameliorate the P-deficient conditions. This dissertation aimed to characterize plant growth, physiology, and tuber quality of potato as influenced by cultivar, P availability, and PGPR. The results revealed a reduction in plant height and biomass by 60–80% under P deficiency compared to P optimum. P deficiency and toxicity conditions also altered the mineral concentration and allocation in plants due to nutrient imbalance. The stress induced by both—P deficiency and toxicity—was evident from an accumulation of proline and secondary metabolites. Furthermore, root metabolite profiling revealed that P deficiency reduced concentrations of sugars and organic acids by 20-90%, but increased amino acids concentrations by 1.5-14.8 times. However, the effect of P toxicity on metabolic changes in roots was less pronounced. These responses were also different between the cultivars. We found the capability of Milva to allocate biomass, P, and sugars to roots under low P supply, causing high P uptake and PUE. In contrast, Lady Claire was not efficient in P uptake under low P levels, but this cultivar was more efficient in P uptake under high P availability. Total P uptake of both cultivars was influenced by modifications of root morphology, which was controlled by P, sugar, and indole-3-acetic acid concentrations in roots. A further comprehensive comparison of six potato cultivars also showed a contrasting response of P-efficient and P-inefficient cultivars under low P availability. P-efficient cultivars—Agria, Milva, and Lilly—possessed substantial plant biomass, tuber yield, and high P uptake efficiency under low P supply. The P-inefficient cultivars—Lady Claire, Sieglinde, and Verdi—lacked the ability to form tubers under P deprivation, as well as the ability to efficiently uptake of P at low P level, but they were efficient in P uptake at high soil P levels. Improved PUpE was important for plant tolerance under limited P availability, allowing efficient use of the supplied P. Although PUtE was impaired under P deficiency, plants attempted to maintain ions and osmolytes in leaves, which are necessary for plants to adapt to the stress caused by P deficiency and mobilize leaf inorganic phosphate to increase internal PUE and photosynthesis. The reduction of plant biomass and tuber yield under P deficiency could be caused by the lower CO_2 assimilation. Furthermore, P deficiency significantly decreased tuber yield, dry matter, and starch concentrations in Agria, Milva, and Lilly. Nevertheless, contents of protein, sugars, and minerals in tubers, as well as antioxidant capacity were enhanced under this condition in these cultivars. To ameliorate P deficiency, the inoculation of five diverse PGPR strains as well as *Bacillus subtilis* addition enhanced root morphology, P uptake, and PUE. Additionally, *Bacillus subtilis* also upregulated P transporter genes (*StPHT1;*1 and *StPHT2;1*) in young leaves. These results highlight the important traits and strategies contributing to potato plant tolerance under P deficiency and toxicity and indicate an opportunity to improve P efficiency and tuber quality of potato under deficient conditions by choosing more efficient cultivars.

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List of publications

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Manuscript under revision and completion for submission:

Chea, L., Alhussein, M., Karlovsky, P., Pawelzik, E, Naumann, M. Effect of phosphorus availability and plant growth-promoting *Bacillus subtilis* on phosphorus efficiency of two potato cultivars.

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Chea, L., Pawelzik, E., Naumann, M. (2021). Effect of limited phosphorus availability on plant growth and quality of potato tubers. **E-poster presentation** at the American Society for Horticultural Sciences, 5-9 August, Denver, Colorado, United States.

Chea, L., Pawelzik, E., Naumann, M. (2019). Biomass partitioning and nutrient allocation for potato in response to phosphorus and plant growth-promoting rhizobacteria in a hydroponic system. **Poster presentation** at the International Conference of the German Society for Plant Nutrition, 25-27 September, Berlin, Germany.

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Declaration

I, hereby, declare that:

- This Ph.D. dissertation has not been submitted in the same or similar form to other examination offices.
- I have not applied for a doctoral degree at any other universities.
- This dissertation was conducted independently and without undue assistance

Göttingen, 16 August 2021

Leangsrun Chea