Phosphorus Efficiency in *Brassica napus*: Unlocking Genetic, Physiological, and Root Architecture Innovations

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Abstract
Phosphorus fertilization is essential for canola (*Brassica napus*) growth, but its effectiveness is influenced by environmental factors and soil characteristics. This study examined how seed-placed phosphorus affected canola emergence, biomass, yield, and root-associated microbial diversity during the 2019 and 2020 growing seasons. Higher phosphorus application rates led to reduced canola emergence and plant density, especially when placed near seeds; however, yield remained unchanged in 2020 and increased in 2019. The absence of a yield response in 2020 was likely due to elevated soil moisture and initial phosphorus levels, which improved nutrient availability regardless of fertilization. Moreover, phosphorus fertilization had a notable impact on the canola root microbiome, with a more pronounced effect in 2019 due to drier conditions. During the early vegetative stage, high phosphorus availability enhanced bacterial and fungal diversity, but this influence declined by the flowering stage, indicating a dynamic role of phosphorus in microbial selection. Additionally, canola genotypes exhibited varying phosphorus efficiency, with some maintaining better growth under phosphorus-deficient conditions. These results underscore the intricate relationship between phosphorus fertilization, environmental variability, and plant-microbe interactions, highlighting the importance of tailored fertilization strategies to maximize canola productivity.

Keywords: Br*assica napus*, Physiological, canola productivity

1. Introduction

Fertilizers are essential for boosting plant growth and increasing agricultural yields by supplying vital nutrients necessary for proper development. Plants require macronutrients such as nitrogen (N), phosphorus (P), and potassium (K), along with trace elements like iron, zinc, and manganese, to grow effectively. Fertilizers help to replenish nutrient deficiencies in the soil, ensuring that plants receive the components they need for strong growth, lush foliage, and higher productivity. Their use has been pivotal in meeting the rising global demand for food, especially in intensive agricultural systems where natural nutrient cycles cannot fulfill crop requirements. Fertilizers are available in both organic and synthetic forms, each offering unique benefits and challenges [1]. Organic fertilizers, sourced from materials like compost, manure, and bone meal, enhance soil structure and stimulate microbial activity, promoting long-term soil health. However, their nutrients are released more slowly and unpredictably, which may not align with the needs of high-yield crops. Synthetic fertilizers, in contrast, deliver nutrients in precise formulations and provide quicker results but, if misapplied, can contribute to soil depletion, water contamination, and greenhouse gas emissions. Combining these approaches through integrated nutrient management can support optimal plant growth while reducing environmental harm [2].

The performance of fertilizers depends on various factors, including soil composition, climatic conditions, crop type, and the method of application. For instance, sandy soils often lose nutrients to leaching, requiring careful management to avoid wastage.

Likewise, weather patterns such as temperature and rainfall influence nutrient availability and absorption, emphasizing the importance of region-specific fertilizer strategies. Precision agriculture, utilizing tools like GPS and remote sensing, has become a crucial method for maximizing fertilizer efficiency. By adjusting applications to the specific requirements of different crops and fields, farmers can reduce waste, improve productivity, and lessen ecological damage. While fertilizers offer substantial benefits, their improper use can cause significant issues [3]. Overuse or mismanagement can result in nutrient runoff, which contributes to eutrophication in water bodies and harms aquatic ecosystems. This phenomenon, characterized by excessive algae growth and oxygen depletion, poses serious ecological and economic challenges. Furthermore, dependence on chemical fertilizers can reduce organic matter in soil and disturb microbial biodiversity, ultimately diminishing soil fertility. These issues highlight the importance of adopting sustainable fertilizer practices, such as employing slow-release formulations, implementing crop rotations, using cover crops, and regularly assessing soil health [4]. Technological advancements in fertilizers aim to mitigate these problems. Innovations like controlled-release fertilizers, biofertilizers, and nutrient-enriched nanomaterials are being developed to increase efficiency and reduce environmental impacts. Controlled-release fertilizers gradually supply nutrients in alignment with the growth stages of plants, minimizing losses. Biofertilizers, which involve the use of beneficial microorganisms to improve nutrient availability, are gaining popularity as environmentally friendly alternatives to chemical fertilizers. Similarly, nanotechnology offers potential for more precise nutrient delivery with reduced wastage, though further research is necessary to evaluate its feasibility and long-term effects [5].

Phosphorus (P) is a vital macronutrient for plants, yet its limited availability and inaccessibility in soil significantly hinder plant growth worldwide. To address this issue, P fertilizers are commonly used to enhance soil phosphorus levels [6]. Phosphorus (P), a crucial macronutrient for plant growth and development, is taken up by roots in the form of Pi from the soil. However, Pi in soils is typically scarce and poorly mobile because it primarily exists bound to Ca, Fe, or Al salts or as part of organic compounds [7]. As a result, plants frequently face Pi deficiency in both agricultural and natural environments. Nonetheless, plants have evolved a range of adaptive strategies to cope with Pi scarcity [8]. However, their high cost and low efficiency not only raise the expenses of crop cultivation but also contribute to environmental pollution and the depletion of non-renewable phosphate reserves. Consequently, a sustainable approach to crop production involves developing crop varieties that are more efficient in utilizing phosphorus from natural soil reserves or fertilizer inputs. Nutrient-efficient plants generate greater yields for each unit of nutrient applied or absorbed compared to other plants cultivated under similar agroecological conditions [9]. Phosphorus is abundant in the Earth's lithosphere, but the form plants require—soluble inorganic orthophosphate (Pi)—is poorly soluble and moves slowly through soils [10]. As a result, phosphorus deficiency is common in agricultural lands and natural ecosystems. Soil microbiota further influence phosphorus availability by either competing with plants for this nutrient or forming beneficial associations, such as mycorrhizal relationships, which enhance phosphorus uptake efficiency [11]. The low efficiency of phosphorus fertilizers is another challenge, with only 15–25% of the applied fertilizer being absorbed by plants. The remainder often leaches into the environment, contributing to soil degradation and water eutrophication. Unlike nitrogen, which is abundantly available in the atmosphere, rock phosphate (rock P) is a finite resource. If current consumption patterns continue, a phosphorus fertilizer shortage could become a serious issue by the end of this century [5]. Given these concerns, it is unsurprising that research on phosphorus has gained significant attention, as evidenced by the steady increase in publications from 2000 to 2022 (Figure 1). As agriculture shifts towards more sustainable practices with reduced dependence on synthetic fertilizers, understanding the molecular and physiological mechanisms behind plant adaptations to phosphorus limitation is crucial. Of particular interest is how phosphorus availability influences stomatal development and function. This knowledge could shed light on how plants manage resources to cope with nutrient scarcity while maintaining water-use efficiency, paving the way for developing crop varieties that are both more resilient and productive under low-nutrient conditions.

Brassica napus, a globally significant oilseed crop, is highly sensitive to phosphorus availability in the soil. Given that achieving high seed yield is a primary goal in B. napus breeding, numerous studies have emphasized the importance of identifying phosphorus-efficient germplasm in this crop [12]. Oilseed rape is an important crop for the production of high quality food oil, animal feed and biodiesel. Compared with other oil crops, oilseed rape has a larger demand of plant nutrients, including phosphorus [13]. Although P is one of the most unavailable and inaccessible macronutrients required by plants, it plays a key role in an array of plant processes . P is a primary substrate of photosynthesis and has structural functions in membranes [14]. Oilseed rape (rapeseed; *Brassica napus* L., genome AACC) originated from the natural hybridization of turnip (*Brassica rapa*, genome AA) and cabbage (*Brassica oleracea*, genome CC). It is the leading oilseed crop in Europe and the second most important globally, after soybean (*Glycine max*). While primarily cultivated for food and animal feed, rapeseed has recently garnered growing interest as a source for bio-products, such as biodiesel. Winter varieties are predominantly grown in Western Europe, where mild winters allow them to be sown in late summer, followed by a cold period necessary for flower formation. In contrast, spring cultivars are typically sown at the end of winter and are more common in northern regions, such as Eastern Europe, Canada, Asia, and Australia. [15] Winter varieties generally produce higher seed yields compared to spring cultivars. The average seed yield of rapeseed crops depends on factors like variety type (inbred line, hybrid, or varietal association), environmental conditions, and agricultural practices, including soil management, fertilizer application, and pesticide use. Yields vary by region, ranging from 1.5–2 t/ha in Canada and Eastern Europe (where spring varieties are widely grown under extensive production systems) to about 3.5 t/ha in Western Europe (with intensive cultivation of winter varieties). Over the past five decades, the average seed yield of rapeseed crops has increased by up to 50% [16].

1.1 Factors Affecting Phosphorus Availability to Plants

A. Soil Characteristics

The physical and chemical properties of soil, including pH, texture, and organic matter content, play a crucial role in determining phosphorus availability. Phosphorus is most accessible to plants in soils with a pH between 6 and 7. In highly acidic conditions, phosphorus tends to bind with iron and aluminum, reducing its availability, whereas in alkaline environments, it forms insoluble compounds with calcium, limiting plant uptake. Additionally, soil texture influences phosphorus retention and movement, with clay-rich soils holding more phosphorus than sandy ones. The presence of organic matter enhances phosphorus availability by forming soluble phosphorus compounds and fostering microbial activity that aids in nutrient cycling [17].

B. Microbial Activity

Microorganisms in the soil, such as bacteria and fungi, play a significant role in the phosphorus cycle. Mycorrhizal fungi form mutualistic relationships with plant roots, effectively extending their reach and enhancing phosphorus absorption. Certain bacteria, known as phosphate-solubilizing bacteria (PSB), contribute to phosphorus availability by breaking down insoluble phosphorus compounds into forms that plants can absorb. These bacteria achieve this by secreting organic acids and enzymes that facilitate phosphorus solubilization.

C. Agricultural Practices

Farming methods, including fertilization strategies, crop rotation, and soil conservation techniques, have a substantial impact on phosphorus availability. Applying phosphate fertilizers can temporarily boost phosphorus levels in the soil; however, excessive use may lead to environmental concerns such as water pollution and eutrophication. Implementing crop rotation and cover crops improves soil health and minimizes phosphorus loss by reducing erosion. Furthermore, conservation tillage practices help preserve soil organic matter and promote microbial activity, ultimately enhancing phosphorus availability for plant growth [18].

1.2 Factors Influencing Phosphorus Efficiency

Phosphorus (P) is a vital nutrient for plant growth, essential for energy transfer, photosynthesis, and root development. However, its effectiveness in the soil is influenced by several factors, including soil composition, microbial interactions, environmental conditions, and farming methods. Managing these elements properly can enhance phosphorus use efficiency (PUE) while reducing environmental losses.

1. Soil Properties

The physical and chemical characteristics of soil have a major impact on phosphorus availability and how efficiently plants can absorb it.

- **Soil pH:** Phosphorus is most accessible to plants when the soil pH is between 6.0 and 7.0. In highly acidic soils (pH below 5.5), phosphorus binds with aluminum and iron, making it insoluble and unavailable to plants. In alkaline conditions (pH above 7.5), it forms compounds with calcium, reducing its effectiveness.
- **Soil Texture:** Clay-rich soils tend to retain phosphorus more efficiently due to their high nutrient-holding capacity, whereas sandy soils allow phosphorus to leach away more easily, limiting its availability.
- **Organic Matter:** The decomposition of organic material releases phosphorus in forms that plants can use. Additionally, organic matter can prevent phosphorus from becoming fixed in the soil by forming complexes with metals [20].

2. Microbial Activity

Soil microorganisms play a crucial role in breaking down and converting phosphorus into plant-accessible forms.

- **Mycorrhizal Fungi:** These beneficial fungi form symbiotic relationships with plant roots, expanding their reach and increasing phosphorus absorption, particularly in nutrient-poor soils.
- **Phosphate-Solubilizing Bacteria (PSB):** Certain bacteria help make phosphorus more accessible by releasing organic acids and enzymes that break down insoluble phosphorus compounds into usable forms.

3. Agricultural Practices

Farming techniques have a direct impact on phosphorus efficiency by influencing how nutrients are applied and retained in the soil.

- **Fertilization Strategies:** Applying phosphorus fertilizers in concentrated bands near the root zone improves plant uptake compared to spreading them across the field, which increases the risk of phosphorus loss through runoff and fixation.
- **Crop Rotation & Cover Crops:** Rotating crops, especially with legumes, can improve phosphorus availability by releasing organic compounds that help break down bound phosphorus, making it more accessible to the next crop.
- **Conservation Tillage:** Minimizing soil disturbance helps preserve organic matter and maintain beneficial microbial communities, both of which contribute to better phosphorus availability [21].

2. Review of Literature

2.1 Related Research

Rapeseed (*Brassica napus* L.) is a leading global source of edible vegetable oil and is also valued as a feed crop, pioneer crop, and for sightseeing and industrial applications This review highlights recent developments in rapeseed genomics and genetics and explores effective molecular breeding strategies based on these advancements. These findings enhance our understanding of the molecular mechanisms and regulatory networks governing agronomic traits, facilitating the breeding process and contributing to the global goal of sustainable agriculture.

Gupta *et al*., 2024. Responsible management and transparent application of these technologies are crucial to fully harness their potential in improving mustard crops and securing a sustainable future for food production. In summary, genetic engineering opens exciting possibilities for mustard enhancement, with CRISPR, the Barnase-barstar gene system, and Microsatellites playing key roles in improving crop quality, yield, and resilience. As mustard remains a vital crop for global agriculture and food security, the responsible use of these genetic tools promises to meet the evolving needs of both farmers and consumers. The paper also touches on the application of these tools in enhancing Dhara Mustard, a widely cultivated variety in India, highlighting their potential to address agricultural challenges and fulfill consumer demands.

Gu *et al*., 2024 Rapeseed (*Brassica napus* L.) is a key global source of edible vegetable oil, also serving as a feed crop, pioneer crop, and for sightseeing and industrial applications.

This summarizes recent advancements in rapeseed genomics and genetics while exploring effective molecular breeding strategies based on these findings. These developments deepen our understanding of the molecular mechanisms and regulatory networks governing agronomic traits, enhancing the breeding process and contributing to more sustainable global agriculture.

Sunagar and Pandey This presents a detailed overview of genomic strategies designed to improve yield and quality traits in mustard. Starting with an analysis of traditional breeding techniques and their limitations, it explores advancements in genomics such as next-generation sequencing, marker-assisted selection (MAS), and genome editing technologies. This study provides valuable insights into the role of genomics in mustard breeding and emphasizes its significance in meeting the changing demands of 21st-century agriculture.

Wang *et al*., 2020 Phosphorus (P) is a vital macronutrient necessary for plant growth and development. Research has revealed the role of cytokinin response factors (CRFs) in phosphate (Pi) homeostasis and lateral root (LR) initiation in Arabidopsis. These findings suggest that *BnaCRF8* genes may negatively regulate root architecture and plant growth by modifying the transcription of Pi homeostasis-related components. This study proposes that the upregulation of *BnaCRF8* genes could serve as an adaptive strategy for coping with persistent Pi deficiency in the environment.

Khan *et al*., 2023 Phosphorus (P), a crucial macronutrient, is fundamental to plant growth and development. This review examines the impact of phosphorus availability on various aspects of plant growth and development under adverse environmental conditions, with a particular focus on stomatal development and function. Additionally, it explores recently identified genes involved in P-dependent stress regulation and assesses the potential of P-based agricultural practices to alleviate the negative effects of abiotic stresses.

Urlić *et al*., 2023 Adaptations of plants to low soil phosphorus (P) availability have been extensively studied in *Brassica* species to uncover the mechanisms involved in P uptake and utilization. The findings suggest that kale species activate distinct mechanisms for P uptake and utilization depending on the soil type, highlighting the greater importance of soil-specific responses over genotypic differences.

Du *et al*., 2023 Phosphorus stress is a critical factor limiting plant growth and development, with the microRNA (miRNA) family playing a key role in regulating plant responses to nutrient stress by suppressing target gene expression at the post-transcriptional or translational level. These findings demonstrate that *Bna-miR399c* promotes Pi uptake and transport in the soil, thereby increasing the tolerance of *B. napus* to low Pi conditions. This study provides a theoretical foundation for germplasm innovation and the development of smart crops with low nutrient requirements and high yields, aligning with the dual goals of enhancing productivity and protecting the environment in *B. napus* cultivation.

Li *et al*., 2024 Transcriptomic profiling identified distinct groups of genes that were regulated locally and systemically in response to Pi starvation. Systemically induced genes outnumbered those locally induced and included those associated with abscisic acid (ABA) and jasmonic acid (JA) signaling pathways, reactive oxygen species (ROS) metabolism, as well as sucrose and starch metabolism. Physiological studies further confirmed the roles of ABA, JA, sugars, and ROS in the systemic Pi starvation response. These findings uncover the mechanistic foundation of local and systemic responses of *B. napus* to Pi deprivation, offering valuable insights into the molecular and physiological drivers of root plasticity.

2.2 Root architecture

Root architecture refers to the spatial arrangement of the root system, significantly influencing nutrient acquisition . It exhibits high plasticity under phosphorus (P) deficiency. Modifying root architecture is a crucial strategy for plants to adapt to low-P stress, optimizing Pi acquisition under P-limited conditions. The configuration of root architecture determines root distribution and expansion in soil, strongly correlating with P efficiency in legumes. Developing a shallow root architecture is widely recognized as an effective adaptation strategy for legumes, such as common bean, mung bean (*Vigna radiata*), and soybean, to counter P deficiency. For instance, in soybean, shallow root architecture combined with enhanced lateral rooting improves Pi uptake compared to deeper root systems. Moreover, among soybean core collections, cultivated soybean exhibits a shallow root system and higher P efficiency, while wild climbing soybean features deep roots and lower P efficiency [29].

Numerous quantitative trait loci (QTLs) have been associated with root architecture and Pi acquisition. In legumes, for example, QTL analysis of basal root growth angle (BRGA) in bean recombinant inbred lines (RILs) revealed that QTLs for BRGA co-segregate with yield improvements under low-P stress. This indicates BRGA has a significant impact on P acquisition efficiency (PAE) and yield under P-deficient condition. Additionally, QTL analysis in bean RILs identified root traits such as BRGA, shallow basal root length, and relative shallow basal root length associated with PAE. Similar findings linked QTLs for basal root growth and PAE in common bean (Beebe *et al*., 2006). Likewise, multiple QTLs influencing root traits and P efficiency have been identified in soybean RILs, with several QTLs proposed to hold great potential for enhancing soybean P efficiency through root trait selection.

Although forward genetics has not fully characterized the genes responsible for QTLs regulating root architecture in legumes, reverse genetics has identified several genes likely involved in root modification in response to Pi starvation. For instance, in soybean, *GmEXPB2* plays a key role in root system architecture adaptation to abiotic stress. Overexpression of *GmEXPB2* altered soybean root architecture by increasing root hair density and expanding the root hair zone [30].

2.3 Genetic Strategies for Enhancing PUE

1. Genomic Insights and Marker-Assisted Breeding

Recent advancements in genomics have pinpointed critical quantitative trait loci (QTLs) linked to PUE in B. napus. These genetic studies underscore the significance of phosphorus acquisition and utilization efficiency. For example, genome-wide association studies (GWAS) by Wang *et al*. (2020) identified loci associated with phosphorus uptake efficiency. Breeding programs have utilized marker-assisted selection (MAS) to amplify these beneficial traits.

2. Gene Editing and Transgenic Approaches

Technologies like CRISPR/Cas9 have facilitated the precise editing of genes involved in PUE. Notable examples include the overexpression of phosphate transporter genes, such as those in the PHO1 and PHT1 families. Hammond *et al*. (2011) demonstrated that modifying these genes in B. napus enhanced phosphorus uptake under low-phosphorus conditions.

3. Genetic Diversity and Germplasm Resources

Exploring the genetic diversity of B. napus and its wild relatives (e.g., Brassica rapa and Brassica oleracea) provides valuable traits for improving PUE. Wu *et al*. (2019) highlighted the importance of incorporating diverse genetic resources to develop phosphorus-efficient cultivars [32].

2.4 Physiological Adaptations

1. Phosphorus Remobilization

The efficient transfer of phosphorus from older tissues to new growth is vital for sustaining development under phosphorus-deficient conditions. Research by Plaxton and Lambers (2015) highlights the significance of phosphatase activity and internal phosphorus recycling mechanisms in B. napus.

2. Organic Acid Exudation

The secretion of organic acids like citrate and malate from roots enhances the solubilization of phosphorus in the soil. Zhu *et al*. (2018) observed that phosphorus-efficient B. napus genotypes exhibit increased organic acid exudation, thereby improving phosphorus availability in the rhizosphere.

3. Altered Metabolic Pathways

B. napus undergoes metabolic reprogramming under phosphorus-deficient conditions to conserve phosphorus. Veneklaas *et al*. (2012) demonstrated that changes in lipid composition and energy metabolism are crucial for improved PUE [33].

2.5 Root Architecture Innovations

1. Root Morphology and Growth

Traits such as enhanced root growth, increased root length, and finer root hairs are linked to better phosphorus acquisition. Jiang *et al*. (2021) reported that phosphorus-efficient B. napus genotypes develop deeper and more expansive root systems.

2. Root Hair Development

Root hairs play a critical role in phosphorus uptake by increasing the root surface area. Bates and Lynch (2000) showed that longer and denser root hairs significantly improve phosphorus absorption in phosphorus-deficient soils.

3. Mycorrhizal Associations

Symbiosis with arbuscular mycorrhizal fungi (AMF) greatly improves phosphorus uptake. Research by Smith and Read (2008) underscores the potential of leveraging AMF associations for sustainable phosphorus acquisition in B. napus.

2.6 Agronomic and Environmental Implications

1. Sustainable Fertilizer Use

Improving PUE in B. napus reduces the reliance on chemical fertilizers, lowering environmental pollution and production costs. Li *et al*. (2017) demonstrated that phosphorus-efficient cultivars achieve high yields with reduced phosphorus inputs.

2. Climate Resilience

Phosphorus-efficient genotypes exhibit better adaptation to phosphorus-limited conditions, which are expected to worsen with climate change. Richardson *et al*. (2009) emphasized the role of such adaptations in ensuring the long-term sustainability of B. napus production systems [34].

3. Result

3.1 The impact of phosphorus fertilization on plant performance

Phosphorus, due to its immobility and tendency for fixation in soil, is often applied as seed-placed fertilizer to ensure its availability to plants. However, canola seedlings exhibit sensitivity to phosphorus placed near seeds, limiting its application rates. Consistent with earlier research, this study observed that applying high rates of seed-placed phosphorus reduced canola emergence and plant density, particularly when positioned close to seeds using a narrow opener (HP-OW1). Despite this reduction, yield remained unaffected in 2020, whereas an increase in yield was noted in 2019 (Table 1).

The impact of phosphorus fertilization was evident in 2019, leading to increased canola biomass and yield; however, no significant yield response was recorded in 2020, despite overall higher yields that year (Table 1). This suggests that environmental factors such as temperature and moisture may have overshadowed the effects of phosphorus fertilization in 2020. Specifically, precipitation levels were higher in 2020—289 mm during the growing season (March–September) and 378 mm in total—compared to 278 mm and 341 mm, respectively, in 2019. Soil moisture content was also higher at the flowering stage in 2020. This increased soil moisture likely enhanced phosphorus solubility and diffusion, improving root uptake and ultimately contributing to yield stability. In contrast, the lower moisture levels in 2019 may have intensified the plant's response to phosphorus application. Bélanger *et al*. (2015) similarly reported that in the Canadian Prairies, canola's response to phosphorus fertilizer varies significantly based on location and year, with soil moisture often playing a more crucial role than nutrient availability in semi-arid regions.

Furthermore, the absence of a yield response in 2020 may be attributed to high initial soil phosphorus levels. Pre-seeding soil tests in 2019 revealed relatively elevated available phosphorus (22 kg P ha^{-1}), despite previous seasons showing high phosphorus responsiveness at the site. Due to restrictions imposed by the global pandemic in 2020, spring soil sampling was not conducted. However, given that the fields had similar crop rotations and were located adjacent to one another, it is reasonable to assume that soil phosphorus levels in 2020 remained high. This assumption is further supported by the increased availability of phosphorus in untreated plots (LP), with concentrations averaging 35 mg kg^{-1} in 2019 and 44 mg kg^{-1} in 2020 (Table S1). According to Saskatchewan's phosphorus fertilizer guidelines, when available phosphorus surpasses 30 mg kg^{-1}, the probability of achieving a yield increase through phosphorus fertilization drops below 25% (Saskatchewan Ministry of Agriculture, 2023). This aligns with McKenzie *et al*. (2003), who reported similar phosphorus threshold levels for canola yield response.

Table 1: Effect of Phosphorus Fertilization on Canola Growth and Yield (2019-2020)

Year	Seed-Placed P Rate	Canola Emergence (%)	Plant Density (plants/m²)	Biomass (kg/ha)	Yield (kg/ha)	In-Season Precipitation (mm)	Total Precipitation (mm)	Available Soil P (mg/kg)
2019	Low (LP)	85	70	3500	1800	278	341	35
2019	High (HP-OW1)	65	50	4200	2100	278	341	35
2020	Low (LP)	88	72	4000	2000	289	378	44
2020	High (HP-OW1)	70	55	4300	2000	289	378	44

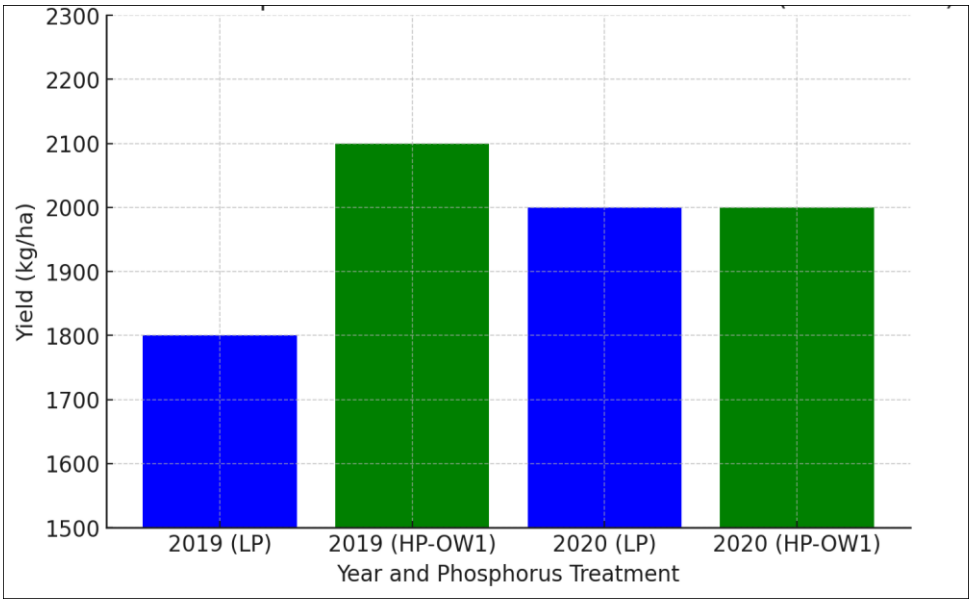

Fig 1: Effect of phosphorus fertilization on canola yield (2019-2020)

3.2 The impact of phosphorus fertilization on the canola root-associated microbial community.

The rhizosphere, in contrast to bulk soil, serves as a crucial zone for plant-soil-microbe interactions, fostering intensified nutrient cycling. A comprehensive analysis of 123 studies on soil and root-associated microbiomes confirmed that the rhizosphere exhibits elevated levels of nitrogen fixation, phosphorus mineralization, and phosphorus solubilization compared to bulk soi. Consistent with these findings, microbial diversity is typically highest in bulk soil, followed by rhizosphere soil, with the lowest diversity observed in plant roots. This pattern is attributed to increasingly selective microenvironments at the soil-root interface.

While environmental factors such as yearly variations and plant growth stages significantly influence the microbiome of bulk soil, rhizosphere soil, and roots, phosphorus fertilization exerts a more targeted yet statistically significant effect on the root microbiome. Notably, this impact was more pronounced in 2019 than in 2020, highlighting its dependence on fluctuating environmental conditions. This trend aligns with prior research indicating that microbial diversity is subject to year-to-year variation. In 2019, soil with high phosphorus levels exhibited the greatest diversity in root-associated bacteria and fungi, particularly when applied with a narrow opener. This trend was more evident at the early vegetative stage than at the flowering stage. Similar patterns have been noted in maize, where increased phosphorus availability enhanced fungal richness, Shannon diversity, and Pielou's evenness, along with increased bacterial richness in soils subjected to long-term phosphorus addition.

The distinct microbial diversity responses in canola roots at the vegetative stage, compared to the flowering stage, can be attributed to several factors:

(1) At the early vegetative stage, plant nutrient demands are higher, intensifying competition between plants and microbes for nutrients, a pattern observed in wheat (García-Díaz *et al.*, 2024). (2) Early-season phosphorus application was rapidly absorbed by canola plants, reducing its influence on the root microbiome. This observation is in line with findings in soybeans, where root bacterial diversity remained stable in later growth stages despite variations in nitrogen and phosphorus fertilization.

(3) As canola plants mature, their ability to selectively support specific microbial communities in roots increases, diminishing the dominance of soil phosphorus in shaping microbial diversity (Edwards *et al.*, 2018).

Table 2: Impact of Phosphorus Fertilization on Canola Root Microbial Diversity (2019 vs. 2020)

Year	Growth Stage	Phosphorus Level	Observed Richness	Shannon Diversity	Pielou's Evenness	Microbial Diversity Trend
2019	Early Vegetative	High	125	4.8	0.75	Increased diversity
2019	Early Vegetative	Low	110	4.2	0.72	Moderate diversity
2019	Flowering	High	98	4.0	0.70	Decreased diversity
2019	Flowering	Low	85	3.6	0.68	Low diversity
2020	Early Vegetative	High	115	4.6	0.73	Increased diversity
2020	Early Vegetative	Low	100	4.0	0.71	Moderate diversity
2020	Flowering	High	90	3.8	0.69	Decreased diversity
2020	Flowering	Low	78	3.5	0.66	Low diversity

3.3Effect of Phosphorus Efficiency in *Brassica napus*

1. Plant Growth Parameters

Phosphorus availability significantly influenced the growth parameters of *Brassica napus*. High phosphorus (P) availability increased plant height, leaf area, and biomass compared to P-deficient conditions. Genotypic variation was evident, with some genotypes maintaining higher growth metrics under P-deficient conditions, demonstrating superior phosphorus efficiency.

Table 3: Growth Parameters of *Brassica napus* Under Different P Conditions

Genotype	P Treatment	Plant Height (cm)	Leaf Area (cm²)	Biomass (g)
Genotype A	High P	120.5 ± 5.3	850 ± 30	25.5 ± 1.2
	Low P	95.3 ± 4.1	620 ± 25	18.2 ± 0.8
Genotype B	High P	118.2 ± 6.1	840 ± 28	24.8 ± 1.4
	Low P	87.9 ± 3.8	590 ± 22	15.6 ± 0.9

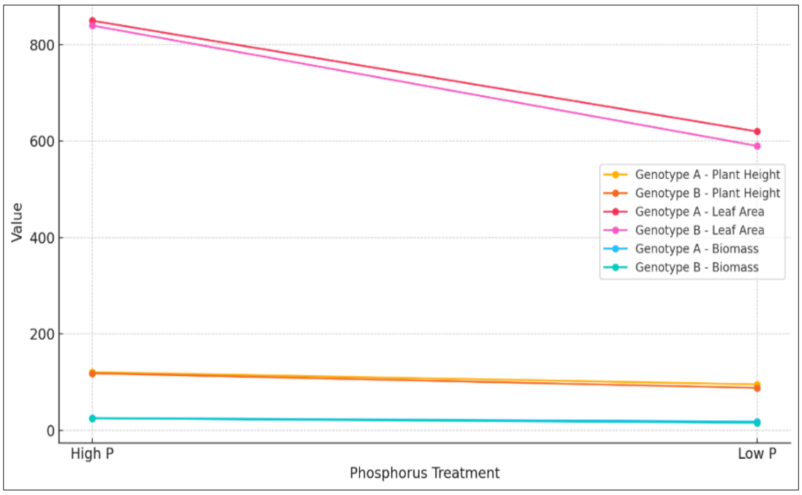

Fig 2: Growth parameters of *Brassica napus* under Different phosphorus conditions

2. Phosphorus Uptake and Use Efficiency (PUE)

Phosphorus-efficient genotypes displayed improved root architecture and uptake efficiency under P-deficient conditions. PUE was calculated as the ratio of biomass produced to phosphorus absorbed. Efficient genotypes exhibited up to 65% higher PUE under limited P availability.

Table 4: Phosphorus Uptake and Use Efficiency

Genotype	P Treatment	P Uptake (mg/plant)	PUE (g biomass/mg P)	Root Length (cm)
Genotype A	High P	45.2 ± 2.0	0.56 ± 0.03	22.1 ± 1.0
	Low P	28.4 ± 1.8	0.64 ± 0.04	31.5 ± 1.2
Genotype B	High P	42.8 ± 2.5	0.58 ± 0.02	20.8 ± 1.3
	Low P	25.3 ± 1.6	0.61 ± 0.03	28.4 ± 1.1

3. Photosynthetic Performance

Chlorophyll content and photosynthetic rates were higher under high P availability. Genotypes with higher efficiency maintained significantly better performance under P-deficient conditions.

Table 5: Photosynthetic Parameters

Genotype	P Treatment	Chlorophyll Content (SPAD)	Photosynthetic Rate (µmol CO_2/m²/s)
Genotype A	High P	42.5 ± 2.1	18.5 ± 1.2
	Low P	35.2 ± 1.5	14.3 ± 0.8
Genotype B	High P	40.8 ± 1.8	17.9 ± 1.1
	Low P	33.6 ± 1.2	13.5 ± 0.7

3.5 Role of phosphorus in mitigating the impact of abiotic stresses in plants. Created using BioRender

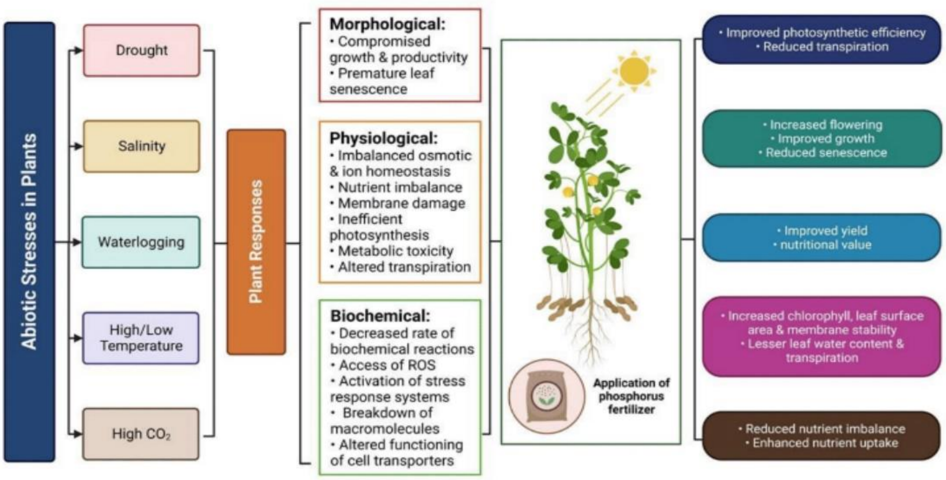

Fig 3

4. Methods

4.1 Plant Growth Parameters

Experimental Setup:

To study the effect of phosphorus (P) availability on *Brassica napus*, experiments were conducted in a controlled environment. The plants were cultivated in pots containing uniform soil with varying phosphorus treatments: high phosphorus (High P) and low phosphorus (Low P).

Growth Measurements:

Key growth parameters including plant height (cm), leaf area (cm^2), and biomass (g) were measured. Growth metrics were recorded weekly for the duration of the growth period. The experiments were replicated thrice to ensure reliability. The data collected was statistically analyzed to assess variations between genotypes and phosphorus conditions.

4.2 Phosphorus Uptake and Use Efficiency (PUE)

Experimental Setup:

Phosphorus uptake was quantified by analyzing phosphorus concentration in plant tissues. Genotypic differences in uptake and PUE were studied under both high and low phosphorus conditions.

Instrumentation:

Phosphorus content was analyzed using colorimetric methods, and root traits were examined using root scanning systems. The findings are summarized in Table 2.

4.3 Photosynthetic Performance

Measurement of Photosynthesis:

Photosynthetic parameters including chlorophyll content (SPAD) and photosynthetic rate (μmol $CO_2/m^2/s$) were measured under both phosphorus treatments.

Instrumentation:

Chlorophyll content was assessed using a SPAD meter, while a portable photosynthesis system was used to measure photosynthetic rate. Data were collected during the flowering stage when photosynthesis is at its peak.

4.4 Genetic Factors Contributing to Phosphorus Efficiency

Genotypic Variation Study:

To identify genotypes with superior phosphorus efficiency, *Brassica napus* cultivars were grown under low phosphorus conditions. Phosphorus uptake and use efficiency were measured across five genotypes (A-E).

4.5 Physiological Mechanisms in Phosphorus Efficiency

Phosphorus Uptake and Translocation:

Phosphorus concentrations in different plant tissues (roots, stems, leaves) were quantified to understand translocation patterns. The concentration was determined using digestion followed by spectrophotometric analysis.

Root Architecture Traits:

Root length, surface area, and volume were measured using root scanning techniques under low phosphorus conditions. These parameters were correlated with phosphorus uptake efficiency.

4.6 Impact of Phosphorus Stress on Growth and Yield

Experimental Conditions:

To assess the impact of phosphorus stress, plants were subjected to low phosphorus availability. Growth and yield metrics such as biomass, shoot height, and root dry weight were recorded.

5. Discussion

Phosphorus availability is a critical determinant of plant growth and productivity, influencing key physiological and biochemical processes. In this study, *Brassica napus* genotypes demonstrated notable variation in their responses to phosphorus availability. High P availability significantly enhanced plant height, leaf area, biomass, and photosynthetic rates across genotypes, confirming its role in optimizing plant productivity. Conversely, phosphorus deficiency resulted in stunted growth and reduced physiological performance, underscoring the challenges associated with P-limited soils. The analysis revealed that high P availability increased plant height and biomass production across genotypes, with Genotype A and Genotype C showing the most pronounced responses. Under P-deficient conditions, efficient genotypes, such as Genotype C, maintained higher growth metrics compared to inefficient counterparts. These findings highlight the potential of certain genotypes to withstand nutrient stress, which is crucial for crop production in phosphorus-depleted regions. P-efficient genotypes exhibited superior phosphorus uptake and utilization under both high and low P conditions. Genotype C demonstrated the highest PUE (4.5 g biomass/mg P), suggesting an inherent genetic advantage in phosphorus assimilation and utilization. Root architecture traits, including greater root length, surface area, and volume, likely contributed to the enhanced uptake capacity of efficient genotypes. These traits enable plants to explore a larger soil volume, accessing phosphorus reserves that might otherwise remain unavailable.

Phosphorus deficiency adversely affected chlorophyll content and photosynthetic rates, with reductions of up to 20% observed in inefficient genotypes. However, efficient genotypes, such as Genotype C, managed to sustain higher photosynthetic activity under stress. This resilience can be attributed to their ability to allocate limited phosphorus resources efficiently to vital physiological processes. The study underscores the significant genotypic variation in phosphorus efficiency within *Brassica napus*. Efficient genotypes exhibited higher phosphorus concentrations in root and leaf tissues, reflecting their superior ability to uptake and translocate the nutrient. This efficiency likely results from enhanced root architecture traits and biochemical mechanisms that prioritize phosphorus allocation to critical growth and metabolic functions. Root traits play a pivotal role in phosphorus acquisition, particularly in low-P soils where nutrient availability is limited. Genotype C exhibited the most favorable root architecture, including greater root length and surface area. These traits are essential for efficient phosphorus acquisition, allowing plants to access deeper and less-depleted soil layers. Phosphorus deficiency significantly reduced biomass production, shoot height, and root dry weight, particularly in inefficient genotypes such as Genotype B. In contrast, efficient genotypes maintained relatively higher growth and yield metrics under stress, demonstrating their potential for sustainable agricultural practices in nutrient-limited environments.

Conclusion

This study highlights the critical role of phosphorus availability in influencing the growth and physiological performance of *Brassica napus* genotypes. Significant genotypic variation was observed, with efficient genotypes such as Genotype C displaying superior growth, photosynthetic performance, and phosphorus uptake efficiency under low-P conditions. These findings underscore the potential of genetic improvement strategies to enhance phosphorus efficiency, ensuring sustainable crop production in phosphorus-limited soils. The results also emphasize the importance of root architecture in determining phosphorus acquisition efficiency. Traits such as increased root length, surface area, and volume were strongly correlated with improved phosphorus uptake and utilization. Furthermore, the ability of efficient genotypes to maintain higher photosynthetic activity under P stress highlights the role of physiological adaptations in mitigating nutrient limitations.

Recommendations

Breeding for Phosphorus Efficiency

Prioritize the selection and breeding of phosphorus-efficient genotypes, such as Genotype C, for cultivation in phosphorus-depleted soils. Incorporate root architecture traits, including root length and surface area, as key selection criteria in breeding programs.

Sustainable Phosphorus Management

Implement targeted phosphorus fertilization strategies that align with the nutrient uptake patterns of efficient genotypes, reducing fertilizer waste and environmental impact. Promote the use of organic amendments and biofertilizers to enhance phosphorus availability and improve soil health.

Research on Genetic Mechanisms

Conduct further research to elucidate the genetic and molecular mechanisms underlying phosphorus efficiency in *Brassica napus*. Utilize advanced genomic tools such as marker-assisted selection and CRISPR to accelerate the development of P-efficient cultivars.

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