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**ANÁLISIS DEL DESARROLLO RADICULAR, ACTIVIDAD
BIOLÓGICA DE LA RIZOSFERA Y SU EFECTO EN LA
DISPONIBILIDAD DE NUTRIENTES DE VARIEDADES DE
TRIGO HARINERO DE HÁBITO INVERNAL LIBERADAS EN
CHILE ENTRE LOS AÑOS 1965 Y 2020**

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RESUMEN

A pesar de los avances en la genética del trigo, el impacto sobre la estructura de las raíces y la biología de la rizosfera aún no se conoce suficientemente. En este estudio, investigamos la exudación de carboxilatos, la colonización por hongos micorrícicos arbusculares, la actividad microbiana y la arquitectura de las raíces en variedades de trigo de invierno liberadas entre 1965 y 2020. Se establecieron catorce variedades diferentes de trigo invernal con cuatro repeticiones en un suelo Andisol con pH 5,6. Se extrajeron sistemas radiculares completos y muestras de suelo hasta 60 cm de profundidad. Los resultados obtenidos mostraron que las variedades hasta el año 1990 tenían una mayor exudación de carboxilatos, siendo succinato 76 % más exudado que los otros carboxilatos. La variedad Tukan (1993), fue la variedad con mayor exudación total de carboxilatos con un 57 %. La colonización micorrícica fue inversamente proporcional a las concentraciones de fósforo y todas las variedades mostraron una mayor actividad microbiana en antesis. Las raíces más largas se encontraron en las variedades liberadas después del año 2000, pero no se encontraron diferencias significativas en otros parámetros de la arquitectura radicular. No se observó ningún patrón claro entre la arquitectura radicular y la actividad biológica en función del año de liberación. La morfología de las raíces y la actividad biológica de la rizósfera como la exudación de carboxilatos, la actividad microbiana y la colonización micorrícica, es moderada por la genética de las plantas, pero se vieron afectadas por altas concentraciones de fósforo. El estudio se realizó en condiciones de campo, considerando las interacciones planta-microorganismos, que a menudo resultan complejas de analizar por la dificultad para extraer los sistemas radiculares.

SUMMARY

Despite advances in wheat genetics, the impact on root structure and rhizosphere biology is still poorly understood. In this study, we investigated carboxylate exudation, colonization by arbuscular mycorrhizal fungi, microbial activity, and root architecture in winter wheat varieties released between 1965 and 2020. Fourteen different winter wheat varieties with four replicates were established in Andisol soil with pH 5.6. Complete root systems and soil samples up to 60 cm depth were extracted and results showed that varieties up to 1990 had higher carboxylate exudation, with succinate being 76 % more exuded than the other carboxylates. The Tukan (1993) variety was the variety with the highest total carboxylate exudation with 57 %. Mycorrhizal colonization was inversely proportional to phosphorus concentrations and all varieties showed higher microbial activity at anthesis. The longest roots were found in varieties released after 2000, but no significant differences were found in other parameters of root architecture. No clear pattern was observed between root morphology and biological activity as a function of release year. Root architecture and rhizosphere biological activity such as carboxylate exudation, microbial activity, and mycorrhizal colonization are moderated by plant genetics but were affected by high phosphorus concentrations. The study was conducted under field conditions, considering plant-microorganism interactions, which are often complex to analyze because of the difficulty to extract from root systems.

CAPÍTULO 1

INTRODUCCION GENERAL

Las asociaciones benéficas entre microorganismos y las raíces de las plantas son de gran importancia ya que favorecen el desarrollo y crecimiento de los cultivos agrícolas sin hacer uso excesivo de agroquímicos. Es por eso, que gran parte de la agricultura actual busca fomentar estas interacciones, debido a los beneficios que aportan a la sustentabilidad de los ecosistemas (Romagnoli et al., 2017). La mayor cantidad de microorganismos se encuentran en la rizosfera, esta zona se encuentra influenciada por exudados radiculares que generan un ambiente con una alta concentración de nutrientes, como metabolitos primarios y secundarios (azúcares, ácidos orgánicos, compuestos fenólicos, vitaminas, enzimas, mucílagos, entre otros) (Oburger y Jones, 2018), desencadenando complejas y dinámicas interacciones entre diversos microorganismos del suelo (Zhalnina et al., 2018). Este dinamismo también favorece la agregación de los suelos, movilización de nutrientes, desintoxicación de metales y contaminantes orgánicos, el intercambio de señales plantas/microorganismos y, además, el aumento de la composición y la actividad de la comunidad microbiana del suelo (Oburger y Jones, 2018). Debido a esto, hoy en día, toman relevancia las mediciones de parámetros bioquímicos del suelo, considerados como indicadores biológicos, los cuales son monitoreados mediante la actividad microbiana (respiración y biomasa), exudados radiculares y actividad enzimática del suelo (Bünemann et al., 2018). La respiración de los suelos es una actividad realizada por los microorganismos que viven allí, en la cual el carbono es incorporado en los ecosistemas terrestres para posteriormente, ser devuelto a la atmósfera como CO₂ (Paolini, 2017). Por otro lado, la actividad enzimática del suelo juega un rol importante para la disponibilidad de nutrientes, influye y ayuda a comprender gran parte de los procesos del suelo, tales como, mineralización de materia orgánica, degradación y cambios del medio ambiente del suelo, los cuales son influenciados por factores edafoclimáticos (temperatura,

pH, estacionalidad, entre otros) (Gajda et al., 2018). Parte sustancial de la actividad biológica de la rizosfera son las interacciones simbióticas con microorganismos del suelo, las más estudiadas son las que se dan entre plantas y hongos micorrícicos arbusculares (HMA). Esta relación consiste en la unión entre ciertos hongos del suelo y las raíces de las plantas formando relaciones de mutuo beneficio (Nazareno et al., 2020). La simbiosis entre los HMA y la planta, se inicia cuando el hongo, percibe señales provenientes de las raíces (Oldroyd, 2013), estas señales son fitohormonas terpenoides llamadas estrigolactonas generadas en la pre-simbiosis (Feng et al., 2019) las cuales promueven la germinación de esporas y ramificación de hifas (Ramírez et al., 2017). Cuando el hongo coloniza la raíz de la planta hospedera, obtiene hidratos de carbono sintetizados por la planta y a la vez, este le ayuda a la planta a obtener fósforo, nitrógeno y otros nutrientes minerales (Padrón et al., 2020). Esta relación mutualista se presenta en aproximadamente el 70% de las plantas de diversos ecosistemas (Romagnoli et al., 2017) siendo fundamentales también en cultivos alimentarios de interés agronómico.

El mejoramiento genético, mediante cambios en la arquitectura de las raíces, y la aplicación de agroquímicos, son dos factores que pueden generar cambios en las interacciones entre las raíces de las plantas y el microbioma del suelo (Jacquiod et al., 2021). Sin embargo, es la aplicación de agroquímicos la principal causa de la pérdida de la biodiversidad en el mundo, ya que afectan las interacciones simbióticas naturales de las plantas (Porter y Sachs, 2020). Jacquiod et al. (2021) determinaron en sus estudios que la aplicación de agroquímicos disminuye la diversidad microbiana de la rizosfera. Algunos autores como Porter and Sachs (2020), señalan que las relaciones mutualistas entre microorganismos y plantas han sido reemplazadas por las aplicaciones de fertilizantes sintéticos, pesticidas y otros productos químicos, a pesar de que aún está la habilidad biológica de realizar simbiosis. Peiffer et al. (2013), demostraron que los genotipos de las plantas hospederas tienen una influencia significativa en la composición y diversidad de la comunidad microbiana de la rizosfera. Como es el caso de las interacciones con rizobios o con HMA, los cuales están potencialmente influenciados por la

domesticación (Milla et al., 2015). Por otro lado, los genotipos de plantas hospederas influyen en el desarrollo, crecimiento e inmunidad de las plantas (Ober et al., 2021; Lemanceau et al., 2017). Los cultivos domesticados, suelen estar bajo prácticas agrícolas intensivas en búsqueda de una mayor producción (Qin et al., 2018), como es el caso del trigo, el cual es el cereal más sembrado en Chile, principalmente en las regiones del sur del país (INE, 2020) y tiene una gran importancia socio económica a nivel mundial (Qin et al., 2018). A partir de la domesticación del trigo, se han realizado una serie de modificaciones genéticas, creando nuevas variedades. A esto se suma la aplicación de agroquímicos, todo con el objetivo de aumentar rendimiento y otros aspectos productivos, sin prestar atención a cómo podría afectar al desarrollo radicular y a las interacciones entre los microorganismos del suelo y las raíces de las plantas. Sumado a lo anterior, la domesticación del trigo se ha enfocado en la selección de órganos aéreos, con escasos estudios orientados a la selección de los rasgos de las raíces, debido a las dificultades prácticas para analizar los sistemas radiculares (Heřmanská et al., 2015). Algunos estudios han demostrado que las nuevas variedades de trigo presentan menor biomasa radicular que las variedades antiguas (Aziz et al., 2017). Investigaciones recientes plantean que la disminución de la biomasa radicular en trigo disminuye su capacidad para reclutar comunidades microbianas benéficas del suelo y así obtener por sí misma los nutrientes para su crecimiento y salud (McGrail y McNear, 2021). Valente et al. (2020), encontraron que las variedades de trigo antiguo tenían una mayor capacidad de interactuar con rizobacterias beneficiosas para el crecimiento de las plantas que las variedades modernas. Estudios realizados en la domesticación del girasol demostraron que dicho proceso afectó a la composición de los hongos de la rizosfera (Leff et al., 2017). Sin embargo, esto no ha sido demostrado conclusivamente en nuevas variedades de trigo. Ober et al., (2021) señala que los sistemas radiculares profundos son importantes para el anclaje y la absorción de agua y, de acuerdo a lo anterior juegan un rol fundamental en la absorción de nutrientes de la solución del suelo, sobre todo cuando los recursos son limitados. Severini et al. (2020) han

demostrado en trigo, que raíces profundas y densas tienen un alto rendimiento en condiciones óptimas de cultivo.

De acuerdo a lo descrito anteriormente, es fundamental ampliar y enfocar el conocimiento en los sistemas radiculares de las plantas, las cuales se relacionan con los microorganismos del suelo promoviendo el crecimiento y desarrollo de cultivos derivados de mejoramiento genético, sometidos a constantes aplicaciones de agroquímicos.

HIPÓTESIS

Variedades de trigo de invierno liberadas después de la década del 70, tienen un menor desarrollo radicular, lo cual se asocia a una menor interacción con la actividad biológica de la rizosfera.

OBJETIVO GENERAL

Evaluar y comparar variedades de trigo de invierno antiguas y modernas liberadas desde el año 1965 hasta el año 2020 según la actividad biológica de la rizosfera y el desarrollo radicular y aéreo y su efecto en la disponibilidad de nutrientes.

OBJETIVOS ESPECIFICOS

-Evaluar la actividad microbiana y enzimática del suelo de las variedades de trigo invernal en relación a tres importantes etapas fenológicas del trigo invernal (macolla, antesis y madurez fisiológica).

-Evaluar y comparar en función de la profundidad del perfil de suelo, la arquitectura radicular de variedades de trigo invernal y la colonización micorrícica arbuscular en relación a la disponibilidad de fósforo.

-Evaluar y comparar los exudados radiculares de las plantas de trigo invernal y su relación con las características del suelo.

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CAPÍTULO 2

Root architecture parameters and rhizosphere biology in fourteen winter wheat varieties released in Chile between 1965 and 2020

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ABSTRACT

Despite advancements in wheat genetics, the impact on root structure and rhizosphere biology is still inadequately understood. In this study, we investigated the exudation of carboxylates, colonization by arbuscular mycorrhizal fungi, microbial activity, and root architecture in winter wheat varieties released between 1965 and 2020. We have sown fourteen different winter wheat varieties with four replicates on acidic Andisol. Complete root systems and soil samples were extracted up to 60 cm depth. Our findings showed that varieties up to 1990 had higher carboxylate exudation, with succinate and oxalate being significantly higher by 21% and 4%, respectively. Mycorrhizal colonization was inversely proportional to phosphorus concentrations and all varieties showed higher microbial activity at anthesis. The longest roots were found in varieties released after the year 2000, but no significant differences were found in other root architecture parameters. There was no clear pattern observed in root architecture or biological activity as a function of the year of release. Plant genetics moderated root architecture, carboxylate exudation, microbial activity, and mycorrhizal colonization, all of which are affected by high phosphorus concentrations. The study was conducted at field conditions, considering the interactions of plant-microorganisms, which are often neglected due to the difficulty of analyzing root systems. Our results analyzed the architecture and biological activity of different varieties, highlighting those that have the potential to produce crops with more efficient roots.

Keywords: Andisol; Carboxylate; Arbuscular mycorrhizal; *Triticum aestivum*

1. INTRODUCTION

Winter wheat (*Triticum aestivum* L.) is a vital cereal crop with widespread global significance, playing a crucial role in food security. In Chile, the genetic improvement of winter wheat started in 1959 at the Instituto de Investigaciones Agropecuarias (INIA) Carillanca, with the creation of F1 hybrids that incorporated the dwarfing genes of the Norin 10 cultivar (Del Pozo et al. 2021). This cereal is the most extensively planted type of wheat in the world (Qin et al. 2019).

In the pursuit of improving wheat production, various genetic modifications have been made to the crop, including selection of aerial organ traits and application of agrochemicals. This has resulted in the creation of new wheat varieties with improved yield and productivity (McGrail and McNear 2021). However, little consideration has been given to the impact of these advancements on root exudation and interactions with arbuscular mycorrhizal fungi (AMF).

The release of modern wheat varieties has been accompanied by a decrease in root biomass over time (Aziz et al. 2017). This trend is likely due to the incorporation of dwarfing genes in the 1960s (Matus et al. 2012; McGrail and McNear 2021) and the application of inorganic fertilizers in excess of the plant's needs. These factors result in a high availability of nutrients, so the plant does not need to invest resources in developing its root system to intercept these nutrients (Jacquiod et al. 2022; McGrail and McNear 2021).

The relationship between plants and microorganisms is shaped by the variations in root morphology and traits within the plant genotype, such as root length and axial development, as well as the characteristics of the microorganisms themselves (Campos et al. 2018; de Souza Campos et al. 2021). Plant cultivars have functional traits in their root systems that enable them to efficiently obtain phosphorus (P) from the soil. These include root exudation of carboxylates, colonization by AMF (Ober et al. 2021), and modifications in root morphology, such as increased depth, density, and diameter. Deeper, denser, and larger diameter roots lead to higher yields due to their greater efficiency in nutrient acquisition (Severini et al. 2020; Wen et al. 2022). P

is a nutrient that tends to bind to soil clays, hindering its mobility and having to be absorbed by the roots through the diffusion mechanism (de Souza Campos et al. 2021), where AMF colonization favors this process, occurring mainly under P-limited conditions since high P application limits AMF community structure (Qin et al. 2020) and the action of some enzymes essential for P mineralization (Liu et al. 2023). On the other hand, when P is scarce, plants increase the release of organic compounds such as carboxylates to dissolve inorganic P in the soil (Pi). This can also be achieved indirectly by signaling to the arbuscular mycorrhizal fungi (AMF) through the distribution of photosynthates (Wen et al. 2022), This is further reinforced by the work of enzymes such as acid phosphatase (P-ase), which play a role in the mineralization of organic phosphorus (Po) (Liu et al. 2023; Nazareno et al. 2020). P can also be obtained from organic matter. However, in soil with low organic P, inorganic sources are a significant source of P. In Andisols, P is bound to aluminum and iron. Furthermore, Chilean Andisols have high levels of total P, organic matter, and acidity. Andisol are the least extensive soil order (Soil Survey Staff 2014) and occupy only 0.7% of the earth's land surface or just below 963,000 km². In Chile, volcanic soils support the bulk of agricultural and forestry production, covering more than 5.3x10⁶ hectares and representing nearly 50-60% of the country's arable land (Borie and Rubio 2003).

The study of carboxylates is becoming increasingly important due to their effectiveness in dissolving P. It has been demonstrated that certain genetically modified plants are capable of increasing the release of organic anions in environments with a shortage of P (Wang et al. 2013a). The efficiency of P solubilization by carboxylates is given by: citrate > oxalate > malate, being citrate and malate, most studied in wheat (Wang et al. 2013c; Wang et al. 2017). As a result, these exudates improve the uptake of nutrients that are otherwise difficult to dissolve and also stimulate the growth and activity of the soil microorganisms. This, in turn, enhances root exploration and leads to better nutrient absorption (Bünemann et al. 2018; Kuzyakov et al. 2000). However, this rhizosphere biological activity can increase or decrease depending on the nutritional needs of crop phenological stages

(Wang et al. 2017) which are closely related to root development and growth (Dharmateja et al. 2021).

Research has shown that during the anthesis stage, there is a rise in the number of AMF in wheat roots. This is because the leaves are growing rapidly and producing a high rate of photosynthesis, generating essential carbohydrates for the nutrition of the mycorrhizae. On the other hand, during the physiological maturity stage, the leaves start to age and photosynthesis decreases, leading to a decrease in AMF colonization (Naseer et al. 2022; Zhu et al. 2017). The goal of this study was to compare and evaluate fourteen winter wheat varieties that were released in Chile between 1965 and 2020, with a focus on their rhizospheric biological activity and root and aerial development at the tillering, anthesis, and physiological maturity stages, under conventional field conditions.

2. Results

2.1. Soil biological properties

The analysis of FDAse activity provides information on the activity of the microbial community present in wheat varieties at tillering, anthesis, and physiological maturity stages. Figure 1a displays the experimental results of FDAse compound measurement, which showed a statistically significant increase ($p < 0.05$) at tillering and anthesis stages compared to physiological maturity. There was a decrease of 59 % in FDAse activity during the physiological maturity stage compared to the other two stages. However, no significant differences were observed among the fourteen wheat varieties at any of the three phenological stages (Figure 1b) or during the entire season (Figure 1c).

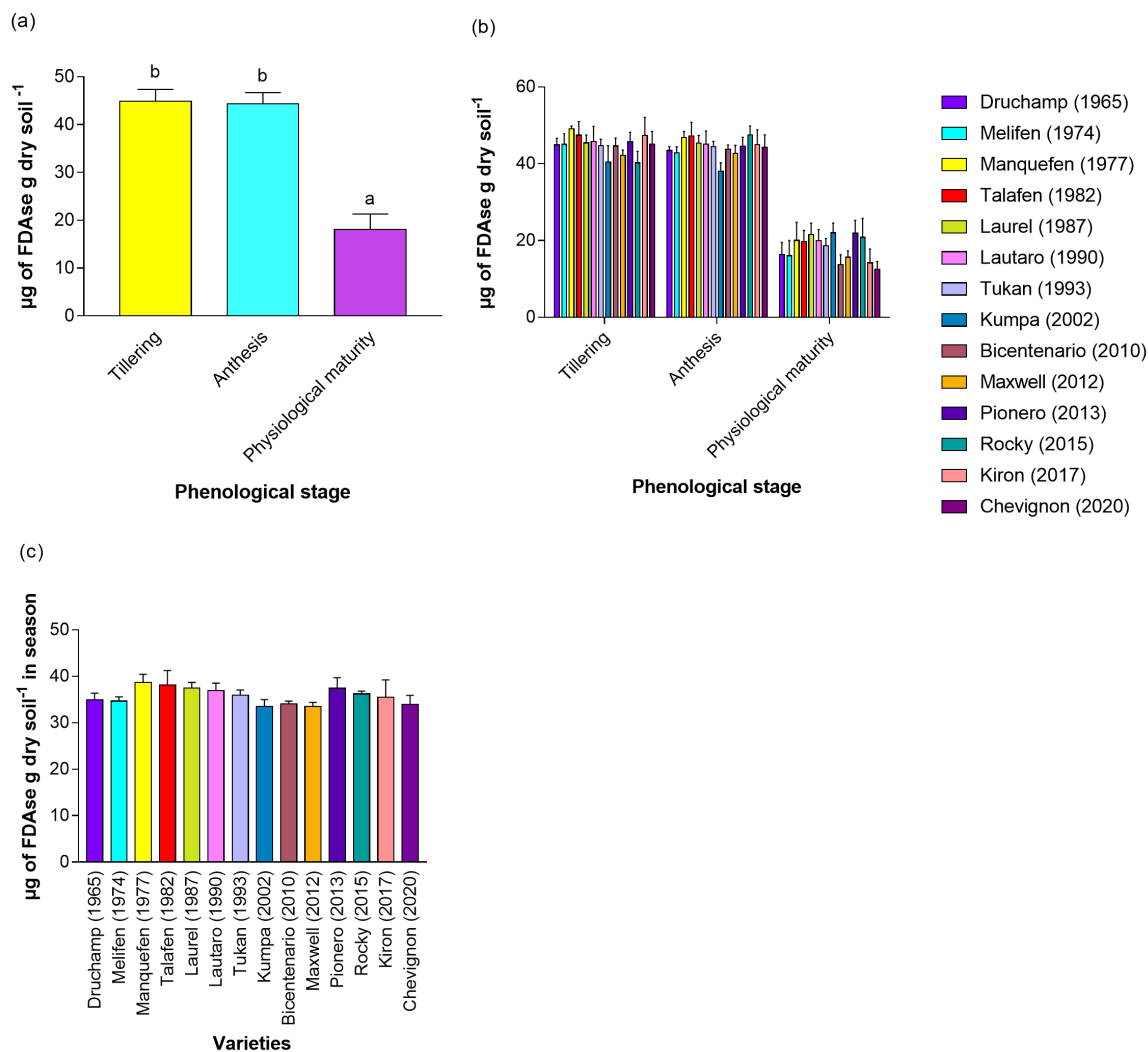


Figure 1. (a) Mean values of FDase activity of the soil ($\mu\text{g of FDase g dry soil}^{-1}$) present at tillering, anthesis, and physiological maturity phenological stages, (b) mean values of FDase activity ($\mu\text{g of FDase g dry soil}^{-1}$) of each wheat variety at tillering, anthesis and physiological maturity phenological stages, and (c) mean values of FDase activity ($\mu\text{g of FDase g dry soil}^{-1}$) of each wheat variety during the whole season. Means \pm SEM is indicated (n=56). Different letters indicate significant differences among phenological stages, as revealed by Fisher's LSD test ($p > 0.05$).

Fuente: Elaboración propia

The analysis and comparison of basal soil microbial respiration in the different phenological stages, tillering, anthesis and physiological maturity, were statistically significant ($p>0.05$) in the tillering and anthesis stages, being 40 % higher than in the physiological maturity stage (Figure 2a). As for the varieties, Figure 2b shows significant differences ($p>0.05$) among varieties in the tillering stage, with the variety Laurel (1987) the one that obtained the highest basal soil respiration, being 35 % higher than the rest of the varieties. However, the statistical test showed no significant differences among varieties at the stages of anthesis and physiological maturity, nor during the whole season (Figure 2c).

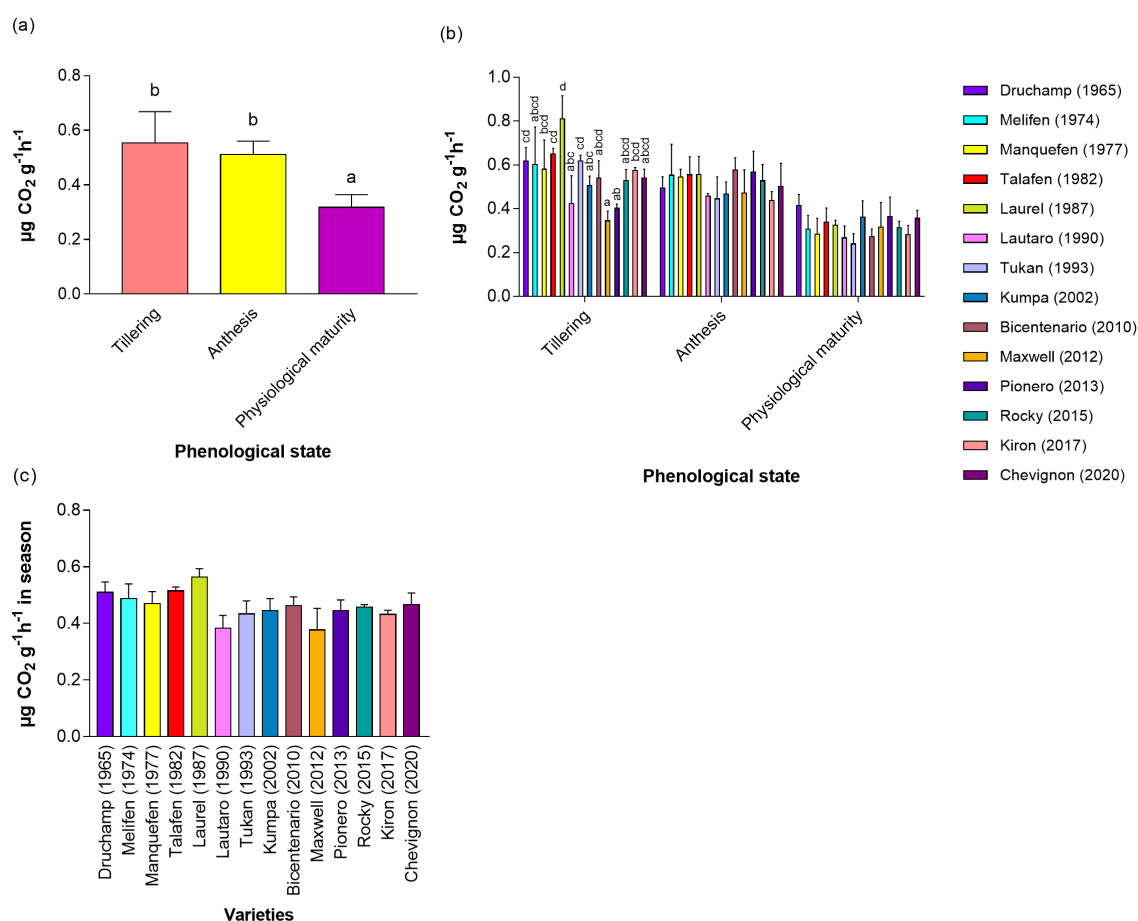


Figure 2. (a) Mean values of soil microbial respiration of the soil ($\mu\text{g CO}_2 \text{ g}^{-1} \text{ h}^{-1}$) at tillering, anthesis, and physiological maturity phenological stages, (b) mean values of soil microbial respiration ($\mu\text{g CO}_2 \text{ g}^{-1} \text{ h}^{-1}$) of each wheat variety at tillering, anthesis

and physiological maturity phenological stages and (c) Mean values of soil microbial respiration ($\mu\text{g CO}_2 \text{ g}^{-1} \text{ h}^{-1}$) during the whole season. Means \pm SEM is indicated (n=56). Different letters indicate significant differences among varieties and phenological stages, as revealed by Fisher's LSD test ($p>0.05$).

Fuente: Elaboración propia

The measurement of the P-ase enzyme activity, represented by PNP in the three phenological stages, showed significantly different values ($p>0.05$), where the anthesis stage was 31 % higher than the tiller stage and 18 % higher than maturity stage physiological (Figure 3a). Figure 3b shows the activity of the P-ase enzyme in each wheat variety concerning their phenological stages, The varieties presented significant differences ($p>0.05$) only in the tillering stage, with Pionero (2013) being approximately 46 % higher than the other varieties. However, no significant differences were found between varieties in the stages of anthesis and physiological maturity, nor during the whole season (Figure 3c).

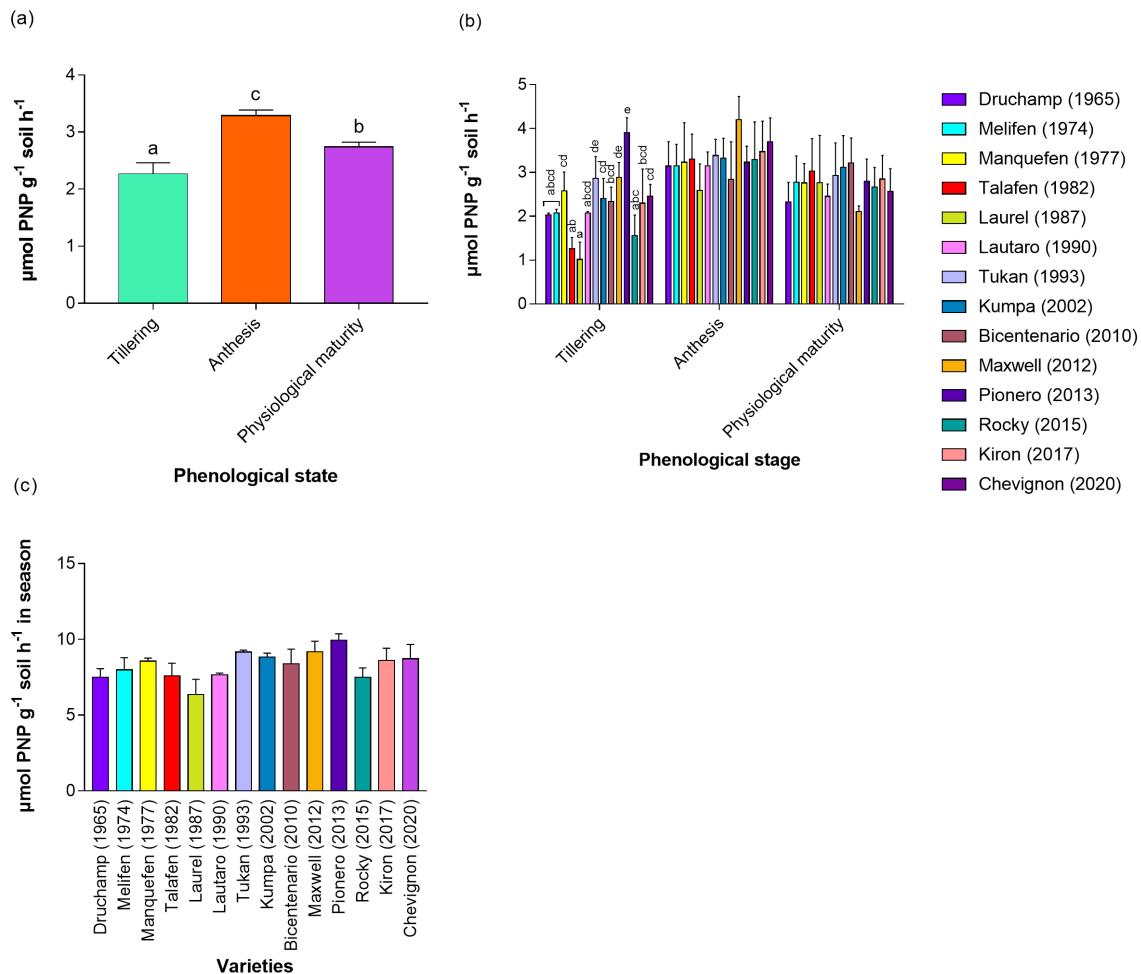


Figure 3. (a) Mean values of acid phosphatase enzyme activity of the soil ($\mu\text{mol PNP g}^{-1} \text{soil h}^{-1}$) at the phenological stages of tillering, anthesis, and physiological maturity, (b) mean values of acid phosphatase enzyme activity of the soil acid phosphatase ($\mu\text{mol PNP g}^{-1} \text{soil h}^{-1}$) of each wheat variety at tillering, anthesis, and physiological maturity phenological stages, and (c) mean values enzyme activity of the soil acid phosphatase ($\mu\text{mol PNP g}^{-1} \text{soil h}^{-1}$) of each wheat variety during the whole season. Means \pm SEM is indicated ($n=56$). Different letters indicate significant differences among varieties and phenological stages, as revealed by Fisher's LSD test ($p>0.05$).

Fuente: Elaboración propia

2.2 Plant analysis

Aboveground biomass

Regarding aerial biomass, as shown in Figure 4a, the weight of the plants at different phenological stages - tillering, anthesis, and physiological maturity - is displayed. The stages of anthesis and physiological maturity were significantly different ($p>0.05$) compared to the tillering stage, with a decrease of 93 %. Figure 4b presents the weight of the wheat varieties at different phenological stages. Statistical analysis revealed significant differences at the anthesis and physiological maturity stages. Specifically, at anthesis, the Druchamp (1965) variety was found to be statistically different ($p>0.05$), displaying a 35 % increase in weight compared to the other varieties. Regarding the state of physiological maturity, the Druchamp (1965) variety differed significantly ($p>0.05$), being around 33 % higher than the varieties Laurel (1987), Lautaro (1990), Tukan (1993), Bicentenario (2010), Pionero (2013), Rocky (2015), Kiron (2017) and Chevignon (2020). Overall, the varieties showed a decrease in weight compared to the Druchamp (1965) variety at both the anthesis and physiological maturity stages. However, no correlation was found between the release years and the decrease in plant weight.

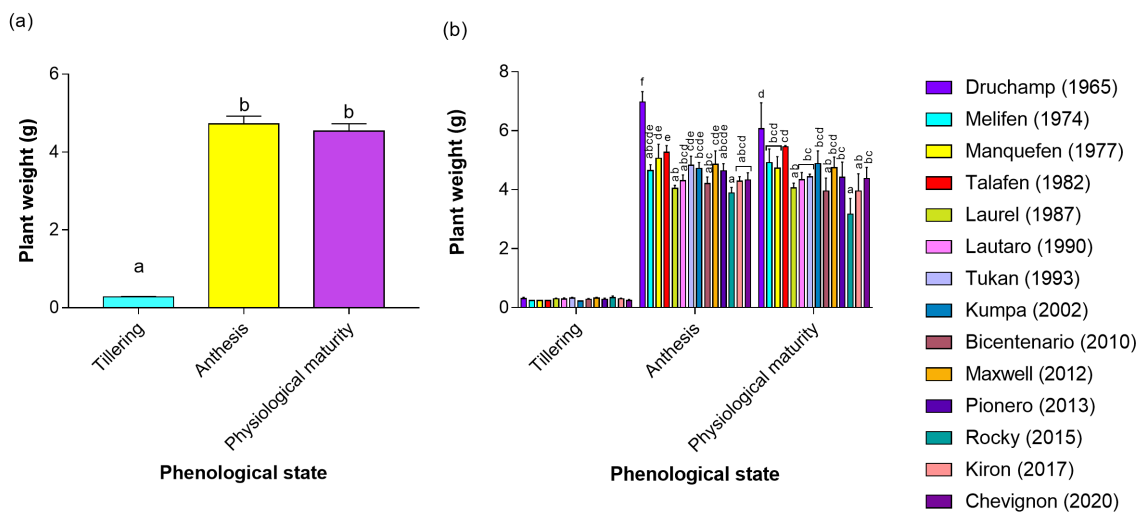


Figure 4. (a) Mean plant weight values (g) at tillering, anthesis, and physiological maturity phenological stages and (b) mean plant weight values of each wheat variety at tillering, anthesis, and physiological maturity phenological stages. Means \pm SEM is

indicated ($n=56$). Different letters indicate significant differences among varieties and phenological stages, as revealed by Fisher's LSD test ($p>0.05$).

Fuente: Elaboración propia

The measurement of plant height at various phenological stages revealed significant differences ($p>0.05$) at the anthesis and physiological maturity stages, which were 87 % taller than the tillering stage (Figure 5a). For wheat varieties, significant differences among varieties were observed at the anthesis and physiological maturity stages. At both stages, Druchamp (1965) was the variety that obtained the greatest height, being significantly different ($p>0.05$) by approximately 30 % from the other varieties (Figure 5b). Generally, at the anthesis and physiological maturity stages, a decrease in height was observed in relation to the Druchamp (1965) variety. At the physiological maturity stage, the varieties released up until the 1990s showed the greatest height, with a 15 % increase compared to the varieties from the 2000s. However, among this group, Chevignon (2020) was one of the tallest varieties, surpassing even Tukan (1993).

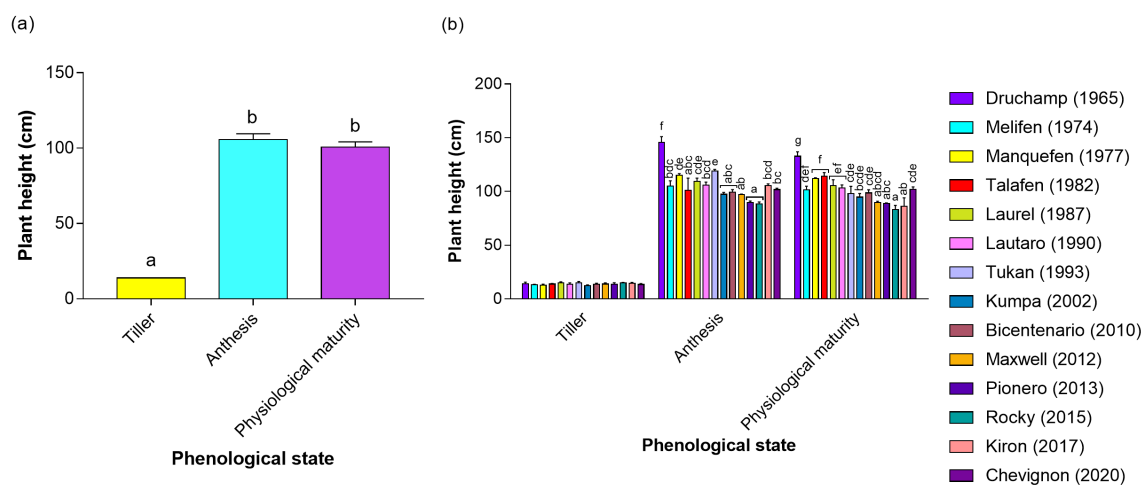


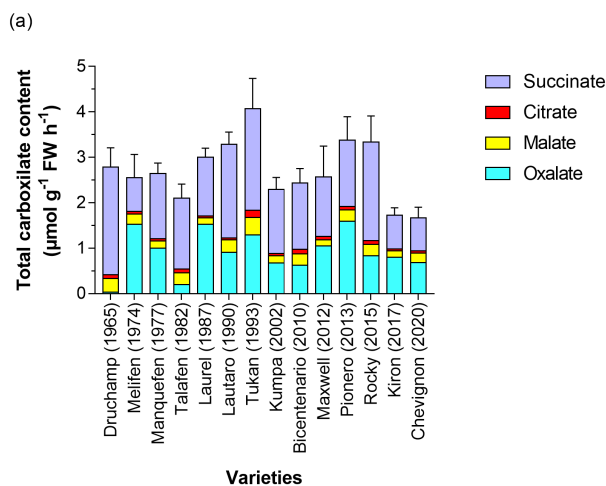
Figure 5. (a) Mean values of plant height at tillering, anthesis, and physiological maturity phenological stages and (b) mean values of plant height of each wheat variety at tillering, anthesis, and physiological maturity phenological stages. Means \pm

SEM is indicated (n=56). Different letters indicate significant differences among varieties and phenological stages, as revealed by Fisher's LSD test ($p>0.05$).

Fuente: Elaboración propia

2.3. Carboxylate exudation

The root systems were found to primarily exude succinate as the main carboxylate, exuding an average of 76 % more than the other carboxylates, which was found to be statistically significant ($p>0.05$) (Figure 6a). Regarding the exudation of each variety (Figure 6b), no significant differences were found for succinate, malate and succinate exudation, except oxalate, where the varieties Druchamp (1965) and Talafen (1982) showed significant differences, exuding approximately 89 % less oxalate than the other varieties.



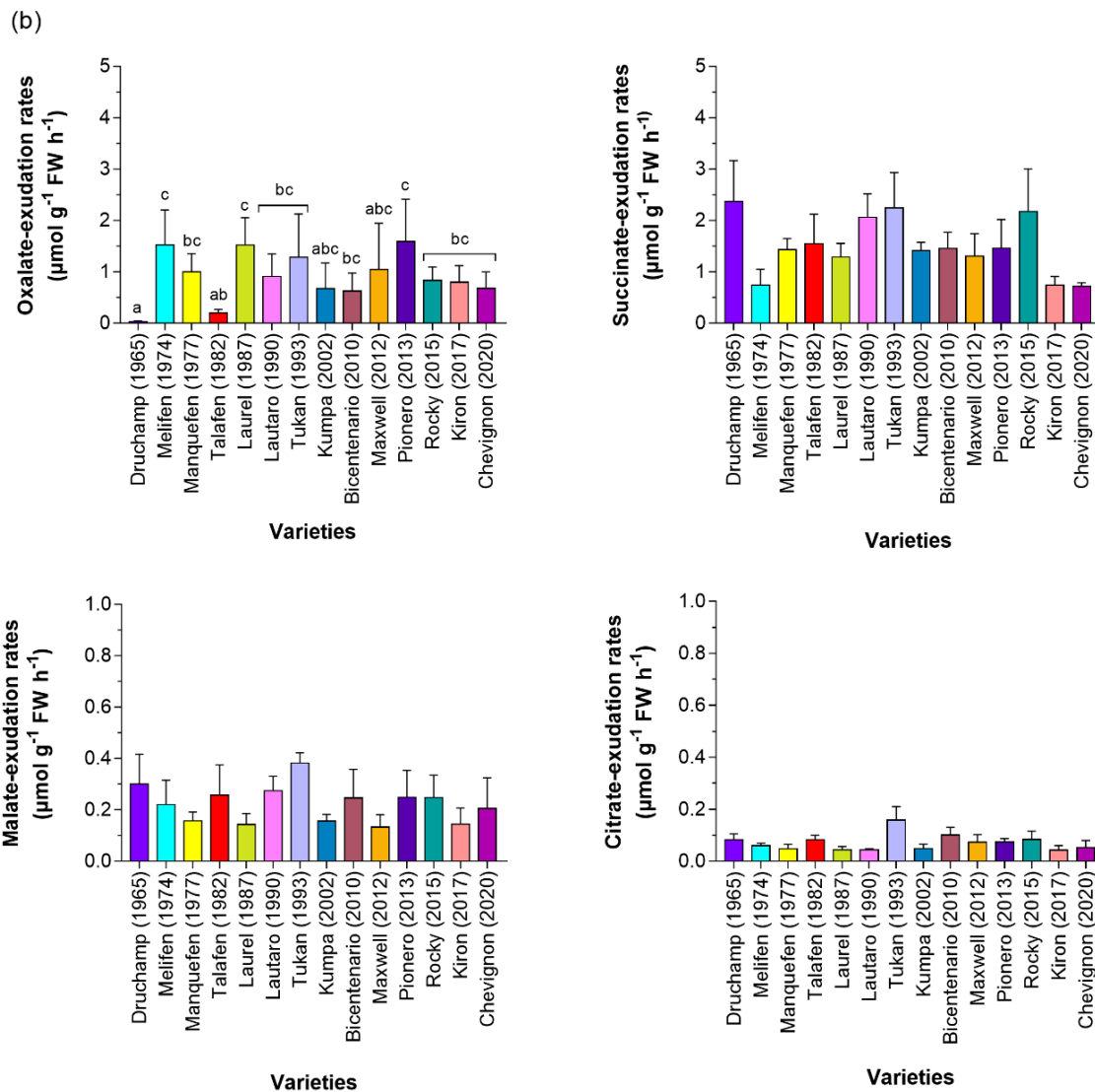


Figure 6. (a) Total carboxylate content exuded per complete root systems of each wheat variety ($\mu\text{mol g}^{-1} \text{FW h}^{-1}$) (b) mean values of oxalate, succinate, malate, and citrate exudation per complete root systems of each wheat variety ($\mu\text{mol g}^{-1} \text{FW h}^{-1}$). Mean \pm SEM is indicated (n=56). Different letters indicate significant differences between varieties as revealed by Fisher's LSD test ($p>0.05$).

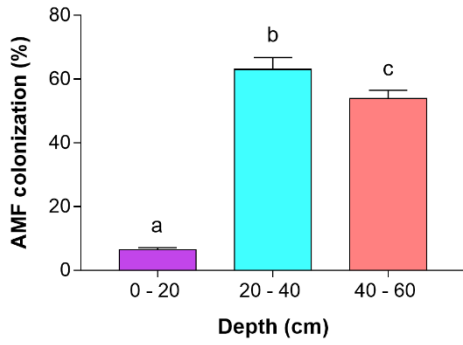
Fuente: Elaboración propia

2.4 Percentage of AMF colonization

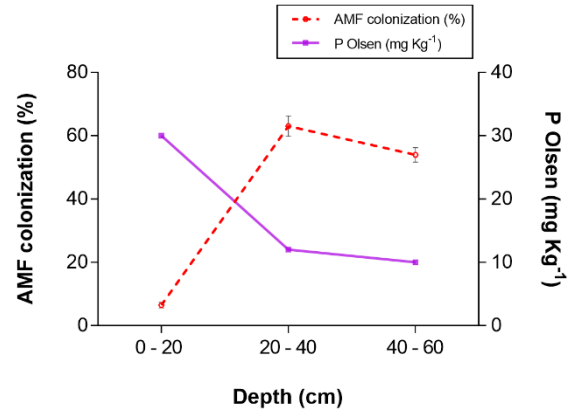
Figure 7a provides an overview of the significant variations in AMF colonization percentages at different soil depths (0-20 cm, 20-40 cm, and 40-60 cm) at anthesis stage. The results indicate that, in general, the highest AMF colonization rate of 63 % was observed at a soil depth of 20-40 cm, and it was found to be statistically significant ($p>0.05$). At 40-60 cm depth, AMF colonization reaches 54 %. However, only 6.5 % AMF colonization was observed at 0-20 cm depth. About this, Figure 7b showcases the concentration of P at various soil depths. The highest concentration of P (30 ppm P-Olsen) was observed at a depth of 0-20 cm, while the lowest concentration (10 ppm P-Olsen) was found at a depth of 40-60 cm. The P concentration was found to have significant effects ($p>0.05$) on the percentage of AMF colonization.

Among the varieties, Laurel (1987) and Maxwell (2012) showed the highest colonization percentages at a soil depth of 0-20 cm, and these results were found to be statistically significant ($p>0.05$). Druchamp (1965), Laurel (1987), Lautaro (1990), Tukan (1993), Bicentenario (2010), Pionero (2013), and Chevignon (2020) showed significant differences ($p>0.05$) and had 29 % higher AMF colonization than the other varieties at 20-40 cm depth. At a soil depth of 40-60 cm, the highest colonization percentages were achieved by the varieties Kiron (1987) and Laurel (2017), and these results were found to be statistically significant ($p>0.05$) compared to the other varieties. Overall, the varieties that showed the highest colonization percentages at a depth of 60 cm were Laurel (1987), Bicentennial (2010), and Druchamp (1965), and these results were significantly different ($p>0.05$) from those obtained by Talafen (1982), Kumpa (2002), and Rocky (2015) (Figure 7d).

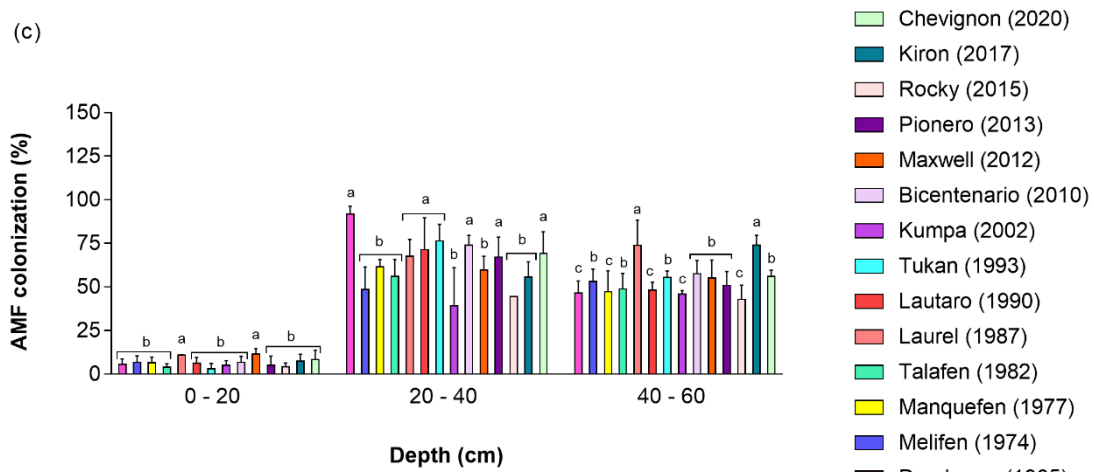
(a)



(b)



(c)



(d)

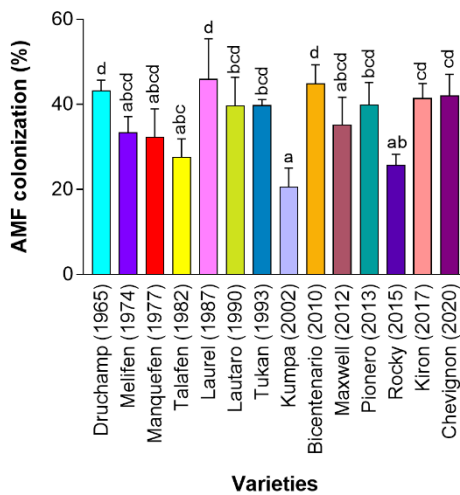


Figure 7. (a) Mean values of AMF colonization (%) in wheat varieties at depths of 0-20 cm, 20-40 cm, and 40-60 cm, (b) mean values of AMF colonization percentage (%) in wheat varieties in relation to phosphorus concentrations analyzed at different depths 0-20 cm, 20-40 cm, and 40-60 cm, (c) Euclidean distance analysis of percent AMF colonization (%) in each wheat variety at depths of 0-20 cm, 20-40 cm and 40-60 cm and (d) mean values of percent AMF colonization (%) in wheat varieties at 60 cm depth. Means \pm SEM is indicated (n=42). Different letters indicate significant differences among varieties and depths, as revealed by Fisher's LSD test ($p>0.05$).

Fuente: Elaboración propia

2.5 Roots analysis

The variety Chevignon (2020) had the longest root length up to a depth of 60 cm, with a length that was 39 % greater than that of the other varieties, and these results were found to be statistically significant ($p>0.05$) with respect to Bicentenario (2010), Laurel (1987), Kiron (2017), Talafen (1982), Maxwell (2012), Druchamp (1965), Lautaro (1990), Pionero (2013), Manquefen (1977) and Melifen (1974) (Figure 8a). The root length results for each variety represent the combined length of all its individual roots. The varieties with the largest root area at a depth of 60 cm were Chevignon (2020), Laurel (1987), and Bicentenario (2010). However, the ANOVA statistical test showed that there were no significant differences in root area compared to the other varieties (Figure 8b). The varieties Laurel (1987), Bicentenario (2010), and Kumpa (2002) obtained the greatest root volume at 60 cm depth, but no statistically significant differences were found with respect to the other varieties (Figure 8c). Regarding root diameter at a depth of 60 cm, the varieties Manquefen (1977), Laurel (1987), and Bicentenario (2010) had the highest values. However, statistical tests did not show significant differences with respect to the other varieties (Figure 8d). At 60 cm depth, the varieties Rocky (2015), Druchamp (1965), and Melifen (1974) showed the greatest root weight. However, statistical analysis did not reveal any significant differences among these varieties and the others (Figure 8e).

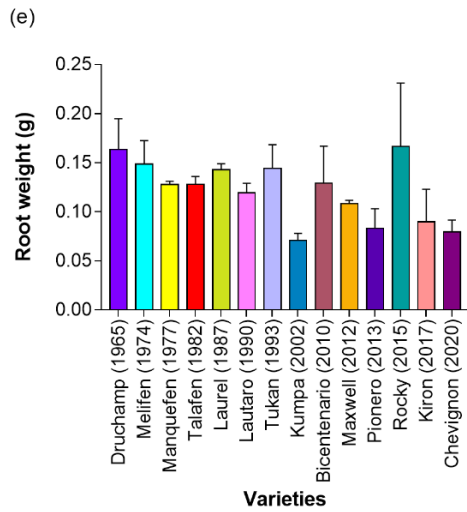
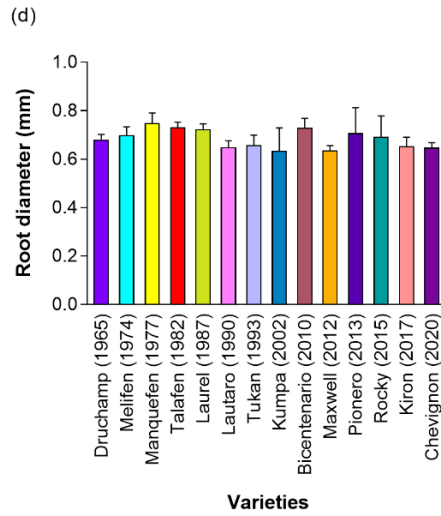
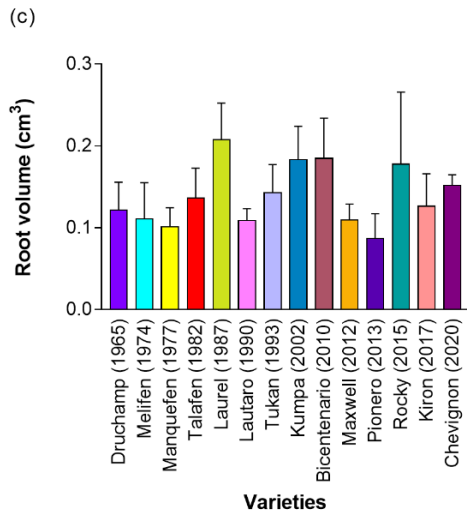
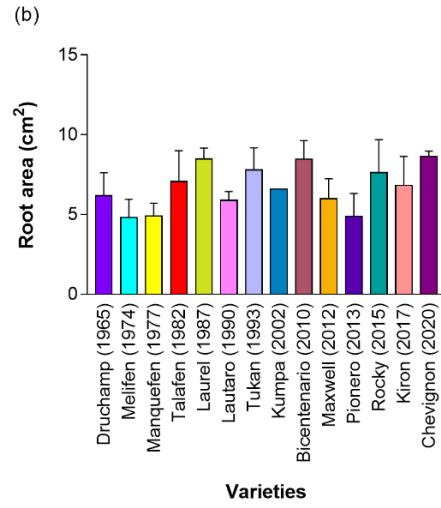
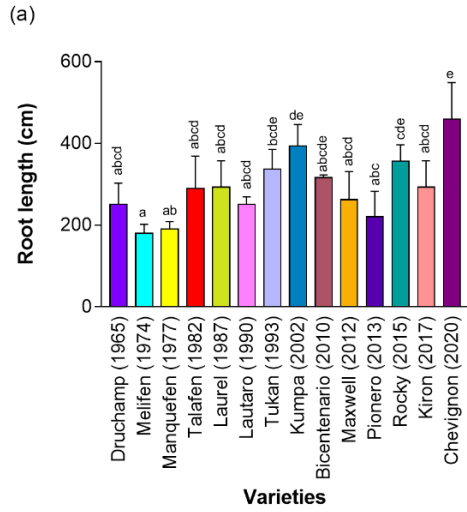


Figure 8. (a) Mean values of root length of wheat varieties at a depth of 60 cm (cm), (b) mean values of root area of wheat varieties at a depth of 60 cm (cm²), (c) mean values of root volume of wheat varieties at a depth of 60 cm (cm³), (d) mean values of root diameter at a depth of 60 cm (mm) and (e) mean values of root weight of wheat varieties at a depth of 60 cm (g). Means \pm SEM is indicated (n=42). Different letters indicate significant differences between varieties as revealed by Fisher's LSD test ($p>0.05$).

Fuente: Elaboración propia

3. Discussion

3.1 Soil microbial activity

In regards to soil microbial activity, the activity of FDAse and microbial respiration were found to be significantly higher for all wheat varieties at the tillering and anthesis stages compared to the physiological maturity stage. This observation aligns with the findings of a field study by Chen et al. (2021) which showed a decrease in soil respiration in wheat during anthesis and physiological maturity. The soil microbial activity was found to be highest at the tillering and anthesis stages of the wheat varieties. This is because the highest nutrient uptake rate (N and P) occurs during these stages. The wheat plant accumulates approximately 75 % of its total N and 85% of its total P during anthesis, after which the nutrient uptake rate decreases and the plant begins to remobilize nutrients from its roots to its aerial organs, such as spikelets and grains. This observation aligns with a study conducted by Berardo and Reussi (2009) which found that the rate of nutrient absorption decreases after anthesis. Plants have a strategy to obtain nutrients from the soil through increased microbial activity. This activity is further enhanced by the rhizodeposition of previous and established crops, where the quantity and quality of C inputs from rhizodeposition play a role in favoring the activity of soil microorganisms (Chaparro et al. 2014; Powlson et al. 2008). One strategy that plants use to obtain nutrients from the soil is the exudation of carbon by roots. This is because exudates serve as an important source of energy for soil microorganisms. However, the amount and composition of

these exudates will vary depending on the plant species and its phenological stage (Gargallo-Garriga et al. 2018). In our study, we observed that the variety Laurel (1987) had higher CO₂ emission in the tillering stage, and this result was statistically significant compared to the other varieties. The variety Laurel (1987) is characterized by good tillering ability (Aguayo 1986), suggesting a higher photosynthetic rate and greater C exudation by roots at the tillering stage. On the other hand, Meier et al. (2023) showed that the addition of P in winter wheat increased the utilization of C sources by microorganisms and, therefore, CO₂ production.

During the early stages of a wheat plant's life cycle, there is a high demand for P. The uptake of P and the development of roots occur before shoot growth (Romer and Schilling 1986). When the plant is in a P-deficient environment, it generates strategies to acquire this element, such as the modification of the root system or the secretion of phosphatase enzymes (also released by P-solubilizing microorganisms), which are essential to mineralize soil Po (de Souza Campos et al. 2021; Liu et al. 2023; Wu et al. 2021). However, our results indicated that phosphatase activity was significantly lower during the tillering stage. In general, the results of phosphatase activity were high compared to those obtained by Schoebitz et al. (2020). Unlike that study, our wheat crops received a high dose of triple superphosphate fertilizer prior to sowing and initially had a high concentration of P in the soil. In relation to this, a study by Liu et al. (2023) determined that high P inputs (200 kg ha⁻¹ yr⁻¹) reduce the relative abundance of genes involved in the P cycle because high doses of P block the signal transduction pathway among the main genes encoding the regulation of the P starvation response (phoR and phoP) and regulating the high-affinity phosphate-specific transport system (pst). However, their results showed that P-ase genes (phoN, aphA, and olpA) increased with long-term P influx and alkaline phosphatase (P-al) genes (phoA and phoD) decreased. On the other hand, Meier et al. (2023) cultivated wheat varieties in an Andisol with low and high P concentrations (4 and 30 mg P kg⁻¹), with water stress and abundant irrigation, and the results obtained differentiated among genotypes. The results of P-ase activity differed among the wheat varieties, as seen in the study conducted by Meier et al. (2023). The Druchamp

(1965) variety displayed stable P-use activity regardless of changes in P doses and water stress, unlike the Rancofén variety, which showed the highest P-use activity in low P conditions. These findings indicate that the impact of high P doses on P-use activity varies among different cultivar varieties (de Souza Campos et al. 2021).

3.2 Analysis of aboveground plant biomass and root architecture

Our findings reveal that the release of wheat varieties between the 1960s and 1990s resulted in a 33 % reduction in weight and a 25 % decrease in height, while the varieties released in the 2000s experienced an 8 % reduction in weight and a 17 % decrease in height. However, concerning the plant weight of wheat varieties, no gradual decrease was found in the years of release, as shown by Lo Valvo et al. (2018) in wheat varieties released between the years 1918 and 2011 in Argentina. Our findings on plant height align with the results reported by Del Pozo et al. (2021), where they observed that the height of the plants decreased with the year of release of the wheat variety. Similarly, a study by Matus et al. (2012) showed that the height of modern wheat cultivars (released after the 1960s) was reduced by 26 % when compared to older cultivars. This is a consequence of the introduction of semi-dwarf genes (Rht1 and Rht2) (Matus et al. 2012), which were incorporated starting in the 1960s and produced greater genetic gain in grain yield, including height reduction (Del Pozo et al. 2021).

Our findings show that the root length at 60 cm depth was greatest in the varieties released in the 2000s. This is because, during the tillering stage, plants allocate more of their photoassimilates towards developing their root system, before focusing on the growth of the above-ground parts. After the anthesis stage, root growth slows down while the above-ground parts continue to grow rapidly until the stage of physiological maturity. (Cabeza and Claassen 2017). This is consistent with the findings of Siddique et al. (1990), who observed that modern wheat varieties had a root penetration rate that was twice that of an older wheat cultivar. However, it's important to note that these results can vary greatly based on the specific plant genotype and soil nutrient conditions. The root system characteristics of 182 wheat varieties were

studied by Dharmateja et al. (2021) in hydroponics under P-limited and non-P-limited conditions. They found that the parameters of root length, area, volume, and diameter showed significant genetic variation. Under P-limited conditions, the total length, total volume, and total root area increased, while the primary root length and average root diameter decreased. Our crops were under high doses of inorganic fertilizers, generating a zone of high nutrient availability. Therefore, the plants would not need to invest resources in the development of their root system to acquire nutrients (McGrail and McNear 2021). These results show the response of the root architecture of different wheat varieties to a high dose of inorganic fertilization in the field.

3.3 Carboxylates and AMF colonization

Carboxylates can solubilize soil P by three mechanisms, chelating metal ions that immobilize P, displacing P from adsorption sites, and changing soil pH (Wang et al. 2013a). Citrate and malate have been the most documented carboxylates in wheat due to their efficiency to solubilize inorganic soil P (de Souza Campos et al. 2021; Wang et al. 2013a; Wang et al. 2017). However, our results showed that, in general, all wheat plants exuded significantly more succinate, followed by oxalate, and to a lesser extent, malate and citrate. A study by Wang et al. (2015) evidenced that in P-deficient environments, plants exude higher amounts of carboxylates, on the contrary, in a high P environment, plants limit the release of carboxylates due to the significant photosynthate cost (5-25 % of total carbon) involved in exudation (Campos et al. 2018; Wen et al. 2022).

Wang et al. (2013c) demonstrated that 200 kg P ha⁻¹ fertilization reduced citrate release, but citrate increased the efficiency of phosphate fertilizer at low fertilization rates (40 kg P ha⁻¹) by increasing crop growth and P uptake. Also, Wang et al. (2017) found that the exudation of citrate, malate, and succinate in the soil by wheat plants was correlated with the levels of P applied and days after emergence, finding higher carboxylates at 29 days to 42 days after emergence, and higher carboxylate accumulation at fertilization of 48 kg P ha⁻¹ compared to no fertilization. Our results showed that wheat plants exuded higher amounts of succinate, followed by oxalate

and then citrate and malate. This is in contrast to the findings of Wen et al. (2019), who reported lower carboxylate exudation in wheat. However, our results are consistent with those of de Souza Campos et al. (2022), who observed higher exudation of oxalates and lower amounts of citrate and malate in wheat genotypes grown in pots with an acid Andisol at low P-Olsen concentration.

Tukan (1993), Pioneer (2013), and Rocky (2015) exuded the highest amounts of carboxylates. However, in general, varieties from 60 to 90 showed higher carboxylate exudation (15 %) compared to those from 2000. Our study revealed that there was no correlation between morphological traits and carboxylate exudation in the Tukan (1994), Pioneer (2013), and Rocky (2015) varieties. This finding is consistent with the results of Iannucci et al. (2021), who observed that despite having similar root characteristics, two durum wheat varieties (one ancient and one modern) showed differences in carboxylate exudation. This is because varieties of the same species may differ in their ability to exude carboxylate depending on their nutritional strategies (Iannucci et al., 2021). In addition, carboxylate exudation may depend on the phenological stage of the plant (Chaparro et al. 2014). However, Tukan (1993) is considered an inefficient genotype in P acquisition due to its low tolerance to Al in acidic Andisol and deficient in P (Seguel et al. 2017). Low Al tolerance, is associated with low citrate exudation (Dong et al. 2004). Our findings indicate that Tukan (1993) exuded a significantly higher amount of citrate compared to other varieties. The varying results in previous studies suggest that the exudation of carboxylates is influenced by the plant genotype and its interaction with the environment. Additionally, multiple processes that take place over time and space affect the plant's requirements (Seguel et al. 2017).

The relationship between high levels of P and low colonization by AMF has been widely documented. This relationship is due to the high energy costs for the plant (Kobae et al. 2016; Qin et al. 2020; Wen et al. 2019). A deeper explanation may be demonstrated by Wang et al. (2013b) who showed that the Ta-PHR1-A1 gene encoding PHR1 protein in wheat was involved in Pi signaling in response to Pi starvation, furthermore, upon expression, it stimulated root growth and thus enhanced

P uptake. Das et al. (2022) demonstrated that rice PHR2 (Phosphate Starvation Response) protein is an important regulator of phosphate starvation response in AMF. In their research they discuss that, at high phosphate levels, PHR2 is repressed by SPX proteins (Lv et al. 2014) in the cytoplasm, preventing PHR2 translocation into the nucleus and consequently, preventing PHR2 binding to the promoters of phosphate starvation-induced genes and genes involved in AMF signaling, exuding a lower amount of strigolactones by the plant and expressing a lower amount of genes sensing AMF Myc-factors.

4. Materials and Methods

4.1 Site and soil description

This study was conducted during the 2021-2022 season at INIA Experimental Station Santa Rosa (36°31'S, 71°54'W), located in the Mediterranean climate region of central Chile. The soil belongs to the Diguillín series, coming from modern volcanic ashes of the Andisol order, its texture is silt loam and the soil is classified as Typic Haploxerands (Soil Survey Staff 2014; Stolpe 2006). The chemical soil properties (0-20 cm) are as follows: organic matter: 5.8%; pH: 5.6; available N: 32 mg kg⁻¹; P-Olsen: 30 mg kg⁻¹; available K: 278 mg kg⁻¹.

4.2 Wheat varieties

Fourteen winter wheats (*Triticum aestivum* L.) varieties released in Chile between 1965 and 2020 were selected. The wheat varieties and their respective release years: Druchamp (1965), Melifen (1974), Manquefen (1977), Talafen (1982), Laurel (1987), Lautaro (1990), Tukan (1993), Kumpa (2002), Bicentenario (2010), Maxwell (2012), Pionero (2013), Rocky (2015), Kiron (2017), Chevignon (2020). Are considered modern cultivars because they were released after 1960 (Matus et al. 2012) and the semi-dwarf genes (Rht1 and Rht2) are incorporated into their germplasm (Del Pozo et al. 2021).

4.3 Experimental procedure

Experimental design was a complete block with four replicates. Plots consisted of nine rows of 2 m in length and 0.20 m distance between rows. Sowing date was may and the seed dose was 180 kg ha⁻¹, previously disinfected with 250 mL of Real@Top (BASF; 166.6 g L⁻¹ thiophanate-methyl, 8.3 g L⁻¹ pyraclostrobin and 83.3 g L⁻¹ triticonazole) and 120 mL of Punto 600 FS (ANASAC Chile; 600 g L⁻¹ imidacloprid) were applied per 100 kg of seed. Fertilizer application consisted of 250 kg ha⁻¹ of triple superphosphate before planting and 230 kg ha⁻¹ of urea (46% N), which was applied at the four-leaf stage, tillering, and first node. At the tillering stage, 100 kg ha⁻¹ of sulphomag (22 % K, 18 % Mg, and 22 % S) and 100 kg ha⁻¹ of muriate of potash (60 % KCL) were also applied. Weed control was carried out in preemergence with the application of 1 L ha⁻¹ of Bacara Forte, Bayer (120 g L⁻¹ flufenacet, 120 g L⁻¹ flurtamone, and 120 g L⁻¹ diflufenican) and in postemergence with the application of 4 g ha⁻¹ of Ally, Dupont (600 g kg⁻¹ metsulfuron-methyl) and 4 kg ha⁻¹ of MCPA (750 g L⁻¹ MCPA-dimethylammonium). For fungal disease control, 6.25 mL L⁻¹ of Juwell Top (BASF; 150 g L⁻¹ phenpropimorph, 125 g L⁻¹ kresoxim-methyl, and 125 g L⁻¹ epoxiconazole) and 0.8 L ha⁻¹ of Priori (Syngenta; 250 g L⁻¹ azoxystrobin) were applied. Plots were furrow irrigated at the tillering, flag leaf just visible, early heading, and medium milk stages.

4.4 Measurements

Samples from each variety were collected at three phenological stages of wheat: tillering (Z30), anthesis (Z65), and physiological maturity (Z92) (Zadoks et al. 1974). The measurements taken during the study included: basal soil respiration (from soil adhered to roots), activity of FDAse and acid phosphatase enzyme (P-ase), root and aerial biomass at tillering, anthesis, and physiological maturity stages. At anthesis, carboxylate exudation was measured in complete root systems at two different soil depths (20 cm to 60 cm), root architecture (area, diameter, volume, length, and weight), and the percentage of AMF colonization.

Soil samples were collected from the central rows of each plot using a tractor-mounted hydraulic sampler tube of 3.5 cm diameter, which reached a depth of 60 cm

(Figure S1). The samples were divided into three fractions based on their depths: 0-20 cm, 20-40 cm, and 40-60 cm.

4.5 FDAse activity

The hydrolysis of fluorescein diacetate (FDAse) was analyzed using the modified methodology by Alef and Nannipieri (1995). This method is based on a colorimetric approach and involves the hydrolysis of FDAse. We used 0.5 g of soil, taking duplicates and leaving one control with 60 % conditioning. The soil sample was added to falcon tubes along with 4.45 mL of sodium phosphate buffer (pH 7.8) and 50 μ L of FDAse. A control was made by adding 5 mL of sodium phosphate buffer (pH 7.8) to the falcon tube. Afterwards, the tubes were placed in a thermostatically controlled bath at 25°C for 1 h and then removed and placed in an ice bath to slow down the reaction. Then, 5 mL of acetone was added to the samples and controls, they were mixed in a vortex mixer, and filtered through a funnel with Whatman N° 40 paper. The filtrate was measured in a spectrophotometer at an absorbance of 490 nm, with a reference to the reagent blank (consisting of 1 mL of distilled water and 1 mL of acetone).

4.6 Basal soil respiration

The basal soil respiration was analyzed using the closed system soil incubation methodology (Alef and Nannipieri 1995). 25 g of 60 % WPFS-conditioned soil was placed in an incubation flask with a test tube containing 7.5 mL of NaOH. The control procedure containing only 7.5 mL of NaOH. The incubation flasks were tightly sealed and placed in an incubation chamber at 22°C for 2 days. After that, 1 mL (1000 μ L) of NaOH was extracted and 2 mL of BaCl₂ was added. Finally, the solution was titrated with HCl until it reached the endpoint at the equivalence point (when the pH reached 8.3).

4.7 Acid phosphatase enzyme activity

For the analysis of phosphatase activity, we used Tabatabai and Bremmer assay (1969): 2 mL of MUB buffer (12.2 g hydroxymethyl aminomethane + 11.6 g maleic acid + 14 g citric acid + 6.28 g boric acid + 488 mL NaOH 1 M and brought to 1 L distilled water) pH 6.5 and 0.5 mL p-nitrophenyl phosphate (PNP) were mixed and incubated at 37°C for 1 h, then cooled in a bath with ice for 10 min to stop the reaction. Next, 0.5 mL of 0.5 M calcium chloride and 2 mL of 0.5 M sodium hydroxide were added to the samples. Parallel blanks were also prepared by adding PNP after the addition of calcium chloride and sodium hydroxide. Both samples and controls were then centrifuged at 3400 rpm/8 min. The relevant dilutions were made and readings in a spectrophotometer at 398 nm. The standard curve was performed with PNP. Once the absorbance was obtained, it was interpolated on the standard curve, obtaining the μmol of PNP g^{-1} soil h^{-1} .

4.8 Root and aboveground biomass

The aboveground biomass was determined by measuring the dry matter. The aboveground part of the plant was harvested and dried in kraft paper bags in an oven set at 70°C/48 h until reaching a constant weight. The total biomass was weighed (Li et al. 2017). For root analysis at different depths, roots were washed to remove adhering soil (Qin et al. 2019). Root parameters such as length, area, volume, and diameter will be determined using the WinRhizo computerized system (Regent instruments Inc, Quebec, Canada) considering the volume of the sample extractor tube and centimeters of depth. Appendices Figure S2 to Figure S15 show exemplary images taken with the WinRhizo program.

4.9 Carboxylate exudation

Complete root systems were collected from the first 20 cm of soil. The roots were washed and incubated in 50 mL CaSO_4 (0.2 mM) at pH 5.5 and shaken on an orbital shaker for 2 h. The solution was filtered with a sterile syringe containing a 0.22 μm filter (PureTech™, FineTech, Taichung, Taiwan) and frozen at -20°C. The frozen solution was lyophilized in a lyophilization apparatus (OPERON, Korea) and

resuspended in 200 μL of analytical grade water. Carboxylates were quantified with an HPLC apparatus (Hitachi Primaide, MERCK, Darmstadt, Germany) and the separation was performed on a reversed-phase column (Kromasil 100-5-C18, Nouryon, Göteborg, Suecia). The mobile phase was carried out according to the method of Cawthray (2003). Citrate, malate, oxalate, and succinate were used as standards and detected at 210 nm. Values were expressed as rate of carboxylates exuded per gram of fresh weight per hour ($\mu\text{mol g}^{-1} \text{FW h}^{-1}$) (Delgado et al. 2013).

4.10 Percentage of AMF colonization

The extent of AMF colonization in roots was determined using a method described by Nicolson (1995) and Read et al. (1976). Root pieces of 1 cm were cut and washed with plenty of water to remove adhering soil, then clarified with KOH (2.5 % w/v) at 120°C/15 min. After that, the KOH was removed and the roots were washed with water, then the roots were covered with HCl (1% w/w) and left for 1 day to remove excess KOH. Subsequently, the HCl was discarded, and the roots were washed with plenty of water and covered with trypan blue (0.05 % w/v) for 1 day. Finally, the dye was carefully discarded and the roots were washed again with water. With this method, AMF structures (hyphae, arbuscules, and vesicles) were visualized in root tissues. For the quantification of root colonization, we used the line intercept method, the roots were randomly distributed on a grid plate and observed with a microscope at 40X, subsequently, all root intersections with horizontal lines were counted (Giovannetti and Mosse 1980).

4.11 Statistical analysis

The statistical analysis was carried out using the INFOSTAT program. Normality and homogeneity assumptions were checked using the Shapiro-Wilk and Levene tests. The results were analyzed using an analysis of variance (ANOVA) and were considered statistically significant at a p-value of less than 0.05. Mean comparison was conducted using Fisher's LSD test. To analyze the percentage of AMF

colonization at different depths (0-20 cm, 20-40 cm, and 40-60 cm), a Euclidean distance cluster analysis was applied.

5. Conclusions

Our findings reveal that there is substantial genotypic variation among winter wheat varieties with regards to their rhizosphere biology and root architecture. This suggests that there are varieties with more efficient nutritional strategies, such as carboxylate exudation, microbial activity, and root growth, which are not necessarily dependent on the year of their release. Our findings indicate that there is no clear pattern of variation in root architecture parameters (such as volume, area, diameter, and length) and rhizosphere biological activity with respect to the year of release of the wheat variety. This field experiment shows that there is no direct correlation between root architecture characteristics and rhizosphere biological activity. Instead, the level of biological activity in the rhizosphere and root architecture is influenced by various factors including P fertilization, environmental conditions, and the crop's growth stage. Further research is required to examine the root architecture, microbial activity and nutrient utilization efficiency under low levels of fertilization.

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Author Contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Paula Paz-Vidal, Dalma Castillo-Rosales, María Dolores López, Iván Matus, Felipe Noriega and Mauricio Schoebitz. The first draft of the manuscript was written by Paula Paz-Vidal and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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