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Caracterización fisiológica, productiva y de calidad en arroz negro (*Oryza sativa* spp. *japonica*) bajo condiciones hídricas contrastantes

Physiological, productive, and quality characterization of black rice (*Oryza sativa* spp. *japonica*) under contrasting water conditions

Tesis para optar al grado de Doctora en Ciencias de la Agronomía

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RESUMEN

El arroz (*Oryza sativa* L.) es uno de los cultivos más importantes a nivel mundial y se considera un alimento básico para gran parte de la población. En Chile, su producción se concentra principalmente en las regiones del Maule y Ñuble, donde las condiciones ambientales resultan más favorables. No obstante, el cultivo enfrenta diversas limitaciones, como bajas temperaturas, disponibilidad limitada de agua y suelos de baja fertilidad, lo que limita las opciones de manejo y de rotación para la industria arrocera.

El arroz se cultiva tradicionalmente en condiciones de inundación, lo que requiere un elevado volumen de agua para mantener la sostenibilidad y la productividad del cultivo. En los últimos años, los efectos del cambio climático se han intensificado, evidenciándose principalmente en la disminución de la frecuencia y la cantidad de las precipitaciones, lo que ha provocado una creciente escasez hídrica que afecta directamente la producción arrocera. Frente a este escenario, resulta fundamental desarrollar nuevos cultivares capaces de tolerar el déficit hídrico y de utilizar el agua de manera más eficiente, sin comprometer la calidad del grano.

El arroz blanco es el más producido y consumido a nivel mundial; sin embargo, dentro de la especie *Oryza sativa* existen otros tipos de arroz, denominados pigmentados, que presentan tonalidades que van desde el rojo hasta el púrpura oscuro, siendo este último conocido como arroz negro. Originario de Asia, el arroz negro se distingue por su alta concentración de antocianinas en el pericarpio, responsables de su coloración característica. Estudios comparativos entre arroces pigmentados y no pigmentados han evidenciado una mayor tolerancia de los primeros al déficit hídrico.

El Programa de Mejoramiento Genético de Arroz (PMGA) del Instituto de Investigaciones Agropecuarias (INIA) de Chile ha concentrado sus esfuerzos en la evaluación de genotipos para determinar su rendimiento en condiciones de cambio climático y en el desarrollo de cultivares adaptados a ambientes variables. Sin embargo, los genotipos de arroz negro solo han sido caracterizados en condiciones de inundación, por lo que aún se desconoce su desempeño frente al estrés hídrico en términos de rasgos agronómicos, productivos y de calidad. En este contexto, el presente estudio evaluó las características agronómicas, fisiológicas, de calidad industrial, nutricional y funcional en líneas avanzadas de arroz negro desarrolladas por el PMGA, cultivadas bajo regímenes hídricos contrastantes.

Mediante ensayos multiambiente realizados durante tres temporadas, se evaluaron 19 genotipos de arroz negro y un cultivar de arroz blanco (Zafiro-INIA) utilizado como referencia, bajo dos condiciones de manejo hídrico: inundación tradicional (IND) y riego sin inundación (NI). Se estimaron componentes de varianza, valores genotípicos, heredabilidad e interacción genotipo × ambiente para los distintos rasgos evaluados. El tratamiento NI redujo significativamente la floración, la calidad del grano y el rendimiento. En promedio, los genotipos de arroz negro mostraron un 31 % menos de rendimiento que el cultivar blanco y obtuvieron 2,4 veces más producción bajo condiciones de inundación que bajo riego sin inundación. Se observaron fuertes correlaciones genéticas entre el rendimiento de grano y características como los días a antesis, el peso del grano, así como la blancura y la translucidez del grano, todas ellas con alta heredabilidad en sentido amplio ($H^2 > 0,9$). La mayoría de los rasgos evaluados presentaron una heredabilidad elevada ($H^2 > 0,7$), lo que indica una alta estabilidad genética. El rendimiento de grano se correlacionó negativamente con el porcentaje de esterilidad de la panícula ($r = -0,84$) y presentó una heredabilidad de 0,76. A pesar de este alto valor de heredabilidad, las condiciones de cultivo tuvieron un efecto significativo en el rendimiento.

Se seleccionaron dos líneas avanzadas de arroz negro (Quila 279101 y Quila 292008), que, junto con el cultivar Zafiro-INIA, fueron evaluadas en dos localidades bajo ambas condiciones hídricas (IND y NI). A los genotipos se les evaluaron diversos rasgos de calidad, incluidos parámetros industriales, nutricionales y funcionales, con énfasis en la composición fenólica y la actividad antioxidante. Los genotipos de arroz negro presentaron altos niveles de contenido fenólico total (TPC), con un promedio de 74,8 mg GAE 100 g^{-1}, y una capacidad antioxidante significativamente superior a la del cultivar de arroz blanco, atribuida principalmente al elevado contenido de cianidina-3-*O*-glucósido en los granos pigmentados. El perfil fenólico varió entre el grano integral y el grano pulido, mientras que la composición mineral dependió de la localidad y del régimen de riego. Se observó una alta correlación entre el color del grano, el TPC y la actividad antioxidante. La calidad del grano estuvo influida por el ambiente, tanto en sus características industriales como en sus propiedades nutricionales, y se detectaron diferencias significativas entre el grano integral y el pulido.

Adicionalmente, se caracterizaron funcionalmente los granos de arroz en términos de digestibilidad, capacidad antioxidante y citotoxicidad. En los granos cocidos de arroz negro se observó una mayor liberación de grupos amino durante todas las fases del proceso de digestión. Entre los péptidos identificados, destacó RA05_ORYSJ por su perfil funcional. No se detectó citotoxicidad del arroz negro en células intestinales Caco-2 ni en células neuronales SH-SY5Y bajo condiciones de estrés oxidativo inducido. Los genotipos de arroz negro redujeron significativamente (>50%) la producción de especies reactivas de oxígeno. Asimismo, el arroz negro disminuyó la actividad de la β-galactosidasa en más de un 65%, lo que sugiere un posible efecto protector frente a la senescencia celular.

Estos resultados destacan la importancia de establecer criterios y herramientas de selección basados en la caracterización integral del arroz negro, que permitan orientar el mejoramiento genético hacia la obtención de cultivares resilientes frente a la variabilidad climática, con alto rendimiento y elevado valor nutracéutico, contribuyendo a diversificar y agregar valor al sector arrocero en Chile, mediante la producción de granos con potencial para el desarrollo de alimentos funcionales.

ABSTRACT

Rice is traditionally cultivated under flooded conditions, which requires a high volume of water to maintain crop sustainability and productivity. In recent years, the effects of climate change have intensified, primarily manifested in reduced rainfall frequency and amount, leading to increasing water scarcity that directly impacts rice production. Under these circumstances, it is essential to develop new cultivars that tolerate water deficit and use water more efficiently, without compromising grain quality.

White rice is the most produced and consumed type worldwide; however, within the *Oryza sativa* species, there are other types of rice, known as pigmented rice, which range in color from red to dark purple, the latter commonly referred to as black rice. Native to Asia, black rice is distinguished by its high anthocyanin content in the pericarp, which gives it its characteristic color. Comparative studies between pigmented and non-pigmented rice have shown that pigmented varieties exhibit greater tolerance to water deficit.

The Rice Breeding Program (RBP) of the Institute of Agricultural Research (INIA) in Chile has focused on evaluating genotypes to assess their performance under climate change conditions and on developing cultivars adapted to variable environments. However, black rice genotypes have been characterized only under flooded conditions, and their performance under water stress remains unknown for agronomic, productive, and quality traits. In this context, the present study evaluated agronomic, physiological, industrial, and nutritional quality, as well as functional characteristics of advanced black rice lines developed by the RBP, cultivated under contrasting water regimes.

Through multi-environment trials conducted over three seasons, 19 black rice genotypes and a white rice cultivar (Zafiro-INIA) were evaluated under two water management conditions: traditional flooding (IND) and non-flooded irrigation (NI). Variance components, genotypic values, heritability, and genotype × environment interactions were estimated for the evaluated traits. The NI treatment significantly reduced flowering, grain quality, and yield. On average, black rice genotypes produced 31% less than the white rice cultivar and yielded 2.4 times more under flooded

conditions than under NI. Strong genetic correlations were observed between grain yield and traits such as days to anthesis, grain weight, chalkiness, and translucency, all of which showed high broad-sense heritability ($H^2 > 0.9$). Most evaluated traits exhibited high heritability ($H^2 > 0.7$), indicating strong genetic stability. Grain yield correlated negatively with panicle sterility percentage ($r = -0.84$) and had a heritability of 0.76. Despite the high heritability, growing conditions had a significant impact on yield.

Two advanced black rice lines (Quila 279101 and Quila 292008) and the cultivar Zafiro-INIA were assessed at two locations under both water regimes (IND and NI). The genotypes were evaluated for various quality traits, including industrial, nutritional, and functional traits, with a focus on phenolic composition and antioxidant activity. Black rice genotypes exhibited a high total phenolic content (TPC), averaging 74.8 mg GAE/100 g^{-1}, and significantly higher antioxidant capacity than the white rice cultivar, primarily due to their elevated cyanidin-3-*O*-glucoside content. The phenolic profile varied between whole and polished grains, whereas the mineral composition depended on location and irrigation regime. High correlations were observed between grain color, TPC, and antioxidant activity. Grain quality was influenced by the environment, affecting both industrial and nutritional characteristics, and significant differences were observed between whole and polished grains.

Additionally, the functional properties of rice grains were characterized by evaluating protein digestibility, antioxidant capacity, and cytotoxicity. Cooked black rice grains showed higher release of amino groups throughout all digestion phases. Among the identified peptides, RA05_ORYSJ stood out for its functional profile. No cytotoxicity was detected in black rice using Caco-2 intestinal cells or SH-SY5Y neuronal cells under induced oxidative stress. Black rice genotypes significantly (>50%) reduced reactive oxygen species production. Moreover, black rice decreased β-galactosidase activity by more than 65%, suggesting a potential protective effect against cellular senescence.

These results highlight the importance of establishing selection criteria and tools based on the comprehensive characterization of black rice, enabling the breeding of resilient cultivars with high yield and high nutraceutical value, thereby contributing to the diversification, and added value of the rice sector in Chile through the production of grains with potential for functional food development.

I. INTRODUCCIÓN GENERAL

El arroz (*Oryza sativa* L.) es una de las plantas más cultivadas del mundo y se considera un alimento básico, aportando alrededor del 25% de las calorías y proteínas necesarias para la población mundial (Kusano et al., 2015; Ito y Lacerda, 2019; Xinkang et al., 2023). El grano entero incluye una capa de salvado (6-7% de su peso total), germen (2-3%) y endospermo amiláceo (90-91%).

En 2023, se produjeron 800 millones de toneladas de arroz en 144 países y se cultivaron aproximadamente 168 millones de hectáreas (FAOSTAT, 2023). Sin embargo, en años recientes, la producción de arroz ha disminuido debido a la creciente frecuencia de temperaturas extremas, sequías e inundaciones, lo que ha provocado un déficit para satisfacer la creciente demanda (Li et al., 2019; Wang y Han, 2022).

El sistema tradicional de producción del arroz se basa principalmente en la inundación, lo que requiere grandes volúmenes de agua y plantea un desafío en un contexto de escasez hídrica a nivel mundial y nacional (Espinosa y Farías, 2017). Con el sistema de cultivo bajo inundación continua, que se mantiene durante la mayor parte del ciclo de crecimiento (Espinosa y Farías, 2017; Orasen et al., 2019), se logra un mayor control de las malezas (Marchesi y Chauhan, 2019), además de prolongar el período de crecimiento de las panículas, ya que la temperatura del agua es mayor a la del aire (Julia y Dingkuhn, 2013).

El cultivo de arroz en Chile se concentra principalmente en las regiones del Maule y Ñuble. La producción nacional se genera con cultivares desarrollados localmente y se destina exclusivamente al mercado interno, cubriendo poco más del 40 % de la demanda total (Donoso et al., 2015). El cultivo se realiza en condiciones de inundación, lo que implica un consumo elevado de agua. El requerimiento hídrico supera los 18.000 m^3 por hectárea (Paredes et al., 2021) y la huella hídrica se estima en más de 1.200 L por kilogramo de arroz producido (Donoso et al., 2015). Adicionalmente, la zona arrocera chilena es la más austral del mundo, lo que implica el desafío de producir bajo condiciones de bajas temperaturas (Paredes et al., 2021). Por esta razón, únicamente se cultiva *Oryza sativa* del tipo japonica templado, debido a su mayor tolerancia al frío (Donoso et al., 2021).

Por otro lado, en las regiones donde se cultiva arroz en Chile predominan suelos de los órdenes Inceptisol, Alfisol y Vertisol. Estos denominados "suelos arroceros" se caracterizan por presentar drenaje deficiente, alto contenido de arcilla y una capa impermeable (Hirzel et al., 2011). Asimismo, presentan deficiencias en nutrientes esenciales como nitrógeno, fósforo y potasio y, en algunos casos, carencias de azufre, boro y calcio (García et al., 2017). Debido a estas condiciones edáficas, los productores, en su mayoría pequeños agricultores, enfrentan limitaciones para diversificar su producción y establecer rotaciones de cultivos (Donoso et al., 2015).

A las problemáticas mencionadas se suma que las preferencias del mercado local se centran en granos de tipo largo-ancho, lo que dificulta la obtención de germoplasma adecuado para el desarrollo de cultivares comerciales adaptados a la producción nacional, dado que no existen otros países con una combinación comparable de requisitos climáticos y de mercado.

El desarrollo de cultivares de arroz adaptados a las condiciones edafoclimáticas locales se lleva a cabo por el Programa Nacional de Mejoramiento del Arroz (PMGA) del Instituto de Investigaciones Agropecuarias de Chile (INIA) (Orona et al., 2013). Este orienta sus esfuerzos al desarrollo de cultivares mediante la integración en el proceso de mejora de rasgos morfológicos, agronómicos, de tolerancia al frío, de rendimiento y de calidad de grano (Paredes et al., 2021).

A las problemáticas antes mencionadas, se suma la disponibilidad cada vez menor del recurso hídrico para el cultivo del arroz. Las precipitaciones en el centro-sur de Chile, donde se encuentra la producción, han disminuido drásticamente en la última década, principalmente debido al cambio climático, lo que ha sido calificado como una "mega sequía" (Garreaud et al., 2019), provocando una escasa disponibilidad de agua para el riego. Las proyecciones climáticas indican que esta situación se prolongará durante las próximas décadas (Morales, 2021). Lo anterior representa una amenaza para el mantenimiento de la productividad y la sostenibilidad del sistema de producción de arroz (Donoso et al., 2015), ya que en condiciones de estrés hídrico se reduce el rendimiento (Manickavelu et al., 2006) y la calidad del cultivo (Khan et al., 2017; Guo et al., 2018; Jaksomsak et al., 2021).

El estrés hídrico es uno de los factores abióticos más perjudiciales (Yadav et al., 2021), ya que afecta a las características fisiológicas, bioquímicas y moleculares de las plantas (Kumar y Sharma, 2018; Usmani et al., 2020) y es el principal factor que limita la productividad vegetal (Balboa, 2019). Este efecto es aún mayor en cultivos como el arroz, que suele cultivarse en condiciones de inundación. Bajo estrés hídrico, las plantas de arroz sufren marchitamiento, aumento de la senescencia como consecuencia de la disminución del contenido de clorofila, disminución del crecimiento debido a la pérdida de turgencia, enrollamiento de las hojas y disminución de la fotosíntesis, lo que da lugar a una disminución en el rendimiento de grano (Farooq et al., 2009; Blum, 2017; Khan et al., 2018; Corso et al., 2020; Yadav et al., 2021). Por lo tanto, el aumento de la inestabilidad climática hace necesario desarrollar cultivares tolerantes al estrés abiótico (Gonzaga et al., 2015; Díaz et al., 2017) que, además, tengan alto rendimiento y calidad del grano (Kushwaha et al., 2020).

La mayoría de los estudios sobre el rendimiento del arroz bajo condiciones de estrés hídrico se han centrado en variedades de arroz blanco tradicional. Sin embargo, investigaciones comparativas entre arroz pigmentado y no pigmentado han mostrado que el arroz pigmentado presenta una mayor tolerancia al déficit hídrico (Hussain et al., 2017), lo que se traduce en una mayor eficiencia en el uso del agua. Considerando esto, junto con las propiedades del arroz negro como alimento funcional (Sui et al., 2016; Ito y Lacerda, 2019), esta variedad podría constituir una alternativa viable para los productores nacionales en el contexto del cambio climático.

Actualmente, el PMGA de INIA se encuentra caracterizando líneas avanzadas de arroz negro bajo cultivo tradicional inundado, con el objetivo de desarrollar cultivares adaptados a las condiciones edafoclimáticas locales. No obstante, aún se desconoce cómo el estrés hídrico afecta los rasgos fisiológicos, agronómicos, productivos y de calidad del grano de estos genotipos. Por ello, se requiere una caracterización profunda del desempeño de estas líneas avanzadas tanto en condiciones de inundación tradicional como de déficit hídrico.

La evaluación de líneas avanzadas de arroz por parte del PMGA se ha centrado en determinar parámetros de estabilidad del rendimiento bajo las condiciones edafoclimáticas locales (Orona et al., 2013). No obstante, para evaluar de manera integral tanto los rasgos productivos como los agronómicos y de calidad, es posible emplear metodologías de análisis multiambiente que consideren, por ejemplo, variaciones de ubicación, año y manejo, incluyendo factores como estrés hídrico, baja disponibilidad de nitrógeno o condiciones óptimas de cultivo (Alvarado et al., 2020).

Los ensayos multiambiente permiten evaluar la consistencia del desempeño genotípico y comprender los mecanismos de interacción genotipo × ambiente. Asimismo, proporcionan información sobre la repetibilidad de las respuestas genotípicas mediante la estimación de la heredabilidad en sentido amplio (Alvarado et al., 2020). La relevancia de una alta heredabilidad en los programas de mejoramiento ha sido ampliamente documentada. Rasgos como tamaño de grano, altura de planta y tiempo de floración han sido estudiados en relación con su estabilidad genética en distintos ambientes. Se ha descrito que los rasgos con alta heredabilidad (H^2) pueden emplearse eficazmente como criterio de selección en el mejoramiento genético (Ortiz et al., 2021). Además, estudios de mejoramiento en arroz han demostrado que características relacionadas con el peso y la calidad del grano suelen presentar alta heredabilidad, lo que las convierte en objetivos idóneos para estrategias de mejoramiento genético (Huang et al., 2013; Nirmaladevi et al., 2015; Qiu et al., 2015; Adjah et al., 2020). El estudio de Hallajian et al. (2024) evidenció que los rasgos con alta heredabilidad suelen estar fuertemente asociados con otros caracteres agronómicos deseables, lo que facilita la selección indirecta de características más complejas.

En el cultivo del arroz, ciertos rasgos desempeñan un papel clave en la estabilidad del rendimiento ante diversas condiciones ambientales. Por ejemplo, la altura de planta se ha correlacionado positivamente con el rendimiento de grano, lo que sugiere que este rasgo podría contribuir a mejorar la estabilidad productiva (Qiu et al., 2015). Por el contrario, la decoloración del grano afecta negativamente su morfología, reduciendo el peso y el rendimiento, lo que repercute directamente en la estabilidad (Nirmaladevi et al., 2015). Estudios realizados en cereales, incluido el arroz, han evidenciado que el rendimiento se asocia positivamente con la biomasa, el índice de cosecha (HI) y el número de granos por metro cuadrado en variedades tolerantes al estrés hídrico (Babu et al., 2003). Asimismo, la pérdida de rendimiento y la disminución del HI bajo condiciones de estrés hídrico se han asociado con la reducción del número de panículas fértiles, de la biomasa y del peso de grano (Pour-Aboughadareh et al., 2020). En conjunto, estos hallazgos resaltan la importancia de integrar rasgos como la altura de planta, el peso de grano, la fertilidad de las espiguillas y el color del grano en los programas de mejoramiento, con el fin de incrementar el rendimiento y garantizar la estabilidad productiva bajo diferentes condiciones ambientales (Huang et al., 2013; Wang et al., 2020).

Para la evaluación de ensayos multiambiente se emplean comúnmente técnicas estadísticas basadas en modelos mixtos. El modelo de máxima verosimilitud restringida (REML) se utiliza para estimar componentes de varianza, mientras que el método de la mejor predicción lineal insesgada (BLUP) permite predecir valores genotípicos, lo que facilita una selección óptima y precisa (Schmidt et al., 2019; Alvarado et al., 2020; Costa et al., 2023). El BLUP permite comparar genotipos provenientes de diferentes poblaciones en múltiples ambientes y simplifica el análisis de conjuntos de datos desbalanceados obtenidos en ensayos experimentales (Ortiz et al., 2021). Además, las observaciones de un genotipo pueden combinarse para generar un único valor fenotípico, desde mediciones de plantas individuales hasta promedios de múltiples plantas del mismo genotipo evaluadas en distintas localidades y a lo largo de varios años (Schmidt et al., 2019).

Dentro de la especie *Oryza sativa* existe una gran diversidad de arroces, que se pueden clasificar en no pigmentados, como el arroz blanco, y pigmentados, que presentan tonos que van del rojo al morado oscuro; este último, conocido como arroz negro (Kushwaha, 2016; Kumar y Prakash, 2020). En el proceso de refinado del grano de arroz blanco se eliminan el salvado y el germen, lo que reduce el contenido de fibra y micronutrientes en comparación con variedades pigmentadas que se consumen como grano integral (Kushwaha, 2016; Wattanavitchkorn et al., 2023). El grano entero conserva una capa de salvado rica en fitoquímicos, fibra y compuestos bioactivos, los cuales contribuyen a la ingesta de componentes esenciales (Reddy et al., 2017; Murali et al., 2020).

El arroz negro destaca por su alto valor nutricional y bioactivo: mayor contenido de fibra dietética, hemicelulosa, minerales (Cu, P, Fe, Mg), ácido fítico, vitaminas E y del complejo B, aminoácidos y una fracción fenólica compuesta por antocianinas, flavonas, fenoles y carotenoides (Kushwaha, 2016; Shao et al., 2018; Ito y Lacerda, 2019; Mbanjo et al., 2020; Jaksomsak et al., 2021). Además, se caracteriza por presentar altas concentraciones de antocianinas en el pericarpio, siendo la cianidina-3-*O*-glucósido la principal, seguida de la peonidina-3-*O*-glucósido (Kushwaha, 2016; Pratiwi y Purwestri, 2017). Ambas pueden representar, en algunos casos, más del 90 % del total de antocianinas presentes en el grano (Zheng et al., 2020; Colasanto et al., 2021; Leonarski et al., 2024).

Se han identificado más de 146 compuestos volátiles distintos en el grano de arroz negro, de los cuales 28 son terpenoides (Thitisut et al., 2020). En estudios de caracterización fenólica se ha determinado la presencia de diversos compuestos bioactivos, entre ellos catequina, ácido siríngico, ácido sinápico, ácido vanílico, ácido 4-hidroxibenzoico, ácido gálico, ácido *p*-cumárico, *γ*-orizanol, ácido ferúlico y triterpenos, además de glucósidos de flavonol como quercetina-3-*O*-glucósido y quercetina-3-*O*-rutinósido (Samyor et al., 2017; Das et al., 2018; Mbanjo et al., 2020). Asimismo, se han identificado carotenoides como luteína, licopeno, zeaxantina y β-caroteno (Pratiwi y Purwestri, 2017; Melini et al., 2019).

Pese a la abundancia de compuestos fenólicos en el grano de arroz negro, estos son altamente sensibles a luz, pH, iones metálicos y procesamiento térmico, causando una disminución de su contenido y capacidad antioxidante en matrices alimentarias (Pedro et al., 2016; Colasanto et al., 2021). Sin embargo, una breve cocción del arroz en microondas ha demostrado que preserva los compuestos bioactivos del grano. Por lo tanto, comprender el efecto del procesamiento sobre la retención de antocianinas es clave para entender la funcionalidad del grano (Thuengtung y Ogawa, 2020; Colasanto et al., 2021).

Una parte de los compuestos fenólicos en el grano está ligada a la fibra dietética, por lo que su bioaccesibilidad depende, entre otros factores, del tamaño de partícula y de la dinámica de la digestión enzimática (Murali et al., 2020; Shi et al., 2022; Hu et al., 2024). Por otro lado, gracias a métodos de digestión gastrointestinal *in vitro*, se ha podido monitorear la liberación de compuestos, como aminoácidos libres, lo que permite una evaluación más precisa del potencial nutricional en condiciones fisiológicas simuladas (Lu et al., 2021; Shi et al., 2022).

Las antocianinas y los ácidos fenólicos son reconocidos por su notoria actividad antioxidante (Kushwaha, 2016; Zaidi et al., 2019; Murali et al., 2020). Estos compuestos poseen la capacidad de neutralizar las especies reactivas de oxígeno (ROS), contribuyendo así a mantener la homeostasis redox y a mitigar el daño ocasionado por el estrés oxidativo (Krishnanunni et al., 2015; Mohidem et al., 2022; Saleh et al., 2024). Además, se ha demostrado que la actividad de la β-galactosidasa constituye uno de los indicadores de envejecimiento asociados a la senescencia celular, ya que permite cuantificar el impacto del estrés oxidativo y evaluar la capacidad protectora de los extractos pigmentados frente al daño oxidativo (Lumba et al., 2017). En este contexto, la evaluación conjunta de los niveles de ROS y de la actividad de la β-galactosidasa proporciona un marco útil para comprender la relación entre la composición química y las propiedades funcionales de las variedades de arroz negro.

El desarrollo de nuevos cultivares de arroz negro adaptados a condiciones de déficit hídrico es fundamental para promover una agricultura sostenible en el contexto actual de escasez de agua. Para ello, se requieren estrategias que permitan aprovechar la variabilidad genética y enfrentar los desafíos asociados al cambio climático (Viana et al., 2019). La escasez hídrica constituye un estrés ambiental significativo que afecta el desarrollo, el rendimiento y la calidad del cultivo. En este sentido, la generación de genotipos de arroz tolerantes al estrés hídrico que, además, mantengan un alto rendimiento y calidad del grano representa un desafío complejo (Varshney et al., 2018; Abd-El-Aty et al., 2023). Esto se debe a que muchos rasgos productivos y de calidad presentan variación fenotípica continua o cuantitativa y están controlados por múltiples genes con distintos efectos, que pueden verse influenciados por las condiciones ambientales (Wang et al., 2020). En consecuencia, la evaluación de distintos genotipos en condiciones tanto óptimas como limitantes de agua resulta esencial para determinar su desempeño, comprender cómo el ambiente influye en la productividad y la calidad del grano, y favorecer el desarrollo de cultivares resilientes frente a condiciones climáticas variables. Asimismo, profundizar en la caracterización de la calidad nutricional y funcional del grano permitirá desarrollar nuevos cultivares de arroz negro con mejores atributos nutracéuticos.

Por lo tanto, este estudio se propuso integrar la evaluación agronómica, productiva y de calidad industrial y funcional de líneas avanzadas de arroz negro desarrolladas por el PMGA, cultivadas bajo regímenes hídricos contrastantes en condiciones edafoclimáticas locales de Chile. Lo cual permitirá establecer criterios de selección que orienten el mejoramiento genético y el desarrollo de cultivares resilientes, de alto rendimiento y de elevado valor nutracéutico. Estos cultivares de arroz negro podrían constituir una alternativa de diversificación para los productores nacionales, al agregar valor al arroz chileno mediante granos de alta calidad y potencial funcional y, al mismo tiempo, contribuir a la adaptación del sector arrocero ante la escasez hídrica y los efectos del cambio climático.

HIPÓTESIS

- Los rasgos fisiológicos, agronómicos, productivos y de calidad del grano en genotipos de arroz negro cultivados bajo condiciones de inundación y déficit hídrico permiten identificar aquellos con alto rendimiento y calidad en ambientes de escasez hídrica, así como determinar rasgos útiles como criterios de selección en programas de mejoramiento genético.
- Las propiedades funcionales y bioactivas de los compuestos del arroz negro contribuyen a reducir el estrés oxidativo y los procesos de senescencia celular.

OBJETIVO GENERAL

- Evaluar el desempeño de genotipos de arroz negro en condiciones hídricas contrastantes en base a rasgos agronómicos, productivos y de calidad del grano.

OBJETIVOS ESPECÍFICOS

- Evaluar los rasgos agronómicos, productivos y de calidad del grano en genotipos de arroz cultivados en condiciones de inundación tradicional y de déficit hídrico.
- Determinar el efecto de la interacción genotipo x ambiente sobre los rasgos agronómicos, productivos y de calidad del grano en genotipos de arroz cultivados en condiciones de inundación tradicional y de déficit hídrico.
- Caracterizar el perfil fenólico y la capacidad antioxidante del grano de arroz negro.
- Evaluar el efecto de la digestión del arroz negro sobre la biodisponibilidad de proteínas y polifenoles, así como su potencial antioxidante.

II. CAPÍTULO I: BLACK RICE PERFORMANCE UNDER WATER DEFICIT CONDITIONS AND GENOTYPE X ENVIRONMENT INTERACTIONS

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ABSTRACT

Rice is a staple food grown worldwide. While white rice varieties have been extensively studied, there is limited information on the performance of pigmented rice genotypes and their tolerance to water deficit. This study evaluated nineteen black rice genotypes and one white cultivar over three years under contrasting water regimes: traditional flooding and non-flood irrigation (NFI). Genotype-environment interactions and their impact on agronomic, yield, and grain quality traits were assessed. Black genotypes under NFI showed reduced flowering and grain quality. The average yield was 31% lower than the white cultivar. Significant genetic correlations were found between grain yield and days to anthesis (DSA), grain weight (TGW), chalkiness (CHA), and translucency (TRAN), with high broad-sense heritability (H2 > 0.9). Most traits exhibited high heritability (H2 > 0.7), indicating strong genetic stability. Grain yield (GR) was highly and negatively correlated with percent sterility (PS) (r = -0.84) and had a heritability of 0.76. Environmental conditions significantly impacted yield despite high genetic control. The results highlight the need to develop black rice genotypes with increased tolerance to water stress and optimized agronomic management to ensure sustainable production. This study is the first to evaluate the performance of a diverse set of black rice genotypes across multiple seasons under contrasting water regimes in a Mediterranean environment.

Keywords: grain yield, grain quality, non-flooding irrigation, heritability.

1. INTRODUCTION

Rice (Oryza sativa L.) is one of the most widely cultivated crops globally. In 2023, 800 million tons of rice were produced across 143 countries, with a cultivated area of approximately 168 million hectares [1]. As a key staple food, this cereal accounts for about 25% of the calories and protein consumed by the global population [2,3].

Black rice, a variety of pigmented rice, is notable for its elevated levels of phenolic compounds, particularly anthocyanins, which are present in the outer layer of the grain, giving it a unique deep purple hue. Furthermore, this rice contains additional bioactive compounds, including flavanones, phenolic acids, vitamins, fatty acids, and carotenoids [4,5]. It is often regarded as a functional food because these phytochemicals provide significant health benefits [6,4]. For this reason, traditional pigmented rice varieties have been used for medicinal purposes in countries such as China and India [7,6]. In addition to its nutritional benefits, black rice is described as a versatile ingredient, as it can be transformed into various products, including bread, cookies, noodles, and beverages, which is an attractive feature for consumers [8,6]. Moreover, extracts rich in anthocyanins from black rice serve as a natural food pigment [6]. In fact, pure black rice powder is utilized as a colorant (E163) and in dietary supplements, a market that is experiencing rapid expansion [9].

Water stress is the main limiting factor for plant growth, influencing physiological, biochemical, and molecular processes, as well as biomass production and overall yield [10,11,12,13]. This abiotic stress is particularly detrimental to the productivity of rice, which is typically grown in flooded conditions [14]. While different management practices and water conservation techniques worldwide can lead to high rice production [15], they are typically implemented in areas where cold temperatures are not a limiting factor. In rice plants, water stress causes several adverse effects, including wilting, increased senescence, stunted growth, leaf rolling, and diminished photosynthesis, ultimately resulting in lower grain yields [16,17,12]. Furthermore, drought conditions significantly affect several grain quality traits, such as milling rate [18], protein and amylose content [19], antioxidant capacity [20,21,22], mineral nutrient levels [23,24,25], and cooking quality [18].

Proper water management is crucial for successful rice cultivation [26,27,28]. In Chile, rice is traditionally grown under flooded conditions, requiring over 18,000 m^3 of water per hectare [14]. However, local rice production faces significant challenges in crop sustainability, including increasing water scarcity, primarily driven by climate change [29], which underscores the need to develop new rice cultivars that can achieve higher yields with improved water efficiency. Additionally, adopting innovative irrigation practices, such as non-flooding irrigation (NFI), could play a key role in conserving water resources [14], contributing to maintaining crop productivity and sustainability.

In the current water scarcity scenario, the development of black rice cultivars that are adapted to water-limited conditions is essential for ensuring resilient and sustainable crop production. Therefore, it is necessary to employ strategies that leverage genetic variability as a tool for adaptation to climate change [30]. Therefore, evaluating different genotypes under optimal and water-scarce conditions is essential to assess yield and understand how the environment affects productivity and grain quality. Many productive and quality traits exhibit continuous or quantitative phenotypic variation and are typically controlled by multiple genes with diverse genetic effects and/or influenced by the environment [31], which makes the development of high-yielding drought-adapted rice genotypes a complex task [32,13].

Specific traits in rice have been identified as significantly influencing yield stability when evaluated in contrasting environments. According to Qiu et al. [33], plant height can improve yield stability because this trait is positively correlated with grain productivity. Additionally, grain discoloration negatively affects grain morphology, decreasing grain weight and overall yield, thus impacting yield stability [34]. Studies in cereals, including rice, have shown that grain yield is positively associated with biomass, harvest index, and grains per square meter in drought-resistant varieties [35]. Furthermore, yield loss and reduction in harvest index under drought stress have been associated with decreased fertile panicle rate, biomass, and grain weight [36]. These findings highlight the significance of breeding programs in enhancing grain yield through the integration of traits such as plant height, thereby ensuring yield stability under variable environmental conditions [37,31].

A key aspect of successful breeding programs is the use of statistical methods to identify genotypes that can perform well under different environmental conditions. To do this, genotypes can be evaluated through trials conducted in multiple environments, considering variations in location, year, and management factors such as water stress, low nitrogen availability, and climatic conditions. Multi-environmental trials help evaluate the consistency of genotypic performance and uncover genotype x environment interaction mechanisms. Moreover, they offer insights into the repeatability of genotypic responses by estimating broad-sense heritability [38].

The importance of high heritability in breeding programs has been well documented. Traits such as grain size, plant height, and flowering time have been extensively studied for their genetic stability across environments. It has been described that traits with high heritability (H^2) can be effectively selected for improvement [39]. In contrast, rice breeding studies have shown that traits related to grain weight and quality often exhibit high heritability, making them ideal targets for genetic improvement strategies [37,34,33,40]. Hallajian et al. [41] demonstrated in a genetic correlation study that traits with high heritability are strongly associated with other desirable agronomic traits, thus allowing for indirect selection.

Understanding how complex trait correlations respond to environmental variation is crucial for developing resilient rice cultivars that are adapted to climate variability. In this context, a three-year field study was conducted to assess the performance of nineteen black rice genotypes under two contrasting water management systems (conventional flooding and non-flooding irrigation) in a Mediterranean zone of Chile.

The analysis included one white rice cultivar to provide a comparative reference. The evaluation focused on agronomic, productive, and grain quality traits, with an emphasis on the role of genotype × environment interactions.

2. RESULTS

2.1. Effect of Genotype and Environment on Agronomic, Productive, and Quality Traits

The analysis of agronomic, productive, and quality traits in relation to the environment (water regime × year) revealed statistical differences ($p < 0.05$) among genotypes for DSA, GY, FGP, TGP, ST, PL, WG, TGWPa, TGWPo, CHA, and TRAN. The cultivar Zafiro-INIA exhibited the highest mean values for most traits, including DSA, GY, FGP, TGWPa, TGWPo, CHA, and TRAN. The interaction between genotype and environmental factors was also statistically significant for ST, PL, WG, TGWPa, TGWPo, and CHA (Tables 1 and 2).

Regarding flowering time, significant differences were observed between Zafiro and Quila 297901, with a delay of 19 days in the former genotype. Additionally, the genotypes Quila 297901 and Quila 299801 flowered at 110 and 116 DSA, respectively, significantly earlier than Zafiro, which flowered at 129 DSA. Interestingly, despite their earlier flowering, pigmented rice genotypes achieved an average yield of 4.17 tons per hectare, which was 31% lower than the grain yield of Zafiro. The reduced yield of black genotypes in this study was largely attributed to a high percentage of floral sterility, which averaged 41.9%, compared to 30.3% in Zafiro (Table 1). Quila 292001 and FLQuila 93 showed sterility percentages of 35.4% and 37.7%, respectively, indicating some variability within the pigmented genotypes. In addition, the thousand-grain weight of pigmented genotypes was, on average, 18% lower than that of Zafiro, both for paddy rice and polished grains (Table 2), which also contributes to the lower yield of black rice genotypes compared to white rice. In terms of grain quality in pigmented genotypes, WG decreased by 2.2%, while ADD increased by 1.6%, compared to Zafiro. However, as expected, there were notable differences in grain appearance; CHA and TRAN decreased by 34.3% and 52%, respectively, in pigmented genotypes (Table 2). The application of NFI resulted in significant changes in rice growth and productivity, affecting all the evaluated traits except PH and ADD (Tables 1 and 2). On average, grain yields were 6.04-ton ha^{-1} under flooding and 2.5-ton ha^{-1} under NFI, representing a 2.4-fold decrease. This reduction in GY under NFI was accompanied by an 18.2 % increase in DSA, ranging from 111.7 to 132.1 days under flooding and NFI, respectively. The decrease in GY under NFI was directly linked to reductions in FGP, TGP, TGWPa, and TGWPo, which decreased by 24.3%, 12.7%, 8.4%, and 8.1%, respectively. In addition, the reduction in yield and its components under NFI was accompanied by a 23.3% increase in ST. On the other hand, NFI increased WG, CHA, and TRAN by 14.8%, 2.3%, and 4.5%, respectively.

During the 2022 season, there was a notable decrease in yield under flooded conditions, reaching only half of that obtained in the 2021 and 2023 seasons. Nevertheless, the yields in 2022 were still higher than the average observed under NFI conditions. PL increased by 16.1% in the same season, TGP rose by 15.4%, and FGP decreased significantly by 32.5%. Additionally, ST was twice as high as the average observed in the other two seasons under flooded conditions. Grain weight showed a slight variation, with TGWPa and TGWPo decreasing by 0.8% and 1.34%, respectively, compared to the average of the other two years (Tables S1 and S2).

Table 1. Analysis of variance of agronomic and productive traits evaluated in 19 black rice genotypes and the white cultivar Zafiro grown under six different environments resulting from the combination of water management (conventional flooding (F) and non-flooding irrigation (NFI)) and three years (2021, 2022 and 2023).

	DSA (days)		**PH (cm)**		**GY (ton ha^{-1})**		**FGP**		**TGP**		**ST (%)**	
Genotype (G)												
FLQuila 93	123.56	±3.17^{bcde}	91.72	±2.36^{ab}	3.82	±4.11^{cde}	48.76	±2.24^{abc}	79.80	±3.39^{cde}	37.67	±2.98^{de}
Quila 279101	125.33	±2.87^{b}	85.17	±2.30^{ab}	4.57	±5.14^{abc}	49.50	±4.72^{abc}	91.64	±4.39^{ab}	45.82	±4.69^{b}
Quila 291602	123.56	±3.13^{bcd}	90.19	±2.79^{ab}	4.60	±7.20^{bcd}	47.60	±3.20^{bc}	82.12	±4.99^{bcde}	40.71	±3.27^{bcde}
Quila 292001	122.72	±2.95^{bcde}	80.61	±2.33^{a}	4.73	±6.28^{abc}	49.98	±3.98^{abc}	77.63	±4.79^{def}	35.44	±3.64^{ef}
Quila 292003	120.83	±3.39^{bcde}	76.42	±3.22^{ab}	4.01	±5.90^{cde}	51.41	±3.69^{abc}	86.58	±5.13^{abcd}	38.94	±3.92^{cde}
Quila 292008	123.50	±3.01^{bcd}	74.39	±2.03^{ab}	3.14	±4.46^{e}	44.92	±2.33^{cd}	78.72	±2.03^{cdef}	42.18	±3.45^{abcd}
Quila 292009	123.28	±2.80^{bcd}	73.64	±2.74^{ab}	4.74	±6.25^{ab}	38.89	±3.19^{de}	69.52	±4.42^{f}	44.60	±2.89^{abc}
Quila 292010	121.83	±2.93^{bcdef}	81.94	±2.11^{ab}	4.27	±5.75^{bcd}	49.70	±4.52^{abc}	87.76	±3.35^{abc}	42.78	±4.84^{abcd}
Quila 292011	124.33	±3.25^{bc}	89.89	±2.59^{b}	4.29	±6.17^{bcd}	45.54	±3.50^{cd}	82.65	±3.05^{bcde}	44.94	±3.70^{abc}
Quila 292012	121.78	±2.75^{cdef}	80.08	±2.20^{a}	3.25	±4.94^{e}	35.36	±3.13^{de}	68.93	±3.32^{f}	48.15	±4.02^{a}
Quila 292013	121.11	±3.11^{bcedf}	79.81	±2.63^{ab}	4.14	±5.88^{bcd}	51.00	±3.66^{abc}	83.52	±3.42^{abcde}	39.25	±3.20^{bcde}
Quila 292014	123.44	±2.98^{bcd}	84.44	±2.56^{ab}	4.20	±5.68^{bcd}	46.24	±3.15^{cd}	85.93	±3.04^{abcd}	45.15	±4.20^{abc}
Quila 292015	122.06	±3.24^{bcde}	81.81	±1.97^{ab}	4.41	±6.03^{abcd}	44.19	±3.76^{cd}	75.56	±4.77^{ef}	41.71	±3.14^{abcde}
Quila 292017	118.94	±3.10^{ef}	76.47	±2.41^{ab}	4.18	±4.80^{bcd}	56.06	±5.16^{a}	93.50	±4.50^{a}	40.71	±4.46^{bcde}
Quila 292018	120.67	±3.11^{def}	81.06	±2.34^{ab}	3.53	±4.96^{de}	48.11	±3.91^{abc}	82.99	±3.03^{bcde}	42.39	±3.68^{abcd}
Quila 297901	110.00	±3.02^{g}	83.89	±2.53^{ab}	3.81	±5.82^{cde}	48.52	±3.91^{abc}	87.12	±2.98^{abcd}	45.08	±3.51^{abc}
Quila 299801	116.33	±2.59^{f}	100.61	±2.33^{b}	4.17	±3.76^{abcd}	45.03	±3.12^{cd}	77.23	±3.86^{def}	41.11	±3.35^{bcde}
Quila 299802	123.44	±2.96^{bcd}	84.36	±2.53^{a}	4.94	±5.73^{ab}	50.16	±4.16^{abc}	82.21	±3.40^{bcde}	39.51	±4.18^{bcde}
Quila 299803	121.78	±3.22^{bcde}	88.08	±2.03^{ab}	4.46	±4.62^{abc}	52.17	±4.78^{abc}	87.18	±4.67^{abcd}	40.90	±3.65^{bcde}
Zafiro	129.11	±3.02^{a}	88.78	±2.65^{ab}	6.04	±8.90^{a}	54.47	±3.44^{ab}	77.76	±1.76^{cdef}	30.28	±3.73^{f}
Environment (E)												
F2021	120.13	±0.77^{d}	95.25	±1.50^{a}	7.12	±2.38^{a}	54.49	±1.40^{b}	80.63	±1.19^{bc}	32.46	±1.40^{e}
F2022	116.15	±0.60^{e}	90.78	±0.96^{a}	3.62	±1.57^{b}	41.26	±2.02^{cd}	96.03	±2.26^{a}	57.73	±1.62^{a}
F2023	98.85	±0.50^{f}	87.86	±1.01^{a}	7.37	±1.93^{a}	67.72	±1.58^{a}	85.74	±1.57^{b}	20.94	±1.21^{f}
NFI2021	135.75	±0.71^{a}	74.54	±1.46^{a}	2.03	±1.24^{c}	42.08	±1.22^{cd}	78.93	±1.24^{c}	46.28	±1.53^{c}
NFI2022	133.72	±0.64^{b}	73.69	±0.89^{a}	2.21	±0.75^{c}	38.39	±1.23^{d}	77.77	±1.76^{cd}	50.45	±1.31^{b}
NFI2023	126.68	±0.67^{c}	79.89	±1.08^{a}	3.25	±1.26^{b}	43.32	±2.36^{c}	72.41	±3.35^{d}	40.33	±1.55^{d}
p-value												

G	**0.0000**	0.6718	**0.0002**	**0.0002**	**0.0000**	**0.0001**
E	**0.0000**	0.9996	**0.0000**	**0.0000**	**0.0000**	**0.0000**
G x E	0.9653	1.0000	0.9969	0.1641	0.8199	**0.0035**

DSA: days from sowing to anthesis, PH: plant height, GY: grain yield, FGP: filled grain per panicle, TGP: total grain per panicle, ST: sterility percentage, and PL: panicle length. Different letters in the same column indicate statistical differences according to the LSD Fisher test ($p \leq 0.05$).

Table 2. Analysis of variance of productive and quality traits evaluated in 19 black rice genotypes and the white cultivar Zafiro-INIA grown under six environments resulting from the combination of water management (conventional flooding (F) and non-flooding irrigation (NFI)) and three years (2021, 2022 and 2023).

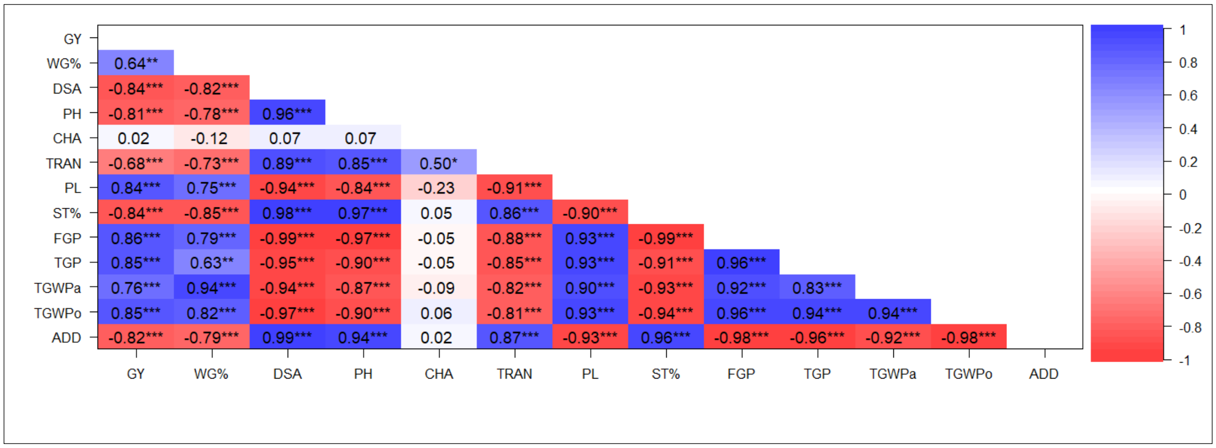
	WG (%)		**TGWPa (g)**		**TGWPo (g)**		**CHA**		**TRAN**		**ADD**	
Genotype (G)												
FLQuila 93	63.36	±0.52^{ab}	30.44	±0.51^{bcd}	22.26	±0.32^{c}	22.89	±0.92^{g}	1.55	±0.10^{ghi}	5.88	±0.15^{abc}
Quila 279101	52.53	±3.11^{abc}	27.13	±0.65^{ghi}	18.21	±0.37^{jk}	13.30	±0.89^{j}	0.57	±0.06^{k}	6.04	±0.18^{abc}
Quila 291602	63.19	±1.53^{a}	31.48	±0.45^{b}	22.88	±0.37^{b}	33.43	±0.44^{b}	2.59	±0.06^{b}	4.61	±0.21^{bc}
Quila 292001	58.29	±2.26^{abc}	29.02	±0.60^{de}	20.34	±0.31^{efg}	27.00	±0.70^{cd}	1.84	±0.09^{def}	6.01	±0.14^{a}
Quila 292003	59.10	±2.42^{ab}	28.66	±0.71^{ef}	20.52	±0.33^{ef}	26.98	±0.74^{cd}	1.87	±0.08^{de}	5.92	±0.15^{abc}
Quila 292008	58.90	±1.92^{c}	25.79	±0.56^{ij}	18.32	±0.41^{jk}	18.09	±0.74^{i}	0.96	±0.06^{j}	5.71	±0.17^{abc}
Quila 292009	63.72	±1.01^{abc}	25.25	±0.49^{j}	17.91	±0.30^{k}	18.39	±0.79^{i}	1.13	±0.11^{hi}	5.97	±0.17^{abc}
Quila 292010	58.94	±2.58^{bc}	29.26	±1.48^{cde}	19.94	±0.37^{gh}	27.29	±0.67^{cd}	1.84	±0.08^{d}	5.91	±0.14^{abc}
Quila 292011	60.88	±1.20^{bc}	30.54	±0.52^{bc}	21.98	±0.31^{cd}	26.39	±0.75^{cde}	1.89	±0.09^{de}	5.87	±0.18^{abc}
Quila 292012	62.64	±1.03^{ab}	28.89	±0.58^{e}	21.67	±0.40^{d}	23.71	±0.96^{fg}	1.54	±0.10^{fgh}	5.76	±0.17^{abc}
Quila 292013	57.90	±1.74^{bc}	26.25	±0.39^{hij}	18.63	±0.37^{ij}	20.33	±0.84^{h}	1.13	±0.07^{ij}	5.54	±0.18^{abc}
Quila 292014	60.29	±1.51^{bc}	26.96	±0.51^{ghi}	19.82	±0.33^{gh}	25.90	±0.77^{cde}	1.73	±0.08^{de}	6.09	±0.17^{ab}
Quila 292015	57.46	±2.09^{bc}	27.08	±0.66^{ghi}	19.98	±0.34^{fgh}	25.64	±0.54^{de}	1.67	±0.06^{def}	5.82	±0.16^{abc}
Quila 292017	54.46	±2.19^{bc}	25.34	±0.74^{j}	18.46	±0.31^{jk}	22.74	±0.97^{g}	1.26	±0.08^{hi}	5.22	±0.24^{abc}
Quila 292018	56.98	±1.81^{bc}	25.89	±0.40^{hij}	18.17	±0.32^{jk}	20.88	±1.02^{h}	1.13	±0.10^{hij}	5.68	±0.18^{abc}
Quila 297901	59.97	±1.45^{ab}	28.35	±0.55^{efg}	19.91	±0.35^{gh}	32.16	±0.69^{b}	2.26	±0.10^{c}	2.93	±0.26^{c}
Quila 299801	57.38	±1.33^{c}	27.27	±0.60^{fgh}	19.67	±0.35^{h}	26.43	±0.91^{cde}	1.76	±0.11^{efg}	5.73	±0.17^{a}
Quila 299802	62.37	±1.33^{ab}	28.91	±0.38^{e}	20.67	±0.30^{e}	25.00	±1.01^{ef}	1.61	±0.11^{efg}	6.14	±0.17^{a}
Quila 299803	52.37	±2.21^{c}	26.26	±0.48^{hij}	19.09	±0.36^{i}	27.38	±0.75^{c}	1.96	±0.10^{d}	5.69	±0.17^{abc}
Zafiro	60.32	±2.08^{abc}	33.93	±0.31^{a}	24.25	±0.28^{a}	37.15	±0.48^{a}	3.34	±0.07^{a}	5.52	±0.17^{abc}
Environment (E)												
F2021	48.88	±1.41^{c}	28.63	±0.34^{b}	20.61	±0.26^{bc}	24.72	±0.82^{bc}	1.71	±0.09^{bc}	6.13	±0.11^{a}
F2022	62.28	±0.54^{b}	29.21	±0.55^{b}	20.79	±0.26^{b}	25.38	±0.71^{b}	1.76	±0.08^{b}	6.02	±0.14^{a}
F2023	53.77	±0.94^{c}	30.27	±0.34^{a}	21.55	±0.23^{a}	24.20	±0.79^{cd}	1.46	±0.08^{d}	4.97	±0.08^{a}
NFI2021	62.67	±0.50^{b}	26.35	±0.41^{d}	19.25	±0.26^{d}	23.11	±0.83^{ef}	1.45	±0.09^{d}	5.91	±0.13^{a}
NFI2022	62.87	±0.60^{ab}	26.68	±0.40^{d}	18.23	±0.23^{e}	29.22	±0.72^{a}	2.15	±0.07^{a}	5.31	±0.14^{a}
NFI2023	63.84	±0.57^{a}	27.67	±0.37^{c}	20.38	±0.26^{c}	23.70	±0.77^{de}	1.55	±0.08^{cd}	5.28	±0.10^{a}

p-value						
G	**0.0299**	**0.0000**	**0.0000**	**0.0000**	**0.0000**	0.5263
E	**0.0000**	**0.0000**	**0.0000**	**0.0000**	**0.0000**	0.9996
G x E	**0.0298**	**0.0023**	**0.0000**	**0.0002**	0.9980	1.0000

WG: whole-grain yield percentage, TGWPa: thousand-grain weight of paddy rice, TGWPo: thousand-grain weight of polished rice, CHA: grain chalkiness, TRAN: grain translucency, and ADD: average degree of dispersion. Different letters in the same column indicate statistical differences according to the LSD Fisher test ($p \leq 0.05$).

2.2. Correlation Analysis

Correlation analyses were performed between the agronomic, productive, and quality traits evaluated under water management conditions (flooding and NFI). Under flooded conditions, GY showed a high correlation with most evaluated traits, except for some quality traits. High positive correlations were observed between GY and TGP, FGP, PL, and TGWPo (r ≥ 0.84), while high negative correlations were found be-tween GY and DSA, PH, ST, and ADD (r ≤ -0.81) (Figure 1). In addition, DSA showed high positive correlations with ST, PH, ADD, and TRAN (r ≥ 0.89), as well as high negative correlations with yield and its components (TGP, FGP, thousand-grain weight), PL, and WG (r ≤ -0.82) (Figure 1).



	GY	WG%	DSA	PH	CHA	TRAN	PL	ST%	FGP	TGP	TGWPa	TGWPo	ADD
GY													
WG%	0.64**												
DSA	-0.84***	-0.82***											
PH	-0.81***	-0.78***	0.96***										
CHA	0.02	-0.12	0.07	0.07									
TRAN	-0.68***	-0.73***	0.89***	0.85***	0.50*								
PL	0.84***	0.75***	-0.94***	-0.84***	-0.23	-0.91***							
ST%	-0.84***	-0.85***	0.98***	0.97***	0.05	0.86***	-0.90***						
FGP	0.86***	0.79***	-0.99***	-0.97***	-0.05	-0.88***	0.93***	-0.99***					
TGP	0.85***	0.63**	-0.95***	-0.90***	-0.05	-0.85***	0.93***	-0.91***	0.96***				
TGWPa	0.76***	0.94***	-0.94***	-0.87***	-0.09	-0.82***	0.90***	-0.93***	0.92***	0.83***			
TGWPo	0.85***	0.82***	-0.97***	-0.90***	0.06	-0.81***	0.93***	-0.94***	0.96***	0.94***	0.94***		
ADD	-0.82***	-0.79***	0.99***	0.94***	0.02	0.87***	-0.93***	0.96***	-0.98***	-0.96***	-0.92***	-0.98***	

Figure 1. Pearson correlation analysis of agronomic, productive, and quality traits evaluated in 19 black rice genotypes and the white cultivar Zafiro grown under conventional flooding conditions over three consecutive years. * p ≤ 0.05, ** p ≤ 0.01, and *** p ≤ 0.001. GY: grain yield, WG: whole-grain yield percentage, DSA: days from sowing to anthesis, PH: plant height, CHA: grain chalkiness, TRAN: grain translucency, PL: panicle length, ST: sterility percentage, FGP: filled grain per panicle, TGP: total grain per panicle, TGWPa: thousand-grain weight of paddy rice, TGWPo: thousand-grain weight of polished rice, and ADD: average degree of dispersion. n= 60

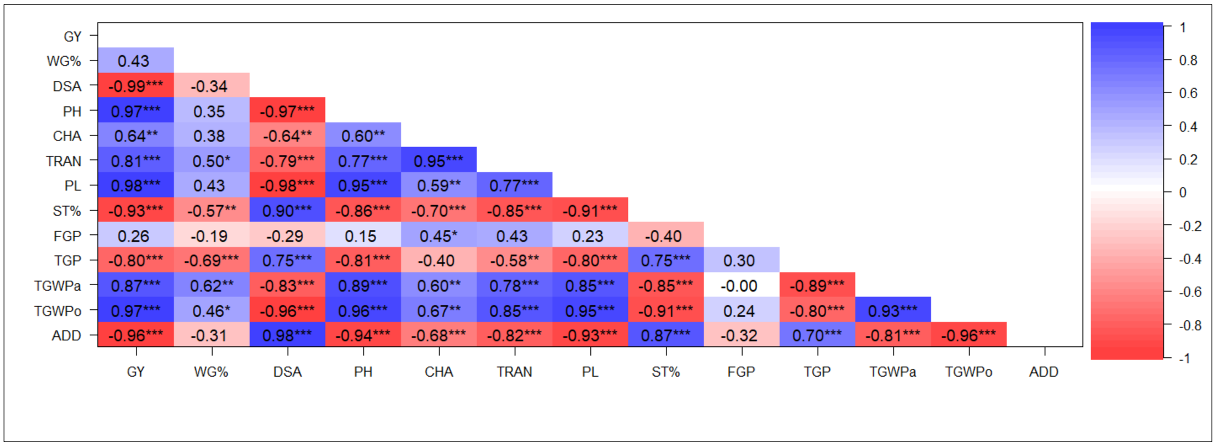


Figure 2. Pearson correlation analysis of agronomic, productive, and quality traits evaluated in 19 black rice genotypes and the white cultivar Zafiro grown under non-flooding irrigation conditions over three consecutive years. * $p \leq 0.05$, ** $p \leq 0.01$, and *** $p \leq 0.001$. GY: grain yield, WG: whole-grain yield percentage, DSA: days from sowing to anthesis, PH: plant height, CHA: grain chalkiness, TRAN: grain translucency, PL: panicle length, ST: sterility percentage, FGP: filled grain per panicle, TGP: total grain per panicle, TGWPa: thousand-grain weight of paddy rice, TGWPo: thousand-grain weight of polished rice, and ADD: average degree of dispersion. n= 60.

Under NFI conditions, grain yield (GY) showed a high correlation with most of the trials evaluated (Figure 2). As with flooding, high positive correlations were found between yield per hectare and thousand-grain weight (TGWPa and TGWPo) and PL ($r \geq 0.87$), while high negative correlations were observed with DSA, ST, and ADD ($r \leq -0.93$). However, unlike flood conditions, yield also showed high positive correlation with PH and TRAN ($r \geq 0.81$), a high negative correlation with TGP, and a weak association with FGP. In addition, DSA showed a strong positive correlation with ST and ADD ($r \geq 0.9$), as well as high negative correlations with PL and thousand-grain weight ($r \leq -0.83$); however, unlike flooding, DSA showed a high positive correlation with TGP ($r = 0.75$) and high negative correlations with PH and TRAN ($r \leq -0.79$). Under NFI conditions, ST was strongly correlated with GY, demonstrating more pronounced association compared to flooded conditions. Furthermore, ST showed a high positive correlation with TGP ($r = 0.75$) and strong negative correlations with PH and TRAN (r

≤ -0.85). The association of ST with FGP, while still negative, was weak under NFI (Figure 2).

2.3. Principal Component Analysis (PCA)

Principal component analysis (PCA) was conducted to establish the relationships between genotypes and agronomic, productive, and quality traits evaluated under different irrigation methods (Figures 4 and 5). The PCA for flooding conditions explained 92.4% of the total variance, with key characteristics such as DSA, FGP, ADD, ST, TGWPo, PL, PH, TGWPa, and TGP accounting for 50% of the variability (Figure 3). The black rice genotypes were grouped into three distinct clusters, classifying Zafiro as a separate genotype. Among the pigmented rice genotypes, Quila 292001, Quila 292003, Quila 2912010, and Quila 291602 were closely associated with TRAN. Genotypes Quila 292008, Quila 292009, Quila 292011, and Quila 292012 formed a cluster linked to ADD, ST, PH, and DSA. The third cluster consisted of lines Quila 292014, Quila 292015, Quila 292017, Quila 292018, Quila 299801, Quila 299802, and Quila 299803. It was strongly associated with productivity-associated traits, including WG, PL, TGWPa, TGP, FGP, GY, and TGWPo.

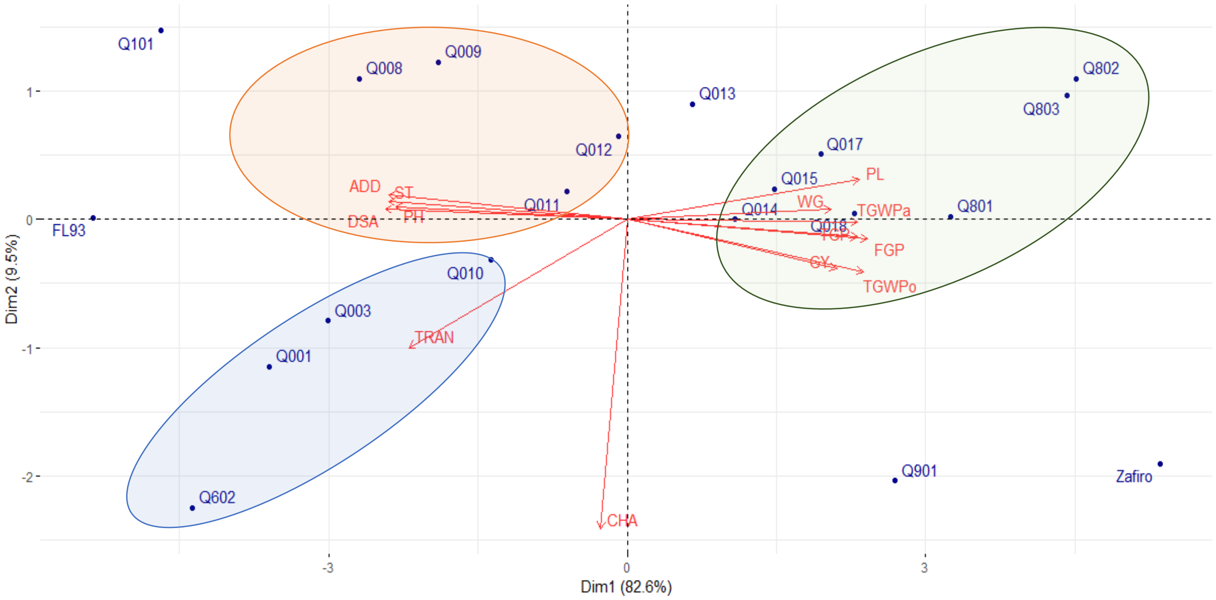


Figure 3. Principal components analysis (PCA) biplot of agronomic, productive, and quality traits of nineteen black rice genotypes and a white rice cultivar assessed under

conventional flooding conditions over three consecutive years. GY: grain yield, PH: plant height, DSA: days from sowing to anthesis, WG: whole grain percentage, CHA: grain chalkiness, TRA: grain translucency, PL: panicle length, TGP: total grain per panicle, FGP: filled grain per panicle, ST: sterility percentage, TGWPa: thousand-grain weight of paddy rice, TGWPo: thousand-grain weight of polished rice, and ADD: average degree of dispersion. Black rice genotypes; FL93: FQuila, 93, Q602: Quila 291602, Q001: Quila 292001, Q003: Quila 292003, Q008: Quila 292008, Q009: Quila 292009, Q010: Quila 292010, Q011: Quila 292011, Q012: Quila 292012, Q013: Quila 292013, Q014: Quila 292014, Q015: Quila 292015, Q017: Quila 292017, Q018: Quila 292018, Q901: Quila 297901, Q101: Quila 279101, Q801: Quila 299801, Q802: Quila 299802, Q803: Quila 299803. White rice cultivar: Zafiro.

The PCA under NFI conditions revealed a different grouping pattern than those observed under flooding (Figure 4). This analysis explained 87.5% of the variance in data using the first two principal components. The traits TGWPo, GY, DSA, PL, PH, ST, ADD, TGWPa, and TRAN accounted for 50% of the variability. Similarly, three distinct clusters were identified, with the white rice cultivar Zafiro being separated from all groups. The black rice genotypes Quila 292011, Quila 292012, Quila 292013, Quila 292014, and Quila 292015 were closely grouped and associated with WG and TGWPa. ADD and DSA primarily influenced lines Quila 292001, Quila 292003, and Quila 292008. Finally, genotypes Quila 292017, Quila 292018, Quila 299801, Quila 299802, Quila 299803, and Quila 297901 formed a separate cluster strongly linked to PH, PL, TGWPo, GY, TRAN, and CHA (Table S2).

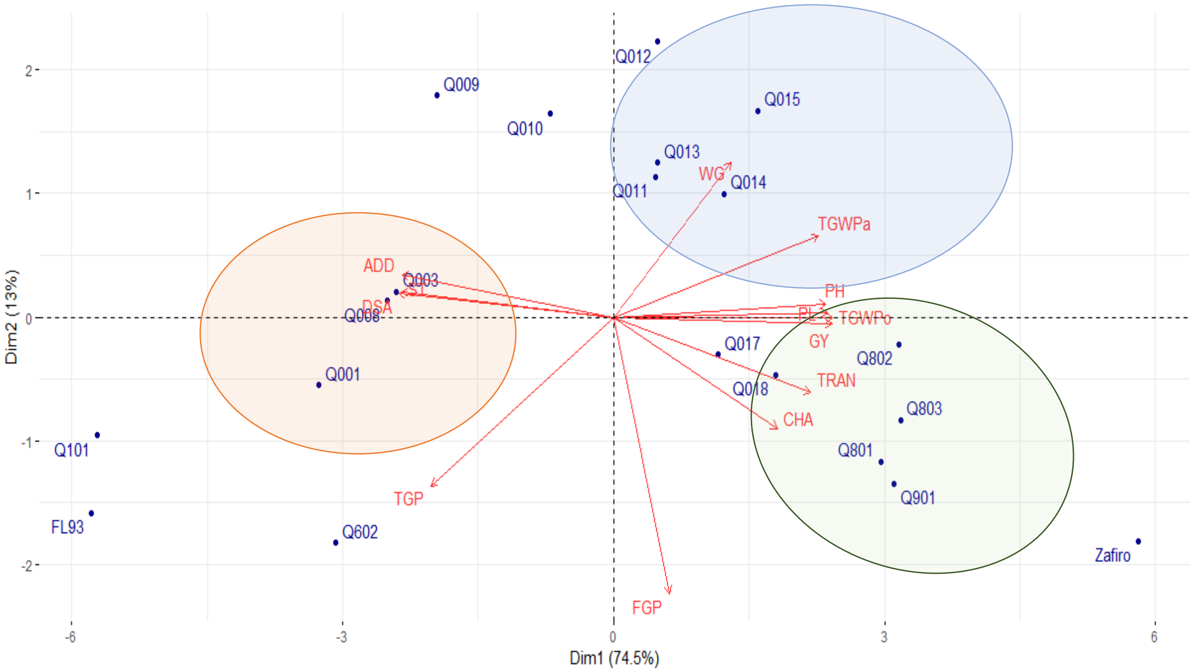


Figure 4. Principal components analysis (PCA) biplot of agronomic, productive, and quality traits of nineteen black rice genotypes and a white rice cultivar assessed under non-flooding irrigation conditions over three consecutive years. GY: grain yield, PH: plant height, DSA: days from sowing to anthesis, WG: whole grain percentage, CHA: grain chalkiness, TRA: grain translucency, PL: panicle length, TGP: total grain per panicle, FGP: filled grain per panicle, ST: sterility percentage, TGWPa: thousand-grain weight of paddy rice, TGWPo: thousand-grain weight of polished rice and, ADD: average degree of dispersion. Black rice genotypes; FL93: FQuila, 93, Q602: Quila 291602, Q001: Quila 292001, Q003: Quila 292003, Q008: Quila 292008, Q009: Quila 292009, Q010: Quila 292010, Q011: Quila 292011, Q012: Quila 292012, Q013: Quila 292013, Q014: Quila 292014, Q015: Quila 292015, Q017: Quila 292017, Q018: Quila 292018, Q901: Quila 297901, Q101: Quila 279101, Q801: Quila 299801, Q802: Quila 299802, Q803: Quila 299803. White rice cultivar: Zafiro.

Regarding the association between traits and environments, DSA, WG, and ST showed strong correlations (Figure S4) and were linked to NFI conditions. In contrast, PH, TGP, TGWPa, and TGWPo also exhibited a high correlation. However, their relationship was opposite to that of DSA, WG, and ST, resulting in a negative correlation between the two trait groups. The flooding conditions for 2021 and 2023 were closely clustered, suggesting similar effects influenced by FGP and GY. However, the association with these traits was not observed in 2022.

2.4. Genotypic and Phenotypic Correlations Among Traits

Genotypic correlations between yield and the other evaluated traits were generally low, except for the correlation with ST (-0.84) (Table 3). Furthermore, GY showed positive correlations (r = 0.46 – 0.57) with DSA, FGP, and TGWPa, as well as with the grain quality traits TRAN and CHA, with correlation coefficients of 0.48 and 0.59, respectively. DSA was positively correlated with ADD (0.72) and negatively correlated with ST (-0.53). The ST also correlated negatively (r = -0.51 – -0.67) with FGP, TGWPa, and TGWPo, as well as with CHA and TRAN, with regression values of -0.64 and -0.73, respectively (Table 3).

Grain weight showed positive correlations (0.61 – 0.82) with grain quality traits (TRA, CHA, and WG) and negative correlations with ST, specifically -0.67 and -0.61 for TGWPa and TGWPo, respectively. Additionally, TRA and CHA were negatively correlated with ST, with values of -0.73 and -0.74, respectively.

Table 3. Genotypic (above diagonal) and phenotypic (below diagonal) correlation among agronomic, productive, and quality traits of nineteen black rice genotypes and a white rice cultivar assessed under conventional flooding and non-flooding irrigation over three years (2021, 2022 and 2023).

Trait	GY	WG	DSA	PH	CHA	TRAN	PL	ST	FGP	TGP	TGWPa	TGWPo	ADD
GY		0.02	0.56	0.23	0.48	0.59	0.04	-0.84	0.46	-0.05	0.57	0.43	0.16
		ns	*	ns	*	**	ns	***	*	ns	**	ns	ns
WG	0.07		0.15	0.01	0.38	0.40	-0.20	-0.12	-0.63	-0.76	0.61	0.71	-0.09
	ns		ns	ns	ns	ns	ns	ns	**	***	**	***	ns
DSA	0.50	0.11		-0.02	-0.09	0.07	0.21	-0.53	0.05	-0.25	0.40	0.37	0.72
	*	ns		ns	ns	ns	ns	*	ns	ns	ns	ns	***
PH	0.32	-0.08	-0.05		0.43	0.45	0.20	-0.21	0.09	0.02	0.50	0.50	-0.06
	ns	ns	ns		ns	*	ns	ns	ns	ns	*	*	ns
CHA	0.43	0.32	-0.08	0.46		0.98	-0.14	-0.64	0.32	-0.04	0.74	0.74	-0.46
	ns	ns	ns	*		***	ns	**	ns	ns	***	***	*
TRAN	0.54	0.34	0.07	0.47	0.98		-0.09	-0.73	0.30	-0.12	0.82	0.80	-0.37
	*	ns	ns	*	***		ns	***	ns	ns	***	***	ns
PL	0.04	-0.12	0.16	0.23	-0.09	-0.05		0.40	-0.36	-0.21	0.10	0.14	0.53
	ns	ns	ns	ns	ns	ns		ns	ns	ns	ns	ns	*
ST	-0.65	0.06	-0.33	-0.12	-0.49	-0.54	0.16		-0.51	0.07	-0.67	-0.61	-0.13
	**	ns	ns	ns	*	ns	ns		*	ns	**	**	ns
FGP	0.43	-0.52	0.03	0.09	0.28	0.26	-0.15	-0.64		0.85	0.21	0.04	-0.18
	ns	*	ns	ns	ns	ns	ns	**		***	ns	ns	ns
TGP	-0.01	-0.60	-0.20	0.05	-0.03	-0.10	-0.09	0.01	0.75		-0.13	-0.30	-0.25
	ns	**	ns	ns	ns	ns	ns	ns	***		ns	ns	ns
TGWPa	0.54	0.43	0.36	0.44	0.75	0.82	0.12	-0.45	0.16	-0.12		1.00	-0.11

	*	ns	ns	ns	***	***	ns	*	ns	ns		***	ns
TGWPo	0.47	0.50	0.33	0.44	0.8	0.85	0.15	-0.40	0.04	-0.24	0.95		-0.09
	*	*	ns	ns	***	***	ns	ns	ns	ns	***		ns
ADD	0.15	-0.10	0.67	-0.10	-0.44	-0.36	0.39	-0.08	-0.13	-0.21	-0.14	-0.13	
	ns	ns	**	ns	ns	ns	ns	ns	ns	ns	ns	ns	

* $p \leq 0.05$, ** $p \leq 0.01$, and *** $p \leq 0.001$. GY: grain yield, WG: whole-grain yield percentage, DSA: days from sowing to anthesis, PH: plant height, CHA: grain chalkiness, TRAN: grain translucency, PL: panicle length, ST: sterility percentage, FGP: filled grain per panicle, TGP: total filled grain per panicle, TGWPa: thousand-grain weight of paddy rice, TGWPo: thousand-grain weight of polished rice, ADD: Average degree of dispersion. n= 60.

Considerably high correlations were found between TRA and CHA (0.98) and TGP and FGP (0.85). Phenotypic correlations generally followed a similar pattern, exhibiting comparable or lower values than genetic correlations.

2.5. Variance Components and Heritability

The combined analysis of variance revealed significant effects of genotype, environment, and genotype × environment interaction (Table 4). The genotypic variance was statistically significant for all traits evaluated except for ST ($p < 0.05$). Similarly, environmental variance was important for all traits except for CHA. Genotype x environment interaction variance was insignificant for DSA, PL, FGP, and TGP. Several traits exhibited very high heritability ($H^2 > 0.9$), including grain weight (TGWPa and TGWPo), quality traits (CHA, TRA, and ADD), as well as DSA and PH. GY demonstrated moderate heritability (0.76). Instead, WG showed moderately high heritability. The lowest heritability values were observed for ST and PL, with values of 0.46 and 0.51, respectively (Table 4).

Table 4. Variance components and heritability of agronomic, productive, and quality traits of nineteen black rice genotypes and a white rice cultivar assessed under conventional flooding and non-flooding irrigation over three consecutive years.

	σ^2_g		σ^2_{env}		σ^2_{ge}		σ^2_ε	H^2
GY	30.74	***	562.65	***	24.60	***	97.82	0.76
WG	7.22	***	35.71	***	17.80	***	14.30	0.66
DSA	13.38	***	183.51	***	1.39	ns	10.62	0.94
PH	39.62	***	76.18	***	12.99	***	28.10	0.91
CHA	29.48	***	3.36	ns	2.94	***	2.83	0.98
TRAN	0.36	***	0.06	**	0.03	***	0.04	0.98
PL	0.41	*	1.22	***	0.21	ns	6.32	0.51
ST	7.42	ns	171.65	***	19.06	**	100.99	0.46
FGP	13.4	**	120.67	***	10.4	ns	148.25	0.57
TGP	29.98	***	57.61	**	0.00	ns	213.23	0.72
TGWPa	4.70	***	2.11	***	0.99	**	4.52	0.92

TGWPo	2.95	***	1.38	***	0.31	***	0.66	0.97
ADD	0.48	***	0.22	***	0.05	*	0.36	0.94

* $p \leq 0.05$, ** $p \leq 0.01$, and *** $p \leq 0.001$. ns: not significant. σ^2_g: genotype variance, σ^2_{env}: environment variance, σ^2_{ge}: genotype x environment variance, σ^2_{ε}: residual/error variance, H^2: heritability. GY: grain yield, WG: whole grain yield percentage, DSA: days from sowing to anthesis, PH: plant height, CHA: grain chalkiness, TRAN: grain translucency, PL: panicle length, ST: sterility percentage, FGP: filled grain per panicle, TGP: total grain per panicle, TGWPa: thousand-grain weight of paddy rice, TGWPo: thousand-grain weight of polished rice, ADD: average degree of dispersion.

3. Discussion

3.1. Genotypic and Environmental Influences on Agronomic Performance and Grain Quality

Rice (Oryza sativa L.) is one of the most important staple crops worldwide, feeding more than half the global population. The agronomic performance of this crop is mainly influenced by genetic factors [42,43,14]. However, environmental conditions such as water availability, temperature fluctuations, and soil properties are critical in determining the final yield and quality of the crop. Parameters like the number of days to flowering play a crucial role in rice performance, particularly in Mediterranean climates like Chile, where water is limited during the flowering and grain development stages (summer). In such environments, earlier-flowering genotypes may hold a relative advantage. On average, black rice genotypes flowered about 8 days earlier than the white rice cultivar (Zafiro) (Table 1). This earlier flowering in pigmented rice alludes to the lower yield potential of black rice compared to white rice [44,45,46], which explains why farmers in various regions are often reluctant to cultivate pigmented rice varieties [44,45]. With these results we demonstrate genetic differences between traditional white rice and pigmented varieties.

Several studies have evidenced the lower yield stability of black rice genotypes across different environments [45,47,48], primarily due to high sterility rates and reduced grain weight. Similarly, Limbongan et al. [49] reported that the highest-yielding white rice genotype, UKIT102-2-056, produced 8.3-ton ha^{-1}; in contrast, the black rice genotype, UKIT104-2-127, reached a significantly lower yield of 3.4-ton ha^{-1} under the same environmental conditions. In addition, the authors emphasized the importance of breeding programs in improving sterility resistance in pigmented rice varieties and enhancing the economic viability of the crop.

In the present study, the thousand-grain weight of the pigmented genotypes was lower than that of Zafiro, providing further evidence that black rice genotypes have lower yields than white rice. The reduced yield of black genotypes can be attributed to a high percentage of floral sterility. In contrast, this lower sterility rate in Zafiro being a temperate O. sativa subsp. japonica cultivar which is adapted to cooler temperatures [50,26]. Zafiro was the first cultivar developed in Chile through population improvement with recurrent selection, with cold tolerance as the primary target. It is particularly well-suited to the local conditions of Chile, the southernmost rice-growing region in the world. However, Quila 292001 and FLQuila 93 showed low sterility rates compared to other pigmented genotypes, which could indicate relatively higher cold tolerance among the pigmented rice genotypes.

In terms of grain quality, slight differences were found between pigmented genotypes and the white rice cultivar, particularly in WG, which is the main industrial quality parameter [51] and in the ADD, a measure of grain gelatinization temperature [52,53,18]. These shifts in WG and ADD suggest that while the visual attributes of pigmented rice differ substantially, its basic industrial processing characteristics might not be as drastically altered compared to white rice. As expected, significant differences were observed in grain appearance, with notable reductions in CHA and TRAN in pigmented genotypes. This highlights the visual distinction of pigmented varieties from white cultivars, which is a primary characteristic that differentiates them and is relevant to consumer preferences and market niches.

Concerning environmental factors, water status is one of the most critical determinants of rice growth, development, and yield, and thus, proper water management is crucial in the cultivation of this crop [27,26,49,28]. Traditional flooding irrigation has been the standard method in many rice-producing regions; however, this type of irrigation results in rice production with a high volume of water, which poses a problem in the face of increasing water scarcity. Therefore, alternative irrigation strategies, such as NFI, have been introduced [54,14].

This delay in the reproductive transition would indicate that this water-saving technique produces an alteration in the physiological processes that control growth and, therefore, has repercussions on grain formation and yield. Additionally, plant height decreased by 7.6%, a response to water deficit that affects cell elongation and internode expansion [28], while the most significant impact of NFI was a substantial decrease in grain yield, which fell by 28% compared to the conventional irrigation method (Table 1). This yield reduction was primarily attributed to a 12.4% increase in floral sterility and a 7.6% decline in filled grains per panicle. The increased sterility suggests that water limitations during reproductive development disrupt successful pollination and grain filling, leading to a higher proportion of unfilled grains [28]. However, grain weight was relatively unaffected, with only a reduction of approximately 2% (Table 1), suggesting grain weight stability and strong genetic control of this trait [34].

Despite its adverse effects on productivity, NFI had a minimal impact on grain quality traits such as CHA and TRAN, while ADD decreased. This indicates high heritability and stability across both water management conditions (Table 2). The CHA is primarily influenced by genetic factors that regulate starch biosynthesis and the grain-filling process. Genes involved in starch synthesis, such as SSIIa (starch synthase IIa) and Wx (waxy gene), play a key role in determining the structure of endosperm starch, which directly affects grain opacity [55]. The stability of CHA under NFI suggests that the expression of genes involved in starch biosynthesis is not highly sensitive to moderate variations in water availability, thus preserving the integrity of endosperm development. Similarly, TRAN, which is linked to the uniform packing of starch granules and the protein matrix within the endosperm, is primarily regulated by genetic factors [56]. The lack of significant variation in TRAN under NFI conditions indicates that water stress does not substantially disrupt the deposition of amylose and amylopectin or the protein-starch interaction within the grain. The ADD reflects the gelatinization temperature of rice starch, which is closely related to the fine structure of amylopectin.

Genetic factors, such as the Wx locus, regulate both amylose content and gelatinization temperature [52]. The results suggest that the use of NFI does not significantly impact amylose content or starch molecular structure, resulting in a stable ADD under both flooding and NFI conditions. Regarding WG, the NFI resulted in a decline of 8.4%. This reduction could be due to a low translocation of assimilates, which alters the regular grain filling process, resulting in empty or immature grains that possess low milling strength and are prone to breakage during post-harvest processing [52].

In general, these findings suggest that NFI can significantly affect yield-related traits due to its effect on reproductive development and grain filling. However, this condition does not substantially alter the biochemical pathways that regulate grain quality traits. A study by Hallajian et al. [41] found that NFI could be an effective water management strategy, as it improves drought resistance. Nevertheless, they emphasized the need for genetic improvements to enhance sterility resistance and prevent yield reductions. Therefore, the development of black rice genotypes with enhanced performance under variable irrigation conditions could make NFI a viable water-saving strategy that preserves grain quality and supports effective postharvest processing and market acceptance.

Temperatures during the reproductive stage are particularly critical in rice cultivation as they can affect flowering and crop yield [57,22,14]. Heat stress exceeding 35°C can cause spikelet sterility, decrease pollen viability, and hinder grain filling, ultimately reducing yields [58]. Moreover, rice genotypes exhibit varying levels of heat tolerance, with certain varieties showing greater resilience through stress-adaptive traits [58].

Rice grain yield in the 2022 season in Chile suffered a notable decrease in performance and a substantial increase in the percentage of sterility compared to the other two seasons, both under flooded and NFI conditions, these decreases were attributed to unfavorable climatic conditions, including low temperatures during germination, insufficient rainfall, and high temperatures during the reproductive stage (January to March) (Figure S1). A correlation analysis between DSA and accumulated degree days revealed that the conditions described above had a negative impact, particularly during the 2022 season (Figure S2). Specifically, a difference in the DSA was observed between years under flooding, with 18 fewer days in the 2022 season compared to the average of the other two seasons. This difference led to a lower accumulation of 123-degree days during the early season (Figure S2). The combination of these conditions and low water availability resulted in fewer effective panicles, negatively impacting yield [22] and making the season particularly challenging for the Chilean rice industry [59]. Therefore, these findings underscore the importance of investigating the mechanisms of stress adaptation, thereby facilitating the selection of genotypes suited to varying environmental conditions [60].

3.2. Correlation Among Agronomic, Productive, and Quality Traits

Understanding the correlation between grain yield and agronomic, productive, and quality traits is crucial for rice breeding as grain yield is a complex trait influenced by multiple genes, while plant phenotype expression is also influenced by the environment [61,62]. Under NFI conditions, GY was highly correlated with most of the evaluated traits (Figure 2), although with some differences in the effect observed compared to conventional flooding.

As expected, grain yield under both conditions was primarily influenced by DSA, which positively affected ST but negatively impacted yield components such as PL and grain weight (Figures 2 and 3). This pattern is well-documented, with early-flowering genotypes typically allocating more assimilates to reproductive structures, resulting in shorter plant height and enhanced grain-filling efficiency [63]. Early flowering has been recognized in crops as an escape mechanism under water stress conditions [64, 54]. However, rice cultivars often delay flowering time [65,66], negatively impacting grain yield. This effect is particularly evident in the strong correlation between GY and DSA under NFI conditions ($r = –0.99$; Figure 2). Research on photoperiod-sensitive rice varieties reveals that flowering delays under stress conditions frequently result in yield losses, primarily due to a shorter grain-filling period and increased sterility [66]. Temperature fluctuations further exacerbate this issue by negatively affecting spikelet fertility [54].

Despite the apparent negative correlation between GY and DSA under both water irrigation methods, the association between GY and PH and between GY and TGP differed. It has been reported that an increase in plant height generally leads to higher yields in rice [63]. In the present study, however, this effect was observed only under NFI (Figure 2), where a strong positive correlation (r = 0.97) was found between GY and PH. The delayed flowering observed under NFI is likely a result of physiological stress responses [22], which can hinder carbohydrate translocation, ultimately affecting grain yield [65], thus leading to smaller and less productive plants.

Water stress and temperature fluctuations in rice have been shown to lead to floral sterility, a reduction in the number of mature pollen grains, and decreases in the number of effective panicles, grains per panicle, and grain yield [54,57,67]. The decline in effective panicles and grain set under NFI conditions may be linked to oxidative stress, as elevated reactive oxygen species (ROS) impair pollen viability and stigma receptivity [54]. This could result in a non-significant correlation between GY and FGP.

The low correlation values of CHA under flooding conditions suggest that this trait is not associated with agronomic and yield characteristics, except for TRAN, which showed a significant positive correlation with CHA. This results in a translucent grain grade, a highly valued characteristic in the rice market [68], significant in premium rice markets of japonica varieties [14]. A different trend was observed under NFI, with CHA showing substantial correlations with most of the evaluated traits. This suggests a water stress effect, even though genetic factors primarily influence CHA [55] and remain consistent across genotypes and environments. On the other hand, the correlation between TRAN and GY demonstrated contrasting effects across different environments. A negative association was observed under flooded conditions, while a positive association was noted under NFI (Figures 1 and 2). This pattern persisted regardless of the slight impact of NFI on grain translucency, although NFI did significantly affect grain yield (Tables 1 and 2).

3.3. Principal Component Analysis of Trait Variation

Principal component analysis (PCA) was performed to explore the relationships between genotypes and agronomic, productive, and quality traits under the different irrigation methods, thereby facilitating an understanding of the underlying data structure [69,70].

A clear positive correlation was observed between grain yield and traits such as PL, TGP, FGP, and grain weight (Figure 3), which are key determinants of crop productivity [14]. These traits are susceptible to environmental conditions since optimal water availability is required for panicle initiation, flowering, and grain development [22,71]. In rice, flooding enhances vegetative growth and reproductive success by sustaining optimal transpiration rates and preventing spikelet sterility [72,73]. However, the present study observed negative associations between GY and DSA, ST, and PH. Delayed flowering in rice typically results in reduced grain yield [65,66], while elevated ST is also linked to lower productivity, particularly in temperate climates, where temperature fluctuations during the reproductive stages can disrupt flowering and diminish crop yield [57,22,14].

As mentioned above, water stress in rice leads to altered carbohydrate metabolism, which negatively affects grain development [54,57,74] and, therefore, grain weight. These results suggest that genotypes under water deficit conditions, which achieve higher biomass production—evident from the positive association between GY with PH and PL— have a greater capacity to achieve grain filling and, consequently, higher yields. As observed under flooded conditions, GY was negatively correlated with DSA and ST (Figure 4) since delayed flowering negatively impacts yield by shortening the grain-filling period and increasing floral sterility [66]. In contrast, under non-flooded conditions, the absence of the thermoregulatory effect of water, combined with low nighttime or high daytime temperatures, adversely affects spikelet fertility [54]. Additionally, Quila 299801, Quila 299802, and Quila 299803 under NFI conditions exhibited relatively high CHA and TRAN values, highlighting a positive correlation between these quality traits and grain yield. Water stress and high temperatures are also known to affect grain quality in rice negatively [75,54]. Reduced water availability during critical grain-filling stages led to incomplete and irregular starch granule formation, which resulted in lower grain quality [14,18]. These results are consistent with previous studies in which chalkiness and translucency were affected under NFI by altering amylose content and protein-starch interactions [52,19]. In contrast, genotypes Quila 292001, Quila 292003, and Quila 292008 form a cluster primarily influenced by DSA, ST, and ADD (Figure 4). Under NFI conditions, these genotypes exhibited yields lower than the average of the black genotypes, with DSA being higher and ADD being like or higher than the average value of the black genotypes (Table S2). This highlights the relationship between water stress in rice and its impact on grain quality, affecting not only chalkiness and translucency but also gelatinization temperature (i.e., ADD) [19,14,18]. Finally, a third cluster, primarily defined by WG and, to a lesser extent, by TGWPa, grouped genotypes Quila 292011, Quila 292012, Quila 292013, Quila 292014, and Quila 292015. This cluster illustrates the positive association between WG and TGWPa, although WG showed only a slight and non-significant correlation with GY under NFI conditions (Figure 2).

The black genotypes Quila 279101 and FQuila 93, and the white genotype Zafiro, were the most responsive based on the evaluated traits in both environments, flooding and NFI (Figures 3 and 4). These genotypes exhibited distinct performances in both environments compared to the other genotypes and were not grouped in any cluster. Under flooded conditions, Zafiro exhibited the highest values for DSA, GY, grain weight, CHA, and TRAN, while it tended to have the lowest values for ST. On the other hand, although FQuila 93 had a particularly low yield, it tended to have the highest WG value. While Quila 279101 had a high yield among the pigmented genotypes, it also tended to show the highest TGP; however, it reached a relatively high ST, while it tended to exhibit the lowest TWGPo, along with a high ADD value (Table S1). Likewise, similar trends were observed in the performance of these genotypes under NFI conditions (Table S2).

3.4. Genotypic and Phenotypic Correlations Among Traits

Information on the variability and heritable traits of the genetic material is crucial for effective yield and grain quality improvement in rice breeding. Understanding the phenotypic and genotypic components of variation, heritability, and correlations among traits provides valuable insights into breeding desirable traits [61,76].

If two traits are genetically positively correlated and selection favors high values for both traits, an evolutionary response is observed [77]. Genotypic correlations between grain yield and the other evaluated traits were generally low, except for the correlation with ST (r = -0.84) (Table 3). The positive correlation between yield and grain quality variables has been similarly described by Tiwari et al. [76] reported strong positive genotypic and phenotypic correlations between grain yield and both days to heading and days to maturity in rice under a rainfed lowland environment. In addition, significant positive genotypic and phenotypic correlations were obtained between GY and grain weight, which agrees with that reported for elite rice lines developed for tropical lowland ecosystems [48].

Conversely, ST exhibited a negative correlation with grain yield and quality traits (Table 3). These findings are consistent with those of Akbar et al. [27], who observed a negative correlation between grain sterility percentage and grain yield and quality traits in double haploid lines of lowland rainfed rice. This suggests that an increase in sterility percentage directly reduces yield, highlighting the importance of selecting genotypes with low sterility, especially under water stress and suboptimal conditions. Conversely, PH exhibited a positive and moderate genetic correlation with grain weight, with values of 0.50 for both TGWPa and TGWPo, contrasting with the findings of Tiwari et al. [76].

On the other hand, although no significant, negative genetic correlations were observed between TGP and grain weight (Table 3). It has been reported that genotypes with a high number of filled grains per panicle exhibit reduced grain weight, likely due to a limited assimilate supply [74], which aligns with the patterns observed in the present study. This phenomenon, often referred to as the "trade-off effect," has been well-documented in cereal crops [78,79]. The trade-off occurs due to limited assimilate availability during grain filling, where an increase in grain number leads to competition for resources, ultimately reducing the individual grain weight [79]. These findings suggest that breeding programs should focus on selecting genotypes with optimal panicle architecture to balance grain number and grain weight.

The strong genotypic and phenotypic correlation between CHA and TRA underscores a close genetic relationship between these key grain quality traits. However, an evident pattern was observed in which the genetic correlation between grain yield and WG was not found to be positive, which is particularly relevant for genetic improvement strategies, since WG is a fundamental industrial quality trait; its reduction through grain breakage significantly decreases its market value [80,14]. Similarly, ADD, which influences gelatinization temperature and cooking behavior, is crucial for determining the technological and market characteristics of rice [18,81]. The absence of a genetic correlation between yield and these two industrial traits suggested a potential trade-off in selection efforts to improve productivity and processing quality in black rice.

3.5. Variance Components and Heritability

Heritability is a predictive function that measures genetic variability, especially in crop improvement, reflecting the effectiveness of genotype selection [61,82]. This index provides valuable information on genetic and environmental factors influencing trait expression in rice breeding programs [83]. High to moderate values of broad-sense heritability ($H^2 > 0.6$) indicate strong genetic control over a trait, with a lesser impact of environmental factors [84].

Differences in genetic variances were found in all traits evaluated, except for ST, indicating the presence of genetic variations within the population [39]. In contrast, the variance of the genotype x environment interaction was not significant in some traits, indicating their stability over the three years of analysis under different water regimes [13,84]. Similar results were reported by Tiwari et al. [76] for early maturing rice in rainfed lowland environments, with broad-sense heritability values of 0.94 and 0.92 for days to heading and days to maturity, respectively, and 0.74 for thousand-grain weight. In addition, using a panel of 100 rice genotypes, Asante et al. [85] reported H^2 values of 0.81 for days to flowering and 0.62 for plant height.

The heritability of grain yield (GY) was high, as this parameter is a quantitative trait influenced by the combined effects of multiple genes (polygenic inheritance) and environmental factors, such as soil fertility, water availability, and temperature fluctuations [71,43]. In this study, the environmental variability accounted for 24%, where NFI significantly reduced yield. Similarly, Li et al. [79] observed that high environmental variation in grain yield was associated with decreased productivity due to water stress. On the other hand, Asante et al. [85] and Adjah et al. [40] reported low heritability for grain yield (0.23 and 0.37, respectively) when using a larger rice accessions pool and under traditional flooded conditions, where greater genetic diversity could result in higher variability in rice performance.

Grain quality traits showed high heritability, indicating strong genetic control. Relatively high H2 values have been recorded for rice in terms of the percentage of chalky grains and the degree of chalkiness, reaching 0.69 and 0.66, respectively, and a low value of 0.15 for the percentage of broken rice grains [40].

The high heritability presented in this study is consistent with the findings of Demeke et al. [86]. They observed that selecting for high-heritability traits, including days to heading and maturity, plant height, and thousand-grain weight, effectively improved yield under diverse environmental conditions. In addition, the PCAs (Figures 3 and 4) further highlighted that those high-heritability traits are key contributors to yield variance across different environments [87].

The correlations between agronomic and quality traits offer a strong basis for indirect selection in breeding programs. High heritability values for traits such as TGWPa, TGWPo, TRAN, and CHA (Table 4) indicate that these traits are predominantly influenced by genetic factors, making them reliable targets for genetic improvement in black rice. The observed correlations between agronomic and industrial quality traits provide a strong basis for indirect selection in breeding programs. Integrating multi-environment trials and genomic prediction models (e.g., genome-wide association studies (GWAS) could enhance selection efficiency by identifying stable genotypes with desirable trait combinations [88].

4. Materials and Methods

4.1. Plant Material, Experimental Design, and Crop Management

Nineteen advanced black rice genotypes and the white rice cultivar Zafiro, developed by the Rice Breeding Program (RBP) of the National Institute of Agricultural Research (INIA), Chile, were evaluated. Genotypes were selected based on their performance in a preliminary screening of black rice lines from the RBP, with an emphasis on grain color and the stability of this trait. On the other hand, Zafiro, which accounts for approximately 70% of the rice cultivated area in Chile, was included as a control genotype. The trial was conducted in the INIA experimental rice fields in San Carlos (36°25′49” S, 72°0′25” W; 161 m.a.s.l.), Ñuble region. The germplasm was evaluated over three years (2021, 2022, and 2023) using two water management techniques: conventional flooding and non-flooding irrigation (NFI) (Figure 5). A completely randomized block design with three replicates (blocks) was used. Each plot consisted of seven 3-m-long rows spaced 0.3 m apart, resulting in a plot area of 6.3 square meters.

Conventional soil management practices were implemented prior to trial establishment, including chemical fallowing with glyphosate (2 L ha^{-1}), leveling, and soil preparation. For optimal plant growth, NPK fertilizer was applied before sowing. Urea (CH4N2O) was used at a rate of 22 kg ha^{-1}, triple superphosphate ($Ca(H_2PO_4)_2H_2O$) at 130 kg ha^{-1}, and muriate of potash (KCl: NaCl) at 150 kg ha^{-1}. Irrigation was initiated one day after planting, with subsequent water applications promoting seedling emergence and supporting plant establishment.

Once the plants reached the 2.5-leaf stage and until grain filling was complete, plants under the flooding regime remained flooded, while those under NFI were irrigated every eight days at field capacity. Nitrogen was applied in three additional phenological stages: 66 kg ha^{-1} of urea at the 2.5-leaf stage, 88 kg ha^{-1} at tillering, and 44 kg ha^{-1} at panicle initiation. Weed control was carried out using Ricer® (210 cc ha^{-1}) and Clincher® (1.5 L ha^{-1}) to manage narrow-leafed weeds and Bentax (2.5 L ha^{-1}) and MCPA (1 L ha^{-1}) for controlling wide-leafed weeds.

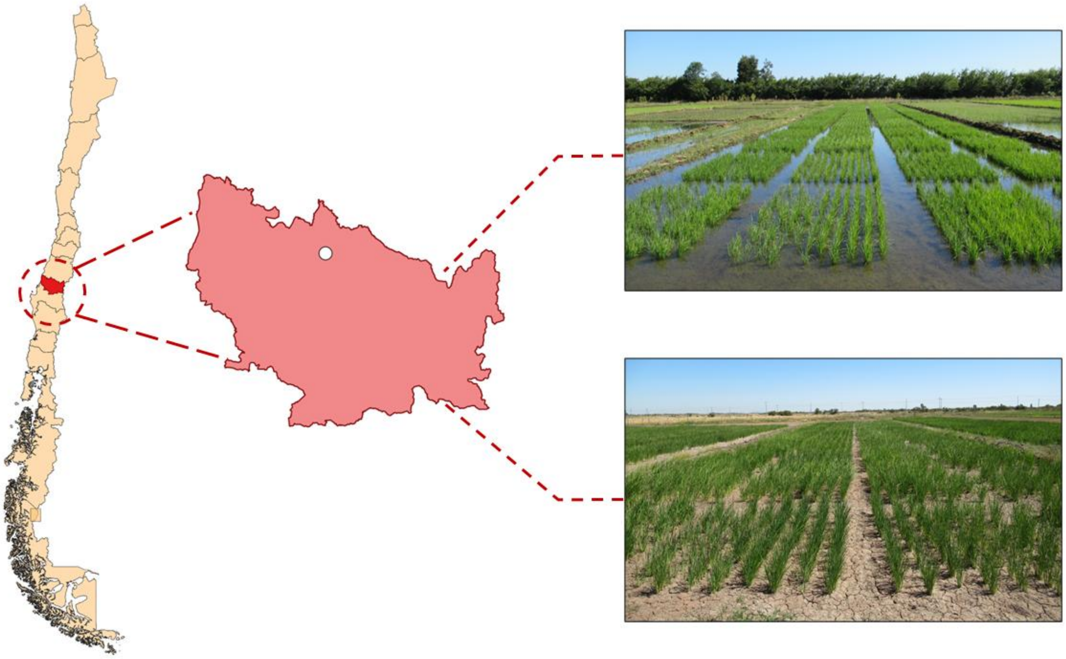


Figure 5. Location of the San Carlos site in the Ñuble Region, and experimental plots under traditional flooding (top) and non-flooding irrigation (bottom).

4.2. Assessment of Agronomic, Productive, and Quality Traits

Days from sowing to anthesis (DSA) and plant height (PH) were recorded at anthesis. At harvest, panicle length (PL), total number of grains per panicle (TGP), number of filled grains per panicle (FGP), panicle sterility percentage (ST), thousand-grain weight of polished (TGWPo) and paddy (TGWPa) rice, and grain yield (GY) at 14% moisture content were evaluated.

To determine the whole grain percentage (WG), 100 grains were polished using a Suzuki MT test mill. Grain chalkiness (CHA) and translucency (TRAN) were measured using an MM1D milling meter (Satake, Japan), with three readings taken per sample. For the average degree of dispersion (ADD) in the alkali reaction, ten representative whole grains were incubated for 23 h at 30°C in a 1.7% KOH solution, after which the gelatinization temperature was measured.

4.3. Linear Mixed Models and Genotype X Environment Interaction

Agronomic, productive, and quality traits were analyzed using linear mixed models (LMM), with water management and year combinations representing six distinct environments. The data were subjected to a combined analysis of variance to assess the genetic variability of rice genotypes across the environments, according to the following equation [38]:

$$\mathrm{Y}_{ijk} = \mu + \mathrm{Gen}_i + \mathrm{Env}_j + \mathrm{Rep}_k(\mathrm{Env}_j) + \left(\mathrm{Gen}_i \mathrm{\ x\ } \mathrm{Env}_j\right) + \varepsilon_{ijk}$$

Y_{ijk} is the observation for the ith genotype in the kth replication in the jth environment (water management x year); μ is the gran mean; Gen_i is the effect of the ith genotype considered as fixed; Env_j is the effect of the jth environment considered as random; $\mathrm{Rep}_k(\mathrm{Env}_j)$ is the effect of the kth replication within the jth environment; $\left(\mathrm{Gen}_i \mathrm{\ x\ } \mathrm{Env}_j\right)$ is the random effect of the interaction between ith genotype with the jth environment; ε_{ijk} is the random effect of the error associated with the ith genotype, jth environment and kth replication, which is supposed to be independent ε ~ N(0, σ2).

The broad-sense heritability based on the means in the multi-environment model was estimated as follows:

$$H^2 = \frac{\sigma_g^2}{\sigma_g^2 + \frac{\sigma_{ge}^2}{n\mathrm{Env}} + \frac{\sigma_\varepsilon^2}{n\mathrm{Env\ x\ }n\mathrm{Rep}}}$$

Where σ_g^2, σ_{ge}^2, and σ_ε^2 represent the variances of genotype, genotype x environment interaction, and error, respectively. $n\mathrm{Env}$ and $n\mathrm{Rep}$ denote the number of environments and replications, respectively.

The genetic correlation between traits was determined using the following equation:

$$\rho_{\mathrm{g}} = \frac{\overline{\sigma_{g(\mathrm{jj'})}}}{\overline{\sigma_{g(\mathrm{j})}\,\sigma_{g(\mathrm{j'})}}}$$

Where $\overline{\sigma_{g(\mathrm{jj'})}}$ is the arithmetic mean of all pairwise genotypic variances between traits j and j' and $\sigma_{g(j)}\ \sigma_{g(j')}$ represents the arithmetic average of all pairwise means of the genotypic variance components for the traits.

4.4. Statistical analysis

Statistical analysis, including the calculation of broad-sense heritability and genetic and phenotypic correlations, was performed using META-R (Multi-Environment Trial Analysis using R) software, version 6.0 [89]. Correlation and principal component analysis (PCA) were conducted in RStudio, version 4.2.1 [90].

5. Conclusions

This study offers new insights into the complex interplay between genetic factors, environmental variability, and water management strategies that influence yield and grain quality in black rice, which is particularly significant given the limited research on black rice performance under water-limited conditions and the scarcity of studies conducted in Mediterranean climates, such as those found in central-southern Chile.

As expected, the yield of pigmented genotypes was 31% lower than that of Zafiro under NFI conditions, highlighting the susceptibility of black rice to water stress and the importance of tailored breeding and management approaches. Significant differences were observed among genotypes and across environments (water management × year) for most agronomic and quality traits, including days from sowing to anthesis (DSA), grain yield, yield components, whole grain percentage, chalkiness (CHA), and translucency (TRAN). However, plant height and average degree of dispersion (ADD) showed no significant differences due to genotype or environmental factors. A strong influence of season-specific climatic conditions on genotype performance, yield, and quality traits was evidenced. The year 2022 was marked by fluctuating temperatures early in the season and during flowering, along with lower accumulated degree days. These factors significantly affected plant performance, resulting in reduced yield, a lower number of filled grains per panicle, and an increased sterility percentage (ST), particularly under flooding conditions.

Pearson correlation and principal component analysis (PCA) revealed distinct associations between traits and genotypes depending on the irrigation method (conventional flooding or NFI). This indicates a clear impact of the water environment and highlights differences in genotype performance under both contrasting conditions. However, the white rice cultivar did not cluster with any other genotype in the PCA under flooding or NFI conditions. This underscores the differences between Zafiro, a white rice cultivar developed for local conditions, and the experimental black rice genotypes.

The multi-environment analysis revealed significant genotypic and phenotypic correlations between yield and ST, DSA, thousand-grain weight of paddy rice (TGWPa), and TRAN, emphasizing the negative impact of ST and the positive influence of grain weight on rice yields. Additionally, the high broad-sense heritability values for DSA, plant height, CHA, TRAN, TGWPa, thousand-grain weight of polished rice (TGWPo), and ADD indicate their stability, making them less sensitive to climatic fluctuations. Therefore, these traits could serve as valuable targets for selection in black rice breeding programs to develop cultivars that are more resilient to environmental variability.

This study underscores the importance of an integrated approach that combines genetic selection, optimized agronomic practices, and strategies to enhance water use efficiency and maintain the sustainability of rice cultivation. It also highlights the importance of future research focusing on identifying genetic markers associated with stress tolerance and resistance to sterility by incorporating genomic selection and multi-environment testing, which could contribute to the development of high-yielding, high-quality, and climate-resilient black rice cultivars.

Supplementary Materials: The following supporting information can be downloaded at:

Figure S1: Air temperature (maximum, minimum, and average) and accumulated rainfall recorded in San Carlos in the 2020/21, 2021/22, and 2022/23 seasons. Figure S2: Linear correlation between days from sowing to anthesis (DSA) and accumulated degree days (DDA) of contrasting environments (traditional cultivation: flooding and non-flooding irrigation: NFI) for the a) 2021, b) 2022, and c) 2023 seasons. Figure S3:

Principal Component Analysis (PCA) biplot based on grain yield of nineteen black rice genotypes and one white rice cultivar evaluated under contrasting environments - conventional flooding (F) and non-flooding irrigation (NFI) - over three years (2021, 2022, and 2023). Figure S4: Principal Component Analysis (PCA) biplot of agronomic, productive, and quality traits of nineteen black rice genotypes evaluated in nineteen black rice genotypes and one white rice cultivar evaluated under contrasting environments - conventional flooding (F) and non-flooding irrigation (NFI) - over three years (2021, 2022, and 2023). Table S1: Analysis of variance of agronomic, productive, and quality traits evaluated in twenty rice genotypes under conventional flooding over three years (2021, 2022, and 2023). Table S2: Analysis of variance of agronomic, productive, and quality traits evaluated in twenty rice genotypes under non-flooding irrigation over three years (2021, 2022, and 2023).

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Data Availability Statement: All data were generated during the study. They are not publicly available but can be accessed through the following Google Drive link: (accessed on X September 2025).

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III. CAPÍTULO II: ASSESSING GRAIN QUALITY CHANGES IN WHITE AND BLACK RICE UNDER WATER DEFICIT

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ABSTRACT

Rice is an essential component of the diet for a significant portion of the population worldwide. Due to the high-water demand associated with rice production, improving water use efficiency and grain quality is critical to increasing the sustainability of the crop. This species includes rice varieties with diverse pigmentation patterns. Grain quality traits of two black rice genotypes and a commercial white rice cultivar were evaluated under different locations and water conditions. Industrial, nutritional, and functional quality traits were evaluated. Flood irrigation produced higher grain weight compared to alternate wetting and drying irrigation. A high correlation was found between grain color, total phenolic content (TPC), and antioxidant activity. The black rice genotypes showed higher TPC levels and antioxidant capacity, mainly due to higher cyanidin 3-*O*-glucoside content. Phenolic profile varied between whole and polished grains, while mineral composition varied as a function of locality and irrigation regime. Grain quality was influenced by the environment in both industrial and nutritional characteristics, and there were significant differences in quality between whole and polished grains. The study provides information on the genotype x environment relationship in rice and its effect on grain quality, which could contribute to the selection of genotypes for an appropriate environment.

Keywords: bioactive compounds; phenolic compounds; antioxidant activity; mineral composition; water stress; functional food.

1. INTRODUCTION

Cultivated rice (Oryza sativa L.) plays a crucial role as a staple food globally. In fact, approximately 25% of calorie and protein intake of the world's population comes from rice [1], representing up to 75% of the calories consumed by Asian countries [2]. In Chile, rice is grown in the region located between 35° and 36° S, which is the southernmost rice production area in the world [3]. Locally, yield reached 4.86 t ha^{-1} in an area of 20.7 thousand ha in the 2021/2022 season [4]. Due to the peculiar climatic conditions of the Chilean rice region, locally adapted cultivars are needed.

White rice O. sativa is the most widely consumed species worldwide, but in nature, there is a wide range of pigmented rice, ranging in color from brown to red to deep purple-black, which belongs to this species [5]. Black rice, predominantly cultivated in Asia, is recognized as "forbidden rice" or "king's rice" in China, its leading producer. It is also cultivated in neighboring countries, such as Sri Lanka, India, the Philippines, and Thailand [5,6]. The deep purple hue of the grain is due to the accumulation of phenolic compounds, mainly anthocyanins, in the pericarp [7,8]. At a global scale, pigmented rice accounts for only 0.1% of the total rice production, which is attributed to poor awareness of this rice variety, relatively low yield, and consumer preferences for polished white rice [9].

Pigmented rice contains several bioactive and nutritional compounds, such as anthocyanins, flavanones, phenolic acids, vitamins, fatty acids, and minerals [10]. Unlike white rice, black rice genotypes are rich in anthocyanins [11,12], mainly cyanidin 3-O-glucoside and peonidin 3-O-glucosid. However, other compounds, such as pelargonidin 3-O-glucoside and cyanidin 3-O-arabinoside, have also been identified. Compared to white rice, pigmented rice grains have higher contents of phenolic acids [12], including ferulic acid, p-coumaric acid, and vanillic acid [12,13], as well as higher levels of γ-oryzanol [14] and carotenoids, namely lutein and zeaxanthin [11,15]. Furthermore, black rice has been reported to offer greater nutritional benefits compared to white rice, with higher levels of protein, minerals, and amino acids. However, the extent of these benefits depends on environmental factors and production management [5,8]. Black rice contains niacin and eighteen amino acids, and it is also a reliable source of thiamine and fatty acids, with a high concentration of essential elements (Fe, Zn, Ca, K, Mg, and Mn) [5,13,15]. Comparative studies have also shown that black rice contains more dietary fiber (21–52%) and total protein content (7–24%), and less total soluble carbohydrates (29–35%) than white rice [6,15].

Several studies have documented the nutraceutical properties of black rice and underscored its potential health benefits, including the prevention of cardiovascular diseases, obesity, type II diabetes, and different types of cancer [5,10,16,17]. These attributes, along with the substantial potential of black rice for functional food production [6,10,16], have resulted in an increasing consumer interest and demand for black rice in European, North American, and Australian markets [5]. In Chile, there is also great interest in this type of rice, and the Black Rice Breeding Program (BRBP) was recently implemented at the Institute of Agricultural Research (INIA) in order to develop cultivars suitable for local conditions.

Rice cultivation in Chile is predominantly based on flooding methods, posing challenges to sustainability and productivity derived from increasing water scarcity, mainly attributed to climate change [4,18]. Water deficit affects plant growth, development, and yield [19]. In this sense, several studies have indicated that rice quality traits, such as milling rate [20,21], protein and amylose content [22], antioxidant content [23,24], mineral nutrients [25,26], and culinary quality [4,27], are also affected by water stress. Most of these studies were conducted on traditional white rice.

Considering the current scenario of water scarcity and the effects of water deficit on rice cultivation, the recently established BRBP aims at developing cultivars with increased performance and grain quality under water-limiting conditions. The objective of this study is to compare the grain quality and nutritional traits of two black rice genotypes and a commercial white rice cultivar developed by INIA under contrasting water conditions.

2. RESULTS

2.1. Appearance and Sensorial Quality Traits

The commercial white rice cultivar Zafiro-INIA showed the highest thousand-grain weight in both paddy and polished rice, differing significantly from Quila 279101 and Quila 292008 (Table 1). The interaction of genotype (G) × location (L) × water regime (W) was statistically significant for thousand-grain weight in paddy rice, while the interaction of L × W was statistically significant in polished rice. All the interactions were significant for grain length and length/width ratio except for location and water regime. Meanwhile, only G × W was significant for grain width.

Differences between locations were detected. The highest grain weight was obtained in Parral, with increases of 4.9% and 2.1% in paddy and polished rice, respectively. Differences in grain weight were also observed for water regime, being higher in the flooded plants and reaching 6.1% and 2.7% in paddy and polished rice, respectively.

Whole-grain (WG) yield showed differences between genotypes, locations, and water regimes (Table 2). No significant interactions between factors were observed in WG except for G × W. Regarding genotypes, the highest WG value was obtained in Zafiro-INIA, being 27% higher than that of Quila 279101, but no statistical differences were found between Quila 292008 and Zafiro-INIA. San Carlos yielded 11.5% more WG than Parral, while this parameter was 41.2% higher under AWD irrigation compared to the yield obtained under flooded conditions.

Table 1. Analysis of variance of grain-weight and grain-size traits of three rice genotypes grown in different locations and under different water regimes.

	TGW-Pa (g)		TGW-Po (g)		LENGTH (mm)		WIDTH (mm)		L/W	
Genotype G)										
Quila 279101	27.83	b	18.54	b	6.12	a	2.40	a	2.55	ab
Quila 292008	24.90	c	17.54	c	6.07	a	2.30	b	2.64	a
Zafiro	34.48	a	24.54	a	6.07	a	2.47	a	2.45	b
Location (L)										
San Carlos	28.34	b	19.99	b	6.12	a	2.40	a	2.56	a
Parral	29.79	a	20.42	a	6.05	a	2.38	a	2.54	a
Water regime (W)										
Flooding	29.98	a	20.48	a	6.18	a	2.44	a	2.54	a
AWD	28.16	b	19.93	b	5.99	a	2.34	b	2.56	a
p-value										
G x L	0.1950		0.3438		**0.0038**		0.1323		**0.0007**	
G x W	0.2917		0.3823		**0.0058**		**0.0454**		**0.0005**	
L x W	0.5028		**0.0073**		0.5192		0.2501		0.1374	
G x L x W	**0.0337**		0.4700		**0.0038**		0.1703		**0.0020**	

TGW-Pa: thousand-grain weight of paddy rice, TGW-Po: thousand-grain weight of polished rice, L/W: ratio length/wide of polished grain. AWD: alternate wetting and drying. Data are mean values ± standard error (n = 3). Means with different letters are statistically different according to the LSD Fisher test. Bold p-values indicate significant interactions between factors ($p \leq 0.05$). There were no statistical differences in grain length between genotypes, locations, or water regimes (Table 1). However, grain width was significantly smaller in Quila 292008, with no differences between the white rice cultivar and the other black rice genotype, being 4.1% wider in plants under flooding. Regarding the grain length/width ratio, significant differences were found between Quila 292008 and Zafiro-INIA, but no differences were detected between Quila 279101 and Quila 292008 or Zafiro-INIA.

Table 2. Analysis of variance of grain quality traits of three rice genotypes grown in different locations and under different water regimes.

	WG (%)		**CHA**		**TRAN**		**WB** (%)		**ADD**	
Genotype (G)										
Quila 279101	41.07	b	12.32	c	0.56	c	15.67	b	6.48	a
Quila 292008	52.53	a	15.61	b	0.80	b	23.83	a	6.17	ab
Zafiro	56.19	a	36.53	a	3.50	a	7.17	c	5.78	b
Location (L)										
San Carlos	52.97	a	21.82	a	1.57	a	17.44	a	6.05	a
Parral	46.88	b	21.16	a	1.66	a	13.67	a	6.24	a
Water regime (W)										
Flooding	36.96	b	22.47	a	1.67	a	21.67	a	6.34	a
AWD	62.89	a	20.50	b	1.56	a	9.44	b	5.95	b
p-value										
G x L	0.8333		0.7814		0.2574		0.4935		0.7068	
G x W	**0.0121**		0.6876		0.2066		**0.003**		0.1177	
L x W	0.0612		0.8629		0.2072		0.841		0.437	
G x L x W	0.7243		0.1621		0.0819		0.137		0.2913	

WG: whole-grain yield, CHA: chalkiness, TRAN: transparency, WB: white-belly rate, and ADD: average degree of dispersion of alkaline reaction. AWD: alternate wetting and drying. Data are mean values ± standard error (n = 3). Means with different letters are statistically different according to the LSD Fisher test. Bold p-values indicate significant interactions between factors ($p \leq 0.05$).

As expected, chalkiness and transparency were statistically different between white and black rice. Similarly, differences were found between black rice genotypes, with Quila 292008 having higher values for chalkiness and transparency compared to those of Quila 292008 (Table 2). There were no differences in these traits between cultivation sites. As for water regimes, grains grown under flooded conditions showed 8.8% higher chalkiness. In addition, none of the interactions between factors were significant for chalkiness and transparency.

There were significant differences in the presence of white belly (WB) in the grain between genotypes and water regimes but not between locations (Table 2). The highest WB values were observed in black rice genotypes, with Quila 279101 and Quila 292008 being 2.2- and 3.3-fold higher, respectively, compared to Zafiro-INIA. Under flooded conditions, WB was more than twice as high as in AWD. No differences in WB were detected between factors except for G × W. The same was true for WB; alkaline dispersion (ADD) showed significant differences between genotypes and water regimes, but no differences were found between growing sites. The highest ADD value was observed in Quila 279101, which was 10.8% higher than in Zafiro-INIA. For flooding, ADD was 6.2% higher than in AWD.

2.2. Nutritional Quality Traits

Phenolic concentration was higher in pigmented rice grains and, to a greater extent, in whole grains (Tables S3 and S4). Both black rice genotypes showed a mean concentration of 2.57 mg g^{-1} in polished grains, although the concentration in Quila 279101 was 37.1% higher than in Quila 292008. On the other hand, phenolic compounds in whole Zafiro-INIA grains averaged 0.105 mg g^{-1}, which was 24.5 times lower than levels in pigmented grains (Table S3). Regarding locations, pigmented genotypes grown in Parral averaged 2.6 times more compounds than those grown in San Carlos, recording concentrations of 3727 mg g^{-1} and 1418 mg g^{-1}, respectively. However, the concentration of compounds in whole black grains showed less variation due to water management, with 2360 and 2785 mg g^{-1} under flooding and AWD regimes, respectively. In black rice grains, the highest concentrations of compounds corresponded to cyanidin 3-O-glucoside followed by apigenin derivative and quercetin, accounting for 78.72%, 5.32%, and 2.90% of the sum of phenolic compounds, respectively. In white rice grains, neither cyanidin 3-O-glucoside nor quercetin was detected, while the most predominant compound was chlorogenic acid, accounting for 39.37% of the sum of phenolic compounds (Table S3).

The total mean phenolic concentration in polished pigmented grains was 0.76 mg g^{-1} (Table S4), which was 3.4 times lower than that in whole black grains (Table S3). Polished Quila 292008 recorded the highest concentration, regardless of location and water regime, with a mean of 1.24 mg g^{-1}, which was 4.3 times higher than that of polished Quila 279101. On the other hand, polished grains from Zafiro-INIA showed a mean value of 0.04 mg g^{-1}, which was 20.6 times lower than that of pigmented grains.

In addition, location had an impact on whole grains; the mean value of pigmented rice from San Carlos was 2-fold that of Parral. For water regimes, polished black grains showed the same trend as whole black rice, with a minor difference in phenolic concentration between flooding and AWD irrigation of 0.72 and 0.81 mg g^{-1}, respectively (Table S4). Similar to what was observed in whole grain, the main compounds in polished black grains were cyanidin 3-O-glucoside and apigenin derivative, which accounted for 76.85% and 6.52% of the total concentration, respectively. In white rice grains, caffeic, vanillic, and chlorogenic acids recorded the highest values, accounting for 82.58% of the total phenolic content (TPC) (Table S4).

When water regime and location were analyzed as a combined environment (Figure 1), statistical differences were observed between genotypes for all phenolic compounds except for chlorogenic acid. Regarding the effect of the environment on the TPC, the most significant differences were found in the concentration of Vad*, VA, AGNd*, and QCT in Quila 292008 (Figure 1. A, B, E, F). Few differences were found between environments for the other two genotypes. However, Quila 279101 showed higher concentrations in most compounds except for cyanidin 3-O-glycoside; Quila 292008 showed a higher concentration of this compound, especially in the AWD-SC environment (Figure 1G). Similarly, the latter environment affected the total concentration of phenolic compounds (Figure 1H). The Zafiro-INIA genotype showed the presence of VA, CA, and AGNd* concentrations in all the environments, while only CGA concentration was detected in AWD-PA (Figure 1C).

The TPC and antioxidant capacity of polished grain extracts are shown in Table 3. Significant differences were observed in the G × L × W interaction in TPC, ORAC, and DPPH. All of them showed similar differences between genotypes, which were significantly higher in black rice compared to white rice. The highest values were observed in Quila 279101, being 3.8, 3.6, and 8.7 times higher for TPC, ORAC, and DPPH, respectively, with respect to Zafiro-INIA. No differences were observed between location and water regimes.

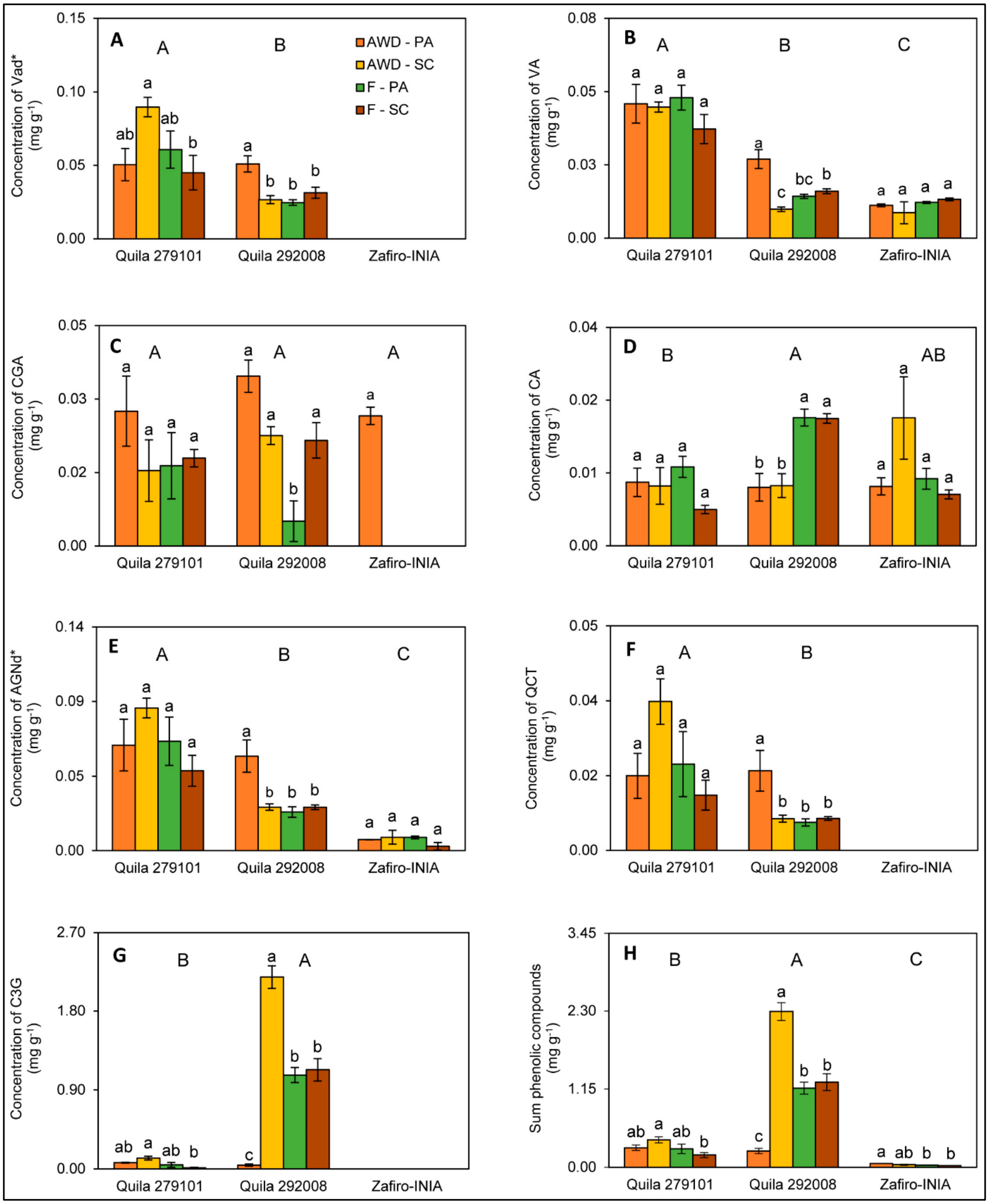


Figure 1. Concentration of phenolic compounds in polished grains of three rice genotypes (Quila 279101, Quila 292008, and Zafiro-INIA) grown in different environments (AWD-PA, AWD-SC, F-PA, and F-SC). (A) vanillic acid derivative, (B)

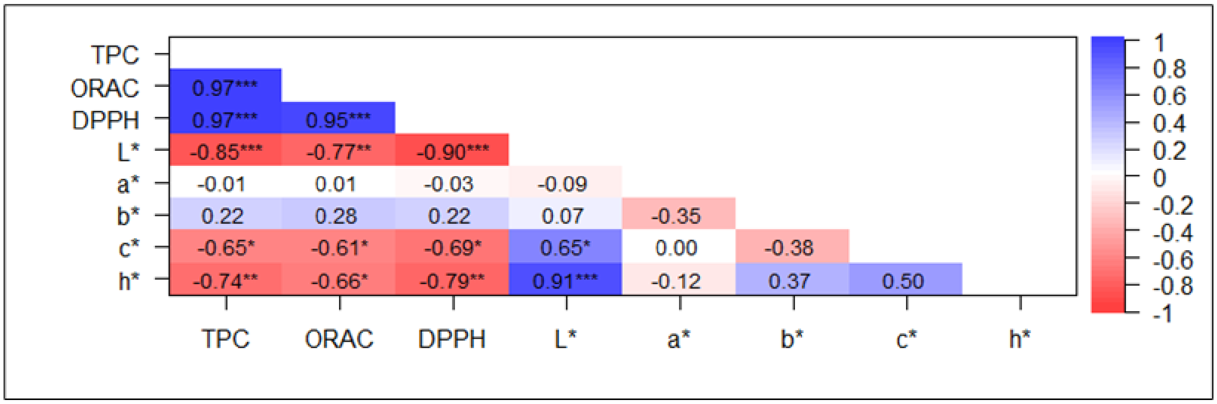
vanillic acid, (C) chlorogenic acid, (D) caffeic acid, (E) apigenin derivative, (F) quercetin, (G) cyanidin 3-O-glucoside, and (H) Sum of phenolic compounds. AWD: alternate wetting and drying, F: flooding. PA: Parral, SC: San Carlos. Data are mean values ± standard error (n = 3). Means with different letters are statistically different according to the LSD Fisher test ($p \leq 0.05$). Capital letters indicate differences among genotypes. Lowercase letters indicate differences between environments for each genotype.

Table 3. Analysis of variance of grain polyphenolic content and antioxidant capacity of three rice genotypes grown in different locations and under different water regimes.

	TPC (mg GAE 100 g^{-1})		**ORAC** (µmol TE 100 g^{-1})		**DPPH** (µmol TE 100 g^{-1})	
Genotype (G)						
Quila 279101	98.37	a	2605.63	a	510.38	a
Quila 292008	51.14	b	1343.05	b	290.87	b
Zafiro	26.05	c	728.34	c	58.92	c
Location (L)						
Parral	58.57	a	1554.18	a	260.09	a
San Carlos	58.47	a	1563.83	a	313.35	a
Water regime (W)						
Flooding	48.22	a	1511.25	a	260.87	a
AWD	68.82	a	1606.76	a	312.57	a
p-value						
G x L	0.3649		0.4454		0.7376	
G x W	0.8585		0.6144		0.1910	
L x W	0.3072		0.7362		0.3225	
G x L x W	**0.0099**		**0.0413**		**0.0314**	

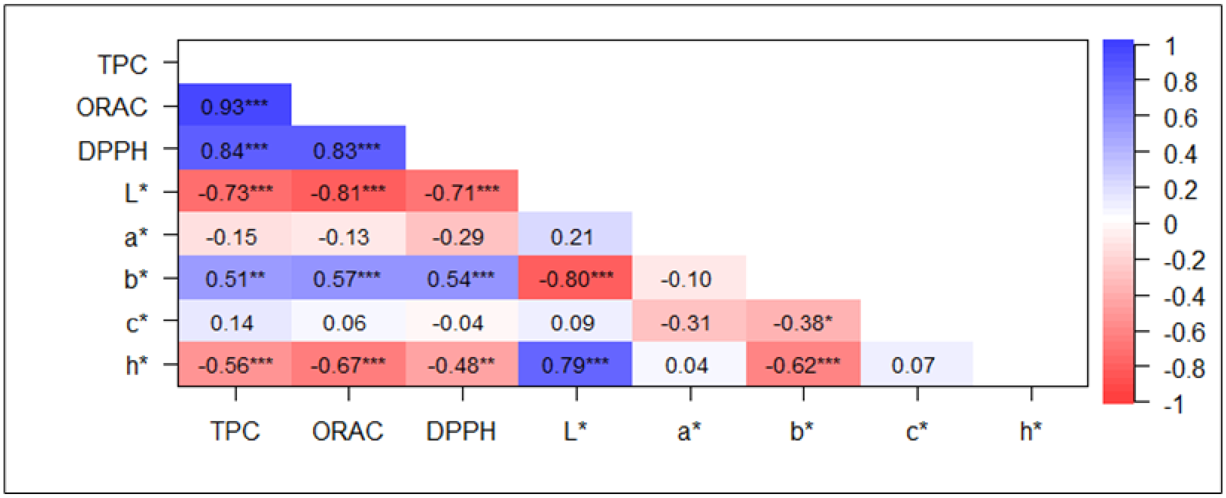
TPC: total polyphenolic content, ORAC: oxygen radical absorbance capacity assay, DPPH: 2,2-diphenyl-1-picrylhydrazyl assay, GAE: gallic acid equivalent, TE: Trolox equivalent, AWD: alternate wetting and drying. Data are mean values ± standard error (n = 3). Means with different letters are statistically different according to the LSD Fisher test. Bold p-values indicate significant interactions between factors ($p \leq 0.05$).

Correlations between antioxidant capacity and TPC of whole and polished rice grains were high and positive (Figure 2 and Figure 3), particularly in whole grains, reaching values higher than 0.95. Among the color parameters, L* showed the highest correlations with TPC, ORAC, and DPPH, ranging between −0.769 and −0.898 and between −0.705 and −0.805 in whole and polished grains, respectively. The c* and h* parameters showed moderately high correlations with antioxidant capacity and TPC in whole grains, ranging from −0.611 to −0.793; h* behaved similarly in polished grains but to a lesser extent, being in the range of −0.480 and −0.665.



	TPC	ORAC	DPPH	L*	a*	b*	c*	h*
TPC								
ORAC	0.97***							
DPPH	0.97***	0.95***						
L*	-0.85***	-0.77**	-0.90***					
a*	-0.01	0.01	-0.03	-0.09				
b*	0.22	0.28	0.22	0.07	-0.35			
c*	-0.65*	-0.61*	-0.69*	0.65*	0.00	-0.38		
h*	-0.74**	-0.66*	-0.79**	0.91***	-0.12	0.37	0.50	

Figure 2. Pearson correlation analysis between total phenolic content (TPC), antioxidant capacity (ORAC and DPPH), and color parameters (L*, a*, b*, c*, and h*) in whole rice grain. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$. L*: luminosity, a*: a measure of redness, b*: a measure of yellowness, c*: chroma, saturation, h*: hue angle. n = 12.

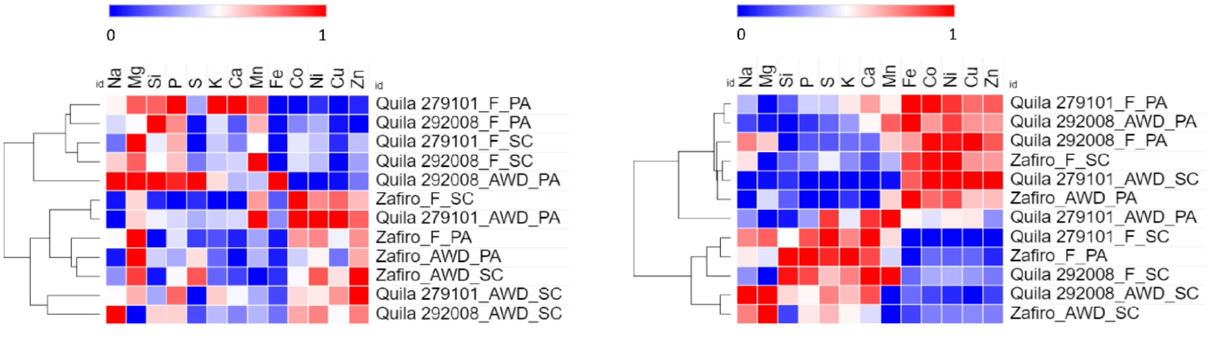


	TPC	ORAC	DPPH	L*	a*	b*	c*	h*
TPC								
ORAC	0.93***							
DPPH	0.84***	0.83***						
L*	-0.73***	-0.81***	-0.71***					
a*	-0.15	-0.13	-0.29	0.21				
b*	0.51**	0.57***	0.54***	-0.80***	-0.10			
c*	0.14	0.06	-0.04	0.09	-0.31	-0.38*		
h*	-0.56***	-0.67***	-0.48**	0.79***	0.04	-0.62***	0.07	

Figure 3. Pearson correlation analysis between total phenolic content (TPC), antioxidant capacity (ORAC and DPPH), and color parameters (L*, a*, b*, c*, and h*) in polished rice grain. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$. L*: luminosity, a*: a measure of redness, b*: a measure of yellowness, c*: chroma, saturation, h*: hue angle. n = 36.

2.3. Relative Mineral Composition

The mineral composition in processed and whole grains was subjected to a heat map and cluster analysis, and the diverse patterns observed reveal distinct element distributions among different genotypes and environments (Figure 4). Segregation based on water regime and grain type was observed in polished grains (Figure 4a). One main cluster included black rice genotypes cultivated under flooded conditions and Quila 292008-AWD-PA. All genotypes grown under AWD irrigation, except for Quila 292008-AWD-PA, and all Zafiro-INIA were included in the second cluster, regardless of irrigation regime and location, indicating differences regarding water conditions and rice types. Compared to AWD rice, flooded rice had lower relative contents of Co, Ni, Cu, and Zn, whereas levels of Mn, P, and Si tended to increase. On the other hand, the levels of relative Fe remained persistently low despite the variations observed in genotypes, water regimes, and locations. Conversely, Mg tended to reach a high relative content.



(a) (b)

Figure 4. Heat map and cluster analysis of mineral relative content in rice with different genotypes (Quila 279101, Quila 292008, and Zafiro-INIA), locations (San Carlos, SC,

and Parral, PA), and irrigation regimes (flooding, F, and alternate wetting and drying, AWD). (a) Polished grain (n = 36) and (b) whole grain (n = 12). The mineral relative content (KeV) data were normalized to the 0–1 scale for the analysis.

The first main cluster in whole grains comprised all genotypes cultivated in Parral except for Zafiro-F-PA. This cluster included Zafiro-F-SC and Quila 279101-AWD-SC. The second cluster grouped the remaining genotypes from San Carlos and Zafiro-F-PA, indicating potential environmental variations due to locations (Figure 4b). The first cluster showed increased levels of Fe, Co, Ni, Cu, and Zn compared to those of Ca, K, S, P, and Si. The second group, mostly consisting of grain from San Carlos, exhibited an opposing trend. Unlike polished grains, no separation was observed between pigmented and white grain genotypes.

Significant differences were observed in the relative contents of P, K, Ca, and Mn between Quila 279101 and Quila 292008 as well as in Zafiro-INIA. The Na content in Quila 292008 significantly differed from Zafiro-INIA, but there were no differences with respect to Quila 279101. In addition, no differences were found in terms of relative mineral contents for the growing site or water regime except for levels of Zn, as higher values were observed under AWD irrigation (Table S1).

2.4. Principal Component Analysis (PCA) of Grain Quality Traits

A PCA was developed to establish relationships between whole and polished grain quality traits with genotype, irrigation regime, and location (Figure 5 and Figure 6). In general, an apparent separation was observed between the black genotypes and the white rice cultivar and, to a greater extent, in polished grains. The separation was mainly due to significant differences in color and higher grain weight of the white rice. The PCA performed for the polished grains explained 57.7% of the variance in the data (Figure 5). All Zafiro-INIA samples clustered closely, regardless of water regime and location. This clustering was determined by TGW-Po and grain appearance traits, such as TRAN, CHA, h*, and L*, and to a lesser extent by WIDTH, a*, WG, and relative mineral content of Zn, Ni, Cu, and Co. All black rice genotypes were in the opposite quadrants of the PCA except for Quila 292008-AWD-SC. The separation of pigmented rice genotypes was associated with the concentration of phenolic compounds such as QCT, CGA, VA, Vad*, AGNd*, AGNd*, and AGNd*; biological activity (TPC, ORAC, DPPH); color parameters, i.e., b*; relative contents of K, P, Ca, and Si; and ADD.

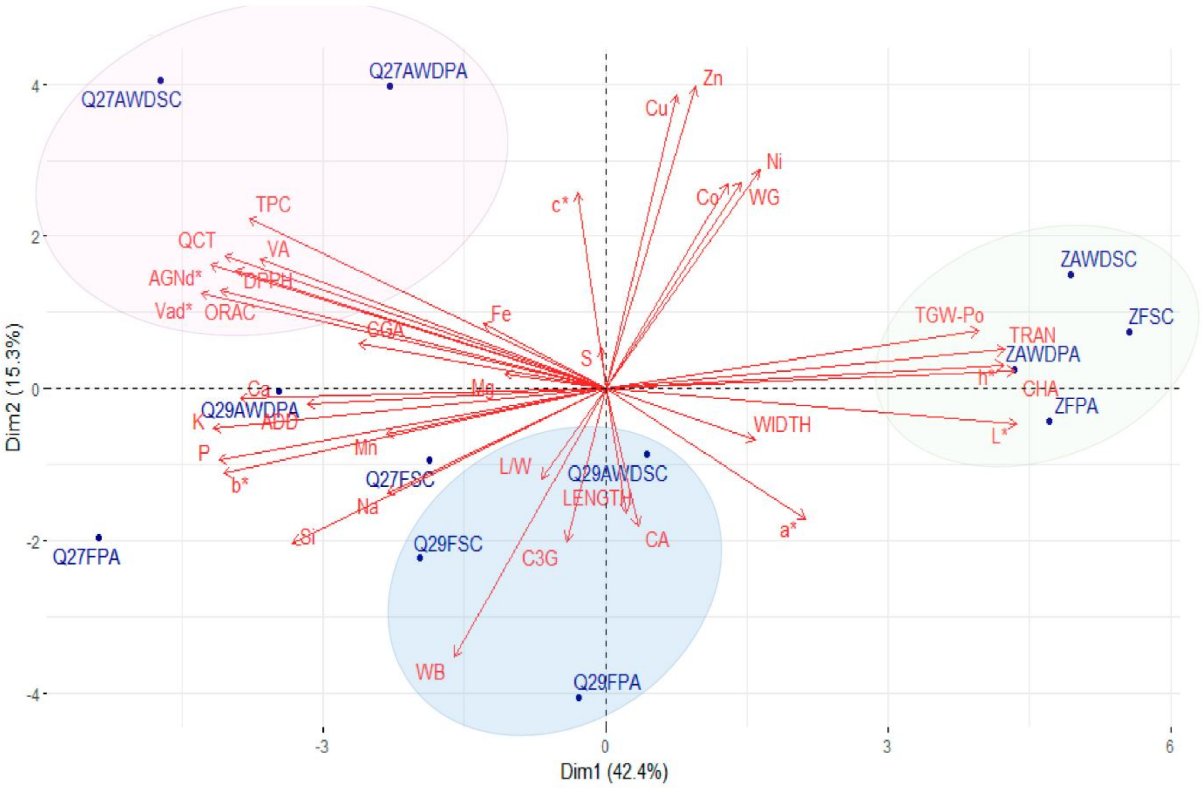


Figure 5. Principal component analysis (PCA) biplot of quality traits of polished rice grain of three genotypes (Quila 279101, Q27; Quila 292008, Q29; and Zafiro, Z) cultivated in two locations (San Carlos, SC; and Parral, PA) under two irrigation regimes (flooding, F; and alternate wetting and drying, AWD).

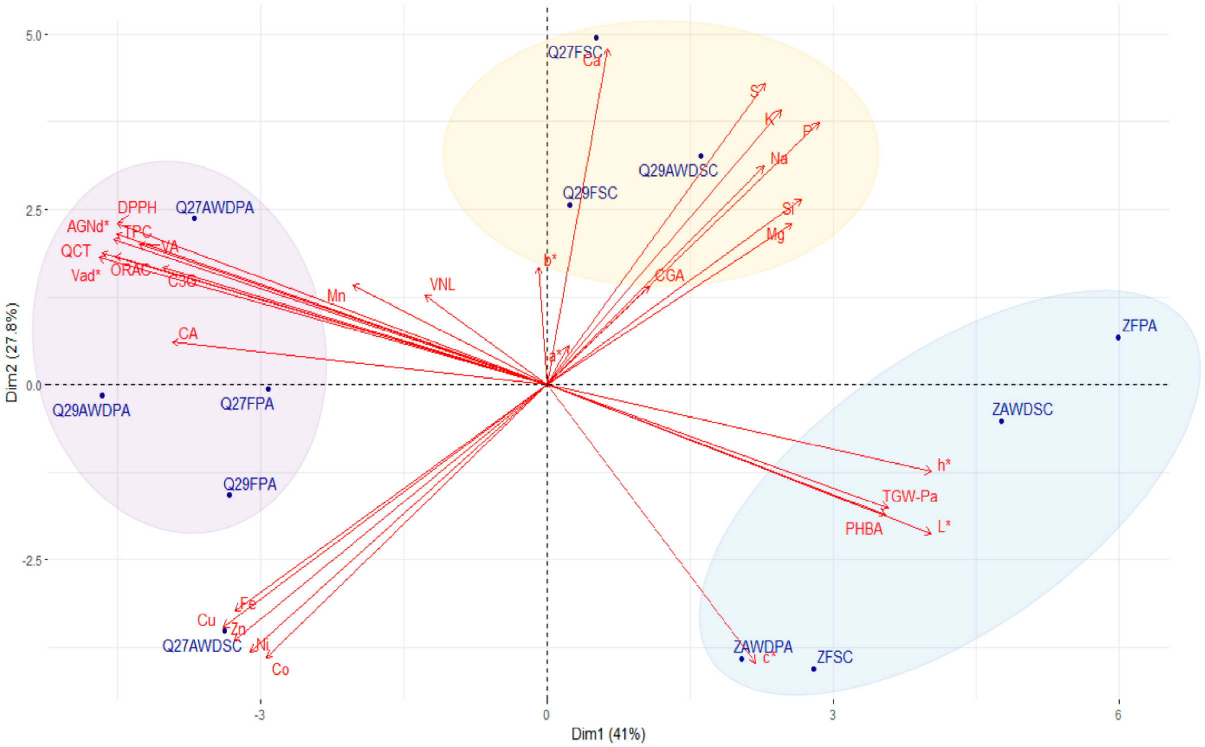


Figure 6. Principal component analysis (PCA) biplot of quality traits of whole rice grain of three genotypes (Quila 279101, Q27; Quila 292008, Q29; and Zafiro, Z) cultivated in two locations (San Carlos, SC; and Parral, PA) under two irrigation regimes (flooding, F; and alternate wetting and drying, AWD).

In contrast to white grains, pigmented grains showed a much broader distribution in the PCA, with no clear grouping by genotype, water regime, or location (Figure 5). However, Quila 279101 under AWD irrigation was separated from the rest, irrespective of the growing site, which was mainly determined by the biological activity traits TPC, DPPH, and ORAC and the concentration of metabolites such as QCT, AGNd*, Vad*, and VA. Quila 279101 grown under flooded conditions clustered in the lower quadrant. On the other hand, Quila 292008 tended to cluster, regardless of water regime and location, which was influenced by the presence of WB and, to a lesser extent, by C3G and CA concentration, LENGTH, and L/W ratio.

The PCA for whole grains explained 68.8% of the data variance of the first two components (Figure 6). The traits Vad*, QCT, AGNd*, ORAC, TPC, DPPH, VA, h*, L*, C3G, CA, TGW-Pa, PHBA, and Cu had the highest contribution to the first two components. As for polished grains, Zafiro-INIA clustered but less tightly, and interestingly, no association with water regime or locations was observed. Likewise, grain weight (TGW-Pa), p-hydroxybenzoic acid (PHBA), and color parameters h* and L* played a significant role in this clustering. Black rice genotypes exhibited a clear tendency to cluster by location, regardless of genotype and water regime, except for Q279101-AWD-SC, which was separated from the San Carlos group. This group was determined by the relative content of minerals such as Ca, S, K, Na, P, Si, and Mg. In contrast, the Parral group was associated with the concentration of phenolic compounds (QCT, VAd*, AGNd*, VA, and C3G) and biological activity (TPC, ORAC, and DPPH).

3. DISCUSSION

3.1. Appearance and Sensorial Features

Grain quality is critical for breeders, producers, and consumers [20,28]. Traditional rice quality parameters encompass aspects related to grain appearance, including weight, size (length and width), shape (length/width ratio), whiteness, and transparency [4,28,29]. On the other hand, culinary and sensory quality parameters include factors such as gelatinization temperature, gel consistency, and cooking method [4,27], which collectively influence consumer preferences [28].

There is a growing interest in black rice due to its high nutritional value and functional quality [5,6,8,10,14]. However, farmers in many countries are unwilling to grow this species because it has a lower yield potential compared to white rice [5]. This is reflected in the significant differences in grain weight between Zafiro-INIA and black rice genotypes (Table 1). In addition, rice grain formation and filling are negatively affected by water-deficit conditions [26,30] such as AWDI irrigation, resulting in decreased grain weight. The TGW-Pa values obtained under flooded and AWD conditions exceeded those reported for pigmented rice by Rungrat and Poothab [30] by 11.2% and 5.5%, respectively. Furthermore, a two-year study conducted by Wang et al. [31] evaluated two japonica rice varieties under water deficit, reporting average values for TGW-Pa ranging from 23.6 to 25.2 g and from 22.9 to 24.4 g under mild and severe drought stress, respectively. These values were notably lower than those obtained in the present study.

The value of rice is related to the percentage of whole grain, since the higher the percentage of whole grain, the higher the commercial value [4]. The white rice cultivar showed the highest whole-grain percentage. However, this did not differ from that of Quila 292008. This may be associated with the highest ADD values of these genotypes (Table 2) since gelatinization promotes the recovery of cracked grain [32]. In addition, the percentage of whole grain significantly increased under AWD irrigation. Some studies have suggested that inducing soil desiccation can promote greater carbon remobilization and root expansion, thus optimizing nutrient uptake during the grain-filling stage, ultimately resulting in increased crop yields [33,34]. These processes could favor the increase in whole-grain yield under AWD conditions.

In terms of grain dimensions, all grains obtained were classified as medium-grain rice based on L/W ratio (2.1–3.0) and as long-grain rice based on the combination grain length (≥6.0 mm) and L/W ratio (2.0–3.0) [35], regardless of genotype, location, or water regime. Grain length showed no differences among genotypes, growing sites, or water regimes. Interestingly, all interactions among factors were statistically significant except for L × W. The environment affected grain width, which decreased in the AWD condition. However, this did not result in differences in the L/W ratio, which had an effect similar to length, with all the factor combinations being significant except for L × W (Table 1). The length, width, and L/W values among genotypes are similar to those reported by Noori et al. [28] in NERICA 4 and IR28 varieties. The hull, degree of filling, and endosperm development are the primary factors determining rice grain size [36]. In this sense, the effect of water deficit on grain filling may have influenced these parameters.

Chalkiness and transparency are essential quality parameters related to the appearance of rice grains. According to FAO [35], brown rice has a chalkiness value of about 20, while well-polished rice has a value of about 40. In this study, only the chalkiness level of the processed grain was determined. A chalkiness value close to optimum (36.5) was obtained in the white rice cultivar. In contrast, values were significantly lower (<16) in the black rice genotypes (Table 2), which is related to the pigment content in the grain of these genotypes. The growing site did not affect grain chalkiness, but levels decreased under water-deficit conditions. Excessive whitening in the abdominal region of grains during the grain-filling period is reported when the crop is exposed to water-deficit conditions [37]. Another study suggests that elevated night temperatures during critical grain-filling stages may influence the increase in rice chalkiness [38]. These observations highlight the complex influence of environmental and climatic factors on rice quality.

Grain transparency is associated with the appearance of a white core within the grain. Consequently, rice can be categorized into two distinct types: chalky, characterized by grains with white cores or bellies, and translucent [39]. The morphology of their endosperm distinguishes these grains. White-bellied rice exhibits numerous globular protein bodies dispersed around the starch granules, resulting in interstitial spaces. In contrast, translucent grains have tightly packed starch granules [22,39]. Due to late plantings, white-belly production has been associated with elevated temperatures and a shorter grain-filling period [4,28]. In the present study, white rice had the most translucent grain and the lowest percentage of white-bellied grains compared to black grain genotypes. In the latter, the percentage of white belly increased significantly, especially in Quila 292008. In addition, a significant G × W interaction effect was observed since rice grains produced under flooding had more than twice the percentage of white bellies compared to those produced under AWD irrigation (Table 2). As there is a genotype–environment effect of high temperature on the activation of alpha-amylase genes in starch accumulation in the grain-filling process, the stress caused by water deficit in AWD was expected to increase the percentage of white belly [40] (Figures S1 and S2), but an opposite effect was observed. However, there is scarce information on the relationship between white-belly formation and water stress.

Gelatinization temperature (GT) is a critical indicator of rice cooking quality, as it influences both cooking time and energy expenditure [27] and significantly affects the culinary quality and palatability of rice [41]. Gelatinization occurs when starch granules absorb water and lose their crystalline structure. Starch degradation can be quantified using Graham's [42] alkaline dispersion table, which is inversely related to GT [41,43]. In this study, the black genotypes had a dispersion grade 6, corresponding to dispersed grain, neck fusion, high alkaline digestion, and low GT [43]. In contrast, Zafiro-INIA scored 5.8, being classified as fragmented grain with perfect or wide neck, intermediate alkaline digestion, and intermediate GT [43] (Table 2). In both cases, gelatinization was moderate to low [27,43]. However, it should be noted that the Zafiro-INIA grains would require a higher amount of water and a longer cooking time. These low GT values are typical of the temperate japonica rice variety [44] to which the evaluated genotypes belong. Under AWD, alkali dispersion was reduced, leading to grains with higher GT values and longer cooking times [27]. It has been reported that GT variation is explained by genotype rather than environmental factors [43,44].

3.2. Anthocyanins, Flavonols, and Other Phenolic Compounds

As expected, TPC was significantly higher in pigmented grains compared to the white rice cultivar. Similarly, whole rice showed higher concentrations of phenolic compounds than polished rice (Tables S2 and S3, Figures S3 and S4) because most phenolic compounds are concentrated in the aleurone layer of the grain and pericarp [5]. In this study, white and black rice genotypes tended to increase antioxidant concentrations in whole and polished grains in response to stress exposure. This response is particularly evident in the increased accumulation of anthocyanins [26,45], as observed in both black genotypes. Regardless of location and water regime, the average cyanidin 3-O-glucoside concentration of both black rice genotypes reached a value of 0.59 mg g^{-1} in polished rice, which was lower than that reported by Pereira-Caro et al. [11]. In whole rice, the mean concentration was 2.03 mg g^{-1}, which was approximately 1.79 times lower than that reported by Colasanto et al. [46] for black rice grains. This lower anthocyanin concentration could be determined by genotypic differences and genotype–environment interaction [5], which influences the transcription of genes encoding anthocyanidin synthase [7].

In black rice, cinnamic acid is transformed into vanillic acid through an alternative pathway [10]. In this study, vanillic acid in whole-grain black rice had a mean concentration of 0.08 mg g^{-1}, which is higher than that reported by Quan et al. [47] but lower than the values obtained by Das et al. [48].

Quercetin levels were notably higher in Quila 279101, in whole rice, averaging 0.08 mg g^{-1}. This does not agree with Tyagi et al. [21], who reported a lower value for black rice. These results indicate that different black rice varieties have unique genetic profiles, resulting in variations in the expression and content of phenolic compounds [49]. It is well-known that secondary metabolite production increases under water stress [50], which corresponds to a mechanism of plant protection and acclimation in response to stress [47,50]. The accumulation of C3G, CA, VNL, and Vad* increased by 17.6, 29.6, 16.0, and 15.7%, respectively, in the whole grain of black rice due to water deficit. Similarly, in polished black grains, there were increases in QCT, CGA, AGNd*, and Vad* of 39.7, 39.1, 29.6, and 25.7%, respectively. On the other hand, whole white rice grains exhibited a 52.1% increase in AGNd* concentration, while polished grains showed increases of 36.7% in CA and 27.5% in AGNd* under AWD conditions. The increase in these phenolic compounds may directly enhance the antioxidant capacity of the plant under water-deficit conditions. Cinnamic acid and vanillin have been noted for their potent drought-tolerance properties in rice [47].

3.3. Total Phenolic Content (TPC) and Antioxidant Capacity (ORAC and DPPH)

The TPC analysis globally quantifies the total number of specific classes of phenolic compounds in the grain. The results of the spectrophotometric analysis were consistent with the phenolic compound identification by HPLC, revealing that TPC was significantly higher in polished black rice compared to the amount of phenolic compounds in white rice. Specifically, TPC was approximately 3.8 and 2 times higher in Quila 279101 and Quila 292008 than in Zafiro-INIA, respectively. It was described that TPC is directly associated with grain color [21], which is confirmed by our results.

There was a direct correlation between the concentration of TFC and grain color, with a Quila 279101 recording the highest value of 98.37 mg GAE 100 g^{-1} DW due to its darker grains [21].

Although no statistically significant differences were observed between water regimes, there was a trend towards higher TPC under AWD irrigation. The TPC in the AWD treatment was 29.9% higher than that obtained in grains under flooding. This could be attributed to the high concentrations of phenolic compounds, such as C3G, CGA, CA, Vad*, or QCT in polished grains of black rice and CA and AGNd* in polished grains of the white rice cultivar, as mentioned above.

Under water-stress conditions, reactive oxygen species (ROS) accumulate in excess in plant cells, generating oxidative stress and causing DNA, protein, and membrane damage [51]. Hence, the increase in polyphenol content may represent an acclimation response since these compounds participate in scavenging excess ROS during water stress [23,52]. Through their hydroxyl groups, phenolic compounds can donate electrons, enabling them to engage in antioxidant activity [53,54]. In addition to their role in ROS scavenging, it has been suggested that phenolic compounds promote secondary cell wall thickening during water stress, enhancing resistance to oxidative damage [52,53] and contributing to improved plant acclimation under such conditions [9]. Black rice is rich in bioactive compounds, primarily anthocyanins such as cyanidin 3-O-glucoside, responsible for its distinctive coloration and potent antioxidant properties [5,6,8].

The ORAC and DPPH methods were used to evaluate antioxidant activity. As expected, the same effect observed in TPC was obtained with both methods. The antioxidant activity was higher in polished grains of black rice genotypes compared to that of Zafiro-INIA, ranging from 1.8 to 3.6 and 4.9 to 8.7 according to ORAC and DPPH assays, respectively. Following the same trend as TPC, the antioxidant capacity showed no statistical differences between water regimes. However, values tended to be higher under AWD irrigation compared to flooding, with increases of 5.9 and 16.5% according to ORAC and DPPH assays, respectively. This increase in antioxidant activity could be a clear response of plant acclimatization to water-deficit conditions.

A previous study in pigmented rice reported DPPH values ranging from 2670 to 5810 µmol TE 100 g^{-1} in the free fraction and from 340 to 1100 µmol TE 100 g^{-1} [54] in the conjugated fraction, which agrees with the values observed in Quila 279101. In contrast, Rocchetti et al. [55] determined an ORAC value of 33.998 µmol TE 100 g^{-1} in black rice flour, which is notably higher than the value recorded in the present study. On the other hand, ORAC values between 60 and 106 µmol TE 100 g^{-1} in the free fraction and between 207 and 534 µmol TE 100 g^{-1} in the combined free/conjugated fraction were described in white rice [56], which are lower than those observed in Zafiro-INIA.

3.4. Correlation of Total Phenolic Content (TPC), Antioxidant Capacity, and Color Parameters in Whole-Grain and Polished Rice

The grains of the evaluated genotypes varied in color, being dark purple in Quila 279101, purple in Quila 292008, and white in Zafiro-INIA. These variations in grain color, whether whole or polished, are associated with phenolic content and antioxidant activity. Polished rice is relatively light in color because part of the bran, which contains phenolic compounds of high antioxidant activity, is removed in processing [6,21].

Color parameters, including lightness (L*), redness (a*), and yellowness (b*), serve as robust indicators of the bioactive components in pigmented rice [14]. In this study, significant positive correlations between phenolic content and antioxidant capacity were observed in both polished and whole grains (Figure 2 and Figure 3), which is consistent with the findings reported by Shen et al. [57]. These traits showed a negative correlation with L* and h* in both polished and whole grains, coinciding with the results reported by Shao et al. [13] for insoluble-free, soluble-conjugated, and insoluble-bound fractions. The h* value, which considers both a* and b* values, was initially considered a potentially better indicator of color than a single-color attribute like L* [58]. However, our findings revealed a strong association between TPC, ORAC, and DPPH values and L*. Shen et al. [57] noted a negative correlation of TPC and antioxidant capacity with b* and c*. We detected a similar relationship for c* in whole grains. However, a moderately positive correlation of TPC and antioxidant capacity with b* was found in polished grains. The color and tone of rice grain pericarp are associated with TPC [57,58].

3.5. Relative Mineral Composition

The distribution of elements in rice grain occurs from the outer to the inner layer, i.e., husk > bran > whole rice > polished rice [59]. No clear separation was observed between pigmented and white genotypes in whole grains, suggesting no significant differences in element content between the different types of grains. However, Chen et al. [60] reported that black rice had higher concentrations of Mg, Ca, Fe, Mn, and Cu compared to white rice. Under stress conditions, specific enzymes involved in free radical scavenging, which use Fe, Zn, or Cu as electron acceptors in antioxidant processes, are activated [61]. This activation could enhance the uptake of these elements [60]. Furthermore, drought stress reduces the uptake of elements such as K, Ca, Mg, Mn, and B [62]. Similarly, rice exposed to salt stress conditions shows an increase in Fe and Zn and a decrease in Ca levels, as reported by Zhang et al. [59]. In rice, the high variability of grain Fe content associated with the degree of milling, genotypes, growing conditions, and fertilization was reported [63].

In polished grains, AWD irrigation increased the relative content of Co, Ni, Cu, and Zn while causing a decrease in Mn, P, and Si levels. Aerobic conditions enhance soil Zn availability [64], potentially explaining the elevated Zn content observed under AWD conditions. In addition, it should be noted that Zn content was linked to Cu assimilation [60], and Cu was associated with mitigating the effects of drought stress [62]. Interestingly, despite the decrease in Si levels observed in polished grains under water-deficient conditions, it is widely accepted that Si plays an essential role in enhancing drought resistance in crops, including rice [63].

Soil nutrients directly affect plant mineral content [38], and their uptake depends on genotype, environmental conditions, and agronomic practices [64]. The few differences observed in the relative mineral content suggest an effect of the genotype rather than of the environment.

3.6. Principal Component Analyses

In black genotypes, clustering was most evident in whole grains, tending to separate according to growing site. The analysis indicates that the environment has a significant impact on grain quality. This is further reinforced by the fact that phenolic compounds and their biological activity mainly characterize the Parral group. At the same time, the mineral composition of Mg, Si, Na, P, K, and Ca largely determines the San Carlos group.

Polished grains exhibited a trend towards genotype clustering, particularly in Quila 292008, primarily attributed to its significantly higher percentage of white belly. On the other hand, Quila 279101-AWD from both cultivation sites were grouped due to their correlation with the concentrations of phenolic compounds, including QCT, AGNd*, Vad*, and VA, along with the TPC and antioxidant activity. The b* color parameter in black genotypes indicated high bioactive compound concentration [14] in polished grain. Meanwhile, the Zafiro-INIA cultivar was consistently associated with transparency and chalkiness, which in turn are related to grain color [4,28,29]. The clear differences between genotypes are mainly responsible for the variations in the content of bioactive compounds within the grains. In addition, metabolite and mineral contents and other grain quality characteristics depend on a combination of genetic factors and environmental conditions [10,24].

4. MATERIALS AND METHODS

4.1. Plant Material, Trial Design, and Crop Management

Two black rice genotypes, advanced lines Quila 279101 and Quila 292008, from the RBP of INIA, which were selected based on their performance in a preliminary study with 286 lines grown under water-deficit conditions, and the white rice cultivar Zafiro-INIA were evaluated (Figure 7). The latter represents approximately 70% of the cultivated area of rice in Chile. The trials were established during 2021 in the experimental rice fields of INIA located in San Carlos (36°25′49″ S, 72°0′25″ W; 161 m.a.s.l.) and in Parral (36°04′37″ S; 72°00′13″ O; 142 m.a.s.l.) in the Ñuble and Maule regions, respectively. The germplasm was evaluated under traditional cultivation (flooding) and alternating wetting and drying (AWD) irrigation (Figure 8). The trials were established in the first week of October in both locations. In San Carlos, the length of the growing cycle was 193 (on average) and 186 days under AWD and flooding, respectively. In Parral, AWD and flooding had an average cycle of 178 days.

Figure 7. Rice grain appearance of the black rice genotype Quila 292008 (top) and the white rice cultivar Zafiro-INIA (bottom). On the left, paddy rice; in the center, whole grain; and on the right, polished grain.

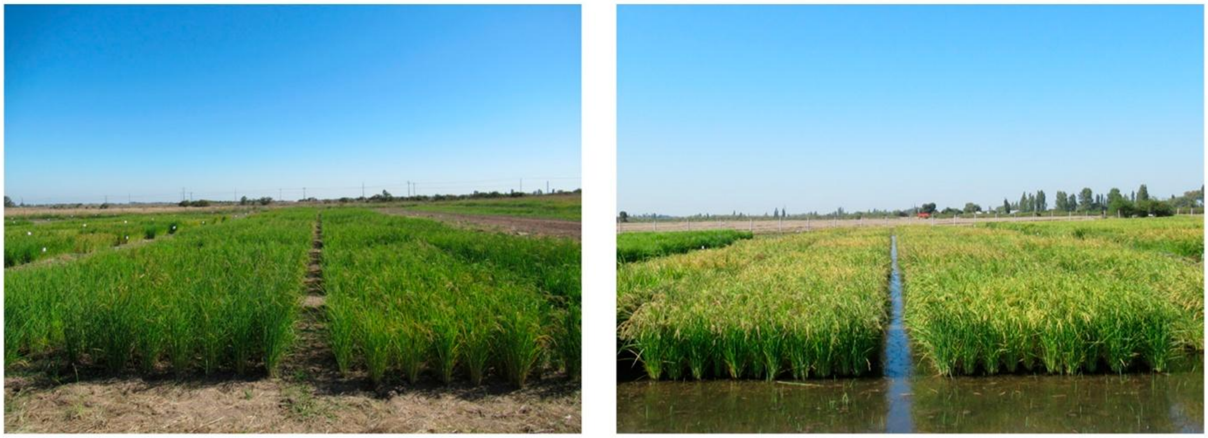


Figure 8. Rice trials in Parral in the 2021 season using different irrigation regimes. (Left): alternate wetting and drying, and (Right): flooding.

The genotypes were arranged in a complete randomized block design with three replicates. Each plot was composed of seven rows. The plot was 3 m long and 2.1 m wide, with a row spacing of 0.3 m. Before the establishment of the trials, conventional soil management was conducted, including chemical fallowing using glyphosate (2 L ha^{-1}), leveling, and soil preparation. Soil was prepared during the dry season to eliminate weeds and improve quality. A shallow plowing and a transverse harrowing were carried out. Contour lines with a height difference of 10 cm were then drawn to form the parapets [3].

The base application of NPK fertilizer was conducted using urea (CH_4N_2O) 22 Kg ha^{-1}, triple superphosphate ($Ca(H_2PO_4)2 \cdot H_2O$) 130 Kg ha^{-1}, and muriate of potassium (KCl: NaCl) 150 Kg ha^{-1}. Irrigation was initiated one day after sowing, and subsequent irrigation was adjusted based on plant emergence and the prescribed water regime. When plants reached 2.5 leaves and until grain filling was complete, plants under traditional growing conditions remained flooded throughout the growing cycle, while plants in the AWD trial were irrigated every seven days at field capacity. Nitrogen was applied in three more doses: 66 Kg ha^{-1} of urea at 2.5 leaves, 88 Kg ha^{-1} at tillering, and 44 Kg ha^{-1} at panicle initiation. Weed control was performed using Ricer® (210 cc ha^{-1}) and Clincher® (1.5 L ha^{-1}) to control narrow-leafed weeds and Bentax (2.5 L ha^{-1}) and MCPA (1 L ha^{-1}) to control wide-leafed weeds.

4.2. Appearance and Sensorial Quality Traits

The percentage of polished whole grain (WG) was obtained from one hundred clean grains using a test mill (MT, Suzuki Co., Shizuoka, Japan). The weights of one thousand grains of polished rice (TGW-Po) and of paddy rice (TGW-Pa) were determined using an analytical balance. The dimensions of polished grains (length and width) were obtained from grain images with GrainScan 1.0.4 software (CSIRO, Canberra, Australia); subsequently, the length/width ratio (L/W) was calculated. Chalkiness and transparency were assessed with an MM1D milling meter (Satake, Hiroshima, Japan). The percentage of white belly (WB) was calculated as the sum of the values corresponding to grades 1 to 5, which provided the overall percentage of white belly in the sample. The mean degree of dispersion (GDD) was determined from ten representative whole grains incubated for 23 h at 30 °C in 1.7% KOH solution and a subsequent measurement of the gelatinization temperature.

4.3. Nutritional Grain Quality Traits

For identification and quantification of anthocyanins and phenolic compounds, as well as for the evaluation of antioxidant capacity, three biological samples (replicates) of each genotype, location, and water condition were used for polished grains, while a composite sample of the three replicates of each genotype, location, and water condition was used for whole grains. In all cases, three technical replicates were evaluated.

4.4. Identification and Quantification of Anthocyanins, Flavonols, and Other Phenolic Compounds

Whole and polished grains were ground using an IKA A10 mill (IKA®-Werke GmbH & CO., Staufen im Breisgau, Germany). Chemical extraction was performed according to López-Belchí [65] with some modifications. Samples of 0.5 g of the obtained rice powder, 430 nm particle size, were used for metabolite extraction with a 25:24:1 organic solvent mixture (methanol: water: formic acid). Sample mixtures were placed in ultrasound for 1 h, kept at −20 °C overnight, and then ultrasonicated for 1 h.

The resulting extracts were centrifuged (12,000 rpm, 5 min), and the supernatant was collected, filtered through a 0.22 μm PVDF membrane (Millex V13, Millipore, Burlington, MA, USA), and stored at −20 °C until use. Anthocyanins, flavonols, and other phenolic compounds were identified and quantified by chromatography using a Hitachi primaide HPLC-DAD equipped with a diode array detector (Hitachi technologies, Merck, Darmstadt, Germany). The chromatographic system was fitted with a Kromasil C18 column (250 × 4.6 mm, particle size 5 μm) (Nouryon AB., Göteborg, Sweden) to separate metabolites. Chromatographic separation was performed with 1% formic acid and acetonitrile as mobile phases and a flow rate of 1 mL min^{-1} [66]. Chromatograms were recorded at 280, 320, 360, and 520 nm. Standards were used to quantify vanillin, vanillic acid, and p-hydroxybenzoic acid at 280 nm; chlorogenic acid, caffeic acid, and coumaric acid at 320 nm; quercetin hydrate and quercetin 3-O-glucoside at 360 nm; and cyanidin 3-O-glucoside at 520 nm (Sigma-Aldrich, St. Louis, MO, USA). All solvents used in the extractions were of analytical grade and were obtained from Merck (Darmstadt, Germany). Analyses were performed in triplicate, and the results were expressed as mg g^{-1} DW.

4.5. Total Phenolic Content (TPC)

TPC was determined using the Folin–Ciocalteu assay with modifications for microscale adaptation as described by Gu et al. [67]. Amounts of 25 µL of 0.5 N Folin–Ciocalteu reagent; 25 µL of the sample, blank or standard; and 200 µL of distilled water were added to each well of the microplate. The plate was then shaken for 30 s and incubated for 5 min at 25 °C in the dark. Finally, 25 µL of 10% Na2CO3 was added, and the absorbance of the samples was measured at 765 nm in a Synergy H1 multimodal hybrid microplate reader (Biotek, Winooski, VT, USA). Results were expressed as mg gallic acid equivalents per 100 g of sample (mg GAE 100 g^{-1} sample).

4.6. Antioxidant Activity

The antioxidant capacity of the extracts was evaluated with DPPH (2,2-diphenyl-1-picrylhydrazyl) and ORAC (oxygen radical absorbance capacity) assays. The chemicals, including Trolox, fluorescein, and 2,2-azobis-2-amidinopropane dihydrochloride (AAPH), were from Sigma-Aldrich (Sigma-Aldrich, USA). In the first method, the antioxidant activity was determined by measuring the scavenging of the DPPH radical. For this purpose, 25 µL of the extract was mixed with 200 µL of a DPPH solution and incubated in darkness for 1 h. Subsequently, the absorbance was recorded at 515 nm, and the results were expressed as µmol Trolox equivalents per 100 g of rice powder weight (µmol TE 100 g^{-1}) [67,68].

The ORAC assay was adapted to the microscale according to the method described by Noriega et al. [66] with modifications. Black 96-microwell plates (Nunc, Roskilde, Denmark) and a Synergy H1 multimodal hybrid microplate reader (BioteK, Winooski, VT, USA) were used. In the first microplate columns, 25 µL of Trolox standard was added at 70, 60, 40, 20, 10, and 5 µM. In the following columns of the microplate, 25 µL of sample diluted in 75 mM phosphate buffer pH 7.4 and 25 µL of blank were added. An amount of 150 µL of fluorescein solution was added to each well, and the plate was incubated at 37 °C for 30 min. Then, 25 µL of AAPH was added to each well. Fluorescence reading was performed every 1 min for 60 min with excitation and emission wavelengths of 485 and 520 nm at 37 °C. The calculation of the antioxidant capacity was obtained by the difference of the area under the curve (AUC) between the sample and the blank. Three replicates per Trolox standard, sample, and blank were performed, and the results were expressed as µmol Trolox 100 g^{-1} dry weight (µmol TE 100 g^{-1}) [65].

4.7. Grain Color

The grains were placed in a Petri dish, and color parameters CIEL*a* b*, C* (color saturation), and H (hue angle, $\tan^{-1}$ (b/a)) of grains were measured with a PCECSM 2 colorimeter (Hunter Lab®, Murnau am Staffelsee, Germany). Three measurements were taken for each sample.

4.8. Relative Mineral Composition of Grain

The method of Cardoso et al. [69] with modifications was used. Spectral signatures of elements in rice grain samples were obtained using a micro-X-ray fluorescence spectrometer (µ-XRF) (M4 Tornado PlusTM, Bruker, Bremen, Germany). A total of 48 rice samples (12 whole grains and 36 polished grains) were analyzed, with six technical replicates per sample. Milled and sieved grain samples were placed in 96-well flat-bottom microplates. A stainless-steel rod was used to compact the sample in the well. For the measurements, the X-ray was set at 50 kV and 30 µA with a spot size of 160 µm and an exposition time of 20 ms per spot. Photons were collected with an energy-dispersive silicon drift detector and under atmospheric pressure. Data were expressed as kiloelectronvolts (KeV). The multipoint measurement tool in the Bruker EspritTM 1.2 software was used for measurements and data processing.

4.9. Statistical Analysis

The statistical analysis of grain quality traits was performed by analysis of variance (ANOVA), in which genotypes, location, and water regime were considered fixed factors, and replications were random factors. The least significant difference (LSD) test with a 95% confidence level was used to compare means. Correlation analysis and principal component analysis (PCA) were also performed. These analyses were accomplished in Rstudio version 4.2.1 [70]. The relative mineral composition of the grains was normalized to the 0–1 scale for heat map analysis. Hierarchical clustering was implemented using the Euclidean distance method. The analysis was performed using the Morpheus tool (https://software.broadinstitute.org/morpheus/ (accessed on 12 September 2023)).

5. CONCLUSION

This study evaluated the effect of genotype, location, and water regimen on rice quality traits. The genotypes under study behaved differently. The commercial white rice cultivar exhibited significantly higher grain weight, chalkiness, and transparency than the black rice genotypes and had a higher whole-grain yield. On the other hand, the black rice genotypes showed a higher percentage of white belly. The phenolic compound profiles were markedly different, with cyanidin-3-O-glucoside predominating in the pigmented grain and chlorogenic acid in the white grain. In addition, black rice grains showed a significantly higher concentration of phenolic compounds, which in turn resulted in a significantly higher antioxidant capacity.

Water regimen and location influenced the grain quality of rice. Under flooded conditions, heavier grains were obtained, with a significantly higher presence of white belly. Although several grain quality traits were negatively affected by AWD irrigation, the concentration of phenolic compounds tended to increase under this water regimen, probably due to plant acclimatization to water stress. However, no significant differences were found in total phenolic content or antioxidant activity. Furthermore, there were differences in grain relative mineral content due to water regime, especially in polished grains. Higher grain weight was obtained in Parral, where the concentration of phenolic compounds showed an upward tendency. Nevertheless, there were no differences in total phenolic content or antioxidant activity between growing sites. In addition, higher whole-grain yields were obtained in San Carlos, while the relative mineral content also varied between locations.

As expected, black rice genotypes showed marked differences in phenolic content and antioxidant activity. However, no differences were observed in total phenolic content or antioxidant activity due to water regime or location, suggesting that the production and accumulation of these compounds in the grain are strongly genetically determined. This study provides insights into the relationship between genotype, growing location, and water regime on rice quality. Nevertheless, further research is needed to understand the environmental impact on rice grain quality.

Supplementary Materials

The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/plants12244091/s1, Table S1: Analysis of variance of grain yield of three rice genotypes grown in different locations and under different water regimes. Table S2: Mineral concentration in polished rice of three genotypes (Quila 279101, Quila 292008, and Zafiro-INIA) grown in two locations (San Carlos and Parral) and under two water regimes (flooding and AWD). Table S3: Concentration of phenolic compounds (mg g^{-1}) in extracts of whole-grain composite samples of three rice genotypes (Quila 279101, Quila 292008, and Zafiro-INIA) grown in two locations (San Carlos and Parral) and under two water regimes (flooding and alternate wetting and drying). Table S4: Concentration of phenolic compounds in polished grain extracts of three rice genotypes (Quila 279101, Quila 292008, and Zafiro-INIA) grown in two locations (San Carlos and Parral) and under two water regimes (flooding and alternate wetting and drying). Figure S1: Agrometeorological data of air temperature (maximum, minimum, and average) and accumulated rainfall recorded in San Carlos in the 2020–2021 season. Figure S2: Agrometeorological data of air temperature (maximum, minimum, and average) and accumulated rainfall recorded in Parral in the 2020–2021 season. Figure S3: Chromatogram of a polished grain sample of the Quila 279101 genotype measured in HPLC at 320 nm. Figure S4: Chromatogram of a whole-grain sample of the cultivar Zafiro-INIA measured in HPLC at 320 nm.

Author Contributions

Conceptualization, M.D.L.-B., K.C.-L., S.F. and M.G.; methodology, M.D.L.-B., K.C.-L. and M.G.; software, A.B.-L., F.N., R.A.C. and P.C.; validation, M.D.L.-B.; formal analysis, A.B.-L., F.N., P.C. and M.G.; investigation, A.B.-L. and M.G.; resources, M.D.L.-B., K.C.-L., M.G. and R.A.C.; data curation, A.B.-L., K.C.-L. and P.C.; writing—original draft preparation, A.B.-L. and M.G.; writing—review and editing, A.B.-L., M.D.L.-B., F.N., R.A.C., S.F. and M.G.; visualization, A.B.-L., M.D.L.-B. and M.G.; supervision, M.D.L.-B. and M.G.; project administration, A.B.-L., M.D.L.-B. and M.G.; funding acquisition, K.C.-L., M.G. and R.A.C. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement

All data were generated during the study. They are not publicly available but can be accessed through the following GoogleDrive link: https://docs.google.com/spreadsheets/d/17nidj8qAv5MNQJHvrhau-Y92K0IFriod/edit?usp=sharing&ouid=108551625874148646272&rtpof=true&sd=true (accessed on 5 April 2023).

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Conflicts of Interest

The authors declare no conflict of interest.

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IV. CAPÍTULO III: EFFECT OF DIGESTION ON RICE PROTEIN AND POLYPHENOL BIOAVAILABILITY AND THEIR POTENTIAL AGAINST FREE RADICALS AND SENESCENCE IN A CELL MODEL.

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ABSTRACT

Black rice (*Oryza sativa* L.) has attracted growing attention due to its resistant starch content and phytochemical profile, particularly its abundance of anthocyanins, compounds recognized for their role in regulating glucose metabolism and antioxidant benefits. This study compared protein digestion and antioxidant activity of black and white rice. The release of free amino groups during digestion was quantified, with greater release observed in black rice at all phases. Exclusive peptides and proteins with differences in coverage and intensity were identified, with RA05_ORYSJ standing out for its functional profile. No cytotoxicity was observed in intestinal Caco-2 and neuronal SH-SY5Y cells, under induced oxidative stress. Black rice genotypes significantly reduced (>50%) reactive oxygen species production. In addition, black rice decreased β-galactosidase activity by more than 65%, suggesting a protective effect against cellular senescence. These results underscore the functional potential of black rice as both an antioxidant and a cell protector, demonstrating its capacity to promote human health and well-being while supporting its inclusion in a nutritionally balanced diet.

Keywords: bioactive compound, functional food, phenolic compound, antioxidant activity, digestion, cell culture.

1. INTRODUCTION

Rice (*Oryza sativa* L.) is a significant source of carbohydrates for a large portion of the global population, particularly in Asia[1]. Its whole grain composition includes a bran layer (6-7% of its total weight), germ (2-3%), and starchy endosperm (90-91%)[2,3]. However, the predominant form consumed is white polished rice[4,1]. The refining process, which removes the bran and germ, significantly reduces essential nutrient and fiber content, compared to unpolished white rice or pigmented rice varieties[4,3].

Comparative studies show that pigmented rice varieties offer superior nutritional quality to white rice, due to the retention of their bran layer rich in phytochemicals, which is preserved by consuming them in the whole-grain (unpolished) form. This layer is rich in fiber and bioactive compounds, which significantly contribute to dietary intake of these essential components[5,6,1]. However, a significant portion of phenolics compounds is bound to dietary fiber, which limits their bioavailability[7]. The bioaccessibility of these compounds is a crucial initial step for their bioavailability, and it is influenced by factors such as particle size, which can affect both enzymatic digestion and release of bioactive compounds[8,7]. *In vitro* gastrointestinal digestion methods have proven to be effective tools for monitoring the release of compounds such as free amino acids, thereby enabling a more accurate assessment of the nutritional potential of foods under simulated physiological conditions[9,8]. Despite this recognized high nutritional value and efforts to promote pigmented rice consumption, broader consumer preferences remain heavily skewed toward white polished rice, which offers a significantly lower nutritional profile[10,11,12].

Among pigmented rice varieties, black rice has attracted considerable attention due to its high content of bioactive compounds and associated health benefits[2,13,11,14]. Unlike white rice, black rice is characterized by dark purple pericarp due to a higher concentration of anthocyanins[4,6]. Cyanidin-3-*O*-glucoside is the predominant anthocyanin in black rice, followed by peonidin-3-*O*-glucoside, constituting more than 90% of the total anthocyanin content[13,15,16]. However, anthocyanins are highly sensitive to environmental factors such as heat, light, pH, and metal ions, and thermal processing has been shown to reduce phenolic content and antioxidant capacity in food matrices[17,6,15]. Interestingly, studies have demonstrated that microwave cooking with shorter durations enables complete water absorption in black rice, thereby preserving higher levels of bioactive compounds and antioxidant activity[18,15]. Considering the thermolability of anthocyanins and their antioxidant relevance, understanding how different processing methods affect their retention is critical. This becomes especially relevant when evaluating functional properties such as oxidative stress modulation in cellular systems.

Beyond their role as natural pigments, anthocyanins and phenolic acids concentrated in black rice bran have been recognized for their antioxidant activity. These compounds can neutralize reactive oxygen species (ROS), thereby contributing to redox homeostasis and reducing the risk of oxidative stress-related damage [19,2,20,21,22]. An imbalance in the redox state induces oxidative stress, which in turn contributes to cellular damage and the development of chronic degenerative diseases. In this context, pigmented rice extract has demonstrated a strong anti-aging function by attenuating oxidative stress, improving cell viability, and inhibiting lipid peroxidation[23]. One of the key indicators of cellular aging is β-galactosidase activity, which is widely used to assess senescence-associated processes in response to oxidative damage, as evidenced by its increased expression[24]. Therefore, the assessment of ROS levels and β-galactosidase activity is a key indicator for determining oxidative damage and senescence in cellular systems[25,24,26].

This study aims to evaluate the functional properties of different black rice genotypes, specifically the free amino acid release during grain digestion, and their effects on cellular viability, ROS scavenging, and cellular senescence. These findings contribute to the comprehensive characterization of black rice genotypes and support the development of new black rice cultivars with improved nutraceutical attributes, thereby facilitating broader consumer acceptance.

2. MATERIALS AND METHODS

2.1. Plant Materials and Field Trial

Three rice genotypes from the Rice Breeding Program (RBP) of the National Institute of Agricultural Research (INIA, Chile) were evaluated: ‘Onix’, a black rice cultivar; ‘Quila 292008’, an advanced black rice experimental line; and ‘Zafiro’, a white rice cultivar that accounts for approximately 70% of the rice cultivated in Chile and was included for comparison purposes.

The field trial was conducted at the INIA experimental rice station in San Carlos (36°25′49″ S, 72°0′25″ W; 161 m.a.s.l.), located in Ñuble, Chile, under non-flooded conditions. A completely randomized complete block design with three replicates was implemented. Each experimental plot comprised seven rows, each 3 meters in length and spaced 0.3 meters apart, resulting in a total plot area of 6.3 m^2. Each plot was harvested at maturity (14% grain humidity percentage).

2.2. Rice Milling and Grain Fractionation

Two hundred grams of grains from each genotype were milled using a Chopin CD1 experimental mill (Chopin Technologies, France). The milling process involved two passes through three fixed slotted cylinders and particle separation using a centrifugal rotary sieve. Flour collected from the first hopper (right side; see Fig. S1, S2) using a 160 µm sieve was used for analysis (first flour). For raw whole flour, 20 g of grains were ground in a Retschs ZM1 ultracentrifugal mill (Retsch, GmbH, Haan, Germany) equipped with a 250 µm sieve. Flours were stored in airtight containers at -20 °C until use, while whole rice grains intended for cooking were kept unground under the same storage conditions.

2.3. Microwave Cooking Treatment in Rice

To assess rice digestibility, 5 g samples of whole rice grains from the 'Onix' and 'Zafiro' genotypes were weighed in triplicate for cooking. Each sample was cooked in a Balay 3WGB2018 microwave oven (Balay, Spain) at 600 W. Optimal cooking times were 9 min for 'Onix' black rice and 10 min for 'Zafiro' white rice (see Table S1).

For the cooking process, the 'Onix' and 'Zafiro' genotypes were selected (Table 1). This selection was based on a previous study[27], which reported that the extract of whole grain 'Onix' exhibited a high polyphenol content of 98.37 mg GAE 100 g^{-1}. Once cooked, samples of the two genotypes were used to evaluate the release of amino acids through the digestive phase of cooked rice and for peptidomic analysis.

Table 1. Characterization and evaluations performed on the rice genotypes used in this study.

Evaluation	Genotypes	Sample format
Amino Acid Release Across Digestive Phases in Cooked Rice	'Onix', and 'Zafiro'	Whole grain cooked
Cell viability or cytotoxicity (MTT)	'Onix', and 'Zafiro'	Freeze-dried First flour
Reactive oxygen species (ROS)	'Onix', and 'Quila 282008'	Freeze-dried First flour

Senescence model by β-galactosidase activity	'Onix', and 'Quila 282008'	Freeze-dried First flour
Peptidomics	'Onix', and 'Zafiro'	Whole grain cooked

2.4. *Static in Vitro Digestion*

To evaluate the behavior of cooked rice after ingestion and assess the bioaccessibility of nutrients, peptides, and bioactive compounds, an *in vitro* static gastrointestinal digestion was performed following the INFOGEST protocol[28], with specific modifications. Digestion was carried out under the following pH conditions: oral phase, pH 7; gastric phase, pH 3; and intestinal phase, pH 7 (Tables S2, S3, and S4).

One gram of each cooked black and white rice sample was weighed in duplicate. Separate tubes were used for each sampling time. Enzymatic treatments included salivary amylase (replaced with 1 mL of human saliva), pepsin (2000 $U \cdot mL^{-1}$), pancreatin (100 $U \cdot mL^{-1}$), and bile salts (10 mM). A protein- and fat-free cookie was used as a digestion control. After completion of the oral, gastric, and intestinal phases, samples were centrifuged at 5000 × g for 20 min. Aliquots of 1 mL from each digestion phase were collected and lyophilized. The remaining pellets and digests were frozen in liquid nitrogen and stored at –20 °C for further analysis.

2.5. *Amino Acid Release Across Digestive Phases in Cooked Rice*

Protein digestibility and free amino acid concentrations in the supernatant were quantified using the *O*-phthaldialdehyde (OPA) assay. Lyophilized digestion samples were resuspended in 0.1 M sodium tetraborate ($Na_2B_4O_7$) solution. For each well, 8 µL of sample, blank, or standard was added in duplicate, followed by 232 µL of OPA reagent. Microplates were incubated in the dark at 30 °C for 10 min. A glutamic acid standard curve (0.25 – 8 mM) was prepared for quantification. Absorbance was measured at 340 nm using a microplate reader. Results were expressed as micromoles of glutamic acid equivalents (GluAE) per gram of cookie or white rice.

After each digestion phase (oral, gastric, and intestinal), the remaining pellets and digests were lyophilized to obtain a stable powder. The weight of the freeze-dried samples was recorded accurately; these powders consisted of the rice matrix combined with the non-volatile components of the digestion fluids (enzymes and salts).

2.6. Extraction and Quantification of Anthocyanins

2.6.1. Extraction of Anthocyanins from Black Rice Samples

Anthocyanin extraction was carried out on all rice ‘Onix’ samples (raw whole flour, cooked whole grain, and freeze-dried *in vitro* samples). For each sample, 50 mg was weighed, and 2 mL of extraction solution (methanol: water (50:50) with 1% formic acid) was used. When necessary, the extraction volume was adjusted proportionally to maintain a concentration of 25 mg mL^{-1}. Samples were then vortexed for 30 min, sonicated for 15 min, and then centrifuged at 10,000 rpm and 4°C for 10 min. The supernatant was collected into a fresh 2 mL Eppendorf tube. The pellet was extracted two additional times with 500 µL of extraction solution each. The combined supernatants were centrifuged, and 1.5 mL was transferred to amber vials for HPLC analysis.

2.6.2. Identification and Quantification of Anthocyanins

Anthocyanins were identified and quantified using a modified HPLC method[29,30]. Analysis was performed on an Agilent 1200 HPLC system (Agilent Technologies, Santa Clara, CA, USA) equipped with a quaternary pump (G1311A) and a diode array detector (Agilent G1315B) for UV-VIS detection. Separation was performed on a Phenomenex Luna C18 column (5 µm, 4.6 mm x 150 mm) operated at 25°C. The mobile phase consisted of water/formic acid (99.9:0.1%, v/v) (A) and acetonitrile/formic acid (99.9: 0.1%, v/v) (B), using an elution gradient: 90% A/10% B (0-30 min), 70% A/30% B (30-35 min), 65% A/35% B (35-45 min), 60% A/40% B (45-50 min), and 90% A/10% B (50-60 min). The flow rate was maintained at 0.6 mL min^{-1}, and the injection volume was 5 µL for both samples and standards. Quantification was performed by interpolation using a calibration curve of cyanidin 3-*O*-glucoside (Sygma Chemical Co, St. Louis, MO, USA). Results were expressed as mg per 100 g^{-1} of sample. For the digestion phases, results were expressed as mg 100 g^{-1} of freeze-dried solid to account for variability in mass recovery at each stage of digestion.

2.7. Peptidomics

Aliquots of freeze-dried intestinal digestion samples from black and white rice genotypes (‘Onix’ and ‘Zafiro’) were used for peptidomic analysis. Each sample was weighed, and 0.2% trichloroacetic acid (TCA) was added to reach a final concentration of 25 µg $µL^{-1}$. The mixture was vortexed for 1 minute to ensure homogenization. To reduce sample turbidity, the solution was subsequently diluted to a final concentration of 12.5 µg $µL^{-1}$ and vortexed again. Samples were centrifuged at 15,000 rpm for 5 minutes, and the supernatant was carefully collected. A 20 µL aliquot was mixed with 80 µL of 0.2% TCA, resulting in a final concentration of 2.5 µg $µL^{-1}$ (250 µg in 100 µL total volume).

2.7.1. Sample Purification by Solid Phase Extraction

Sample purification was carried out following the protocol described by Agilent Technologies[31], with minor modifications. Two solutions were used for conditioning and washing of the ZipTip® (Bond Elut OMIX SCX): a washing solution of water containing 0.2% trifluoroacetic acid (TFA) and a conditioning solution consisting of a 50:50 mixture of acetonitrile and water containing 0.1% TFA.

The purification procedure involved several sequential steps. First, the Zip tip was conditioned by aspirating and discarding 100 µL of the conditioning solution twice, followed by equilibration with 100 µL of the washing solution, also repeated twice. For sample binding, 25 µL of the pretreated sample was aspirated and dispensed through the tip in five cycles to maximize interaction with the resin, with up to ten cycles performed if necessary. The bound sample was then purified and desalted by washing the tip four times with 100 µL of the washing solution. Finally, analytes were eluted for LC/MS analysis by aspirating and dispensing 100 µL of the conditioning solution three times.

2.7.2. Micro-Flow LC-MS/MS Analysis

The methodology was adapted from Thermo Scientific[32]. Following purification, samples were resuspended in 62.5 µL of the initial HPLC mobile phase (water with 0.1% formic acid, v/v). The resuspended samples were then injected into a microflow LC-MS/MS system comprising an Orbitrap Exploris 240™ mass spectrometer (Thermo Fisher Scientific, USA) coupled to a Vanquish™ Duo UHPLC system (Thermo Fisher Scientific, USA).

Chromatographic separation was carried out on a nanoViper™ C18 column (15 cm × 75 µm, 2 µm particle size, Thermo Scientific™) at a constant flow rate of 0.1 µL min^{-1}. A linear gradient elution was applied, ranging from 5% to 35% mobile phase B (acetonitrile with 0.1% formic acid, v/v) over 64 minutes, followed by a 5-minute wash at 90% B and a 10-minute re-equilibration at 5% B. Mobile phase A consisted of water with 0.1% formic acid (v/v). Most peptides eluted between 30 and 40 minutes, with a peak signal observed at 36 minutes.

The mass spectrometer operated in data-independent acquisition (DIA) mode, using positive ionization at 1700 V. MS1 scans were acquired over an m/z range of 350–1500, and MS2 spectra were collected at a resolution of 15,000. A dynamic exclusion window of 45 seconds was applied to minimize repeated fragmentation of the same precursor ions.

2.7.3. Data Processing and Bioinformatic Analysis

Peptidomic data from intestinally digested black and white rice samples were visualized using Bioware Peptigram following UHPLC analysis[33]. Identified peptide sequences were then matched against the UniProtKB/Swiss-Prot database[34]. Finally, functional coupling, including potential regulatory interactions and participation in shared biological processes, was explored using FunCoup[35].

2.8. Biological Activity, Cell Culture Models.

2.8.1. Cell Culture and Differentiation

The human colon adenocarcinoma cell line (Caco-2) and neuroblastoma (SH-SY5Y) were used for the *in vitro* experiments. Caco-2 lines were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 1% antibiotic (penicillin-streptomycin (5000 U·mL^{-1})), and 1% non-essential amino acids. The SH-SY5Y lines were maintained in the same basal medium but without non-essential amino acids and supplemented with 1% antibiotic. All cultures were incubated at 37 °C in a humidified atmosphere with 5% CO_2 until reaching 80–90% confluence. The culture medium was renewed every 2 to 3 days.

The SH-SY5Y cell line was differentiated following a previously described methodology[29], with slight modifications. The differentiation medium consisted of DMEM supplemented with 1% FBS, 1% penicillin/streptomycin, and 10 μM all-trans retinoic acid (RA) (Sigma-Aldrich, St Louis, MO, USA). Cells were cultured, with medium renewal on days 2 and 4. Differentiation was considered complete after 5 days, at which point the assay was conducted.

2.8.2. Cell Viability or Cytotoxicity (MTT)

The cytotoxicity of freeze-dried first flour fractions from different rice genotypes, along with a commercial standard of cyanidin 3-*O*-glucoside, was evaluated using the MTT assay (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide), following a standard protocol[36] with modifications.

Caco-2 and differentiated SH-SY5Y cell lines were seeded at densities of 2.4×10^4 and 5×10^3 cells per well, respectively, and incubated for 24 hours at 37 °C in a humidified atmosphere with 5% CO_2. After incubation, the culture medium was replaced with either the treatment solutions or the commercial standard. Treatments were prepared in serum-free DMEM at concentrations ranging from 31.25 to 500 μg mL^{-1}. Cyanidin 3-*O*-glucoside was diluted in serum-free DMEM containing 0.1% dimethyl sulfoxide (DMSO) to final concentrations of 12, 25, and 50 μg mL^{-1}.

After 24 hours of incubation under the same conditions, the medium was removed, and the cells were washed with phosphate-buffered saline (PBS). Subsequently, 200 μL of serum-free DMEM and 20 μL of MTT solution (5 mg mL^{-1} in 0.2 μm-filtered PBS) were added to each well. Plates were incubated for 2.5 hours at 37 °C in 5% CO_2. After incubation, the MTT-containing medium was discarded, and 200 μL of DMSO was added to each well to solubilize the formazan crystals.

Control wells consisted of untreated cells in DMEM (for treatments) and DMEM with 0.1% DMSO (for the standard), both used as 100% viability references. Absorbance was at 570 nm using a microplate reader PowerWaveTM XS (BioTek Instruments, Inc., Winooski, VT, USA). Cytotoxicity was expressed as the percentage of cell viability relative to the corresponding control.

2.8.3. Reactive Oxygen Species (ROS)

The formation of reactive oxygen species was assessed using the 2',7'diclorodihydrofluorescein diacetate (DCFH-DA) method[29] with modifications. Caco-2 cells were seeded in 24 wells (5 x 10^4 cells/well) and incubated for 24 hours at 37° C under 5% CO_2. Two genotypes of freeze-dried first flour were used, which were diluted in basal DMEM at a concentration of 100 μg mL^{-1}. Cyanidin 3-*O*-glucoside, used as a standard, was prepared in DMEM containing 0.1% DMSO at concentrations of 25 and 50 μg mL^{-1}. After 3 hours of treatment, the medium was replaced with DMEM with 250 mM ethanol, and cells were incubated for 16 h. The medium was then discarded, and cells were washed with PBS. Subsequently, 500 μL of DMEM and 12 μL of DCFH-DA solution (0.62 mM in DMSO) were added to each well and incubated for 60 minutes. Excess probe was discarded, and cells were washed with PBS before adding 500 μL of DMEM containing the pro-oxidant agent tert-butyl-hydroperoxide (t-BOOH, 1mM).

Oxidation controls included: i) a positive oxidative control (C+), consisting of 250 mM ethanol and 1 mM t-BOOH without antioxidants, and ii) a basal cellular control without pro-oxidant treatment (DMEM only). Cells were incubated for 150 min, and fluorescence was measured at λex = 485 nm and λem = 530 nm in a microplate reader Synergy™ MX Multi-Mode (BioTek Instruments, Inc., Winooski, VT, USA). For reference purposes, an additional positive control was prepared using 250 mM ethanol without t-BOOH (C (+)). The area under the curve (AUC) was calculated using the trapezoidal method[37], with modifications, to obtain the fluorescent units per minute in treatments with and without the pro-oxidant agent.

$$\text{AUC} = \sum_{i=1}^{n-1} \left(\frac{F_i + F_{i+1}}{2}\right) \times (t_{i+1} - t_i)$$

Where F_i is the average fluorescence over time, t_i. t_i the time of each measurement point (150, 170, 190, and 210 minutes), and n the total number of points over time.

2.8.4. Senescence Model by Β-Galactosidase Activity

The activity of β-galactosidase, a marker of cellular senescence, was assessed using freeze-dried extracts of two whole black rice genotypes and a commercial standard of cyanidin 3-*O*-glucoside, following the principle of a previously described method[38], with modifications to optimize performance in a 96-well fluorescence-based format.

Caco-2 cells were seeded at a density of 1.36×10^4 cells per well in 96-well microplates. Ethanol was applied at concentrations ranging from 12.5 to 1,000 mM to evaluate its effect as a senescence inducer. Treatments included black rice fractions at 25, 75, and 100 µg mL^{-1}, and cyanidin 3-*O*-glucoside at 6, 12, 25, and 50 $\mu g{\cdot}mL^{-1}$. Ethanol at 50 mM was selected as the minimum concentration with a significant effect on β-galactosidase activity, with no cytotoxic effect compared to the untreated control.

Cells were pre-treated with ethanol for 3 hours, followed by replacement of the medium with ethanol-free culture medium containing the respective treatments. Incubation continued for an additional 16 hours under standard conditions (37 °C, 5% CO_2).

After treatment, the culture medium was removed, and 50 µL of 0.8 mM 4-methylumbelliferyl-β-D-galactopyranoside (MUG) substrate and 100 µL of 0.1 M citrate-phosphate buffer (pH 4.4) were added to each well. Calibration curves were generated using a range of substrate concentrations (0.012 – 0.75 mM) and the fluorescent reaction product 4-methylumbelliferone (4-MU; 3 – 95 µM). Fluorescence (λ_{ex} = 360 nm, λ_{em} = 450 nm) was measured 120 minutes after substrate addition using a multimode microplate reader Synergy™ MX Multi-Mode (BioTek Instruments, Inc., Winooski, VT, USA).

Background fluorescence was corrected by subtracting the signal from untreated control wells, thereby ensuring that the measured fluorescence exclusively represented β-galactosidase activity linked to cellular senescence.

3. Statistical analysis

Statistical analysis of biological activity, ROS levels, and β-galactosidase activity was conducted using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test at a 95% confidence level to compare group means. Data normality was previously verified using the Kolmogorov–Smirnov test. All statistical procedures were performed in RStudio 4.2.1[39].

4. RESULTS

4.1. Free Amino Acid Formation in Cooked Whole Grain Rice

4.1.1. Undigested Samples

The OPA assay allowed quantification of free amino group release from cooked whole grain rice samples across the simulated static digestive phases. Significant differences were observed between undigested samples and those subjected to gastric and intestinal digestion. In undigested cooked samples, the average concentration of free amino groups was 3.62 μM GluAE g^{-1} for the black rice 'Onix' and 1.05 μM GluAE g^{-1} for white rice 'Zafiro', indicating a higher baseline availability of amino groups in black rice before digestion. In contrast, the cookie sample used as a control showed no detectable free amino groups in its undigested state (Table 2).

Table 2. Quantification of free amino groups by OPA assay in cooked whole grain rice.

Samples	Phase	Glutamic acid equivalents	CV

		(μM GluAE g^{-1})		(%)
Cookie	Gastric	21.82	± 5.11	23.43
	Intestinal	262.22	± 30.99	11.82
Black rice	Gastric	275.11	± 16.37	5.95
	Intestinal	850.41	± 45.83	5.39
White rice	Gastric	257.30	± 17.00	6.61
	Intestinal	709.94	± 53.84	7.58

Black rice: cooked ‘Onix’ grains. White rice: cooked ‘Zafiro’ grains. Cookie: food control without protein intake. Data are mean values ± standard deviation. CV: coefficient of variation.

4.1.2. Gastric

After gastric digestion, a marked increase in free amino group content was observed in both rice varieties, reaching average values of 275.11 ± 16.37 μM GluAE g^{-1} for black rice and 257.30 ± 17.00 μM GluAE g^{-1} for white rice, with coefficients of variation of 5.95% and 5.39%, respectively. The cookie control, included to account for enzymatic contributions, exhibited a free amino group concentration of 21.82 μM GluAE g^{-1} in the gastric phase.

4.1.3. Intestinal

In the intestinal phase, both rice samples showed a substantial increase in free amino group release, reaching 850.41 ± 45.83 μM GluAE g^{-1} for black rice and 709.94 ± 53.84 μM GluAE g^{-1} for white rice (Table 2).

4.1.4. Corrected Values

To estimate the specific contribution of each rice variety, values were corrected by subtracting the enzymatic background determined from the cookie control. The calculations were based on a protein content of 2.7% for both rice varieties. After correction, free amino group release in the gastric phase reached 253.30 μM GluAE g^{-1} for black rice and 235.48 μM GluAE g^{-1} for white rice. In the intestinal phase, corrected values increased to 588.19 μM GluAE g⁻¹ for black rice and 447.72 μM GluAE g⁻¹ for white rice (Table 2). Additionally, the net release of free amino groups in undigested samples was 3.62 μM GluAE g⁻¹ for black rice and 1.05 μM GluAE g⁻¹ for white rice. Direct comparison between the two cooked whole grain genotypes showed a difference in free amino group release of 17.82 μM GluAE g⁻¹ in the gastric phase, which increased to 140.47 μM GluAE g⁻¹ in the intestinal phase (Table 2).

4.2. Anthocyanins

4.1.2. Raw and Cooked

The raw (undigested) whole grain black rice flour 'Onix' contained 101.55 mg cyanidin 3-*O*-glucoside and 46.75 mg peonidin 3-*O*-glucoside per 100 g of sample (Figure 1 and Figure S3). After cooking, cyanidin 3-*O*-glucoside decreased by 43.74% and peonidin 3-*O*-glucoside by 6.55%.

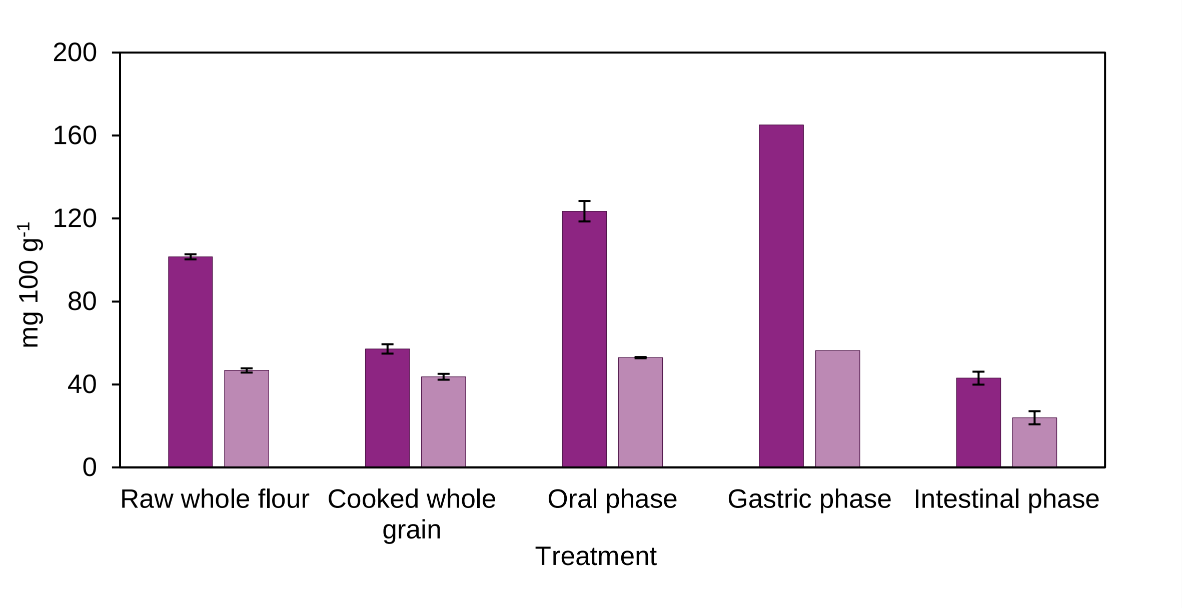

Figure 1. Anthocyanin content in raw whole flour, cooked whole grain, and *in vitro* digestion phases of the 'Onix' genotype. The data represent the content of cyanidin 3-*O*-glucoside (dark purple bars) and peonidin 3-*O*-glucoside (light purple bars) expressed as mg 100 g^{-1}. For the digestion phase, values are reported per 100 g of freeze-dried solid. Results for raw flour and cooked grain are presented as average ±

standard error (n = 3), while digestion phases represent the average ± standard error (n = 2). The gastric phase corresponds to a single data point, as no additional replicates were available.

4.2.1. Digestion

Similar variations in anthocyanin content were observed during the simulated static digestion stages. Both compounds increased during the oral and gastric phases; however, in the intestinal phase, cyanidin 3-*O*-glucoside and peonidin 3-*O*-glucoside decreased by 73.95% and 57.46%, respectively, relative to the oral phase (Figure 1 and Figure S3).

4.3. Peptidomic Analysis

Peptidomic analysis was performed using micro-flow LC-MS/MS, and peptide profiles were visualized with Peptigram. Venn diagram analysis revealed that 57 peptides were shared between the intestinally digested black and white rice samples, representing 45.2% of the total identified peptides. Additionally, 51 peptides were unique to white rice, whereas 18 were exclusive to black rice (Figure 2).

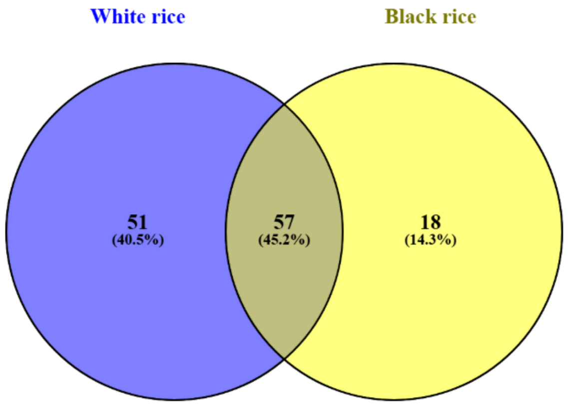

Figure 2. Venn diagram shows unique and shared peptides identified in cooked, intestinally digested samples of white and black rice.

However, Venn diagram analysis of this representation does not account for the number of protein accessions associated with each peptide. For instance, the peptide

ADEIFPF was linked to two distinct accessions: Q6K508|GLUD1_ORYSJ and Q6T726|Q6T726_ORYSJ. Consequently, the Peptigram matrix included a total of 152 data points (Figure S4).

The analysis identified 23 proteins corresponding to *Oryza sativa* subsp. japonica (Table 3). However, one protein, T1T6C4_ORYSI, was associated with subsp. indica. The relative abundance of the identified proteins, based on mass spectrometry signal intensity, ranged from 157,000 to 10,000,000. Among the detected proteins, GLUA3_ORYSJ, Q0E2D2_ORYSJ, and Q0E2D3_ORYSJ exhibited the highest number of unique peptides, with 15 peptides each. Additionally, they showed the highest sequence coverage – 13%, 11%, and 11% – compared to the other proteins identified (Table 3).

Table 3. Proteins identified by proteomic analysis in cooked whole grain black rice (‘Onix’) and white rice (‘Zafiro’) samples in the intestinal digestion phase.

Protein	Organism	UniProt id	# peptides	Max intensity	Coverage
A3A5D5_ORYSJ	Oryza sativa subsp. japonica	A3A5D5	8	10.100.000	7%
GLUA1_ORYSJ	Oryza sativa subsp. japonica	P07728	6	10.100.000	5%
GLUA2_ORYSJ	Oryza sativa subsp. japonica	P07730	7	10.100.000	5%
GLUB4_ORYSJ	Oryza sativa subsp. japonica	P14614	9	10.100.000	7%
GLUA3_ORYSJ	Oryza sativa subsp. japonica	Q09151	15	10.100.000	13%
Q0E2D2_ORYSJ	Oryza sativa subsp. japonica	Q0E2D2	15	10.100.000	11%
Q0E2D3_ORYSJ	Oryza sativa subsp. japonica	Q0E2D3	15	10.100.000	11%
Q0E2G5_ORYSJ	Oryza sativa subsp. japonica	Q0E2G5	4	10.100.000	4%
GLUB5_ORYSJ	Oryza sativa subsp. japonica	Q6ERU3	9	10.100.000	7%
Q6ESW6_ORYSJ	Oryza sativa subsp. japonica	Q6ESW6	5	10.100.000	4%
GLUD1_ORYSJ	Oryza sativa subsp. japonica	Q6K508	5	10.100.000	5%
GLUD1_ORYSJ	Oryza sativa subsp. japonica	Q6T726	5	10.100.000	5%
Q84X94_ORYSJ	Oryza sativa subsp. japonica	Q84X94	4	10.100.000	4%
T1T6C4_ORYSI	Oryza sativa subsp. indica	T1T6C4	9	10.100.000	9%
RA05_ORYSJ	Oryza sativa subsp. japonica	Q01881	10	2.510.000	15%
Q9ZWJ8_ORYSA	Oryza sativa	Q9ZWJ8	2	1.150.000	1%
RA16_ORYSJ	Oryza sativa subsp. japonica	Q8H4L8	4	491.000	12%
PROA_ORYSJ	Oryza sativa subsp. japonica	Q0DN94	1	438.000	4%
RAG2_ORYSJ	Oryza sativa subsp. japonica	Q01882	3	270.000	7%

RAG1_ORYSJ	Oryza sativa subsp. japonica	Q01883	2	270.000	7%
Q40653_ORYSJ	Oryza sativa subsp. japonica	Q40653	3	270.000	7%
AI172_ORYSJ	Oryza sativa subsp. japonica	Q7X8H9	3	226.000	8%
SSG1_ORYSJ	Oryza sativa subsp. japonica	Q0DEV5	8	157.000	8%

Protein: identified protein, organism: from which the protein originates, UniProt: unique identifier from the universal database for this protein, peptides: number of peptides associated with the protein, Max. intensity: maximum intensity of the precursor ion signal of the peptides associated with that protein, coverage: percentage of the protein sequence covered by the identified peptides.

For visualization of peptides per protein, those with high intensity, coverage, and number of identified peptides were selected. Based on these criteria, six proteins stood out: GLUA3_ORYSJ, Q0E2D2_ORYSJ, Q0E2D3_ORYSJ, RA05_ORYSJ, T1T6C4_ORYSI, and GLUB4_ORYSJ (Figure 3). Most of the filtered proteins averaged approximately 500 amino acids in length (from N- to C- terminus).

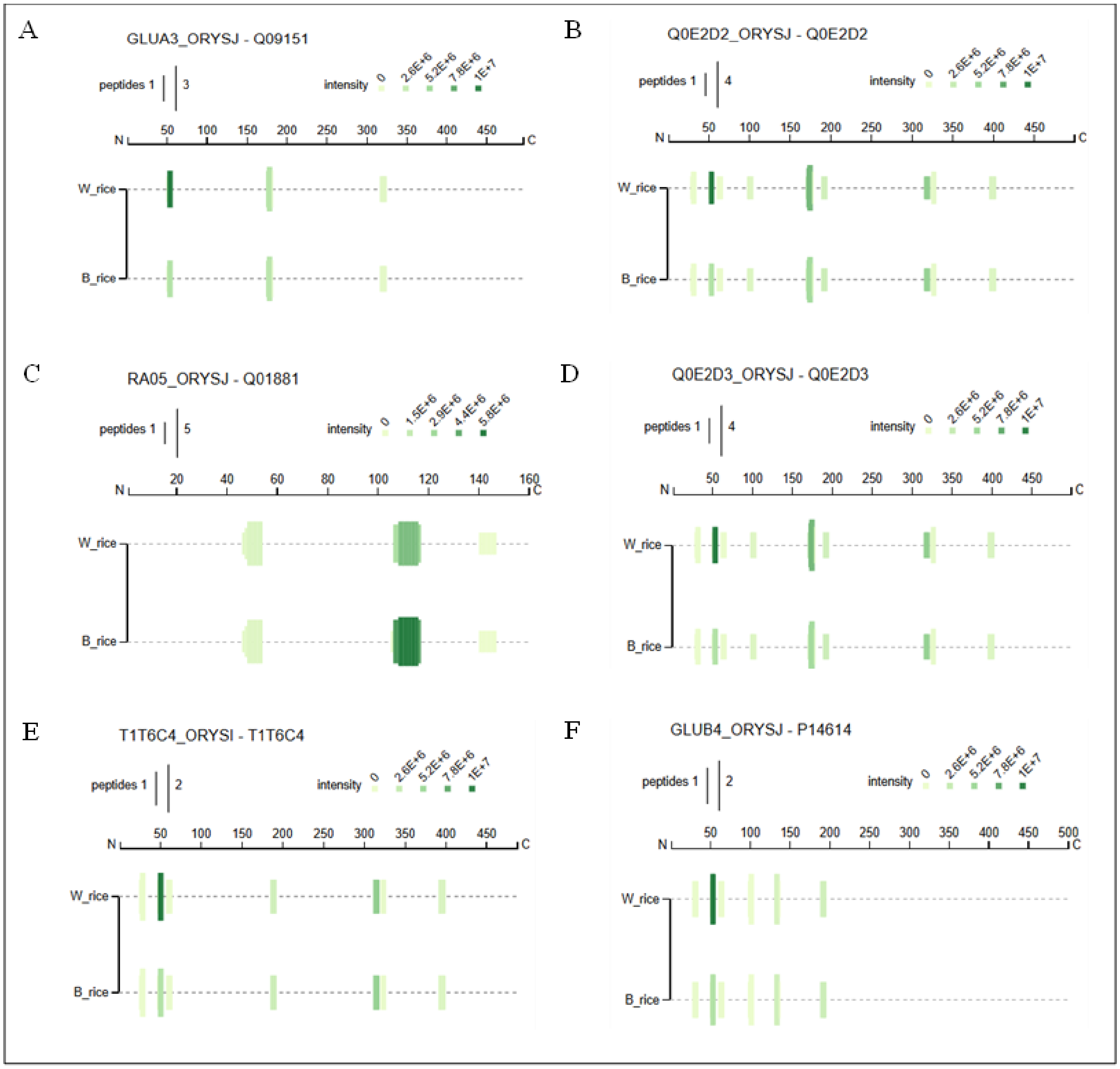

Figure 3. Peptide mapping of intestinally digested rice proteins filtered by intensity, sequence coverage, and peptides count using Peptigram. The graph depicts the relative abundance of peptides along the amino acids sequence of each protein. Samples include white rice (W_rice) and black rice (B_rice). Dark green bars represent regions of higher relative peptide abundance; light green bars indicate lower relative abundance. Bar height reflects the number of overlapping peptides at each position. Intensity refers to the maximum intensity observed for peptides at a given position. Peptides denotes the number of unique peptides identified.

In the white rice samples, a higher relative abundance of peptides was observed around the amino acid position 50 for most proteins (indicated by dark green bars), whereas black rice samples showed no significant accumulation or overall increase in peptide intensities at that position (Figure 3). The RA05_ORYSJ protein was the exception, displaying a different pattern in this region (Figure 3C). This protein was 160 amino acids in length, with one unique peptide identified for approximately every five amino acids. In both samples, peptides covering similar regions of the protein were visualized, specifically between positions 40–60, 100–120, and 140–160. The highest relative peptide abundance was in the middle region of the protein, specifically between amino acids 100 and 120, with greater intensity in black rice compared to the white rice (indicated by dark green and overlapping bars) (Figure 3C).

Table S5 summarizes the proteins identified in the proteomic analysis of cooked rice samples. Most of the detected proteins were glutelin-type storage proteins. However, several proteins classified as allergens were also identified, including UniProt entries Q01881, Q01882, Q01883, and Q40653. These allergenic proteins exhibited high relative abundance within the 100–120 amino acid region, with greater fluorescence intensity overlaps observed in black rice samples, as indicated by the darker green bars in Figure S5, compared to white rice.

Most of the proteins were identified by mass spectrometry and are classified as having protein-level evidence (Figure S4; Table S5). Functional annotation revealed that these proteins are primarily involved in molecular and biological processes related to nutrient storage and seed development. Although some proteins are annotated as allergens, their functional roles include IgE binding and serine-type endopeptidase inhibitor activity, rather than direct allergenic function (Table S5).

Given the relevance of the RA05_ORYSJ protein (UniProt Q01881) – not only due to its atypical abundance in black rice but also because of its, previously reported, allergenic potential – an in-depth characterization of its interactions was performed. Using the FUNCOUP functional coupling database, RA05_ORYSJ was found to participate in a network of interactions and functional associations, represented by a larger, intense blue node compared to other UniProts (Figure 4). The UniProts displaying similar characteristics included Q2QZZ6, Q7XKM1, Q2R1I6, Q2QMA4, Q8HL8, A0A0P0XXH9, Q01882, A0A0P0Y6T1, and A0A0N7KMT7. The remaining UniProts were represented by smaller nodes and lighter colors (Figure 4).

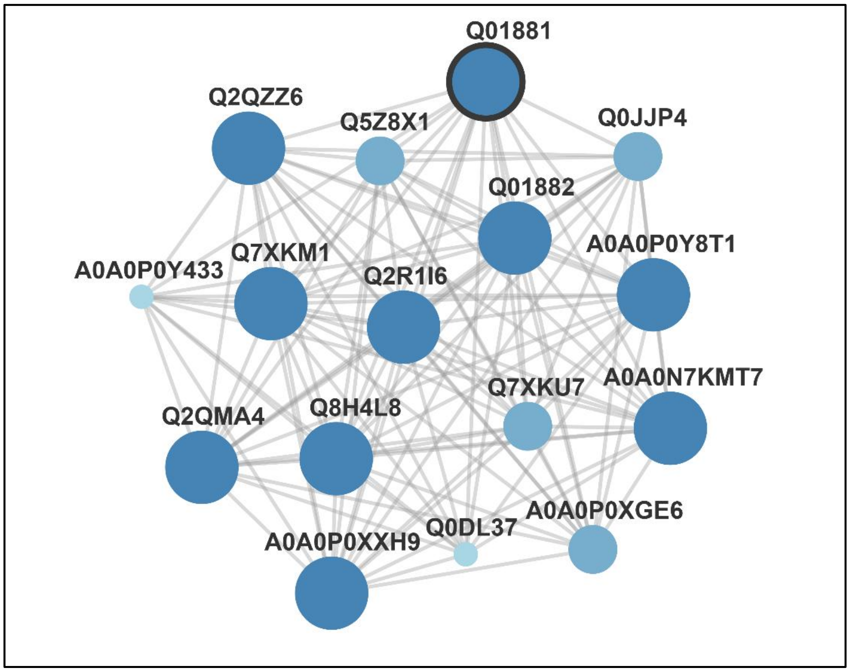

Figure 4. Protein – protein interaction network centered on the RA05_ORYSJ protein (UniProt Q01881). The network visualizes predicted functional associations and direct or indirect interactions involving RA05_ORYSJ. Each node represents a protein, and each edge denotes an interaction or functional link. Node size and color indicate connectivity: large, dark blue nodes correspond to proteins with high interactions degree, while small light blue nodes proteins with fewer interactions.

4.4. Cell Viability (MTT) in Caco-2 and SH-SY5Y Cells

Cell viability was assessed using the MTT assay in Caco-2 intestinal epithelial cells to evaluate the potential cytotoxic effects of the first flour fraction from white and black rice genotypes. At the highest concentration tested, ‘Onix’ extract (500 μg mL^{-1}) reduced cell viability by 27.86% compared to the control, indicating an effect at high dose. In contrast, 12 μg mL^{-1} cyanidin 3-*O*-glucoside increased cell viability above controls levels ($p \leq 0.05$) (Figure S6).

Citotoxicity in the SH-SY5Y cell line was evaluated in the two black rice genotypes, 'Onix' and 'Quila 292008'. For both genotypes, no statistically significant differences in cell viability were observed compared to the control ($p > 0.05$) (Figure S7). For 'Onix', treatment at 25 μg mL^{-1} increased viability by 2.82% relative to the control and by 1.32% relative to cyanidin 3-*O*-glucoside at 12 μg mL^{-1}. In contrast, higher concentrations of 'Onix' (75 and 100 μg mL^{-1}) reduced viability by an average of 9.5%, although these changes were not statistically significant ($p > 0.05$) (Figure S7).

4.5. ROS Generation in Cellular Oxidative Stress

Oxidative stress in Caco-2 cells was induced using tert-butyl hydroperoxide (t-BOOH) to evaluate the effects of the treatments on intracellular ROS production. The cells were induced with t-BOOH and monitored from 150 to 210 minutes. At 150 minutes, the positive control (C(+)) exhibited significantly higher fluorescence values than the negative control (C(–)), the DMEM control, the reference treatments with cyanidin 3-*O*-glucoside (25 and 50 μg mL^{-1}), and the black rice genotypes 'Onix' and 'Quila 292008' (100 μg mL^{-1}) ($p \leq 0.05$) (Figure 5). No significant differences were observed among treatments ($p > 0.05$) across time points (170 – 210 minutes; Figure S8).

The relative percentage change in fluorescence for each treatment was calculated with respect to C(+). At 150 minutes, all treatments showed a decrease of more than 50%, specifically 'Quila 292008' 100 μg mL^{-1} (-57.14 %), 'Onix' 100 μg mL^{-1} (-51.58 %), cyanidin 3-*O*-glucoside at 25 μg mL^{-1} (-57.15 %), and 50 μg mL^{-1} (-52.31%) (Figure 6). At 210 minutes, reductions remained consistent (average 58.34%) (Figure 6).

Regarding cumulative ROS production (AUC), treatments were compared with the positive control (C(+)) and ethanol 250 mM to assess their capacity to mitigate oxidative stress. Under ethanol-only conditions, black rice treatments showed increases of 17.40% and 34.05% ('Quila 292008' and 'Onix' at 100 μg mL^{-1}), while the cyanidin standards exceeded 20%. Under oxidative stress (t-BOOH), all treatments markedly reduced ROS levels relative to C(+). 'Quila 292008' and 'Onix' at 100 μg mL^{-1} decreased ROS by 65.04% and 60.61%, respectively, like cyanidin 3-*O*-glucoside at

25 and 50 μg mL^{-1}. These results were further supported by AUC comparisons between conditions with and without t-BOOH, where C(+) exhibited a 295.11% increase in ROS, whereas black rice treatments and cyanidin standards showed much smaller increases, averaging 16.88% and 21.17%, respectively.

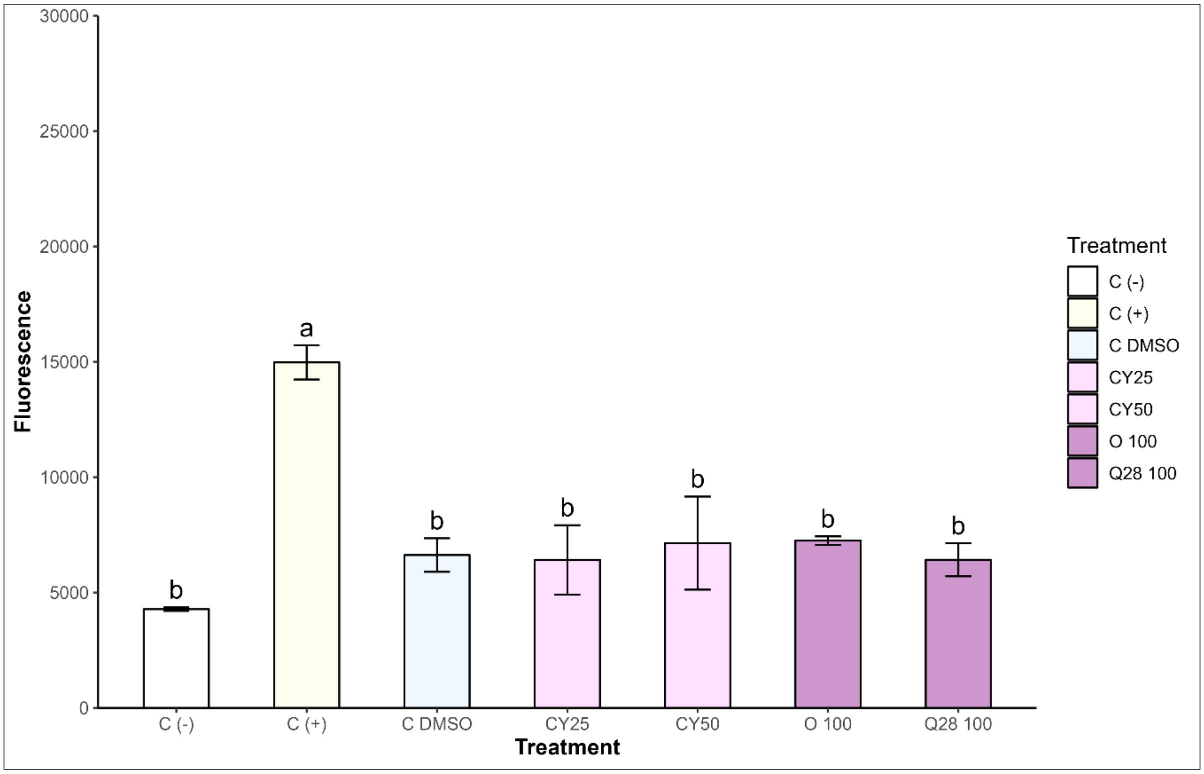

Figure 5. Reactive oxygen species (ROS) production in Caco-2 treated with first flour extracts from two black rice genotypes 'Onix' (O) and 'Quila 292008' (Q28), measured using a fluorescent probe (DCFH-DA) after 150 min. C(-): Control DMEM, C(+): Ethanol 250 mM + t-BOOH 1mM, C DMSO: Control DMSO, CY25: cyanidin 3-*O*-glucoside 25 μg mL^{-1}, CY50: cyanidin 3-*O*-glucoside 50 μg mL^{-1}, O: 'Onix' 100 μg mL^{-1}, Q28: 'Quila 292008' 100 μg mL^{-1}. Data represents the mean ± standard error. Different letters indicate statistically significant differences between treatments ($p \leq 0.05$).

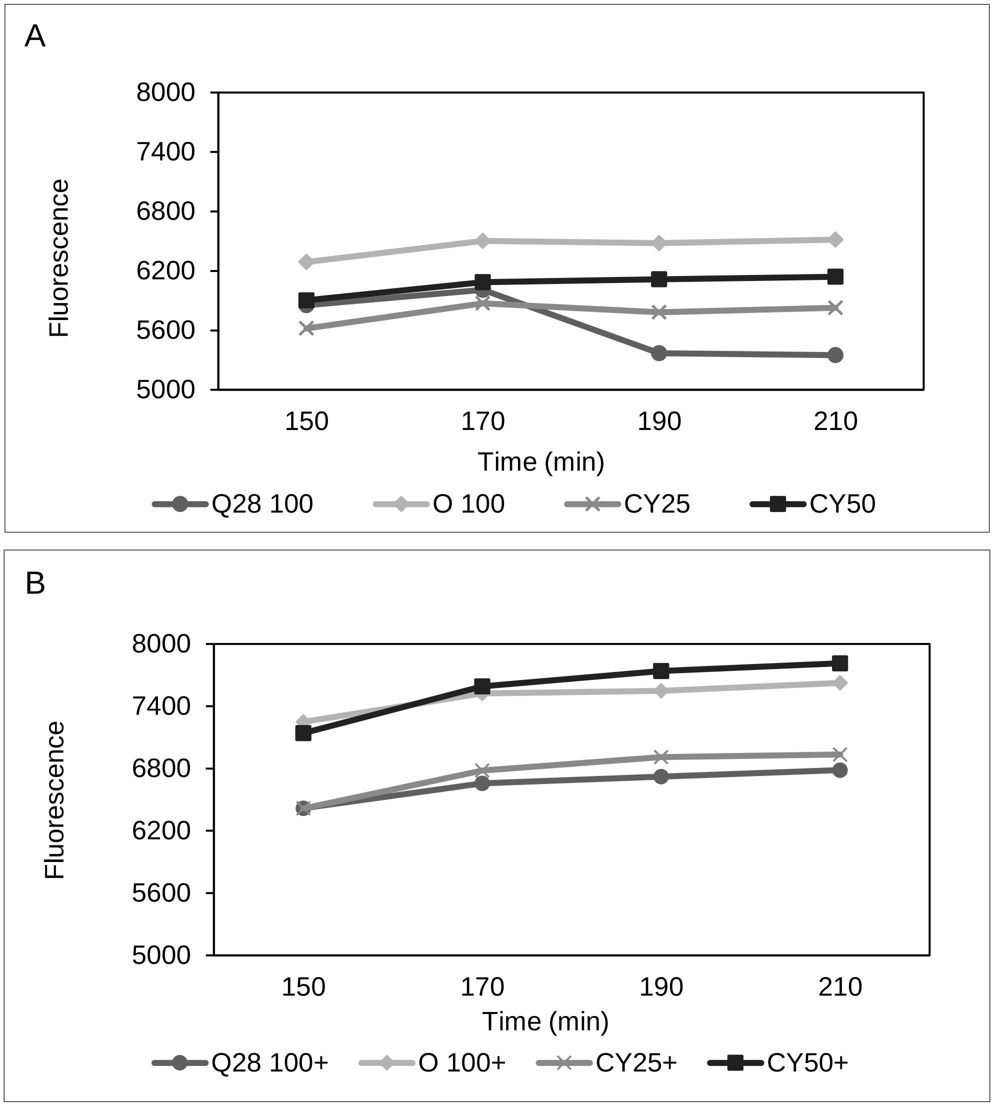

Figure 6. Time-course analysis of reactive oxygen species (ROS) levels in Caco-2 cells under different treatments, monitored via fluorescence from the oxidized DCFH-DA probe between 150 and 210 minutes. Two experimental conditions are shown: A) Cells treated with 250 mM ethanol (cell senescence inducer and ROS generator); and B) Cells treated in the presence of 250 mM ethanol + t-BOOH 1mM (oxidative stress inducer). Fluorescent curves represent the following treatments: 'Onix' and 'Quila 282008' (O and Q28), flour extracts from black rice genotypes 'Onix' and 'Quila 292008' at 100 µg mL^{-1}; CY25 and CY50, cyanidin 3-*O*-glucoside at concentrations of 25 and 50 µg mL^{-1}, respectively.

4.6. Senescence Model Based on B-Galactosidase Activity

The enzymatic activity of β-galactosidase was assessed by quantifying the formation of 4-methylumbelliferone (4-MU) expressed as nmol produced over 120 minutes. The ethanol-free control showed an average of -0.02 nmol, whereas the 50 mM ETOH treatment, used as the senescence positive control, yielded an average of 0.40 nmol. For the cyanidin 3-*O*-glucoside standards, mean values of 0.17, 0.21, 0.19, and 0.20 nmol of 4-MU were obtained at concentrations of 6, 12, 25, and 50 µg mL^{-1}. Among black rice extracts, 'Quila 292008' at concentrations of 100, 75 and 25 µg mL^{-1} showed averages of 0.13, 0.18, and 0.16 nmol. Meanwhile, 'Onix' extracts, at the same concentration exhibited average values of 0.13, 0.27, and 0.22 nmol of 4-MU, respectively (Figure S9).

At 120 minutes, the 50 mM ETOH control exhibited a β-galactosidase activity of 1.005 nmol of 4-MU, corresponding to a 325.56% increase compared with the ethanol-free control, which produced 0.236 nmol 4-MU. This difference was statistically significant ($p \leq 0.05$) (Figure S10). In contrast, treatments with black rice extracts and cyanidin standard attenuated ethanol-induced β-galactosidase activity. Specifically, 'Quila 292008' at 100 µg mL^{-1} reduced 4-MU formation by 72.20%, which was 4.34% higher than the reduction observed for 'Onix' at the same concentration (67.84%). However, this effect was not statistically significant ($p > 0.05$) (Figure S9 and Figure S10).

During 120 minutes of incubation, treatments with 'Quila 282008' at 75 and 25 µg mL^{-1} resulted in an average reduction of 69.66% in β-galactosidase activity, while 'Onix' exhibited a lower average decrease of 48.29%, representing a difference of 21.37% relative to 'Quila 282008' at the same concentration. Regarding the cyanidin standard at different concentrations, an average reduction of 53.32% was observed, comparable to that for 'Onix' at 25 µg mL^{-1}. No statistically significant variation was found between cyanidin concentrations, between black rice treatments, or when comparing black rice with the cyanidin standards ($p > 0.05$). However, all treatments showed a significant reduction compared with the ethanol-induced senescence control (ETOH 50 mM) ($p \leq 0.05$) (Figure S9 and Figure S10).

5. DISCUSSION

This study assessed the functional and bioactive properties of whole grains and the first flour fraction from different rice genotypes, with a particular focus on the differential responses of contrasting black and white rice genotypes during *in vitro* digestion, and their effects on cellular parameters related to oxidative stress and senescence.

During simulated static digestion, both rice types showed a progressive increase in the release of free amino groups, reaching their maximum in the intestinal phase. After correction with the enzymatic control (cookie), black rice demonstrated a higher net release of amino groups compared to white rice at each digestion phase, suggesting a distinct pattern of protein hydrolysis, possibly linked to differences in protein composition or protein-phenol interactions. Black rice is characterized by high levels of nutrients, vitamins such as B1, B3 and E, iron, magnesium, phosphorus, and dietary fiber[4,6,40]. This high concentration of dietary fiber is expected to slow the release of nutrients and bioactive compounds, as they are physically entrapped within its structure, limiting enzymatic activity due to increased viscosity of gastric fluids[8,9]. However, in the present study, black rice showed higher release of free amino groups compared to white rice throughout digestion, suggesting that other factors such as protein composition, matrix structure, or protein-phenol interactions may have outweighed the potential barrier effect of fiber[40].

In addition, the stability of the main anthocyanins presents in black rice, cyanidin 3-*O*-glucoside and peonidin 3-*O*-glucoside, was evaluated throughout the digestive process. A relative increase in anthocyanin levels was observed in the early stages of digestion compared to undigested samples. No increase in anthocyanin was observed in the oral phase compared to the intestinal phase because, in the oral cavity, anthocyanins undergo partial degradation influenced by salivary enzymes and oral microbiota, with losses of up to 50% described ex vivo; however, the short time spent in the mouth limits net accumulation[41].

The food matrix significantly influences the digestibility of anthocyanins[40,42]. An *in vitro* study comparing whole red cabbage with its anthocyanin-rich extract determined that the stability of these compounds during digestion depends on the matrix. The recovery of anthocyanin was significantly higher in whole cabbage (18–113%) compared to the extract (3–31%)[43]. In addition, it has been shown that the food matrix delays intestinal degradation, exerting a protective effect on anthocyanins[40,41]. However, it is important to note that the stability of these compounds is influenced by pH[44], as observed in a study with blackberries (*Rubus* spp.) subjected to simulated gastrointestinal digestion, where a 74% reduction in anthocyanins was observed[45]. These results show that stability depends on both pH and matrix[17, 43].

The findings of this study are consistent with a previous observation on bread food matrices, which demonstrated that during simulated digestion the bread matrix exerts a protective effect on free anthocyanins[46]. Given that black rice is particularly rich in starch and dietary fiber, these components may act as a natural encapsulation matrix, limiting anthocyanins accessibility and modulation their release during gastrointestinal transit[42].

It should be noted that the samples were prepared using a conventional microwave oven at 600 W, with grain cooking times of 9 minutes for whole black rice and 10 minutes for whole white rice, reflecting previously reported differences in gelatinization temperatures between these genotypes[27]. Although microwave cooking may induce thermal effects that contribute to anthocyanin degradation – since heating is known to affect their stability[18] – no significant reduction in peptide or anthocyanin release was observed under these conditions. In fact, black rice exhibited high levels of anthocyanins after digestion, suggesting that the heat treatment did not compromise the bioaccessibility of its bioactive compounds.

Peptide characterization revealed that white rice contained a higher number of unique peptides, whereas black rice exhibited peptides concentrated in specific regions, particularly between residues 100 – 120, within proteins of potentially relevant functions. One notable protein was RA05_ORYSJ, whose fragmentation in this region was more intense in black rice. This protein was identified as an α-amylase/trypsin inhibitor allergen previously described in rice[47,48], known for its ability to bind to IgE in patients with food allergies. It has demonstrated IgE reactivity and stability after heat processing[49], underscoring its relevance as an allergenic component in rice.

In this context, the increased release of peptides derived from RA05_ORYSJ in cooked black rice could reflect heat-induced alterations in protein structure, which enhance the exposure of specific regions and increase accessibility to digestive enzymes during simulated intestinal digestion[50]. These results suggest that, beyond its allergenic potential, this protein functions as an inhibitor of serine-type endopeptidases, indicating a modulatory function on digestive enzyme activity[51]. Given that serine protease inhibitors have been associated in other models with physiological processes such as inflammatory modulation, wound healing, apoptosis, antitumor activity, and antiviral effects, it is plausible that, together with the phytochemical compounds of black rice, RA05_ORYSJ contributed to the functional properties previously described[51,52].

Additionally, functional network analysis revealed that RA05_ORYSJ closely interacts with a group of proteins with defense functions, including a γ-thionin-type protein, alpha amylase/trypsin inhibitor RA16, and other proteins associated with cell signaling pathways and stress responses. This network suggests that RA05_ORYSJ does not act in isolation but instead functions as part of a protein complex, potentially involved in the immune responses and the regulation of cellular processes, thereby reinforcing its proposed biological role in pigmented varieties such as black rice.

At the cellular level, black rice flour extracts did not show cytotoxicity in Caco-2 and SH-SY5Y cell lines, even at high concentrations. These findings are consistent with a previous study that also reported no cytotoxic effects of black rice at similar or higher concentrations[53]. Under oxidative stress induced by t-BOOH and ethanol, extracts from the ‘Onix’ and ‘Quila 292008’ black rice genotypes, as well as the cyanidin 3-*O*-glucoside standard, significantly reduced ROS production, both at specific time points and cumulatively (AUC). At 150 minutes, rice extracts and cyanidin-3-*O*-glucoside reduced ROS-associated fluorescence by more than 50% relative to the positive control, demonstrating remarkable antioxidant capacity. These results align with prior findings[29], which attribute ROS inhibitory effect to anthocyanins such as cyanidin 3-*O*-glucoside in red fruit extracts.

Similarly, the same extracts that reduced ROS levels also significantly decreased β-galactosidase activity by more than 65% relative to the control, highlighting the anti-aging potential of the phenolic compounds in these grains. As β-galactosidase is a recognized biomarker of cellular senescence, the observed reduction suggests that black rice may attenuate processes associated with cellular aging[24]. These findings are consistent with previous research in which black rice methanol extract double the WI-38 cell population in an induced senescence model, thereby delaying aging and attenuating oxidative stress[54]. Complementary studies have shown that black rice bran intake, combined with physical exercise, exerts modulatory effects on aging and longevity in experimental models[55]. Furthermore, black rice extracts have been reported to attenuate H_2O_2-induced damage in PC12 cells *in vitro* by suppressing ROS accumulation, and *in vivo* studies have shown lifespan extension in *Caenorhabditis elegans*[56]. Collectively, the results of this study support the potential of black rice as a functional food with anti-aging and antioxidant properties.

This study demonstrated that black rice genotypes possess a distinctive functional profile, characterized by enhanced peptides and amino group release during digestion, significant antioxidant activity, and potential anti-aging effects. By identifying and characterizing specific peptides generated during intestinal digestion, this research highlights the multifunctional bioactivities of black rice. These provide a foundation for developing functional food ingredients derived from this cereal, while also supporting its consumption as part of a balanced diet.

Author Contributions

The manuscript was written through the contributions of all authors. All authors have approved the final version of the manuscript. ‡These authors contributed equally.

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V. CONCLUSIÓN GENERAL

Este estudio permitió comprender la interacción entre factores ambientales, genéticos y de manejo hídrico sobre el rendimiento, la calidad y las propiedades nutrifuncionales en líneas avanzadas de arroz negro. Los resultados confirmaron que el genotipo es el principal determinante en la acumulación de compuestos fenólicos, de la cual depende la capacidad antioxidante del grano, mientras que el ambiente y el régimen hídrico modulan los rasgos agronómicos, productivos y de calidad industrial.

En el estudio multiambiente se analizó la compleja interacción genotipo × ambiente, reflejada tanto en el rendimiento como en la calidad del grano, la cual mostró diferencias significativas entre los ambientes y los genotipos. Se evidenció la susceptibilidad del arroz negro al estrés hídrico en climas mediterráneos del centro-sur de Chile, con un rendimiento promedio 31 % inferior al del arroz blanco (Zafiro) bajo condiciones de no inundación. Los altos valores de heredabilidad en sentido amplio (H^2) de caracteres como DSA, PH, CHA, TRAN, TGWPa, TGWPo y ADD indican estabilidad genética, lo que sugiere que estos rasgos podrían servir como referencia para la toma de decisiones en la selección de genotipos en programas de mejoramiento de arroz negro.

No obstante, las condiciones climáticas estacionales ejercieron un impacto significativo, afectando directamente el rendimiento en el año 2022, lo que se tradujo en una reducción de la producción y un incremento del porcentaje de esterilidad floral, especialmente bajo condiciones de inundación. Estas amenazas, asociadas a un clima cambiante, resaltan la necesidad de continuar investigando la adaptación del arroz a futuros escenarios de cambio climático.

La evaluación comparativa de los genotipos de arroz negro frente al arroz blanco, cultivados en condiciones tradicionales de inundación, demostró que el cultivar comercial presenta mayores rendimientos y granos más pesados, aunque con una elevada presencia de panza blanca. Esto evidencia que, pese a alcanzar un buen rendimiento, la calidad del grano se ve afectada por su apariencia, lo que reduce la consistencia en la gelatinización y, por tanto, puede repercutir en la aceptación por parte de los consumidores, debido a una textura menos uniforme o más quebradiza. Por otro lado, bajo condiciones de déficit hídrico, aunque el rendimiento fue menor que en el genotipo de arroz blanco, la concentración de compuestos bioactivos tendió a incrementarse, lo que evidencia un mecanismo adaptativo frente al estrés hídrico. Los granos de arroz negro presentaron elevadas concentraciones de compuestos fenólicos, lo que se tradujo en una mayor capacidad antioxidante, especialmente en los genotipos Quila 279101 y Quila 292008. No obstante, esta acumulación no mostró diferencias significativas entre localidades ni según el régimen hídrico, lo que sugiere que la síntesis de estos compuestos está predominantemente determinada por el control genético.

A nivel funcional, este estudio demostró que los genotipos de arroz negro evaluados presentan un perfil bioquímico distintivo, caracterizado por una elevada liberación de péptidos con propiedades biológicas y funcionales, así como de grupos amino libres durante la digestión, además de una elevada actividad antioxidante y de efectos antienvejecimiento. Estos hallazgos posicionan al arroz negro como una fuente de alimento funcional y como una alternativa de diversificación para los productores nacionales.

Finalmente, este estudio resalta la importancia de futuras investigaciones enfocadas en la identificación de marcadores genéticos asociados a la tolerancia al estrés y a la esterilidad floral, con el objetivo de contribuir al desarrollo de cultivares de arroz negro de alto rendimiento, de calidad superior y resiliencia frente a condiciones climáticas adversas.

VI. PROYECCIONES FUTURAS

La identificación y cuantificación detalladas de los compuestos bioactivos presentes en el grano, junto con la evaluación de su bioaccesibilidad, permitirán establecer la calidad nutrifuncional de los diferentes genotipos evaluados. Esta información, combinada con los datos disponibles sobre rasgos fisiológicos, agronómicos, productivos y de calidad industrial del grano bajo condiciones de inundación y déficit hídrico, facilitará la selección de genotipos de arroz negro con alta productividad, calidad de grano y mayor eficiencia en el uso del agua. De este modo, se contribuirá al desarrollo de variedades comerciales adaptadas a las condiciones edafoclimáticas locales.

Estas variedades podrán constituir una alternativa de diversificación para los productores de arroz, agregando valor al arroz nacional mediante la producción de granos de alta calidad y con potencial nutrifuncional, abriendo nuevas oportunidades en la industria de alimentos funcionales y ampliando la oferta de estos productos en el mercado chileno. Asimismo, ofrecerán opciones de cultivos más tolerantes al déficit hídrico, favoreciendo la adaptación de los productores ante la escasez de agua y los efectos del cambio climático, contribuyendo así a la seguridad alimentaria y a la sostenibilidad de la industria arrocera en Chile.

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