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Metabolic and antioxidant effects of inoculation with arbuscular mycorrhizal fungi in crops of flesh-coloured *Solanum tuberosum* treated with fungicides

Running title: *Solanum tuberosum* crops under mycorrhizal and fungicide treatments

Sebastián Alarcón[1,2], Gonzalo Tereucán[1,2,] Pablo Cornejo[1,2], Boris Contreras[3], and Antonieta Ruiz[1,2]*,

[1]Centro de Investigación en Micorrizas y Sustentabilidad Agroambiental, CIMYSA, Universidad de La Frontera, Avda. Francisco Salazar 01145, 4811230 Temuco, Chile

[2]Departamento de Ciencias Químicas y Recursos Naturales, Scientific and Technological Bioresource Nucleus BIOREN-UFRO, Universidad de La Frontera, Avda. Francisco Salazar 01145, 4811230 Temuco, Chile.

[3]Novaseed Ltda. and Papas Arcoiris Ltda., Loteo Pozo de Ripio s/n, Parque Ivian II, Puerto Varas, Chile.

*Corresponding author: maria.ruiz@ufrontera.cl

Abstract

BACKGROUND: *Solanum tuberosum* tubers have higher contents of phenolic compounds such as hydroxycinnamic acid derivatives (HCAD) and anthocyanins in coloured genotypes. The use of fungicides for crops is common, but there are few studies regarding the interaction of fungicides and arbuscular mycorrhizal fungi (AMFs). Here, the AMF-plant interactions and the metabolic responses of three potato genotypes with different tuber colourations (VR808, CB2011-509 and CB2011-104) inoculated with *Claroideoglomus claroideum* (CC), *Claroideoglomus lamellosum* (HMC26) or *Funneliformis mosseae* (HMC7) were studied together with the use of the fungicides MONCUT (M) and ReflectXtra (R). Mycorrhizal traits, phenolic compound profiles and antioxidant activity (AA) were evaluated.

RESULTS: Two HCADs were identified, with 5-caffeolquinic acid being the most abundant, whereas four anthocyanins were detected only in purple potato genotypes. The anthocyanin and HCAD profiles, as well as AA, showed that the CB2011-104 genotype had better characteristics than the other genotypes, while VR808 and CB509 showed similar responses. The responses were dependent on the specific combinations

of genotypes, fungicide and the AMF strain and generally showed better responses when colonized by AMFs.

CONCLUSION: The three potato genotypes had differential responses depending on the inoculated AMFs and the fungicide applied before sowing, where the optimal combinations for antioxidant response, mycorrhization degree and performance were HMC26/R for VR808, HMC7/M for CB2011-509 and HMC26/M for CB2011-104. Our results suggest the existence of functional compatibility that can be registered even at the genotypic level of the host regarding a specific AMF strain.

Keywords: *Solanum tuberosum*, arbuscular mycorrhizal fungi, fungicides, anthocyanins, antioxidants

INTRODUCTION

Plant food consumption is important worldwide because the nutritional composition of plants is essential for the population.[1] From a functional point of view, fruits and vegetables are characterized by being metabolism-regulating foods, where daily consumption and varied rates promote health and help to prevent chronic diseases such as hypertension or diabetes.[2] One of the foods that stands out for its nutritional value is potato (*Solanum tuberosum*) tubers. Their energy contents are mainly attributed to polysaccharides such as starch, and they are an excellent source of fibre, which promotes intestinal motility and colon protection.[3] Potato tubers are rich in vitamins such as ascorbic acid, B1, B3, folate, riboflavin and pantothenic acid and contain minerals such as potassium, magnesium and phosphorus, as well as bioactive molecules such as carotenoids and anthocyanins.[4] Potatoes are important large-scale agronomic crops due to their high demand, with a global production of approximately 388,191,000 tons in 2017, making them the fourth most produced food of plant origin after corn, rice and wheat.[5]

Southern Chile provides a great variety of potato genotypes that present higher contents of phenolic compounds, such as anthocyanins and hydroxycinnamic acid derivatives, in the pulp and peel.[6] In the case of the flesh-coloured genotypes, the most representative anthocyanins correspond to acylglycosil derivatives of petunidin, pelargonidin, peonidin and malvinidin, whereas three derivatives of hydroxycinnamic acid have also been detected, the trans 3-, 4- and 5-isomers of caffeoylquinic acid.[6,7] Anthocyanins are responsible for the red, purple and blue colour of some plant foods, and these antioxidant pigments have shown great benefits for the treatment of cardiovascular diseases, inflammatory processes,

obesity and type II diabetes.[8] There is also evidence of chemopreventive effects of anthocyanins on breast and colorectal cancers in preclinical and clinical trials.[9] Due to their benefits, anthocyanins have shown interesting applications in the food industry,[10] are an important source of natural colorants and have been applied as indicators of food expiration in smart packaging systems due to their sensitivity to pH changes.[11,12]

The development of new agronomic techniques has involved the use of biologically active agents to improve crop productivity and reduce the use of agrochemicals, where arbuscular mycorrhizal fungi (AMFs) have shown positive effects on the physiology and metabolism of their host.[13] AMFs promote the quality of the final crop product by improving the contents of beneficial molecules because the association produced, known as arbuscular mycorrhizae, positively alters the synthesis of secondary metabolites in the host plant. For instance, Sarmiento et al[14] reported an increase in phenolic compounds in *Stevia rebaudiana* leaves of plants colonized by AMFs. In artichoke leaves of plants colonized by *Claroideuglomus claroideum* and *Funneliformis mosseae*, significant increases in total phenols and antioxidant activity were observed compared to those of noncolonized plants.[15] In weedy species such as *Solanum nigrum*, usually used for medicinal purposes, positive effects of the concentrations of total phenols and anthocyanins in leaves of plants colonized by *F. moseeae* were reported.[16] Similarly, the association with AMFs improved the antioxidant capacity in *Fragaria ananassa* fruits due to an increase in the concentration of total phenols.[17]

One of the most important problems that affects *Solanum tuberosum* crops is diseases related to pathogenic fungi; the most commonly found species are *Rhizoctonia solani* and *Phytophthora infestans*, which are responsible for large production losses.[18,19] The application of fungicides is an important aspect of crop management, with some of the most commonly used commercial formulations including MONCUT 40 SC, based on fluotanil specific to basidiomycetes (*R. solani*), and REFLECT® XTRA, a compound of isopyrazam and azoxystrobin that is effective against a wide range of fungal pathogens; both are long-acting and have curative and preventive actions. However, these active compounds can be harmful at the recommended doses for certain strains of AMFs.[20] Diedhiou et al.[21] reported a decrease and inhibition of mycorrhizal activity after the irrigation application of azoxytrobin and kresoxim-methyl in corn crops. Buysens et al.[20] reported that the application of fluotanil to potato crops did not affect AMF spore germination or the formation of extraradical mycelium but did decrease root colonization and development.

Due to the importance of the potato tuber, it is relevant to study the effects of fungicidal agents on the mycorrhizal interaction in *Solanum tuberosum* plants and, in the same way, to evaluate the contributions of AMFs to biomass production, profiles and concentrations of antioxidant compounds and antioxidant activity in potato tubers. The results of this study will provide new evidence regarding the role of AMFs in potato production and the combinations of AMF strains and fungicide treatments most adequate for the improvement of nutraceutical characteristics, especially in flesh-coloured tubers.

MATERIALS AND METHODS

Samples, experimental design and growth conditions

Tubers of three potato genotypes developed by the company Papas Arcoíris Ltda. (Puerto Varas, Los Lagos Region, Chile) were provided, which were distinguished by the colour of the peel and pulp. The genotype with yellow pulp and skin was encoded as VR808, the genotype with full purple flesh-coloured tubers was encoded as CB2011-104, and the genotype with intermediate colouration, purple skin and partially pigmented pulp was encoded as CB2011-509 (Suppl. Mat 1). Three AMF isolates were used: *Claroideoglomus claroideum* (CC), *Claroideoglomus lamellosum* (HMC26) and *Funneliformis mosseae* (HMC7). Moreover, uninoculated treatments were also considered. The commercial fungicides for the treatments were MONCUT 40 SC (M) Nihon Nohyaku Co. Ltd. (Tokyo, Japan) and REFLECT® XTRA (ReflectXtra (R) – Syngenta Limited Grangemouth Manufacturing Centre (Stirlingshire, UK)); control treatments (without fungicide) were also considered. The total number of combinations was 36, and each treatment was carried out in triplicate (n = 3), which resulted in a total of 108 experimental units. The crops were maintained in open air outside the Department of Chemical Sciences and Natural Resources of the Universidad de La Frontera (Temuco, Chile) under a mesh that provided 50% shading. The sowing was carried out on Thursday, September 12, 2019, and the harvest was carried out during March 2020.

The seeds were exposed to indirect light for two weeks to induce shoot production. Sterilized peat, which was autoclaved for 20 min at 121°C and 1 atm of pressure, was used as the substrate for the 108 11-L pots. For sowing, the pots were loaded with sterilized peat, and then the potato tubers (in all cases, one tuber per pot was used) treated with only AMF inoculum were sown at a depth between 5 and 10 cm, where 2 g of AMF inoculum was placed in contact with the tubers. For the treatment with MONCUT 40 SC, the commercial product was diluted in distilled water (at a ratio of 250 mL per ton of seed) and was then

sprayed onto the tubers. REFLECT® XTRA was applied to the surface of the substrate diluted in distilled water (at a ratio of 5 L per hectare). After the application of fungicide, the tubers were sown in the same way as described above.

The cultivation of the different potato genotypes lasted approximately 180 days from the start of sprout production. All the shoots emerged approximately one month after sowing due to the low temperatures recorded in late September and early October. However, all the experimental units showed 100% germination. The crop was fertilized with the doses of nitrogen, phosphorus and potassium recommended in the INIA potato cultivation manual.[22]

To evaluate the influence of the different treatments on the production of tubers, the number and weight of tubers per plant were recorded.

Microbiological analysis of the rhizosphere

For the determination of AM colonization, root samples were cut into 1-cm segments, thoroughly washed with tap water and stained with trypan blue according to Phillips and Hayman.[23] AM colonization was evaluated by microscopic visualization (40-90X) and quantified using the grid intersection method.[24] The evaluation of the extraradical hyphae was carried out according to the method used by Borie and Rubio[25], where the intersections between the extraradical mycelium and the nitrocellulose filter grid were quantified under stereomicroscopy (100-400X) and the density of the AMF mycelium was measured by the formula used by Newman.[26] To evaluate the spore density, 100 g of sample was used, and quantification was carried out by the method of wet sieving and decanting described by Sieverding.[27]

Extraction of antioxidant compounds from tubers

The extraction was carried out according to Ruiz et al.[6] with some modifications. Briefly, 1 g of mashed potato was mixed with 3 mL of methanol:formic acid (97:3 v/v) extraction solvent, and then ultrasound was applied for 60 seconds at a 40% amplitude. Maceration was carried out on an orbital shaker at 200 rpm for 10 min, and then the extract was centrifuged at 4°C for 10 min at 3000 xg. The extraction was carried out two times. Finally, the extract was filtered with 0.45-µm filters and stored at -20°C in darkness for subsequent analysis.

Identification and quantification of phenolic compounds

This analysis was performed according to the method optimized by Ruiz et al.[6] The technical specifications of the equipment were as follows: a Shimadzu HPLC-DAD system (Tokyo, Japan) equipped with an LC-20AT quaternary pump with a DGU-20A5R degassing unit, a CTO-20A oven and a diode array detector (SPD-M20A). Identity assignments were performed using an HPLC-DAD system coupled to an Applied Biosystem MDS Sciex system QTrap3200 LC/MS/MS mass spectrometer (Foster City, CA, USA). A C_{18} column (Kromasil 250 x 4.6 mm, 5 µm) equipped with a C_{18} guard column (Novapak, Waters, 22 x 3.9 mm, 4 µm) with an oven temperature of 40°C was used. Mobile phase A was water/acetonitrile/formic acid 87/3/10 (v/v/v), and mobile phase B was water/acetonitrile/formic acid 40/50/10 (v/v/v). The chromatographic analysis was performed by a gradient as follows: mobile phase B from 6 to 30% in 15 min, from 30 to 50% at minute 15, then from 50 to 60% for 5 min and from 60 to 6% in 6 min, with a final stabilization for 10 min. The detection of anthocyanins and HCAD was carried out at 520 nm and 320 nm by external calibration using petunidin-3-glucoside (98.95% purity) and chlorogenic acid (>95% purity) as standards, respectively.

Antioxidant activity determinations

The concentration of total phenols (Folin-Ciocalteu method), the antioxidant capacity equivalent to Trolox (TEAC), the reducing antioxidant capacity of the cupric ion (CUPRAC) and the antioxidant activity of the radical DPPH were determined according to Parada et al.[17] The ferric reducing antioxidant power (FRAP) assay was carried out according to the method developed by Jiménez et al.[28] All determinations were carried out by spectrophotometry adapted to 96-well microplates in Epoch UV-visible equipment from BioteK (Winooski, USA) using TROLOX as a standard. The results are expressed as Trolox equivalents (TE).

Statistical analysis

For data treatment, a two-way analysis of variance was performed, followed by a factorial analysis with extraction of principal components, where the fixed variables considered were the three types of potato, the different AMF isolates (and control) and the two fungicides (and control), with the aim of observing which types of interaction were statistically significant. The means were compared by Tukey's multiple range tests,

and a significance level of $p < 0.05$ was established for all cases. The software used for the analysis was IBM SPSS Statistics v. 23 (IBM Corp., New York, United States).

RESULTS AND DISCUSSION

Arbuscular mycorrhizal traits

Microbiological analysis of the rhizosphere in the three potato genotypes was carried out to evaluate AM root colonization. Data regarding control treatments (without AMF inoculation) are not reported since they did not show significant values, indicating that the sterilization of the substrate was carried out successfully. In the VR808 genotype (Figure 1A), the colonization percentages ranged from 3.3 to 24.0%. CC presented the highest colonization, followed by the HMC7 isolate (23.3%), both without fungicide application. In both cases, it was observed that the presence of fungicides negatively affected AM colonization, which was more evident with ReflectXtra. HMC26 colonized at a rate of 20.7% under treatment with R, where it was observed that this isolate increased its colonization in the presence of fungicides. The total mycelium (Figure 1B) showed values between 0.31 and 0.75 m g^{-1}, where the highest density was observed in treatments with fungicides, such as CC/M, followed by HMC7/R and HMC26/R. The other treatments presented lower hyphal lengths. The spore density (Figure 1C) showed results that varied between 214 and 307 spores in 100 g of substrate, where the highest values were recorded in the HMC7, HMC7/R and CC/M treatments. The CB2011-509 genotype presented AM colonization (Figure 1D) between 6.0 and 35.3%, where the highest percentage was observed with HMC26, followed by CC, whereas fungicides also negatively influenced the colonization rate. For the total mycelium length (Figure 1E), the highest densities were obtained in the CC and CC/R treatments. Values between 154 and 247 spores in 100 g of substrate were registered (Figure 1F), with a decreasing trend with fungicide application.

For the CB2011-104 genotype (Figure 1G), the HMC7 isolate without fungicide showed the lowest degree of colonization (10.7%), while the HMC7/M treatment showed the highest percentage (28.7%). In all cases, treatment with M increased the percentage of AM colonization. Regarding the total mycelium length (Figure 1H), the results varied between 0.45 and 0.82 m g^{-1}, where the treatments without fungicide showed the highest density, while fungicides slightly affected the hyphal content. The density of spores (Figure 1I) varied between 175 and 322 units per 100 g of substrate, where the highest value was observed in HMC26/M; however, no clear trends were detected.

Bharadwaj et al.[29] reported colonization values between 35 and 41% in *Solanum tuberosum* plants inoculated with soils from monocultures with the presence of AMFs, mainly from the *Glomus* genus. These results are similar to those presented here; however, in other crops, such as artichoke (*Cynara scolymus*) (83%) or sunflower (*Helianthus annuusy*) (79%), with multiple AMF strains, higher values have been reported. In lettuce crops (*Lactuca sativa L*), *CC* presented 61% colonization, while the plants inoculated with *F. mosseae*, *C. lamellosum* and *Diversispora celata* showed 67% colonization.[15] This variability is attributed to differential levels of colonization between varieties of the same species.[30]

Moreover, the application of fungicides influences key stages of the fungus-host interaction, such as the presymbiotic phase, which involves the germination of spores, elongation of the germ tube, branching of hyphae and contact with the root of the host plant.[31] The stage of active symbiosis with the host and the interaction with the surrounding soil are also affected, while the impact degree of pesticides depends on the type of substance, the dose and the specific AMF isolate used as the inoculant.[31] Diedhiou et al.[21], working with corn crops inoculated with AMFs and treated with high-spectrum fungicides derived from strobilurin (azoxytrobin and kresoxim-methyl), showed that foliar application did not affect the development of symbiosis, which was only affected by direct application to the soil. MONCUT is composed of flutolanil, a specific fungicide for *Basidiomycota*, and in experiments that evaluated the life cycle of *Rhizophagus irregularis* (an AMF) in potato crops, treatment with flutolanil affected the intraradical phase of the fungus[20] and was thus classified as a toxic pollutant for AMFs.

Determination of biomass

The yield was determined by the count and weight of the tubers for each experimental unit. Regarding the crop yield of the three potato genotypes, it was not possible to detect clear differences with respect to the treatments, but certain positive trends in the application of AMFs without the presence of fungicide were observed in some cases, such as in VR808 (Figure 2A). For both the number of tubers per plant and biomass, the inoculation with CC and HMC26 led to mean values higher than those in the control, with performance tending to decrease in the presence of fungicides (Figure 2B). In CB2011-509, a similar trend was observed with respect to fungicides, specifically in the treatment with CC (Figure 2C-D). For the CB2011-104 genotype, there were no significant differences between the treatments (Figure 2E-F); however, it was observed that this genotype presented the highest yields, although the size of the tuber was

much lower than that of the other evaluated genotypes. Tuber production and weight yield have been reported to vary highly between genotypes, where morphological characteristics, genetic differences, and exposure to different environments influence tuber production.[32]

In field trials, Hijri[33] reported significant increases in commercial potato yields with *Rhizophagus irregularis* inoculation. However, in uncoloured genotypes of potato from India, Pathak et al.[34] also reported average production levels between 3.2 and 10.3 units per plant in pots, with biomass yields that varied between 66.7 and 96.7 g per plant. These results demonstrated that plant height, root length, weight yield, and the number of tubers increased with the presence of AMFs along with other beneficial microorganisms such as plant growth-promoting rhizobacteria. These results agree with those observed for CB2011-509 and VR808 with respect to the number of tubers, but there were much lower values in terms of the highest biomass values recorded in our experiment, indicating the greater size and weight of the tubers in our study. Regarding the use of fungicides, research has focused on the efficacy of these compounds to improve yields, which are strongly affected by pathogenic fungi such as *Phytophthora infestans*, *Rhizoctonia solani* or *Fusarium proliferatum* in wheat and potato crops;[35-37] however, there is no information about interactions with individual AMF isolates. Therefore, it is not possible to corroborate whether the decrease in performance due to the fungicide observed in some cases is related to AM characteristics, such as the percentage of colonization or the density of other fungal structures.

Phenolic composition and antioxidant activity of potato tubers

Chromatographic analysis of potato genotypes revealed the presence of six phenolic compounds, two of which corresponded to HCADs and the remaining four to anthocyanins. The HCADs identified in potato tubers (Suppl. Mat. 2 and 3) corresponded to two derivatives of chlorogenic acid: i) 5-caffeoylquinic acid (peak 1), present in the three genotypes, and ii) caffeoylquinic acid isomer (peak 2), present in only VR808 and CB2011-509, which have been reported at very low concentrations compared to those of 5-caffeoyquinic acid.[6] HCAD quantification was performed by external calibration using 5-caffeoylquinic acid as a standard, with the equation y=70814x+1913.5, a detection limit (DL) of 0.27 mg L^{-1}, a quantification limit (QL) of 0.91 mg L^{-1} and a linear range (LR) between 0.91 and 100 mg L^{-1}. Regarding anthocyanins, the highest-intensity signal was observed for the CB2011-104 genotype (Suppl. Mat. 3 and 4), corresponding to petunidin-3-coumaroylrutinoside-5-glucoside (peak 4), two more derivatives of petunidin (peaks 3 and 5)

and a derivative of malvidin, corresponding to 3-coumaroylrutinoside-5-glucoside (peak 6). The CB2011-509 genotype presented similar anthocyanin profiles as CB2011-104 (Suppl. Mat 4 and 5), except for peak 3, while the presence of anthocyanins was not detected in VR808 (unpigmented). In genotypes lacking pigmentation, such as VR808, the main components with antioxidant capacity are the carotenoids of the xanthophyll family.[38] In coloured genotypes, it has been reported that the most abundant anthocyanins include pelargonidin, peonidin, petunidin and malvidins, glycosylated and acylated with hydroxylated aromatic acids,[39] whereas in red-fleshed genotypes, the predominant aglicon is pelargonidin, and in purple-fleshed genotypes, the most abundant aglycon is petunidin.[40] For the quantification of anthocyanins, a calibration curve was constructed using petunidin-3-glucoside as an external standard with the equation y=101546x-307.4 and the following analytical parameters: DL 0.057 mg L^{-1}, QL 0.0191 mg L^{-1} and LR between 0.0191 and 100 mg L^{-1}.

The VR808 genotype presented the lowest concentration of HCAD, in the range of 3.4 to 27.9 mg kg^{-1}, whereas the highest concentrations were obtained in the control treatment and HMC26/R (Figure 3A). The total phenol concentrations were also the lowest in this genotype, showing the highest concentrations with the HMC7 isolate without the presence of fungicides (Figure 3B). In the CB2011-509 genotype, a partially pigmented tuber, the total anthocyanin concentrations ranged from 8.4 to 46.0 mg kg^{-1}, where the treatment with the highest concentration was CC/R (Figure 4A). For HCADs, the results showed variations between 12.5 and 103.3 mg kg^{-1}, whereas the highest concentration was also observed in the CC/R treatment (Figure 4B). The total phenols (Figure 4C) varied between 471.3 and 722.9 mg kg^{-1}, where the HMC7 isolate without fungicide showed the highest concentration and was significantly different from the other treatments. In the CB2011-104 genotype, which was strongly pigmented, the highest concentrations of anthocyanins were detected, ranging between 414.7 and 1437.9 mg kg^{-1}, where the highest concentration was detected under HMC26/M treatment (Figure 5A). The HCADs ranged between 324.0 and 1036.1 mg kg^{-1}, where the CC isolate without fungicides showed the highest concentration, although HMC26/M showed similar results (Figure 5B). The concentration of total phenols ranged between 2510 and 4486 mg kg^{-1}, where the HMC26 inoculum produced the highest concentration (Figure 5C). Notably, the CB2011-104 genotype had the highest levels of phenolic compounds, reaching an anthocyanin concentration approximately 32 times higher than that of CB2011-509, considering the highest values recorded in each case.

The highest concentration of anthocyanins reported in red tubers by Ruiz et al.[6] was 212 mg kg^{-1} in the CR2002 genotype, several times lower than that registered in the present study, which indicates that purple-pigmented genotypes such as CB2011-104 have a higher content of antioxidant pigments. In the case of HCADs, the CB2011-104 genotype, with only one chlorogenic acid derivative, also presented a high concentration with respect to the other genotypes with two HCADs, whereas VR808 presented the smallest amount. Valiñas et al.[41] studied Andean genotypes and showed that pigmented tubers have much higher concentrations of chlorogenic acid, which represents the main antioxidant compound of nonpigmented tubers. The authors demonstrated that the coloured genotypes presented high expression levels of genes in the phenylpropanoid pathway responsible for synthesizing anthocyanins and chlorogenic acid. This also suggests strong expression of this metabolic pathway in the CB2011-104 genotype, given its complete pigmentation. In the CB509 genotype, the presence of HCAD predominates compared to anthocyanins, which may be related to the partial pigmentation of tubers. In all genotypes, it was observed that the concentration of total phenols was much higher with respect to the amounts of anthocyanins and HCADs, which may indicate that phenols in polymeric forms are the major compounds. In this sense, Ruiz et al.[6] reported the same trend and showed that these polymers tend to be released, causing an increase in the concentration of HCAD after the potatoes are exposed to high temperatures, as in the frying process.

The antioxidant activity ranges were similar in VR808 and CB2011-509 (Figures 3C-F and 4D-G), while CB2011-104 (Figure 5D-G) showed the highest activity, which was strongly related to the concentration of phenolic compounds. Pigmented tubers tended to show higher antioxidant activity with the CUPRAC method. Regarding the AMF-plant interaction, it was observed that the interaction tended to increase the antioxidant activity and the concentrations of compounds of phenolic origin. In *Fragaria ananassa*, Parada et al.[17] reported significant increases in antioxidant activity in plants inoculated with *C. claroideum* using the TEAC, DPPH and CUPRAC methods. Avio et al.[42] also reported that the inoculation of two AMF isolates in oak leaf lettuce crops caused changes in the composition of secondary metabolites, whereas inoculation with *Rhizopagus irregularis* and *F. mosseae* notably increased the antioxidant activity and the concentrations of phenolic compounds compared to those in the controls. Measurements are also positively affected by the use of fungicides. For example, Mohamed & Akladious[43] indicated that the use of different commercial fungicides, including MONCUT, increased the content of phenolic compounds in cotton plants. It has been reported that the use of high-spectrum fungicides can lead to some oxidative damage in

plants, which can activate defence systems and increase the expression levels of genes that encode proteins involved in the synthesis of phenolic compounds.[44]

Multivariate associations

Principal component analysis (PCA) was carried out considering all the evaluated parameters. In the CB2011-104 genotype (Figure 6 A, B), fungal characteristics did not show a higher influence on tuber yield. Furthermore, all antioxidant methods, except TEAC, were positively correlated with antioxidant compounds and total phenols, whereas CC showed the highest influence on the mycorrhizal characteristics and the antioxidant response. Although the absence of fungicides presented better results for some characteristics, the CC treatment together with MONCUT was optimal for this genotype. For the CB2011-509 genotype (Figure 6 C, D), all antioxidant activity detection methods were strongly correlated with anthocyanins and hydroxycinnamic acid derivatives (PC1), where the characteristics of AM colonization, yield and total phenols were also positively grouped (PC2). Moreover, the HMC7 isolate, with or without the presence of fungicide M, produced the best results. In the VR808 genotype (Figure 6 E, F), it was observed that the most representative variables of PC1 were the phenolic compounds and antioxidant measurements, where 5-caffoylquinic acid was the main compound within the total HCAD; there were also high correlations between total phenols, TEAC, CUPRAC and DPPH. Furthermore, it was observed that the FRAP antioxidant activity did not represent the content of phenolic compounds. The most representative variables for PC2 were the fungal characteristics AM colonization, mycelium and spores. These variables were related to yield (NT and FW), indicating that AMF-plant interactions are beneficial for crop yield. The plants inoculated with HMC26 in the presence of fungicide R induced the best antioxidant response, highest yield and most mycorrhization.

Among the most relevant observations in this study, it can be emphasized that the magnitude of mycorrhizal traits and the antioxidant responses of the secondary metabolites of phenolic origin for each of the genotypes are the major determinants of the global behaviour of the crop. In addition, the three potato genotypes had differential responses depending on the AMF isolate used as the inoculum and the fungicide applied during pre-sowing, making it possible to identify optimal factors that allow increases in yield and nutritional quality related to antioxidant compounds. Finally, our results suggest that the interactions occur even at the host genotype level, providing guidance for further studies on how the different AMF strains can

present different levels of functional compatibility and performance, which is especially well justified in species with high global consumption such as potato.

CONCLUSIONS

The study of the effects of fungicides on three *Solanum tuberosum* genotypes inoculated or not with different arbuscular mycorrhizal fungus (AMF) strains showed noticeable differences in the yield, antioxidant activities and phenolic profiles of tubers. Certain combinations positively affected root colonization by AMFs in potato plants and improved the yield and content of antioxidant compounds of tubers. The analysis of antioxidant compounds by HPLC-DAD demonstrated the presence of two derivatives of hydroxycinnamic acids, with 5-caffeoylquinic acid identified in all genotypes. In addition, four anthocyanins were identified only in the flesh-coloured tubers, corresponding to one derivative of malvidin and three derivatives of petunidin, with petunidin-3-coumarylrutinoside-5 glucoside being the most abundant. The antioxidant activity and total phenol content were evidently higher in the most pigmented genotype and had a direct relationship with the concentrations of anthocyanins and HCADs, confirming the great nutraceutical value of this potato genotype. Moreover, the multivariate analysis allowed us to identify the most effective combinations for antioxidant response, mycorrhization degree and increase in yield: for the VR808 genotype, the optimal treatment was the *Claroideoglomus lamellosum* inoculum with the fungicide ReflectXtra; for CB2011-509, the optimal treatment was *Funneliformis mosseae* with the fungicide MONCUT; and CB2011-104 revealed a better response without fungicide but inoculated with *C. claroideum*. However, since the use of fungicides is a common practice for the agronomic management of potato crops, the *C. lamellosum* inoculum in the presence of MONCUT fungicide is optimal for this last genotype.

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CONFLICT OF INTERESTS

The authors declare that they have no conflicts of interest.

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Figure Captions:

Figure 1. Quantification of arbuscular mycorrhizal fungal (AMF) traits in *Solanum tuberosum* growing with or without the supply of fungicide. (A,D,G) AMF root colonization, (B,E,H) extraradical AMF hyphae length, (C,F,I) AMF spores in the substrate. Where CB104: CB2011-104 potato genotype, CB509: CB2011-509 potato genotype, VR808: VR808 potato genotype, CC: *Claroideoglomus claroideum*, HMC7: *Funneliformis mosseae,* HMC26*: Claroideoglomus lamellosum*, WF: without fungicide, M: MONCUT, R: ReflectXtra. Different letters indicate significant differences according to Tukey's multiple range test (P <0.05).

Figure 2. Yield parameters of *Solanum tuberosum* growing with or without the supply of fungicide. (A,C,E) Number of tubers per plant, (B,D,F) tubers weight per plant.
Where CB104: CB2011-104 potato genotype, CB509: CB2011-509 potato genotype, VR808: VR808 potato genotype, CC: *Claroideoglomus claroideum*, HMC7: *Funneliformis mosseae,* HMC26*: Claroideoglomus lamellosum*, WF: without fungicide, M: MONCUT, R: ReflectXtra. Different letters indicate significant differences according to Tukey's multiple range test (P <0.05).

Figure 3. Total phenolic compound concentrations and antioxidant activity (AA) in tubers of *Solanum tuberosum* from VR808 genotype growing with or without the supply of fungicide. A) Total hydroxycinnamic acids by HPLC-DAD, B) Total phenols determined by the Folin-Ciocalteu method, C) AA determined by the TEAC (Trolox equivalent antioxidant capacity) method, D) AA determined by the DPPH (2,2-diphenyl-1-picrylhydrazyl) method, E) AA determined by the CUPRAC (copper reducing antioxidant capacity) method and F) AA determined by the FRAP (ferric reducing antioxidant power) assay.
Where VR808: VR808 potato genotype, CC: *Claroideoglomus claroideum*, HMC7: *Funneliformis mosseae,* HMC26*: Claroideoglomus lamellosum*, WF: without fungicide, M: MONCUT, R: ReflectXtra. Different letters indicate significant differences according to Tukey's multiple range test (P <0.05).

Figure 4. Total phenolic compound concentrations and antioxidant activity (AA) in tubers of *Solanum tuberosum* from CB2011-509 genotype growing with or without the supply of fungicide. A) Total anthocyanins by HPLC-DAD, B) Total hydroxycinnamic acids by HPLC-DAD, C) Total phenols determined by the Folin-Ciocalteu method, D) AA determined by the TEAC (Trolox equivalent antioxidant capacity)

method, E) AA determined by the DPPH (2,2-diphenyl-1-picrylhydrazyl) method, F) AA determined by the CUPRAC (copper reducing antioxidant capacity) method and G) AA determined by the FRAP (ferric reducing antioxidant power) assay.

Where CB509: CB2011-509 potato genotype, CC: *Claroideoglomus claroideum*, HMC7: *Funneliformis mosseae,* HMC26*: Claroideoglomus lamellosum*, WF: without fungicide, M: MONCUT, R: ReflectXtra. Different letters indicate significant differences according to Tukey's multiple range test (P <0.05).

Figure 5. Total phenolic compound concentrations and antioxidant activity (AA) in tubers of *Solanum tuberosum* from CB2011-104 genotype growing with or without the supply of fungicide. A) Total anthocyanins by HPLC-DAD, B) Total hydroxycinnamic acids by HPLC-DAD, C) Total phenols determined by the Folin-Ciocalteu method, D) AA determined by the TEAC (Trolox equivalent antioxidant capacity) method, E) AA determined by the DPPH (2,2-diphenyl-1-picrylhydrazyl) method, F) AA determined by the CUPRAC (copper reducing antioxidant capacity) method and G) AA determined by the FRAP (ferric reducing antioxidant power) assay.

Where CB104: CB2011-104 potato genotype, CC: *Claroideoglomus claroideum*, HMC7: *Funneliformis mosseae,* HMC26*: Claroideoglomus lamellosum*, WF: without fungicide, M: MONCUT, R: ReflectXtra. Different letters indicate significant differences according to Tukey's multiple range test (P <0.05).

Figure 6: Scores of the principal component (PC) for the experimental variables (left) determined in tubers of three genotypes of *Solanum tuberosum* and the grouping of the samples according to the PCs distribution (right). CB2011-104 A and B, CB2011-509 C and D, VR808 E and F. Were M: MONCUT, R: ReflectXtra and 0: without fungicide treatments; and regarding the AMF 0: uninoculated treatments, CC: *Claroideoglomus claroideum*, HMC7: *Funneliformis mosseae,* HMC26*: Claroideoglomus lamellosum* in the subfigures at right. Experimental variables (left subfigures) are HCAD1: 5-caffeoylquinic acid, HCAD2: caffeoylquinic acid isomer, A1: Petunidin-3- caffeoylrutinoside-5-glucoside, A2: Petunidin-3-coumaroylrutinoside-5-glucoside, A3: Petunidin-3-feruloylrutinoside-5-glucoside, A4: Malvidin-3-coumaroylrutinoside-5-glucoside, THCAD: total hydroxycinnamic acid derivatives, TA: total anthocyanins, TP: concentration of total phenols according to Folin-Ciocalteu method, TEAC: Trolox equivalent antioxidant capacity, CUPRAC: reducing antioxidant capacity of the cupric ion, DPPH antioxidant activity of the DPPH

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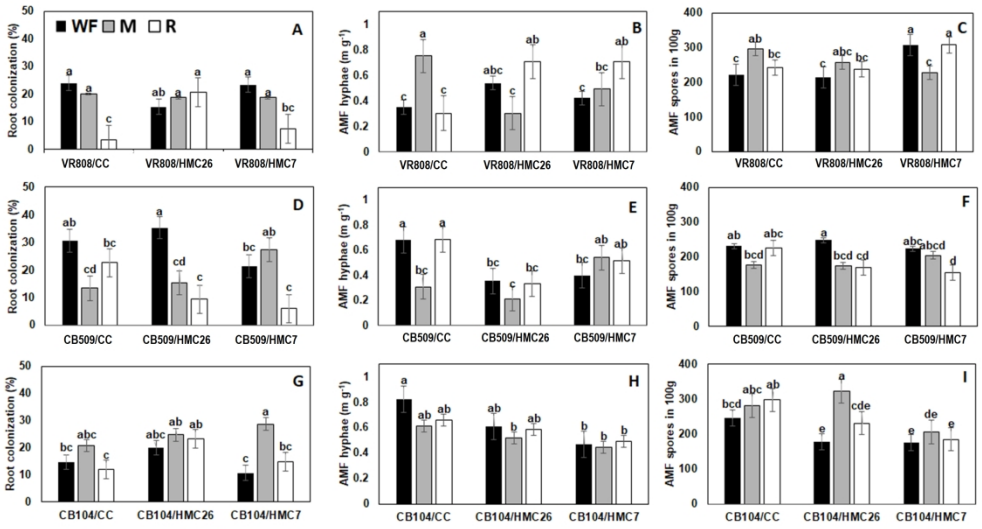

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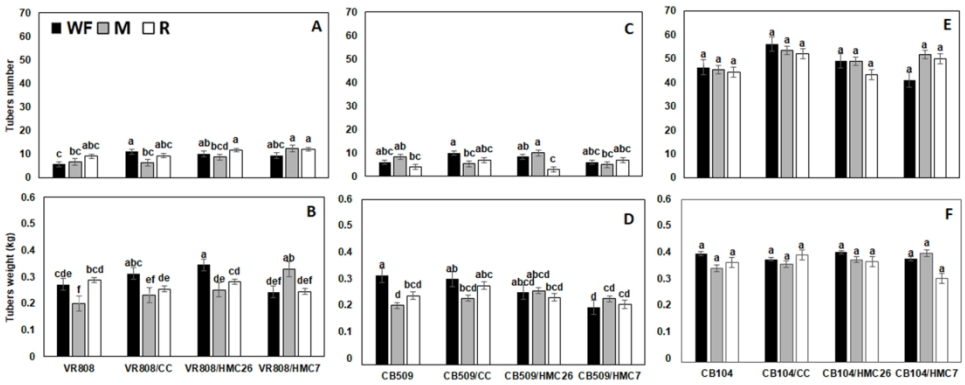

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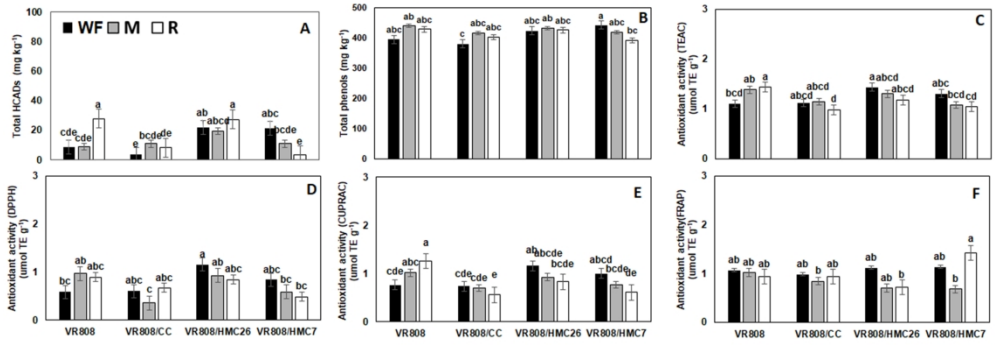

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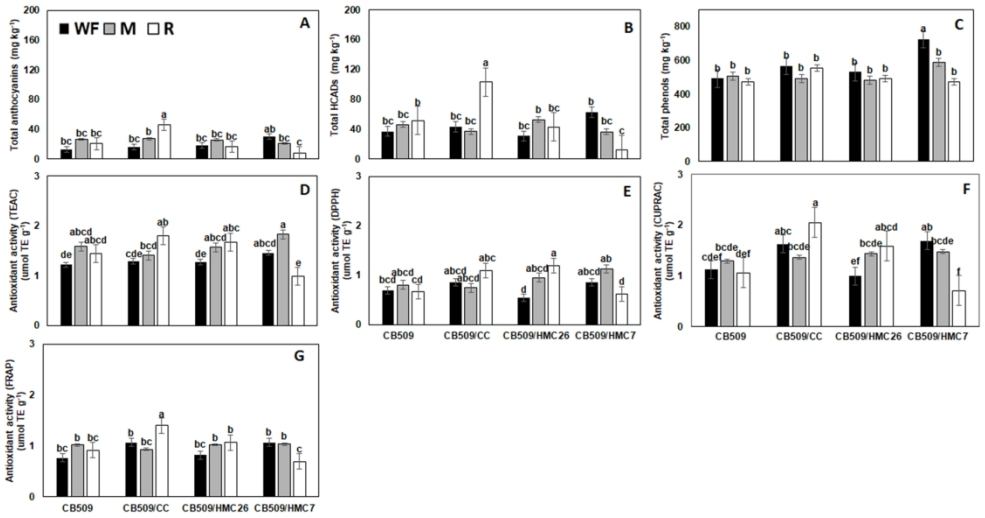

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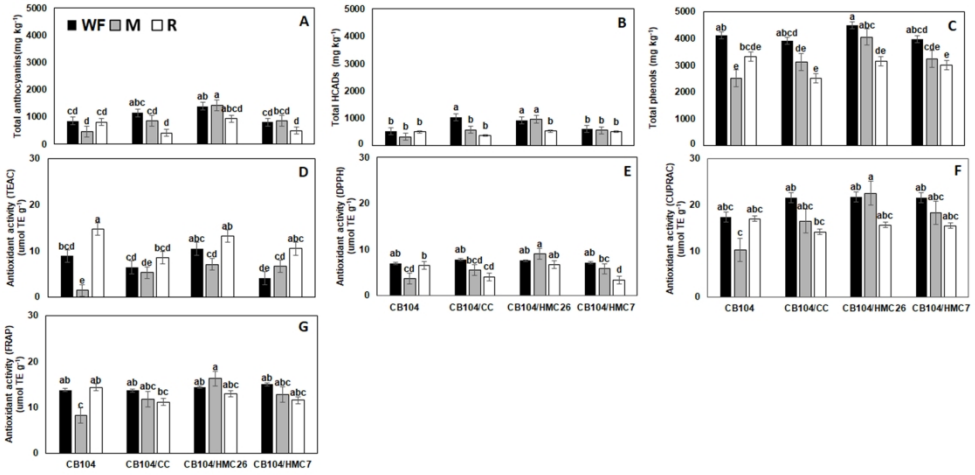

Figure 5. Total phenolic compound concentrations and antioxidant activity (AA) in tubers of Solanum tuberosum from CB2011-104 genotype growing with or without the supply of fungicide. A) Total anthocyanins by HPLC-DAD, B) Total hydroxycinnamic acids by HPLC-DAD, C) Total phenols determined by the Folin-Ciocalteu method, D) AA determined by the TEAC (Trolox equivalent antioxidant capacity) method, E) AA determined by the DPPH (2,2-diphenyl-1-picrylhydrazyl) method, F) AA determined by the CUPRAC (copper reducing antioxidant capacity) method and G) AA determined by the FRAP (ferric reducing antioxidant power) assay. Where CB104: CB2011-104 potato genotype, CC: Claroideoglomus claroideum, HMC7: Funneliformis mosseae, HMC26: Claroideoglomus lamellosum, WF: without fungicide, M: MONCUT, R: ReflectXtra. Different letters indicate significant differences according to Tukey's multiple range test ($P < 0.05$).

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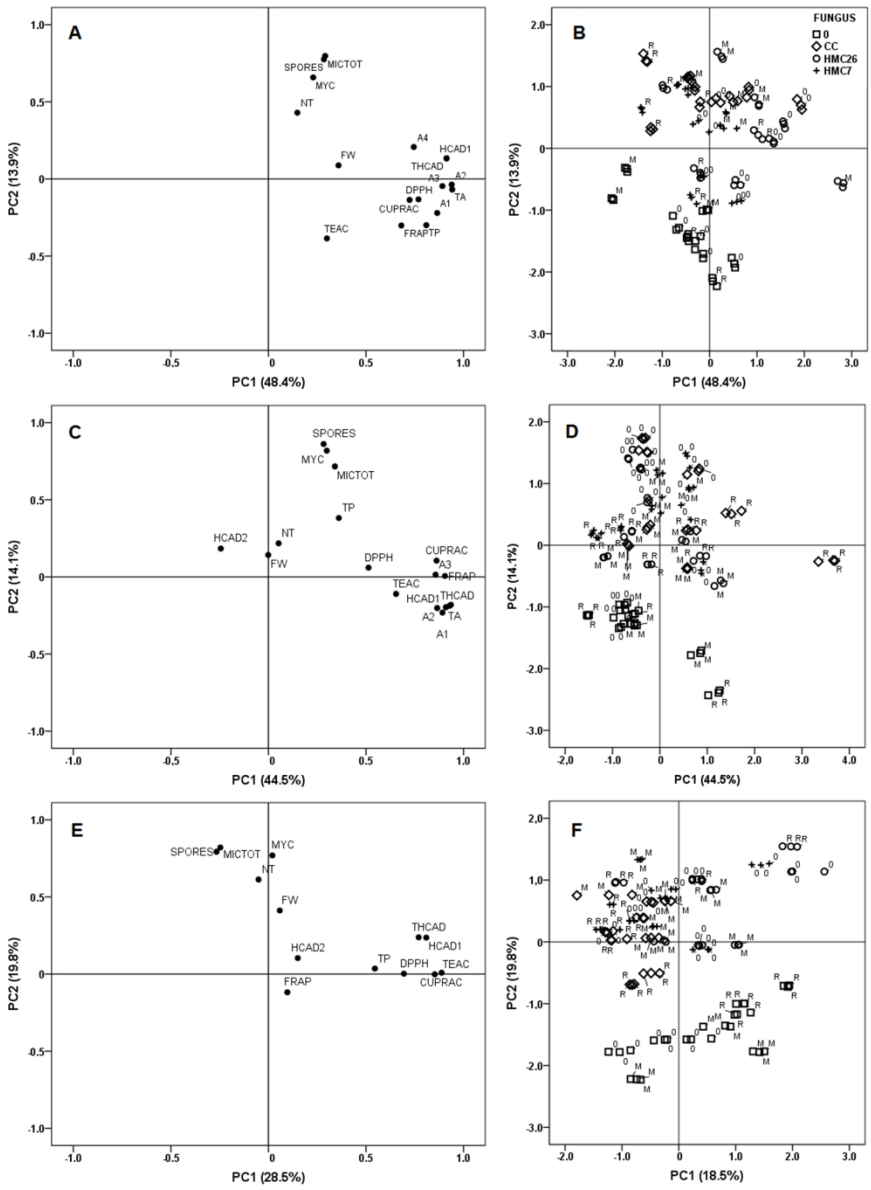

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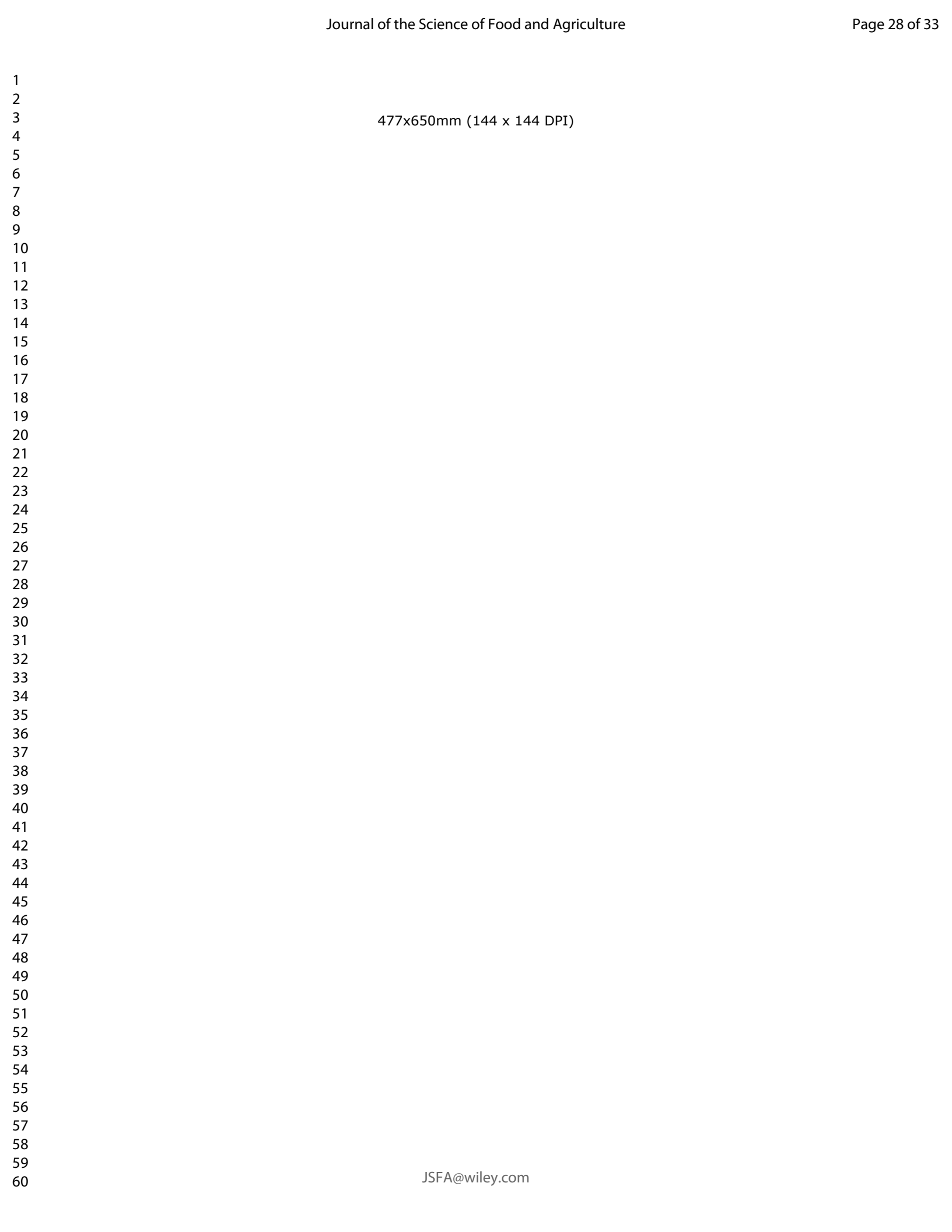
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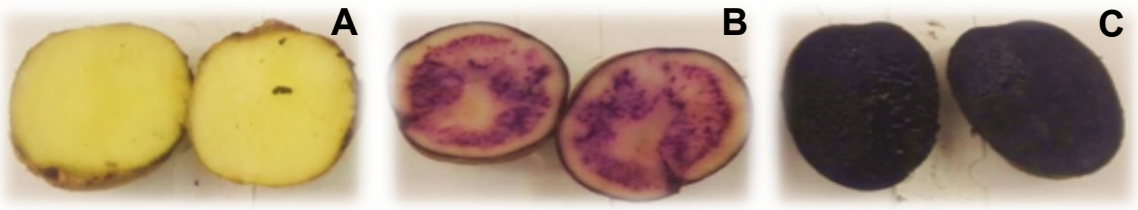
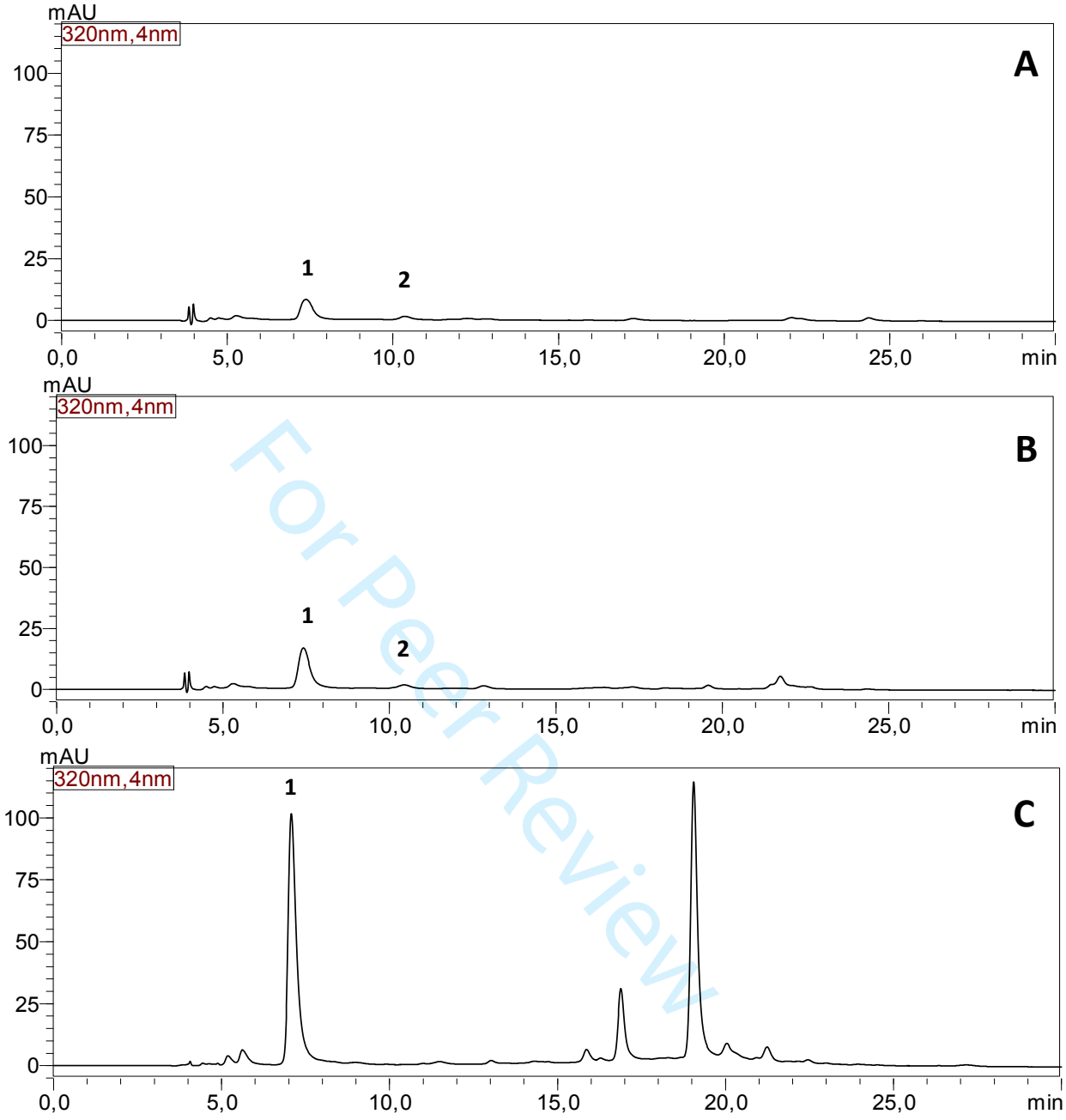


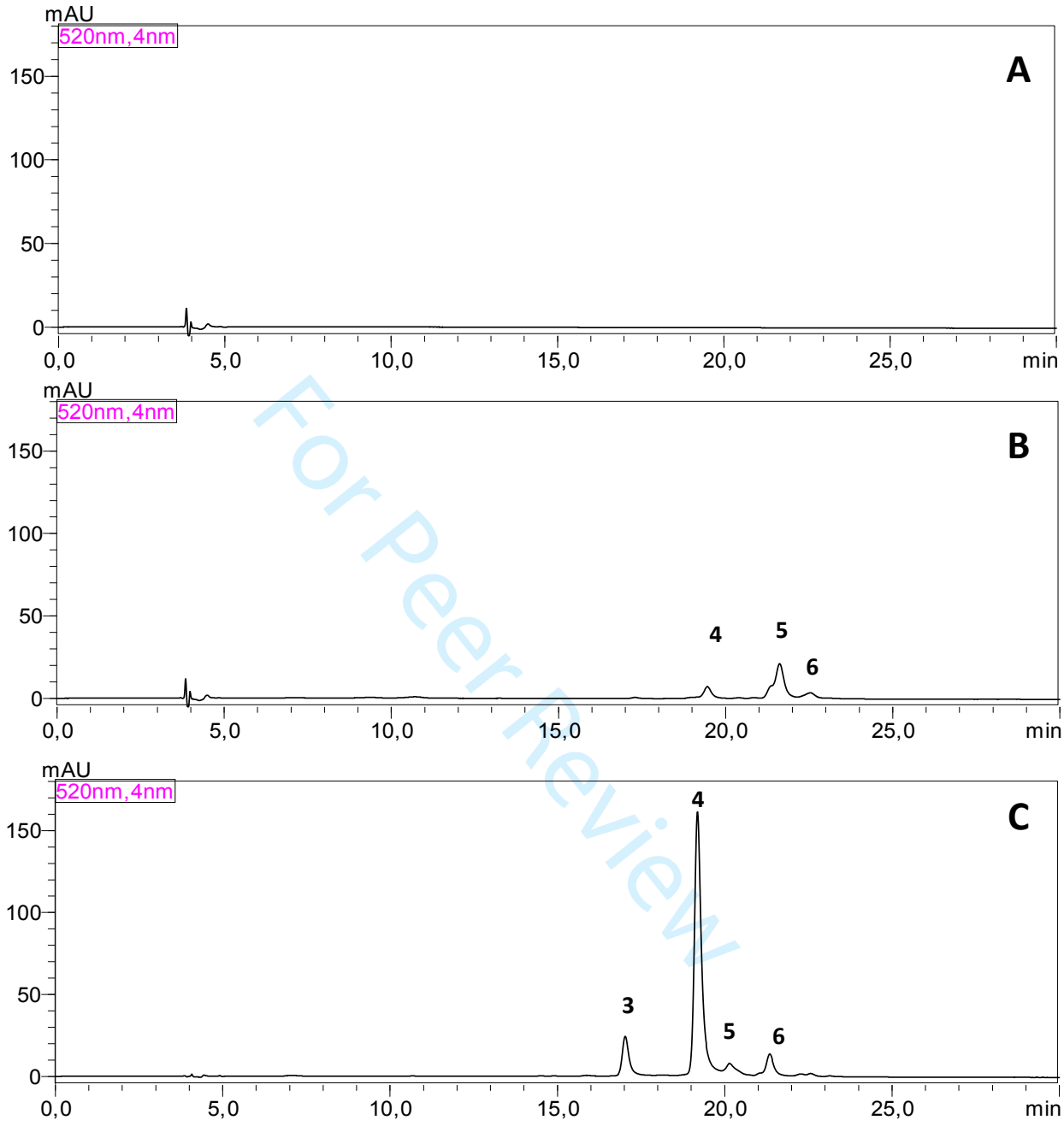
Supplementary material 1: Potato genotypes used in this study. A) VR808 genotype B) CB2011-509 genotype C) CB2011-104 genotype.



Supplementary material 2: HPLC-DAD chromatogram at 320 nm of hydroxycinnamic acid derivatives profiles in potato tubers. A) VR808 genotype, B) CB2011-509 genotype and C) CB2011-104 genotype. Where 1) 5-caffeoylquinic acid and 2) caffeoylquinic acid isomer.

Supplementary material 3: Identification of anthocyanins and hydroxycinnamic acid derivatives in potato tubers by HPLC-DAD-ESI-MS/MS

Peak	Rt (min)	Compound	λmax	Pseudomolecular ion $[M]^+$ or $[M-H]^-$	Product ions
1	7.7	5-caffeoylquinic acid	324	353.0	191.0
2	10.2	Caffeoylquinic acid isomer	327	353.0	190.9
3	17.0	Petunidin-3- caffeoylrutinoside-5-glucoside	531	949.4	787.2; 479.0; 317.0
4	19.2	Petunidin-3-coumaroylrutinoside-5-glucoside	531	933.4	771.4; 479.0; 317.0
5	20.2	Petunidin-3-feruloylrutinoside-5-glucoside	531	963,4	479.0; 317.1
6	21.5	Malvidin-3-coumaroylrutinoside-5-glucoside	531	947.5	493.2; 331.2



Supplementary material 4: HPLC-DAD chromatogram at 520 nm of anthocyanin profiles in potato tubers. A) VR808 genotype, B) CB2011-509 genotype and C) CB2011-104 genotype. Where 3) Petunidin-3- caffeoylrutinoside-5-glucoside, 4) Petunidin-3-coumaroylrutinoside-5-glucoside, 5) Petunidin-3-feruloylrutinoside-5-glucoside and 6) Malvidin-3-coumaroylrutinoside-5-glucoside

Supplementary material 5: Individual anthocyanin and hydroxycinnamic acid concentrations (mg kg^{-1} fresh weight) by HPLC-DAD in *Solanum tuberosum* tubers. Identifications Where peak 1: 5-caffeoylquinic acid, peak 2: caffeoylquinic acid isomer, peak 3: Petunidin-3- caffeoylrutinoside-5-glucoside, peak 4: Petunidin-3-coumaroylrutinoside-5-glucoside, peak 5: Petunidin-3-feruloylrutinoside-5-glucoside and peak 6: Malvidin-3-coumaroylrutinoside-5-glucoside, VR808: VR808 potato genotype, CB509: CB2011-509 potato genotype, CB104: CB2011-104 potato genotype, CC: *Claroideoglomus claroideum*, HMC7: *Funneliformis mosseae*, HMC26: *Claroideoglomus lamellosum*, WF: without fungicide, M; Moncut, R: ReflectXtra. Different letters in a column indicate the presence of statistically significant differences according to the Tukey's multiple range test ($P \leq 0.05$; n = 9). Where nd: not detected.

Treatment	Peak 1	Peak 2	Peak 3	Peak 4	Peak 5	Peak 6
VR808	5.27 ± 2.17 d	3.49 ± 2.71 bc	Nd	nd	nd	nd
VR808/CC	2.72 ± 2.36 d	0.98 ± 1.47 cd	Nd	nd	nd	nd
VR808/HMC26	19.79 ± 3.28 ab	2.30 ± 1.73 cd	Nd	nd	nd	nd
VR808/HMC7	18.29 ± 10.72 abc	2.96 ± 0.1 bcd	Nd	nd	nd	nd
VR808/M	6.8 ± 2.79 cd	2.2 ± 1.7 cd	Nd	nd	nd	nd
VR808/CC/M	8.12 ± 3.82 cd	2.84 ± 1.52 bcd	Nd	nd	nd	nd
VR808/HMC26/M	12.37 ± 6.53 bcd	7.10 ± 2.71 a	Nd	nd	nd	nd
VR808/HMC7/M	7.14 ± 2.62 cd	3.81 ± 3.27 bc	Nd	nd	nd	nd
VR808/ R	24.97 ± 8.98 a	2.94 ± 0.90 bcd	Nd	nd	nd	nd
VR808/CC/R	4.73 ± 1.42 d	3.57 ± 2.38 bc	Nd	nd	nd	nd
VR808/HMC26/R	21.53 ± 18.67 ab	5.70 ± 0.97 ab	Nd	nd	nd	nd
VR808/HMC7/R	3.02 ± 1.10 d	0.41 ± 0.61 d	Nd	nd	nd	nd
CB509	35.04 ± 6.91 bc	2.17 ± 1.22 abc	0.55 ± 0.83 c	10.87 ± 1.56 bc	1.67 ± 0.18 b	nd
CB509/CC	40.94 ± 18.52 bc	2.59 ± 1.19 ab	2.05 ± 0.51 bc	11.90 ± 5.70 bc	2.37 ± 1.18 b	nd
CB509/HMC26	27.39 ± 1.69 bc	3.42 ± 1.17 a	2.16 ± 0.65 bc	13.78 ± 5.16 bc	2.47 ± 0.41 b	nd
CB509/HMC7	60.32 ± 13.71 b	2.50 ± 1.91 ab	5.73 ± 2.39 ab	22.08 ± 6.97 ab	2.77 ± 0.51 b	nd
CB509/M	43.09 ± 18.86 bc	2.90 ± 0.25 a	3.85 ± 1.45 bc	20.15 ± 7.52 abc	2.40 ± 0.76 b	nd
CB509/CC/M	33.30 ± 5.51 bc	3.60 ± 2.58 a	2.20 ± 0.70 bc	22.07 ± 12.02 ab	2.75 ± 1.46 b	nd
CB509/HMC26/M	52.15 ± 28.41 b	0.51 ± 0.77 bc	5.36 ± 3.53 b	18.36 ± 9.97 abc	2.03 ± 0.80 b	nd
CB509/HMC7/M	33.42 ± 14.86 bc	3.21 ± 0.88 a	2.17 ± 0.94 bc	16.07 ± 8.18 bc	2.74 ± 0.88 b	nd
CB509/R	50.16 ± 43.09 b	1.66 ± 1.24 abc	4.90 ± 5.21 b	13.88 ± 11.22 bc	1.95 ± 0.60 b	nd

CB509/CC/R	103.33 ± 41.91 a	0.00 ± 0.00 c	9.70 ± 5.40 a	30.91 ± 15.26 a	5.34 ± 2.66 a	nd
CB509/HMC26/R	39.90 ± 23.41 bc	3.18 ± 1.95 a	3.11 ± 1.57 bc	11.37 ± 1.87 bc	2.20 ± 0.40 b	nd
CB509/HMC7/R	11.02 ± 8.32 c	1.53 ± 1.16 abc	0.54 ± 0.80 c	6.82 ± 4.48 c	1.07 ± 0.80 b	nd
CB104	522.55 ± 182.67 b	nd	143.17 ± 42.49 abc	621.09 ± 143.41 bcd	46.18 ± 23.58 c	37.68 ± 4.94 def
CB104/CC	1036.09 ± 341.24 a	nd	157.15 ± 56.63 abc	849.79 ± 172.37 abc	79.56 ± 20.40 ab	63.32 ± 2.60 ab
CB104/HMC26	925.63 ± 158.94 a	nd	247.09 ± 42.34 a	1007.88 ± 278.81 ab	84.66 ± 11.67 a	56.93 ± 16.56 abcd
CB104/HMC7	603.65 ± 131.36 b	nd	138.21 ± 71.57 abc	581.40 ± 197.62 cd	52.15 ± 19.25 bc	33.21 ± 8.07 ef
CB104/M	324.04 ± 249.77 b	nd	54.46 ± 36.29 c	348.26 ± 227.30 d	24.44 ± 20.15 cd	38.65 ± 18.48 cdef
CB104/CC/M	580.92 ± 135.26 b	nd	117.50 ± 67.98 bc	630.41 ± 298.36 bcd	37.75 ± 9.42 cd	58.47 ± 10.96 abc
CB104/HMC26/M	972.13 ± 257.02 a	nd	241.79 ± 182.46 ab	1040.10 ± 413.78 a	84.58 ± 31.72 a	71.41 ± 3.44 a
CB104/HMC7/M	562.49 ± 128.45 b	nd	121.72 ± 51.36 abc	627.37 ± 218.25 bcd	53.39 ± 17.10 bc	51.54 ± 21.27 abcde
CB104/R	505.02 ± 48.61 b	nd	143.16 ± 68.28 abc	581.17 ± 45.36 cd	36.99 ± 25.40 cd	45.44 ± 2.07 bcdef
CB104/CC/R	378.10 ± 168.35 b	nd	46.42 ± 22.75 c	326.75 ± 150.01 d	13.31 ± 4.10 d	28.26 ± 10.04 f
CB104/HMC26/R	531.99 ± 243.01 b	nd	162.52 ± 127.11 abc	701.40 ± 384.00 abcd	26.35 ± 14.43 cd	42.48 ± 16.76 cdef
CB104/HMC7/R	519.28 ± 15.83 b	nd	60.42 ± 49.46 c	379.52 ± 210.88 d	15.00 ± 3.93 d	36.57 ± 18.81 def