Field Crops Research 315 (2024) 109456

Contents lists available at ScienceDirect

Field Crops Research

journal homepage: www.elsevier.com/locate/fcr

Plant genotype and inoculation with indigenous arbuscular mycorrhizal (AM) fungi modulate wheat productivity and quality of processed products through changes in the frequency of root AM fungal taxa

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ARTICLE INFO

Keywords:
Indigenous AMF inoculum
AMF root diversity
Nutrient uptake
Nutraceuticals
Rheological flour properties

ABSTRACT

Context: Arbuscular mycorrhizal fungi (AMF) have been extensively applied as biofertilizers in wheat to promote crop productivity. However, variability in AM root colonization, grain yield, and nutrients was observed among wheat genotypes and according to AM genotype and environment.
Objectives: We hypothesized that wheat response to AM inoculation is more affected by genotype than environment; the response is driven by increases in AM abundance and community structure changes, and not by modification of composition.
Methods: We inoculated an indigenous AM consortium on four old genotypes (Bianco Nostrale, Andriolo, Abbondanza, Sieve) and one modern variety (Bologna) of bread wheat for two years. The effect was evaluated by assessing grain yield, nutrients, and quality of processed products (flour and breadsticks), while the AM abundance and the community composition and structure in roots were characterized, at two plant growth stages, using morphological and molecular tools.
Results: The functional traits of AMF and plant were better explained by inoculation than by genotype or environment (33 %, 17 %, 4 % of total explained variance), although significant interactions environment x genotype and genotype x inoculation were highlighted. Consistent increases in AM abundance in Sieve and Bologna were associated with positive changes in grain yield and nutrients, supporting the good responsiveness of these genotypes with inoculated AMF, while the plant response of other genotypes was shaped by air temperature and rainfall. However, we did not find significant correlations between changes in AM colonization and mycorrhizal response ratio, with the exception of P and K. After inoculation, AM community composition was similar in all wheat genotypes, but the structure greatly differed among genotypes in interaction with inoculation and plant growth stage. These changes were significantly related to wheat productivity. A *Septoglomus* taxon, present in the inoculum, was the best predictor of wheat performance. The characterization of the community structure at early crop development and maturity allowed the identification of fast and latest active AM colonizers. Our results showed for the first time that AM inoculation affect the rheological parameters and nutraceuticals of processed products, although the response was modulated by genotype.
Conclusions: The selection of responsive wheat genotypes is fundamental for the positive outcome of inoculation. The positive effects on wheat productivity and field persistence of the inoculated AMF support the use of indigenous consortia that have low impacts on resident AMF.
Significance: Our findings advance the understanding of the facilitative mechanisms that underlie compatibility between AMF and wheat genotypes.

1. Introduction

Bread wheat (*Triticum aestivum* L.) is one of the top five cereals with 808 million tonnes of grain harvesteved in 2022 (FAO, 2023a). This crop provides approximately 20 % of the word's intake of food calories and proteins (Shiferaw et al., 2013). It is cultivated in a wide range of agricultural areas, over 200 million hectares (Singh et al., 2022), from Scandinavia and Russia to Argentina, including elevated tropical and

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https://doi.org/10.1016/j.fcr.2024.109456
Received 26 January 2024; Received in revised form 20 April 2024; Accepted 2 June 2024
Available online 8 June 2024
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subtropical regions (Shewry, 2009). China, India, and the Russian Federation are the top three producers accounting for 43 % of the global production (FAO, 2023b).

Global wheat production has increased by 38 % since 2000, due to a considerable increase in yield per unit of area and a slight decline in the cultivated area (FAO, 2023a). Growth in wheat productivity was made possible through breeding programs aiming to develop semi-dwarf, high-yielding varieties, having a broad spectrum of resistance to pests and diseases and highly responsive to fertilizers (Reynolds and Braun, 2022). However, in the recent years, wheat production has suffered due to reduced inputs, threats due to climate change and emerging diseases, and a decline in soil fertility (Shiferaw et al., 2013; Reynolds and Braun, 2022). Actually, the global average rate of increase per year is slow or static and is considered insufficient to meet the demand of the growing population of the world (Shiferaw et al., 2013; Singh et al., 2022). Thus, modern technologies should be applied to develop new wheat varieties that improve yield resilience and sustainability, as well as grain quality, along with innovation in agronomic practices that increase productivity, improve resource use efficiency, and reduce environmental risks (Balyan et al., 2016; Tadesse et al., 2016; Pinke et al., 2022).

Beneficial microbes, such as arbuscular mycorrhizal (AM) fungi (Glomeromycota; Tedersoo et al., 2018), have been applied as biofertilizers and biostimulants in wheat (Pellegrino et al., 2015; Zhang et al., 2018). Field application of AM fungal (AMF) inoculants worldwide was estimated to increase by 5 % and 20 % straw and grain yield, compared to controls not inoculated. Additionally, AMF inoculation increased the grain N and Zn content by 31 % and 13 %, respectively. However, a considerable variability in the AMF root colonization of wheat was observed among varieties and according to the AMF genotype and environmental conditions (e.g., soil fertility, climatic parameters), and mutualistic benefit in terms of host biomass and yield greatly differed (Hetrick et al., 1992, 1993, 1996; García de Léon et al., 2020; Nahar et al., 2020; de Souza Campos et al., 2021; Sawers et al., 2017; Elliott et al., 2021). Great differences in wheat AMF root colonization have been reported among wheat genotypes (Hetrick et al., 1992, 1993, 1996; Kapulnik and Kushnir, 1991), and these differences may be correlated or not with plant response in terms of biomass and P acquisition (Lekberg and Koide, 2005; Lehmann et al., 2012; Sawers et al., 2017). Earlier works have reported a greater mycorrhizal dependency for old wheat cultivars, landrances (before 1950), and ancestors compared to modern cultivars (Hetrick et al., 1992, 1993). However, in a meta-analytical synthesis of published experimental results on different annual crops, Lehmann et al. (2012) demonstrated that new cereal varieties are more mycorrhiza-responsive in terms of biomass than old genotypes and ancestors, but no significant trend could be found according to the year of release (Lehmann et al., 2012). Another meta-analysis carried out to measure the effect of domestication on mycorrhizal responsiveness in 27 species of crops and their wild progenitors showed that wild progenitors benefited from AM symbiosis regardless of availability of P, while domesticated crops only profited under P-limited conditions (Martín-Robles et al., 2018). However, the magnitudes and directions of response were diverse among the 27 crops. These results were explained by the fact that new cultivars were bred to grow faster and produce higher yield under high input of fertilizers, due to lower root lengths, less branched root system, and lower root-to-shoot ratio than their older relatives. Recent field studies comparing old and new bread and durum wheat genotypes reported a range of mycorrhizal responses (MR), from no response to positive effect on grain yield and macro and micronutrients, and no consistent trend with the year of release (Pellegrino et al., 2020; De Santis et al., 2022). In a comprehensive study to identify genotypic differences in bread wheat with respect to AMF root colonization (i.e., 94 genotypes different in the year of release), significant effects were detected for the interactions of genotype x environment and genotype x year, with moderate repeatability between years or environments (Lehnert et al., 2017). This demostrates a moderate heritability for AMF root colonization.

The outcome of symbiosis is modified not only by the interaction between wheat genotype and year/environment, but also by the interaction between wheat and AMF genotype (Lehnert et al., 2017; Pellegrino et al., 2015; Zhang et al., 2018). A high variation in the outcome of the symbiosis was reported for different associations between wheat genotypes and AM fungal taxa (Pellegrino et al., 2015), although the variance explained by AMF fungal species was lower (20 %) than that explained by soil (60–90 %) or climatic parameters (72 %). These results are mainly derived from single-species AMF inocula tested in the field (i.e., *Entrophospora etunicata*, *Funneliformis caledonium*, *Funneliformis mosseae*, *Gigaspora margarita*, *Rhizophagus fasciculatus* and *Rhizophagus intraradices*), while multiple-species inocula were rarely tested and do not include more than three species.

Since inoculation with exotic AMF can decrease the diversity of resident microbial communities (Schwartz et al.,2006), indigenous AMF inoculants (single or mixed) were encouraged and successfully applied for the cultivation of many field crops (e.g., Egyptian clover, alfalfa, chickpea, maize, sunflower, barley) (Jansa et al., 2008; Pellegrino et al., 2011, 2022; Pellegrino and Bedini, 2014; Jerbi et al., 2022; Arcidiacono et al., 2024). Benefits in plant biomass, yield, and nutrient uptake were observed when indigenous AMF consortia were applied, and these benefits were comparable to those of exotic AMF inoculants. In alfalfa, the benefits (i.e., forage dry weigh, N, P and fatty acid content) were explained by molecularly detected increases in abundance of an indigenous AMF taxon (Pellegrino et al., 2022). Similarly, comparable benefits were observed in barley (e.g., shoot biomass, shoot P, Fe, Cu, Zn, Ca, Na, and K content), after inoculation with indigenous and exotic mixed AMF inoculants, and the pattern of variability was explained by soil abudance of AM fungi (Jerbi et al., 2022).

Previous experiments, testing the effect of field inoculation with exotic AM fungi, led to a manipulation of abundance, community composition, and structure simultaneously (Ercoli et al., 2017b; Pellegrino et al., 2020). In this work, we inoculated an indigenous AMF consortium in five bread wheat genotypes for two years of cultivation to dissect the effect of the interaction of the wheat genotype and the environment on the symbiotic outcome, removing the effect of the change in AMF composition due to the inoculation of exotic AM fungi (Fig. S1). The benefits of AM inoculation were evaluated by assessing grain yield, nutrient concentration, and quality of processed products (i.e., flour and breadsticks), while the abundance and the community composition and structure of AMF in the roots were evaluated, at different plant growth stages, using morphological and molecular tools. The composition indicates which AMF species are present, while the structure adds the information on the frequencies/abundances of the species constituting the AMF community. The indigenous inoculum was composed of many AM fungal species, isolated from soil located in the same agricultural area where the experiment was carried out. We hypothesized that: (i) the wheat genotype exerts greater control over the response of the plant to AMF inoculation than the environment; (ii) the response of wheat to the inoculation is driven by the increases in AM fungal abundance and changes in the structure of the AM fungal community, and not by the modification of the composition of AMF.

2. Material and methods

2.1. Fungal and plant material

The AM fungi used as inoculum were a consortium of AMF taxa originating from a local field site (Pellegrino and Bedini, 2014). The AM fungal inoculum was composed of 14 species belonging to five families: *Acaulospora cavernata*, *Acaulospora spinosa*, *Acaulospora* spp., *Diversispora spurca*, *Funneliformis coronatum*, *Entrophospora etunicata* (syn. *Claroideoglomus etunicatum*), *Funneliformis geosporum*, *Funneliformis mosseae*, *Glomus* spp., *Rhizophagus clarus*, *Rhizophagus irregularis*, *Scutellospora aurigloba*, *Scutellospora calospora* and *Septoglomus viscosum*. Five wheat genotypes were tested: Bianco Nostrale, Andriolo,

Abbondanza, Sieve (old tall genotypes registered in the Tuscany Germoplasm Bank and in the Italian Register of Agricultural and Food Interest Biodiversity) and Bologna (a modern dwarf variety registered in the Italian Agricultural Information System) (Table S2).

2.2. Experimental field site

The experiment was carried out in the 2020 and 2021 growing seasons at the organic farm 'Azienda Agricola Grappi Luchino', Pienza, Siena, Tuscany (43 ° 03'35" N-11 ° 42'07" E; 355 m above sea level) in two adjacent fields. The soil of 2020 and 2021 showed a similar texture and low nutrients availability (Table S1). Averaged over the two fields, the soil texture was silty clay (11.4 % sand, 43.6 % silt and 45.0 % clay; USDA-NRCS, 1996). The soil had a low content of organic carbon (C), 13.9 g kg^{-1} in 2020 and 19.1 g kg^{-1} in 2021 (Walkley-Black; Nelson and Sommers, 1982), the pH indicated moderate alkaline, 8.4 in 2020 and 8.2 in 2021, (deionized water 1:2.5 w/v; McLean, 1982). The concentrations of nutrients in the soil was in 2020 and 2021: 1.10 and 1.16 g total N kg^{-1} (Kjeldahl; Bremner and Mulvaney, 1982), 8.0 and 12.9 mg available P kg^{-1} (Olsen) (Olsen and Sommers, 1982), both indicating a low nutrient status; 10.0 and 15.6 mg available Fe kg^{-1} (diethylene--triamine-penta acetic acid method, DTPA; Lindsay and Norvell, 1978), indicating an optimum level; 0.6 and 1.9 mg available Zn kg^{-1} (DTPA method; Lindsay and Norvell, 1978), indicating a low/adequate level. The C/N ratio was 7.5 in 2020 and 9.5 in 2021. Furthermore, the cation exchange capacity (CEC) was equal to 23.1 and 23.5 in 2020 and 2021, respectively. The climate of the site is cold and humid Mediterranean (Csa), according to the Köppen–Geiger climate classification (Kottek et al., 2006) with a 10-year average of annual precipitations of 791 mm, a mean annual maximum and minimum daily air temperatures of 19.2 °C and 10 °C, respectively. During the wheat cropping cycle in 2020 (January-July), mean maximum and minimum temperatures and total precipitation were 19.2 °C, 9.6 °C and 260 mm, and in 2021 18.6 °C, 8.9 °C and 257 mm (Fig. S2).

2.3. Experimental set-up and sampling

A complete factorial experimental design with three factors was adopted with two years of cultivation (2020 and 2021), five wheat genotypes, and two AMF inoculation treatments (inoculated with the AMF consortium, +M; mock-inoculated/control, -M). The experiment was arranged in a completely randomized design with three replicate plots (8 m x 25 m = 200 m^2) (Fig. S3). The inoculum, produced as described by Pellegrino and Bedini (2014), was a micronized mixture of mycorrhized roots of sorghum (*Sorghum halepense* L.), spores, hyphal fragments, and bentonite as carrier. The inoculum was distributed at sowing by manual application to seeds that had been previously moistened with water. The rate of the AMF inoculum was 0.24 g m^2 (2.4 kg ha^{-1}) (1.2 kg of inoculum 100 kg^{-1} seeds, about 750 spores ha^{-1}). The mock inoculum (control) consisted of the same dose of steam-sterilized AMF inoculum (121 °C for 25 min on two consecutive days). To ensure a common microflora, both inocula received 0.05 L kg^{-1} of a filtrate obtained by filtering through a Whatman no. 1 filter paper the AMF consortium. The seed rate was 200 kg ha^{-1}, corresponding to approximately 450 viable seeds per m^2, distributed in rows 14 cm apart. Bread wheat was seeded with a pneumatic seeding machine (Gaspardo Pinta) on 23 January 2020 and 19 January 2021. Before the experimental setup, the preceding crop for both fields was clover (*Trifolium alexandrinum* L.), cultivated following the organic agriculture procedure. Soil tillage was carried out in autumn by mouldboard ploughing at a depth of 25 cm soil, and by harrowing at a depth of 15 cm immediately before seeding. No organic/chemical fertilizer was applied. No weed and pest/pathogen control treatments were applied. In both years of cultivation at stages of two-leaves unfolded (GS12) (Zadoks et al., 1974) and at physiological maturity (GS90), ten plants, randomly selected in each replicate plot, were excavated with their root system to determine AMF abundance and diversity. Furthermore, in GS12, shoots from ten plants were randomly collected in each replicate plot. Bread wheat was harvested in each replicate plot by a combine harvester (Laverda, Vincenza, Italy) on 16 July 2020 and 20 July 2021.

2.4. Mycorrhizal abundance in bread wheat roots

At each sampling, fresh roots from each replicate plot were combined and cleaned from the attached soil by soft washing with tap water. Mycorrhizal abundance was measured by the percentage of root length containing arbuscules and vesicles and the percentage of AM fungal root colonization. These AMF root traits were assessed under an optical microscope after root clearing and staining (Phillips and Hayman, 1970) and using the modified grid-line intersect method (McGonigle et al., 1990). Root sub-samples were stained as follows: cleared with 10 % KOH in a 90 °C water bath for 30 min, neutralized in 1 % aqueous HCl and stained with 0.05 % trypan blue in lactic acid. The roots were mounted on microscope slides and covered with 40 × 22 mm coverslips. Sixty root fragments for each sample were aligned parallel to the long axis of the slides and observed with a light microscope (Leitz Laborlux S, Wetzlar, Germany) at a magnification of x125–500. Each slide was moved along the long axis five times and the negative and positive intersections were counted by a grid line intersect ocular. Negative intersections were those without fungal material in roots, whereas positive interactions were those with arbuscules, vesicles, or hyphae. A total of 200 intersections were evaluated for each slide.

2.5. Grain yield and nutrient uptake

At GS12, the concentration of P in the shoots was determined according to the ammonium-molybdophosphoric blue color method (Chapman and Pratt, 1961). At physiological maturity, the grain yield was determined by oven drying at 65 °C up to a constant weight. The concentration of N and P in grains was determined by the Kjedahl method (Jones et al., 1991) and the ammonium-molybdophosphoric blue color method (Chapman and Pratt, 1961), respectively. Furthermore, the concentrations of potassium (K), calcium (Ca), magnesium (Mg), copper (Cu), Fe, manganese (Mn) and Zn in the grains were determined by a microwave-assisted acid digestion system (COOLPEX Smart Microwave Reaction System, Yiyao Instrument Technology Development Co., Ltd., Shanghai, China) and a microwave plasma atomic emission spectroscopy (4210 MP-AES, Agilent Technologies, Santa Clara, CA, USA). Details of the nutrient analysis methods are given in Supplementary Methods S1. Host benefits were calculated as:

$$\frac{(\text{MGY or MNutriC} - \text{NMGY or NMNutriC})}{\text{NMGY or NMNutriC}} \text{x}100$$

where: MGY: grain yield in inoculated plants; MNutriC: nutrient concentration in inoculated plants; NMGY: grain yield in not inoculated plants; NMNutriC: nutrient concentration in not inoculated plants.

2.6. Milling and breadsticks making

Before milling, the grain was conditioned to ~15 % moisture content for 15 h. The grain was ground using a stone grinding mill (EPOM-600, Partisani, Forlì-Cesena, Italy) and three fractions were produced: whole-mill flour (type 1, which contains germ and bran), semolina and bran. The whole-mill flour was obtained using a 300 μm diameter mesh. The flour was then stored at 6 °C until further analysis. Breadsticks were produced at the 'Forno Moderno' (Certaldo, Tuscany, Italy) from Bianco Nostrale whole-meal flour obtained from the 2020 and 2021 years of cultivation. The breadstick making recipe consisted of flour (1.5 kg), 40 g of dry yeast (*Saccharomyces cerevisiae*), 30 g of salt and 1 L of water. The ingredients were mixed with a professional planetary kneading machine (Planetary Kneading; Sammic, Bergamo, Italy). The doughs were processed with an automatic sheeter to obtain breadsticks

(Industrial Breadstick Machine; Prim Italia Srl, Milan, Italy). The breadsticks were automatically placed on stainless steel baking trays. After the leavening phase of 30 min at 32°C, the breadsticks were cooked in an electric oven at 168 °C for 27 min, cooled to room temperature, and finally packaged in a transparent polypropylene film, each containing approximately 50 g. The breadsticks were stored at −20 °C until analysis.

2.7. Rheological determinations in flour

The Chopin alveograph test was carried out on whole-meal flour (1 kg) obtained from the 2020 field experiment. Three alveographic indices were recorded for each sample of the alveographac curve: P that corresponds to the height (mm) of the alveograph curve and is related to the tenacity of the dough; L that corresponds to the length of the alveograph curve (mm) and is an index of dough extensibility; W ($J\times10^{-4}$) that corresponds to the total area of the alveograph curve and is related to the strength of the dough. The swelling index (G in ml) was calculated as the square root of the volume of air necessary to inflate the dough bubble until it ruptures ($G=2.226 \times L^{1/2}$) (Bordes et al., 2008). The ratio P/L was also calculated. Furthermore, the gluten content (g dry gluten 100 g^{-1} dw flour) was determined as described in the AACC Approved Methods 38–10.01 and 38–12.02 (Finnie and Atwell, 2016).

2.8. Nutraceutical determinations on flour and breadsticks

The total phenol content (TPC) and antioxidant activity were determined in the flour of all treatments (n=30) obtained in the 2020 field experiment and in the Bianco Nostrale flour (n=6) obtained in 2021. Furthermore, TPC and antioxidant activity were determined on breadsticks obtained from the Bianco Nostrale flour of the 2020 and 2021 experiments (n=6 in both years). Before analysis, breadsticks were manually ground using a mortar and a pestle and homogenized. Phenolics were extracted by applying a modification of the method described by Moore et al. (2005). Briefly, 4 g of whole-mill flour or ground breadstick was mixed with 40 ml of 50 % acetone. The mixture was centrifuged at 2500 g for 10 min. The supernatant was filtered through a 0.45 μm filter, and stored at −20 °C until analysis. The total phenolic content (TPC) of each extract was estimated using the Folin-Ciocalteu (F-C) reagent by the method described by Singleton et al. (1999) with some modifications. The reaction mixture contained 1 ml of pure water, 100 μL of sample extract, 100 μL of the 2 N solution of the F-C reagent, and after 20 min it was neutralized with 800 μL of Na_2CO_3 75 g L^{-1} solution. After 60 min at room temperature and darkness, the absorbance of the solution was measured at 760 nm against a blank reagent. Gallic acid was used as the standard and TPC was expressed as mg of gallic acid equivalence (GAE) per g of flour (fresh weight). For calibration, standard solutions of gallic acid (range 0–150 μg ml^{-1}) were prepared. The determination was carried out in triplicate.

The antioxidant activity was determined using the oxygen radical absorbance capacity (ORAC) assay kit (Cell Biolabs Inc., USA), according to the manufacturer's protocol. The assay was performed in black-walled 96-well plates (NuncTM black microwell, Japan). The antioxidant standard curve was prepared using the water-soluble vitamin E analog TroloxTM. Each well contained 25 μL of 50 % acetone extracts or Trolox standard (range 2.25–50 μM) and 150 μL fluorescein (final concentration 1 M) which were incubated at 37 °C for 30 min. After incubation, 25 μL Free radical Initiator solution was added to each well. Fluorescence intensity was measured using a fluorescence microplate reader (Wallac Victor 3 Multilabel Plate Reader, PerkinElmer Inc., Waltham, MA) at an excitation of 485 nm and an emission of 520 nm for 20 cycles per min at 37 °C. The ORAC results were expressed as μmol Trolox equivalents per 100 g of fresh weight. The determination was made in triplicates.

2.9. Mycorrhizal diversity in bread wheat roots

At both growth stages (GS12 and GS90), a root subsample per each replicate plot of the field experiment carried out in 2021 was prepared for DNA extraction employing a combination of washing and ultrasound treatments to simultaneously separate the rhizospheric fraction (1 mm root soil attached) from roots and the roots colonized by endophytes (Bulgarelli et al., 2015). Briefly, roots were placed in a sterile flask with 50 ml of sterile Phosphate buffered saline solution (PBS; pH 7.0) (130 mM NaCl, 7 mM Na_2HPO_4, 3 mM Na_2HPO_4, 0.02 % Silwet L-77, Merk, Germany) and stirred/washed for 20 min at 180 rpm on a shaking platform in order to clean all soil (rhizospheric compartment) from root surfaces. The roots were then transferred to a new sterile falcon tube and subjected to a second washing treatment (in 25 ml of PBS solution). The doubled-washed roots were then transferred to a 15 ml sterile falcon tube and sonicated for 10 min at 40 kHz (Branson 2200 Ultrasonic Cleaner) for 10 intervals of 30 s pulse and 30 s pause to enrich microbes that flourish in close association with root tissues (endophytes) and remove the root surface tightly adhering microbes (rizhoplane microbes). The roots were then removed from the PBS solution and stored at −20 °C in 15 ml sterile falcon tubes until molecular analysis.

Genomic DNA was extracted from root samples (1 g of fresh weight) using the Dneasy Plant Mini Kit (Qiagen, Germany) (five wheat genotype x three replicate plot x wo AMF inoculation level x two growth stages = a total of 60 samples). DNA extracted was quantified by a spectrophotometer (NanoDrop Technology, Wilmington, DE), verified by Qubit fluorimetric quantification (Thermo Fisher Scientific, USA), and then stored at −20 °C until analysis. The small subunit ribosomal RNA (SSU) fragments were amplified using a nested PCR approach with two pair of primers. The forward primer AML1 (5′-ATC AAC TTT CGA TGG TAG GAT AGA-3′) and the reverse primer AML2 (5′-GAA CCC AAA CAC TTT GGT TTCC-3′) were used in the first step, while the forward primer WANDA-ill (5′-**TCG TCG GCA GCG TCA GAT GTG TAT AAG AGA CAG ANN NHN NNW NNN HG**C AGC CGC GGT AAT TCC AGCT-3′) (Dumbrell et al., 2011) and the reverse primer AML2- ill (5′-**GTC TCG TGG GCT CGG AGA TGT GTA TAA GAG ACA G**GA ACC CAA ACA CTT TGG TTT CC-3′) were used in the second step (in bold, the adaptors for the Illumina reaction) (Lee et al., 2008). PCRs were generated from 10 ng μl^{-1} genomic DNA in volumes of 25 μl with 0.125 U μl^{-1} of GoTaq® Hot Start Polymerase (Promega Corporation, WA, USA), 0.5 μM of each set of primers for AMF, 0.2 mM of each dNTP, 1 mM of $MgCl_2$ and 1x reaction buffer, using a S1000 Thermal Cycler™ (BioRad, Hercules, CA, USA). For both steps, the PCR cycle consisted of an initial denaturation at 95 °C for 2 min followed by 25 cycles of 94 °C for 30 s, 59 °C for 45 s, 72 °C for 1 min 30 s and 72 °C for 10 min. The quality of all PCR products was examined by gel electrophoresis through a 2 % agarose gel in 1 × TBE buffer, then purified with magnetic beads (Agencourt AMPure® XP, Beckman Coulter, USA) and freshly prepared 80 % ethanol. Then, DNA concentration was quantified by fluorescence with the Quant-iTTM dsDNA High-Sensitivity Assay Kit (Invitrogen by Thermo Fisher Scientific, CA, United States), following the manufacturer's instructions. The cleaned and quantified amplicons of each library (a total of 30 amplicons per each growth stage) were adjusted in an equimolar ratio (10 ng μl^{-1}) for the addition of dual-index barcodes using the Nextera® XT DNA library preparation kit (Illumina Inc., CA, United States), and the generated metabarcoding libraries were sequenced on an Illumina MiSeq sequencer (2 × 300 bp paired-end reads) at the University of York (UK), loading a 12-pM final library concentration with 20 % PhiX library spike-in (Illumina) and an Illumina MiSeq V3 600 cycle sequencing kit.

2.10. Statistical analysis and bioinformatics

A three-way analysis of variance (ANOVA) was performed to test the effect of year of cultivation (Y), wheat genotype (G) and AM fungal inoculation (Inoc) on mycorrhizal abundance in wheat roots, grain yield

and nutrient uptake. G and Inoc were considered as fixed factors and Y as a random factor. A two-way ANOVA was performed to test the effect of G and Inoc on the rheological parameters and nutraceuticals of flour obtained from the 2020 field experiment. Furthermore, a one-way ANOVA was performed to test the effect of Inoc on the nutraceuticals of the Bianco Nostrale breadsticks obtained from the 2020 and 2021 experiments, and on the nutraceuticals of the Bianco Nostrale flour obtained from the 2021 experiment. Data were transformed if necessary (e.g., log10, arcsen). Differences between means were determined using the post-hoc Tukey-B procedure. The data shown in the box plots are for untransformed data. The solid black line in the box plots represents the median of the data, the box represents the range between the first and third quartiles, and the lines outside the box represent the minimum and maximum values of the data set. All analyzes were performed using the SPSS 25.0 software package (SPSS Inc., Chicago, IL, USA) and plotted using the ggplot2 package (Wickham and Chang, 2008) in R.

A multivariate approach based on permutational analysis of variance (PERMANOVA) was performed to test the effect of year of cultivation (Y), wheat genotype (G) and AMF inoculation (Inoc) and their interactions on the functional parameters of the plant and AMF. Data in the matrix were square root transformed, standardized, and Euclidean distance matrices were calculated. Since PERMANOVA was statistically significant, principal coordinate analysis (PCO) was performed to visualize the most relevant patterns in the data. In the PCO biplot, the overlay of vectors is reported. Since PERMANOVA is sensitive to differences in multivariate location (average community composition of a group) and dispersion (within-group variability), the analysis of homogeneity of multivariate dispersion (PERMDISP; Anderson, 2006) was performed to check the homogeneity of dispersion among groups (beta-diversity) (Anderson et al., 2006).

Raw sequence data generated from the Illumina MiSeq sequencing run of the 60 samples (2021 year of cultivation: 30 samples per growth stage) were processed and analyzed using the QIIME2 (2018.11) pipeline and plugins (Bolyen et al., 2019). Demultiplexed forward and reverse paired-end reads were joined using the '-fastq_mergepairs' of the USEARCH plugin (Edgar, 2010). From 2021 MiSeq sequencing, out of the 71,760 reads exposed to merging, 84 % (60,203 reads) were successfully merged, and 19 % (13,543 reads) were aligned with zero differences. The primer sequences were trimmed from the sequences using the cutadapt plugin 1.18 with Python 3.5.5, and 60,089 valid sequences were obtained after optimization. The average read length was approximately 300 base pairs (bp) based on the maximum expected error (MaxEE). The command USEARCH 'fastq_eestats2' was used to check sequence quality and, based on the percentage MaxEE, the reads were truncated at the drop-off point of 280 bp using the USEARCH 'fastq_filter' command. Quality-filtered reads were dereplicated using the USEARCH 'fastx_uniques' command and operational taxonomic units (OTUs) were generated by clustering reads at a 91.3 % similarity threshold using the USEARCH 'cluster_otus' command. During the process, chimeric sequences and singletons were also removed. The resulting OTUs were assigned to virtual taxa (VTXs) using the MaarjAM database (https://maarjam.botany.ut.ee). All representative newly generated sequences were deposited in the NCBI Sequence Read (SRA) database as SUB14096345 (accession numbers from OR978407 to OR978439). Representative sequences were aligned with NCBI sequences of closely related AM fungal species (33 and 24 sequences, for a total of 57 sequences), using the MAFFT online service (Katoh et al., 2019), and a Neighbor-Joining (NJ) tree was built using MEGA11 (Tamura et al., 2021), following the bootstrap test of phylogeny with 1000 bootstraps. The substitution model used was the Kimura 2-parameter with uniform rates among sites, pairwise deletion, and 7 threads. The evolutionary analysis was conducted in MEGA11 (Tamura et al., 2021), and the NJ tree was edited using Adobe Illustrator 2022.

The AMF richness (S) was calculated as the number of VTXs per sample. Shannon index (H') and Simpson index (λ) were calculated as follows: $H' = SUM(Pi*Log10(Pi))$ and $1- \lambda' = 1-SUM(Ni*(Ni-1)/(N*(N/1))$. In the formulas Pi is the proportion of individuals belonging to the i^{th} VTX, Ni is the number of individuals to the i^{th} VTX, and N is the total number of individuals of all VTXs. To test the effect of G, Inoc, and growth stage (GS) on S, H' and λ, a three-way ANOVA was performed. G, Inoc, and GS were considered as fixed factors. The data were transformed (i.e., log10). The differences between means were determined using the post-hoc Tukey-B procedure. Data given in the box plots are for untransformed data. All analyzes were performed using the SPSS 25.0 software package (SPSS Inc., Chicago, IL, USA) and plotted by ggplot2 package (Wickham and Chang, 2008) in R.

PERMANOVA was then performed to test the effect of wheat genotype (G), AMF inoculation (Inoc), and growth stage (GS) and their interactions on the AMF community structure (relative abundances of AMF Virtual Taxa, VTX). The relative abundance of the AMF in each sample was calculated based on the ratio between the number of sequences affiliated with each VTX and the total number of sequences obtained from the clone library. Data from the AMF matrix were fourth-root transformed and the Bray-Curtis matrix of similarity was calculated. The P-values in PERMANOVA [P(perm)] were calculated using the Monte-Carlo test (999 permutations) (Anderson and Braak, 2003). The explained variance was calculated and divided among the sources of the variation. PERMDISP (Anderson, 2006) was performed to check the homogeneity of dispersion among groups (beta-diversity) (Anderson et al., 2006). Data were plotted, according to the significance of PERMANOVA, using a non-metric multidimensional scaling (nMDS). In the 3-D nMDS plot the overlay of vectors and only the AMF VTXs with a strong correlation ($r > 0.6$) are displayed. The datasets were also used to generate Venn diagrams, representing the OTUs unique and shared to each treatment interaction (i.e., composition; data are expressed as percentages), according to the significance of PERMANOVA. Venn diagrams were generated using InteractiVenn (Heberle et al., 2015) and edited by Adobe Illustrator 2022.

To understand the relationship between the structure of the AM fungal community and plant parameters and to identify the main AMF taxa responsible for plant functional changes, a multivariate statistical approach (RELATE analysis) was applied to determine the strength of the correlation between two matrices in rank-order patterns of dissimilarity (Clarke and Warwick, 2001). The analysis was based on the Spearman rank and 999 permutations with ρ equal to 1 representing the perfect relationship, and the result was plotted as a graph. Since RELATE was significant, we displayed the structure of the AM fungal community at GS90 using a two-dimensional nMDS plot and the plant parameters using a PCO biplot. To find the best descriptor of the relationship, the BEST analysis, based on BioEnv methods (all combinations), Spearman rank, and 999 permutations, was applied (Clarke et al., 2008). All multivariate analyzes were performed using PRIMER 7 and PERMANOVA + software (Clarke and Gorley, 2006, 2015; Anderson et al., 2008).

3. Results

3.1. AM fungal abundance and agronomic effectiveness under field inoculation of wheat

At GS12, AM fungal root colonization and percentage of arbuscules were significantly affected by the G x Inoc interaction. Averaged over two years of cultivation, AM fungal root colonization increased in response to inoculation only in Sieve and Bologna (+38 % and +28 %, respectively), while the percentage of arbuscules increased by +25 % and +21 %, respectively (Fig. 1a,b; Table S3). Furthermore, at GS12, in both years the percentage of vesicles was similar for all genotypes except Sieve, showing higher rates in 2020 than in 2021 (+5 %).

Similarly, at GS90, percentage of vesicles did not vary across years in all genotypes, except for Bianco Nostrale, showing a higher value (+6 %) in 2020 than in 2021 (Fig. S4a,b; Table S3). At GS90, AMF root colonization greatly differed among varieties in 2020, with Abbondanza

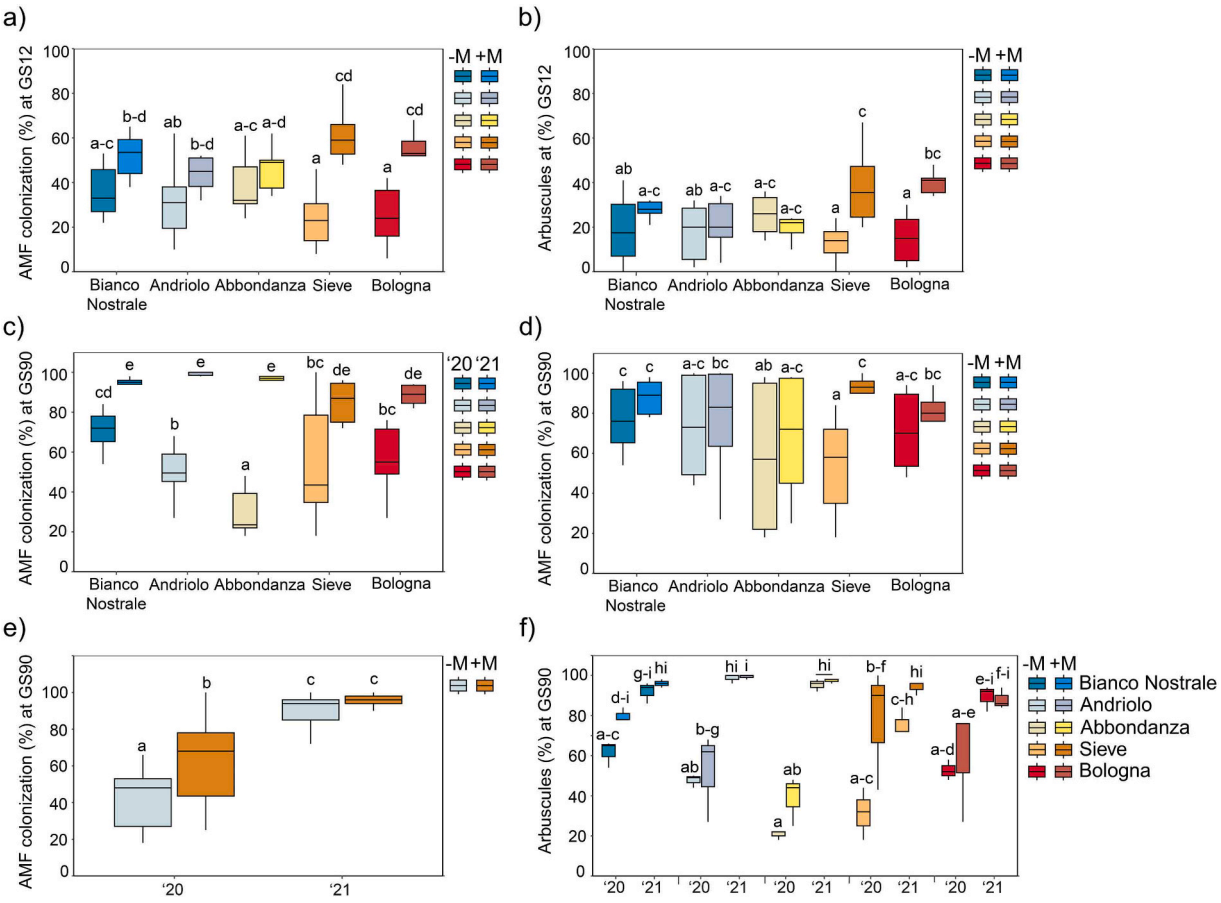

Fig. 1. Effect of the interactions among year of cultivation (Y), wheat genotype (G) and arbuscular mycorrhizal fungal (AMF) inoculation (Inoc) on AMF abundance in roots (Table S3). Effect of the significant interactions: between G and Inoc on the AMF root colonization at GS12 and GS90 (a, d), and on the percentage of root length containing arbuscules at GS12 (b); between Y and G on AMF root colonization at GS90 (c); between Y and Inoc on AMF root colonization at GS90 (e), and among Y, G and Inoc on percentage of root length containing arbuscules at GS90 (f). The years of cultivation were: 2020 ('20) and 2021 ('21). Five bread wheat genotypes: Bianco Nostrale, Andriolo, Abbondanza, Sieve (old tall genotypes registered in the Tuscany Germoplasm Bank and in the Italian Register of Agricultural and Food Interest Biodiversity), and Bologna (a modern dwarf variety registered in the Italian Agricultural Information System, SIAN) (see Table S2). The AMF inoculation treatments were: inoculated with of 14 AMF species originating from a indigenous agricultural site (+M), and mock-inoculum as control (-M). AMF abundance in roots was measures at two growth stages: two-leaves unfolded stage (GS12; Zadoks et al., 1974) and physiological maturity (GS90). The output of the boxplots is based on three replicate plots for each treatment. Different letters highlight statistically significant differences according to Tukey-B-test.

displaying the lowest value (about 30 %) and Bianco Nostrale, Sieve, and Bologna the highest (on average 61 %), while in 2021 it increased up to saturation in all varieties (on average 93 %) (Fig. 1c; Table S3). At the same growth stage, the AMF root colonization, averaged over two years, was not affected by inoculation in all genotypes, except Sieve that showed an increase after inoculation (+32 %) (Fig. 1d; Table S3).

Finally, AMF root colonization was affected by the interaction Y x Inoc, with lower values in 2020, but a significant promotion due to

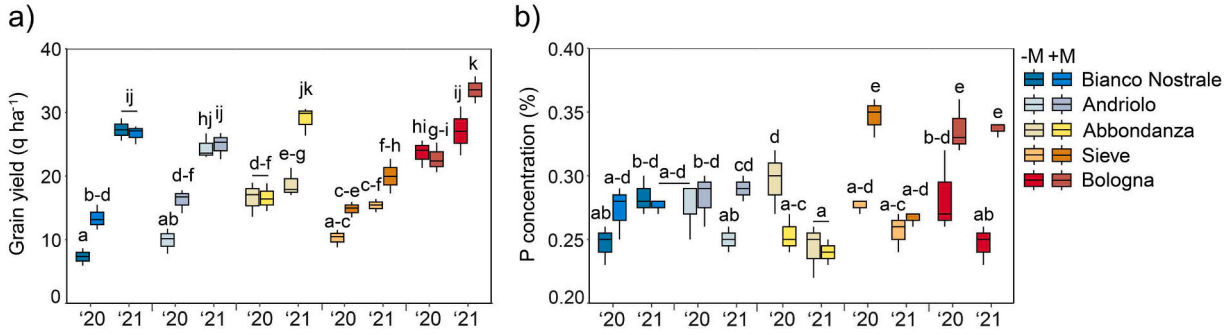

Fig. 2. Effect of the significant interactions among year of cultivation, wheat genotype and arbuscular mycorrhizal fungal (AMF) inoculation on grain yield (a) and P concentration in grain (b) at the physiological maturity (GS90; Zadoks et al., 1974) (Table S3). The years of cultivation were: 2020 ('20) and 2021 ('21). Five bread wheat genotypes: Bianco Nostrale, Andriolo, Abbondanza, Sieve (old tall genotypes registered in the Tuscany Germoplasm Bank and in the Italian Register of Agricultural and Food Interest Biodiversity), and Bologna (a modern dwarf variety registered in the Italian Agricultural Information System, SIAN) (see Table S2). The AMF inoculation treatments were: inoculated with of 14 AMF species originating from a indigenous agricultural site (+M), and mock-inoculum as control (-M). The output of the boxplots is based on three replicate plots for each treatment. Different letters highlight statistically significant differences according to Tukey-B-test.

inoculation (+19 %) (Fig. 1e; Table S3). At GS90, the percentage of arbuscules was affected by the interaction Y x G x Inoc (Fig. 1f; Table S3). In general, the percentage of arbuscules was lower in all inoculated and not inoculated genotypes in 2020 than in 2021, and increased significantly by inoculation only in Bianco Nostrale (Fig. 1f; Table S3). Finally, at GS90, the percentage of vesicles was promoted in inoculated wheat compared to controls in both years of cultivation (2020: 3.7 ± 0.7 *vs* 1.0 ± 0.3; 2021: 16.2 ± 1.8 *vs* 10.7 ± 1.9) (Table S3).

Wheat genotypes responded differently to inoculation in the two years of cultivation in terms of yield and P concentration in the grain (Table S3). In 2020, when the grain yield of all wheat genotypes was generally lower than in 2021, the Bianco Nostrale and Andriolo values increased significantly by inoculation (83 % and 63 %, respectively) (Fig. 2a). On the contrary, the grain yield of the other genotypes did not respond to inoculation. In 2021, only the grain yields of Abbondanza and Bologna were positively affected by inoculation.

At GS12, the concentration of P in the shoots, averaged over years of cultivation and wheat genotypes, was promoted by AMF inoculation (+80 %) (Fig. S4c). In addition, averaged over years and inoculation, P shoot concentration was the highest in Bologna and Bianco Nostrale, intermediate in Adriolo and Sieve, and lowest in Abbondanza (Fig. S4d). Moreover, the P concentration in the grain did not change due to inoculation in Bianco Nostrale, while it increased in Bologna in both years (+19 % in 2020 and +36 % in 2021), in Sieve in 2020 (+25 %), and in Andriolo in 2021 (+16 %). No changes were reported in the other

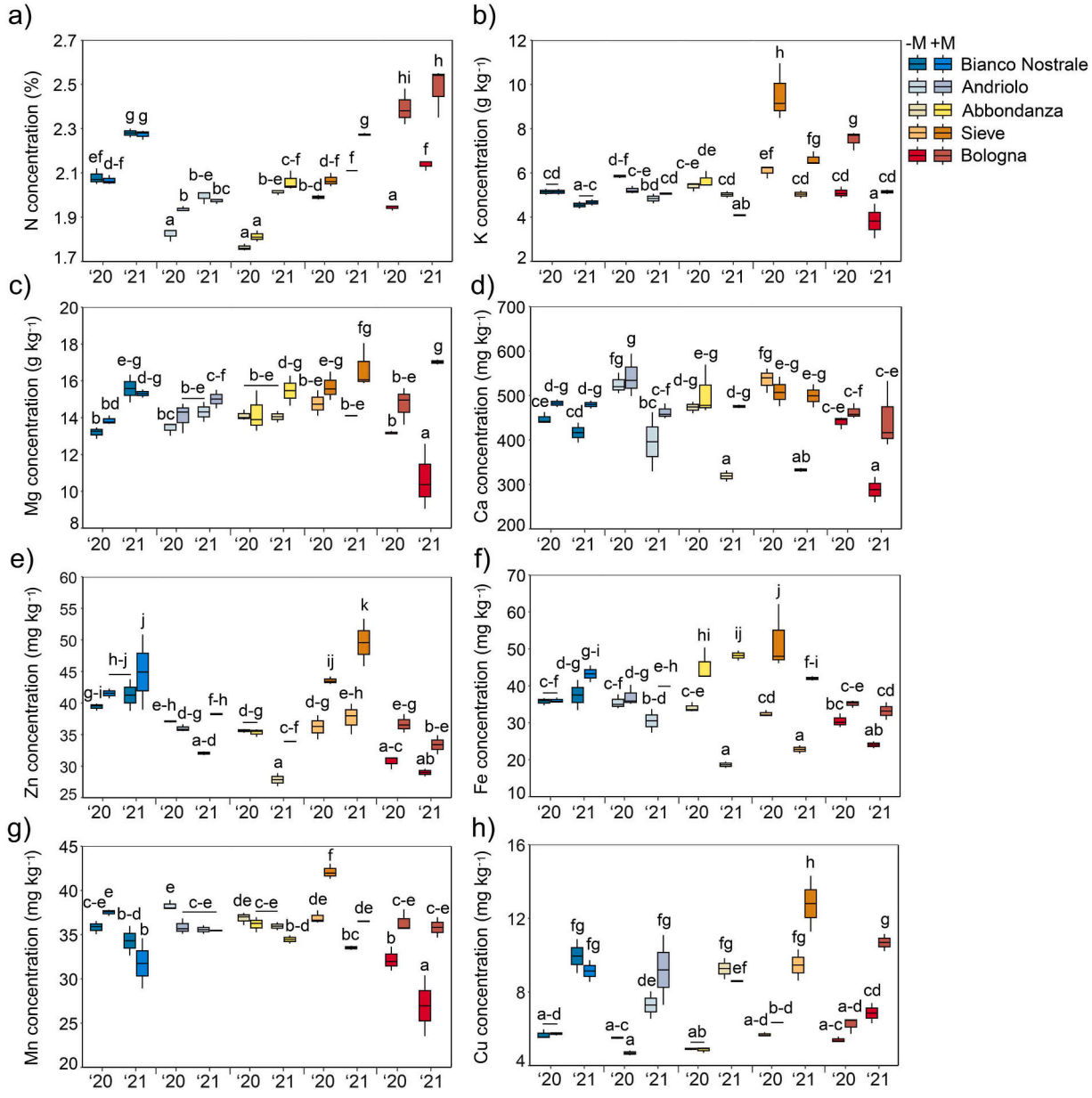

Fig. 3. Effect of the significant interaction among year of cultivation, wheat genotype and arbuscular mycorrhizal fungal (AMF) inoculation on grain nutrient concentration at the physiological maturity (GS90; Zadoks et al., 1974) (Table S3). Grain nutrients are: N (a), K (b), Mg (c), Ca (d), Zn (e), Fe (f), Mn (g), and Cu (h). The years of cultivation were: 2020 ('20) and 2021 (21'). Five bread wheat genotypes: Bianco Nostrale, Andriolo, Abbondanza, Sieve (old tall genotypes registered in the Tuscany Germoplasm Bank and in the Italian Register of Agricultural and Food Interest Biodiversity), and Bologna (a modern dwarf variety registered in the Italian Agricultural Information System, SIAN) (see Table S2). The AMF inoculation treatments were: inoculated with of 14 AMF species originating from a local agricultural site (+M), and mock-inoculum as control (-M). The output of the boxplots is based on three replicate plots for each treatment. Different letters highlight statistically significant differences according to Tukey-B-test.

cases, except for an unexpected reduction in P concentration under AMF inoculation in the Abbondanza grain in 2020.

Similarly, other mineral elements in wheat grain genotypes were differently affected by inoculation in the two years of cultivation (Fig. 3; Table S3). Generally, inoculation increased the grain N concentration of Bologna in both years of cultivation, and of Andriolo in 2020 and Sieve in 2021 (Fig. 3a). Inoculation promoted the grain Zn concentration of Sieve in both years of cultivation and of Bologna in 2020 and in Andriolo in 2021 (Fig. 3e). Similarly to Zn, inoculation positively affected the grain Fe concentration of Sieve in both years of cultivation and in Bologna and Andriolo in 2021 (Fig. 3f). Furthermore, the concentration of Zn in the Abbondanza grain in 2021 and the concentration of Fe in both years of cultivation was increased by inoculation (Fig. 3e,f). A positive effect of inoculation was also observed in the concentration of Mn in the Sieve and Bologna grains in both years of cultivation (Fig. 3g). Significant increases in the concentration of Ca, Mg and Cu after inoculation were detected in Sieve and Bologna in 2021, and of K in Sieve and Bologna in both years (Fig. 3b,c,d,h). Furthermore, in 2021 the Ca concentration in the Abbondanza grain and the Cu concentration in Andriolo were significantly increased by inoculation, while the K was reduced in Abbondanza (Fig. 3b,c,d).

However, our data did not allow us to find a significant relationship between the change in mycorrhizal colonization (ΔAM) (ΔAM = AM_{+AMF}− $AM_{control}$ expressed as log_{10}) and the mycorrhizal response ratio for grain yield and neither for nutrients ($MR_{yield/grain\ nutrient}$ = Yield or Grain Nutrient $Concentration_{+AMF}$/Yield or Grain Nutrient $Concentration_{control}$) with the exception of P and K (R^2=0.179, P=0.028; R^2=0.284, P=0.004, respectively) (Fig. S5).

PERMANOVA allowed us to summarize the pattern of plant and AMF parameters and to highlight statistically significant interactions between genotype and inoculation and between year of cultivation and genotype

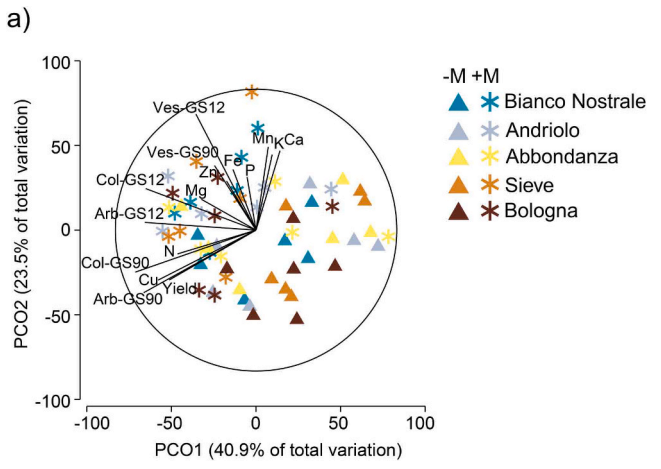

Fig. 4. Principal coordinate analysis (PCO) biplot on the significant effect of the interaction between wheat genotype and arbuscular mycorrhizal fungal (AMF) inoculation on plant and AMF functional parameters (Table S4). The plant functional parameters were: yield and nutrient concentration in grain (i.e., N, P, K, Mg, Ca, Zn, Fe, Mn, Cu). The AMF functional parameters were: AMF root colonization (Col), percentage of root length containing arbuscules (Arb) and percentage of root length containing vesicles (Ves) at the two-leaves unfolded stage (GS12; Zadoks et al., 1974); Col, Arb and Ves at the physiological maturity (GS90). Data were obtained from two years of cultivation: 2020 and 2021. Five bread wheat genotypes: Bianco Nostrale, Andriolo, Abbondanza, Sieve (old tall genotypes registered in the Tuscany Germoplasm Bank and in the Italian Register of Agricultural and Food Interest Biodiversity), and Bologna (a modern dwarf variety registered in the Italian Agricultural Information System, SIAN) (see Table S2). The AMF inoculation treatments were: inoculated with of 14 AMF species originating from a local agricultural site (+M), and mock-inoculum as control (-M). The PCO is based on the Euclidian distance matrix of similarity calculated on the square root and standardized parameters of three replicate plots for each treatment.

(Table S4). The PCO biplot (Fig. 4) showed that when inoculation is applied, all wheat genotypes positively responded to inoculation. This was supported by the PERMANOVA pairwise comparisons (Table S5), and more differences among genotypes were recorded when wheat was not inoculated. Wheat genotypes performed differently in the two years of cultivation, irrespective to inoculation (Fig. S6a), and looking at the PERMANOVA pairwise comparisons in 2020 greater variability among genotypes was recorded (Table S6). The PERMDISP output showed similar patterns in data dispersion among wheat genotypes and between inoculation levels, but it showed a significant difference in data dispersion between years of cultivation (Table S4). In fact, the distances among the samples and their centroids were significantly different, and a less variable pattern of response was observed in the year 2021.

3.2. Bread wheat flour and breadstick quality

Inoculation significantly modified the rheological parameters of the flour obtained from the wheat genotypes (Fig. 5b,d,f). In detail, as expected values of dough strength (W), dough tenacity (P) and the ratio P and dough extensibility (P/L) were significantly higher in the modern variety Bologna compared to the old genotypes both inoculated and not inoculated (+26 %, +22 %, +30 %, respectively). On the contrary, inoculation did not affect dough extensibility (L), swelling index (G) and gluten content (P=0.303, P=0.250 and P=0.897, respectively), while L, G and gluten content varied among genotypes (Fig. 5a,c,e; P<0.001). Interestingly, Sieve flour showed the highest L, similar to Bologna (average: 73 mm) that did not differ from Bianco Nostrale, while the flour obtained from the other genotypes (i.e., Andriolo and Abbondanza) showed statistically lower values of L (on average: 59 mm) (Fig. 5c). Similarly, Sieve and Bologna showed the highest values of G (on average: 19 ml), Andriolo and Abbondanza the lowest values (on average: 17 ml) and Bianco Nostrale the intermediate values (Fig. 5e). Finally, the Bianco Nostrale flour showed the highest gluten content values and Abbondanza the lowest values, while the other genotypes did not show any differences (Fig. 5a).

In general, in 2020, inoculation did not significantly affect the total phenolic content (TPC) of the flour, with the exception of Bianco Nostrale for which a significant increase in TPC was recorded (+17 %; P<0.001) (Fig. 6a). Similarly, antioxidant activity (i.e., ORAC) was largely promoted by inoculation in the flour of Bianco Nostrale and Andriolo (+165 %; P<0.001) (Fig. 5e).

To examine whether the effect of the inoculation observed in Bianco Nostrale persists in the flour and after the transformation of the flour into breadsticks, TPC and antioxidant activity were studied in the flour obtained in the second year of cultivation and in the breadsticks of both years. In this genotype, the TPC and antioxidant activity of the flour were consistently and positively affected by inoculation (+4 % and 5 %, P=0.001 and P=0.029, respectively). Furthermore, breadsticks made with Bianco Nostrale flour, obtained from inoculated wheat for the two years, showed significantly higher TPC and antioxidant activity than those obtained from not inoculated wheat (2020: + 14 % and +70 %, P=0.013 and P=0.001, respectively; 2021: +15 % and +13 %, P=0.002 and P=0.013, respectively) (Fig. 6b,c,g,h).

3.3. AMF diversity in wheat under on-farm inoculation

To investigate how the AMF diversity (i.e., composition and community structure) in the roots of the wheat genotypes changes according to AMF inoculation, we focused our investigation on the 2021 year of cultivation and we also evaluated the effect of the wheat growth stage. The choice of data obtained in 2021 was driven by the fact that this year the functional response of wheat genotypes to inoculation was less variable among replicate plots. Finally, we dissected the role of the AMF taxa, found in roots at plant maturity, in shaping wheat productivity.

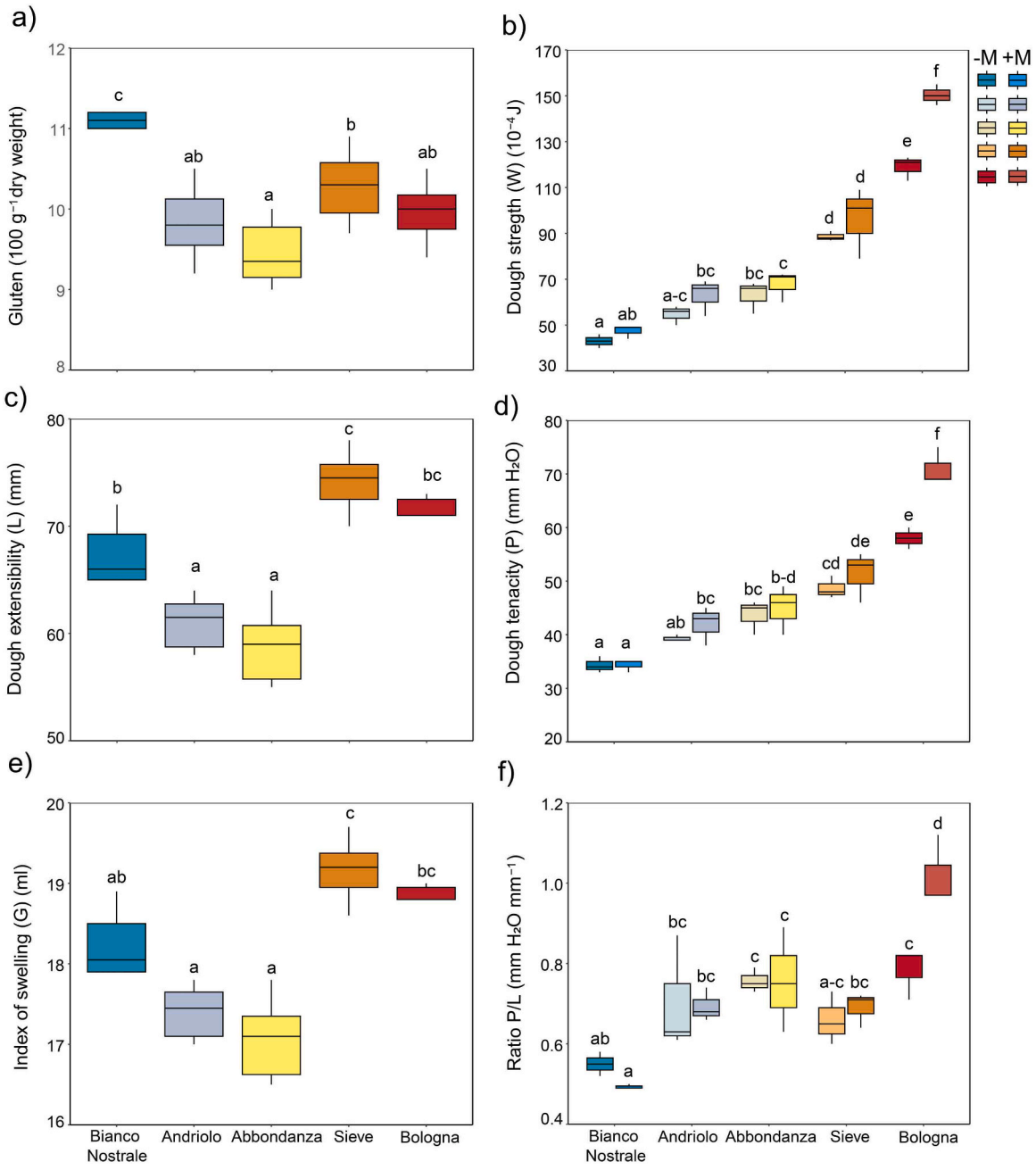

Fig. 5. Significant effect of the wheat genotype (G) on: gluten content (a), dough extensibility (L) (c), and index of swelling (G) (e) ($P<0.001$; n=6). Significant effect of the interaction between G and arbuscular mycorrhizal fungal (AMF) inoculation (Inoc) on: dough strength (W) (b), dough tenacity (P) (d), and P/ L (f) ($P=0.015$, $P<0.001$, $P=0.031$, respectively; n=3). Rheological properties of the dough are measured on flour obtained from grains of the 2020 year of cultivation. The output of the boxplots is based on three replicate plots for each treatment (G and Inoc). Different letters highlight statistically significant differences according to Tukey-B-test. The five bread wheat genotypes are: Bianco Nostrale, Andriolo, Abbondanza, Sieve (old tall genotypes registered in the Tuscany Germoplasm Bank and in the Italian Register of Agricultural and Food Interest Biodiversity), and Bologna (a modern dwarf variety registered in the Italian Agricultural Information System, SIAN) (see Table S2). The AMF inoculation treatments were: inoculated with of 14 AMF species originating from a local agricultural site (+M), and mock-inoculum as control (-M).

3.3.1. *Illumina sequencing output, AMF richness and alpha-diversity*

After curation of the AMF sequences, 52,839 reads, ranging from 86 to 2711 reads per sample, were retrieved and assigned to 33 VTXs (Fig. S7; Table S7). The 33 AMF VTXs belonged to five orders (i.e., Archaeosporales, Diversisporales, Entrophosporales, Glomerales and Paraglomerales) and seven families (i.e., Archaeosporaceae, Acaulosporaceae, Gigasporaceae, Entrophosporaceae, Glomeraceae and Paraglomeraceae). The accumulation curves and rarefaction analyses of AMF confirmed that the Illumina sequencing effort was sufficient for the analysis (data not shown).

In 2021, regardless of the wheat genotype, inoculation differently affected the richness of AMF (S) and the alpha-diversity (H' and λ) in wheat roots in the two growth stages (G12 and GS90) (Table S8). At GS90, a slight but significant increase in S was recoderded in inoculated wheat compared to not inoculated one (S=13 *vs* 10; +23 %), while no variation was reported at GS12 (S=11) (Fig. S8a). Furthermore, at GS12, inoculation tended to decrease alpha-diversity (H' and λ) (-18 %), while at GS90 no differences were recorded (Fig. S8b,c).

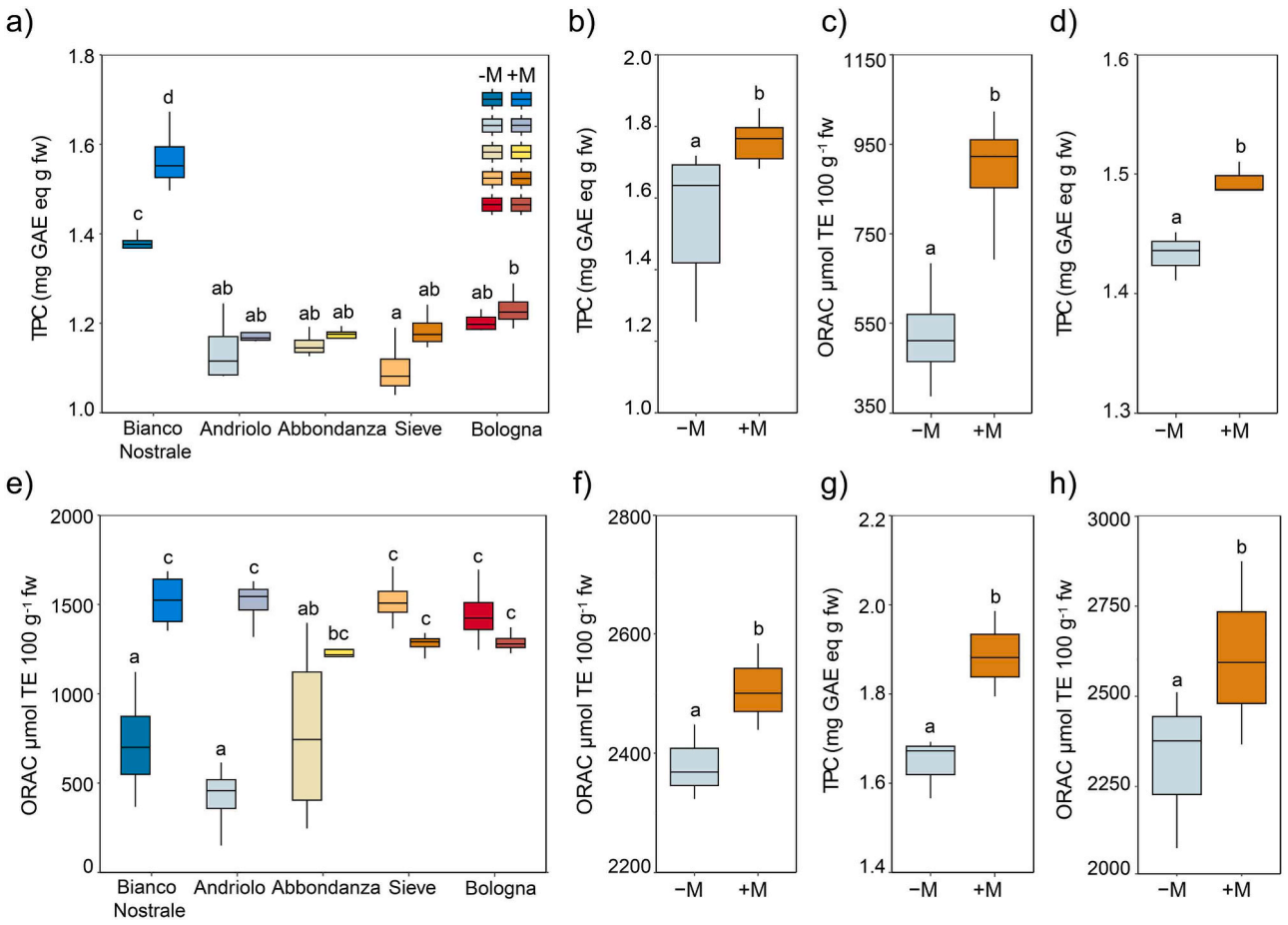

Fig. 6. Significant effect of the interaction between wheat genotype (G) and arbuscular mycorrhizal fungal (AMF) inoculation (Inoc) on: total phenolic content (TPC) (a) and antioxidant activity (oxygen radical absorbance capacity, ORAC) (e) in the wheat flour obtained from grains of the 2020 year of cultivation ($P<0.001$; n=3). The five bread wheat genotypes are: Bianco Nostrale, Andriolo, Abbondanza, Sieve (old tall genotypes registered in the Tuscany Germoplasm Bank and in the Italian Register of Agricultural and Food Interest Biodiversity), and Bologna (a modern dwarf variety registered in the Italian Agricultural Information System, SIAN) (see Table S2). The AMF inoculation treatments were: inoculated with of 14 AMF species originating from a local agricultural site (+M), and mock-inoculum as control (-M). Significant effect of Inoc on: TPC (b) and ORAC (c) in breadsticks made from flour obtained from grains of Bianco Nostrale genotype cultivated in the 2020 year of cultivation ($P=0.013$ and $P=0.001$, respectively; n=3). Significant effect of Inoc on: TPC (d) and ORAC (f) in flour ($P=0.001$ and $P=0.029$; n=3), and TPC (g) and ORAC (h) in breadsticks of Bianco Nostrale genotype ($P=0.002$ and $P=0.013$, respectively; n=3) cultivated in 2021. Different letters highlight statistically significant differences according to Tukey-B-test.

3.3.2. Community composition and structure

On average, in wheat genotypes, 67 % of AMF taxa (VTXs) were shared between inoculated and not inoculated treatments, as shown by Venn diagrams (Fig. 7b). Shared AM fungal taxa ranged from 56 % in Abbondanza to 74 % in Bianco Nostrale. As example, shared taxa belonging to *Acaulospora* spp., *Entrophospora* spp., *Funneliformis* spp., *F. mossea*, *Glomus* spp., *Rhizophagus* spp., *R. irregularis*, *Scutellospora* spp. and *Septoglomus* spp. were retrieved in inoculated and not inoculated treatments.

Despite this high number of common AMF taxa, some were unique to certain inoculation treatments in each genotype. As an example, among a total of 19 VTXs, in Bianco Nostrale, the taxon VTX00238 (*Paraglomus occultum*) was only retrieved in the inoculated treatment (+M), while four taxa (i.e., *Acaulospora* sp. VTX00030, *Glomus* sp. VTX00098, *Glomus* sp. VTX00365 and *Entrophospora* sp. VTX00357) were unique to the non-inoculated treatment (-M) (Fig. 7a; Table S7). However, in all wheat genotypes, the taxa unique to +M and -M showed a low relative abundance (mean: 0.76 ± 0.03; min: 0.02; max: 5.2) (Fig. 7a). Across the wheat genotypes, the unique taxa in the inoculated treatments were consistently similar (i.e., *Rhizophagus* sp. VTX00072; *Septoglomus* sp. VTX00064; *Acaulospora* sp. VTX00030), as well as in the non-inoculated treatments (i.e., *Archaeospora* sp. VTX00009; *Glomus* sp. VTX00153, VTX00103, VTX00202). However, the Bologna variety slightly differed in this pattern, since it consistently showed unique taxa in the inoculated and not inoculated treatment (i.e., VTX00030 and VTX00202/VTX0009, respectively), while this consistency was not observed for other taxa (i.e., +M: VTX00098, VTX00365; -M: *Rhizophagus* sp. VTX00363, *Diversispora* sp. VTX00354). Finally, we could not generalize a consistent behavior among genotypes in the number of unique taxa in +M and -M, since in Bianco Nostrale and Andriolo they were less in +M, while in Sieve they were more in +M, and in Abbondanza and Bologna they were similar.

Looking in depth at the AMF community structures in the roots of the wheat genotypes visualized in the nMDS plot of Fig. 7c, we can observe the interaction between wheat genotype (G) and AMF inoculation (Inoc). As an example, Bologna showed a large presence of *Enthrophospora lamellosum* VTX00193 in the not inoculated plots, and a large presence of *R. irregularis* VTX00105 in the inoculated plots. On the contrary, Sieve and Abbondanza showed a higher presence of *E. lamellosum* VTX00193 in inoculated plants, and a similar presence of *R. irregularis* VTX00105 between inoculated and not inoculated plots. This is supported by the results of the PERMANOVA that highlighted the significance of the interaction G x Inoc (Table S9), suggesting that the wheat genotypes had different AMF community structures depending on

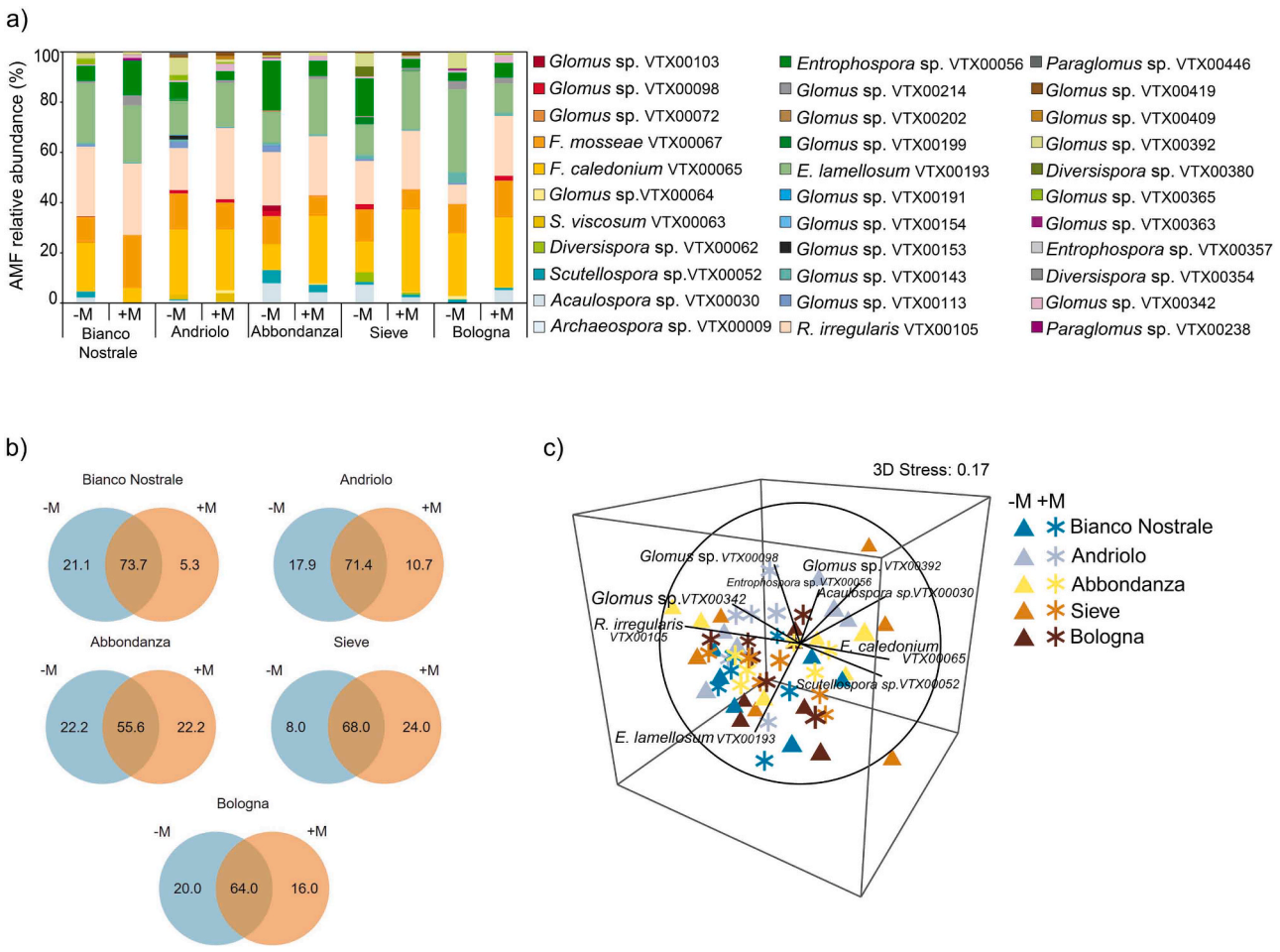

Fig. 7. Relative abundance (a) of the arbuscular mycorrhizal fungal (AMF) virtual taxa (VTX) found in the roots of five wheat genotypes inoculated with an AMF inoculum (+M) (14 species originating from local agricultural site) and mock-inoculated (control; -M). (b) Venn diagrams of the AM fungi (i.e., VTX) uniquely retrieved and shared between +M and -M in each wheat genotype (data are expressed as percentages). The relative abundances used in (a) and (b) are means of two growth stages of the 2021 year of cultivation (two-leaves unfolded stage, GS12; physiological maturity, GS90; Zadoks et al., 1974). Information about VTX is reported in Table S7. (c) Three-dimensional non-metric multidimensional scaling (nMDS) plot on the significant effect of the interaction between wheat genotype and AMF inoculation on the AMF community structure in the roots of wheat (Table S9). The nMDS is based on the Bray-Curtis similarity matrix calculated on the fourth-root of the relative abundances of the AM fungi (i.e., VTX). In the plot the overlay of vectors is reported and only the AMF taxa with a strong correlation ($r > 0.6$) are displayed. The data matrix is built using the AMF abundances found in 2021 at GS12 and GS90 (n=60). The five bread wheat genotypes are: Bianco Nostrale, Andriolo, Abbondanza, Sieve (old tall genotypes registered in the Tuscany Germoplasm Bank and in the Italian Register of Agricultural and Food Interest Biodiversity), and Bologna (a modern dwarf variety registered in the Italian Agricultural Information System, SIAN) (see Table S2).

inoculation or not. Furthermore, more differences among genotypes were recorded when wheat was not inoculated according to the PERMANOVA pairwise comparisons (Table S10). The PERMDISP output showed similar patterns in the dispersion of the data among wheat genotypes, but a significant difference in the dispersion of the data between the inoculation levels (Table S9). In fact, a less variable pattern of response was observed in the inoculated treatments (Fig. 7c).

On average, over wheat genotypes, 69 % of AMF taxa were shared between GS12 and GS90, as shown by the Venn diagrams (Fig. S6c). The percentage of shared AMF taxa was high, ranging from 64 % in Sieve to 74 % in Abbondanza. As an example in Bianco Nostrale among 19 VTXs, the taxon VTX00357 (*Entrophospora* sp.) was only found at GS90, while five taxa (i.e., *Paraglomus occultum* VTX00238, *Rhizophagus* sp. VTX00363, *Paraglomus* sp. VTX00446, *Glomus* sp. VTX00365, *Acaulospora* sp. VTX00030) were recovered at GS12 (Fig. S6b; Table S7). However, in all wheat genotypes, the taxa unique to GS12 and GS90 showed a low relative abundance (mean: 0.73 ± 0.17; min: 0.02; max: 4.09) (Fig. 7a). Bianco Nostrale and Andriolo showed a similar pattern for the the taxa that were uniquely found in each growth stage (i.e., GS12: VTX00446, VTX00365; GS90: VTX00357), Abbondanza and Sieve showed a more similar pattern (i.e., GS90: VTX00357, *Glomus* sp. VTX00202), while Bologna consistently showed a different pattern (e.g., GS12: VTX00357).

Looking in depth at the AMF community structures in the roots of wheat genotypes that are visualized in the nMDS plot of Fig. S6d, we can observe the interaction between wheat genotype (G) and growth stage (GS). As an example, *Enthrosphospora* sp. VTX00056 was largely recovered at GS90 in Bologna, while in the other wheat genotypes it was abundant at GS12. This is supported by the PERMANOVA results that highlighted a significant interaction (G x GS) (Table S9). This interaction means that AMF community structures changed during the growth cycle of wheat genotypes, and more differences among genotypes were recorded at GS12, according to PERMANOVA pairwise comparisons (Table S11). Similarly, the PERMDISP output highlithed a significant difference in the dispersion of data between growth stages (Table S9).

3.3.3. *Relationship between AMF and wheat productivity*

In disagreement with our first hypothesis, there was no significant relationship between the traits of AMF abudance in roots and wheat productivity (RELATE: ρ=0.023; P=0.359). On the contrary, we proved

our hypothesis that there was a significant relationship between the root changes in the AMF community structures and the wheat productivity. This was supported by the RELATE analysis reporting a ρ equal to 0.35 (P=0.001) (Fig. 8c). Furthermore, this can be visualized by the distribution pattern of the samples in the nMDS plot of the AMF community structure in the roots of the wheat genotypes at GS90, and the corresponding pattern in the PCO biplot of the yield and nutrient uptake (Fig. 8a,b). The BEST analysis allowed us to highlight that the best predictors of wheat productivity (ρ=0.47, P=0.01) (Fig. 8d) was the taxon *Septoglomus* sp. VTX00064, considering a model based on one decriptor (r=0.421), or the taxa VTX00064 and *R. irregularis* VTX00105, considering a model based on two decriptors (r=0.427). These BEST predictors were also highlighted in the nMDS plot (Fig. 8a).

4. Discussion

4.1. AM fungal abundance and agronomic effectiveness of field inoculation

The proof of the success of the inoculation by the indigenous AMF consortium was given by the increase in P concentration in the wheat shoot at the early growth stage GS12. The effectiveness of AM fungi in increasing host P uptake has been largely related to inoculum potential (e.g., Sanders and Tinker, 1971; Sanders et al., 1977). Indeed, AM fungi are well known to promote the absorption of phosphate by roots in soils with low phosphate status and transporters involved in P uptake were described (e.g., transmembrane phosphate transporter GvPT) (Bonfante et al., 1998; Ezawa and Saito, 2018).

The success of inoculation was associated with an improvement in the presence of vesicles in the roots at both plant growth stages. This can be explained by the large presence in the applied AMF consortium of AMF taxa known to be able to form vesicles (Schenck and Pérez, 1990; https://invam.ku.edu). In a two-year field inoculation study on

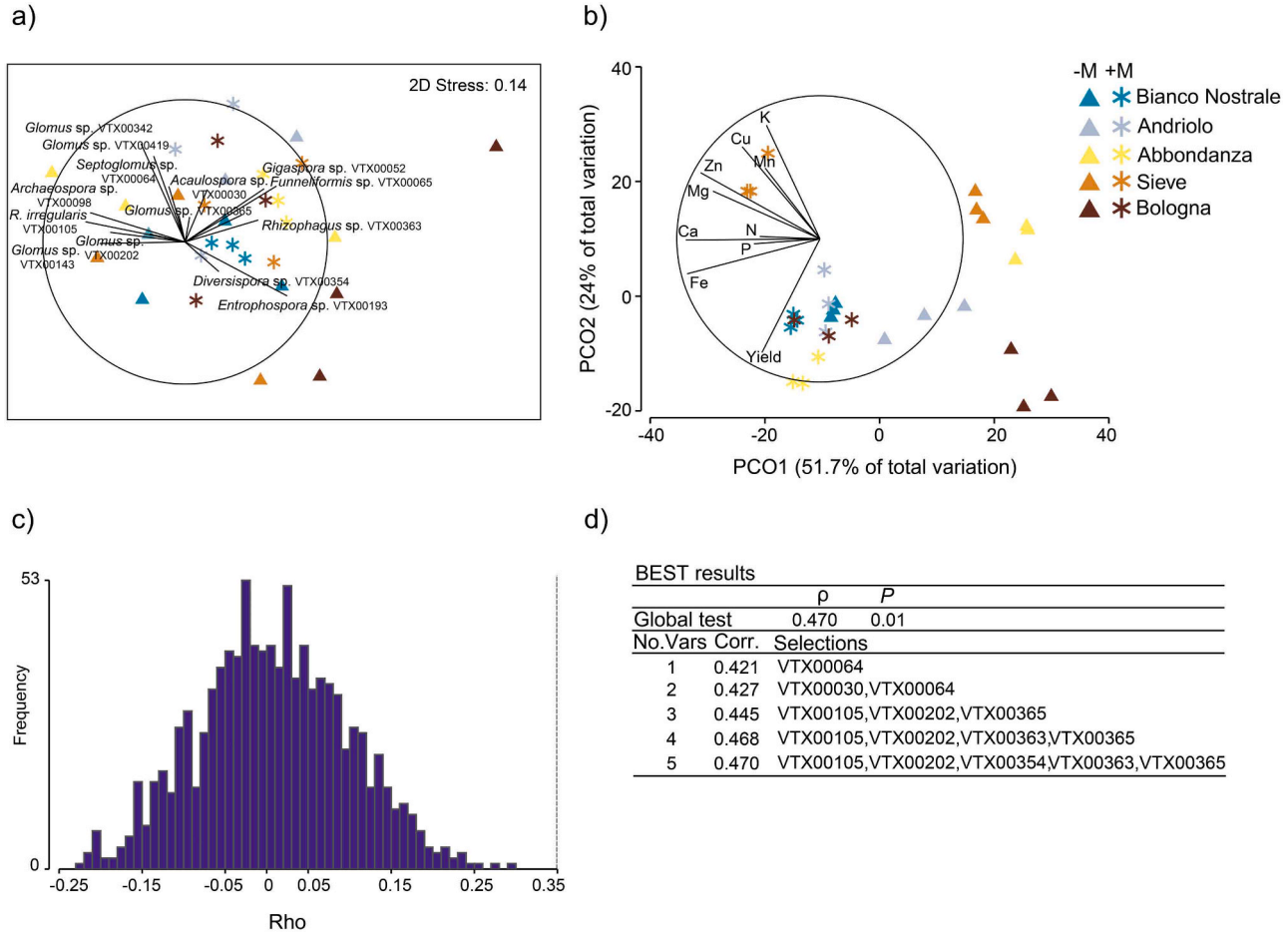

Fig. 8. Two-dimensional non-metric multidimensional (nMDS) plots (a) on the effect of the wheat genotype and arbuscular mycorrhizal fungal (AMF) inoculation on the AMF community structure in the roots of wheat. In the plot the overlay of vectors is reported and only the AMF taxa with a good correlation (r > 0.4) are displayed. The data matrix is built using the abundances of the AMF virtual taxa (VTX) found at physiological maturity (GS90; Zadoks et al., 1974) in 2021 (n=30). Principal coordinate analysis (PCO) biplot (b) on the effect of wheat genotype and AMF inoculation on plant functional parameters (i.e., yield and grain nutrient concentrations). The PCO is based on the Euclidian distance matrix calculated on the square-root and standardized plant parameters measured at GS90 in 2021. The five bread wheat genotypes are: Bianco Nostrale, Andriolo, Abbondanza, Sieve (old tall genotypes registered in the Tuscany Germoplasm Bank and in the Italian Register of Agricultural and Food Interest Biodiversity), and Bologna (a modern dwarf variety registered in the Italian Agricultural Information System, SIAN) (see Table S2). The AMF inoculation treatments were: inoculated with of 14 AMF species originating from a local agricultural site (+M), and mock-inoculum as control (-M). (c) RELATE analysis based on Spearman rank and 999 permutations for testing the significance of the relationship between the two matrices: matrix of the AMF community and matrix of the plant parameters (ρ=0.35; P=0.001) (Clarke and Warwick, 2021). Results of the BEST analysis (d) based on BioEnv methods (all combinations), Spearman rank and 999 permutations: ρ and P values of the Global Test and BEST descriptor(s) of the relationship together with the correlation values (Clarke et al., 2008).

sunflower, carried out in an area close to the one where this experiment was set up and applying the same inoculum, the frequency of vesicles was similarly promoted in the roots of the inoculated plots (Arcidiacono et al., 2024). This supports the fact that when inocula composed of many AM fungi forming vesicles are applied, this trait can be appropriate to trace the establishment and development of AM fungi in roots, as well as early acquisition of P from the plant.

In both years of cultivation at GS12, the abundance of AMF, in terms of root colonization and percentage of arbuscules, increased by inoculation only in the old genotype Sieve and in the modern variety Bologna. At plant maturity (GS90), AMF abundance was promoted only in Sieve. Thus, Sieve and Bologna can be considered highly compatible to inoculation, while the other genotypes were less compatible. The climatic conditions in 2021 favored the development of AM fungi in the roots of all genotypes and the AMF colonization approached saturation at maturity, while in 2020 the values were much lower, similarly to the results of other studies (Ercoli et al., 2017a; García de Léon et al., 2020). These strong increases in Sieve and Bologna partially supported our hypothesis that AMF inoculation in low fertile soils, as our field sites, increases the abundance of AMF in roots, while the response of the other genotypes suggest a great influence by environmental conditions.

In earlier studies testing field AMF inoculation, at early crop stages old and new varieties of wheat responded positively in terms of AMF root colonization (Pellegrino et al., 2020; Ercoli et al., 2017b). By contrast, at plant maturity the effect of inoculation was no longer perceptible and similar values were observed in inoculated and not inoculated treatments. Previously, Lekberg and Koide (2005) reported an increase of AMF colonization by 29 %, averaging over grass and non-grass plants, grown in pot and field conditions. Zhang et al. (2018) reported similar increases in field inoculated cereals. Additionally, among ancestors, old, and new crop varieties, Lehmann et al. (2012) found similar positive changes in AMF colonization, after inoculation by two AMF species (*R. irregularis* and *F. mosseae*).

The differences we found among AMF colonization in the roots of wheat genotypes could be due to variability in the genetic basis of susceptibility to AMF inoculation. Significant genotypic differences among 94 bread wheat genotypes in the ability to form symbiosis were previously detected (Lehnert et al., 2017). Moreover, 30 significant markers associated with root colonization, representing six QTL regions, were proposed. However, despite considerable efforts to clarify the role of signalling molecules and plant hormones during different stages of development of AM symbiosis (i.e., pre-symbiotic signalling, root-internal fungal development and root-external mycelium growth), the mechanistic basis of variation in cereal response to AM symbiosis has not yet been fully elucidated (Lehnert et al., 2017; De Vita et al., 2018; Sawers et al., 2017; Floss et al., 2013). Therefore, we can only speculate that Sieve and Bologna differ from the other genotypes in the genes involved in the signaling or formation of symbiosis.

The increases in abundance of AMF in the roots of Sieve, after inoculation, determined positive changes in the concentration of Zn, K, Mn, Fe, P, N, Ca, Mg, and Cu in grain, but not in grain yield. On the contrary, in Bologna, increases in AMF root abundance due to inoculation determined the promotion of yield in 2021 and of the concentration of P, N, K, Mn, Zn, Fe, Mg, Ca, and Cu in grain. These results support the good responsiveness of these wheat genotypes with the inoculated indigenous AMF consortium and are in agreement with the general positive effect of AMF on Zn, Cu, and Mn concentration (Lehmann et al., 2014; Lehmann and Rillig, 2015). However, our results support the general relationship found between ΔAM and mycorrhizal response ratio (MR) for P concentration, but are in contrast to the positive relationship observed in wheat and other crops between ΔAM and MR for grain yield (Lekberg et al., 2005; Pellegrino et al., 2015). Thus, in our study, we were unable to verify our hypothesis that the increase in AMF abundance in roots, after inoculation with an indigenous AMF consortium, is directly related to the benefits of the plant. The lack of significance in this correlation was also verified by powerful multivariate approaches that took into account the whole functional response of the plant.

Since in all wheat genotypes the composition of the core community and the diversity of the roots did not vary after inoculation, other factors have driven the overall recorded benefits of wheat. Furthermore, we can only speculate on the post-inoculation occurrence in roots of the 14 AM fungal species, constituting the indigenous inoculant. Indeed, in this study, the length of the amplified fragment and the target gene do not fully allow to reach a resolution at AM fungal species. Advancements were recently done using a long-fragment as target (i.e., SSU, ITS1, 5.8 S, ITS2 and large subunit ribosomal RNA) and applying the Real-Time (SMRT) Sequencing provided by Pacific Biosciences (PacBio) that enables the sequencing of single DNA molecules of long read lengths longer (Kolaříková et al., 2021). However, this technique requires high PCR cycle numbers that may bias the relative abundances of the detected AM fungal taxa (Castaño et al., 2020), and the long fragment includes several places susceptible as chimeric breakpoints (Kolaříková et al., 2021).Changes in root AMF community structure induced by inoculation were identified as responsible for the outcome of the symbiosis. Similar to previous works on alfalfa and sunflower (Pellegrino et al., 2022; Arcidiacono et al., 2024), changes in AMF community structure and in particular of few taxa, occurring in the indigenous consortium, were mainly responsible for the outcome of the symbiosis across wheat genotypes. In alfalfa, the stimulation of the proliferation of a single taxon (*R. irregularis*) induced by exotic and indigenous single and mixed inoculants was the main determinant of host benefits (Pellegrino et al., 2022). Furthermore, in sunflowers, inoculated with the same indigenous consortium, no changes in AMF community composition occurred, but changes in the AMF community structure and in particular of a single taxon (*Rhizophagus* sp.) were the best predictors of the plant functional traits (Arcidiacono et al., 2024). These results suggest a selection driven by the environment for highly efficient indigenous AM fungi and support the application of indigenous multiple species AMF inocula, reducing the risk of introducing invasive taxa (Rodriguez and Sanders, 2015; Hart et al., 2017a,b). Furthermore, in our study, the low availability of nutrients in soil, frequently linked with a low mycorrhizal infection potential and number of spores (Oehl et al., 2003; Gosling et al., 2006; Peng et al., 2023), could have boosted multiple benefits of wheat.

Similarly to our results on non-inoculated wheat, in previous studies (Mao et al., 2014; Kavadia et al., 2020; Stefani et al., 2020), plant genotypes within a single plant species showed differential affinity for AMF taxa. Actually, the effect of the host on the AMF community in roots is widely reported (e.g., Vandenkoornhuyse et al., 2003; Öpik et al., 2003, 2009; Montesinos-Navarro et al., 2012; Higo et al., 2015; Ciccolini et al., 2016; Mhlanga et al., 2022). Our findings, based on the characterization of the AM fungal diversity in the root endosphere free of soil residues (Bulgarelli et al., 2015), additionally support that genotypes within a single plant species is a deterministic factor and not only a stochastic mechanism. For bread wheat, genotypes with similar agronomic traits were found to show a similar AMF community structure (Mao et al., 2014). For durum wheat, the different AMF assemblages found at two sites in the rhizosphere of 32 genotypes indicated that genotype together with environmental factors, such as climate, soil properties, and field management, affect AMF communities (Ellouze et al., 2018).

Since in our study we characterized the AMF communities only in one year, we could not discriminate the potential effect of environmental factors on AMF assemblages in the roots of different genotypes of bread wheat. When inoculation was applied as field practice, the differences we found in the AMF root communities of the genotypes were weaker than when wheat was not inoculated, and this can support the previously found significant interaction between durum wheat genotypes and field management (Ellouze et al., 2018).

Finally, the beneficial effect of inoculation on the productivity of Bologna over the two years did not allow us to confirm the hypothesis

that modern crop varieties lost the set of genes required to fully benefit from mutualistic symbioses. Our results demonstrate that old wheat genotypes are positively affected by inoculation with indigenous AM fungi, although performance of the plant was shaped by climatic parameters. Indeed, the grain yield of Bianco Nostrale and Andriolo was increased by inoculation in 2020, while the grain yield of Abbondanza e Bologna increased in 2021.

The pattern of distribution of assimilates from photosynthesis within plant organs varies during the wheat cycle and is greatly affected by climatic conditions (Bidinger et al., 1977). Reserves deposited in vegetative plant parts prior to anthesis can buffer grain yield against conditions adverse to current assimilation during the grain filling period (Palta et al., 1994; Gebbing and Schnyder, 1999; Masoni et al., 2007). In these conditions, the remobilization of assimilates temporarily stored in vegetative plant parts, including roots, is improved (Ercoli et al., 2008, 2010). Thus, our results suggest that in silty clay soils characterized by low nutrient availability and under dry field conditions during grain filling, the AMF community in the roots of Bologna and Abbondanza supported remobilization to grain as recorded in 2021. This resulted in increases in grain yield in the inoculated plants. On the contrary, in similar soil conditions, dry conditions in winter determined poor crop establishment and tillering rate, resulting in low crop production. The AMF community in the roots of Bianco Nostrale and Andriolo, probably early and faster colonizer taxa, were able to support plant growth and nutrient uptake at early crop stages, resulting in increases in grain yields in inoculated plants.

4.2. Interaction between growth stage and wheat genotype on AMF communities in roots

The structure the AMF community in the roots was strongly shaped by the wheat genotype, although the changes varied between growth stages. Furthermore, the variability among genotypes at the early growth stage was greater than at plant physiological maturity. This is in accordance with a previous work carried out under controlled conditions with three cereal species (i.e., bread wheat, oat and barley) inoculated and not with a multi-species AMF consortium (Aguilera et al., 2021). In this work, the AMF communities within the roots were greatly differentiated due to the phenological stage of the plant and not by plant species. Seasonality in AMF communities was reported in species of maple (Helgason et al., 2014), and in several perennial and annual grasses and non-grasses species (e.g., *Trifolium repens*, *Poa annua*, *Digitaria cruciata*) (Lingfei et al., 2005). Furthermore, the abundance of AMF spores under different tillage systems (no-tillage *vs* conventional tillage) varied according to the phenological stage of wheat (Schalamuk et al., 2013). Our study demonstrates, using molecular tools, the dynamic nature of AMF communities in field crops, such as wheat, and the importance of sampling roots throughout the plant growth cycle to obtain a complete characterization of AMF communities.

The variation in the structure of AMF in the roots between the growth stages can be attributed to the mechanism of competition among AM fungi within a community (Chagnon et al., 2013). AMF communities can change with time because of an early occurrence of very fast colonizers that steady increase colonization (i.e., r strategy) with specific effects on plant nutrition. By constrat, the later occurrence of other taxa having delayed reproduction are able to displace the earlier ones due to their higher competitive ability (i.e., K strategy). Indeed, in the work of Jansa et al. (2008), when 3 AM fungi were inoculated together, *F. mosseae* predominated at early stages, while *R. irregularis* and *E. claroidea* developed later. Furthermore, the variation in growth stage in AMF root communities can be modulated by climatic factors, such as temperature and moisture (Giovannetti and Avio, 2002). Soil moisture and temperature, changing during the plant growth cycle, can affect the abundance and activity of AM fungi, differently promoting the germination of the spores belonging to different genera, species, and isolates (Daniels and Trappe, 1980; Koske and Halvorson, 1981; Douds et al., 1991; He et al., 2002).

4.3. Bread wheat flour and breadstick quality

This is the first study investigating the effect of AMF inoculation on the quality aspects of processed wheat products. The fact that the protein and gluten content and the rheological parameters of wheat flour differed among genotypes and that the modern variety has superior baking quality than the old genotypes is not surprising (Bordes et al., 2008; Ghiselli et al., 2016; Migliorini et al., 2016; Cappelli et al., 2020; Bánfalvi et al., 2020). Bordes et al. (2008), studying the grain quality and flour rheology of 372 bread wheat genotypes, reported grain protein concentrations, ranging from 11 % to 19 %. Furthermore, they reported large variations among wheat genotypes in dough strength (W; 46–652 10^{-4} J), tenacity (P; 22–143 mm H_2O), extensibility (L; 32–269 mm), dough swelling (G; 13–36 ml) and ratio P/L (0.1–3.3 mm H_2O mm^{-1}). However, in organic agriculture, the old and new wheat genotypes showed similar grain protein (14 %) and differences in rheological properties, such as P/L (0.6 *vs* 2) and W (45 *vs* 220 10^{-4} J) (Migliorini et al., 2016; Ciccolini et al., 2017; Cappelli et al., 2020; Pellegrino et al., 2020). In our study, in organic farming, the highest values of W and P were found in the modern variety Bologna and the highest values of L and G in the old genotype Sieve and Bologna (up to 80 mm and 20 ml). However, despite the positive effect of inoculation, the flour is not suitable (L $<$ 100 mm; W $<$ 150 10^{-4} J; P $<$ 60 mm H_2O) for transformations that require long leaving.

In addition, Bologna was the unique genotype shown to be responsive to AMF inoculation, as demonstrated by W, P, and P: L. This may be related to its specific response in terms of grain protein content. Therefore, inoculation with AMF, under organic farming systems, enhances the rheological properties of modern genotypes to acceptable bread making values (W: 150 10^{-4} J; P: 71 mm H_2O; P/L: 1). Accordingly, De Santis et al. (2022) showed that AMF inoculation affected the protein composition of the Bologna flour by increasing the proportion of the S-poor storage protein subfractions of polymeric glutenins (i.e., HMW-GS, LMW-GS) and monomeric gliadins (e.g, ω-gliadins).

Our results on the effect of AMF inoculation on the quality of flour and breadsticks in terms of total phenolic content (TPC) and antioxidant activities allow a better understanding of how AMF can modulate the production of health-promoting phytochemicals in crops. Previous works, synthesized by Agnolucci et al. (2020), reported a substantial increase in bioactive compounds in shoot/leaves and seeds of medicinal and aromatic plants inoculated with single AMF species. Increases in antioxidant activity were also reported in food crops, such as onion inoculated with single and multiple AMF isolates and with commercial inocula. Furthermore, great increases in TPC and antioxidant activity were observed in two tomato varieties inoculated before transplanting by AM fungi belonging to different families (Pellegrino et al., 2023). In two genotypes of bread wheat, grown in greenhouse conditions, Nahuelcura et al. (2022) found no changes in grain TPC and antioxidant activity after inoculation with AMF, while Yi et al. (2016) reported increases in some varieties applying a single species (*F. mosseae*) and a commercial inoculum.

These results are in line with our pattern of response of the wheat genotypes to inoculation with AMF. Thus, the unique increase in TPC and antioxidant activity found in Bianco Nostrale flour demonstrates that genotype selection is of great importance for improving the nutraceutical quality of bread wheat flour products in organic farming systems. When the Bianco Nostrale flour was processed, AMF-derived breadsticks consistently showed higher nutraceutical properties, indicating that the transformation process did not affect the quality of the final product. Previously, TPC, antioxidant activity, and lycopene were reported to be strongly reduced in tomato sauce compared to fresh fruits, highlighting that the transformation process can negatively impact health-related compounds with no more detectable effects due to AM fungi (Pellegrino et al., 2023). These results underline the need for large

studies on AMF-derived processed products.

5. Conclusions

Overall, the use in the field of indigenous AMF-based biostimulants improve wheat productivity, in terms of grain yield and nutrients. The selection of responsive plant genotypes is fundamental for the positive outcome of AMF inoculation. However, since the mycorrhizal response was modulated by climatic parameters, the selection of wheat genotypes should be assisted by medium and long-term climatic predictions. As an example, under organic agriculture associated with reduced tillage and in silty clay soils with low content of organic C and low nutrient status (P and N) Bologna and Abbondanza can be suggested when dry conditions are expected during grain filling. Thus, to confirm the stability of the response of wheat genotypes to AMF inoculation in interaction with climatic parameters there is a need of long-term field experiments that allow to gain robust prediction models for a large number of cereal genotypes. Wheat productivity and quality and in field persistence of inoculated AM fungi support the use of indigenous consortia that have low ecological impacts on resident AM fungi. Indeed, the indigenous consortium did not modify the composition of the AMF communities in roots. Under AMF inoculation, the stimulation of the proliferation of an indigenous taxon, such as *Septoglomus* sp., present in the indigenous inoculant, was the main determinant of improved wheat benefits, suggesting an environmental-driven selection for a highly efficient AMF strain. Extensive studies in metagenomics and population genomics are needed to assess the level of interaction between wheat genotypes and AMF symbionts to predict the benefit that each partner will derive from the symbiosis. Finally, the peculiar positive response to AMF inoculation of the old and modern wheat genotype Bianco Nostrale and Bologna, in terms of health-promoting phytochemicals in flour and breadsticks and rheological properties of the flour, underlined that biostimulants based on AM fungi can also be applied to improve the quality of processed products.

Funding

This work was funded by the European Agricultural Fund for Rural Development 2007–2013 for Tuscany (Italy) measure 16.1 (FERTIBIO project; CUP Artea 743548; project leader Dr. Elisa Pellegrino) and measure 16.2 (FERTIBIO project; CUP Artea 828090; project leader Prof. Laura Ercoli. Valentina Marrassini was supported by a PhD scholarship in Agrobiosciences (XXXVI cycle) from the Scuola Superiore Sant'Anna (SSSA, Pisa, Italy).

CRediT authorship contribution statement

Laura Ercoli: Writing – review & editing, Supervision, Resources, Methodology, Funding acquisition, Conceptualization. **Elisa Pellegrino:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Resources, Methodology, Funding acquisition, Data curation, Conceptualization. **Gaia Piazza:** Writing – review & editing, Data curation. **Valentina Marrassini:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data will be made available on request.

Acknowledgments

The authors thank Luchino and Amedeo Grappi for hosting the field experiments on their farm in Pienza, Siena (Italy) and for milling the flour. We alo thank Pasquale La Rossa of 'Forno Moderno' in Certaldo, Florence (Italy) for breadstick making and Dr. Giusto Giovannetti of CCS Aosta for large-scale culturing of the AMF inoculum.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.fcr.2024.109456.

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