Productive and Physiological Response of Organic Potato Grown under Highly Calcareous Soils to Fertilization and Mycorrhization Management

Sara Lombardo | Cristina Abbate | Gaetano Pandino | Bruno Parisi | Aurelio Scavo | Giovanni Mauromicale

1 Department of Agriculture, Food and Environment (Di3A), University of Catania, 95123 Catania, Italy; saralomb@unict.it (S.L.); cristina.abbate@unict.it (C.A.); g.pandino@unict.it (G.P.); g.mauromicale@unict.it (G.M.)
2 Research Centre for Cereal and Industrial Crops (CREA-CI), Via di Corticella, 133-40128 Bologna, Italy; bruno.parisi@crea.gov.it

* Correspondence: aurelio.scavo@unict.it; Tel.: +39-0954783415

Received: 9 July 2020; Accepted: 12 August 2020; Published: 14 August 2020

Abstract: The enhancement of the actual low yields is the most important challenge regarding organic farming management. In this view, a valid tool may arise by the improvement of fertilization management and efficiency. In this regard, arbuscular mycorrhizal fungi (AMF) can play an important role, especially in low fertility soils such as calcareous ones, through a better nutrient uptake and by alleviating abiotic stresses. A replicated-space experiment was carried out to investigate the role of mycorrhizal-based inoculants combined with full or halved fertilizer doses on yield and physiological traits of three early potato cultivars organically grown in highly calcareous and alkaline soils. The results indicate that AMF symbiosis ameliorated, in comparison to the not-inoculated plants, the potato tolerance to limestone stress by enhancing the potential quantum efficiency of photosystem II ($F_v/F_0$) and plant gas-exchange parameters (photosynthesis rate and stomatal conductance). Moreover, a significant improvement of marketable yield (+25%) was observed, mainly due to an increase of the number of tubers plant$^{-1}$ (+21%) and, to a lesser extent, of average tuber weight (+10%). The AMF efficiency was higher applying halved fertilizer doses and in the location where soil conditions were unfavourable for potato growth. Moreover, the qRT-PCR highlighted that AMF colonization was similar in each location, demonstrating their tolerance to limestone, alkalinity and P stresses. These findings outlined that AMF are good candidate to bio-ameliorate calcareous soils and are very useful for improving potato yields under organic farming, limiting external fertilizers supply and environmental pollution.

Keywords: arbuscular mycorrhizal fungi; potato; organic farming; fertilization; calcareous soils; crop physiology; tuber yield; sustainability

1. Introduction

In the latest years, an increasing public interest for environmental safety and food quality has driven a major expansion in the organic farming sector all over the world [1]. In Europe, Spain, Italy and France are the countries with the largest organic agricultural land (2.1, 1.9 and 1.7 Mha, respectively [2]). The entire European Union organic food market is composed of 37% cereal products, 11% dairy products, 11% meat and 41% all other products [3]. Among the arable crops, potato ($Solanum tuberosum$ L.) is successfully organically grown both in Italy [4] and elsewhere [5,6]. The production of organic early potato tubers is of particular economic relevance. They can be sold around 200–250 euros € per tonne in Italy [7]. The high content of minerals such as potassium, phosphorus and calcium [8,9],...
as well as that of polyphenols and carotenoids [10,11], is an attractive feature of the early crop potato (winter–spring cycle; planted from November to January and harvested from March to early-June). In addition, early potato tubers are also useful as feedstock for industrial products [12,13]. Therefore, crop conventional producers often adopt inorganic fertilizers and pesticides [14], which can result in the build-up of undesirable residues in both tubers and soil [15–17]. As a result, the share of organic production of early potato tubers has been increasing. However, yield levels are typically lower in organic systems than in conventional high-input ones [7,18,19]. Indeed, organic restrictions on fertilization mainly cause a reduced N availability [20–22], resulting in a detrimental effect on potato plant growth and tuber development. In addition, the early potato cycle is often characterized by a relatively low temperature, a short photoperiod and limited solar radiation, which are conditions with an appreciable effect on plant growth, substantially modifying the morphological and phenological characteristics of the plants (for example, most potato cultivars do not flower) compared to those cultivated in the common spring–summer cycle [23,24].

In the coastal agricultural areas of the Mediterranean basin, the early crop potato is commonly cultivated under calcareous soils, so containing a high concentration (>15%) of calcium carbonate (CaCO$_3$) and HCO$_3$\(^{-}\) in soil solution, and a reaction included in neutral–alkaline range, always <8.5 [25]. Calcareous soils have been estimated covering more than 30% of the world’s land surface area, resulting in ~800 Mha according to FAO [26] and especially widespread in the arid and semi-arid regions because of the low leaching process. These soils are characterised by crust formation due to an inadequate quality of irrigation water, a high degree of P-fixation and Fe-precipitation, a low availability of nitrogen, magnesium and zinc [25], thus impairing the plant’s mineral nutrition and worsening yield performances.

A reasonable agronomic measure for enhancing early potato organic production is represented by an adequate nutrient management [27] and an improvement of fertilization use efficiency [28]. Particularly, this may be achievable by applying arbuscular mycorrhizal fungi (AMF), obligate symbionts of the vast majority of land plants [29]. The main advantages arising from the application of AMF as inoculum for agricultural purposes are: (i) the increase of root system extension by more than 100-fold; (ii) the enhanced uptake of the soil immobile mineral nutrients; (iii) the reduction of abiotic stresses such as water scarcity and thermal imbalances; (iv) a better soil aggregation, which is important in improving soil structure and preventing soil erosion [29,30]. Therefore, the AMF application may play a key role under organic farming, since plants may particularly benefit by mycorrhizal symbiosis through a better uptake of mineralized soil nutrients present at low concentrations [31]. Black and Tinker [32] firstly reported the interaction between AMF symbiosis and potato in field conditions. After them, other researches have been carried out with different results considering the potato cultivar and the mycorrhizal fungus isolate. In several scientific studies reviewed by Wu et al. [33], potato crop was chosen as a case study for evaluating the impact of AMF on crop production, due to its worldwide diffusion and recognized nutritional value in the human diet. However, to our knowledge, few literature data [34–36] are available concerning the influence of AMF on potato crop performances in a large-scale production system under organic farming. The role of AMF on organic early potato grown in highly calcareous soils is still unknown. Moreover, specific attention must be directed to the cultivar choice, since this has a relevant role in the crop productive and qualitative performances under organic farming [8,37]. In particular, adaptable cultivars for organic production need to show a reliably high yield under low input production system, efficiency in nutrient uptake, fast early ground cover, good level of resistance/tolerance to the common biotic and abiotic stresses and a high suitability to low temperatures of storage [38]. In addition, it is recognized that AMF isolates may show a host genotype-specificity [35]. For example, the symbiosis with Glomus fasciculatum was found to increase the potato yield, contrariwise to G. mosseae, which had not effect [39].

Taking into account all these considerations, the present research was designed (1) to evaluate the influence of AMF application on the yield performances and plant physiology profile of three early potato genotypes organically grown in open-field conditions; (2) to investigate whether it is possible
by using AMF inoculants to halve the organic fertilization rate, while keeping yield reduction to a minimum and having positive effects on crop physiology; (3) to observe the aforementioned effects of AMF application in different highly calcareous soils; (4) to verify the exploitation of the inherent advantages of the quantitative real-time PCR (qRT-PCR) technique in the detection and quantification of AMF Glomus spp. and Gigaspora spp. in soil.

2. Materials and Methods

2.1. Site, Soil and Climate

To consider the influence of soil type and the between-site variability, the research was replicated in space in accordance with Johnstone et al. [40]. The trials were conducted during the 2017 growing season in three different experimental fields (hereafter referred to as location I, II and III) placed on the coastal plain of South Siracusa (36°49′ N, 14°57′ E, 130 m a.s.l., south-eastern Sicily, Italy), a typical area for ‘early’ potato cultivation in the southern Italy. The soil, moderately deep, was Calcixerollic Xerochrepts on the basis of the USDA Soil Taxonomy Classification [41]. A layer, 0.25 m thick (from −0.05 to −0.30 m), where about 90% of active potato roots were located, was considered for the soil analysis. All soil analyses were carried out using the procedures approved by the Italian Society of Soil Science [42]. The three locations were characterized by various soil types, whose characteristics are reported in Table 1.

| Soil Characteristic | Location I | Location II | Location III |
|--------------------|------------|-------------|--------------|
| Sand (%)           | 42.6       | 54.1        | 51.8         |
| Silt (%)           | 38.7       | 24.8        | 22.0         |
| Clay (%)           | 18.7       | 21.1        | 26.2         |
| Total limestone (%)| 68.0       | 44.2        | 65.6         |
| Active limestone (%)| 27.9     | 15.5        | 18.0         |
| Organic matter (%) | 2.18       | 1.7         | 2.6          |
| Organic carbon (%) | 1.27       | 1.0         | 1.5          |
| C/N ratio          | 7.0        | 7.5         | 7.5          |
| Total N (g kg⁻¹)   | 1.8        | 1.3         | 2.0          |
| Assimilable P₂O₅ (mg kg⁻¹) | 28.5 | 66 | 135 |
| Exchangeable K₂O (mg kg⁻¹) | 197 | 455 | 612 |
| pH                 | 8          | 7.8         | 7.5          |
| Electrical conductivity (dS m⁻¹) | 1.7 | 1.32 | 1.14 |
| Cation exchange capacity (meq 100 g⁻¹) | 17.2 | 22.8 | 26.0 |

The active limestone level was high in all the soils, with the highest amount (~28%) in location I, +80% and +55% with respect to location II and III, respectively (Table 1). On the contrary, the lowest amount of P₂O₅ was detected in location I (28.5 mg kg⁻¹), with increasing values in location II (+132%) and III (+374%). Also, the K₂O concentration followed this trend. The three soils showed medium organic matter contents, but are characterised by a high level of organic matter mineralization. The climate of the area including the three locations under study, which are 4–5 km apart, is semi-arid Mediterranean with mild wet winters and common rainless springs. A meteorological station (Mod. Multirecorder 2.40; ETG, Firenze, Italy), located on the experimental field of location I, was used to daily record the air temperatures (minima, maxima and mean) and rainfall during the growing season. Both maximum and minimum monthly average temperature and total monthly rainfall during the early potato crop production (January–May) were calculated (Figure 1). The total rainfall of the growing season (115 mm) was lower compared to 179 mm of 30-year period. February experienced 45% of the rainfall, while April was particularly dry (only 2 mm). Minima temperatures never fell below 7.8 °C during the growing season, while the mean maximum temperature was above 16.4 °C at the plants’ emergence (February) and 20.6 °C at the tuberification stage (April). The mean maximum temperature
(18.8 °C) and the mean minimum temperature (10.4 °C) were consistent with the long-term average (18.7 °C and 9.8 °C, respectively).

### 2.2. Field Experimental Design, Plant Material and Management Practices

In each location, the experiment was arranged in a randomized split-plot design with three replications including three potato cultivars (i.e., Arizona, Mondial and Universa) as the main plots, and three fertilization management treatments as the sub-plots. The fertilization management treatments were summarized in Table 2 and, in particular, they included: (a) plots optimally fertilized and not inoculated, as a control (F100); (b) plots optimally fertilized and mycorrhizal inoculated (F100+M); (c) plots sub-optimally fertilized (with halved fertilizer doses respect to the other treatments) and mycorrhizal inoculated (F50+M).

#### Table 2. Agronomic management treatments of ‘early’ crop potato under organic farming.

| Fertilization Management Treatment (F) | Phenological Stage of Application | Commercial Product | No. of Applications | Dose Rate per Application |
|--------------------------------------|----------------------------------|---------------------|---------------------|--------------------------|
| F100                                 | At sowing                        | Xedaneem Pel®       | 1                   | 1.2 t ha⁻¹               |
|                                      |                                  | Kalisop®            | 1                   | 0.6 t ha⁻¹               |
|                                      |                                  | Fosfonature 26®     | 1                   | 0.4 t ha⁻¹               |
|                                      | After emergence                  | Biosin®             | 3                   | 150 cc hL                |
| F100+M                               | At sowing                        | Xedaneem Pel®       | 1                   | 1.2 t ha⁻¹               |
|                                      |                                  | Kalisop®            | 1                   | 0.6 t ha⁻¹               |
|                                      |                                  | Fosfonature 26®     | 1                   | 0.4 t ha⁻¹               |
|                                      | After emergence                  | Xedaopen®           | 1                   | 40 kg ha⁻¹               |
| F50+M                                | At sowing                        | Xedaneem Pel®       | 1                   | 0.6 t ha⁻¹               |
|                                      |                                  | Kalisop®            | 1                   | 0.3 t ha⁻¹               |
|                                      |                                  | Fosfonature 26®     | 1                   | 0.2 t ha⁻¹               |
|                                      | After emergence                  | Xedaopen®           | 1                   | 40 kg ha⁻¹               |

The optimal fertilization was formulated on the basis of the recommendations provided by Research Institute of Organic Agriculture (FiBL) [43] and Sicily Department of Agriculture (www.regionesicilia.it), while considering both the NPK uptake by potato crop in Sicily with target yield of 20 t ha⁻¹ and average NPK availability of experimental soils. At sowing, N was soil-applied by commercial organic...
sources derived from castor seeds (4% of N, Ricin-Xed®; XEDA Italia s.r.l., Forlì, Italy) and Neem seeds (3% of N, Xedaneem Pel®, XEDA Italia s.r.l., Forlì, Italy) after oil extraction, K₂O by applying a commercial granular product allowed in organic farming (50% of K₂O and 45% of SO₃, K+S KALI GmbH, Verona, Italy) and P₂O₅ by a complex of ‘Pheoflore’ algal origin (26% of P₂O₅ and 41% of CaO, Fosfonature 26®, TIMAC Agro, Milan, Italy). After potato plants’ emergence, a further N organic application was provided in three times by using a commercial liquid product (Biosin®, XEDA Italia s.r.l., Forlì, Italy) with 7.7% of N. In F100+M and F50+M treatments, the mycorrhizal inoculation (40 kg ha⁻¹) was also provided by using a commercially available inoculant (Xedaopen®, Xeda s.r.l., Forlì, Italy), containing 7 active propagules g⁻¹ of the genus Glomus spp. and Gigaspora spp., as guaranteed by the manufacturer. The inoculation was manually carried out by placing the microgranules of 1.5 mm directly beneath the tuber seed at sowing. The cultivars utilized in this research differ for their morphological, biological, physiological and productive characteristics. ‘Mondial’ (Spunta × VE66-295) is a Dutch B cooking type (by EAPR classification) cultivar with high tuberification speed, medium to high vigour and late cycle. ‘Arizona’ (UK 150-19D22 × Mascotte) is a new Dutch AB cooking type cultivar with medium to late cycle, high vigour and low resistance to common scab. ‘Universa’ (Agata × 88F164.1) is a French AB cooking type cultivar, very common in Sicily, with high potential yield, medium vigour and early to medium cycle. All cultivars are skin and flesh yellow coloured, and rather used for production of early’ potato crop.

The experimental fields have been cultivated in a potato–lettuce–carrot rotation over the last twenty years, as commonly in the cultivation area. Obliviously, the previous crop was carrot and the three locations were fertilized with the same dose of NPK (120, 80 and 130 kg ha⁻¹). In the three locations, tillage consists of a 30 cm depth ploughing followed by harrowing in October. Disease-free, no-pre-sprouted “seed” tubers, from a single seed lot, were manually planted on January 6th 2017 in the three experimental fields. Whole tubers were planted at 0.24 m intervals in rows and 0.75 m apart, corresponding to a planting density of 5.55 plants m⁻². Each sub-plot size was 4.2 × 4.2 m and consisted of six rows. The two external rows and two plants on each row-end were used as border to minimize contamination from adjacent treatments. The two middle rows per plot were harvested to assess the yield when about 70% of leaves were dry (121 days after planting, DAP). Drip irrigation was provided once the accumulated daily evaporation rate (derived from measurements of an unscreened class A-Pan evaporimeter) had reached about 30 mm. Over the crop cycle, about 180 mm irrigation water was provided by five applications. Weed and pest control followed current EU regulations (Regulation CE 834/2007, 889/2008, 967/2008, 1235/2008 and 1254/2008) for organic farming.

2.3. Crop Physiology, Measurements and Calculations

The physiological variables detected in the present study were the photosynthetic rate (P₀), the stomatal conductance (g), the chlorophyll (Chl) content and the Chl fluorescence parameters F₀/Fm and Fv/F0. Concerning these ratios, F₀ is the initial fluorescence (the basal emission of Chl fluorescence when redox components of photosystems are fully oxidised), Fm is the maximum fluorescence (the situation under fully saturated irradiance, when the electron acceptor QA is fully reduced) and Fv is the variable fluorescence (the reduction at a given time of the primary electron acceptor, which, in the oxidised state, quenches fluorescence) [44,45], calculated as Fm−F₀. The Fv/Fm ratio is considered a measure of the photochemical efficiency of the electron transport in photosystem II (PSII) and it is well correlated with the quantum yield of net photosynthesis [46]. The Fv/F0 ratio is a more sensitive parameter than Fv/Fm since exhibits a higher dynamic range, given that both components are considered at any time and thus it is very fast in response [47].

In each location, three physiological measurements (each one performed over three consecutive days in the 3 locations) during the potato plant growth were made from the youngest fully expanded leaf (usually the 3rd or 4th leaf from the apex) and at the same hours (10:00–12:00, local solar time). In particular, they were determined at 81, 91 and 99 DAP in location I, at 82, 92 and 100 DAP in location II and at 83, 93 and 101 DAP in location III. For simplicity, measurement date (M) will
be indicated as M1, M2 and M3 for all the locations. At each time point, measurements per each fertilization management treatment and cultivar were taken in duplicate on the same leaves of five plants (10 readings per sub-plot), previously marked for the purpose. Chl fluorescence parameters were detected with a with a portable fluorescence induction monitor (Fi 1500; Alma Group Company, Hoddesdon, Herts, UK) by applying a clip on the terminal of full sun-exposed leaflets after a 20 min dark adaptation period. Chl fluorescence measurements were carried out with saturation irradiance up to 3000 µmol m$^{-2}$s$^{-1}$. Leaf SPAD absorbance readings (correlated to Chl content) were detected by using a portable absorbance-based Chl meter (SPAD-502 model, Konica Minolta, Sakai, Osaka, Japan). $P_r$ and $g$ were measured by a LI-6200 closed gas-exchange system (LI-Cor Inc., Lincoln, NE, USA) previously calibrated according to manufacturer’s instructions. Instantaneous gas-exchange measurements were taken on the same leaves previously used for Chl fluorescence measurements inside a 250 cm$^3$ chamber in the closed-circuit mode. Days on which $P_r$ was measured were typically clear sunny and characterized by a $\text{PAR} < 1800$ µmol photons m$^{-2}$s$^{-1}$. Air temperatures varied only slightly during each measuring hour, but ranged between 18 and 24 °C during the period of measurements.

2.4. Crop Yield and Its Components

In each location, for the determination of yield and its components, tubers (from each sub-plot and replicate) were harvested manually when about 70% of leaves and haulms were fully desiccated (i.e., at 120, 122 and 124 DAP in location I, II and III, respectively), and the number and weight of both marketable and unmarketable tubers per plant were determined. Tubers, which were greened, misshapen or displayed pathological damage were classed as unmarketable, as well as those with weight lower than 20 g. This allowed the calculation of the number of marketable tubers per plant (NMTP), average marketable tuber weight (AMTW) and marketable yield (MY). The yield of unmarketable tubers was very low (below 2.0%) and hence excluded from the data.

2.5. Tuber Dry Matter Determination

In laboratory, for each location, a sub-sample of 15 marketable tubers per replicate was washed with tap water, dried with tissue paper, diced and immediately oven-dried at 65 °C (Binder, Milan, Italy), until a constant weight was reached, in order to determine the tuber dry matter percentage (TDMP).

2.6. Soil Sampling and DNA Extraction

In this study, the qRT-PCR conjugated with the fluorescent SYBR Green I dye was used to quantify the AMF *Glomus* spp. and *Gigaspora* spp. in soil. In each location, three soil samples for each sub-plot [each adjacent (±5 cm distance) to a standing plant and weighting 500 g] were collected from the first 20 cm layer, excluding the outer 3 m of each plot and the non-homogeneous areas, by taking care not to include weeds. Then they were sieved through 2 mm pores and kept frozen at −20 °C for DNA extraction. Each soil sample derived from the composition of three soil cores, giving a total of 81 cores (3 plots × 3 cultivars × 3 locations × 3 cores). The extraction of soil DNA was carried out following Scavo et al. [48]. The purified DNA was stored at −20 °C until RT-PCR amplification. Purified DNA was quantified spectrophotometrically (all with 260:280 ratios above 1.7).

2.7. Real-Time Quantitative PCR Assay of Soil DNA Extracts

The qRT-PCR is a very powerful and sensitive technique to determine the amount of PCR product. The absolute quantification method was used to analyze data from RT-quantitative PCR experiments. Absolute quantification determines the input copy number of the gene of interest, usually by relating the PCR signal to a standard curve [49]. A DNA-binding dye, such as SYBR Green, binds to all double-stranded DNA in PCR, causing fluorescence of the dye. An increase in DNA product during PCR therefore leads to an increase in fluorescence intensity and is measured at each cycle, thus allowing DNA concentrations to be quantified. In qRT-PCR assay, a positive reaction is detected by accumulation of a fluorescent signal. The Ct (cycle threshold) is defined as the number of cycles
required for the fluorescent signal to cross the threshold (i.e., exceeds background level). Ct levels are inversely proportional to the amount of target nucleic acid in the sample.

In this study, a iCycler iQ™ (BIORAD) detection system was used. Reactions were 25-µL volumes using Platinum Quantitative PCR Supermix-UDG (Invitrogen, Carlsbad, CA, USA). Two sets of fungal 28S rDNA primers were used to amplify Glomus spp. and Gigaspora spp. For Glomus spp. Glofor (5′-GAAGTCAGCTACCAACCGGAA-3′) and Glorev (5′-CTCGCAGATCGCAAGGC-3′) oligonucleotides, flanking a 101 bp DNA fragment, were used (Alkan et al., 2006). For Gigaspora spp. the primer pair Gigfor (5′-CTTTGAAAAGAGAGTTAAATAG-3′) and Gigrev (5′-GTCCATAACCCAACACC-3′) was used to generate a DNA product of 272 bp [50].

The conditions for Glomus spp. DNA template amplification were initial denaturation at 95 °C for 10 min followed by 40 cycles at 95 °C for 15 s, 62 °C for 30 s, 72 °C for 30 s. For testing the primers, Glomus mosseae (BEG12) was directly used as a source of DNA template in a 25-µL reaction. The optimized cycling conditions for Gigaspora spp. were established as follows: initial DNA denaturation at 95 °C for 15 min, then 45 cycles each with denaturation at 95 °C for 10 s, annealing at 48 °C for 30 s and elongation at 72 °C for 1 s. For testing the primers, Gigaspora margarita (BEG34) was used. The same strains were used as standard for calibration curves and the subsequent calculation of their amount. Threshold cycle (Ct) values were determined, in triplicate, using 2-µL samples of each soil DNA extract per PCR reaction. The concentrations of fungal genomic DNA in soil experimental treatments were calculated by comparing the Ct values to the crossing point values of the linear regression line of the standard curve.

2.8. Statistical Analysis

Productive, physiological and microbiological data were analysed statistically through analysis of variance (ANOVA) by using the CoStat® computer package version 6.003 (CoHort Software, Monterey, CA, USA). Untransformed data are reported and presented as means ± standard deviation.

Concerning yield data, a three-way ANOVA ‘fertilization management × cultivar × location’ was used and, when needed, two-way ANOVAs were performed at each location. To remedy deviations from the ANOVA basic assumptions, data NMTP were square-root transformed, while an arcsine-square root transformation was applied to tuber dry matter percentage. Then, homoscedasticity was verified with the Bartlett’s test and normality through a graphical inspection of the residuals, which showed not significant deviations. Pairwise mean comparisons were carried out with the Fisher’s protected Least Significant Difference (LSD) test at α = 0.05. Data about potato plant physiology were initially analysed according to a four-way ANOVA factorial model with ‘3 fertilization managements’, ‘3 cultivars’, ‘3 locations’ and ‘3 measurement dates’ as the main factors. Since the four-way ANOVA showed a high significance (p ≤ 0.001) of location for all the variables under study, data were processed according to the Bartlett’s test and homogeneity of variances. One in accordance with Scavo et al. [52], they were log-transformed prior to ANOVA for the homogeneity of variances.

3. Results

3.1. Mycorrhizal Colonization

ANOVA results showed that mycorrhizal colonization was significantly influenced (p ≤ 0.001) by main factors and even their interactions (Table 3).
Table 3. F-values as absolute value of main factors and their interactions resulting from ANOVA of qRT-PCR analysis, yield and its components.

| Source of Variation | df | qRT-PCR Analysis | Yield and Its Components |
|---------------------|----|-------------------|---------------------------|
|                     |    | Gigaspora spp.    | Glomus spp.               |
|                     |    | MY               | AMTW                      |
|                     |    | NMTP             | TDMP                      |
| **Main factors**    |    |                  |                           |
| Fertilization       | 2  | 4641.5 ***       | 3240.5 ***                |
| management (F)      |    | 221.1 ***       | 9.3 ***                   |
| Cultivar (C)        | 2  | 573.1 ***       | 342.7 ***                |
| Location (L)        | 2  | 39.1 ***       | 122.5 ***                |
| Interactions        |    |                  |                           |
| (F) × (C)           | 4  | 171.9 ***       | 30.5 ***                  |
| (F) × (L)           | 4  | 37.1 ***       | 61.5 ***                  |
| (C) × (L)           | 4  | 18.4 ***       | 10.4 ***                  |
| (F) × (C) × (L)     | 8  | 13.8 ***       | 11.0 ***                  |

Values are given as F of Fisher. *** and ** indicate significant at $p \leq 0.001$ and $p \leq 0.01$, respectively, and NS, not significant. MY: Marketable yield; AMTW: Average marketable tuber weight; NMTP: Number of marketable tubers plant$^{-1}$; TDMP: Tuber dry matter percentage.

Overall, the effect of fertilization management accounted for 84.5 and 84.8% of the variance for *Gigaspora* spp. and *Glomus* spp., respectively. In all the locations, DNA extraction by qRT-PCR pointed out that soil samples of F100+M and F50+M were efficiently colonized by both mycorrhiza, which showed a very similar trend. Keeping in mind that Ct levels are inversely proportional to the amount of target nucleic acids in the sample, a decrease of 31.9% and 28.1% of F100+M and F50+M as compared to F100 was observed in location I, of 27.7% and 26.4% in location II, and of 30.7% and 30.9% in location III (Figure 2). Therefore, the trend was also constant in relation to the soil type. It is interesting how the cultivar, explaining 10.3% and 8.9% of the total variance for *Gigaspora* spp. and *Glomus* spp., also significantly affected the mycorrhizal colonization, showing the highest Ct levels (and thus the lowest amount of nucleic acids) for both genera in ‘Universa’.

3.2. Marketable Yield and Its Components

ANOVA demonstrated that the productive response of early crop potato, evaluated by MY and yield components, varied in relation to fertilization management, location’s soil characteristics and cultivar (Table 3). The use of AMF with half fertilizer doses (F50+M) has led to an increase of 25.5 and 15.1% of MY as compared to F100 and F100+M, respectively (Figure 3). The MY increase highlighted by inoculated (F100+M and F50+M) sub-plots than not inoculated ones (F100) was more marked in location I (on average 217%) than in location II (87%) and III (72.8%), as demonstrated by the significance of the ‘fertilization management × location’ interaction ($F = 4.5$). Such MY differences can be attributed to the higher NMTP, observed in inoculated sub-plots (on average 7.4) than not inoculated ones (6.3), and, in location I, also to the higher AMTW (Figure 3). The F50+M also caused a reduction of unmarketable yield in location I and III (data not shown). Location provided the largest contribution (66.7%) to variance, followed by cultivar (16%) (Table 3). Overall, the mean MY values were 2.0 and 2.2 fold higher in location II and III than in location I, respectively (39.8 and 42.3 t ha$^{-1}$ vs. 19.7 t ha$^{-1}$). Although the lowest mycorrhizal colonization detected, ‘Universa’ interestingly had the highest mean MY (40 t ha$^{-1}$), followed by ‘Arizona’ (33 t ha$^{-1}$) and ‘Mondial’ (28 t ha$^{-1}$).
Following the trend of MY, higher AMTWs were observed in F50+M (90 g) than in F100+M (86 g) and F100 (82 g), and mainly in location I with an increase of 66% (Figure 3). Similarly, F50+M showed higher mean NMTP (7.8) than F100 (6.3). Regardless of fertilization management and cultivar, the highest AMTW was observed in location III (100 g), while location II expressed the highest NMTP (8.2). Among cultivars, ‘Universa’ showed the highest AMTW (108 g), while ‘Arizona’ had the highest NMTP (7.6) and ‘Mondial’ the highest TDMP (19.3%). As observed for MY and AMTW, TDMP was higher in F50+M than in F100 (18.8% vs. 17.7%, \( p \leq 0.005 \)) and increased by 10% from location I to location III (\( p \leq 0.005 \)) (data not shown).
Figure 3. Marketable yield (t ha$^{-1}$), average marketable tuber weight (g) and number of marketable tubers plant$^{-1}$ of early crop potato organically grown in highly calcareous soils as affected by fertilization management, soil type and potato cultivar. The Least Significant Difference (LSD) interaction was calculated with the Fisher’s protected LSD test at $\alpha = 0.05$. Each bar means ± standard deviation ($n = 3$). F100: optimal fertilization without mycorrhizal inoculation (control); F100+M: optimal fertilization with mycorrhizal inoculation; F50+M: sub-optimal fertilization with mycorrhizal inoculation; ‘Arizona’, ‘Mondial’ and ‘Universa’: potato cultivars.

3.3. Photosynthesis Rate ($P_r$)

The three-way interaction for $P_r$ was only significant ($p \leq 0.001$) in location I and II (Table 4). In particular, the main factors (namely fertilization management, cultivar and measurement date) significantly affected $P_r$ in the three locations, except for cultivar in location II and fertilization management in location III, with measurement date providing always the major source of variation. The effect of fertilization management and cultivar on $P_r$ was clearly influenced by soil characteristics of each location (Figure 4). In both location I and III, the mycorrhizal inoculation was not consistent. On the contrary, in location II, F50+M determined a significantly higher $P_r$ than F100 at each measurement date. In this location the increase, averaged over all measurement date, was equivalent to 8.3% (11.7 vs. 10.8 $\mu$mol CO$_2$ m$^{-2}$ s$^{-1}$), for ‘Arizona’ in location II (11.3 $\mu$mol CO$_2$ m$^{-2}$ s$^{-1}$) and for ‘Arizona’ in location III (10.1 $\mu$mol CO$_2$ m$^{-2}$ s$^{-1}$). Moreover, regardless of fertilization management, $P_r$ significantly decreased with plant age in each location. Indeed, $P_r$ declined by 32.6–10.2 and 21.3% respectively in location I, II and III passing from M1 to M3.
Table 4. F-values as absolute value of main factors and their interactions resulting from ANOVA of photosynthesis rate (Pr), chlorophyll content (Chl), Chl fluorescence parameters and stomatal conductance (g).

| Main Factors | Location I | | Location II | | Location III |
|--------------|------------|-----------------|-----------------|-----------------|
| Fertilization Management (F) | Cultivar (C) | Measurement Date (M) | (F) × (C) | (F) × (M) | (C) × (M) | (F) × (C) × (M) |
| degrees of freedom | 2 | 2 | 4 | 4 | 4 | 8 |
| Pr | 3.3 * | 8.7 *** | 208.0 *** | 8.6 *** | 3.6 ** | 14.6 *** | 6.8 *** |
| Chl content | 66.1 *** | 0.3 NS | 32.9 *** | 4.9 ** | 18.7 *** | 12.1 *** | 1.6 NS |
| Fv/Fm | 0.1 NS | 7.3 ** | 0.5 NS | 6.7 *** | 1.5 NS | 2.5 * | 1.1 NS |
| Fv/F0 | 2.9 NS | 7.4 ** | 0.9 NS | 14.8 *** | 5.7 *** | 3.3 * | 4.4 *** |
| g | 35.8 *** | 40.3 *** | 94.7 *** | 3.1 * | 3.1 * | 2.7 * | 2.3 * |
| Location II | | | | | | |
| Pr | 21.0 *** | 2.4 NS | 64.2 *** | 6.6 *** | 1.3 NS | 4.1 ** | 12.3 *** |
| Chl content | 11.8 *** | 44.0 *** | 144.2 *** | 29.5 *** | 2.6 * | 10.6 *** | 1.0 NS |
| Fv/Fm | 1.2 NS | 8.2 *** | 1.0 NS | 1.4 NS | 0.6 NS | 1.3 NS | 3.6 ** |
| Fv/F0 | 0.4 NS | 36.2 ** | 5.8 ** | 9.6 *** | 8.9 *** | 12.6 *** | 13.1 *** |
| g | 21.2 *** | 30.3 *** | 33.1 *** | 10.2 *** | 4.3 ** | 10.9 *** | 3.1 ** |
| Location III | | | | | | |
| Pr | 0.2 NS | 6.3 ** | 120.6 *** | 0.8 NS | 2.5 NS | 1.3 NS | 0.8 NS |
| Chl content | 15.1 *** | 96.6 *** | 317.5 *** | 19.9 *** | 6.0 *** | 9.7 *** | 1.7 NS |
| Fv/Fm | 0.3 NS | 2.3 NS | 4.3 * | 0.5 NS | 1.5 NS | 0.7 NS | 0.8 NS |
| Fv/F0 | 4.1 * | 5.1 ** | 9.1 *** | 3.1 * | 4.9 ** | 8.7 *** | 5.4 *** |
| g | 24.7 *** | 26.4 *** | 32.7 *** | 6.3 *** | 5.8 *** | 7.2 *** | 4.4 *** |

Values are given as F of Fisher. ***, ** and * indicate significant at \( p \leq 0.001, p \leq 0.01 \) and \( p \leq 0.05 \), respectively, and NS, not significant.
Figure 4. Photosynthetic rate (μmol CO₂ m⁻² s⁻¹) of leaves of early crop potato organically grown in highly calcareous soils as affected by fertilization management, soil type and potato cultivar. The Least Significant Difference (LSD) interaction was calculated with the Fisher’s protected LSD test at α = 0.05. Each bar indicates means ± standard deviation (n = 5). F100: optimal fertilization without mycorrhizal inoculation (control); F100+M: optimal fertilization with mycorrhizal inoculation; F50+M: sub-optimal fertilization with mycorrhizal inoculation; ‘Arizona’, ‘Mondial’ and ‘Universa’: potato cultivars. M1, M2 and M3: first, second and third measurement date.
3.4. Chl Content and Chl Fluorescence

The effect of fertilization management provided the largest source of variation for Chl content in location I, while becoming less significance for the other two locations in favour of the measurement date as index of plant age (Table 4). At each measurement date, on the average of cultivars, F50+M plants had the lowest Chl content in the three locations (Figure 5). The differences among the studied fertilization management treatments were mainly evident in location I, given that the Chl content, averaged over the measurement dates, was 47.3 for F100, 45.8 for F100+M and 43.1 for F50+M. In accordance with Pr, Chl content declined with increasing plant age in each location. This was particularly highlighted in location III, where from M1 to M3 the Chl values decreased by 20%. The effect of cultivar was not significant in location I, while in the other two locations ‘Universa’ showed the highest Chl content (39.6 and 43.0 in location II and III), followed by ‘Mondial’ and ‘Arizona’ (37.2).

The Fv/Fm ratio was not relevant in location I and III, while showing significant results in location II. In particular, it was higher in the mycorrhizal inoculated plots for each measurement date and particularly for ‘Mondial’.

More clear results were observed for the Fv/F0 ratio, for which the three-way interaction was significant at \( p \leq 0.001 \) in each location (Table 4). Except for location II, it was
significantly higher in F50+M plants respect to F100 ones (+20% in location I and +8% in location III) (Figure 6). Furthermore, location II and III exhibited more than 4-fold higher values of the Fv/F0 ratio than location I, thus pointing out how soil characteristics closely affected this parameter. Cultivar was the main cause of variance in location I (19%) and II (42%), and the second one in location III (12.5%) after the measurement date (22.4%) (Table 4). The performances of the three cultivars were markedly different in relation to soil characteristics for which, averaged over the other factors, ‘Arizona’ had the highest Fv/F0 in location I (0.64), ‘Mondial’ in location II (2.49) and ‘Universa’ in location III (2.12), even with not statistical differences with ‘Arizona’ (Figure 6).

**Figure 6.** Fv/Fm ratio of leaves of early crop potato organically grown in highly calcareous soils as affected by fertilization management, soil type and potato cultivar. The Least Significant Difference (LSD) interaction was calculated with the Fisher’s protected LSD test at $\alpha = 0.05$. Each bar indicates means ± standard deviation ($n = 5$). F100: optimal fertilization without mycorrhizal inoculation (control); F100+M: optimal fertilization with mycorrhizal inoculation; F50+M: sub-optimal fertilization with mycorrhizal inoculation; ‘Arizona’, ‘Mondial’ and ‘Universa’: potato cultivars. M1, M2 and M3: first, second and third measurement date.

### 3.5. Stomatal Conductance (g)

ANOVA showed a high significance ($p \leq 0.001$) of main factors for each location (Table 4). As observed for $P_c$, the measurement date contributed to the largest part of the overall variance (by 52.0–29.3 and 30.3% in location I, II and III, respectively), followed by cultivar (22.1%, 26.8% and 24.6%, respectively). With reference to the effect of fertilization management, the mycorrhizal inoculated plots (i.e., F100+M and F50+M) significantly lowered g in location I (0.34 and 0.29 vs. 0.40 mol H$_2$O m$^{-2}$ s$^{-1}$...
of F100) (Figure 7). On the contrary, F100+M and F50+M caused a significantly higher \( g \) than F100 both in location II and III. Concerning the effect of measurement date, \( g \) changed based on location. In location I, the highest \( g \) was observed at M1, while in location II and III at M2 and M3, respectively. In each location, regardless of fertilization management and cultivar, \( g \) showed an opposite trend than \( P_r \), with increasing values at declining plant age. This was particularly marked in location III, which for \( g \) reported an increase by 53% from M1 to M3. Moreover, \( g \) was higher for ‘Mondial’ in each location (on average 0.44 mol H\(_2\)O m\(^{-2}\) s\(^{-1}\)), while ‘Arizona’ and ‘Universa’ recorded values not statistically different.

4. Discussion

The changes in terms of yield, including its components, and physiological traits in early potato crop organically grown in relation to mycorrhizal colonization and cultivar were studied in three different locations. The latter (namely location I, II and III) were characterized by highly calcareous and alkaline soils, as common in the coastal-areas of the Mediterranean basin. Such conditions are usually referred to affect negatively crop yield by impairing the availability and uptake of minerals, especially
P, also when additionally added by inorganic based-P fertilizers due to the rapid transformation into stable minerals relatively unavailable to crops [53]. Location I was characterised by the highest active limestone content (28%), the highest pH (8.0) and the lowest P₂O₅ (28.5 mg kg⁻¹) and K₂O (197 mg kg⁻¹) levels, thus offering the most detrimental conditions for potato growth. Location III showed opposite characteristics to location I, i.e., lower active limestone content (18%), the lowest pH (7.5) and the highest P₂O₅ (135 mg kg⁻¹) and K₂O (612 mg kg⁻¹) levels, while location II had intermediate conditions.

To our knowledge, few information can be found in literature regarding the AMF effect on potato crop in highly calcareous soil. In our research, the qRT-PCR, carried out on DNA extracted by soil samples, revealed that mycorrhizal colonization occurred with similar percentages in each location, independently from the soil characteristics, demonstrating that in the specific conditions of this research AMF were able to occur in highly calcareous soils, alkaline and with high P levels. Our results are in contrast with those commonly reported in literature indicating a negative relationship between soil P level and mycorrhizal colonization [29], but at the same time, are consistent with Sylvia and Schenc [54] and Alkan et al. [55], who observed that AMF differ in their ability to P-tolerance, with G. mosseae showing a high sensitivity to increasing levels of P. Interestingly, in our study AMF were also detected in not-inoculated sub-plots in accordance to Hijri [36], reporting a dataset of 231 field trials with AMF-potato associations. This should not be surprising since AMF naturally occur in field soils but their abundance, diversity and time needed for the establishment can be negatively affected by crop management practices both directly or indirectly [56]. Generally, mycorrhizal colonization is favoured under organic farming. For instance, in a long-term field trial Mäder et al. [31] found that AM root colonization of vetch-rye, winter wheat and grass-clover crops was 30–60% higher in low-input farming systems than in the conventional ones. However, in some cases (e.g., due to soil with P concentrations too high caused by high P fertilizers doses, excessive tillage for weed control or plant diseases) the performance of AMF is low, likely because the benefit received by modern cultivars from mycorrhizal association is poor [56]. Regarding potato cultivar differences, the lowest level of colonization of both mycorrhizal genera was detected in ‘Universa’ plots due to its high potential yield, while ‘Arizona’ was more colonized by Gigaspora spp. and ‘Mondial’ by Glomus spp.

In accordance with previous findings [34–36], this research outlined a significant increase of potato MY, AMTW, NMTP and TDMP due to mycorrhizal colonization. The mycorrhizal inoculation with halved fertilizers dose (F50+M) showed the best results in terms of yield and its components in all the locations. However, the application of full fertilizer doses (F100+M) reported worse results than halved-inoculated sub-plots, likely due to a negative impact of the full dosage on AMF. The high amounts of organic amendments fertilizers, which are generally high in P, is reported to negatively affect the AMF symbiosis with crops [56], and probably this was amplified in the high P soils of the present research. The average yields obtained here with F50+M (38 t ha⁻¹) were higher than those obtained by Lombardo et al. [7] (~20 t ha⁻¹) and Maggio et al. [4] (16 t ha⁻¹) under organic farming management. Douds et al. [34] reported higher yields and larger tubers with commercial inoculants of G. intraradices than by using conventional chemical fertilizers. The enhancement in potato tuber production by AMF inoculation could be attributable to many reasons, but most of researches indicate the increased nutrient uptake, mainly P due to the ability of mycorrhizal fungal hyphae to acquire P well beyond the limits of the rhizosphere, and the disease resistance to Fusarium spp. [33,57] as the most reasonable ones. In our specific field conditions, we also hypothesize an increased tolerance to active limestone and alkalinity, as found by Romero-Munar et al. [58] for salinity in Arundo donax L., through ion homeostasis, vacuoles-compartmentalization and Na⁺ translocation, as suggested by Ruiz-Lozano and Azcón [59]. Moreover, as originally supposed, the highest MY and AMTW were found in location III, which offered better soil conditions for potato growth and AMF, but the highest yield increase, caused by F50+M, was found in location I (109%), compared to an increase of 8% and 17% in location II and III, respectively. The lower AMF efficiency in location III may be ascribed to its high soil P₂O₅ level, since the potential for a mycorrhiza-mediated growth benefit decreases as soil P
According to our data, AMF showed the highest efficiency applying halved fertilizer doses and in the location where soil conditions were unfavourable for potato growth. It should also be noted that ‘Universa’ reported the highest MY and AMTW, despite the mycorrhizal colonization was the lowest. Cultivar differences in response to AMF inoculation, that in field are attributable to a number of factors, were observed both for potato [34] and other crops such as wheat [60], barley [61], white clover [62], globe artichoke [63], etc.

The beneficial effects of AMF on potato yield and its components were also consistent in terms of physiological traits. Indeed, under the specific conditions in which the experiment was conducted, AMF increased the stomatal conductance in location II and III, enhanced the photochemical efficiency and improved the photosynthesis rate, even if statistical significance was only recorded in location II. The poor response in $P_T$ could be attributable to imbalance in the energetic status of the plant, since the light energy absorbed by chlorophylls can be used for photosynthesis, re-emitted as light-chlorophyll fluorescence or dissipated by heat, and these three processes are competitive to each other’s [64]. Soil abiotic factors are reported to inhibit the photosynthetic processes by over-reducing the reaction centres in PSII or inhibiting specific enzymes involved for the synthesis of photosynthetic pigments, thus causing a reduction in plant chlorophyll content [65]. Among soil abiotic factors, salinity is the most common and discussed in literature [64], but also calcareous soils are reported to be negatively correlated to the plant’s photosynthetic machine [66]. AMF are able to ameliorate salt stress by improving the photosynthetic activity, the photochemical properties, the source-sink ratio or the water use efficiency [64,65]. Mycorrhizal-inoculated rice plants in saline soils were found to present a higher photochemical efficiency for $CO_2$ fixation and solar energy utilization than not-inoculated plants through an increase in actual quantum yield of PSII photochemistry, net photosynthetic rate, stomatal conductance and transpiration rate as well as by stimulating carbohydrate transport and metabolism between source and sink tissues [64,67]. A similar behaviour was found by Hajiboland et al. [68] in tomato. Studying the influence of AMF symbiosis in $A. donax$ grown under low P availability, Romero-Munar et al. [58] indicated that AMF conferred salt tolerance by enhancing nutrient use efficiency rather than nutrient uptake: worse $Na^+$ uptake, $Na^+$ root-to-shoot translocation and $Na^+/K^+$ ratio, and better P and K use efficiencies. The authors also reported that the mycorrhizal symbiosis ameliorated the response of $A. donax$ to combine low P and mild salinization conditions, and that the plant growth was driven by salinity rather than P availability. Under our experimental conditions, there was also an effect of concurrent abiotic stresses on plant growth caused by the high active limestone content, alkalinity, low nutrient efficiency (mainly for P and Fe) and high organic matter mineralization. Since soil colonization by $Gigaspora$ spp. and $Glomus$ spp. was found in the three locations with very similar results, we hypothesize a tolerance of AMF inoculates to the above-mentioned abiotic factors, at least in terms of primary colonization, while the effects on secondary colonizations are unknown.

To better understand the photosynthetic ability and energy conversion efficiency to abiotic stress, the PSII photochemical efficiency has been studied through the $F_v/F_m$ and $F_v/F_0$ ratios. According to Pinior et al. [69], under disturbance of biotic or abiotic stresses, the plant dissipates its redundant energy to avoid damage of tissues, and such dissipation can occur via heat or chlorophyll fluorescence. The potential quantum efficiency of PSII is widely reflected with the $F_v/F_m$ ratio. It did not respond well in this research, probably due to high variability of field conditions. For this reason, we calculated the $F_v/F_0$ ratio which is a more sensitive and dynamic parameter to better investigate the PSII efficiency [70]. Indeed, since $F_m$ represents the sum of $F_v$ and $F_0$, the $F_v/F_m$ ratio is slow when $F_v$ slightly decreases and $F_0$ slightly increases [47]. Its mean values were very low in location I (~0.3–0.8), when the stress conditions were higher and impaired the PSII electron transport, and increased in the other two locations to optimal values. Except for location II, AMF increased $F_v/F_0$ by 11.7% in location I and 8.2% in locations III, indicating a better performance in more detrimental soil conditions, as demonstrated by the higher yield increase in the same location.
5. Conclusions

To summarize, the results obtained in the present research demonstrated that AMF inoculation is a useful tool for enhancing early potato yield and physiological traits in highly calcareous and alkaline soils. These results are particularly noticeable by providing halved fertilization doses to the potato crop. Despite the detrimental soil conditions, the qRT-PCR highlighted that AMF colonized all the experimental soils, showing good tolerance to high active limestone, pH and P levels. Furthermore, AMF ameliorated the early potato tolerance to such abiotic stresses by increasing the plant’s gas-exchange capacity and the PSII photochemical efficiency. These findings are of key importance not only for improving the yield of early potato under organic farming, but also for the sustainable management of fertilization by halving the doses with better results in terms of production and environmental impact, as well as for the possibility of a profitable potato cultivation in the coastal agricultural areas of the Mediterranean basin and other arid or semi-arid regions. To this end, further investigations will be necessary to clarify the mycorrhizal association with potato, particularly the role of indigenous AMF communities in this process, as well as to investigate other possible integrations of mycorrhizal-based inoculants with other agronomic practices.

Author Contributions: Conceptualization, G.M., B.P., and S.L.; methodology, A.S., S.L., C.A., G.P., B.P. and G.M.; investigation, S.L., C.A., A.S., G.P. and G.M.; data curation and statistical analysis, A.S. and S.L.; writing—original draft preparation, A.S., S.L. and C.A.; writing—review and editing, A.S., S.L., G.P. and C.A.; supervision, G.M. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by “Fondi di ateneo 2020–2022, Università di Catania, linea Open Access”.

Acknowledgments: The authors gratefully acknowledge the BIOVERDE S.S. farm (Rosolini, Italy) for hosting the field experimental trials, as well as XEDA Italia s.r.l. (Forlì, Italy) for kindly providing the agrochemical products. Thanks are also due to Umberto Burdieri and Angelo Litrico for their excellent technical assistance in conducting the field trials.

Conflicts of Interest: The authors declare no conflict of interest.

References
1. Yiridoe, E.K.; Bonti-Ankomah, S.; Martin, R.C. Comparison of consumer perceptions and preference toward organic versus conventionally produced foods: A review and update of the literature. Renew. Agric. Food Syst. 2005, 20, 193–205. [CrossRef]
2. FiBL-AMI. Statistics on Organic Agriculture. 2018. Available online: http://www.fibl.org/en/themes/organic-farming-statistics (accessed on 20 April 2020).
3. Willer, H.; Schaack, D.; Lernoud, J. Organic farming and market development in Europe and the European Union. In The World of Organic Agriculture. Statistics and Emerging Trends; Willer, H., Lernoud, J., Eds.; FiBL & IFOAM–Organics International: Nürnberg, Germany, 2019; pp. 217–254.
4. Maggio, A.; Carillo, P.; Bulmetti, G.S.; Fuggi, A.; Barbieri, G.; De Pascale, S. Potato yield and metabolic profiling under conventional and organic farming. Eur. J. Agron. 2008, 28, 343–350. [CrossRef]
5. Bacchi, M.A.; De Nadai Fernandes, E.A.; Tsai, S.M.; Santos, L.G.C. Conventional and organic potatoes: Assessment of elemental composition using k0-INAA. J. Radioanal. Nucl. Chem. 2004, 259, 421–424. [CrossRef]
6. Warman, P.R.; Havard, K.A. Yield, vitamin and mineral contents of organically and conventionally grown potatoes and sweet corn. Agric. Ecosyst. Environ. 1998, 68, 207–216. [CrossRef]
7. Lombardo, S.; Lo Monaco, A.; Pandino, G.; Parisi, B.; Mauromicale, G. The phenology, yield and tuber composition of ‘early’ crop potatoes: A comparison between organic and conventional cultivation systems. Renew. Agric. Food Syst. 2013, 28, 50–58. [CrossRef]
8. Lombardo, S.; Pandino, G.; Mauromicale, G. The influence of growing environment on the antioxidant and mineral content of “early” crop potato. J. Food Compos. Anal. 2013, 32, 28–35. [CrossRef]
9. Lombardo, S.; Pandino, G.; Mauromicale, G. The mineral profile in organically and conventionally grown “early” crop potato tubers. Sci. Hortic. 2014, 167, 169–173. [CrossRef]
10. Buono, V.; Paradiso, A.; Serio, F.; Gonnella, M.; De Gara, L.; Santamaria, P. Tuber quality and nutritional components of early potato subjected to chemical haulm desiccation. J. Food Compos. Anal. 2009, 22, 556–562. [CrossRef]
11. Lombardo, S.; Pandino, G.; Mauromicale, G. The effect on tuber quality of an organic versus a conventional cultivation system in the early crop potato. *J. Food Compos. Anal.* 2017, 62, 189–196. [CrossRef]

12. Izmirlioglu, G.; Demirci, A. Enhanced bio-ethanol production from industrial potato waste by statistical medium optimization. *Int. J. Mol. Sci.* 2015, 16, 24490–24505. [CrossRef]

13. Jagatee, S.; Behera, S.; Dash, P.K.; Sahoo, S.; Mohanty, R.C. Bioprospecting starchy feedstocks for bioethanol production: A future perspective. *JMRR* 2015, 3, 24–42.

14. Ierna, A.; Mauromicale, G. Potato growth, yield and water productivity response to different irrigation and fertilization regimes. *Agric. Water Manag.* 2018, 201, 21–26. [CrossRef]

15. Canali, S.; Ciaccia, C.; Antichi, D.; Barberi, P.; Montemurro, F.; Tittarelli, F. Interactions between green manure and amendment type and rate: Effects on organic potato and soil mineral N dynamic. *J. Food Agric. Environ.* 2010, 8, 537–543.

16. Hepperly, P.; Lotter, D.; Ziegler, C.; Seidel, R.; Reider, C. Compost, manure and synthetic fertilizer influences crop yields, soil properties: Nitrate leaching and crop nutrient content. *Compos. Sci. Util.* 2009, 17, 117–126. [CrossRef]

17. Rosen, C.J.; Allan, D.L. Exploring the benefits of organic nutrient sources for crop production and soil quality. *HortTechnology* 2007, 17, 422–430. [CrossRef]

18. Caliskan, M.E.; Kilic, S.; Gunel, E.; Mert, M. Effect of farmyard manure and mineral fertilization on growth and yield of early potato (*Solanum tuberosum* L.) under the Mediterranean conditions in Turkey. *Indian J. Agron.* 2004, 49, 198–200. [CrossRef]

19. Ierna, A.; Parisi, B. Crop growth and tuber yield of “early” potato crop under organic and conventional farming. *Sci. Hortic.* 2014, 165, 260–265. [CrossRef]

20. Clark, M.S.; Horwath, W.R.; Shennan, C.; Scow, K.M.; Lantini, W.T.; Ferris, H. Nitrogen, weeds and water as yield-limiting factors in conventional, low-input, and organic tomato systems. *Agric. Ecosyst. Environ.* 1999, 73, 257–270. [CrossRef]

21. Lynch, D.H.; Shariff, M.; Hammermeister, A.; Burton, D. Nitrogen management in organic potato production. In *Sustainable Potato Production: Global Case Studies*; He, Z., Larkin, R., Honeycutt, W., Eds.; Springer: Dordrecht, The Netherlands, 2012; pp. 209–231. [CrossRef]

22. Van Delden, A.; Schroder, J.J.; Kropff, M.J.; Grashoff, C.; Booiij, R. Simulated potato yield and crop and soil nitrogen dynamics under different organic nitrogen management strategies in the Netherlands. *Agric. Ecosyst. Environ.* 2003, 96, 77–95. [CrossRef]

23. Ierna, A.; Mauromicale, G. Tuber yield and irrigation water productivity in early potatoes as affected by irrigation regime. *Agric. Water Manag.* 2012, 115, 276–284. [CrossRef]

24. Mauromicale, G.; Signorelli, P.; Ierna, A.; Foti, S. Effects of intraspecific competition on yield of early potato grown in Mediterranean environment. *Ann. J. Potato Res.* 2003, 80, 281–288. [CrossRef]

25. Taalab, A.S.; Ageeb, G.W.; Siam, H.S.; Mahmoud, S.A. Some characteristics of calcareous soils. A review. *Middle East J. Agric. Res.* 2019, 8, 96–105.

26. Di Gregorio, A. *Land Cover Classification System, Classification Concepts and User Manual*, 3rd ed.; FAO: Rome, Italy, 2016.

27. Koch, M.; Naumann, M.; Pawelzik, E.; Gransee, A.; Thiel, H. The importance of nutrient management for potato production Part I: Plant nutrition and yield. *Potato Res.* 2019, 63, 97–119. [CrossRef]

28. Lombardo, S.; Pandino, G.; Mauromicale, G. Optimizing nitrogen fertilization to improve qualitative performances and physiological and yield responses of potato (*Solanum tuberosum* L.). *Agronomy 2020, 10,* 352. [CrossRef]

29. Smith, S.E.; Read, D.J. *Mycorrhizal Symbiosis*, 3rd ed.; Academic Press: London, UK, 2008.

30. Scavo, A.; Abbate, C.; Mauromicale, G. Plant allelochemicals: Agronomic, nutritional and ecological relevance in the soil system. *Plant Soil* 2019, 442, 23–48. [CrossRef]

31. Mäder, P.; Edenhofer, S.; Boller, T.; Wiemken, A.; Niggli, U. Arbuscular mycorrhizae in a long-term field trial comparing low-input (organic, biological) and high-input (conventional) farming systems in a crop rotation. *Biol. Fertil. Soils* 2000, 31, 150–156. [CrossRef]

32. Black, R.L.B.; Tinker, P.B. Interaction between effects of vesicular-arbuscular mycorrhizae and fertilizer phosphorus on yields of potatoes in the field. *Nature 1977,* 267, 510–511. [CrossRef]

33. Wu, F.; Wang, W.; Ma, Y.; Liu, Y.; Ma, X.; An, L.; Feng, H. Prospect of beneficial microorganisms applied in potato cultivation for sustainable agriculture. *Afr. J. Microbiol. Res.* 2013, 7, 2150–2158. [CrossRef]
34. Douds, D.D., Jr.; Nagahashi, G.; Reider, C.; Hepperly, P.R. Inoculation with arbuscular mycorrhizal fungi increases the yield of potatoes in a high P soil. *Biol. Agric. Hortic.* 2007, 25, 67–78. [CrossRef]

35. Duffy, E.M.; Cassells, A.C. The effect of inoculation of potato (*Solanum tuberosum* L.) microplants with arbuscular mycorrhizal fungi on tuber yield and tuber size distribution. *Appl. Soil Ecol.* 2000, 15, 137–144. [CrossRef]

36. Hijri, M. Analysis of a large dataset of mycorrhiza inoculation field trials on potato shows highly significant increases in yield. *Mycorrhiza* 2016, 26, 209–214. [CrossRef] [PubMed]

37. Lombardo, S.; Pandino, G.; Mauronicale, G. Nutritional and sensory characteristics of “early” potato cultivars under organic and conventional cultivation systems. *Food Chem.* 2012, 133, 1249–1254. [CrossRef]

38. Parisi, B.; Govoni, F.; Mainolfi, A.; Baschieri, T.; Ranalli, P. Nuovi cloni italiani per la pataticoltura nazionale. *L’Informatore Agrar.* 2002, 46, 41–45.

39. Graham, S.O.; Green, N.E.; Hendrix, J.W. The influence of vesicular-arbuscular mycorrhizal fungi on growth and tuberization of potatoes. *Mycolgia* 1976, 68, 925–929. [CrossRef]

40. Johnstone, P.D.; Lowther, W.L.; Keoghan, J.M. Design and analysis of multi-site agronomic evaluation trials. *N. Z. J. Agric. Res.* 1993, 36, 323–326. [CrossRef]

41. Soil Survey Staff. *Soil Taxonomy: A Basic System of Soil Classification for Making and Interpreting Soil Surveys*, 2nd ed.; US. Gov. Print. Office: Washington, DC, USA, 1999.

42. Italian Society of Soil Science. *Metodi Normalizzati Di Analisi Del Suolo*; Edagricole: Bologna, Italy, 1985.

43. Dierauer, H.; Siegrist, F.; Weidmann, G.; Commercial Organic Fertiliser as Supplementary Fertilisers in Potato Crop Production. Research Institute of Organic Agriculture-FiBL, Practice Abstract. 2017. Available online: https://www.orgprints.org/31027/ (accessed on 20 April 2020).

44. Mauronicale, G.; Ierna, A.; Marchese, M. Chlorophyll fluorescence and chlorophyll content in field-grown potato as affected by nitrogen supply, genotype, and plant age. *Photosynthetica* 2006, 44, 76–82. [CrossRef]

45. Mauronicale, G.; Lo Monaco, A.; Longo, A.M.G. Effect of branched broomrape (*Orobanche ramosa*) infection on the growth and photosynthesis of tomato. *Weed Sci.* 2008, 56, 574–581. [CrossRef]

46. Maxwell, K.; Johnson, G.N. Chlorophyll fluorescence—A practical guide. *J. Exp. Bot.* 2000, 51, 659–668. [CrossRef]

47. Babani, F.; Lichtenthaler, H.K. Light-induced and age-dependent development of chloroplasts in etiolated barley leaves as visualized by determination of photosynthetic pigments, CO₂ assimilation rates and different kinds of chlorophyll fluorescence ratios. *J. Plant Physiol.* 1996, 148, 555–566. [CrossRef]

48. Scavo, A.; Restuccia, A.; Abbate, C.; Mauronicale, G. Seeming field allelopathic activity of *Cynara cardunculus* L. reduces the soil weed seed bank. *Agron. Sustain. Develop.* 2020, 41. [CrossRef]

49. Livak, K.J.; Schmittgen, T.D. Analysis of relative gene expression data using real-time quantitative PCR and the 2(−Delta Delta C(T)). *Methods* 2001, 25, 402–408. [CrossRef] [PubMed]

50. Thonar, C.; Erb, A.; Jansa, J. Real-time PCR to quantify composition of arbuscular mycorrhizal fungal communities-marker design, verification, calibration and field validation. *Mol. Ecol. Resour.* 2012, 12, 219–232. [CrossRef] [PubMed]

51. Gomez, K.A.; Gomez, A.A. *Statistical Procedures for Agricultural Research*; John Wiley & Sons: New York, NY, USA, 1984.

52. Scavo, A.; Restuccia, A.; Lombardo, S.; Fontanazza, S.; Abbate, C.; Pandino, G.; Anastasi, U.; Onofri, A.; Mauronicale, G. Improving soil health, weed management and nitrogen dynamics by *Trifolium subterraneum* cover cropping. *Agron. Sustain. Develop.* 2020, 40, 18. [CrossRef]

53. Khademi, Z.; Jones, D.L.; Malakouti, M.J.; Asadi, F. Organic acids differ in enhancing phosphorus uptake by *Triticum aestivum* L.—Effects of rhizosphere concentration and counterion. *Plant Soil* 2010, 334, 151–159. [CrossRef]

54. Sylvia, D.M.; Schenck, N.C. Application of superphosphate to mycorrhizal plants stimulates sporulation of phosphorus-tolerate vesicular-arbuscular mycorrhizal fungi. *New Phytol.* 1983, 95, 655–661. [CrossRef]

55. Alkan, N.; Gadkar, V.; Yarden, O.; Kapulnik, Y. Analysis of quantitative interactions between two species of arbuscular mycorrhizal fungi, *Glomus mosseae* and *G. intraradices*, by Real-Time PCR. *Appl. Environ. Microb.* 2006, 72, 4192–4199. [CrossRef]

56. Gosling, P.; Hodge, A.; Goodlass, G.; Bending, G.D. Arbuscular mycorrhizal fungi and organic farming. *Agr. Ecosyst. Environ.* 2006, 113, 17–35. [CrossRef]
57. Ismail, Y.; McCormick, S.; Hijri, M. A fungal symbiont of plant-roots modulates mycotoxin gene expression in the pathogen *Fusarium sambucinum*. *PLoS ONE* 2011, 6, e17990. [CrossRef]

58. Romero-Munar, A.; Baraza, E.; Gulias, J.; Cabot, C. Arbuscular mycorrhizal fungi confer salt tolerance in giant reed (*Arundo donax* L.) plants grown under low phosphorus by reducing leaf Na⁺ concentration and improving phosphorus use efficiency. *Front. Plant Sci.* 2019, 10, 843. [CrossRef]

59. Ruiz-Lozano, J.M.; Porcel, R.; Azcón, C.; Aroca, R. Regulation by arbuscular mycorrhizae of the integrated physiological response to salinity in plants: New challenges in physiological and molecular studies. *J. Exp. Bot.* 2012, 63, 4033–4044. [CrossRef]

60. Al-Karaki, G.; McMichael, B.; Zak, J. Field response of wheat to arbuscular mycorrhizal fungi and drought stress. *Mycorrhiza* 2004, 14, 263–269. [CrossRef] [PubMed]

61. Zhu, Y.G.; Smith, F.A.; Smith, S.E. Phosphorus efficiencies and responses of barley (*Hordeum vulgare* L.) to arbuscular mycorrhizal fungi grown in highly calcareous soil. *Mycorrhiza* 2003, 13, 93–100. [CrossRef] [PubMed]

62. Eason, W.R.; Webb, K.J.; Michaelson-Yeates, T.P.T.; Abberton, M.T.; Griffith, G.W.; Culshaw, C.M.; Hooker, J.E.; Dhanoa, M.S. Effect of genotype of *Trifolium repens* on myconhizal symbiosis with *Glomus mosseae*. *J. Agric. Sci.* 2001, 137, 27–36. [CrossRef]

63. Pandino, G.; Lombardo, S.; Lo Monaco, A.; Ruta, C.; Mauromicale, G. In vitro micropropagation and mycorrhizal treatment influences the polyphenols content profile of globe artichoke under field conditions. *Food Res. Int.* 2017, 99, 385–392. [CrossRef] [PubMed]

64. Porcel, R.; Redondo-Gómez, S.; Mateos-Naranjo, E.; Aroca, R.; Garcia, R.; Ruiz-Lozano, J.M. Arbuscular mycorrhizal symbiosis ameliorates the optimum quantum yield of photosystem II and reduces non-photochemical quenching in rice plants subjected to salt stress. *J. Plant Physiol.* 2015, 185, 75–83. [CrossRef]

65. Sheng, M.; Tang, M.; Chen, H.; Yang, B.; Zhang, F.; Huang, Y. Influence of arbuscular mycorrhizae on photosynthesis and water status of maize plants under salt stress. *Mycorrhiza* 2008, 18, 287–296. [CrossRef]

66. Bavareco, L.; Poni, S. Effect of calcareous soil on photosynthesis rate, mineral nutrition, and Source-Sink ratio of table grape. *J. Plant Nutr.* 2007, 26, 2123–2135. [CrossRef]

67. Tisarum, R.; Theerawitaya, C.; Samphumphuang, T.; Polispatik, K.; Thongpoem, P.; Singh, H.P.; Cha-um, S. Alleviation of salt stress in upland rice (*Oryza sativa* L. ssp. *indica* cv. Leum Pua) using arbuscular mycorrhizal fungi inoculation. *Front. Plant Sci.* 2020, 11, 348. [CrossRef]

68. Hajiboland, R.; Aliasgharzadeh, N.; Laiegh, S.F.; Poschenrieder, C. Colonization with arbuscular mycorrhizal fungi improves salinity tolerance of tomato (*Solanum lycopersicum* L.) plants. *Plant Soil* 2010, 331, 313–327. [CrossRef]

69. Pinior, A.; Grunewaldt-Stöcker, G.; von Alten, H.; Strasser, R.T. Mycorrhizal impact on drought stress tolerance of rose plants probed by chlorophyll a fluorescence, proline content and visual scoring. *Mycorrhiza* 2005, 15, 596–605. [CrossRef]

70. Lima, J.D.; Mosquim, P.R.; Da Matta, F.M. Leaf gas exchange and chlorophyll fluorescence parameters in Phaseolus vulgaris as affected by nitrogen and phosphorus deficiency. *Photosynthetica* 1999, 37, 113–121. [CrossRef]