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Application of Arbuscular Mycorrhizae *Glomus iranicum* var. *tenuihypharum* var. *nova* in Intensive Agriculture: A Study Case

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Abstract: Intensive agriculture contributes to a decrease in microbial biomass and crop yields, while accelerating soil degradation. Arbuscular mycorrhizae associations have direct benefits for plant nutrition, and may be considered a useful tool in modern agriculture. Notwithstanding the widespread knowledge of these benefits, their use in intensive farming systems has until now been ineffectual, because most mycorrhizal species have low tolerance toward high concentrations of nutrients and are poorly adapted to the soil and/or mycorrhizal functioning. The aim of this work was to test the efficacy of an arbuscular mycorrhizal (AM) fungus, *Glomus iranicum* var. *tenuihypharum* on lettuce and table grape crops in different intensive farming systems. The variables studied were root colonization percentage, external mycelium concentration, gas exchange, photosynthetic activity, root starch concentration and plant nutrition. The main finding was that the fungus is tolerant of a wide range of soil pH values, high salinity levels and abundant external mycelium. In lettuce, it produced significant increases in plant physiological activity and productivity (10%-15%); and in table grapes, increases of 12%-45% in yield were achieved for more than three years in Crimson variety, and significant increases in fruit cluster weight, color uniformity and Brix (°Bx). The AM species is protected by two patents and is a component of MycoUp, MycoUp Activ, Resid HC and Resid MG, whose commercial application has spread to more than 30 countries, with increments in crop yields of 8%-45% in lines as varied as leaf vegetables, berries, fruit, olives, grapes, greenhouse crops and cereals.

Key words: Arbuscular mycorrhizal, *Glomus iranicum* var. *tenuihypharum*, intensive agriculture, gas exchange, root starch concentration, photosynthetic activity.

1. Introduction

Intensive agriculture as a production system is characterized by high inputs of capital, fertilizers and pesticides, although such high inputs have allowed a substantial increase in production [1].

However, among its many disadvantages, intensive farming alters the environment in many ways, decreasing microbial populations and promoting soil erosion, while altering the biology of rivers and lakes as well.

In the last decade, new ways of practicing intensive agriculture have emerged, including biointensive agriculture, which focuses on maximizing efficiency compared with conventional practices, and increasing yield per energy and area input. Unfortunately, the yields of many crops often diminish in the long term and the changes involved present new challenges to farmers, who rely on modern farming equipment that is best suited to monocultures [2, 3].

This phenomenon has resulted in a drastic reduction in the microbiota in soils, leading to a reduced microbial population, which is unbalanced and dependent on the above inputs, commonly resulting in fungal or bacterial diseases [4].

Moreover, in the case of highly profitable crops,

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such as berries, leafy vegetables or greenhouse crops, it is common to use soil disinfection programs to eliminate the microbial imbalance generated by this praxis. Consequently, there is a gradual process of enrichment with the microbial populations contained in the irrigation water and in the environment, all random and resulting in a low disease pressure of the soil's phytopathogens, such as phytonematodes or fungi, including *Fusarium* sp., *Rhizoctonia* sp., *Sclerotinia* sp., *Botrytis* sp., *Pythium* sp. and *Oidio* sp., etc. [5, 6].

On the other hand, the world is in transition from an era of food abundance to one of scarcity. With 40% of the planet's land devoted to human food production, up from 7% in 1700, and as the world's demand for food increasing to reach a predicted 70% by 2050, feeding a rapidly growing human population will mean adopting a sustainable food production approach that can run indefinitely with minimized impacts on the environment, animal welfare and human health.

It is therefore necessary to incorporate in these highly productive agricultural systems' biotechnological tools that are compatible with the amount of nutrients required for each specific crop. The global market for bio-inoculants, which is growing at an estimated rate of 10% per annum [7], was valued at $440 million in 2012 and is expected to reach $1,295 million by 2020 [8].

Much information exists concerning microorganisms in plant nutrition, especially with regard to bacteria and arbuscular mycorrhizal fungi (AMF), in the case of commercial products. Despite this, it is still very difficult to determine their effectiveness in promoting plant growth and crop yields, when in most cases the mechanisms responsible for these beneficial effects are unknown and quality control procedures within the industry and accepted standards to allow product comparison, etc. are generally lacking [9]. The aim of this contribution was to show the benefits of the application of *Glomus iranicum* var. *tenuihypharum* on plant physiology, nutrition and productivity under several farming systems and covering several commercial crops, such as table grapes and vegetables. The impact of its application in different countries was also studied.

2. Selection Criteria for Choosing an Arbuscular Mycorrhizal (AM) Strain

The term "mycorrhiza" is derived from the Greek myco-(fungus) and rhiza (root). Mycorrhizae are associated with plant roots through an extensive (extraradical) network of hyphae in the soil, which acts as an extension of the plant's root system.

The efficient exchange of nutrients (sucrose to the fungus and N/P and other nutrients to the plant) is mediated via specialized structures within the roots (e.g., intracellular arbuscules in AMF). AM and ectomycorrhizae are the most widespread and ecologically important types of mycorrhiza and the only ones commercially exploited in agriculture and forestry.

However, major advances in our knowledge of mycorrhizal symbiosis have thrown light on their activity in plant nutrition. Just as the ecological functions of this symbiosis are much better understood, the biodiversity and evolution of the same are no longer a black box, and the genomes of a wide range of mycorrhizal fungi have been sequenced. At the same time, molecular interactions responsible for the symbiosis are starting to be revealed [10].

The success of AM symbiosis depends not only on the plant and fungal genotypes, but also on the conditions of the environment. The functional specificity that exists between plants and AMF has been well documented [11-13]. The soil environment is known to exert a strong selection pressure on AMF [14, 15], but the influence of the soil on AM genotypes is less well understood [16].

The soil is an important key to the effectiveness of AMF, because it not only provides mineral nutrients to the fungi, but also constitutes the chemical, physical and environmental platform, where both

associates live. Liming the soil was seen to decrease root colonization by *Acaulospora laevis*, but to increase root colonization by *G. invermaium* [17], confirming that different AM species have different soil pH optima. The adaptation of AMF to specific soil pH was revealed by AM strains that only existed within a given range of soil pH values [18]. It seems, then, that AM strains may survive and function well only within a certain range of soil environmental conditions [19].

Several studies have shown that plant responses to inoculation vary in different soils [20-23], and until now, this lack of consistency has hindered the efficient use of AM inoculants in plant production. AM strains must not only be highly effective, but also be able to function in the soil environment where they are introduced. Selecting strains based on target soil properties may be the key to consistency in the effect of AM inoculants.

Both nutrient level and the nutrient balance are important factors influencing AM symbiotic development and function [24]. A nutrient imbalance may alter the function of indigenous AMF strains, and soils with an altered nutrient balance may benefit from the introduction of adapted AM strains.

Although AM symbiosis constitutes the most important microbial association in agronomy and has evolved in most terrestrial environments as an efficient system of nutrient uptake in plants [25], factors, such as type of soil, high and unbalanced fertilizations levels, and the incorrect selection of strains [26] will exert a marked influence on the success of the implantation of this technology [27], making it necessary to choose strains according to the type of soil.

3. Performance of AM Inoculants

There are two types of AM inoculants, those containing a mixture of spores and propagules propagated with a host plant on an inert carrier in containers or beds in field conditions [28], and those produced *in vitro* with transformed carrot root organ cultures as host, mainly the strain *Rhizophagus irregularis* or *intraradices.*

The success of commercial AM inoculants may be judged according to economic gain, either through improved yields or reduced applications of inorganic fertilizer, or both. They should not be viewed as a replacement for inorganic fertilizers, but as a potential component of an integrated nutrient management strategy that enhanced soil nutrient acquisition [9].

Some of the peer-reviewed publications that report AM crop application results are listed in Table 1.

Table 1 Peer-reviewed publications that have used commercially available AM products. Species names are as reported within each paper (AMF, formerly known as *Glomus* spp., have recently been renamed).

Product	Crop	Field/lab	Microorganisms	Results	Application rate	References
Mycormax. JH Biotech Inc., Ventura, U.S.	*Zea mays*	Lab	2 *Glomus* species + 5 EM	< 5% of root colonization; increased DMY	1.2 g/L soil	[29]
BEI Bio Organics, Santa Maria, U.S.	*Zea mays*	Lab	6 *Glomus* species + 1 *Gigaspora* + 1 *Paraglomus*	< 5% root colonization; no increased DMY	1.8 g/L soil	[29]
AgBio Endos, AgBio Inc., Westminster, U.S.	*Zea mays*	Lab	6 *Glomus* species + 1 *Gigaspora*	< 5% root colonization; no increased DMY	3.0 g/L soil	[29]
AM 120. Reforestation Technologies Int., U.S.	*Zea mays*	Lab	3 *Glomus* species	< 5% of root colonization; increased DMY	3.0 g/L soil	[29]
BioGrow Endo. Mycorrhizal Applications Inc., U.S.	*Zea mays*	Lab	3 *Glomus* species + 1 *Trichoderma*	< 5% of root colonization; increased DMY	3.0 g/L soil	[29]
EcoMic-Micofert Research Centres, Cuba	Coffee and horticultural crops	Field	*G. intraradices*, *G. fasciculatum*, *G. mosseae*, *G. etunicatum*, *Paraglomus ocultum*	Increased DMY according soil type	4-5 kg/ha	[30]

(Table 1 continued)

Product	Crop	Field/lab	Microorganisms	Results	Application rate	References
Mycor Tree Root Dip Plant Healthcare Inc., U.S.	*Zea mays*	Lab	5 Ecto/Endomycorrhiza	< 5% root colonization; no increased DMY	Mix with water (not specified)	[29]
MYKE PRO SG2 Premier Tech, Canada	*Zea mays*	Lab	*G. intraradices*	No increased DMY	7.5 kg/ha	[31]
MYKE PRO SG2 Premier Tech, Canada	*Solanum tuberosum*	Field	*G. intraradices*	Increased DMY	15 cm/plant	[32]
Aegis Italpollina, Rivoli Veronese, Italy	*Zea mays*	Field	*G. intraradices*	No increased DMY	25 kg/ha	[33]
MycoApply Endo	*Zea mays*	Field	*G. intraradices*	No increased DMY	10 g/pot	[34]
VAM 80	*Zea mays*	Field	*G. intraradices*	No increased DMY	1 tablespoon/pot	[34]
Ascend PB	*Zea mays*	Field	*G. intraradices*	No increased DMY	1 g/pot	[34]
NTC	*Zea mays*	Field	*G. intraradices*	No increased DMY	30 mL/pot	[34]
MycoGrowth Symborg, Spain	Tomato	Field	*G. iranicum* var. *tenuihypharum*	Increased DMY and yield	3kg/ha	[35]
MycoGrowth Symborg, Spain	Lettuce	Field	*G. iranicum* var. *tenuihypharum*	Increased DMY and yield	3 kg/ha	[36]
MycoGrowth Symborg, Spain	Grape	Field	*G. iranicum* var. *tenuihypharum*	Increased DMY and yield	3 kg/ha	[37]
Resid Symborg, Spain	Rice	Field	*G. iranicum* var. *tenuihypharum*	Increased DMY and yield	3 kg/ha	[38]
MycoGrowth Symborg, Spain	Ornamental plant	Field	*G. iranicum* var. *tenuihypharum*	Increased DMY and yield	3 kg/ha	[39]
MycoGrowth Symborg, Spain	Strawberry	Field	*G. iranicum* var. *tenuihypharum*	Increasing firm depend of the variety	3 kg/ha	[40]

DMY = dry matter yield.

Some authors [29] found the number of positive and negative responses to the inoculation of maize with different mycorrhizal inoculants containing different fungal species to be the same. Hart and Forsythe [41] found that a single inoculation with *R. irregularis* increased the host (*Allium porrum* L. and *Plantago lanceolata* L.) nutrient content, irrespective of soil nutrient status, in contrast to mixed AM treatments. Similarly, using soil with high levels of phosphorus (P), Cozzolino et al. [32] recorded a significant increase in total fresh weight of a potato crop using the single strain (Mike Pro SG2) (*R. irregularis*).

For their part, using different mycorrhizal products based on *R. intraradices*/*R. irregularis*, Corkidi et al. [34] always achieved negative results in field trials of maize cultivation, using different inoculation dosages. However, lettuce plants inoculated with *G. iranicum* var. *tenuihypahrum* showed higher nitrogen (N), calcium (Ca) and potassium (K) uptake from saline and non-saline substrates, while the uptake of sodium (Na) was reduced [36]. Under highly saline field conditions, better growth of the ornamental plant *Viburnum tinus* (Laurustinus) was observed [39]. Under greenhouse conditions, tomato plants inoculated with *G. iranicum* var. *tenuihypharum* showed improved water use efficiency (WUE), probably the result of a controlled rate of transpiration, and a higher rate of net photosynthesis. In addition, a close relationship was found between the respiratory activity of AMF and the rate of net photosynthesis, leaf N, P, Fe and K concentrations, and fruit yield. As a result, the overall performance (growth, nutrition and yield) of plants cultivated under an intensive fertigation regime improved [35]. The species has a broad host spectrum covering both monocots, such as *Sorghum* spp., and dicots, such as *Viburnum* spp., *Lactuca* spp. [36, 39]. In strawberry, a positive influence was observed on production [40] and the levels of anthocyans in the variety Fortuna, but not in other varieties.

The efficacy of AMF, when used in inoculants, is

also influenced by numerous soil, crop and environmental factors, including crop species compatibility, size and effectiveness of indigenous microbial populations, soil fertility and management [42]. Despite the successful laboratory results obtained with certain strains of AMF, studies agree that scale-up of their use for agriculture has been slow, probably due to the relative ineffectiveness of the inoculation process [27], or other factors, such as the technical difficulty involved in their application, the degree of soil compatibility [19] and field carrying capacity [35], etc..

In light of the above results, our objective was to select an AM species that could have a stable positive effect, regardless of external conditions, when used as a biological inoculant in intensive agriculture. In this context, the main criteria taken in account were the soil environment and the adaptation of the AMF to a specific range of soil pH levels [18, 19]. The species chosen would have to show constant results in the special conditions required by different intensive agriculture systems and crop families.

4. *G. iranicum* var. *tenuihypharum*: A Study Case

The strain *G. iranicum* var. *tenuihypharum* was previously isolated from a hydromorphic and highly compacted sodium saline soil classified as Solonetz Gley type. Some chemical properties are presented in Table 2. As can be seen, it is a very alkaline soil (pH H_2O 9.5), with high concentrations of Ca, Na and Mg, low C/N ratio and low organic matter level.

Strain Morphology.

Sporocarps: none observed.

Spores: Small spores up to 65-70 μm diameter, usually aggregated in loose to compact clusters. The spore wall consists of two or three layers. The spore wall layer one, forming the spore surface, is mucilaginous, short-lived and stains in Melzer's reagent or is type unique (not divided into sublayers), permanent and does not react in Melzer's reagent (it is not present in Fig. 1). The middle layer (SWL_2) is permanent, 0.5-2.0 μm thick, and the inner layer (SWL_3) laminar (0.5-1.5 μm). Spore contents are pale and guttulate (Fig. 1).

Subtending hyphae are hyaline to pale ochraceous, straight or undulating 2.5-4.5 μm in diameter (mean 3.0 μm), with a cylindrical to slightly funnel shape continuous with spore wall layers; pore open in mature spores.

Germination structure: Germ tube re-grows through the hyphal attachment, forming vesicular-arbuscular mycorrhizae. Extraradical mycelium is abundant. Older spores look somewhat shaggy. SWL_1 exhibits a dextrinoid reaction, when stained with Melzer's reagent, giving young spores a brownish red colour. SWL_2 is permanent, 0.5-2.0 μm thick; SWL_3 laminar (0.5-1.5 μm). Spore contents are pale, guttulate (Fig. 1).

Table 2 Some chemical properties of the Solonetz Gley soil where *G. iranicum* var. *tenuihypharum* was isolated.

Parameters	Concentration
pH (H_2O)	9.5
pH (KOH)	10.1
Organic matter (%)	0.8
N (%)	0.2
C/N ratio	6.5
Calcium carbonates (%)	12.0
Ca (ppm)	5,809.6
Na (ppm)	1,829.4
Mg (ppm)	2,967.04

Fig. 1 Light micrographs of spores of *G. iranicum* var. *tenuihypharum*. (1) Mature spore; spore wall layers SWL_2 and SWL_3 are visible (arrows); (2) typical, loose cluster of spores; (3) open pore towards the subtending hypha.

4.1 Effect of G. iranicum var. tenuihypharum and Other AM Species on Lettuce Crop Production under Standard Fertilization Program

The aim for this study was to determine the effectiveness of *G. iranicum* var. *tenuihypharum* compared with other strains of AMF in lettuce (*Lactuca sativa* L., variety "Capitata") grown under a standard fertilization program.

4.1.1 Material and Methods

4.1.1.1 Experiment Design

This study was conducted at the experimental farm of the Centro de Edafología y Biología Aplicada del Segura (CEBAS-CSIC) in Murcia, Spain (38°07′18″ N, 1°13′15″ W). The experiment lasted 90 d (January-May 2015) and was carried out under controlled conditions in a greenhouse equipped with a cooling system with a relative humidity of 55%-65%, day/night temperatures of 25 °C/12 °C, and a 10 h photoperiod at a maximum photosynthetic photon flux density (PPFD) of 1,500 μmol, measured using an external light meter (LI-Quantum Q-40211, LI-COR Inc., Lincoln, NE, USA) during the gas exchange measurements.

An experimental design consists of four treatments, 10 pots per treatment and one plant per pot were used. A total of 40 pots, each five liters of substrate composed of a 3:2:1 (v/v/v) mix of calcareous soil:sand:vermiculite was used in the experiment. The substrate had a pH of 7.1, with 1.7% (w/w) organic matter, 0.9% (w/w) total organic carbon, 0.1% (w/w) total N and 196.6 mg/kg available P. The substrate was not sterilized in order to simulate the real-life scenario of compatibility and competition between native and inoculated AMF.

The studied treatments were the AMF types and a non-treated control. Three species of AMF were used in this study: *F. mosseae* (Nicholson & Gerdeman) Gerdeman & Trappe, *R. irregularis* (N.C. Schenck & G.S. Sm., 1982) and *G. iranicum* var. *tenuihypharum* var. *nova* from Symborg company.

An irrigation system was installed; each pot was fitted with one pressure-compensated emitter per plant at a flow rate 2 L/h and a device for homogenizing water delivery over the entire surface of the substrate. The lettuce seedlings (*L. sativa* L.) of variety Capitata and iceberg type were transplanted in January-March 2015. One week after transplanting, an AM powder formulation was delivered through the irrigation system at doses equivalent to 3 kg/ha of *F. mosseae* with a propagule concentration of 125 propagules/g, *R. irregularis* at 140 propagules/g and *G. iranicum* var. *tenuihypharum* at 120 propagules/g, according to the most probable number test, as described by Porte [43].

Each treatment received the same standard fertilization. Fertilization consisted of a granulate fertilizer that contained: N 7%, P 4%, K 6%, Ca 25% and sulfur (S) 2%, mixed with the substrate at the

equivalent of 500 kg/ha before filling the pots and, after transplanting, a fertirrigation solution of pH 7.90, electric conductivity 2,106 mmhos/cm, and total soluble solids 0.93 g/L, KNO_3 15,000 g, $Ca(NO_3)_2$ 1,000 g, H_3PO_4 508 g, HNO_3 452 g were applied twice a week during the assay. Water irrigation was applied equally in all treatments (three times per week).

4.1.1.2 Symbiotic Activity

The percentage of mycorrhizal colonization was calculated following the classic gridline intersect method [19], after staining the roots with the method described by Phillips [44], which consisted of clearing washed roots (200 g) in 10% KOH, 20 min at 90 °C in water bath and after staining with 0.05% trypan blue in lactic acid (v/v), for 15 min, keeping the same conditions mentioned before. Extramatrical mycelium was measured by estimating the visual presence in a given area [19]. Easily extractable glomalin was detected by the method described by Wright et al. [45].

4.1.1.3 Biomass Production

Lettuce growth (shoot fresh weight (SFW), shoot dry weight (SDW), root fresh weight (RFW), root dry weight (RDW) and shoot diameter (SD)) were analysed at each sampling time according to: (1) Regulation No. 771/2009 of the European Union Commission, (2) the indications proposed by Ryder [46], and (3) personal communication with landowners. Dry weights (DW) were measured after drying in a forced hot-air oven at 80 °C until a constant weight was reached (approximately 48 h).

The gas exchange parameters (net photosynthesis (A) and stomatal conductance (g_s)) were measured in 10 plants/treatment, using a LICOR LI-6400 Portable Photosynthesis System (LI-COR Inc., Lincoln, NE, USA model. LI-6400). All measurements were performed at solar noon and twice during the assay period of 75 d. WUE was determined from the A/g_s ratio.

Chlorophyll (in SPAD units) was measured in 10 plants twice (days 40 and 75) during the assay, using a portable meter (chlorophyll meter SPAD-502, Konica Minolta), which makes instantaneous and non-destructive measurements of the relative chlorophyll index.

The ions content of leaves was obtained by the ICP-OES technique (Iris Intrepid II XDL, OribaSci.) 75 d after transplanting. The ions measured were N, P, K and Ca, and the trace elements were manganese (Mn), iron (Fe) and zinc (Zn).

4.1.1.4 Data Analysis

Data were analyzed using a one-way analysis of variance, using SPSS V21 for Windows to detect any significant differences between the parameters measured. In addition, when differences were significant, Tukey's range test at the 95% confidence level was carried out for a comparison between treatments. Percentage values of root colonization were arcsine (square root (X)) transformed before statistical analysis.

4.1.2 Results

4.1.2.1 Mycorrhizal Activity

Table 3 showed the mycorrhizal activity of the three strains under study. As can be seen, the AMF led to higher values of every variable studied, compared with untreated plants. The highest colonization values (78%) were obtained with *G. iranicum* var. *tenuihypharum* var. *nova* strain, while *F. mosseae* and *R. irregularis* attained much lower values (21.24% and 24.0%), although in all cases the difference was statistically significant compared with untreated plants.

Surprisingly, extramatrical mycelium showed notable differences among the types of fungal species. The species isolated from the sodium-saline soil (*G. iranicum* var. *tenuihypharum* var. *nova*) showed the highest values of external mycelium, 850 mg/kg soil, followed by *R. irregularis* and both values were higher than that obtained with the untreated plants. In the case of *F. mosseae*, a low level of mycelium was noted (143 mg/kg soil), which were comparable with the obtained with the native strains (122.2 mg/kg soil

in the untreated plants). Similar behavior was observed in the case of glomaline excretion, *G. iranicum* var. *tenuihypharum* var. again being the specie that excreted more protein into the medium than the other two species (Table 3).

4.1.2.2 Physiology Activity

Table 4 showed the measurements of SPAD, *A*, g_s and WUE (A/g_s) at 75 d. *G. iranicum* var. *tenuihypharum* var. *nova* reached the highest *A* and SPAD values, following the same pattern as above. The same species, in contrast, exhibited the lowest g_s value (120 mmol $H_2O/m^2/s$)—even lower than that observed with untreated plants. However, this species had the best WUE (106.33 μmol CO_2/mol H_2O), significantly higher than that observed for the other species, which did not present significant differences between them.

The other strains displayed lower physiological activity than *G. iranicum* var. *tenuihypharum* var. *nova* and, although they had higher photosynthetic activity at 75 d, it was at the expense of increased stomatal conductivity, meaning a less efficient use of water.

The untreated plants reached a WUE value of 67.76, which was not significantly different from the values reached with the other two species of AMF, *F. mosseae* and *R. irregularis* (67.07 and 65.92). The fact that these values are lower than in the control treatment reflects a photosynthetic mismatch, possibly derived from a symbiotic relationship not suitable for this stage of the crop.

Obviously, this analysis of the physiological activity of the evaluated plants is closely related to productivity and the nutritional levels achieved in plants treated with AMF.

4.1.2.3 Biomass Production

Tables 5 and 6 showed the leaf and root fresh dry weights reached in the different treatments at harvest, as well as nutritional elements at 75 d of cultivation.

The analysis of shoot and root biomass illustrates the positive effect of the application of AMF. Due to its proper mycorrhizal functioning and the physiological benefits it provided to the treated plants, *G. iranicum* var. *tenuihypharum* var. *nova* induced the highest biomass values measured as fresh and dry weight (Table 5), while the other species of arbuscular mycorrhizae studied did not produce any significant increase in biomass production.

Table 3 Extramatrical mycelium (mg/kg soil), mycorrhizal colonization (%) and easily extractable Glomaline concentration (mg/g soil) in lettuce plants inoculated with *F. mosseae*, *G. iranicum* var. *tenuihypharum* and *R. irregularis* and untreated after 75 d under a conventional fertilization program.

Treatments	Extramatrical mycelium (mg/kg soil)	Mycorrhizal colonization (%)	Glomaline (mg/g soil)
F. m	143.0 ± 17.8	21.25 ± 1.50	133.0 ± 2.1
G.i.v.t	850.0 ± 19.9	78.7 ± 1.63	467.1 ± 5.6
R. i.	232.0 ± 13.9	24.3 ± 6.70	323.3 ± 5.6
C	122.2 ± 13.2	12.3 ± 1.00	90.00 ± 1.7

F. m: *F. mosseae*; G.i.v.t: *G. iranicum* var. *tenuihypharum*; R. i: *R. irregularis*; C: untreated.

Table 4 SPAD, *A* (μmol $CO_2/m^2/s$), g_s (mmol $H_2O/m^2/s$) and WUE (μmol CO_2/mol H_2O) in lettuce plants inoculated with *F. mosseae*, *G. iranicum* var. *tenuihypharum* and *R. irregularis* and untreated after 75 d with a standard fertilization program.

Treatments	SPAD	*A*	g_s	WUE (A/g_s)
F. m	20.2^b	9.39^b	140^a	67.07^b
G.i.v.t	28.1^a	12.76^a	120^b	106.33^a
R. i.	19.0^c	9.23^b	140^a	65.92^b
C	19.1^c	8.47^b	125^b	67.76^b

Different letters in the same column correspond to significantly different values. F. m: *F. mosseae*; G.i.v.t: *G. iranicum* var. *tenuihypharum*; R. i: *R. irregularis*; C: untreated.

The analysis of foliar nutrient concentrations at 75 d of cultivation confirmed the results obtained for the other variables studied (Table 6). Significant differences were found in most of the elements between the studied treatments except the K and Zn contents. Once again, the highest values were recorded in the treatments that showed the greatest development as regard biomass and physiological activity, i.e., with *G. iranicum* var. *tenuihypharum* var. *nova* species.

It is also important to highlight the high values of the trace elements Fe and Mn, which are essential for photosynthesis and growth activity in general, measured in plants treated with *G. iranicum* var. *tenuihypharum* var. *nova*, which differed significantly from the rest of the plants, treated or not with AMF.

For the other species of mycorrhizal fungi and the untreated plants, the concentrations of nutritional elements did not show any significant differences between them, reflecting the low absorption of these elements under conditions of intensive nutrient fertirrigation.

4.1.3 Discussion

Although irrigation water with a high nutrient content can reduce the colonization capacity, spore germination and the growth of fungal hyphae [47], some AMF can be used under intensive agriculture conditions. The results showed that AMF symbiosis was well established on lettuce roots and that the colonization rates were in close agreement with those observed in other studies [48, 49].

The increased biomass (SDW) observed in plants inoculated with *G. iranicum* var. *tenuihypharum* reflected the high degree of mycorrhization observed in those plants [50]. Mycorrhizal symbiosis in lettuce root might have favored the absorption of water and nutrients [51], increasing biomass. The high values of root development found in the most successful association suggested that the combination probably induced the root system to improve hydraulic conductivity [52] and take more water from the soil.

The higher N concentration detected in plants treated with *G. iranicum* var. *tenuihypharum* can be mainly attributed to: (1) the high NO^{3-} concentration in the nutrient solution and (2) an increase in the nutrient absorption rate, which would improve the nutritional status of the lettuce plants because of a more developed root system as a result of the mycorrhizal symbiosis [53].

Table 5 Biomass of shoot and root expressed as SFW, SDW, RFW and RDW in untreated lettuce plants and plants inoculated with *F. mosseae*, *G. iranicum* var. *tenuihypharum* and *R. irregularis* 75 d after planting and exposed to a conventional fertilization program.

Treatments	SFW (g)	SDW (g)	RFW (g)	RDW (g)
F. m	600.42 ± 0.33	9.18 ± 1.50	122.28 ± 1.60	1.70 ± 1.70
G.i.v.t	800.03 ± 0.62	17.19 ± 0.25	232.13 ± 5.43	2.89 ± 0.44
R. i.	613.68 ± 4.60	10.71 ± 2.30	162.83 ± 9.62	1.60 ± 0.33
C	678.26 ± 3.73	9.12 ± 2.45	179.51 ± 3.21	1.4 ± 0.93

F. m: *F. mosseae*; G.i.v.t: *G. iranicum* var. *tenuihypharum*; R. i: *R. irregularis*; C: untreated lettuce plants.

Table 6 Concentrations of some nutritional elements (ppm) in lettuce plants inoculated with *F. mosseae*, *G. iranicum* var. *tenuihypharum* and *R. irregularis* and untreated plants after 75 d of a conventional fertilization program.

Treatments	N (ppm)	P (ppm)	K (ppm)	Ca (ppm)	Mn (ppm)	Fe (ppm)	Zn (%)
F. m	2.2^{b}	0.12^{b}	5.9	2.3^{b}	130.2^{b}	322^{c}	23.4
G.i.v.t	3.0^{a}	0.15^{a}	6.5	3.2^{a}	161.4^{a}	650^{a}	44.2
R. i.	2.1^{b}	0.11^{b}	5.9	2.4^{b}	132.2^{b}	342^{c}	22.1
C	2.4^{b}	0.10^{b}	6.8	2.2^{b}	136.5^{b}	489^{b}	30.2

Different letters in the same column correspond to significantly different values. F. m: *F. mosseae*; G.i.v.t: *G. iranicum* var. *tenuihypharum*; R. i: *R. irregularis*; C: untreated plants.

Good compatibility in the AM association between the roots and mycorrhizal mycelium can increase the use of different forms of N by plants [54]. Indeed, Johansen et al. [55] concluded that such arbuscular external mycelium as fine rootlets can take up N directly from the soil solution and transfer it toward the host plant at the same time.

Hirrel and Gerdemann [56] indicated that the increased uptake of P by plants inoculated with AMF seems to be one of the key factors responsible for increased vegetable production. In the present experiment, it was found that P uptake and growth only clearly increased in the case of *G. iranicum* var. *tenuihypharum*, but not with the rest of the strains or untreated plants.

K plays an important role in stomatal movements, protein synthesis and in the response to changes in leaf water status [57], but no significant differences in these respects were observed in the present study, while the increase in Ca detected in plants treated with *G. iranicum* var. *tenuihypharum* agreed with the observations of Cantrell and Lindeman [58].

Mycorrhizal plants also enhance the uptake of relatively immobile metal micronutrients, such as Fe and Zn [59]. Ca, Mg, Mn and Fe were higher, and P was within the optimum range calculated by the diagnosis and recommendation integrated system (DRIS) and proposed by Hartz et al. [60]. Different authors suggest great variability in these optimum ranges. For instance, in the case of Ca, Mg and Mn, Jones et al. [61] reported different optimum ranges (Ca 1.5%-3%, Mg 0.36%-0.50% and Mn 25-250 ppm). In the case of phosphate, Hochmuth et al. [62] indicated that optimum values range between 0.25% and 0.50%. Bearing in mind all these assumptions, all macro- and micronutrients were deemed to be within the optimal nutritional ranges.

A higher chlorophyll content (SPAD units) was detected in plants treated with *G. iranicum* var. *tenuihypharum*, Van den Driessche [63] detected a close relationship between some nutrients (N, Mn and Fe) and chlorophyll, and reported that an increase in these nutrients could lead to a stimulation of the synthesis of chlorophyll and hence the photosynthetic capacity. In this sense, the higher concentration of N and Fe measured in the plants could be, to a certain extent, related to the detected increase in *A* [64].

Stable transpiration rates (g_s) and the increased levels of *A*, associated with mycorrhizal plants significantly increase WUE [47]. In this study, the plants treated with *G. iranicum* var. *tenuihypharum* had significantly lower transpiration rates, even below those of untreated plants, but higher *A* levels and WUE. These three positive effects, *A*, g_s and WUE, may also have accounted for the enhanced plant growth of colonized plants, most probably as a result of better CO_2 fixation under salt stress [49].

4.1.4 Conclusions

G. iranicum var. *tenuihypharum* var. *nova* was seen to be very effective under a standard fertilization program, differing not only from the controls, for which a lower response might be expected, but also compared with other species of AMF, which, in the case of some variables, led to poorer results than the control.

4.2 Effective of G. iranicum var. tenuihypharum var. nova on Table Grape Production during a Three-Year Experiment

4.2.1 Introduction

In South-Eastern Spain, table grapes are cultivated under intensive agriculture regimes with drip irrigation. The effectiveness of using an AM inoculant based on *G. iranicum* var. *tenuihypharum* var. *nova* was described here, which was specifically developed to be applied in this system.

Under natural conditions, genus *Vitis* is strongly dependent on the presence of AMF, and there are several studies about different grape varieties and their interaction with AMF [37, 65-67]. Here, the effect of the application of *G. iranicum* var. *tenuihypharum* var. *nova* on the physiology was studied, nutrient

acquisition and yield of the table grape cultivar "Crimson" during three seasons.

4.2.2 Materials and Methods

The experimental design was a randomized complete design, composed of three blocks with three experimental plots per block. The standard plot was made up of 16 plants, located in four adjacent rows. The four central plants of the two middle rows were used for the different measurements and the other 12 acted as guard plants.

During the first year, the following two treatments were performed: non-inoculated Crimson grapevine plants, considered as the control treatment (T_0) (12 plants) and inoculated Crimson grapevine (T_1) (24 plants). During the second and third year, half of the plot that had been inoculated during the first year was used to evaluate the persistent effect of the AMF, so the plants in this half were not re-inoculated during the second year (T_1) (12 plants) and the plants of the other half of the plot were re-inoculated in years 2 and 3 (T_2).

4.2.2.1 Fungal Inoculation

The AMF strain used *G. iranicum* var. *tenuihypharum* var. *nova*, was previously isolated in a sodium-saline soil with a high pH value of 9.5. The inoculum at concentration of 120 propagules/g, calculated according to Porte [43], was supplied through the drip irrigation system (injection pump regulated at 25 L/h) at the beginning of the vegetative growth phase at a dose of 3 kg/ha.

It should be noted that the small size of the particles composing the product (< 100 μm) did not cause clogging problems in the filters, irrigation lines or emitters, and once mixed with water, the inoculum was actively located near young roots, which are the most absorbent [68].

4.2.2.2 Symbiotic Development

At the time of grape veraison, young root samples from rhizospheric soil were collected at a depth of 20-30 cm to assess symbiotic development. Five root samples from eight vines per treatment were used with at least four replicates per root. The percentage of mycorrhizal root colonization was estimated following the gridline intersect method [19] under a microscope (100× magnification) after clearing and staining roots [44].

4.2.2.3 Stem Water Potential

The stem water potential (ψ_{stem}) was measured at noon, using a pressure chamber (Model PMS 3000; Soil Moisture Equipment Corp., USA). Measurements were carried out twice a month during the first year of the experiment but, to summarize the results, only data from the veraison stage (average of two measurements in early and late July) are shown. Before collection and taking measurements, the leaves selected were enclosed in polyethylene bags covered with aluminum foil for at least 2 h [69].

4.2.2.4 Net Photosynthesis (A) and Stomatal Conductance (g_s)

Instantaneous measurements of A and g_s were performed on four leaves per plot (one leaf from each inner tree), using an open gas exchange system (Li-6400; Li-Cor., Inc., Lincoln, NE, USA) with an integrated leaf chamber fluorometer (Li-6400-40; Li-Cor. Inc., Nebraska, USA). All measurements were performed on young, fully expanded leaves, at a photosynthetic photon flux density of 1,500 μmol/m^2/s to ensure light saturation, with a CO_2 concentration in the cuvette of 400 μmol CO_2/mol air. The sampling periods were the same as for ψ_{stem}. Additionally, intrinsic WUE was determined and computed as the A/g_s ratio [70].

4.2.2.5 Leaf-Ion Concentration

An inductively coupled plasma ICP (ICP-ICAP 6500DUO Thermo, UK) was used for the determination of leaf macronutrients (P, K, Ca and Mg) and micronutrients (Fe, Zn, Mn, Cu and B). N was determined following the Dumas method [71]. In each plot, 20 leaves (five leaves from each inner tree) were collected following the criteria described for the selection of leaves used to measure ψ_{stem}. The sampling periods were the same as for ψ_{stem}. Ion

concentration was compared with the macro- and micronutrient ranges determined by DRIS. Because it is difficult to obtain reliable measurements in grape, the present study used the DRIS proposed by García-Escudero et al. [72] for Tempranillo grapevine as a rough guide.

4.2.2.6 Starch Concentration

The starch concentration of grape roots was determined in February 2013, after the first two years of the experiment, using the colorimetric assay described by Zapata et al. [73], but slightly modified. Five root samples (apical and central parts) from eight grapevines per treatment were used with at least four replicates per root (apical and central parts). Then, the apical and central parts were snap frozen with liquid N and extracted with Milli-Q H_2O (1/10, w/v). Extracts were incubated at 100 °C for 15 min, and then centrifuged at 12,000× g for 2 min. The supernatant was mixed with absolute ethanol (1/3, v/v) and centrifuged at 9,600× g for 5 min. The pellet, containing starch, was re-suspended in Milli-Q H_2O and then diluted (1/10, v/v) if necessary (for samples containing high starch concentration). Finally, 1 mL of sample was mixed with 50 µL of Lugol's solution (Sigma-Aldrich, Gillingham, UK) and the optical density was recorded at 595 nm using a Tecan-Sunrise plate reader. The starch concentration was calculated using a standard curve of pure rice starch (Sigma-Aldrich, Gillingham, UK) (0-200 µg/mL).

4.2.2.7 Yield and Quality of Grapes

In light of the natural stepped process of grape maturation, three cuts were performed at commercial maturity as determined primarily by color. The first cut was considered to be of the greatest commercial value. Finally, the total yield was determined as the sum of the yield weighed at each cut. To determine the quality of grapes, 100 bunches and 100 individual grapes were selected randomly from each treatment. Then, the bunch and berry weights (Radwag WLC 1.2/B1, Poland), berry diameter, firmness (Durofel DFT 100 penetrometer, France), soluble solids concentration (ATAGO MASTER-T refractometer, USA) and acidity (Metrohm 785 DMP Titrino + automatic sample changer Metrohm 760, Switzerland) were determined. The maturity index was computed as the ratio of soluble solids concentration to acidity. Coloration of the fruits was determined using a Konica Minolta Sensing CR-10 colorimeter (Singapore) based on lightness (L; overall intensity how light or dark a colour is), angle (Hue; a dimension of color readily experienced when look at color) and chroma (Ch; defined as the strength or dominance of the hue).

4.2.2.8 Statistical Analysis

Data were analyzed using a one-way analysis of variance, using SPSS V21 for Windows to detect any significant differences between the parameters measured. In addition, when differences were significant, Tukey's range test at the 95% confidence level was carried out for a comparison between treatments. Percentage values of root colonization were arcsine (square root (X)) transformed before statistical analysis.

4.2.3 Results

4.2.3.1 Fungal Colonization and Water Status

Table 7 showed only the percentage of root colonization for the first two years of the experiment. Natural AMF proliferation was detected in the control treatment (T_0) in both the first (10%) and the second year (19%). As expected, inoculated grapes presented significantly ($P < 0.05$) higher rates of colonization than the non-inoculated ones. During the first year, the mycorrhizal colonization rate compared with the control treatment was significantly ($P < 0.05$) higher (60%).

This value was notably higher in the second year (colonization increased to 89.0%) for T_1. During this second year, not inoculating the grapevines that had only been inoculated in the first year (T_2) limited the percentage of AM root colonization (43.0%), although a significant ($P < 0.05$) increase of 24% (≈ 2.3 times

Table 7 Percentage of root colonization and stem water potential (ψ_{stem}; MPa) for the three treatments applied in this experiment.

		AMF root colonization (%)		Stem water potential (MPa)	
	Treatments	First year	Second year	First year	Second year
Crimson	T_0	10 ± 2.1*	19 ± 6.2*	-1.1 ± 0.06	-0.8 ± 0.05
	T_1	60 ± 21.6	89 ± 8.2	-0.9 ± 0.06	-0.6 ± 0.04
	T_2	59 ± 19.5	43 ± 9.0	-0.9 ± 0.05	-0.7 ± 0.05

Values correspond to the average ± S.E. of two measurements performed at the time of grape veraison (in early and late July).
* Native AMF.

Table 8 Concentration of macronutrients (N, P, K, Ca and Mg) and micronutrients (Fe, Zn, Mn, Cu and B) in table grape leaves of the Crimson variety at the time of veraison for the two-year experimental period.

		First year		Second year			Optimal range
		T_0	T_1	T_0	T_1	T_2	(DRIS)
Macronutrients (g/kg)	N	32 ± 2.1	27 ± 1.6	29 ± 1.1	26 ± 0.5	28 ± 0.7	21.9-22.9
	P	1 ± 0.3	2 ± 0.2	2 ± 0.2	3 ± 0.1	2 ± 0.3	1.5-1.6
	K	12 ± 2.1	17 ± 1.8	12 ± 0.5	17 ± 1.2	13 ± 0.7	7.6-9.0
	Ca	40 ± 2.5	36 ± 3.1	31 ± 1.5	34 ± 0.7	29 ± 1.8	31.0-33.4
	Mg	4 ± 0.4	4 ± 0.3	4 ± 0.3	4 ± 0.5	4 ± 0.4	3.8-4.5
Micronutrients (mg/kg)	Fe	140 ± 7.2	151 ± 6.5	150 ± 26	160 ± 31	126 ± 24	134-164
	Zn	12 ± 0.5	14 ± 0.4	14 ± 2.1	15 ± 2.7	14 ± 1.9	16-19
	Mn	43 ± 4.2	48 ± 3.2	45 ± 6.1	48 ± 4.3	46 ± 7.2	99-124
	Cu	39 ± 5.3	31 ± 4.5	32 ± 2.2	33 ± 4.4	33 ± 3.8	117-221
	B	61 ± 2.7	56 ± 3.4	58 ± 6.5	50 ± 4.5	53 ± 3.2	34-40

Values correspond to the average ± S.E. of two measurements performed at the time of grape veraison (in early and late July). The optimal macro- and micronutrient ranges calculated for grapevine "Tempranillo" by DRIS [72] are shown.

higher) compared with T_0 was still observed (Table 7).

Stem water potential (ψ_{stem}): During the first year of the experiment, AMF inoculation significantly increased ψ_{stem} levels (from -1.07 MPa to -0.87 MPa). During the second year, a general increase in ψ_{stem} compared to the first year was detected, but the effect of inoculation was only significant when ($P < 0.05$) T_1 (-0.62 MPa) and T_0 (-0.80 MPa) (Table 7) plants were compared.

4.2.3.2 Leaf-Ion Concentration

Table 8 presented the leaf-ion concentration (macro- and micronutrients) for the two-year experimental period. During the first year, inoculated grapevines showed significantly ($P < 0.05$), lower N but higher P and K concentrations compared to the control treatment (N = 27 g/kg vs. 32 g/kg, P = 2 g/kg vs. 1 g/kg and K = 17 g/kg vs. 12 g/kg). In contrast, Ca and Mg remained unaffected (Table 8). A similar trend was observed during the second year, when inoculated grapevines had significantly ($P < 0.05$) reduced N and increased P, K and Ca levels compared with the control treatment (N = 26 g/kg vs. 29 g/kg, P = 3 g/kg vs. 2 g/kg, K = 17 g/kg vs. 12 g/kg and Ca = 34 g/kg vs. 31 g/kg). Overall, the results for T_2 were similar to those observed in T_0. As regard micronutrients, no significant differences were found between treatments regardless of the year under study, and the concentrations of all micronutrients were similar in both the first and second year (Table 8).

4.2.3.3 Net Photosynthesis (A) and Stomatal Conductance (g_s)

During the first year, A and g_s seemed to be dependent on inoculation, as inoculated plants (T_1) presented significantly ($P < 0.05$) higher values (A = 8.3 μmol $CO_2/m^2/s$ and g_s = 112 mmol $H_2O/m^2/s$) (Fig. 2). Increases of 37.2% in A and 34.5% in g_s with respect to T_0 were observed. In contrast, no significant difference were found between treatments T_0 and T_1 in WUE (≈ 70 μmol CO_2/mol H_2O). During the second

year, the highest values of A and g_s were found in T_1 (A = 12.3 μmol $CO_2/m^2/s$ and g_s = 102.2 mmol $H_2O/m^2/s$) (Fig. 2b). These differences meant significant ($P < 0.05$) increases in A and g_s of 92% and 48%, respectively, compared to T_0. In contrast, the lowest A and g_s values were found in both T_0 and T_2, where no significant differences were detected. A significant increase ($P < 0.05$) was also seen for WUE

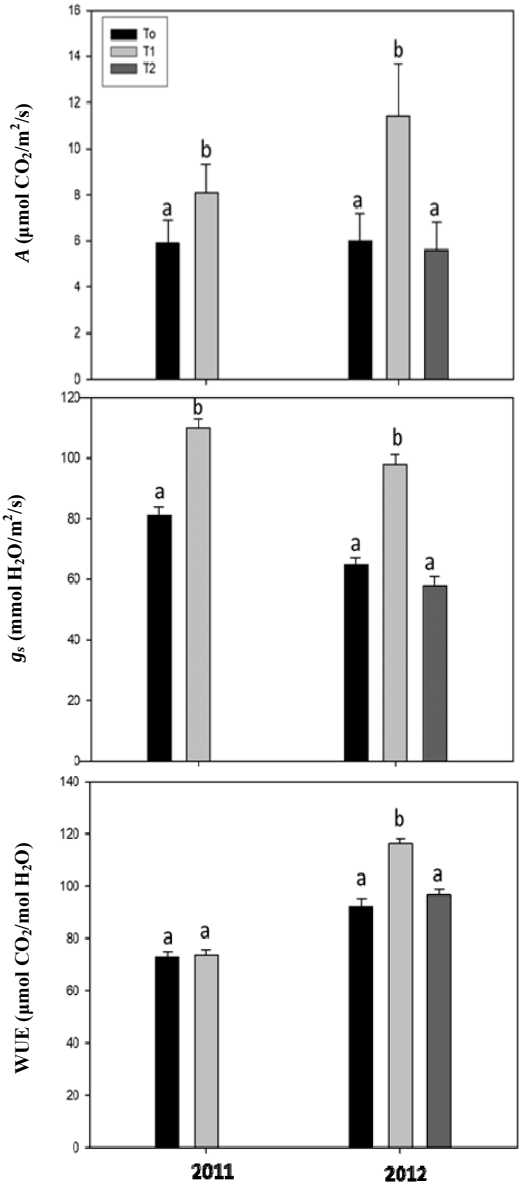

Fig. 2 (a) A (μmol $CO_2/m^2/s$), (b) g_s (mmol $H_2O/m^2/s$) and (c) WUE = A/g_s (μmol CO_2/mol H_2O) for the three treatments applied in this experiment.

Values correspond to the average ± S.E. of two measurements performed at grape veraison (in early and late July).

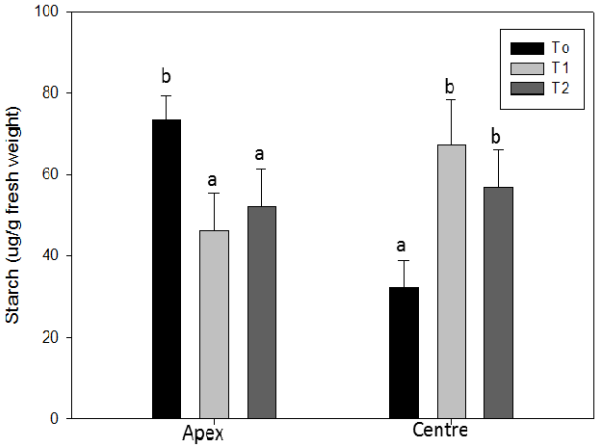

Fig. 3 Starch concentration (μg/g fresh weight) at the apex and the central parts of the roots for the three treatments performed, measured in February 2013, after the first two years of the experiment had finished.

Data show the average value ± S.E.

in T_1 in the second year (120.3 μmol CO_2/mol H_2O), but not in T_2.

4.2.3.4 Starch Concentration

Fig. 3 showed the starch concentration measured at the apex and the central parts of the roots for the three treatments, performed after the first two years of the experiment (February 2013). A similar total starch concentration was measured in T_0, T_1 and T_2 treatments (105.4, 113.6 and 109.2 μg/g, respectively) although there was a slight trend toward increased starch levels with AMF inoculation. In general, this greater accumulation of starch in AMF inoculated grapes agreed with the higher A and WUE data (Fig. 2), as well as with the highest yield (Table 9).

4.2.3.5 Yield and Quality of Grapes

Table 9 presented the yield (t/ha) for the Crimson grape variety for each year of the experiment. At the end of the first year, the yield in the control treatment (T_0) was 20.98 t/ha, but inoculation significantly ($P < 0.05$) increased the total yield by 48.3%, to 31.12 t/ha, with respect to T_0. In inoculated plants (T_1), the yield was unevenly distributed between the first, second and third cut. For T_0 in the first year of the experimental period, half the yield was obtained at the first cut.

In the second year, a general reduction in yield compared to the first year was observed. For instance,

in T_0 and T_1 yield was reduced by 15% and 20%, respectively. However, as expected, inoculation significantly ($P < 0.05$) increased the total yield of grapes, compared with the non-inoculated control (increase of 59.1%). Such increases were also observed in the last cut, as occurred in the first year.

The persistence of AMF, as evaluated in treatment T_2, also increased the total yield significantly ($P < 0.05$) but with a more moderate effect (yield of 25.06 t/ha; an increase of 27.0% with respect to T_0). However, in contrast to T_1, this increase was only observed in the third cut, when most of the yield (c. 80%) was harvested.

In the third year, a general increase compared with the other years was observed. At the end of the third year, the yield in the control treatment (T_0) was 38.18 t/ha, but inoculation significantly ($P < 0.05$) increased this to 51.06 t/ha and 56.00 t/ha in T_1 and T_2, respectively. In the second and third year, treatment T_2 produced the greatest increases in production with 59.11% (25.06 t/ha) and 46.6% (56.00 t/ha), respectively, and in both years the production in the first cut was even greater than in previous years by 35.94% (4.09 t/ha) and 73.00% (17.40 t/ha), respectively.

In treatment T_1, with a single application of *G. iranicum* var. *tenuihypharum*, production was still increased in the second and third year of the trial, confirming the residual effect of the strain applied in the first year of the trial in this treatment. However, global production (in three years) was lower than that achieved when plants were inoculated annually, which

Table 9 Yield (t/ha) of Crimson grape variety for the three-year experimental period for the three treatments performed.

Year	Treatment	1st cut (kg/ha)	2nd cut (kg/ha)	3rd cut (kg/ha)	Total (kg/ha)
2011	T_0	10.47 ± 2.1	7.41 ± 1.3	3.10 ± 0.2	20.98 ± 2.1
	T_1	12.00 ± 1.3	10.30 ± 2.0	8.82 ± 1.6	31.12 ± 3.3
2012	T_0	4.09 ± 0.2	1.87 ± 0.2	9.79 ± 2.1	15.75 ± 2.1
	T_1	3.46 ± 1.2	2.06 ± 0.5	14.49 ± 1.1	20.01 ± 2.2
	T_2	5.56 ± 0.3	9.17 ± 2.1	10.33 ± 0.2	25.06 ± 1.5
2013	T_0	10.02 ± 1.2	14.20 ± 1.2	13.78 ± 0.6	38.18 ± 1.2
	T_1	15.10 ± 1.3	19.70 ± 2.2	16.26 ± 3.0	51.06 ± 2.5
	T_2	17.40 ± 2.0	18.70 ± 2.1	19.90 ± 2.1	56.00 ± 2.1

Data show the average value ± S.E.

Table 10 Quality fruit parameters for Crimson variety at the two cuts: bunch weight (g), firmness of the grain (pressure in N/cm^2), soluble solids concentration (°Brix) and maturity index (°Brix/%Tartaric) for the two-year experimental period for the three treatments applied.

Treatment		1st cut	2nd cut	1st cut	2nd cut
Bunch weight (g)	T_0	493 ± 43	391 ± 60	617 ± 51	537 ± 35
	T_1	560 ± 20	518 ± 58	876 ± 40	830 ± 43
	T_2	ND	ND	654 ± 55	556 ± 47
Firmness (N/cm^2)	T_0	62 ± 1.2	69 ± 4.1	82 ± 1.3	86 ± 1.5
	T_1	64 ± 0.9	69 ± 3.2	88 ± 1.5	87 ± 3.5
	T_2	ND	ND	86 ± 1.6	86 ± 2.9
Soluble solids content (°Brix)	T_0	20 ± 0.6	20 ± 0.3	21 ± 0.4	21 ± 0.6
	T_1	21 ± 0.4	20 ± 0.6	21 ± 0.5	21 ± 0.4
	T_2	ND	ND	21 ± 0.3	21 ± 0.5
Maturity index (°Brix/%Tartaric)	T_0	50 ± 1.1	56 ± 2.1	46 ± 3.1	47 ± 2.9
	T_1	53 ± 1.3	62 ± 1.6	59 ± 2.6	60 ± 3.1
	T_2	ND	ND	52 ± 2.4	55 ± 2.4
		1st year (2011)		2nd year (2012)	

Data show the average value ± S.E.; ND: No data during the first year of trial.

reflected the need for an annual application of the product at the beginning of the growing season.

Table 10 presented the main fruit quality parameters of the harvested grapes during the first two years of the experiment. During the first year, at the first cut, inoculation led to significant ($P < 0.05$) increase in bunch weight (from 493 g to 560 g), berry firmness (from 61.9 N/cm^2 to 64.4 N/cm^2), soluble solids concentration (from 20.1 ºBrix to 21.3 ºBrix) and maturity index (from 50.2 ºBrix/%Tartaric to 52.8 ºBrix/%Tartaric). No significant differences were detected in the equatorial diameter or berry weight (data not shown). In the second cut, significant ($P < 0.05$) increases in T_1 were only observed in the bunch weight and maturity index (Table 10).

During the second year, the quality of grapes was noticeably higher with respect to the first year. In the first cut, AMF inoculation led to significant ($P < 0.05$) increases in bunch weight (617 g to 876 g), berry firmness (82 N/cm^2 to 88 N/cm^2), maturity index (45.8 ºBrix/%Tartaric to 58.5 ºBrix/%Tartaric) and in the equatorial diameter (data not shown). However, the berries showed similar soluble solids concentration and weight with respect to T_0. In T_2, significant increases were found in the berry firmness (from 82.0 N/cm^2 to 86.1 N/cm^2), maturity index (from 55 ºBrix/%Tartaric to 52 ºBrix/%Tartaric) and equatorial diameter (data not shown). In the second cut, a similar trend was observed. Overall, for treatments T_1 and T_2, the increase of soluble solid concentration ranged from 1% to 6% and, additionally, a reduction of acidity ranging from 10% to 20% was observed.

4.2.4 Discussion

The study demonstrated that Crimson grapes are heavily dependent on AMF. The successful establishment of the inoculant was confirmed by the fact that the range of root colonization was similar to that reported in previous research [74] and much higher in the second and third year of the experiment. Of note is the observation that the highest percentage of AMF colonization was reached when inoculation was performed during two consecutive years. However, in the second year of the experiment in treatment T_2, an expected reduction in AMF colonization compared to T_1 was detected, although colonization was still much higher than in the control treatment (T_0). While the high degree of AMF colonization probably meant a high degree of compatibility between the AMF used and plant variety, the reduction in colonization seen in T_2 might have been due to a certain degree of competition between the native and introduced AMF [75].

A high presence of AMF in the root zone has been shown to aid the uptake of water and to contribute to an improved water status in vines [76]. In that sense, the significant increase observed in the current study in ψ_{stem} for T_1 during the first year of the experiment could be attributed to root colonization by AMF. In the second year, the increasing percentage of root colonization, the large amount of water supplied after veraison and improved host P nutrition probably induced the increase observed in ψ_{stem} compared to the first year. In fact, the current study highlights the fact that ψ_{stem} increases as root colonization increases.

The results point to a reduced N concentration in the inoculated treatment T_1, regardless of the year of study, although the concentration was always slightly above the optimal range found for the DRIS norm proposed for grapes by García-Escudero et al. [72]. Several growers have reported that the moment of veraison in Crimson grapes is especially complicated, because fully colored ripe and unripe grapes can coexist at the same time. Therefore, it is widely agreed that the fundamental parameter for determining the optimal harvest moment is grape coloration, for which a suitable N/K balance must be reached. In the inoculated treatments, regardless of the year of study, the reductions in N and increases in K led to a somewhat reduced N/K ratio, but similar to that described by Martín et al. [77]. In all treatments, the concentration of K was always within the optimal

ranges proposed by DRIS [72].

In the current study, the increase in P detected in the inoculated treatment (T_1) was attributed to AMF. In contrast, T_2 did not show a significant increase in P, compared to the control treatment (T_0), which indicates that the reduction in AMF colonization probably conditioned the absorption of this element. As regard the other macronutrients analyzed, Ca (except in the second year for T_1) and Mg, no significant differences were found, which agrees with a previous study [23].

Some authors suggest that certain micronutrients may or may not be affected by inoculation with AMF, although there is research that agrees with the results found in the current study, indicating that AMF has no effect on those elements. Bearing all this in mind, it is very likely that the difference in Zn and Cu, mentioned in the various studies arise from differences in cultivation conditions between studies, as well as from genotypic differences, since the plant response may also change with inoculation of different fungi [78].

At veraison in the first year of the experiment, inoculation increased the A and g_s rates significantly, compared to T_0, although the high fruit load probably limited photosynthetic activity to a certain extent. However, despite these increases, WUE remained stable. In the second year, the good water status of the grapevines, the higher percentage of root colonization and lower fruit load led to considerably higher values of both A and WUE in treatment T_1 [79]. In fact, AMF are known to exert a degree of control over transpiration, allowing plants to maintain high levels of WUE [80]. Such an increase, which agrees with other studies in vines [81], may also have accounted for the enhanced growth of plants colonized with *Glomus* species, most probably as a result of enhanced CO_2 fixation [49].

Zapata et al. [73] reported that the C stored in perennial tissues, such as roots, mainly comprises starch and that its mobilization plays a substantial role in supporting vegetative and reproductive growth. In the current study, it is noticeable that: (1) in non-inoculated grapes, most of the starch was allocated to the apex; (2) in grapes inoculated during the first year but not the second, the starch was equally distributed between the apex and the central part of the root; (3) in grapes inoculated during first and second years, the starch was primarily stored in the central part of the root. In this last case, the decrease in starch at the root apex could indicate that the carbohydrates were destined for the development of new roots and the need to satisfy the energy demand for AMF activity [53]. Also of note is the observation that although plants inoculated only in the first year showed similar A, WUE, leaf nutrient content and water potential to plants to the control plants in the second year, the yield was notably increased, especially in the second and third cuts. This could be explained by the higher concentration of starch stored in the center of the root in the first season with respect to the control treatment, which was remobilized to the shoot, increasing fruit set and hence yield [82].

As expected, effective mycorrhizal symbiosis favored the absorption of water and nutrients [51] and hence increased yield. Additionally, the effect of the persistence of the AMF on yield was still significant in T_2. Overall, the fruit quality parameters evaluated, bunch weight, firmness, soluble solids concentration and maturity index, were also affected positively. Of particular note is how inoculation significantly increased the soluble solids concentrations and reduced the acid concentration more than proportionally.

4.2.5 Conclusions

The selected AMF colonized the Crimson table grape roots satisfactorily. The inoculated grapes had an improved water status, increasing their absorption of some nutrients, such as P, K and Ca and, in addition, both yield and fruit quality were positively affected. Moreover, the ability of AMF to persist in the roots of grape in the second year also produced positive effects. AMF could have favored root development by mobilizing the apex starch reserves, hence, its application led to a higher concentration of

starch in the central part of the root. Finally, this study suggested that the straightforward application of AMF through a drip irrigation system can be considered a favorable technique. Hence, the development of such symbiosis could be useful in intensive agriculture. Moreover, competition between native and non-native AMF to colonize the soil suggests that the percentage of mycorrhizal colonization should be periodically monitored and re-inoculations be applied to achieve all the positive effects evident in the inoculated treatments.

4.3 Effectiveness of G. iranicum var. tenuihypharum in Different Crops Systems

4.3.1 Effect of AM Doses on Tomato Production and Nutrition under Greenhouse Condition

Two different doses of *G. iranicum* var. *tenuihypharum* sp. *nova* were applied through a drip irrigation system to evaluate their effects on the physiological, nutritional and agronomic performance of tomato plants. Trials were conducted in South-Eastern Spain under controlled greenhouse conditions from September to December 2012. The high rate of AMF colonization at both applied doses improved plant growth performance, leading to significant increases in leaf macro- (N, P, K, Ca and Mg) and micro- (Fe, Cu, Zn, Mn and B) nutrient concentrations. The AMF present in colonized plants not only exerted some control over the rate of transpiration (stomatal control), but also maintained a higher rate of *A* and hence improved the intrinsic WUE (computed from the ratio of the rate of A/g_s). In addition, a close relationship was found between the respiratory activity of the AMF and the rate of *A*, leaf N, P, Fe and K concentrations, and fruit yield (expressed as the product of the average number of fruit × the average fruit weight in each treatment). In summary, the application of AMF was effective at improving the performance (i.e., the growth, nutrition and yield) of tomato plants cultivated under an intensive fertigation regime [35].

4.3.2 Protective Effects on *Viburnum tinus* Plants Irrigated with Saline Water

The effect of *G. iranicum* var. *tenuihypharum* on Laurustinus (*V. tinus*), a popular shrub much used in gardening, was evaluated under field conditions using two types of water: control, C with electrical conductivity (EC) < 0.9 dS/m and reclaimed water (wastewater previously treated in a sewage treatment plant) with EC 4 dS/m during a first saline period (11 weeks) and with EC 6 dS/m during a second saline period (25 weeks). The chemical properties of the soil, as well as the physiological behavior, leaf nutrition and aesthetic value of plants were evaluated. Due to the high salinity of the wastewater (6 dS/m), Laurustinus plants decreased their stem water potential values and, to a lesser extent, g_s. Also, the visual quality of the plants was diminished. The inoculated AMF satisfactorily colonized the laurustinus roots and enhanced the structure of the soil by increasing the glomalin and carbon contents. Furthermore, *G. iranicum* var. *tenuihypharum* inoculation decreased the Na and Cl contents, stimulated flowering and improved the stem water potential of the plants irrigated with both types of reclaimed water (RW). The AMF also had a positive effect by simulating plant physiological parameters, including stem water potential and g_s. Effective AMF associations that avoid excessive salinity could provide an outlet for reusing wastewater, especially when plants are grown in heavy soils [39].

4.3.3 Protective Effects on Lettuce Plants Irrigated with Saline Water

The present study also evaluated the effects of inoculation with AMF (*G. iranicum* var. *tenuihypharum* sp. *nova*) on the physiological performance and production of lettuce plants grown under greenhouse conditions and supplied with RW (treated urban wastewater with high EC; 4.19 dS/m). Four treatments, fresh water, fresh water plus AMF inoculation, RW and RW plus AMF inoculation, were applied and their effects were analyzed. Root

mycorrhizal colonization, plant biomass, leaf-ion content, g_s and A were assessed. Overall, the results underline the significance of AMF for alleviating salt stress and its beneficial effects on plant growth and productivity. Inoculated plants showed an increased ability to acquire N, Ca and K from both non-saline and saline media. Moreover, mycorrhization significantly reduced plant Na uptake. Even with RW, inoculated plants also showed improvements in physiological parameters, such as A, g_s and WUE, compared with non-mycorrhizal plants. Additionally, the high concentration of nutrients dissolved in reclaimed water suggests that farmers should adjust fertigation accordingly. Finally, this experiment demonstrated that mycorrhization could be a suitable way to induce salt stress resistance in iceberg lettuce crops, since the plants irrigated with RW satisfied minimum legal commercial size thresholds. Moreover, the maximum values of *Escherichia coli* in the RW were close to, but never exceeded established international thresholds (Spanish Royal Decree 1620/2007; Italian Decree, 2003) and so the lettuces were suitable for sale [36].

4.3.4 Effect on the Nutritional Response of Pepper Plants Grown on Coconut Fiber

An experiment was carried out with pepper plants (*Capsicum annuum* L.) grown on coconut fiber and inoculated with the species *G. iranicum* var. *tenuihypharum*, in order to study how well the fungus establishes itself in this type of substrate and the influence of nutrient availability on plant physiology, yield and crop quality. The results showed that treated plants had a higher number of flowers and lateral shoots, possibly related to the improved hormonal expression pattern of treated plants. In addition, an increase in root development and dry matter production was observed in mycorrhized plants, and the fruits were of better quality (greater firmness, in the three samplings carried out and had higher ºBrix at the last sampling time). The total production of the treatment inoculated with this species was 9.4% higher than that of the untreated plants at the end of the harvesting period, with a significant increase of 33% in mycorrhizal production compared with the control at the last cut (August 1st), coinciding with the time the plants entered a senescence phase caused by the effect of the high temperatures characteristic of this summer period [83].

4.3.5 Effects on Phytochemical Content in Strawberry Fruits

The effect of inoculation date using AMF on the fruit quality and the phytochemical content of strawberry plants grown in a soilless growing system was analyzed. The experiment was performed in Huelva (Spain) and was conducted in a greenhouse on the La Rábida Campus of Huelva University under natural light and temperature conditions from October 2013 to June 2014. Three short-day strawberry cultivars ("Splendor", "Sabrina" and "Fortuna") were grown in polyethylene bags filled with coconut fibers in a randomized block design, with three repetitions and factorial arrangement (3 cultivars × 3 treatments). Each replicate consisted of one bag with 12 plants supporting structures at 40 cm height. The treatments were: T_1 = mycorrhizal inoculation at transplantation, T_2 = mycorrhizal inoculation 30 d after transplantation (DAT) and T_0 = control treatment, without inoculation. AMF inoculation significantly affected the anthocyanin and phenolic contents, both of which were higher when inoculation was performed at transplantation (514 mg/100 g and 235.6 mg/100 g fruit fresh weight, respectively). Mycorrhizal inoculation also decreased the acidity of fruit throughout the growing season and increased firmness, but only during the early stage of production. Similar treatments applied to Fortuna variety led to an even higher anthocyanin content (514.04 mg/100 g fruit fresh weight) [40].

4.3.6 Effects on Root Architecture and Productivity of Melon

The effect of inoculation with the AMF *G. iranicum* var. *tenuihypharum* on the root development and yield

of melon plants (*Cucumis melo* L. cv. Hispano de Nuhnems), grown in soil in an intensive agricultural system for three months, was evaluated by measuring the root length, root volume, area, number of root tips, rhizosphere activity, yield and crop quality. The inoculated melon plants had an increased percentage of colonization, which had reached 75% by the completion of the assay. This meant that the inoculated plants exhibited greater rhizosphere activity, both bacterial and fungal, than the control plants. Inoculation resulted in greater root length, volume and area, and a greater number of tips with a diameter of less than 0.5 mm, indicating greater proliferation of lateral roots and a significant change in the root architecture. In addition, the plants showed higher fruit number and length, yield and fruit sugar content than untreated plants. Therefore, the mycorrhizal association had a strong positive effect on the architecture and root activity, stimulating the capacity for nutrient absorption and resulting in a higher yield and fruit quality (www.symborg.com).

4.3.7 Physiological and Agronomic Influence on Pepper Plants Grown under Greenhouse Conditions

The arbuscular mycorrhiza *G. iranicum* var. *tenuihypharum* significantly ($P < 0.05$) influenced plant physiology (better water status and higher gas exchange levels) and the productivity of a pepper crop cultivated under greenhouse conditions in El Ejido (Almeria, Spain), following the cultural practices of the area. The inoculant was not only efficient during the early stages but throughout the whole experiment, enhancing nutrient uptake and physiological characteristics and improving the plant defense system against stress conditions, such as the low winter temperatures. The cultural practices of the area include keeping the fruits on the plants until midwinter (February), when the product reaches the highest prices in the market (during the 2011 season, pepper price reached 1.5 €/kg). For this reason, although a cut of a few kilos was made in mid-December, most of the peppers (64% of the total) were harvested in midwinter (from February to mid-March). During the investigation period, 40 plants per treatment were randomly selected and studied in two replications from different areas of the greenhouse. Inoculation with the above species resulted in the gradual growth of plants throughout the experiment, reaching 48% during the third harvest, which meant an average growth of 15.29%. The final yield increased from 7.39 kg/m^2 in the control treatment to 8.52 kg/m^2 in the biological treatment (www.symborg.com).

5. *G. iranicum* var. *tenuihypharum*, the Basis of Different Biological Inoculant Products and the Development of a New Market

Symborg is a leader in agricultural biotechnology research, development and innovation. After finding and patenting the new species *G. iranicum* var. *tenuihypharum*, the company has developed a multiplication technology that allows large scale production. The process involves an advanced grinding technique that enables different kinds of application, without losing any of the biological properties of the fungus.

Based on *G. iranicum* var. *tenuihypharum*, Symborg has developed a line of biological inoculants for application in most agricultural and crop systems: MycoUp® and MycoUp Activ®, biological inoculants for irrigation; Resid MG®, biological inoculant in microgranulated form; Resid HC®, biological inoculant for coating seeds.

In seven years, the company has demonstrated constant and effective results, helping growers from all parts of the world to maximize their crop yields, increasing production in almost all crop families (Table 11). The efficacy of the company's products has therefore been shown in most major agricultural areas, climates, soils and agricultural systems, ranging from intensive to extensive (Fig. 4 and Table 11).

G. iranicum var. *tenuihypharum*'s product line is today sold in most major agricultural areas of the

Table 11 Maximum yields achieved using *G. iranicum* var. *tenuihypharum* as biological inoculants, according to crop family, application system and substrate on a global scale.

Continent	Crop family	Substrate	Application System	Product	Máximum yield achieved
Europe	Vegetables	Soil	Irrigation	MycoUp, MycoUp Activ	22%
		Hydropic	Irrigation	MycoUp, MycoUp Activ	33%
	Fruit trees	Soil	Irrigation	MycoUp, MycoUp Activ	28%
	Berries	Soil	Irrigation	MycoUp, MycoUp Activ	7%
		Hydropic	Irrigation	MycoUp, MycoUp Activ	40%
	Cereals	Soil	Planting time	Resid MG	27%
	Cereals	Soil	Seed treatment	Resid HC	15%
	Corn	Soil	Planting time	Resid MG	57%
	Corn	Soil	Seed treatment	Resid HC	18%
	Citrus	Soil	Irrigation	MycoUp	19%
America	Vegetables	Soil	Irrigation	MycoUp, MycoUp Activ	40%
		Hydropic	Irrigation	MycoUp, MycoUp Activ	27%
	Fruit trees	Soil	Irrigation	MycoUp, MycoUp Activ	23%
	Berries	Soil	Irrigation	MycoUp, MycoUp Activ	32%
		Hydropic	Irrigation	MycoUp, MycoUp Activ	21%
	Cereals	Soil	Planting time	Resid MG	16%
	Cereals	Soil	Seed treatment	Resid HC	14%
	Corn	Soil	Planting time	Resid MG	19%
	Corn	Soil	Seed treatment	Resid HC	16%
Africa	Vegetables	Soil	Irrigation	MycoUp, MycoUp Activ	22%
	Berries	Soil	Irrigation	MycoUp, MycoUp Activ	9%
		Hydropic	Irrigation	MycoUp, MycoUp Activ	33%
	Cereals	Soil	Planting time	Resid MG	13%
	Cereals	Soil	Seed treatment	Resid HC	11%
	Corn	Soil	Seed treatment	Resid HC	18%
	Vegetables	Soil	Irrigation	MycoUp	22%
Eurasia	Vegetables	Hydroponic	Irrigation	MycoUp	17%
	Citrus	Soil	Irrigation	MycoUp	19%
	Vegetables	Soil	Irrigation	MycoUp	19%
		Hydroponic	Irrigation	MycoUp	34%

world, creating a new market where demand has been driven by a variety of factors.

Political and social pressure has led to the global agro biostimulant market growing rapidly. In 2014, global sales were around US$1.4bn and are projected to reach US$2.9 billion by 2021 with a CARG of 10% to 12%.

In the USA, where organic agriculture has undergone enormous growth since the early 1980s, sustainable farming practices follow strict standards, with emphasis on the use of biological tools. In the USA, *G. iranicum* var. *tenuihypharum* has therefore been used as biological inoculant since 2013. This regulatory context has led other countries of the area to follow the USA restrictions in order to export its products to the USA.

Such is the case of Mexico, Chili or Peru where *G. iranicum* var. *tenuihypharum* is marketed in its different formulations to cover the demand in extensive and intensive crops.

In Europe, an increasingly strict regulatory climate governing conventional chemical inputs driven by rising health consciousness of society led to the development of a new approach for using biological products. With 72 million tons of cereals produced in Europe, especially in France where 9.6 million ha are

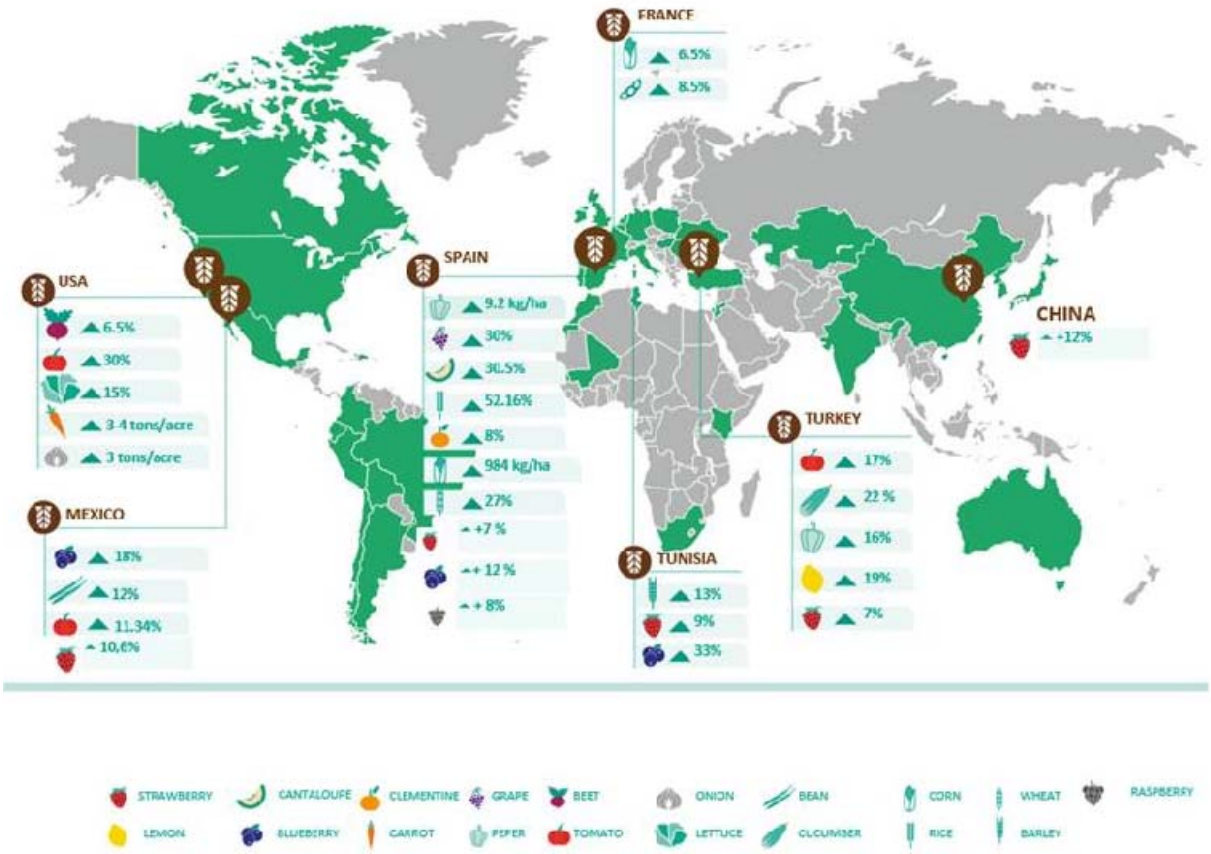

Fig. 4 Global picture and efficacy of MycoUp, MycoUp Activ, Resid MG, Resid HC based on the average of 10 trials.

dedicated to cereal production, *G. iranicum* var. *tenuihypharum*, as a component of Resid MG microgranulated and Resid HC seed treatment formulation are applied on maize, soya and wheat, helping growers to increase their production.

However, worldwide soil deficiency is becoming a major constraint to crop production. Good microbiological management of the soil is essential for growing crops, as it enhances micronutrient translocation to the plant and improves plant immunity. Deficiencies in the microbiological balance affect the quality and quantity of crop production and greater awareness of the problem is leading to the more widespread use of microorganisms in agriculture: the mycorrhizal forming fungus *G. iranicum* var. *tenuihypharum*, by improving the soil's microbiological properties, is therefore well regarded by the market.

Ever diminishing arable land area is also one of the major factors driving the demand for new biological tools, as a result of the greater pressure on farmers to increase crop production to meet the demand of an increasing population. The increasing prices of agricultural inputs are also driving farmers to invest intelligently in agricultural resources. The application of *G. iranicum* var. *tenuihypharum* has helped farmers by increasing the effectiveness of applied micronutrients, thus reducing future expense associated with additional inputs.

The increase usage of modern agricultural practices has contributed to the positive growth seen in the use of *G. iranicum* var. *tenuihypharum* in regions, such as Asia-Pacific and Latin America.

The characteristics of *G. iranicum* var. *tenuihypharum* and its different application forms have led to the Symborg product line based on AMF reaching a global market.

In 2017, *G. iranicum* var. *tenuihypharum* is

marketed and used as biological inoculant in more than 30 countries.

6. Conclusions

Although AM symbiosis constitutes the most important microbial association in agronomy and has evolved in most terrestrial environments as an efficient system of nutrient uptake in plants, factors, such as type of soil, high and unbalanced fertilizations levels, and the incorrect selection of strains, will exert a marked influence on the success of the implantation of this technology, making it necessary to choose strains according to the type of soil.

Despite the successful laboratory results obtained with certain strains of AMF, scale-up of their use for agriculture has been slow, probably due to the relative ineffectiveness of the inoculation process, or other factors, such as the technical difficulty involved in their application, the degree of soil compatibility and field carrying capacity.

G. iranicum var. *tenuihypharum* var. *nova* was seen to be very effective under a standard fertilization program, differing not only from the controls, for which a lower response might be expected, but also compared with other species of AMF, which, in the case of some variables, led to poorer results than the control. In horticultural crops, the application of this species not only produces significant increases in agricultural production between 10% and 15%, but also generates a high physiological activity, through increased photosynthetic activity, and an efficient use of water.

Table grapes showed an improved water status, the increased absorption of some nutrients, such as P, K and Ca and, in addition, both yield and fruit quality were positively affected. Moreover, the ability of AMF to persist in the roots of grape in the second year also had positive effects. AMF could have favored root development by mobilizing the apex starch reserves; hence, its application led to a higher concentration of starch in the central part of the root.

Finally, this study suggested that the straightforward application of AMF through a drip irrigation system can be considered a favorable technique. The development of such symbiosis could be useful in intensive agriculture. Moreover, competition between native and non-native AMF to colonize the soil, suggests that periodic monitoring of the percentage of mycorrhizal colonization should be carried out and that re-inoculation is needed to benefit from all the positive effects evident in the inoculated treatments.

G. iranicum var. *tenuihypahrum* var. *nova* is protected by two patents and is a component of MycoUp, MycoUp Activ, Resid HC and Resid MG, whose commercial application has spread to more than 30 countries, achieving increases in crop yields of 8%-45% in lines as varied as leaf vegetables, berries, fruit, olives, grapes, greenhouse crops and cereals.

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