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DEVELOPMENT OF A BIOFERTILIZATION TECHNOLOGY BASED ON MYCHORIZAE

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Abstract

The importance of mycorrhiza for the plant is not limited to the absorption of water and nutrients from the soil. Symbiotic plants are often more competitive and more tolerant to environmental stress than those without mycorrhizae. Here we can give an example of osmotic stress, mycorrhizae make plants more resistant to cold weather (below 15 degrees). Arbuscular mycorrhizal fungi colonize the roots of most monocotyledons and dicotyledons despite their different root architecture and cell patterning. Key Result Large lateral roots are preferentially colonized, and fine lateral roots are immune to arbuscular mycorrhizal colonization. Fungal preference for large lateral roots also occurred in sym mutants that block colonization of the root beyond rhizodermal penetration.

***Key words**: biotechnology, mycorizae, wheat, roots.*

INTRODUCTION

Farmers and agricultural experts are under pressure to increase crop yield due to the rising demand for food and the shrinking amount of arable land. To increase agricultural output, this has resulted in an overuse of chemical pesticides and fertilizers. The symbiotic relationship between rooted plants and arbuscular mycorrhiza (AM) is one of the earliest known symbiotic relationships in nature. About 80% of the species of terrestrial plants are colonized by a fungus called arbuscular mycorrhiza. In exchange for carbon sources, these plants take nutrients from the earth and transfer them to hosts plants. Indeed, AM fungi play a significant role in sustainable agriculture by protecting plants from biotic and abiotic stressors. Due to the negative effects of chemical-based fertilizers, the AM fungi are crucial for biofertilizers because of these characteristics.

Arbuscular mycorrhizal fungi (AMF or AM fungi) are an important link between plants and the mineral nutrients in the soil. As a result, interest in them as natural fertilizers is growing. According to Schüßler et al. (2001), AMF are obligatory symbionts that are members of the phylum Glomeromycota and develop mutualistic symbioses with roughly 80% of land plant species, including a number of commercial crops. They exchange water and mineral nutrients for photosynthetic byproducts from the host plant (Smith and Read, 2008). When the AMF mycelium emerges from the root system, it can access portions of the soil where roots cannot get to get nutrition (Smith et al., 2000). Additionally, fungal hyphae are significantly thinner than roots and can therefore pierce smaller pores because of this (Allen, 2011). Afterward, carbohydrates and mineral nutrients

Mycorrhizae are fungi that form mutualistic symbioses with plant roots, providing them with nutrients and food necessary for growth and development. Mycorrhiza-based products are used in intensive agriculture to improve soil quality and increase plant productivity.

Arbuscular Mycorrhizal Fungi (AMF) are obligate root biotrophs that trade mutually beneficial effects with roughly 80% of plants. Since they give the host water, nutrients, and protection from pathogens in return for photosynthetic products, they are regarded as natural biofertilizers. AMF are hence essential biotic soil components that, when lacking or depleted, might result in an ecosystem working less effectively. In order to promote sustainable agriculture, the process of restoring the natural level of AMF richness can be a viable substitute for traditional fertilization techniques. Direct reinoculation of AMF propagules (inoculum) into a target soil is the major tactic that can be used to accomplish this purpose. Initially, AMF were proposed as agriculturally acceptable since they were widely considered as lacking host- and niche-specificity.

The fungi of the genus *Glomus*, including *Glomus intradiceps*, form mutualistic symbioses with plant roots by means of mycorrhizal hyphae, which penetrate the roots and extend through them. In this process, fungi use enzymes to fix nitrogen from the air in the form of ammonia (NH_3) and nitrites (NO_2^-), which are then converted into organic compounds, such as nitrates (NO_3^-), used by plants as sources of nitrogen. In this way, *Glomus intradiceps* mushrooms provide plants with the nitrogen necessary for growth and development, in exchange for carbohydrates and other nutrients provided by plants. This symbiosis is beneficial for both plants and fungi, which are protected by the growth and development of plant roots and by access to sources of energy and nutrients. It is important to mention that the process of nitrogen fixation is dependent on many factors, including soil pH, the availability of other essential nutrients, such as phosphorus and potassium, and climatic conditions. Therefore, it is important to monitor these factors and to control them adequately to ensure an efficient symbiosis between *Glomus intradiceps* and the host plants.

MATERIALS AND METHODS

The soil taken into analysis from the geographical area of Muntenia, Romanian Plain, more precisely Berceni commune, Ilfov county is located within the physical body 150 noted as soil profile P1. The soil profile P1 framed as argic cherniosome presents a profile of the type Amp-Am-AB-Bt-Ck, being formed by loessoid deposits. The groundwater is located at a depth of over 5 m. The soil was formed by an illuviation process of the clay from the upper horizon.

Experimental work was carried out both on the field of the experimental field and in the collaborating laboratories of Agricola Berceni SRL. It was used to multiply the *Glomus intraradices* fungus, on a nutrient broth type solid culture medium. After Multiplication, they were stabilized in suitable solutions, later arriving on the sample land cultivated with the wheat host plant. In the experimental field, two experimental wheat plots were established. One plot was cultivated as a control, while the second plot was cultivated and treated with mycorrhizae. For the establishment of the wheat culture, the technology according to this culture was executed, without producing a rebate from any operation.

The abiotic factors that have an impact on mycorrhiza were studied. The light. The energy source of the symbiont fungus is in the plant and depends directly on the way it carries out its photosynthesis process and on its ability to translocate the products of photosynthesis to the root (Varma, 2008). The lack of the light source produces a restriction for the development of the fungus, so its evolutionary process is slowed down, sporulation no longer occurs, and the expansion of the mycelium in the soil and in the root is reduced.

Temperature. From the point of view of the processes of spore germination, root penetration by hyphae and their proliferation inside the cortical cells, temperature can be a factor with a limiting effect (Gavito et al., 2005) soil pH. The efficiency of the fungus-plant association is determined by the adaptability of the fungal partner to a certain soil pH level. The pH affects both spore germination and their development. The relationship between soil pH and the effects of mycorrhizae depends on the host species, the type of soil, the forms of phosphorus and the species of fungi involved Salinity. In the case of high salinity, a decrease in the production of propagation structures (propagules) and in the colonization of vesicular-arbuscular fungi was observed (Pfeiffer and Bloss, 1988).

A well-developed root system means a good capacity for the absorption of nutrients from the soil followed by the sustained development of the aerial part of the plant, the increase of the vegetative mass and the final increase of the quality of the harvested vegetables. The addition of phytohormonal solutions can add to the growth of the plant.

In most types of mycorrhizae, the movement of carbohydrates, produced during photosynthesis, is done from the host plant (autotrophic partner) to the symbiotic fungus (heterotrophic partner). In the case of absorption of nutrients from the soil, the transfer has an inverse direction, from the fungus to the host plant (Jakobsen, 1999). The contribution of vesicular-arbuscular fungi to the assimilation of nutrients is the absorption of nutrients (especially phosphorus) from the soil, with the help of extraradicular hyphae - especially from those parts of the soil to which the plant did not have access. The hyphae of the fungus act similarly to the absorbent hairs on the root of the plant; After comparing the diameter of the absorbent hairs (5-20 µm) with that of the mushroom hyphae (3-7 µm), the absorbent hairs would gain the cause, but comparing the length and density of the mushroom hyphae with that of the absorbent hairs - the fungus would be, because it exceeds the possibilities of expansion of the plant by 10 to 100 times more.
Variant 1. Mixture based on mycorrhizae: with a rooting effect was used to fertilize the crop. The fertilizer was added to the surface of the culture substrate when the wheat crop is in the needle phase. Fertilizer was added in one pass. The same treatment options were applied for the three analyzed varieties.
The mycorrhizal fertilizer was added in order to determine the rapid development of the root system and to process food from the soil with the help of fungi. A well-developed root system means a good capacity to absorb nutrients from the soil followed by the sustained development of the aerial part of the plant, the growth of the vegetative mass and finally the increase in the quality of the harvested grains.
Variant 2. At sowing, it was fertilized by incorporating NPK complex (15-15-15 active substance) N 45 kg active substance, P_2O_5 45 kg active substance, K_2O 45 kg active substance. Straw cereals have average specific consumption of nutrients (Cs = kg N, P_2O_5, K_2O/1 t production), but they are extremely demanding on fertilization, given the fact that they have a poorly developed root system and have a low capacity to solubilize substances nutrients from soil reserves, especially wheat.

RESULTS AND DISCUSSIONS

The need to fertilize crops and the principles of rational fertilization are summarized in the fertilization plan, which represents the control and management tool for fertilizers. The fertilization plan is based on a foundation made up of the combination of the following parameters: crop rotation, the genetic production potential of the crop, the availability of the soil nutrient reserve, the water resource and the dose of applied fertilizers. The dose of applied fertilizers is the result obtained from the calculation of the system made up of three equations: the genetic production potential of the crop, the availability of the soil nutrient reserve and the water resource.
Basic fertilization is carried out with organic fertilizers and complex chemical fertilizers that provide the plants with the necessary nutrients, which they need for the desaturation of the vegetative cycle with a satisfactory result.
The expected productions for sustainable agriculture cannot be based on the nutrients provided by the environment. Fertilization technology is based on two classes of nutrients: macroelements and microelements, both showing the same degree of importance in obtaining a sustainable production. The macronutrient class has 3 major nutrients and the micronutrient class consists of 6 major elements, which plants need to go through a healthy vegetative cycle.

The terminology of macroelements and microelements, respectively, does not represent the nutritional importance to the plant, but strictly the quantities needed for a plant to produce the maximum. Both macroelements and microelements are equally important, being key elements in the biochemical processes carried out in the plant growth cycle. Macroelements are N (nitrogen), P (phosphorus), K (potassium), and microelements are Ca (Calcium), Mg (Magnesium), S (Sulfur), Fe (Iron), Mn (Manganese) and Zn (Zinc), adding another series of microelements: Ni (Nickel), Mo (Molybdenum), Co (Cobalt), Cu (Copper), B (Boron), etc. which plants need in relatively small quantities, but which are essential to life. These elements are part of the composition of

many enzymes that catalyze biochemical processes.
The advantage brought by the existence of mycorrhizal symbioses for plant nutrition, highlighted the influence that this association has on the growth and development of plants. The existence of mycorrhizal fungi was demonstrated about 400 million years ago, the first discoveries being the fossils of Aglaophyton major plants that showed traces of shrubs, these being considered edifying transfer structures for the vesicular-arbuscular endomycorrhizal type.
Mycorrhizae are present in mature ecosystems, ecosystems that show a cyclical and unitary evolution of the components between the biotic and abiotic unit, when mycorrhizal associations have the role of regulating the assimilation of food resources for the plants with which they are associated.
In this association, hyphae play an important role in the nutrient cycle, having the function of stopping losses in the ecosystem
From the data analysis, it is observed that in the variant treated with phytohormone fertilizer, the highest gluten accumulation was recorded for all 3 varieties of wheat, the differences between the varieties being characteristic of the genetic potential (Table1).

Table 1. Determination of gluten from the obtained production

No	R	V.M (g %)		V. 1 (g %)		V. 2 (g %)	
1. Glosa	1.1	24.02	24.06	29.01	29.03	28.02	27.97
	1.2	24.05		28.98		27.98	
	1.3	24.1		29.1		27.91	
2. Apache	2.1	22.97	22.99	27.41	27.32	26.8	26.91
	2.2	23.01		26.98		27.02	
	2.3	22.99		27.56		26.91	
3. Arnold	3.1	32.01	32.07	38.92	38.94	38.74	38.83
	3.2	32.11		39.01		38.95	
	3.3	32.09		38.89		38.81	

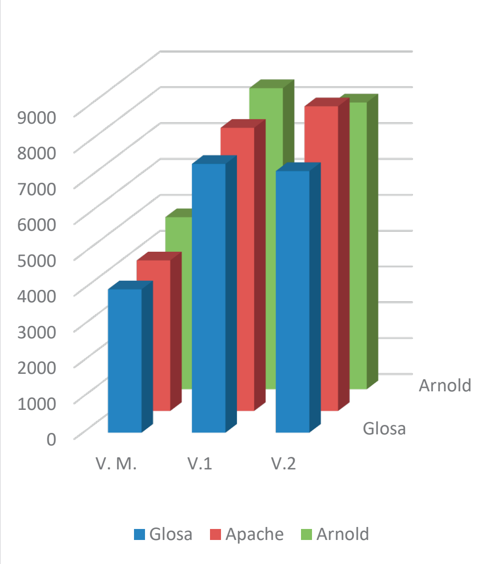

Figure 1. Influence on production

Analysis of the results revealed variations in protein content between the two fertilization options for all 3 wheat varieties. The results obtained are presented in Figure 1. Increases in protein content are observed for the variants treated with fertilizers compared to the control variant with differences between 0.8 and 2.88%.
In conclusion, the mycorrhizal fertilizer determined the most pronounced increase in terms of protein synthesis. In terms of protein content, the leading variety is Arnold followed by Glosa at a difference of 2.47% (Table 2).
In the case of the Glosa wheat variety, the ratio between the protein content and the production achieved is directly proportional. In the case of the first variant of fertilization with mycorrhizae, which had a rooting effect, they stimulated the increase in the protein content of the production obtained by 1.06% compared to the second variant of fertilization, which has both a lower protein content and a lower production.
Thus differentiating the two variants, a fact that highlights that a plant with a well-developed root system, a system that maintains the plant throughout the vegetative cycle, can exploit the genetically coded productivity characteristics much more efficiently.

Table 2. Determination of wheat proteins

No.	R	V.M (g %)		V. 1 (g %)		V. 2 (g %)	
1.Glosa	1.1	12.23	12.17	13.85	13.91	13.12	13.18
	1.2	12.17		13.91		13.25	
	1.3	12.11		13.98		13.17	
2.Apache	2.1	11.98	11.75	12.54	12.55	12.01	12.07
	2.2	11.52		12.46		12.11	
	2.3	11.74		12.65		12.09	
3.Arnold	3.1	13.76	13.50	16.41	16.38	15.84	15.82
	3.2	13.23		16.35		15.91	
	3.3	13.52		16.39		15.71	

Increasing protein content has two associated components, namely quantity and quality. Both are directly related to the amount of nitrogen assimilated by the plant in accordance with the vegetative cycle. A high yield is believed to decrease the grain protein content, so it is essential to determine the expected yield as the crop approaches the grain filling phase. When protein quality is desired, mainly to mimic high molecular weight, long chain gluten proteins. Gluten proteins (gliadin, glutenin, albumin and globulin) give wheat products unique extensibility and processing properties.

CONCLUSIONS

The need to use AMF as a biofertilizer to create sustainable agriculture is becoming more and more critical since proper management of these simbiotic fungs may lessen the need for agrochemicals. Inoculating AMF propagules in a concentrated solvent is the primary approach taken to achieve this goal.
Unfortunately, AMF are required to be symbiotic and cannot be grown in pure cultures without the use of their host plants' gasd. This limiting characteristic makes producing a long scare of AMF inoculation very challenging and complex.
There are three primary types of AMF vaccinations. First off, because it contains colonized fragments, AMF spores, and hife in a typical manner, soil from an AMF-hosting plant's zone may be used as an immunization.
The general conclusion of the research is to identify the intake of nutrients brought by mycorrhiza for wheat cultivation. The mycorrhizal relationship leads to the solubilization of minerals, the production of plant growth stimulants and the control of pathogens.
The cumulative benefit brought to the plant leads to a high production by substituting chemical fertilizers
It is concluded that it brings a major benefit in the transport of nutrients for plants. In most types of mycorrhizae, the movement of carbohydrates, produced during photosynthesis, is done from the host plant (autotrophic partner) to the symbiotic fungus (heterotrophic partner). In the case of absorption of nutrients from the soil, the transfer has an inverse direction, from the fungus to the host plant (Jakobsen 1999). The contribution of vesicular-arbuscular fungi to the assimilation of nutrients is the absorption of nutrients (especially phosphorus) from the soil, with the help of extraradicular hyphae - especially from those parts of the soil to which the plant did not have access.
The nutritional variants applied differentially influenced the three varieties of wheat used in the experiment, so that both the accumulation of all biocomponents, expressed as protein, and the particular accumulation of gluten was made according to the variety and according to the treatment.
Here are some of the benefits that wheat can get from symbiosis with *Glomus intraradices*:
1. Increasing nutrient absorption: *Glomus intraradices* can increase the absorption surface of wheat roots and help more efficient absorption of nutrients such as nitrogen, phosphorus and potassium. This can improve plant health and increase crop yield.
2. Stress tolerance: *Glomus intraradices* can help increase the wheat plant's tolerance to abiotic stress such as drought, high temperature or nutrient poor soils. It can help increase the plant's resistance to harsh environmental conditions and improve the plant's survival under such conditions.

3. Disease Protection: *Glomus intraradices* can help protect the wheat plant against diseases and fungal infections. It can increase the immunity of the plant and help reduce the incidence of diseases.

4. Improving soil quality: *Glomus intraradices* can help improve soil structure and fertility. This can help reduce soil erosion, improve the soil's ability to retain water and nutrients, and increase soil microbial biodiversity.

The symbiotic relationship between *Glomus intradiceps* and wheat is manifested by the formation of a mycorrhizal collet between the wheat roots and the fungal filaments. In this collet, the fungi provide the plants with nutrients, including nitrogen, phosphorus and other minerals, obtained from the soil, while the plants provide the fungi with carbohydrates produced through photosynthesis.

This mycorrhizal collet can grow and develop in the wheat roots, thus improving its ability to absorb nutrients from the soil. In addition, mushrooms help to improve the structure of the soil, reducing its compaction and increasing the permeability of water and air. Therefore, the symbiotic relationship between *Glomus intradiceps* and wheat can lead to better growth of wheat, with higher productivity and increased resistance to water stress and pests.

Nitrogen in the air is fixed by fungi and cyanobacteria, transforming it into nitrogen compounds, such as nitrates and ammonia.

Plants absorb these nitrogen compounds from the soil and use them to build proteins and other substances necessary for growth. Animals eat plants and accumulate proteins and other nitrogenous substances in their bodies. When animals defecate or die, organic residues with nitrogen are released into the soil. Fungi in the soil break down organic residues and release nitrogen in the form of ammonia, which can be fixed again and used by plants.

The process continues, allowing the transfer of nitrogen between different components of the ecosystem. It is important to mention that this natural circuit can be disturbed by human activities, such as the excessive use of synthetic fertilizers or soil pollution. This can have negative effects on the health of ecosystems and on agricultural productivity.

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