

## Brief Overview of Arbuscular Mycorrhiza Research Conducted in Russia Over the XX<sup>TH</sup> Century

Andrey P. Yurkov<sup>1</sup>; Lidija M. Jacobi<sup>2</sup>; Alexey A. Kryukov<sup>3</sup>; Lidija G. Perevedentseva<sup>4</sup>; Maria F. Shishova<sup>5</sup>

<sup>1</sup>All-Russia Research Institute for Agricultural Microbiology, Russia.

<sup>2</sup>All-Russia Research Institute for Agricultural Microbiology, Russia.

<sup>3</sup>All-Russia Research Institute for Agricultural Microbiology, Russia.

<sup>4</sup>Perm State University, Russia.

<sup>5</sup>Saint-Petersburg State University, Russia.

### Abstract

*Arbuscular mycorrhiza (AM) is one of the most common terrestrial plant-microbe symbioses. The interest in studying AM is caused by its importance for the growth and nutrition of plants and its ecological role in preserving biological diversity. At present, 92% of plant families are known to form AM. Fungi in AM are obligate symbionts and belong to a monophyletic group – Glomeromycotina subdivision. Some researchers believe that formation of AM-symbiosis with fungi by ancient plants allowed them to inhabit land, which has led to the formation of the biosphere as we know it. AM is studied by researchers from around the world including Russia. This overview presents in brief the results of AM research in Russia during the Soviet period which did not receive international coverage due to its classified nature; the most prominent research schools and works of AM researchers are identified; main stages and lines of this research are listed, such as: distribution of AM in plant communities and different climatic zones, mycorrhiza anatomy and morphology, fungi species composition on different soils, collection of AM fungi and growing AM in vitro, physiological role of AM for agricultural plants, the impact different ecological factors have on AM fungi species composition and AM development, conditions for effective use of Am fungi to increase productivity and quality of agricultural plants as well as to perform biological recultivation of toxic soils and restore the fertility of degraded soils.*

**Key-words:** Arbuscular Mycorrhiza, Plant Mycosymbiotrophism, Plant Productivity, Physiology, Ecology, Agrotechnology, Symbiotic Effectiveness, Symbiogenetics.

## 1. Introduction

Arbuscular mycorrhiza (AM) is one of the most wide-spread plant-fungus associations formed by most higher terrestrial plant species (75-90% by different estimates) with monophyletic fungi from Glomeromycotina subdivision of Mucoromycota Division (Spatafora et al., 2016) that consists of 240-348 species (Stockinger et al., 2014; Öpik et al., 2014). According to I. Karatygin, AM constitute at least 20% of cycle of matter in terrestrial ecosystems (Karatygin, 1993). The first description of mycorrhiza in Russia was provided by F. Kamenskiy (Kamenskiy, 1886). Studying the anatomy of a herbaceous plant *Monotropa* (*Monotropa hypotitis*) covered by thick mycelium, Kamenskiy supposed that this was a symbiosis between a plant and a fungus. In 1911, studying the morphology of *Fungi hypogaei* that formed endomycorrhiza on herbaceous plants, F. Bukhgoalts suggested that the fungi belonged to Phycomycetes of Endogonaceae family (Bukhgoalts, 1911). The range of AM symbiosis research is very wide and includes the role of AM in the evolution and biodiversity of plant communities; physiological studies of the AM role in mineral nutrition and regulation of metabolic processes in host plants, in photosynthesis and adaptation to adverse environmental conditions; AM role in soil formation processes, cycle of matter, ecosystem sustainability plant adaptation to biotic and abiotic stress factors as well as genetic studies of the formation and functioning of plant-microbe systems (PMS) which gave rise to an independent discipline – symbiogenetics. The modern science considers the formation of PMS to be a result of coevolution of interrelated organisms which lead to parallel convergence of partners of a symbiosis (Karatygin, 1993; Provorov, 2001). It was the symbiosis with AM fungi that allowed the early Rhyniophytina plants expand beyond their water environment and form a new ecological niche (Pirozynski, Malloch, 1975; Malloch, Pirozynski, 1980; Karatygin, 1990; Karatygin, 1993). Mycelium is believed to have performed the role of roots and provided the plants with water and minerals. AM is an ancestral form of symbiosis between fungi and terrestrial plants and as a mutualistic biotrophic intracellular association is one of the most successful evolutionary strategies which is proved by the fact that it exists for more than 400 million years and is the most widely spread PMS (Karatygin et al., 2006; Smith, Read, 2008, Provorov, Stark, 2014). Plants used many molecular genetic mechanisms of AM plant-microbe interaction in symbioses that emerged at later stages of evolution, for example, a symbiosis between Fabaceae and Rhizobia. In accordance with the hypothesis of their coevolution with host plants, AM fungi have always been obligate mycotrophs. Any other ancestor of AM fungi, for example parasites or saprophytes, has not been yet found (Pirozynski, Malloch, 1975; Koide, Mosse, 2004). Close

coevolution of plants and AM fungi proves that AM played the decisive role in the formation of the biosphere in its present form.

The AM role in the development of plants can hardly be overestimated. I. Selivanov believed that AM main function should be considered its participation in the phosphorus cycle (Selivanov, 1976). The importance of research of AM role in plant nutrition was proved as early as in 1933 when S. Kostychev wrote that without systematic biochemical investigation of mycotroph nutrition mechanisms our understanding of root nutrition would not be full (Kostychev, 1933). At present, AM is an important area of research for a number of fundamental questions of PMS evolution and root nutrition of mycorrhizal plants. AM economic role has also become obvious for agriculture and forestry as well as for land recultivation performed to improve plant mineral nutrition and increase their productivity. This gave rise to a promising research and development area – the creation of biological products and selection of AM plants characterized by high symbiotic activity.

## **2. Overview of Research of I. Selivanov's School**

AM started to be actively studied in the second half of the XX century. From 1950 to 1990 plant mycosymbiotrophism was studied in international science from the following points: morphological, mycological, geographic, ecological and physio-biochemical. The most common is the research of mycorrhiza morphology, geography of distribution and its role in ecosystems. During the post-Soviet period in Russia (from 1990s) an actively developed field was molecular-genetic research of AM.

In the Soviet Union, a systematic research of mycosymbiotrophism in vegetation cover began in 1957 and was led by Ivan Selivanov (Perm, Perm State Pedagogical Institute, PSPI, department of botany); in 1963 I. Selivanov created the first Russian scientific school The Study of Consortive Relations and Population Structures in Natural and Anthropogenic Ecosystems. Each of his undertakings was supported by his wife and companion – Lyutsiana Krüger. Selivanov engaged students in research and organised scientific expeditions to different parts of the USSR. One doctoral dissertation scientific advice to: T. Boyko (Sentyabova) (study of the intensity of mycorrhiza formation in fir seedlings depending on the growth conditions), N. Golubinskaya (growing arbuscular mycorrhiza in a laboratory), N. Yeleusenova, K. Yeropkin, L. Ivashkina, V. Loginova, A. Lusnikova, L. Mekhonoshin, T. Maksimova, O. Namestnikov, L. Nozadze, V. Perevedentsev, L. Perededentseva, E. Shkaraba, L. Utemova (study of Liliaceae and Iridaceae mycorrhiza, general questions regarding mycosymbiotrophism), V. Shavkunova (mountain-meadow phytocenosis), L. Shirinkina (description

of mycosymbiotrophism and other forms of consortive relations in wheat agrocenoses of the Central Cis-Urals). Since 1975 all data on mycorrhiza research has been concentrated in a Perm periodical, republican interuniversity collection of studies titled *Mycorrhiza and Other Consortive Relations* of which I. Selivanov was appointed an editor-in-chief at the II All-Union convention dedicated to consortive relation research. From 1978 to 1994, 9 editions of the collection were published, of which the last one was deposited. In 1981 Nauka publishing house released I. Selivanov's monograph awarded with a Moscow Society of Naturalists Award. The monograph summarized and analyzed all available work on mycosymbiotrophism over 1829–1977 period (1,007 sources including 494 foreign ones). One doctoral dissertation and ten PhD theses were defended under Selivanov's guidance. Since 1960s, the members of the department of botany of Perm State Pedagogical University (Selivanov, Shirinkina, Perevedentseva, Khrushcheva and Talatina) including undergraduate and postgraduate students have actively studied mycosymbiotrophic consortive relations in natural and agricultural biocenoses. A detailed analysis of symbiosis between higher plants and AM fungi was undertaken. This form of consortive relations was studied in all landscapes and geographic zones of the USSR and main vertical climate zones: in the Ural and Caucasus Mountains, some mountain systems in Central Asia (Gissar and Chatkal Ranges, Tian Shan and Kopet Dag). A description of mycorrhizae in more than 4,000 Cryptogams, Gymnosperms and Angiosperms was performed; mycosymbiotic parameters of the key natural biocenoses were calculated; some aspects of ecology of mycorrhiza formation were studied experimentally, in particular, the dynamics of mycorrhiza formation and development and the influence of soil conditions and light intensity on mycorrhiza formation (Selivanov, 1983). Along with these studies, terminology and classification of mycorrhizae were elaborated, main types of mycorrhizae were indentified and solutions to methodological problems were developed (Selivanov, 1973). Since 1970, the results of this research have been published in dozens of annual proceedings of the Perm State Pedagogical Institute – *Mycorrhiza and Other Consortive Relations*, *Plant Mycorrhiza*, *Role of Consortive Relations in the Formation of Biogeocenoses* – as well as in I. Selivanov's monograph (Selivanov, 1981). Let us briefly review some of the results of this research.

Extensive study conducted in tundra, forests, steppes and deserts have shown that arbuscular mycorrhizae dominate in vegetation cover of these landscapes. It was determined that the landscapes and geographic zones under study differ in the frequency of occurrence of mycotrophic plants. Thus, the proportion of mycotrophic species was 42.2% in tundra, 77.9% – in forests and 60.9% – in deserts (Selivanov, 1976b). The proportion of AM plants to all studied plants was: 32.8% in tundra, 63.3% – in forests, 82.0% – in steppes and 60.1% – in deserts. The proportion of AM plants to

mycotrophic plants (of all types of mycorrhizae) was 70.0%, 97.8% and 96.0% respectively (Selivanov, 1976b).

The first results of morphological AM research were obtained using optical microscopy in 1957. In particular they showed that: 1) AM fungi do not form hyphal sheath typical for ectomycorrhizas but can form a dense net of hyphae with episodic chlamydospores or sporocarps that contain chlamydospores; 2) AM fungi penetrate the root through epidermis and – less frequently – through fibrils although the latter is frequently observed in grains (Krüger, 1957; Selivanov et al., 1961; Selivanov, Utemova, 1968); 3) AM almost does not change the appearance of the roots, and a slight change of root color into brownish or greenish-yellow in spots where the fungus is concentrated is displayed only by some plant species, such as the onion, corn, wheat, pea, tomatoes and other Solanaceae, which quickly disappears in the sun; hyphae can grow intra- and intercellularly; during the active growth of hyphae the mycelium is non-septate, however, if the conditions are adverse, it may form occasional septa; the fungus grows in the root cortex without penetrating the endodermis, stele and root meristem; chloroplast-bearing cells are not infected by the AM fungus; arbuscules are formed as a result of repeated dichotomous branching of the hyphae tips, and the branches that resemble a "tiny tree goes on until they fill all spaces between the cells, the last branches being very thin, less than 1 micrometer, and are hardly observable through the optical microscope (Selivanov, 1975a). Arbuscules later degrade and turn into "grainy mass" which is then lyzed (digested) by the host plant. Based on this, I. Selivanov Classified AM as phycomycetic tamniscophagous endomycorrhiza (Selivanov, 1975c).

Analysis of the morphology and anatomy of mycorrhizal roots of herbacious plants from different landscape and geographic areas (the Urals, the Kola Peninsula, the Caucasus, Crimea, Central Asia, the Pamir Mountains, forests and steppes of the Trans-Urals, Western Siberia and Moscow Region) as well as greenhouse and house plants, among which tropical species were predominant, allowed to conclude that these mycorrhizae were formed by the same or very similar fungi species, supposedly, of the genus *Endogone*. The same conclusion was made from a study of introduced and acclimatized plants of Batumi, Tashkent, Novosibirsk and Perm botanical gardens. Thus, the members of the department of botany of Perm State Pedagogical Institute who studied AM in different lanscapes and geograpical zones found the relation between AM and phycomycetes from the family Endogonaceae (Selivanov, 1975b).

Along with the research of AM distribution, morphology and anatomy, the following problems were studied in 1960–1970s: ways of mycorrhizal infection transmission (through seeds, from soil or other media); AM host plants specialization; the ability of different exophytes and

endophytes (along with phycomycetes) to form AM. The study of location of endophyte fungi in a number of plants has shown that fungi do not form mycorrhiza in plant's reproductive organs (Krüger, Selivanov, 1976). It was proven experimentally (on wheat and pea) that plant do not display cyclic mycorrhizal infections, and that AM occurs when roots are infected in the soil (Krüger et al., 1973). A series of experiments where wheat and seedlings grown in sterile conditions were infected using mycorrhizal roots of different herbaceous plants and fungal spores isolated from soil proved that phycomycetic rhizomycetes (lower unicellular fungi that are found in the plant rhizosphere) do not have a narrow host plant specialization (Krüger et al., 1973).

The literature on the biological nature of AM fungi available at that time was controversial. According to E. Khrushcheva, for example, such micromycetes as *Fusarium oxysporium* Schecht and *F. javanicum* var *radicicola* Wr. create in wheat roots a picture similar to endotrophic mycorrhiza (Khrushcheva, 1955; Shirinkina, 1978; Selivanov, 1983). However, in other cases these very species of the genus *Fusarium* behave as parasites. Experiments in which L.endophytic fungi Krüger and her colleagues (Krüger et al., 1973) infected wheat and pea seedlings with exophytic and endophytic fungi (*F. javanicum*, *Rhizoctonia* sp., *Gilmaniella humicola*, *Rhizopus nigricans*, *Chaetomium globosum* Kunze, *Alternaria tenuis*, *Penicillium verticilloides*, *Hypholoma fasciculate*) showed that they do not form AM but rather behave as parasites affecting xylem (like *F. javanicum*) and phloem vessels (like *Rhizoctonia* sp.) or as saprophytes. Others, for example *Chaetomium giobosum*, *Penicillium verticilloides* penetrate the roots and settle in cortex rather than in stele

Studies of AM ecological functions in natural ecosystems in Russia began as early as in 1950s when its active role in plant growth and development was identified. A number of studies of mycorrhiza formation in bread grains grown on chernozem, solonetz, forest-steppe, grey forest and sod-podzolic soils was conducted (Khrushcheva, 1955; Khrushcheva et al., 1986; Bulayeva, 1965; Selivanov, Utemova, 1968; Shirinkina, 1978); a primary identification of AM plant species, assessment of AM distribution in different geographical zones and influence of the environmental conditions on AM formation were performed. The key ecological factors that influence mycorrhiza formation were identified, such as: humidity, water and air conditions, pH level, amount of organic and mineral matter, agrotechnologies used to cultivate plants, air temperature, amount of light and soil type (Khrushcheva, 1961; 1980; Khrushcheva, Sogina, 1968; Golubinskaya, 1980a, 1980b). The research usually took place in the field, i.e. mycorrhiza formed by indigenous fungi was studied. Inoculation with AM fungi was not performed. The AM effectiveness was estimated using indirect evidence, for example, by comparing the productivity of slightly and highly mycorrhizal plants or the results of correlation analysis of plant productiveness and mycotrophism. As a fungal inoculum for

laboratory experiments on sterile substrates, mycorrhizal roots of different plants were used growing in the wild or in greenhouses or soil from mycorrhizal plants as well as fungal spores isolated from the soil.

It was E. Khrushcheva who has first noticed the positive effect of mycorrhiza on the growth of corn growing on light-grey forest soil with a small amount of mineral fertilizers (Khrushcheva, 1961). The conclusions were based on the positive correlation between the corn height and the amount of AM in the roots. The author explains this by better mineral nutrition of plants due to AM and faster synthesis processes, in particular, faster protein synthesis in leaves (Khrushcheva, Sogina, 1968). L. Shirinkina's research has shown that mycosymbiotrophism of wheat largely depends on the amount of mineral nutrients in the soil, in particular – phosphorus. When agricultural conditions are favorable and agrotechnologies are advanced, wheat grows well and shows high yield with very slight relation with mycorrhizal fungi (the intensity of mycorrhizal infection varying from 1 to 4%) and absence thereof. When the soil has a smaller amount of phosphorus and the agricultural technologies are of an average level, the wheat yield is almost twice lower although all plants show AM, and the degree of mycotrophism is many times higher (14-38%) (Shirinkina, 1978). L. Shirinkina also studied the relation between mycorrhiza formation in wheat and climatic conditions (Shirinkina, 1985). Three-year long observation in the field has shown that the amount of precipitation and diurnal air temperature during the period before the sowing influenced the intensity of mycorrhiza formation at the early stages of plant vegetation and the dynamics of its further development. The maximum AM occurrence at the beginning of tillering (~70%) was observed in the year when both factors – temperature and air humidity were relatively high (relatively humid conditions and moderately warm weather). Low air humidity and warm weather resulted in much rarer AM occurrence (~40%), as well as humid and cool weather (~35%). Further observation showed that the intensity of mycorrhiza formation in wheat increased towards the ripening stage, with AM developing best in warm and considerably humid weather.

E. Khrushcheva and her coauthors (Khrushcheva et al., 1980) studied the development of AM in some grains (wheat, rye, oats and corn) and determined the impact it has on the plant growth and yields depending on the time of plowing (early in July or late in October) and the soil moisture level especially at an early stage of AM formation. Thus, when the soil moisture was sufficient and the plowing was late, 67% of the plants formed AM (by the three-leaf stage), and the wheat yield was 16,500 kg/ha higher than when the plowing occurred early. A positive correlation was found between the plant mycotrophism and the grain weight, which may be a proof that AM increases the wheat yield. When the plowing occurred early and the soil moisture was the same, only 6% of the plants

formed AM, and the difference in the height between AM and non-AM plants was insignificant. The authors suggested that the difference in mycotrophism of the plants was determined by the degree of organic matter decomposition in the soil which was not only the source of energy for AM-fungi but also a place where fungus primordia – mycelium and spores – were stored. When the plowing was late, these organic residues decomposed slower than when it was early, which, supposedly, resulted in better survival of AM fungi and, consequently, intense formation of mycorrhiza in wheat during spring. When the summer was dry and the plowing occurred late, the yield was 77 kg/ha lower than when the plowing occurred early. Thus, different times of plowing of the grass cover and different levels of soil moisture create different conditions of plant soil nutrition, which in turn influences the interaction between AM fungi and higher plants (Khrushcheva et al., 1980).

N. Golubinskaya studied the influence of temperature and nitrogen nutrition on the growth, yield and mycotrophism of the pea (*P. sativum*) and red clover (*Trifolium pratense*) in a vegetation experiment on sterile substrates – vermiculite and quartz sand (Golubinskaya, 1980a). It was found that a higher amount of nitrogen fertilizers led to lower AM occurrence in the clover roots and lower symbiotic effectiveness. An optimum temperature for AM development in the clover and pea was determined (+17°C). N. Golubinskaya also provides data on the influence of different light intensity on mycotrophism of self-pollinated corn lines (Golubinskaya, 1980b). It was shown that both in a greenhouse (with the illuminance of 1,200 and 580 lux) and in a phytotron (with the illuminance 4,600 and 2,800 lux), higher light intensity resulted in 2-4 times lower corn mycotrophism.

L. Shirinkina studied consortive relations between wheat and micromycetes – Am fungi and a pathogenic fungus *Ustilago tritici* that causes the "loose smut" disease (Shirinkina, 1975; Shirinkina, 1986). Having compared the level of mycorrhiza infection development in healthy plants and plants infected with loose smut at the milky stage, Shirinkina 1) mycorrhiza is formed in both healthy and infected wheat heads; 2) healthy and infected plants differ in the intensity of mycorrhiza infection development – it is developed faster in infected plants (with more grey mass and vesicles and fewer arbuscules) than in healthy plants; 3) AM fungi could not prevent the development of the pathogenic infection as it occurred when the plants were in bloom and was transmitted with seeds. L. Shirinkina also assessed the effect of a complex of agricultural activities on the species composition and mycorrhiza formation of weed plants in wheat plantings in the Perm Region (Shirinkina, 1979). The results of the study showed a relatively low mycotrophism of weed plants in agrocenoses. The author suggests that this is the result of favorable conditions for soil autotrophic plant nutrition. A total of 54 species of weed plants were identified, of which 30 formed mycorrhizas, the anatomical and morphological pattern of which was largely similar to that of wheat mycorrhizas and is characteristic

of the fungi of the family. Endogonaceae. Mycorrhizae did not form in the species of the Caryophyllaceae, Chenopodioideae, Equisetophytina, Fumarioideae, Polygonaceae and Rubiaceae. A relatively high intensity of mycorrhiza formation in the roots was observed in Compositae, Labiatae and Fabaceae. The development by plants of symbiotic relations largely depended on their lifetime: more fungi were observed in perennial species. Annual and biennial plants make up the structural basis of weed sinuses in wheat crops (72%), of which 54% are non-mycorrhizal, and the rest are slightly and medium-mycotrophic plants. Perennial weeds are medium-mycotrophic species.

L. Shirinkina found that consorts in agrocenoses found in the same soil are affected by the ecological situation, which is formed under the influence of different seeding rates and, consequently, the area of nutrition. In the Central Cis-Urals alluvial soils, where mycorrhiza forms relatively well, when the planting is thinned to amount twice lower than the standard rate, the number of AM fungi increases significantly, whereas, when the planting thickens, it, on the contrary, decreases. In cultivated sod-slightly podzolic light loamy soils, where the process of mycorrhiza formation is less intense, the thinning of plantings does not affect consortive relations, whereas, when plantings thicken, the relations between plants and AM fungi are enhanced (Shirinkina, 1978). The same field experiments showed that, with the seed rate in the same soil being equal, mycorrhiza formation depends on the productivity of individual plants. Thus, among the production crops, well-developed plants show 31% intensity of mycorrhizal infection, medium-developed – 26%, and underdeveloped – 20%. They account for 19%, 73% and 8% of the yield respectively. Therefore, the major part of the yield (92%) is produced by more mycotrophic, medium-developed and well-developed plants.

Thus, from 1960s to 1980s Russian researchers identified the main abiotic factors (and their parameters) that affect the development of AM in some crops and managed to show a positive correlation between the level of mycorrhiza development and the crop yield. The study of reasons of the AM symbiotic effectiveness began. In this respect, I. Selivanov saw the main contribution of phycomycetic endomycorrhizas in the fact that they play an essential role in the phosphorus nutrition of plants, being a link that also takes part in the phosphorus cycle in the biosphere (Selivanov, 1976b). Unlike nitrogen and sulfur, phosphorus is found in the tissues in an oxidized form. Mineralization of organophosphorus compounds usually occurs via hydrolytic cleavage of phosphoric acid from the corresponding organic compounds. In the soil this process is carried out with the help of bacteria (mainly *Bacillus mesenteries*, *B. megaterius*, *Pseudomonas sp.*) and microscopic fungi (for example, species of the genera *Rhizophagus*, *Penicillium*, *Aspergillus*, *Trichothecium*, *Alternaria* and *Rhodotorula*). Among soil bacteria, there are narrowly specialized strains that have adapted to the decomposition of organic substances high in phosphorus (*B. megaterium* var. *Phosphaticum*). As a

result of microbiological decomposition of organophosphorus compounds, phosphoric acid is formed, which immediately binds to the bases of the soil and transforms for the most part into salts of calcium, magnesium and iron, which are poorly soluble and therefore ill-suited for plant nutrition. Consequently, phosphorus for some time does not take part in a biological cycle of organic matter. According to I. Selivanov, its subsequent inclusion into a cycle of matter occurs microbiologically – with an input of carbon dioxide, that acidifies the soil and thereby increases the solubility of phosphates (Selivanov, 1976b). This is also achieved by the activity of nitrifying bacteria and sulfur bacteria. But a more universal way is, perhaps, the formation of AM that utilizes poorly soluble phosphorus compounds (Selivanov, 1976b). I. Selivanov puts forward the following hypothesis about the mechanism of phosphorus fixation during the formation of AM. The fungi that form AM in most plants are, by their nature, low-pathogenic organisms. They cause a "chronic root disease," which to some extent proves useful to the host plant (Selivanov, 1976b). As a rule, the higher plant reacts to the introduction of a foreign body by more intense respiration (Kuprevich, 1947; Selivanov, 1976b). If mycorrhizal plants have more intense respiration, then more carbon dioxide is released, which facilitates the transformation of poorly soluble phosphorus compounds into forms that can be used by plants. Of great importance is probably also the fact that a significant number of connections between the endophytic (intra-root) mycelium and exophytic mycelium (mycelium on the surface of the root) and between the exophytic mycelium and soil increases the adsorption area of endomycorrhizas and the permeability of the cortical cells of the root. I. Selivanov reports that phycomyctic vesicular-arbuscular endomycorrhizae are an ancient type of mycorrhizal symbiosis, which has been common in the biosphere since the Paleozoic Era; as a result of a long evolution, the higher plants developed defense mechanism to the introduction of endophytes limiting the distribution of AM fungus to a relatively small absorption area (Selivanov, 1976b). One of the external manifestations of such mechanisms is the lysis of endophytic mycelium by the host plant root cells and the formation of the so-called "digestion zone" in endomycorrhizae. Due to the active reactions of the host plant, the AM fungus remains functionally young for a long time: it actively branches on the root surface and forms arbuscules in tissues. A long-term balance is established between the host plant and the AM fungus whereby the fungus is not so strong as to become a real pathogen, but the plant can not destroy the AM fungus in its tissues either. Such AM equilibrium is proves useful for both symbionts. The fungus lives off metabolic by-products of the host plant but, by activating its physiological processes, in particular, respiration, it facilitates more intense absorption of phosphorus from the soil by mycorrhizal roots compared to non-AM roots. The host plant apparently also benefits by lysing the AM fungus that penetrates its root tissues. When the lysis occurs, the fungus protoplast enters the

root cells and, supposedly, the nutrients from the lysed fungal cell can be used by the higher plant. Thus, as the author believes, phycomycetic vesicular-arbuscular mycorrhizae have formed in the course of evolution as a means of active extraction of phosphorus excluded from the biological cycle of organic matter and as a way to include it in the nutrient cycle of green plants (Selivanov, 1976b).

An interesting fact is the detection of fixation of atmospheric nitrogen by AM fungi (Selivanov, 1975d), but these data were found false because of the inability to detect *nif*-genes encoding nitrogenase in eukaryotic chromosomes. In the 21st century, however, a hypothesis was proposed about the AM ability to increase the nitrogen nutrition of plants based on the presence in AM fungi of symbiotic bacteria (similar to the genus *Burkholderia*) that have *nif*-genes (Minerdi et al., 2001; Bianciotto et al., 2003). The number of such gram-negative bacteria (*Candidatus Glomeribacter gigasporarum*) in *Gigaspora margarita* can be up to 250,000 cells per spore, which indicates the effectiveness of endosymbiont transmission during AM fungus reproduction.

The need to study the role of AM in plant nutrition was recognized as early as in 1933 by S. Kostychev who wrote: "It would be highly advisable to subject the problem of mycotrophic plant nutrition to systematic biochemical investigation: for it is one of the most fundamental problems of soil nutrition of plants, and we can rightfully say that, until this problem is solved, our understanding of root nutrition will not be full." (Kostychev, 1933; Lobanov, 1963). Solution of these problems required, at least, to develop methods to "synthesize mycorrhizae" in laboratory, i.e. to obtain strains of AM fungi on the host plant in vitro. To accomplish this task, N. Golubinskaya used as inoculum for apple and clover seedlings mycorrhizal clover roots whose surface was sterilized with 0.2% solution of the mercury (variant # 1), unsterilized clover roots (variant # 2) and spores of unidentified fungus of the genus *Endogone* (variant # 3) (Golubinskaya, 1979). When growing plants on sterile substrates, Golubinskaya managed to synthesize variant # 2 and variant # 3 of the typical AM. AM plants were both higher and heavier than the control non-AM plants. Thus, following the lead of foreign researchers (Nicolson, 1952; Mosse, 1961), methodological approaches to study AM in controlled conditions were developed.

### **3. The History of Research of G. Muromtsev's School**

Cultivation of AM fungi using the method of open-pot culture (OPC; Mosse, 1961; Gilmore, 1968) in Russia began in the early 1980s at the All-Union (now All-Russian) Research Institute for Agricultural Microbiology – ARRIAM (Leningrad, now Saint Petersburg) at the Laboratory of Ecology and Physiology of Soil Microorganisms directed by the VASKhNIL (Lenin

All-Union Academy of Agricultural Sciences) academician Ggeorgiy Muromtsev. A number of postgraduate students from Muromtsev's research group have successfully defended their theses dedicated to AM: I. Sarkina, E. Guseva, M. Dobronravova, N. Serebrennikova, A. Klyuchnikov, N. Kozlova and E. Krayeva.

I. Sarkina studied the mycosymbiotrophism of the main tree landscape-forming species and possible ways of using the data for biological monitoring of ecosystems, restoration of degraded landscapes and green construction (Sarkina, 1988). Inventory of mycorrhizae of the key tree landscape-forming species with an estimation of mycotrophism has been carried out on the Southern coast of the Crimea (Nikitsky Botanical Garden); the anatomical and morphological features of endotrophic and ectotrophic mycorrhizae, as well as the species composition of mycorrhizal fungi and the density of their populations in natural plant communities with different degrees of disturbance have been studied. The studies have shown that the main tree landscape-forming species in this region are mycotrophs, which differ significantly in the nature and intensity of mycorrhiza formation processes and are represented by both ecto- and endomycorrhizal species. I. Sarkina showed that the most common endomycorrhizal fungi for the Southern coast of the Crimea are fungi of the genus *Glomus*, in particular *G. mosseae*. Based on the results of the vegetation tests, it was concluded that inoculation with AM fungi gives the best results when applied to the seedlings of the Crimean pine (*Pinus pallasiana* D.Don.) in containers and to young plants during their transplantation from the nurseries into the natural environment. I. Sarkina's observations of two types of mycorrhizae on the same plant is an interesting fact from the point of view of the fungi interaction that represent different types of symbiosis. Sarkina showed that within a single root system, competition relations can arise between ecto- and endomycorrhizal fungi. Similar studies had already been conducted before (Golubinskaya, Selivanova, 1975). Cases of double mycorrhizal infection had been observed in *Rhododendron aureum* Georgi, *R. dahuricum* L., and especially in *R. luteum* Swett. N. Golubinskaya and I. Selivanova reported that this most likely occurs particularly often in soils that have long not been covered with forest or in greenhouses where there is not enough ectomycorrhizal fungi, and if they are there, they are in inactive form (Golubinskaya, Selivanova, 1975). However, based on the results of I. Sarkina's research (Sarkina, 1988) on the synthesis of mycorrhiza using AM fungi on tree species seedlings, it became possible to talk about the alternation of mycorrhizae: at the early stages of plant growth, AM is formed, which is later replaced with ectomycorrhiza formed with eumycetes.

E. Guseva studied the development of AM fungi in toxic sulfide-containing (pH 2.8) dark-grey sandy loam soil above the coal seam near the Moscow brown-coal basin of the Tula Region (Muromtsev et al., 1988). It was shown that the effectiveness of plant symbiosis with AM fungi

depends on the combination of the inoculated mycobiont, soil and climatic conditions, fertilization and liming. The use of the indigenous AM fungus *G. mosseae* adapted to the conditions of sulfate salinization in the field and vegetation experiments had a stable positive effect on the biological productivity of plants, compared with strains of fungi isolated from non-saline soils. Inoculation with AM with fungi proved the most effective when N<sub>120</sub>P<sub>84</sub>K<sub>120</sub> were accompanied by liming. The yield of the vetches and oats in the field increased 1.5-2 times, compared to the control area (uninoculated plants). E. Guseva found negative effect of plants that did not form AM (mustard, lupine, rapeseed) and soil resting on the number of indigenous AM fungi spores (3-11 spores per 100 g of soil) in sod-podzolic cultivated soil (Leningrad Region). This caused the development of the introduced endophyte in the roots of the vetches and oats. When mycotrophic plants (oats, barley, ryegrass, clover) were grown, the number of indigenous fungi spores was up to 100-220 spores per 100 g of soil.

M. Dobronravova studied the biological characteristics of arbuscular-vesicular mycorrhizae in the phytocenoses of the Stavropol Territory and tried to find effective inducers of mycorrhiza formation in grains (Dobronravova, 1998).

N. Serebrennikova studied the formation of microbial communities in sulphide-containing soils above the coal seam during their restoration with the aim of developing effective methods of biological recultivation (Zolnikova, Serebrennikova, 1990). N. She found that in the fertile layer of soil recultivated by the application of chernozem to sulphide-bearing rocks, a complex of microorganisms adapted to the stressful conditions of sulfate salinization was formed. A method of biological recultivation of sulfide-containing rocks was developed, whereby a complex of microorganisms in the form of a soil-root mixture containing the AM-fungus *G. mosseae* was introduced into the soil above the coal seam together with the seeds after amelioration, which increased the number of microorganisms and activated microbiological processes in the recultivated soils. This technique helped improve the survival of alfalfa on recultivated soils and increase the yield of green mass by 19.4-47.1% during the 3 years of the experiment.

A. Klyuchnikov studied the influence of AM fungi of the genus *Glomus* and rhizosphere bacteria on the development of the potato (*Solanum tuberosum* L.); the effect of *Azotobacter chroococcum* and *Pseudomonas fluorescens* bacteria on the development of AM fungus *G. mosseae* *in vitro*; he estimated the impact of AM on the number of certain groups of bacteria, for example, gram-negative tricalcium phosphate-solubilizing bacteria with high phosphatase activity in potato rhizosphere (Klyuchnikov, Kozhevin, 1990). Klyuchnikov showed that the association with *Azospirillum brasilense* and *P. fluorescens* increases the occurrence of mycorrhizal infection in the

potato, as well as the effectiveness of AM at the early stages of plant development. It was established that AM changes the structure of the rhizosphere bacteria complex, increasing the number of phosphate-mobilizing and nitrogen-fixing bacteria. The development of AM resulted in 2-5 times higher nitrogenase activity of the bacterial complex in the mycorrhizosphere as compared to the non-AM rhizosphere.

N. Kozlova searched for new methods of AM fungi identification. For this purpose, she proposed immunochemical methods and obtained specific antibodies for water-soluble proteins (Kozlova, 2001). In the course of the study, the method of isolating the vesicles of *G. intraradices* endomycorrhizal fungi from the roots of *Plectranthus australis* was optimized. For the first time polymorphism of protein antigens for AM fungi has been analyzed using the method of two-dimensional electrophoresis. These techniques could be used to study biodiversity and identify AM fungi.

E. Krayeva was the first Russian researcher to perform an ultra-cytomorphological analysis of the AM Sudan grass (*Sorghum vulgare* var. *sudanense*) that included the identification of 7 effective *Glomus sp.* fungi to species level (Krayeva, Lebsky, 1991) using diagnostic "Vesicular-arbuscular mycorrhizal fungi test" (Schenck, Pérez, 1988). As a result, the fungus was identified as *G. intraradices*. The technique of AM root samples preparation for electron microscopy was modified, and the ultrastructure of the intra-root mycelium, arbuscules, vesicles and AM-fungal spores at different stages of plant ontogeny was studied. X-ray microanalysis showed that the characteristic spectrum of the AM fungus sporoderm contains 7 elements: Si, P, S, Cl, K, Ca and Mg. The outer layer of the sporoderm contained a significant amount of Ca. The predominant element of the spore cytoplasm was P (Kraeva, Lebsky, 1991). The results of microscopy helped discover a previously unknown out-of-the-root stage of development of *G. intraradices*, namely, the formation of spores in the seeds of the nonmycotrophic *Chenopodium album* resting in the soil.

At ARRIAM, overseen by G. Muromtsev, effective strains of AM fungi were isolated and cultivated using the OPC method (Muromtsev et al, 1985a, 1985b). It became possible to obtain inoculation material for AM studies in the field and in laboratory. The main line of research of G. Muromtsev's group became the study of the influence of AM formed with the participation of AM fungi selected strains on the yield and mineral nutrition of agricultural plants at different levels of fertilizer application (Zolnikova, 1993). Studies aimed at obtaining a preparative form of AM fungi have been conducted. For this purpose, clay minerals – clinoptilolites –from different deposits were studied as substrates for AM fungi reproduction using the OPC method. The most suitable substrate from the point of view of its physico-chemical properties proved to be clinoptilolite from the

Sokirnitsky deposit in Ukraine (Jacobi et al, 1992). In 1986, under the editorship of O. Berestetskiy, an ARRIAM Bulletin was published. The materials published there allow us to identify the following groups of AM research in Russia: 1) evaluation of mycosymbiotrophism of wild and cultivated plants in different regions of the USSR (Krüger, Selivanov, 1986; Maksimova, 1986; Sarkina, 1986; Utemova, 1986; Shirinkina, 1986; Shkaraba, 1986; Shubin, 1986); 2) assessment of the effect of phosphorus levels in the soil on the AM development in agricultural plants (Golubinskaya, 1986; Mindiashvili, 1986; Khrushcheva et al., 1986); 3) evaluation of the effect of AM fungi inoculation on the mineral nutrition of agricultural plants (Samoshkin, Postnikova, 1986); 4) evaluation of the effect of AM fungi inoculation on plant growth in recultivated soils (Zolnikova, Muromtsev, 1986); 5) modification of the techniques for quantitative assessment of plant mycosymbiotrophism, in particular, the new term "mycorrhiza usefulness index" ("M<sub>u.i.</sub>") was introduced as the difference between the dry mass of AM and non-AM plants (Selivanov, Krüger, 1986). The authors point out that this index largely depends on the amount of phosphorus in the soil, as much as the AM symbiotic effectiveness. This index is introduced to assess the effectiveness of AM in in-vivo studies without AM fungi inoculation.

#### **4. The Formation of the Academician I. Tikhonovich's School**

In the 1990s ARRIAM began research on the molecular-genetic foundations of the formation and functioning of mutually beneficial PMS using model Fabaceae. By 2003, these studies formed a school "Molecular-genetic foundations of the formation and functioning of mutually beneficial plant-microbial systems", led by the academician of the Russian Academy of Sciences I. Tikhonovich and sponsored by grants of the President of the Russian Federation (project # NSh-1103.2003.4, etc.). More than a hundred academic papers have been published as a result of these studies in top-rated journals, for example (Tsyganov et al., 1994; Provorov et al., 2002; Jacobi et al., 2003; Borisov et al., 2004; Yurkov et al., 2015), as well as a number of monographs, including (Tikhonovich, Provorov, 1998; 2009; Provorov et al., 2010; Provorov, Vorobiev, 2012). New data have been obtained regarding the mechanisms of AM initiation and development, identification, assessment of the functional significance and location of genes that control different stages of AM development and plant-fungal signaling of this symbiosis were carried out. I. Tikhonovich and N. Provorov formulated the idea of the genetic integration of interacting organisms, which formed the basis of the "symbiogenetics" concept (Tikhonovich, Provorov, 1998; 2003). Symbiogenetics has become a new discipline that studies manifestations of the laws of variation and heredity in superorganisms

(Tikhonovich, Provorov, 2009). Detailed studies in this area have proved that, despite the diversity of PMS, such as Fabaceae-Rhizobium symbiosis, symbiosis between plants and cyanobacteria, mycorrhizal fungi, growth-promoting bacteria and endophytic fungi and bacteria, they have much in common both at the functional level and at level of molecular-genetic regulation of PMS development (Tikhonovich, Provorov, 2009).

## **5. Conclusion**

I. Selivanov, G. Muromtsev and I. Tikhonovich have formed the key Russian research groups to study the biodiversity of AM fungi, mycorrhizae formed by them, the influence of environmental factors on the development of effective AM and the molecular genetic, physiological and biochemical foundations of effective AM formation. In the framework of the I. Selivanov's school much attention was paid to the theory of consortia in the system of biotic relationships in biogeocenoses. AM has been defined by researchers as a key element in the consortive relations of terrestrial ecosystems. A large-scale approach to research allowed the authors to analyze the mycotrophism of a significant number of cultivated and wild plants in natural zones ranging from tundra to deserts in Russia and neighboring countries. Under the G. Muromtsev's guidance, applied studies of AM fungi were extended using the OPC method: recultivation techniques for sulfide-bearing soils using AM inoculums and alfalfa plants as phytomeliorants was developed; the first assessment of the influence of AM on the structure of the rhizosphere bacteria complex was performed; the studies of tree plant mycorrhizae were carried out, in which the possibility of a double mycorrhizal infection (with ecto- and endomycorrhizal fungi) was demonstrated, and the first detailed ultrastructural analysis of the development of the AM fungus and AM formed by it was conducted in Russia.

As the molecular genetic methods evolved, I. Tikhonovich founded a new scientific discipline at ARRIAM – symbiogenetics, a considerable part of which is the research of the mechanisms of AM initiating and development. Symbiosis started to be seen as a superorganism with its own manifestations of the laws of variation and heredity.

## **Acknowledgements**

The research of A. Yurkov and M. Shishova was sponsored by the grant of the Russian Foundation for Basic Research (RFBR) # 19-29-05275 MK, the research of A. Kryukov and A. Gorbunova – by the grant of RFBR # 20-016-00245 A.

## References

- Bulaeva L. Dependence of mycorrhiza formation on the biological nature of the higher symbiont. *Collection of scientific works of the Perm Branch of the All-Union Botanical Society*. 1965, vol. 2. 3642
- Buchholz F. *New data on the morphology and cytology of underground fungi*. Part 1. Genus *Endogone* Link. Riga, 1911.
- Golubinskaya N. Mycosymbiotrophism of corn at the early stages of growth, depending on the dose of mineral nitrogen, 2,4-Dichlorophenoxyacetic acid and the illuminance. *Mycorrhiza and other forms of consortive relations in nature*, 1980b, p. 89-95.
- Golubinskaya N. Some methods of regulation of endophytic mycorrhizal associations. *Mycorrhiza and other forms of consortive relations in nature*, 1980a, Perm: Perm State Pedagogical Institute, p. 48-54.
- Golubinskaya N. Growing vesicular-arbuscular fungi in laboratory. *Plant mycorrhiza*. Perm: Perm State Pedagogical Institute, 1979, p. 7-15.
- Golubinskaya N. The development of mycorrhizae and nodules in clover at different levels of phosphate. *Bulletin of the All-Union Scientific Research Institute of Agricultural Microbiology*. Ed. by O. Berestetskiy, 1986, no. 44, p. 14-17.
- Golubinskaya N., Selivanova I. On the double mycorrhizae in tree and shrub species of the Central Siberian Botanical Garden. *Plant mycorrhiza*. Perm: Perm State Pedagogical Institute, 1975, p. 48-50.
- Dobronravova M., Brykalov A., Zolnikova N. A study of the mycotrophism of some medicinal plants growing in the Stavropol Territory. *Bulletin of the Botanical Garden of Professor I. Kosenko*. Krasnodar, 1998, 48 pp.
- Zolnikova N., Muromtsev G. Effect of endomycorrhizal fungi on the yield of plants on recultivated soils. *Bulletin of the All-Union Scientific Research Institute of Agricultural Microbiology*. Ed. by O. Berestetskiy, 1986, no. 44, p. 3-7.
- Zolnikova N., Serebrennikova N. The development of microbiocenoses during the chemical recultivation of soils in the brown-coal basin of the Moscow Region. *Proceedings of the All-Russian Scientific Research Institute of Agricultural Microbiology*. V. 60, Leningrad, 1990, p. 73-86.
- Zolnikova N. *Development of ecological safety of farming methods: proceedings of the Russian-Finnish symposium of the Russian Academy of Agricultural Sciences. Branch on non-chernozem zone of the Russian Federation*. St. Petersburg, 1993, p. 125-137.
- Kamenskiy F. O On the symbiotic relation between the fungal mycelium and the roots of higher plants. *Proceedings of St. Petersburg Society of Naturalists*. 1886, 17: 34.
- Karatygin I. *Co-evolution of fungi and plants*. Saint Petersburg.: Gidrometeoizdat, 1993, 115 pp.
- Karatygin I.V. Co-evolution of fungi and plants: ecological and phylogenetic consequences. *Botanical Journal*. 1990, v. 75, no. 8, p. 1049-1060.
- Klyuchnikov A., Kozhevnikov P. The dynamics of populations of *Pseudomonas fluorescens* and *Azospirillum brasilense* during the formation of vesicular-arbuscular mycorrhizae. *Microbiology*. 1990, no. 4, p. 651-655.

Kostychev S. Plant physiology. Part 1. Chemical physiology. M.-L.: Association of State Book and Magazine Publishing Houses (OGIZ) – Selkhozgiz, 1933, 528 pp.

Krayeva E., Lebsky V. Ultrastructural and elemental analysis of sporoderm of endomycorrhizal fungi. *Byulleten VNIISKHM. Bulletin of All-Russian Research Institute for Agricultural Microbiology*. 1991, no. 54, p.59-61.

Krüger L. K. On the issue of studying mycorrhiza in the most important meadow grasses. *Proceedings of the Agricultural Institute named after academician D. Pryanishnikov*. 1957, issue 15, p. 167-174.

Krüger L., Perevedentseva L., Safiullina S. Perevedentseva L.G., Safiullina S.N. Experiments on the synthesis of mycorrhizae in wheat and pea. *Plant mycorrhiza*. Perm: Perm State Pedagogical Institute, 1973, p. 45-53.

Krüger L., Selivanov I. Fungi that form arbuscular-vesicular mycorrhizae and methods of their study. 1976. *The role of consortive relations in the organization of biogeocenoses*. 1976. *Scientific notes of Perm State Pedagogical Institute*. V. 150, p. 187-193.

Krüger L., Selivanov I. Endomycorrhizae in wild and cultivated Fabaceae. *Bulletin of the All-Union Scientific Research Institute of Agricultural Microbiology*. Ed. by O. Berestetskiy, 1986, no. 44, p. 36-38.

Kuprevich V. *Physiology of the infected plant in connection with general issues of parasitism*, M.-L. Academy of Sciences of the USSR Publ. 1947, 300 pp.

Lobanov N. *Plant mycorrhizae*. Moscow: Publishing house of agricultural literature, magazines and posters, 1963, 431 pp.

Maksimova T. Mycorrhizae of alpine plants of Kuznetsk Alatau of Khakassia. *Bulletin of the All-Union Scientific Research Institute of Agricultural Microbiology*. Ed. by O. Berestetskiy, 1986, no. 44, 54-57.

Mindiashvili Zh. Influence of phosphorus fertilizers on mycotrophism, growth and development of some cultivated plants. *Bulletin of the All-Union Scientific Research Institute of Agricultural Microbiology*. Ed. by O. Berestetskiy, 1986, no. 44, p. 17-24

Muromtsev G., Guseva E., Zolnikova H. Effect of endomycorrhizal fungi on productivity, nitrogen, phosphorus and potassium nutrition of vetches and oats in recultivated soils. *Agrochemistry*. 1988, no. 6, p. 64-75.

Muromtsev G., Marshunova G., Pavlova V., Zolnikova N. The role of soil microorganisms in the phosphorus nutrition of plants. *The achievements of microbiology*. 1985a, v. 20, p. 174-198.

Muromtsev G., Jacobi L., Marshunova G. Increase in the yield of oats and the amount of phosphorus in it under the influence of endomycorrhizal fungi. *Reports of the Academy of Agricultural Sciences*). 1985b, no. 3, p. 14-17.

Provovarov N., Shtark O. Directed evolution of fungi and plants in symbiotic systems. *Mycology and phytopathology*. 2014, v. 48, p. 151-157.

Provorov N., Vorobiev N. *Genetic foundations of the evolution of plant-microbial symbiosis*. Ed. by I. Tikhonovich, Saint Petersburg: Inform-Navigator Publ., 2012, 400 pp.

Samoshkin V., Postnikova O. Effect of endomycorrhizal vesicular-arbuscular fungi inoculation on the mineral nutrition of soy. *Bulletin of the All-Union Scientific Research Institute of Agricultural Microbiology*). Ed. by O. Berestetskiy, 1986, no.44, p. 7-10.

Sarkina I. Dependence of the inoculation potential of the soil on the ecological conditions and species of the higher plant. *Collection of scientific works of the Nikitsky Botanical Garden*. 1988, v. 104, p. 62-72.

Sarkina I. Mycorrhiza of the juniper is high in the Cape Martyan Reserve. *Bulletin of the All-Union Scientific Research Institute of Agricultural Microbiology*. Ed. by O. Berestetskiy, 1986, no.44, p. 46-49.

Selivanov I. Issues of terminology and classification of mycorrhizae and mycorrhizal associations. *Plant mycorrhizae. Scientific notes of Perm State Pedagogical Institute*. 1973, v. 112, p. 3-44.

Selivanov I. Materials on physiology and ecology of mycotrophic plant nutrition. The role of mycorrhizal fungi in soil nutrition of plants. *Uchenyye zapiski PGPI Problems of botany, ecology and physiology of plants. / Scientific notes of Perm State Pedagogical Institute*. 1975d, p. 141, p. 3-32.

Selivanov I. *Mycosymbiotrophism as a form of consortive relations in the vegetation cover of the Soviet Union*. Moscow.: Nauka Publ., 1981, 232 pp.

Selivanov I. Problems of studying consortive relations in natural and anthropogenic ecosystems. *Mycorrhiza and other forms of consortive relations in nature*). Perm: Perm State Pedagogical Institute, 1983, p. 3-12.

Selivanov I. The structure of phycomycetic tamniscophagous (vesicular-arbuscular endomycorrhizae). *Plant mycorrhizae. Scientific notes of Perm State Pedagogical Institute*. V. 142, 1975a, p. 60-68.

Selivanov I. Theoretical and practical problems of studying phycomycetic mycorrhizae. *The role of consortive relations in the organization of biogeocenoses. 1976b. Scientific notes of Perm State Pedagogical Institute*. V. 150, p. 201-213.

Selivanov I. Endogone fungi in culture and methods of their study. *Plant mycorrhizae. Scientific notes of Perm State Pedagogical Institute*. V. 142, 1975b, p. 96-110.

Selivanov I. Endogone fungi in culture and methods of their study. *Plant mycorrhizae. Scientific notes of Perm State Pedagogical Institute*. 1975c, p. 96-110.

Selivanov I., Vereshchagina V., Melnikova S., Salamatova N. Materials for the study of mycorrhizae in some grasses of the steppe belt. *Collection of scientific papers*. Perm, Perm State Pedagogical Institute, 1961, v. 18, no. 3, p. 3-10.

Selivanov I., Krüger L. On the need to clarify the methodology for quantitative assessment of plant mycosymbiotrophism. *Bulletin of the All-Union Scientific Research Institute of Agricultural Microbiology*. Ed. by O. Berestetskiy, 1986, no. 44, p. 30-33.

Selivanov I., Utemova L. Materials for the characteristics of mycorrhizal grasses. *Collection of scientific papers*. Perm, Perm State Pedagogical Institute, 1968, v. 64, p. 302-310.

Tikhonovich I., Provorov N. *Genetics of symbiotic nitrogen fixation and the basics of selection*. Saint Petersburg.: Nauka Publ., 1998, 194 pp.

Tikhonovich I., Provorov N. Symbiogenetics of microbial-plant relations. *Ecological genetics*. 2003, V.1, no. 0, p. 36-46.

Tikhonovich I., Provorov N. *Symbiosis between plants and microorganisms: molecular genetics of agroecosystems of the future*. Saint Petersburg: Publishing House of St. Petersburg University, 2009, 210 p.

Utemova L. The dynamics of mycorrhizal associations of wild grasses at different stages of their individual development and seasonal functioning. *Bulletin of the All-Union Scientific Research Institute of Agricultural Microbiology*. Ed. by O. Berestetskiy, 1986, no. 44, p. 49-54.

Khrushcheva E. On some morphological properties of corn mycorrhiza. *Agrobiology*, 1961, no. 4, p. 595-598.

Khrushcheva E., Aleksandrova E., Sogina I., Vorobyova T., Talatina G. The development of mycorrhizae and root rot in grasses having different amounts of available elements of mineral nutrition. *Bulletin of the All-Union Scientific Research Institute of Agricultural Microbiology*. Ed. by O. Berestetskiy, 1986, no. 44, p. 27-29.

Khrushcheva E., Aleksandrova E., Sogina I., Talatina G. The role of endomycorrhiza in the formation of yield of some grasses. *Mycorrhiza and other forms of consortive relations in nature*. 1980, p. 55-58.

Khrushcheva E., Sogina I. On the carbohydrate metabolism of mycotrophic corn plants. *Proceedings of the State Agricultural Institute*, 1968, V. 26, p 97-102.

Khrushcheva E. The effect of soil conditions on the formation of mycorrhizae in agricultural plants. *Collection of scientific papers. Gorky, State Agricultural Institute*, 1955, v.7, issue. 1, p. 67-76.

Shirinkina L. The intensity of mycorrhizal infection in healthy wheat heads and those infected with loose smut. The role of consortive relations in the organization of biogeocenoses. *Scientific notes of Perm State Pedagogical Institute*. 1975, v. 150, p. 196-200.

Shirinkina L. The intensity of mycorrhiza formation in spring wheat based on some bioecological factors. *Bulletin of the All-Union Scientific Research Institute of Agricultural Microbiology*. Ed. by O. Berestetskiy, 1986, no. 44, p. 33-35.

Shirinkina L. On the dynamics of mycorrhiza formation in spring wheat. *Mycorrhiza and other forms of consortive relations in nature*, Perm: Perm State Pedagogical Institute, 1985. p. 54-58.

Shirinkina L. *Wheat mycorrhiza in different agricultural environments*. Mycorrhiza and other forms of consortive relations in nature. Republican collection of scientific works. Perm: Perm State Pedagogical Institute, 1978, p. 42-51.

Shirinkina L. *Mycorrhiza in weed plants in wheat plantings*. Mycorrhiza of plants. Perm: Perm State Pedagogical Institute, 1979, p. 44-52.

Shkaraba E. Population approach to the study of mycorrhizae. *Bulletin of the All-Union Scientific Research Institute of Agricultural Microbiology*. Ed. by O. Berestetskiy, 1986, no. 44, p. 41-43.

Shubin V. Prospects of the use of plant mycosymbiotrophism in taiga forestry. *Bulletin of the All-Union Scientific Research Institute of Agricultural Microbiology*. Ed. by O. Berestetskiy, 1986, no. 44, p. 39-41.

Jacobi L. Endomycorrhizal fungi: mycorrhiza formation and influence on the productivity of oats depending on the conditions of mineral nutrition. *Bulletin of All-Russian Research Institute for Agricultural Microbiology*, 1987, no. 47, p. 11-17.

Jacobi L., Muromtsev G., Patyka V., Marshunova G., Bayrakov V., Andreeva V. USSR author's certificate No. SU 1828088 A1 for the invention Strain of endomycorrhizal fungus *Glomus mosseae* for the production of a fertilizer for agricultural plants. Application No. 4922392, priority from 22.01.1991, publ. 10/13/1992. Applicant: All-Russian Research Institute for Agricultural Microbiology. Uzhgorod: Patent Production and Publishing Complex, 1992, 5.

- Bianciotto V., Lumini E., Bonfante P., Vandamme P. Candidatus glomeribacter gigasporarum gen. nov., sp. nov., an endosymbiont of arbuscular mycorrhizal fungi. *Int. J. Syst. Evol. Microbiol.* 2003. V. 53(1): P. 121-124. doi: 10.1099/ijs.0.02382-0.
- Borisov A.Y., Danilova T.N., Koroleva T.A., Naumkina T.S., Pavlova Z.B., Pinaev A.G., Shtark O.Y., Tsyganov V.E., Voroshilova V.A., Zhernakov A.I., Zhukov V.A., Tikhonovich I.A. Pea (*Pisum sativum* L.) regulatory genes controlling development of nitrogen-fixing nodule and arbuscular mycorrhiza: fundamentals and application. *Biologia.* 2004. V. 59, N 13. P. 137-144.
- Gilmore A.E. Phycomycetous mycorrhizal organisms collected by open pot culture methods. *Hilgardia.* 1968. 39 (4): P. 87-105.
- Jacobi L.M., Petrova O.S., Tsyganov V.E., Borisov A.Y., Tikhonovich I.A. Effect of mutations in the pea genes *Sym33* and *Sym40*. I. Arbuscular mycorrhiza formation and function. *Mycorrhiza.* 2003. V. 13. P. 3-7.
- Karatygin I.V., Snigirevskaya N.S., Demchenko K.N. Species of the Glomites as plant mycobionts in Early Devonian ecosystems. *Paleontol. J.*, 2006. V. 40, N5. P. 572-579.
- Koide R.T., Mosse B. A history of research on arbuscular mycorrhiza. *Mycorrhiza.* 2004. V. 14. P. 145-163.
- Kozlova N.V., Strunnikova O.K., Labutova N.M., Muromtsev G.S. Production and analysis of specificity of polyclonal antibodies against soluble proteins from the arbuscular mycorrhizal fungus *Glomus intraradices*. *Mycorrhiza.* N10. 2001. P. 301-305.
- Malloch D.W., Pirozynski K.A., Raven P.H. Ecological and evolutionary significance of mycorrhizal symbiosis in vascular plants. *Proc. Nat. Acad. Sci. USA.* 1980. V. 77. P. 2113-2118.
- Minerdi D., Fani R., Gallo R., Boarino A., Bonfante P. Nitrogen fixation genes in an endosymbiotic *Burkholderia* strain. *Appl Environ Microbiol.* 2001. V. 67(2): P. 725-732. doi: 10.1128/AEM.67.2.725-732.2001.
- Mosse B. Experimental techniques for obtaining a pure inoculum of an *Endogone* sp., and some observations on the vesicular-arbuscular infections caused by it and other fungi. // *Rec. Adv. Bot.*, V. 2. 1961. P. 1728-1732.
- Nicolson T.H. Vesicular-arbuscular mycorrhiza – a universal plant symbiosis. // *Science Progress.* 1952. V. 55. P. 561-581.
- Öpik M., Davison J., Moora M., Zobel M. DNA-based detection and identification of Glomeromycota: the virtual taxonomy of environmental sequences. // *Botany.* 2014; 92(2):135-147. doi: 10.1139/cjb-2013-0110.
- Pirozynski K.A., Malloch D.W. The origin of land plants: a matter of mycotrophism. // *BioSystems.* 1975. V.6. P. 153-164.
- Provorov N.A., Borisov A.Y., Tikhonovich I.A. Developmental genetics and evolution of symbiotic structures in nitrogen-fixing nodules and arbuscular mycorrhiza // *Journal of Theoretical Biology.* 2002. V. 214: 215-232.
- Provorov N.A., Shtark O.Y., Zhukov V.A., Borisov A.Y., Tikhonovich I.A. *Developmental genetics of plant-microbe symbioses.* NOVA Science Publishers Inc., New York, 2010, 151.
- Schenck N.C., Pérez Y. *Manual for the identification of VA mycorrhizal fungi.* 2nd Edition. Gainesville: INVAM, University of Florida. 1988. 241 p.
- Smith S., Read D. *Mycorrhizal Symbiosis.* 3rd Ed. NY: Academic Press. 2008. 800 p.

Spatafora J.W., Chang Y., Benny G.L., Lazarus K., Smith M.E., Berbee M.L., Bonito G., Corradi N., Grigoriev I., Gryganskyi A., James T.Y., O'Donnell K., Roberson R.W., Taylor T.N., Uehling J., Vilgalys R., White M.M., Stajich J.E. A phylum-level phylogenetic classification of zygomycete fungi based on genome-scale data. *Mycologia*. 2016. V. 108(5): P. 1028–1046. doi: 10.3852/16-042.

Stockinger H., Peyret-Guzzon M., Koegel S., Bouffaud M.-L., Redecker D. The largest subunit of RNA polymerase II as a new marker gene to study assemblages of arbuscular mycorrhizal fungi in the field. *PLoS One*. 2014;9(10):e107783. doi: 10.1371/journal.pone.0107783.

Tsyganov V.E., Borisov A.Y., Rozov S.M., Tikhonovich I.A. New symbiotic mutants of pea obtained after mutagenesis of laboratory line SGE. *Pisum Genet.* 1994. V. 26. P. 36–37.

Yurkov A.P., Jacobi L.M., Gapeeva N.E., Stepanova G.V., Shishova M.F. Development of arbuscular mycorrhiza in highly responsive and mycotrophic host plant – black medick (*Medicago lupulina* L.). *Russian Journal of Developmental Biology*. 2015. V.46, N5. p. 263-275.