

## BIOGENIC AND ANTHROPOGENIC MAGNETIC NANOPARTICLES IN THE PHLOEM SIEVE TUBES OF PLANTS

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### ABSTRACT

The samples of leaves and roots of *Nicotiana tabacum*, the stems and tubers of *Solanum tuberosum* and the stems of pea *Pisum sativum* were examined by scanning probe microscopy in atomic force and magnetic force modes after cultivation without and with addition of magnetite nanoparticles in soil. Chains of both biogenic and anthropogenic magnetic nanoparticles are detected in the wall of the phloem sieve tubes i.e., the vascular tissue of plants. Such a localization of biogenic magnetic nanoparticles supports the idea that the chains of magnetic nanoparticles in different organs of plants have common metabolic functions. Stray gradient magnetic fields about several thousand Oe, which are created by chains of both biogenic and anthropogenic magnetic nanoparticles, can significantly affect the metabolic processes through the influence on mass transfer of vesicles, granules, organelles and other components. In view of the results of this investigation, both BMNs and anthropogenic magnetite nanoparticles chains can significantly affect the processes of mass transfer of vesicles, organelles, structural elements of the membrane and others components because they create stray magnetic fields and gradient magnetic forces.

**Keywords:** Biogenic magnetic nanoparticles; magnetotaxis; magnetoreception

### INTRODUCTION

More than five decades ago, strong natural nanoscale magnets (biogenic magnetic nanoparticles (BMNs)) were revealed in teeth of mollusks (Lowenstam, 1962). Later BMNs were detected in magnetotactic bacteria in the form of chains of intracellular particles (Blakemore, 1975), the synthesis of which is genetically programmed and carried out by microorganisms by themselves. To date, BMNs has been experimentally detected in algae (Torres de Araujo et al., 1986) and protozoa (Monteil et al., 2018), plants (Chaffee et al., 2015), worms (Cranfield et al., 2004), snails (Suzuki et al., 2006), ant and butterflies (Gould et al., 2006), honey bees (Hsu et al., 1978), termites (Maher, 1998), lobsters (Lohmann, 1984), tritons (Brassart et al., 1999), migratory and non-migratory fish (Kirschvink, 1989; Gorobets et al., 2018c), turtles (Walcott et al., 1979), birds (Falkenberg et al., 2010), bats (Holland et al., 2008), dolphins and whales (Zoeger et al., 1981), pigs (Gorobets et al., 2017b) and humans (Grassi-Schultheiss et al., 1997; Gorobets et al., 2018b). In humans, BMNs is detected both in norm and in pathologies, for example, BMNs are found in neurodegenerative diseases (Dobson, 2001), oncological diseases (Brem, 2006), heart aneurysms (Darmenko et al., 2018), atherosclerosis (Alexeeva et al., 2018). The methods of comparative genomics have shown that the genetic apparatus of the BMNs biosynthesis is unique in the representatives of all kingdoms of living organisms and is based on genes that originate from a common ancestor before the appearance of multicellular organisms (Gorobets et al., 2014; Gorobets et al., 2017a). Besides BMNs, the anthropogenic magnetic nanoparticles accumulate in organs and tissues of different organisms due to environmental pollution or to use of artificial magnetic nanoparticles in many branches from medicine to agriculture.

There are a complicated problems of distinguishing biogenic from anthropogenic magnetic nanoparticles (Gieré, 2016) and revealing the mechanisms of influence of both biogenic and anthropogenic magnetic nanoparticles on metabolism. The investigations in this field of research have received especially rapid development in agriculture. Magnetic nanoparticles play a key role in the growth and improvement of plant yields, from seed germination to the accumulation of reserve nutrients, and all metabolic processes of plants are stimulated by the addition of magnetic nanoparticles (Zia-ur-Rehman et al., 2018; Claudio et al., 2017). For example, under the influence of magnetite nanoparticles, the accumulation of chlorophyll and vegetative growth of chrysanthemums increases (Banijamali et al., 2019). The mechanisms of influence of magnetic nanoparticles on plant growth are not fully understood. It is important for understanding of this mechanism that the gradients of magnetostatic stray fields of the BMNs have a sufficient value of dynamic factor  $\frac{\sqrt{H}^2}{2} \approx (10^{10} \div 10^{12}) Oe^2/cm$  to influence the transport of

vesicles, granules and other components (Gorobets et al., 2014; Gorobets & Gorobets, 2012; Gorobets et al., 2013a; Gorobets, 2015; Gorobets & Gorobets, 2000). The influence of gradient magnetic forces on the displacement of intracellular amyloplast was revealed experimentally even under the influence of an external gradient magnetic field with a significantly lower dynamic factor  $\frac{\sqrt{H}^2}{2} \approx (10^9 \div 10^{10}) Oe^2/cm$ , and, as a consequence of the distortion of the seedlings of the barley *Hordeum vulgare* was observed in the direction of the gradient of the magnetic field (Kuznetsov & Hasenstein, 1997).

In this regard, the purpose of this work is to reveal experimentally plant tissues where both biogenic and anthropogenic magnetic nanoparticles are localized and to reveal structural organization of magnetic nanoparticles in plants.

### MATERIAL AND METHODS

The presence of BMNs was performed by means of scanning probe microscope Solver PRO-M. During the measurement the "two pass" technique was used which consists of the microscopy of two types: atomic-force microscopy (AFM) and magnetic-force microscopy (MFM). The semicontact and magnetic scan regimes were used. The AFM and MFM images were processed by program Grain Analysis v2.2 (NT-MDT). The method for obtaining magnetite nanoparticles and their microanalysis is described in details in a book (Gorobets, 2018a). The average size of the magnetic nanoparticles is about 10 nm.

The study of BMNs in plants was carried out on the example of tobacco *Nicotiana tabacum*, as the most studied model organism among plants, as well as pea *Pisum sativum* and potato *Solanum tuberosum*.

Tobacco grown in accordance with the methodology (Murashige & Skoog, 1962) on the nutrient medium Murasige-Scuga which has not contained magnetite nanoparticles. Such a choice of nutrient medium is due to the fact that it does not contain magnetite nanoparticles, in contrast to the vast majority of soils, which usually contain a concentration of magnetite nanoparticles of about 0.1% by weight of soil (Maher & Taylor, 1988).

The features of the growth of pea *Pisum sativum*, grown on soils without the addition of magnetite and on soils with the addition of magnetite nanoparticles in a magnetic fluid with an average size of nanoparticles of 11 nm, a minimum particle size of 2 nm and a maximum size of 23 nm. The magnetic fluid is obtained by the method (Gorobets & Gorbyk, 2018). Magnetite nanoparticles were used at concentrations of 1 mg/ml and 0.1 mg/ml (concentration close to the content of magnetite in the soil) (Lalonde et al., 2004). Magnetite nanoparticles were added to the soil daily, for 3 weeks in a quantity of 1 ml for each plant.

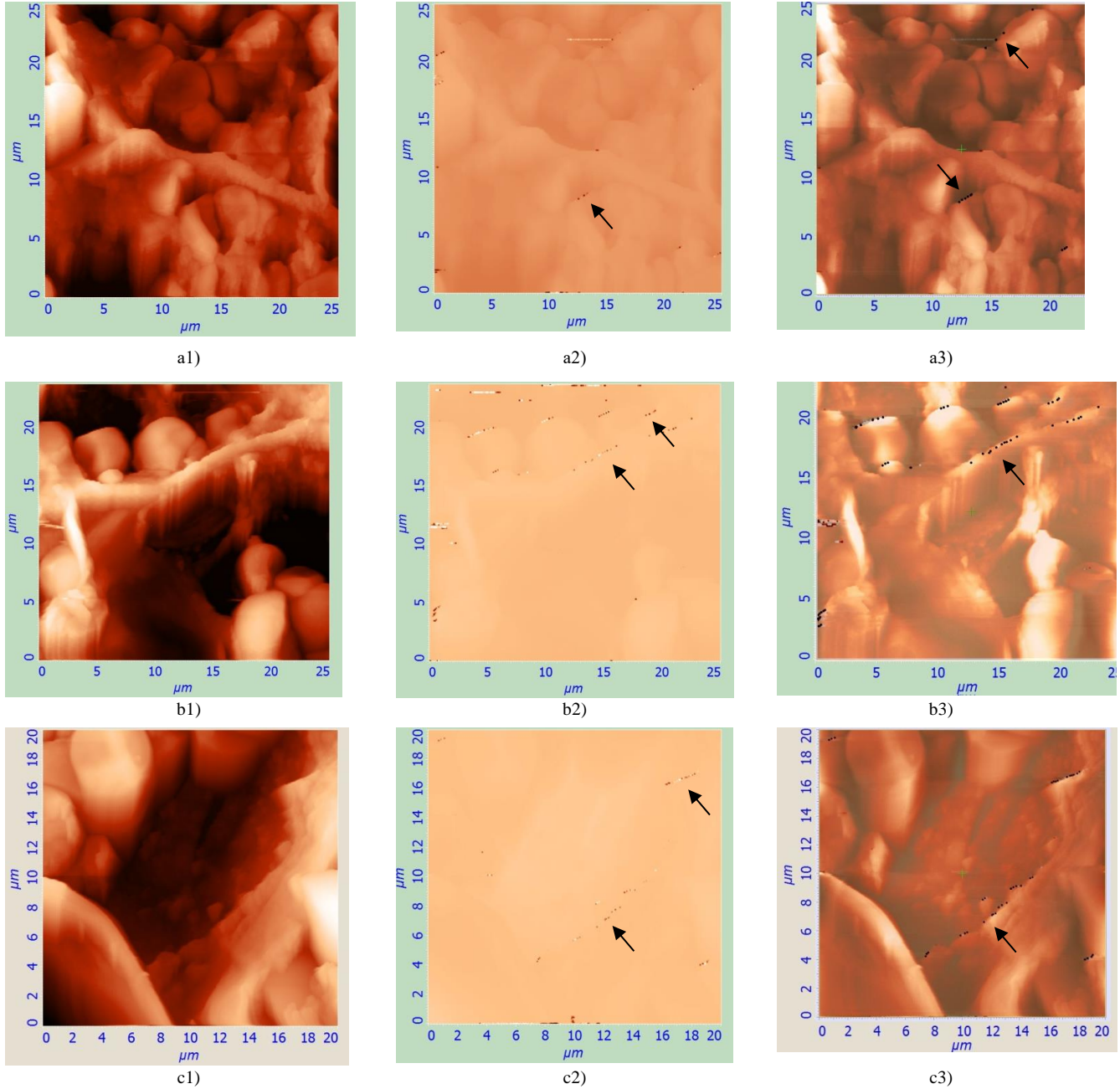
**RESULTS AND DISCUSSION**

Methods of atomic force microscopy and magnetic force microscopy were used for experimental study of plant tissues for the presence of BMNs. The study of BMNs in plants was carried out on the example of tobacco *Nicotiana tabacum*, as the most studied model organism among plants, as well as pea *Pisum sativum* and potato *Solanum tuberosum*.

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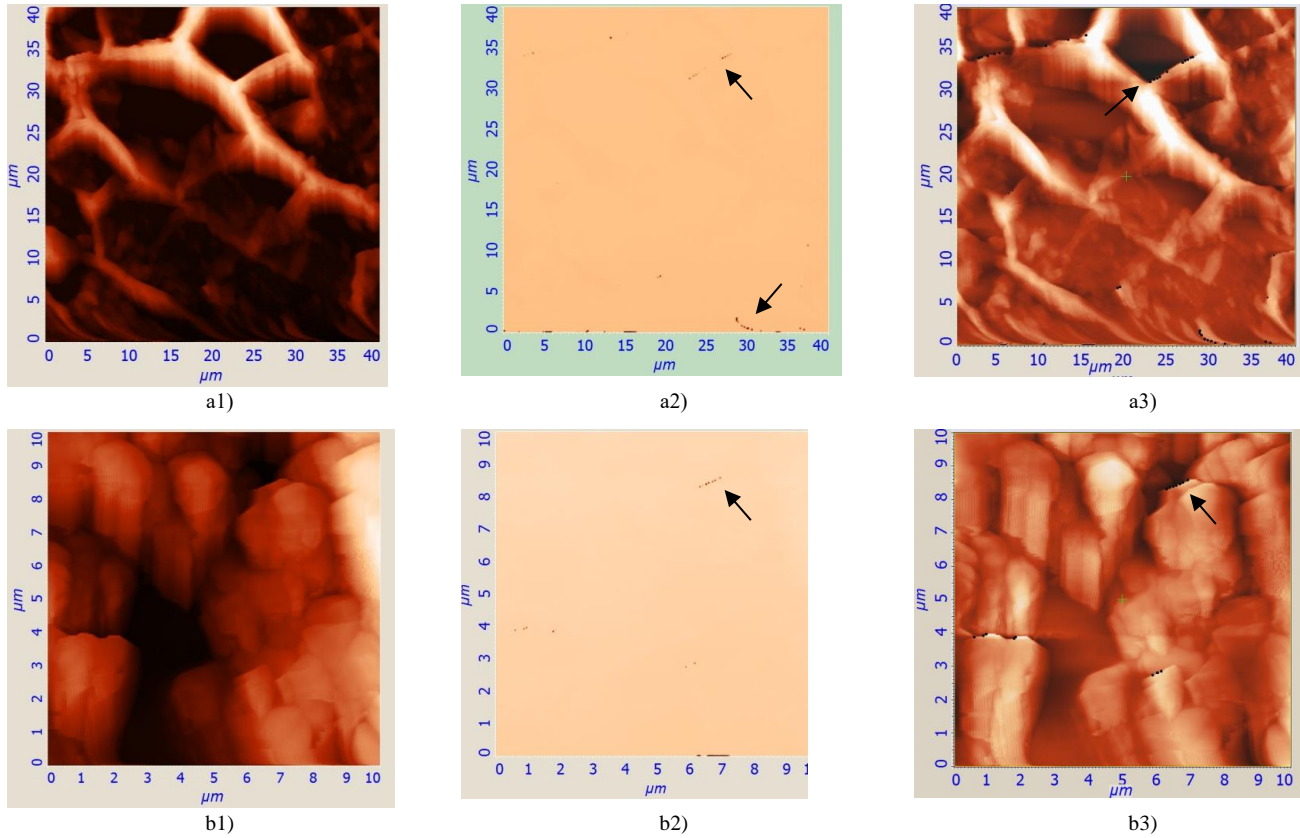
A leaf, a leaf vein and a root were investigated in the tobacco to check the presence of BMNs. BMNs are located on the membrane of sieve tubes of phloem in a leaf, a leaf vein and a roots of tobacco. Results are shown in the **Figure 1**. The phloem is a vascular tissue of plants that forms a network of sieve tubes through which the transport of organic substances synthesized by leaves during photosynthesis is provided to all organs of the plant (Lalonde et al., 2004), in contrast to the vascular tissue of plants – xylem, which provides transport of water and mineral substances from the soil (Chaffey, 2008). The sieve tubes of the tobacco leaf, that are shown in **Figure 1**, have typical morphology and dimensions as described in (Ding, 1988).



**Figure 1** Scanning probe microscopy of tobacco *Nicotiana tabacum*: a1) – AFM image of tobacco leaf, a2) – MFM image of tobacco leaf (BMNs are shown with arrows), a3) – combined AFM and MFM images of tobacco leaf (arrow starches indicate pores of sieve tubes); b1) – AFM image of a vein of tobacco leaf, b2) – MFM image of a vein of tobacco leaf (BMNs are shown with arrows), b3) – combined AFM and MFM images of a vein of tobacco leaf (arrow starches indicate membranes of sieve tubes); c1) – AFM image of a root of tobacco, c2) – MFM image of a root of tobacco (BMNs are shown with arrows), c3) – combined AFM and MFM images of a root of tobacco (arrow starches indicate phloem)

A similar localization of BMNs is observed in samples of potato. Results are shown in the **Figure 2**. It can be seen from **Figure 2** that BMNs in the stem and potato tubers are associated with short chains located on the boundary of the vascular

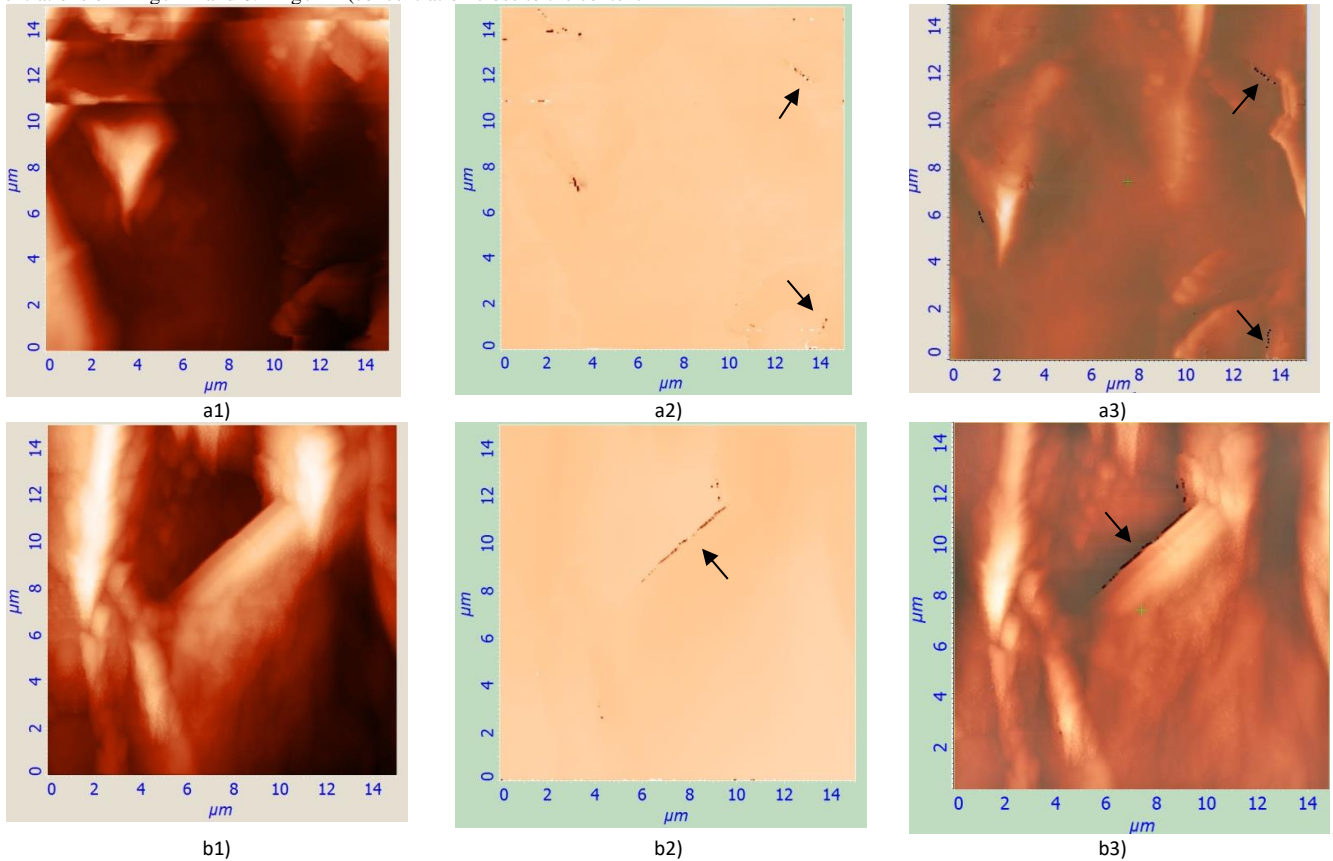
tissue in the potato stem and along the boundaries of starch grains and sieve tubes in potato tubers.

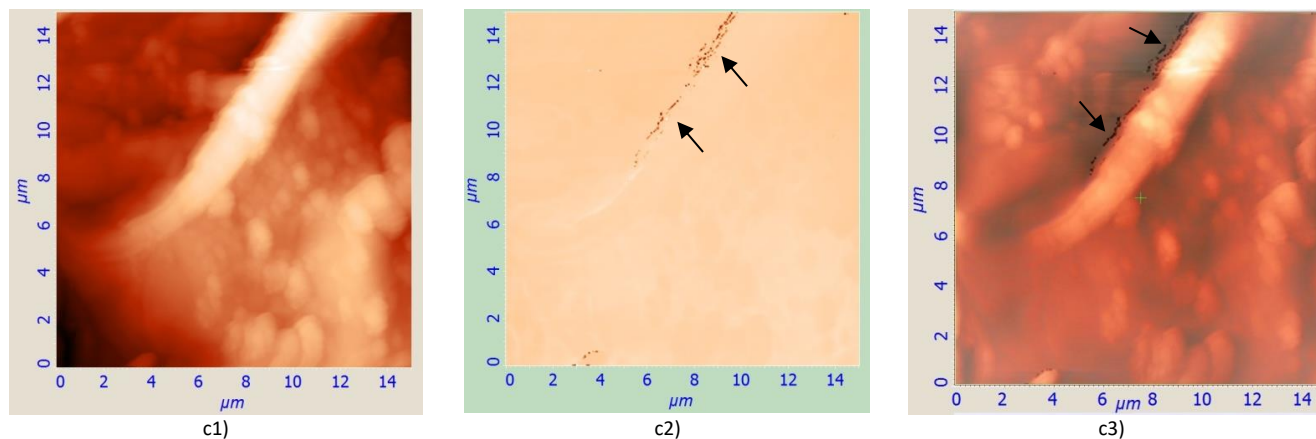


**Figure 2** Scanning probe microscopy of potato *Solanum tuberosum*: a1) – AFM images of a stem of potato; a2) – MFM images of potato stem (BMNs are shown with arrows), a3) – combined AFM and MFM images of potato stem (arrows indicate sieve tubes); b1) – AFM image of a tubers of potato, b2) – MFM image of tubers of potato (BMNs are shown with arrows), b3) – combined AFM and MFM images of potato tubers (black arrows indicate starch grains and orange arrows indicate sieve tubes)

The features of the growth of pea *Pisum sativum*, grown on soils without the addition of magnetite and on soils with the addition of magnetite nanoparticles in a magnetic fluid with an average size of nanoparticles of 11 nm, a minimum particle size of 2 nm and a maximum size of 23 nm. The magnetic fluid is obtained by the method (Gorobets & Gorbyk, 2018). Magnetite nanoparticles were used at concentrations of 1 mg / ml and 0.1 mg / ml (concentration close to the content

of magnetite in the soil (Maher & Taylor, 1988). Magnetite nanoparticles were added to the soil daily, for 3 weeks in a quantity of 1 ml for each plant. BMNs in pea *Pisum sativum* (Fig. 3) are located on the membrane of phloem sieve tubes, as well as in the samples of tobacco *Nicotiana tabacum* (Fig. 1) and potato *Solanum tuberosum* (Figure 2).





**Figure 3** Scanning probe microscopy of the stem of pea *Pisum sativum*: a1) – AFM images of a stem of pea grown on the soil without the addition of magnetite; a2) – MFM images of a stem of pea grown on the soil without the addition of magnetite (BMNs are shown with arrows), a3) – combined AFM and MFM images of pea stem grown on the soil without the addition of magnetite (arrows indicate sieve tubes); b1) – AFM image of a stem of pea grown on the soil with the addition of magnetite (concentration of 0.1 mg/ml), b2) – MFM image of a stem of pea grown on the soil with the addition of magnetite (concentration of 0.1 mg/ml) (BMNs are shown with arrows), b3) – combined AFM and MFM images of a stem of pea grown on the soil with the addition of magnetite (concentration of 0.1 mg/ml) (black arrows indicate starch grains and orange arrows indicate sieve tubes); c1) – AFM image of a stem of pea grown on the soil with the addition of magnetite (concentration of 1 mg/ml), c2) – MFM image of a stem of pea grown on the soil with the addition of magnetite (concentration of 1 mg/ml) (BMNs are shown with arrows), c3) – combined AFM and MFM images of a stem of pea grown on the soil with the addition of magnetite (concentration of 1 mg/ml) (black arrows indicate starch grains and orange arrows indicate sieve tubes)

The location of BMNs in plants in the form of chains at the boundary of the vascular tissue is similar to the location of BMNs, in the samples of fungi on the cell walls of hyphae (Gorobets et al., 2021; Gorobets et al., 2020), in the tissues and organs of animals (including humans) on the walls of the capillaries (Darmenko et al., 2018).

Based on the results of AFM and MFM, the maximum size of the BMNs or their clumps (as the average distance between adjacent black or white areas in Figure 1

and Figure 2) and the amount of BMNs were estimated in the chain of examined tissues of tobacco and potato (Table 1). Comparing the results of AFM and MFM, the maximum size of BMNs and the amount of BMNs in the chain of investigated organisms were estimated for comparison with the relevant data for other organisms (Table 1).

**Table 1** Sizes of BMNs in tobacco and potato and comparison with literature data for other organisms

Organism	Estimation of the maximum size of BMNs, nm	Number of particles in chains
Tobacco leaf	110-220	7±3
Tobacco root	80-185	8±2
Potato stem <i>Solanum tuberosum</i>	60-120	6±2
Potato tuber <i>Solanum tuberosum</i>	35-60	5±3
Mushroom <i>Agaricus bisporus</i>	70±15 (Gorobets et al., 2021)	4±3 (Gorobets et al., 2021)
Magnetotaxis bacteria	10-40, 35-120	4-200
<i>Magnetospirillum gryphiswaldense</i> MSR-1	(Richter, 2007)	(Richter, 2007)

Based on experimental data and theoretical calculations, it can be supposed that gradient magnetic forces in the vicinity of the BMNs are sufficient for the accumulation of vesicles, granules, liposomes (Mikeshyna, 2018; Gorobets et al., 2014; Gorobets et al., 2013b), amiloplasts (Kuznetsov & Hasenstein, 1997). The energy of the magnetodipole interaction of the BMNs with the vesicles in the cell is sufficient to hold the vesicles in the vicinity of the BMNs chain for BMNs sizes from 20 nm to 150 nm and the size of vesicles greater than 100 nm, that is, near

the membrane. Since the size of vesicles and BMNs in plants are in this range, it can be shown that the BMNs perform the same function in the plant organism as in human and animals, namely the function of concentrators of vesicles and granules including influence on vesicular transport. The maximum size of the BMNs and the amount of BMNs in the chain in the stem of the experimentally grown pea *Pisum sativum* were estimated.

**Table 2** The maximum size of the BMNs and the amount of BMNs in the chain

Conditions for growing pea <i>Pisum sativum</i>	Estimation of the maximum size of the BMNs, nm	Number of particles in chains, pcs	Number of chains of artificial magnetite nanoparticles parallel to chains of BMNs, pcs
Soil without addition of magnetite nanoparticles	103±1	5±2	–
Soil with addition of magnetite nanoparticles, 0,1 mg/ml	97±2	10±3	–
Soil with addition of magnetite nanoparticles 0,1 mg/ml	97±3	10±4	1-2

It is evident from Figure 3 and Table 1 that when the concentration of magnetite in the soil increases, the amount of BMNs in the chain increases, which proves that an artificial magnetite can be embedded in a chain of biogenic magnetic nanoparticles. We observe not only chains of biogenic magnetite, located on walls of sieve tubes and also parallel chains of artificial nanoparticles at concentration of magnetite 1 mg/ml. The formation of chains of artificial magnetite nanoparticles parallel to the BMNs chain leads to a change in the spatial distribution of magnetostatic fields in the vicinity of natural BMNs (Gorobets et al., 2020). With significant accumulation of artificial nanoparticles of magnetite, they can serve as a magnetic circuit and close the magnetic fields of natural BMNs, which is

correlated with the results of inhibition of plant growth (Table 3 and Figure 4). Besides the inhibition of plant growth at high concentrations of magnetite nanoparticles (for example, 1 mg/ml) can be explained also by blockage of sieve tubes due to agglomeration of magnetite nanoparticles similar to the results of the paper (Gorobets et al., 2021) demonstrating effects of mushroom cultivation with addition of magnetite nanoparticles.



**Figure 4** Morphology of roots of pea *Pisum sativum* (from left to right): the plant grown on the soil without the addition of magnetite (control), the plant grown on the soil with the addition of magnetite (concentration of 0.1 mg/ml), the plant grown on the soil with the addition of concentrated magnetite (concentration of 1 mg/ml)

**Table 3** Influence of different concentrations of magnetite on the morphology of pea *Pisum sativum*

Average values	Control	0.1 mg/ml	1 mg/ml
Length of stems	25,2±1,2	33,8±0,8 (*34%)	26,8±0,9 (*6%)
Length of roots	9,4±0,2	8,6±0,3 (*-9%)	13,1±1,1 (*39%)
Length of plants	35±2	42,3±1,6 (*22%)	39,8±1,9 (*15%)
Length of leaves	1,3±0,3	1,9±0,4 (*46%)	1,1±0,3(*-16%)
number of lateral roots	8±1	31±2 (*287%)	4±2 (*-50%)
number of leaves	24±2	29±1 (*20%)	29±2 (*20%)
mass of plants	8,64	11,54	9,68
mass of roots	1,75	2,3	1,7

\* Increase in length, weight of plants and the number of roots and leaves in relation to control

Morphological differences of plants of pea *Pisum sativum*, grown on the soil with the addition of artificial nanoparticles of magnetite at a concentration of 0.1 mg/ml are similar to morphological changes occurring in plants with an increase in the intensity of the synthesis of phytohormones auxin, which affects the formation and growth of roots (Tanimoto, 2005). Results are shown in the Figure 2 and Table 3. Namely, there is a shortening of the main root and the development of lateral roots in both cases. It is known that phytohormone auxin is transported by a plant with vesicles measuring 180-220 nm (Niemietz & Tyerman, 2000).

Thus, it is likely that with considerable accumulation of artificial nanoparticles of magnetite and closure of magnetic fields, gradient magnetic forces in the vicinity of the BMNs, acting on vesicles, granules and liposomes, will be significantly reduced, which affects the growth of plants. Results are shown in the Figure 4 and Table 3).

## CONCLUSION

Experimental studies of BMNs and anthropogenic magnetic nanoparticles in plant samples, carried out in this work by AFM and MFM methods, showed that both BMNs and anthropogenic magnetic nanoparticles in plants form chains and are located in the wall of the vascular tissue, namely, in the wall of the sieve tubes of the phloem. As the vascular tissue of phloem of plants serves for the transfer of organic substances, hormones, etc. (Lalonde et al., 2004), the same localization of BMN chains in different organs of higher plants cannot be occasional, taking into account that the genetically engineered biosynthesis mechanism of BMNs appeared at the beginning of evolution (Glansdorff & Xu, 2008). Besides, the analogous localization of both BMNs and anthropogenic magnetic nanoparticles in vascular tissues of plant and mushrooms is in accordance of the experimental results about similar influence of magnetic nanoparticles on metabolism of plants and mushrooms, i.e. inducing acceleration of their growth at moderate concentrations of magnetite nanoparticles and inhibition of their growth at high concentrations (Gorobets et al., 2020; Gorobets et al., 2021). In view of the results of this investigation, both BMNs and anthropogenic magnetite nanoparticles chains can significantly affect the processes of mass transfer of vesicles (Mikeshyna et al., 2018; Gorobets et al., 2014), organelles, structural elements of the membrane and others components because they create stray magnetic fields and gradient magnetic forces.

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