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PROTECTIVE ACTION MECHANISM OF *ECOSTIM* NANOCOMPOSITE FOR PRESOWING SEED TREATMENT

The state of water in KCl hydrated powder, in KCl/AM1/H_2O composite system and with hydrophobic organic compounds added has been studied in the air environment by low-temperature NMR spectroscopy. The presence of hydrophobic nanoparticles in composite system consisting of KCl hydrated salt powder and AM1-300 hydrophobic nanosilica has been established to significantly increase water binding to the surface. As a result, water retention in the composite is significantly higher than in the powder fertilizers. An additional increase in interfacial water binding has been reported for the case when KCl/AM1/H_2O composite system contacts with hydrophobic substance imitating hydrophobic areas of seed surface. It is likely explained by an increase in free energy as a result of water cluster split in nanoscale systems with hydrophobic and hydrophilic components.

Keywords: NMR spectroscopy, strongly- and weakly bound water, water clusters, composite system, mineral fertilizers, and seeds.

Mineral fertilizers are widely used in all areas of crop farming. They are introduced to the soil either simultaneously with the seeds or as plant nutrition in the form of mineral solutions or dry powders. A substantial portion of the nutrients do not fall within the zone of absorption of plant roots and the soil is washed away by rain which reduces the fertilizer efficiency. A set of methods for seed pelleting and creating on their surface a thin layer (1—5% of seed weight) of a mixture of fertilizer and hydrophobic silica [3—5] has been developed [1, 2]. This significantly increases the uptake of chemical substances by plants. As of today, *Ecostim* nanocomposite (TI 03291669-006-2013) that stimulates a 20% increase in the yield of many crops has been developed and tested at many farms.

Pictures of corn seed samples sprouted without and in the presence of *Ecostim* are showed in Fig. 1.

The formation of aqueous poly-associates at the interface of nanocomposite components and root growing zone has been suggested to be one of the main factors determining the high efficiency of *Ecostim*. These poly-associates have a structure of hydrogen bond grid, which is very different from that existing in liquid water [3, 5]. As a result, soil moisture can be bound at the surface of the seeds, with the solubility of minerals and organic matter in interfacial water significantly differing from the bulk one, which ensures their optimal uptake by plants at the early stages of growth.

This research is aimed at determining the capacity of *Ecostim* type nanocomposite system to retain moisture on the seed surface and at studying the moisture-retaining mechanisms in the presence of mineral fertilizers and hydrophobic nanosilica. The basic research method is low-temperature ^{1}H NMR spectroscopy [6–9] which allows the researchers to determine the amount of strongly and weakly bound water based on changing NMR signal intensity during the thawing of samples and to

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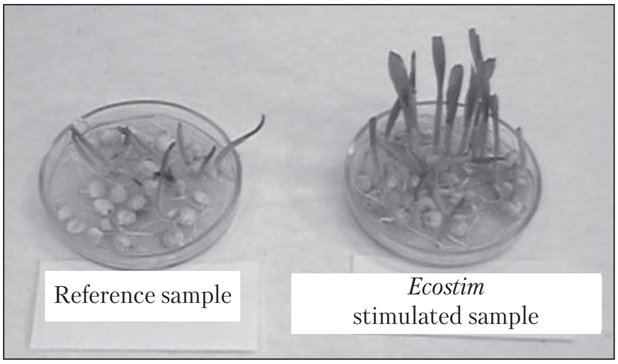


Fig. 1. Effect of *Ecostim* on the state of five-day corn seed sprouts

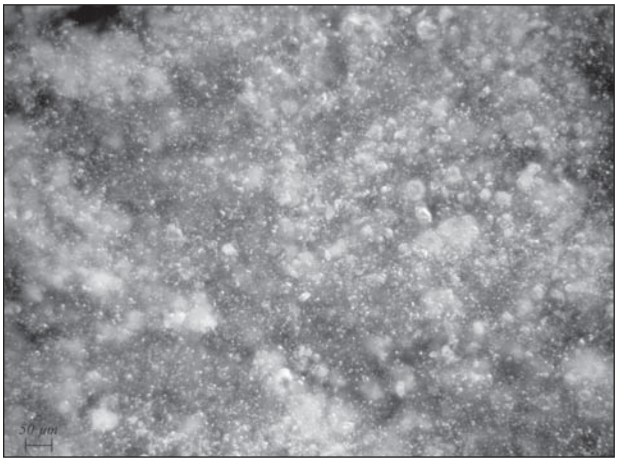


Fig. 2. Micro-pictures of KCl/AM1 nanocomposite powder made in reflected light (x100 magnification)

find the distribution of the radii of freezing water clusters using the Gibbs-Thomson equation [10, 11]. Based on the chemical shift of water one can calculate the average degree of association of water molecules in poly-associates taking into consideration the fact that the protons of non-associated (weakly associated) water have a chemical shift δ_H = 1–1.5 ppm, the ice-like structures typical for hexagonal ice have a shift δ_H = 7 ppm [12], while for the liquid water, δ_H = 4.5–5 ppm is reported.

PREPARATION OF SAMPLES

Before the measurements, the samples of potassium chloride (KCl) were carefully ground in an agate mortar to get a fine powder with a particle size of 1–10 microns. Then, a required amount of H_2O was added to the samples. AM1-300 methylated silica with a specific surface of 285 m^2/g made at the Kalush Experimental Plant of the Chuiko Institute of Surface Chemistry was used as hydrophobic silica. The composite samples consisting of equal weights of methylated silica and KCl were prepared by co-grinding the components in agate mortar during 10 minutes to get a homogeneous composite material with a bulk density of 300 mg/ml. Pure tetrachloromethane (CCl_4), deuterated chloroform ($CDCl_3$) and *n*-decane were used as organic medium.

LOW TEMPERATURE ^{1}H NMR

The NMR spectra were recorded on a high-resolution NMR spectrometer (*Varian Mercury*) with an operating frequency of 400 MHz. Eight 60°probing pulses having of 1 μs duration at 20 kHz bandwidth were used. Temperature was controlled by *Bruker VT-1000* temperature controller with an accuracy of ±1°. The signal intensity was measured as the area of peaks by decomposing the signal into its components assuming the Gaussian waveform and optimization of the zero line and the phase with an accuracy of, at least, 5% for the well-separated signals and ±10% for the overlapping signals. To prevent overcooling of water in the test samples the concentration of non-freezing water was measured when heating the samples precooled to a temperature of 210 K. The temperature dependences of NMR signal intensity were measured in an automated cycle for the sample held during 9 minutes at a constant temperature. The time of measurement was 1 minute. The measurements were made in standard 5 mm ampoules. The powders were photographed using a *Prim Star* microscope (*Carl Zeiss*, Germany) with ×100 magnification.

RESULTS AND DISCUSSION

Micro-photographs of nanocomposite powder in reflected light with ×100 magnification are showed in Fig. 2.

The nanosilica particles have a shape close to spherical and are present as aggregates of a size ranging within 1–5 microns. The KCl microcrystals are evenly distributed over the sample volume, with the majority of them having submicron dimensions. They mechanically contact with AM1-300 particles.

The ^{1}H NMR spectra of atomized KCl powder containing 286 mg/g H_2O captured at different temperatures are given in Fig. 3, *a*. The sample was a wet powder with visually unidentified liquid phase of KCl solution. This sample simulates the behavior of powdered fertilizer in moist soil. On the thermograms of thawing sample, the water signal was recorded at $T \geq 240$ K. The concentration dependence of water freezing temperature is showed in Fig. 4, *a*. Within the temperature range $240<T<280$ water concentration varies slightly. The samples were thawing according to the Raoult law. The drop in freezing point of the electrolyte solution is determined by a decrease in the density of saturated water vapor above its surface. As a result of freezing, water crystallizes out the solution as hexagonal ice crystals and solid KCl crystals germinate. Accordingly, the drop in the freezing point of water [6—9] is determined by variation in free energy of ice caused by the formation of solution of the individual components (water and KCl) (Fig. 4, *b*). During the thawing, solution forms again, but for this liquid water is required. The solid water in the form of nanocrystals whose size is determined by the Gibbs-Thomson equation [9–11] melts at $T<273$ K. The distribution of water nano-drops radii during the thawing is showed in Fig. 4, *c*. This distribution implies that the water wetting the powder KCl freezes mainly in the form of crystals with a radius $R = 2$ nm. The chemical shift of water associated with potassium chloride varies within the range $4.5 <\delta_H<6$ ppm. This value is slightly higher than that for liquid water [7]. Therefore, one can assume that KCl dissolved in water has a cosmotropic effect [14–18] that manifests itself in a more ordered grid of hydrogen bonds of aqueous poly-associates.

In composite system KCl/AM1/H_2O (Fig. 3, *b*), the chemical shift of water signal remains practically the same, whereas the range of water freezing temperatures moves downward. Presumably, the hydrophobic nanosilica particles can interact with aqueous salt solution through van der Waals mechanism. As a result, the probability of formation of continuous solution film decreases. This film freezes in accordance with the Raoult law, with a significant portion of the solution transforming into nanoscale clusters separated by mineral particles. In this case, for this portion of the solution, the free energy decreases significantly as a result of its interaction with the area of interface of mineral particles (Fig. 4, *b*) (a section corresponding to large variations of Gibbs free energy on the ($\Delta G\,(C_{uw}$) dependence). The distribution of water cluster radii during the thawing has maxima at R=1 and R=20 nm (Fig. 4, *c*).

A thin composite film well retained by the surface appears at the interface of the surface of most types of seeds with the composite materials based on methyl silica and fertilizers. This is caused by the presence of hydrophobic sites with high affinity to methyl silica on the seed surface. One can assume that the salt solution interface with hydrophobic sites on the seed surface is also able to effect significantly the state of water in the composite. Such interactions were simulated by adding to the KCl/AM1/H_2O composite system a small amount of aliphatic hydrocarbon. Fig. 3, *c* features ^{1}H NMR spectra of KCl/AM1/H_2O composite with 70 mg/g of *n*-decane added.

In addition to the strongly associated water (SAW) signals, signals of methyl and methylene groups of *n*-decane appear in the spectra (0.9 and 1.25 ppm, respectively) as a fused peak (because of a large width). In the same spectral band, a signal of weakly associated water (WAW) is expected as well [6–9]. In Fig. 3, *c*, it is observed as a weak signal with a chemical shift $\delta_H = 1.7$ ppm. When comparing the data in Figs. 3, *b* and 3, *c*, one can conclude that even small amount of hydrophobic agent can cause significant changes in the spectral characteristics of bound water. The range of existence of unfreezing water expands up to T=210 K, with the SAW chemical shift at low temperature moving towards $\delta_H = 7.5$ ppm, which

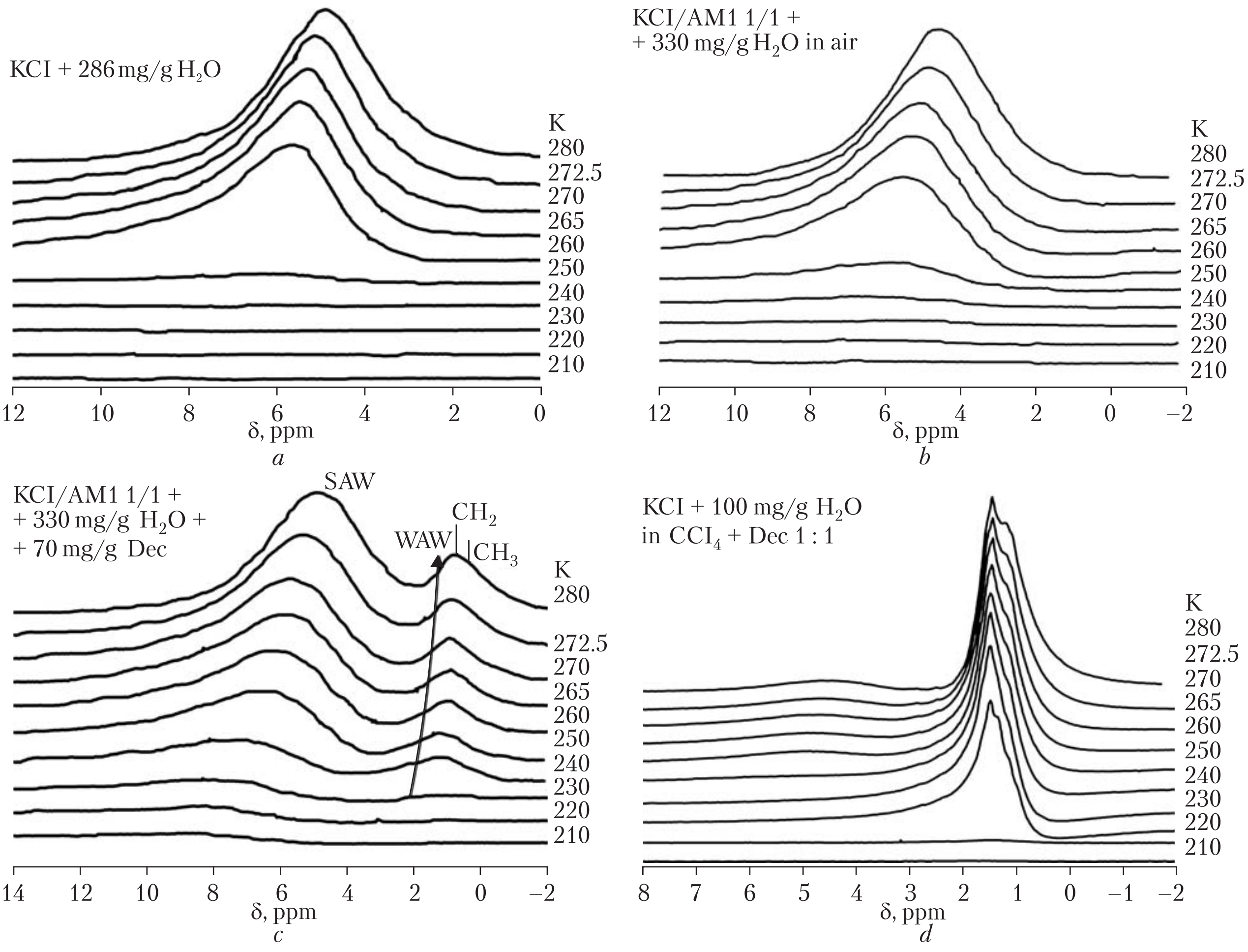


Fig. 3. ^{1}H NMR spectra of KCl hydrated powder and KCl/AM1/H_2O composite system in the presence of organic admixtures recorded at various temperature

corresponds to the enhancement of cosmotropic effect and the formation of mainly ice-like grid of hydrogen bonds in poly-associates of bound water. The lowering freezing point of water indicates the transition of its large part in poly-associate state where the free energy decreases due to the interaction with internal boundaries of interface (solid salt, mineral particles). The distribution of water cluster radii during the thawing changes accordingly (Fig. 3, *c*): it has maxima of similar intensity corresponding to water poly-associates with $R = 2$ and $R = 8$ nm.

Fig. 3, *d* shows that the continuous hydrophobic organic environment can have a significant impact on the state of water (aqueous solution of KCl) in the KCl/H_2O binary system. However, in this case, a chaotropic impact of the environment on bound water is reported. The chemical shift of water decreases, and at $T = 280$ K, it reaches a shift $\delta_H = 4$ ppm. In addition, the signal of protons within the band corresponding to the protons of *n*-decane and WAW becomes more complex, which can be interpreted as increase in signal intensity of WAW or as formation of heterogeneous system in which one part of *n*-decane is dissolved in liquid CCl_4, while the other one is adsorbed on the surface of solid KCl particles. Three maxima corresponding to water structures with $R = 1$, 4, and 30 nm are reported on the radii distribution of water poly-associates (Fig. 4, *c*).

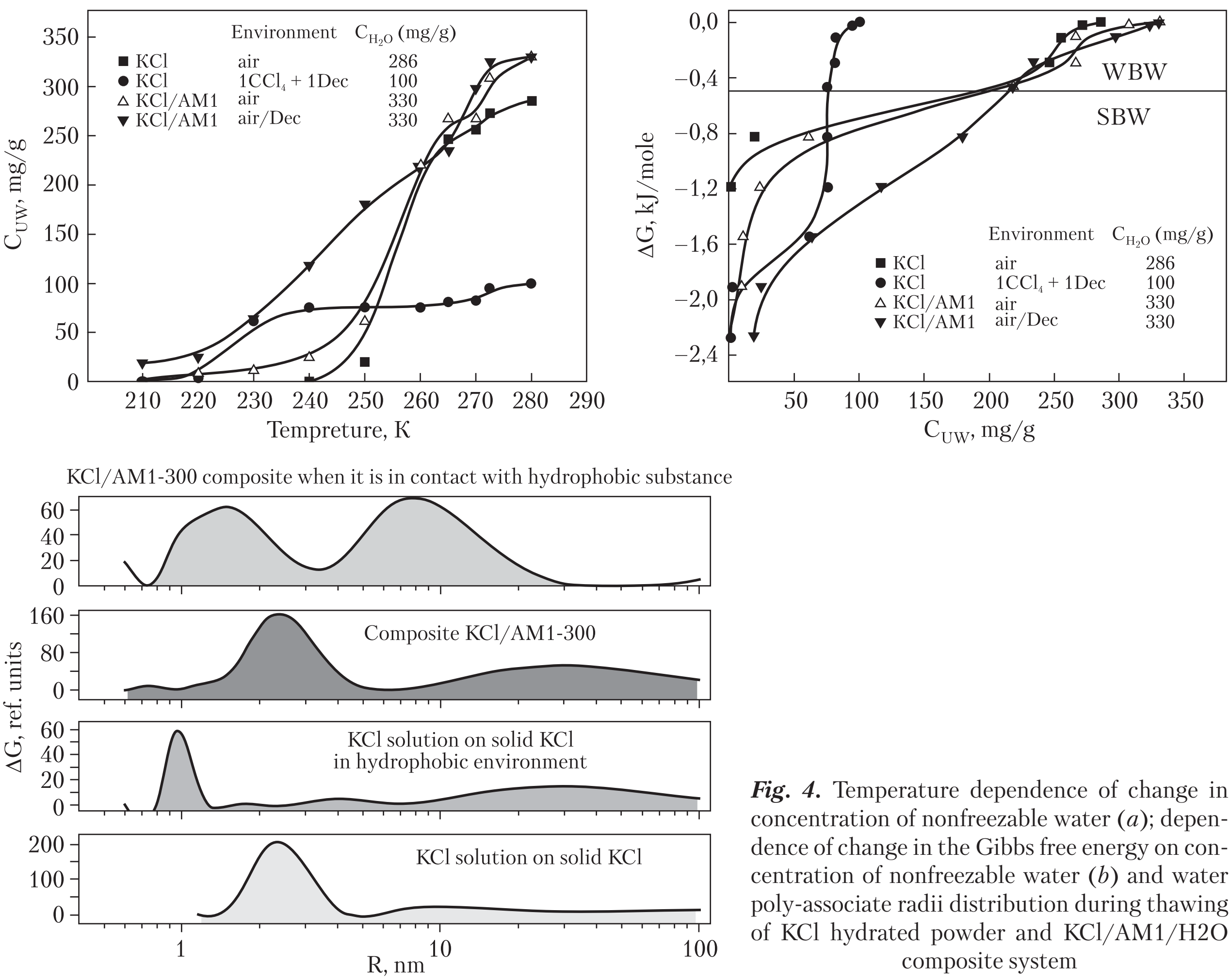


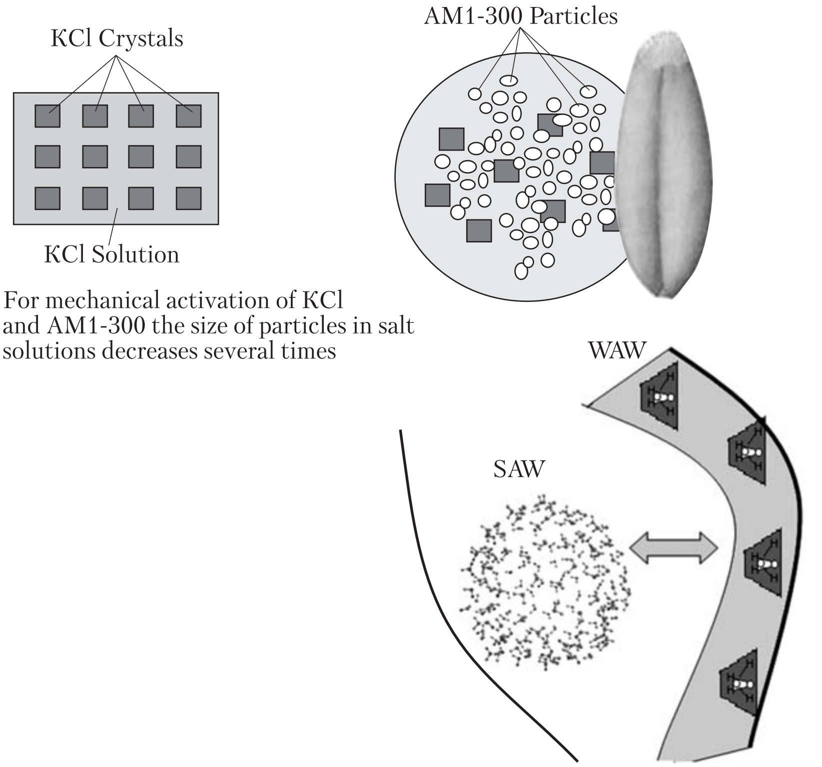
Fig. 4. Temperature dependence of change in concentration of nonfreezable water (*a*); dependence of change in the Gibbs free energy on concentration of nonfreezable water (*b*) and water poly-associate radii distribution during thawing of KCl hydrated powder and KCl/AM1/H2O composite system

Concentrations of strongly and weakly bound water (C_{uw}^{s} and C_{uw}^{w}, respectively), maximum decrease in Gibbs free energy (ΔG_{max}), and interfacial energy (γ_S) calculated on the basis of Fig. 4, *b* according to the method described in detail in [6–9] for KCl wet powders and KCl/AM1/H_2O composite system are given in Table below.

For the hydrated KCl powder, when the heterogeneous system contains only solid salt covered with a film of saturated solution, ΔG_{max} determines maximum change in the free energy of salt ion hydration by water molecules at a con-

Properties of Nonfreezable Water Layers in Wet KCl Powders and in KCl/AM1/H2O Composite System

System	Environment	C_{H2O}, mg/g	C_{uw}^{s}, *mg/g*	C_{uw}^{w}, mg/g	ΔG_{max}, kJ/mole	γ_{S}, J/g
KCl	Air	286	180	106	−1.2	9.0
KCl	1CCl$_4$+1Dec	100	75	25	−2.4	7.1
KCl/AM1	Air	330	180	150	−2.4	11.1
KCl/AM1	Air/Dec	330	210	110	−3.2	17.3



For mechanical activation of KCl and AM1-300 the size of particles in salt solutions decreases several times

On the interface between the nanocomposite and the seed surface, nanoclusters of salt solutions and WAW are formed. They ensure an active transport of nutrient chemicals to the kernel

Fig. 5. Transformation of mineral salt aqueous solutions during the preparation of *Ecostim* and its contact with seed surface

centration C_{H2O} = 286 mg/d. Since, as a result of freezing, the whole water transforms into hexagonal ice crystals, γ_S determines the total value of free energy of hydration, which (according to the Hess Law) is equal to the energy of dispersion of bulk ice over nanocrystal system (Fig. 4, *c*).

Thus, the inclusion of hydrophobic nanosilica that seemingly weakly interacts with water and with KCl ionic crystals into composite system still significantly effects the characteristics of interfacial water as for similar hydration of samples, ΔG_{max} increases twice (from 1.2 to 2.4 kJ/mole), while the interfacial energy grows from 9 to 11.1 J/g. This growth is explained by increasing amount of WAW. The difference is caused by contribution of interaction between water poly-associates and *solid-liquid* interfaces (adsorption layer) to the change in Gibbs free energy, in addition to the effect of ion hydration. Adding a small amount of hydrophobic agent (e.g., *n*-decane) that can simulate contact with surface of biological objects (seeds) stimulates even stronger binding of water, as ΔG_{max} reaches 3.2 kJ/mole whereas γ_S comes up to 17.3 J/g, mainly, due to increasing SAW contribution. The difference in the values of γ_S for hydrated KCl powder and its composite with AM1-300 silica in the presence of hydrophobic organic agent shows a gain in the free energy of binding of water that can be expected for using the protective and stimulating nanocomposites based on mechanically activated mix of hydrophobic silica and fertilizers in the crop farming.

The transformation of aqueous solutions of mineral salts during the creation of nanocomposites such as *Ecostim* and the mechanism of its contact with the seed surface are showed in Fig. 5. As size of water poly-associates containing mineral salt solutions decreases, the retention of water in the area of contact with the seed surface gets stronger, which significantly reduces the probability of drying of kernel. In addition, in the nano-sized droplets of water, the retention of ions gets weaker, which increases the possibility of their diffusion into the seed kernel. The formation of WAW layer in the zone of contact of solution with the kernel also helps to optimize the access of nutrients to the root system.

CONCLUSIONS

The presence of nanoparticles in the composite system consisting of hydrated KCl powder and AM1-300 hydrophobic nanosilica (a model of protective and stimulating nanocomposite *Ecostim*) has been established to significantly increase the binding of water to the surface. As a result, the effectiveness of water retention in the composite is much higher than that in the powder fertilizer.

For the composite system KCl/AM1/H_2O contacting a hydrophobic substance (e.g., *n*-decane) the binding of interfacial water gets stronger due to growing contribution of SAW. This may be induced by decreasing size of water poly-associates as a result of increase in van der Waals interactions in the nanoscale systems with hydrophobic and hydrophilic components. Some portion of water in the area of contact with hydrophobic sites of heterogeneous system can transform into the state of WAW.

The research results explain the ability of protective and stimulating composites such as *Ecostim* to retain a significant amount of water contained in mineral fertilizer solution in the zone of contact with seeds.

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МЕХАНІЗМ ЗАХИСНОЇ ДІЇ НАНОКОМПОЗИТНОЇ СИСТЕМИ «ЕКОСТИМ» ДЛЯ ПЕРЕДПОСІВНОЇ ОБРОБКИ НАСІННЯ

Методом низькотемпературної ^{1}H ЯМР спектроскопії у повітряному середовищі вивчено стан води в гідратованому порошку KCl, композитній системі KCl/AM1/H_2O та з добавками гідрофобних органічних речовин. Виявлено, що в композитній системі, яка складається з гідратованого порошку солі KCl і гідрофобного нанокремнезему AM1-300, присутність гідрофобних наночастинок істотно збільшує зв'язування води з поверхнею. В результаті утримування води в композиті значно вище, ніж в порошках мінеральних добрив. При контакті композитної системи KCl/AM1/H_2O з гідрофобною речовиною, що імітує гідрофобні ділянки поверхні насіння, спостерігається додаткове зростання зв'язування міжфазної води, яке, ймовірно, відбувається завдяки виграшу у вільній енергії в результаті дроблення кластерів води в нанорозмірних системах, що мають гідрофобні і гідрофільні складові.

Ключові слова: ЯМР-спектроскопія, сильно- і слабкозв'язана вода, композиційна система, мінеральні добрива.

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МЕХАНИЗМ ЗАЩИТНОГО ДЕЙСТВИЯ НАНОКОМПОЗИТНОЙ СИСТЕМЫ «ЭКОСТИМ» ДЛЯ ПРЕДПОСЕВНОЙ ОБРАБОТКИ СЕМЯН

Методом низкотемпературной ^{1}H ЯМР спектроскопии в воздушной среде изучено состояние воды в гидратированном порошке KCl, композитной системе KCl/AM1/H_2O и с добавлением гидрофобных органических веществ. Обнаружено, что в композитной системе, которая состоит с гидратированного порошка соли хлорида калия и гидрофобного нанокремнезема AM1-300, присутствие гидрофобных наночастиц существенно увеличивает связывание воды с поверхностью. В результате содержание воды в композите значительно выше, чем в порошках минеральных удобрений. При контакте композитной системы KCl/AM1/H_2O с гидрофобным веществом, имитирующим гидрофобные участки поверхности семян, наблюдается дополнительный рост связывания межфазной воды, которое, вероятно, происходит благодаря выигрышу в свободной энергии в результате дробления кластеров воды в наноразмерных системах, имеющих гидрофобные и гидрофильные составляющие.

Ключевые слова: ЯМР-спектроскопия, сильно- и слабкосвязанная вода, кластеры воды, гидрофобные вещества, минеральные удобрения, семена.

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