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Effect of inoculation with diazotrophs and treatment with solution of trace elements obtained by nanotechnology on wheat

Dmytro KIRIZIY, Sergii KOTS, Oleg STASIK, Petro PUKHTAIEVYCH (✉), Kateryna KUKOL, Lilia RYBACHENKO

Institute of Plant Physiology and Genetics, National Academy of Sciences of Ukraine, 31/17 Vasylkivska St., Kyiv, 03022, Ukraine

✉ Corresponding author: azotfixation@gmail.com

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ABSTRACT

Currently, the importance of using new biotechnologies in crop cultivation, in particular the introduction of microbial inoculants and products obtained by nanotechnology, is increasing. However, the peculiarities of the interaction of microorganisms with plants when using such products have not been studied enough. The work aimed to study the effects of seed inoculation with a complex of rhizospheric diazotrophs (CRD) and treatment with a trace elements solution (TES) obtained by nanotechnology on CO_2 net assimilation (A_n) and yield of winter wheat. Plants were grown in a pot experiment with the following treatments: 1 – control; 2 – seeds inoculation with diazotrophs (CRD); 3 – seeds treatment with trace element solution (TES); 4 – seeds treatment with CRD + TES seeds treatment; 5 – TES plants treatment at the heading stage; 6 – CRD seeds inoculation + TES plants treatment at the heading stage. The obtained results showed that the nitrogenase activity in the wheat plants' rhizosphere under CRD inoculation was significantly higher by 25% on average compared to the control and treatments with only TES. For all treatments, A_n at ripening stage exceeded the control level by 18–34%, and the plant grain productivity – by 17–27%. Significant correlations (r = 0.86–0.90) were found between the A_n at the ripening stage and the grain weight from the whole plant. It was concluded that the optimal combination was CRD seeds inoculation + TES seeds treatment, where the highest main shoot and the whole plant grain productivity were observed. The seed treatment with the trace element solution was more effective in increasing the main shoot productivity, while the treatment at the heading stage was more effective for the whole plant's productivity.

Keywords: *Triticum aestivum* L., associative nitrogen fixation, CO_2 assimilation, transpiration, productivity

INTRODUCTION

Currently, in order to reduce environmental pollution, the importance of using new biotechnologies in crop cultivation, in particular, the introduction of microbial inoculants, including diazotrophs (bacteria and archaea) that fix gaseous nitrogen in the atmosphere into a more usable form such as ammonia, is increasing (Volkohon et al., 2020; Dheeman and Maheshwari, 2022). The potential of using diazotrophs as biofertilizers has been demonstrated for various non-legumes, including wheat (Bageshwar et al., 2017). Since the phylogenetic diversity of diazotrophic microorganisms that form associations with different crops is quite wide, complex bacterial mixtures containing various species of microorganisms are used for seed treatment (Nag et al., 2020). In addition to improving nitrogen nutrition, associative nitrogen-fixing microorganisms produce physiologically active substances (hormones, vitamins, amino acids, plant growth regulators, etc.) and activate the absorption capacity of the root system. Also, beneficial microorganisms colonizing the roots prevent infection of plants by phytopathogens (Mawarda et al., 2020). To optimize the interaction between associative microorganisms and non-legumes, various biotechnological approaches are used (Nag et al., 2020; Thiebaut et al., 2022).

Now, the use of nanotechnologies in agriculture is highly relevant (Kolbert et al., 2022), especially in relation to trace elements (Rizwan et al., 2021). The trace elements fertilizers obtained by nanotechnology have several advantages compared to ones, based on inorganic salts – they are completely soluble in water and more reactive than conventional fertilizers, they are stable in a wide range of soil pH and do not bind to sparingly soluble compounds (Elemike et al., 2018). Due to the changed interactions between molecules, nanomaterials exhibit new properties (Kolbert et al., 2022). It has been shown that fertilizers obtained by nanotechnology provide an increase in resistance to adverse weather conditions and an increase in the yield of almost all food and industrial crops (Dadarwal et al., 2022). At the same time, their effects occur in a lower concentration, while higher doses have a negative impact on plants, which is characteristic of biologically active substances (Kolbert et al., 2022). Nanopreparations affect both plants and microorganisms. However, the peculiarities of the interaction of microorganisms with plants when using such products have not been studied enough (Elemike et al., 2018; Kolbert et al., 2022).

The work aim was to study the effect of wheat seed inoculation with a complex of rhizospheric diazotrophs and treatment with a trace elements solution obtained by nanotechnology on the CO_2 net assimilation rate and yield of winter wheat plants.

MATERIALS AND METHODS

Materials

The experiment was carried out with plants of winter wheat (*Triticum aestivum* L.) cv. Bohdana (originated from the Institute of Plant Physiology and Genetics of the National Academy of Sciences of Ukraine).

For pre-sowing inoculation of seeds, rhizospheric diazotrophs strains *Enterobacter aerogenes* Tw2, *E. hormaechei* Tw3, *E. cloaceae* T3 were used, which was obtained by analytical selection from the rhizosphere of spring and winter wheat at the Department of Symbiotic Nitrogen Fixation of the IPPG NAS of Ukraine. Cultures grown on a liquid nutrient medium were mixed in equal proportions. The titer of microbial cells in the inoculant was 10^8 cells/mL.

Trace elements solution (TES) was provided by the company AVATAR LLC (Ukraine, Kyiv). The complex product contained five trace elements chelated with natural carboxylic acid. They were obtained in two stages: 1 – an aqueous colloidal solution of microelements nanoparticles by dispersing in deionized water the highly purified granules of the corresponding metals by electric current pulses; 2 – metal carboxylates by the direct reaction of the obtained nanoparticles with food carboxylic acid (citric). In the complex solution, the trace elements content was: Co – 0.001%; Fe – 0.05%; Ge – 0.001%; Cu – 0.01%; Mo – 0.005%. The biogenic microelements – Co, Fe, Cu, Mo are cofactors of many important enzymes. In particular, Mo and Fe are included in nitrogenase, the key enzyme for the fixation of atmospheric nitrogen. Germanium (Ge) activates the antioxidant defense mechanisms in plants and increases their stress tolerance and productivity (Liu et al., 2015).

Experimental conditions

After overwintering under natural conditions, the wheat plants in the spring tillering stage were transplanted into pots in early April (10 kg of 3:1 mixture of gray podzolic soil – pH salt (KCl) 5.8, organic matter content 1.8 %, nitrogen 30 mg/kg, phosphorus 25 mg/kg, potassium 30 mg/kg, and sand). Plants were grown all the time at optimal soil moisture (60–70% of soil field capacity). Nitrogen, phosphorus, and potassium were additionally added to the pots at the rate of 160 mg per kg of soil for each element. The pots were placed on a shelving with a transparent polyethylene film roof. The temperature and light were natural. The plants were grown until the grain reached full ripeness (early August).

The experimental treatments were as follows: 1 – control (without treatment); 2 – seeds inoculation with a complex of rhizospheric diazotrophs (CRD); 3 – seeds treatment with a trace elements solution (TES); 4 – CRD seeds inoculation + TES seeds treatment; 5 – TES plants treatment at the heading stage (BBCH 58–59); 6 – CRD seeds inoculation + TES plants treatment at the heading stage.

Number of repetitions

For each treatment, 4 pots with 20 plants per each pot were set up (in total 80 plants for each treatment). Thus, the number of biological repetitions for each treatment was 4-fold. Biometric indices – plant dry weight and grain productivity were determined in 10-fold replication, the CO_2 net assimilation (A_n), transpiration (T) rates, and the nitrogenase activity (NA) – in 4-fold.

Measurement methods

NA of plant rhizosphere was determined by the acetylene method (Hardy et al. 1968) on gas chromatograph Agilent GC system 6850 (Santa Clara, USA). Net assimilation (A_n) and transpiration (T) rates were recorded on flag leaves of main shoots under controlled conditions (temperature 25 °C, photosynthetically active radiation 1800 µmol/m^2/s) with the gas analyzer EGM-5 (PP Systems, Amesbury, USA). Conditioned air (humidity 10 mbar, CO_2 concentration 400 ppm) was blown through the chamber at a rate of 1 L/min. Gas exchange parameters were calculated according to Laisk and Oja (1998).

Statistical analysis

The data obtained were processed by methods of variation statistics using Microsoft Excel. The significance of the differences between the treatment's mean values were assessed using ANOVA with Bonferroni's correction, they were considered significant at $P < 0.05$. The Figures and the Table show the arithmetic mean and standard error of the mean.

RESULTS AND DISCUSSION

Nitrogenase activity in the plant rhizosphere

At the anthesis stage (BBCH 65–69), NA in the treatments with the CRD inoculation was higher by an average of 25% compared to the control and treatments with TES only (Figure 1). The treatments with TES had almost no effect on the NA. At the ripening stage (BBCH 83–85), a nearly twofold drop in NA was observed in all treatments compared with the corresponding indices at the anthesis stage.

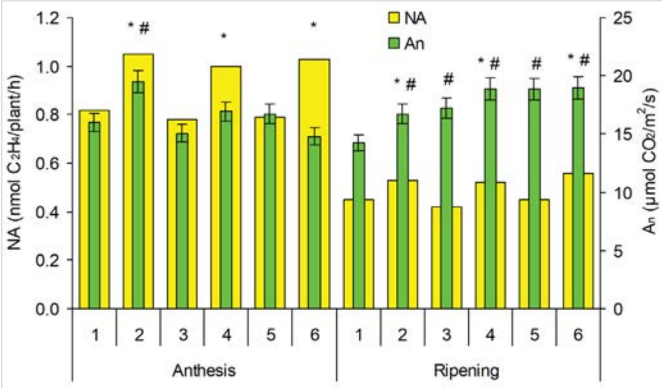

Figure 1. Nitrogenase activity (NA) in the rhizosphere of wheat and the CO_2 net assimilation rate (A_n) of leaves after seeds inoculation with a complex of rhizospheric diazotrophs (CRD) and treatment with trace elements solution: 1 – control (without treatment); 2 – CRD seeds inoculation; 3 – TES seeds treatment; 4 – CRD seeds inoculation + TES seeds treatment; 5 – TES plants treatment at the heading stage; 6 – CRD seeds inoculation + TES plants treatment at the heading stage. The vertical error bar represents the standard error of the mean (n = 4), *significant difference for NA compared to control at P < 0.05; #significant difference for An compared to control at P < 0.05

It is known that the main source of energy for rhizospheric diazotrophs is the organic matter of plant root exudates (Dheeman and Maheshwar, 2022). The root's exudative activity and, accordingly, the quantity and activity of rhizospheric diazotrophs, depend on the amount of photoassimilates coming from leaves, and increases in the presence of their certain excess (Luo et al., 2022). In wheat, such excess is accumulated during the anthesis stage, when the photosynthetic apparatus already formed, but the main sink (grain) has not formed yet (Morgun et al., 2022). Therefore, it can be supposed that a significant decrease in NA in the wheat rhizosphere at the ripening stage is caused by a decrease in the root's exudative activity as a result of redistribution of all newly synthesized and already available assimilates to the grain filling.

There no correspondence has been observed between A_n in the leaves and NA in the plant rhizosphere (Figure 1). It has been noticed that in plants that are capable of symbiotic nitrogen fixation (legumes, in particular soybean), there was a fairly close positive correlation between the nitrogen-fixing activity of nodules and the leaf CO_2 net assimilation rate (Kiriziy et al., 2022). This is due to the decisive role of nodules in providing legumes with nitrogen, which is critical for the photosynthetic apparatus functioning. However, the nitrogenase activity of rhizospheric diazotrophs is lower by 2–3 orders of magnitude than that of nodule bacteria, which explains the lack of such correlation in the current experiment.

CO_2 net assimilation rate

At the anthesis stage, the highest A_n was observed in treatment 2 (CRD seeds inoculation), while other treatments and control differed insignificantly (Figure 1). At the ripening stage, plants of all treatments with TES and CRD significantly exceeded the control in this parameter. The effect was most pronounced in treatments 4 (CRD seeds inoculation + TES seeds treatment), 5 (TES plants treatment at the heading stage) and 6 (CRD seeds inoculation + TES plants treatment at the heading stage). The A_n values in the treatment with only CRD inoculation (treatment 2) and the treatment of seeds with only TES (treatment 3) were somewhat lower but still exceeded the control ones.

It should be noted that at the ripening stage, the A_n in control plants decreased compared to the anthesis stage, and in other treatments, it remained at the same level or was higher (except treatment 2 – CRD seeds inoculation). The decrease in A_n in wheat after the anthesis stage is explained by the remobilization of nitrogen-containing compounds from the leaves into the grain, and the gradual aging of leaves. Both the CRD inoculation and TES treatment slowed down these processes: CRD – due to a stimulating effect on the root system, which could contribute to the additional absorption of mineral nitrogen from the soil and producing physiologically active substances (Shabanamol et al., 2018; Kumar et al., 2022), and the TES treatment – due to a positive regulatory effect on physiological and biochemical processes, in particular antioxidant defense systems (Kabata-Pendias et al., 2010; Zhou et al., 2023). At the same time, the TES was more effective in the plant treatment at the heading stage (treatment 5 and 6) as well as in the combined use with CRD for seed inoculation (treatment 4). In the latter case, a positive effect of TES can be assumed not only on seeds but also on microorganisms concerning their stimulating effect on the root system during further functioning in the plant rhizosphere (because the effect only of CRD inoculation or only TES treatment of seeds was less) (Dhayalan et al., 2021).

The relationships between A_n and plant productivity

It is known that wheat grain productivity is determined by the photosynthetic apparatus activity mainly during the grain-filling period (Murchie et. al., 2023). Both CRD inoculation and TES treatment had a positive effect on A_n at the ripening stage and grain productivity, which for the whole plant in all treatments exceeded the control by 17–27% (Table 1). The highest grain productivity of both the main shoot and the whole plant (including lateral shoots) was observed in treatment 4 (CRD seeds inoculation + TES seeds treatment). Since the parameters of gas exchange were measured on the flag leaves of the main shoot, the relationship between A_n and its productivity deserves a separate analysis. Figure 2 shows that, except two pairs of A_n values measured at the anthesis stage (treatments 3 – TES seeds treatment, and 4 – CRD seeds inoculation + TES seeds treatment) (Figure 2A), and at the ripening stage (treatments 5 – TES plants treatment at the heading stage, and 6 – CRD seeds inoculation + TES plants treatment at the heading stage) (Figure 2B), for the remaining treatments there was a close correlation between A_n both at the anthesis stage and ripening stage and grain productivity of the main shoot. At the same time, there was no correlation between the CO_2 assimilation rate measured at the anthesis stage and the grain productivity of lateral shoots. However, a fairly close correlation was observed between the assimilation rate measured at the ripening stage and the grain productivity of lateral shoots (Figure 2C).

An analysis of the relationship between the A_n at the anthesis stage and the whole plant grain productivity did not reveal any correlation between these parameters. There was also no correlation between the CO_2 assimilation rate at the anthesis stage and the number of productive lateral shoots (as one of the components of the grain productivity of the whole plant).

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Table 1. Productivity of wheat plants after seed inoculation with rhizospheric diazotrophs (CRD) and treatment with trace elements solution (TES)

Treatment	Total weight (g)	Grain weight (g)	Grains number (pcs.)	1000 grains weight (g)	Harvest index	Productive shoots (pcs.)
			Main shoot			
(1) Control (without treatment)	3.03 ± 0.13^{a}	1.53 ± 0.07^{a}	33.0 ± 1.6^{a}	45.9 ± 2.1^{a}	–	–
(2) CRD inoculation of seeds	3.34 ± 0.12^{*b}	1.75 ± 0.07^{*b}	36.2 ± 1.5^{a}	48.3 ± 1.9^{*b}	–	–
(3) Seeds treatment with TES	3.41 ± 0.12^{*b}	1.76 ± 0.07^{*b}	38.3 ± 1.6^{*b}	45.6 ± 2.1^{a}	–	–
(4) CRD inoculation of seeds + seeds treatment with TES	3.51 ± 0.15^{*b}	1.84 ± 0.07^{*b}	38.6 ± 1.7^{*b}	47.6 ± 2.2^{a}	–	–
(5) Treatment of plants with TES at the heading stage	3.11 ± 0.12^{a}	1.54 ± 0.07^{a}	34.3 ± 1.3^{a}	45.0 ± 2.1^{a}	–	–
(6) CRD inoculation of seeds + treatment of plants with TES at the heading stage	2.93 ± 0.11^{a}	1.43 ± 0.05^{a}	31.0 ± 1.34^{a}	46.1 ± 1.9^{a}	–	–
			Whole plant			
(1) Control (without treatment)	4.03 ± 0.16^{a}	2.01 ± 0.09^{a}	44.7 ± 2.1^{a}	45.0 ± 2.1^{a}	0.50	2.52 ± 0.10^{a}
(2) CRD inoculation of seeds	4.52 ± 0.20^{a}	2.35 ± 0.10^{*b}	48.8 ± 2.2^{*b}	48.4 ± 2.3^{a}	0.56	2.57 ± 0.11^{a}
(3) Seeds treatment with TES	4.91 ± 0.21^{*b}	2.38 ± 0.09^{*b}	54.1 ± 2.6^{*c}	44.0 ± 2.1^{a}	0.51	2.65 ± 0.10^{a}
(4) CRD inoculation of seeds + seeds treatment with TES	5.43 ± 0.22^{*c}	2.55 ± 0.11^{*c}	55.8 ± 2.9^{*c}	45.7 ± 2.1^{a}	0.52	2.83 ± 0.12^{*b}
(5) Treatment of plants with TES at the heading stage	5.01 ± 0.20^{*b}	2.37 ± 0.10^{*b}	55.5 ± 2.5^{*c}	42.7 ± 1.9^{a}	0.48	2.92 ± 0.12^{*b}
(6) CRD inoculation of seeds + treatment of plants with TES at the heading stage	4.92 ± 0.21^{*b}	2.36 ± 0.10^{*b}	54.3 ± 2.4^{*c}	43.5 ± 2.0^{a}	0.48	2.97 ± 0.11^{*b}

Legend: (x ± standard error, n = 10; *significant difference compared to the control at $P < 0.05$; different letters indicate significant differences between the treatments separately for the main shoot and the whole plant). CRD – complex of rhizospheric diazotrophs, TES – trace elements solution.

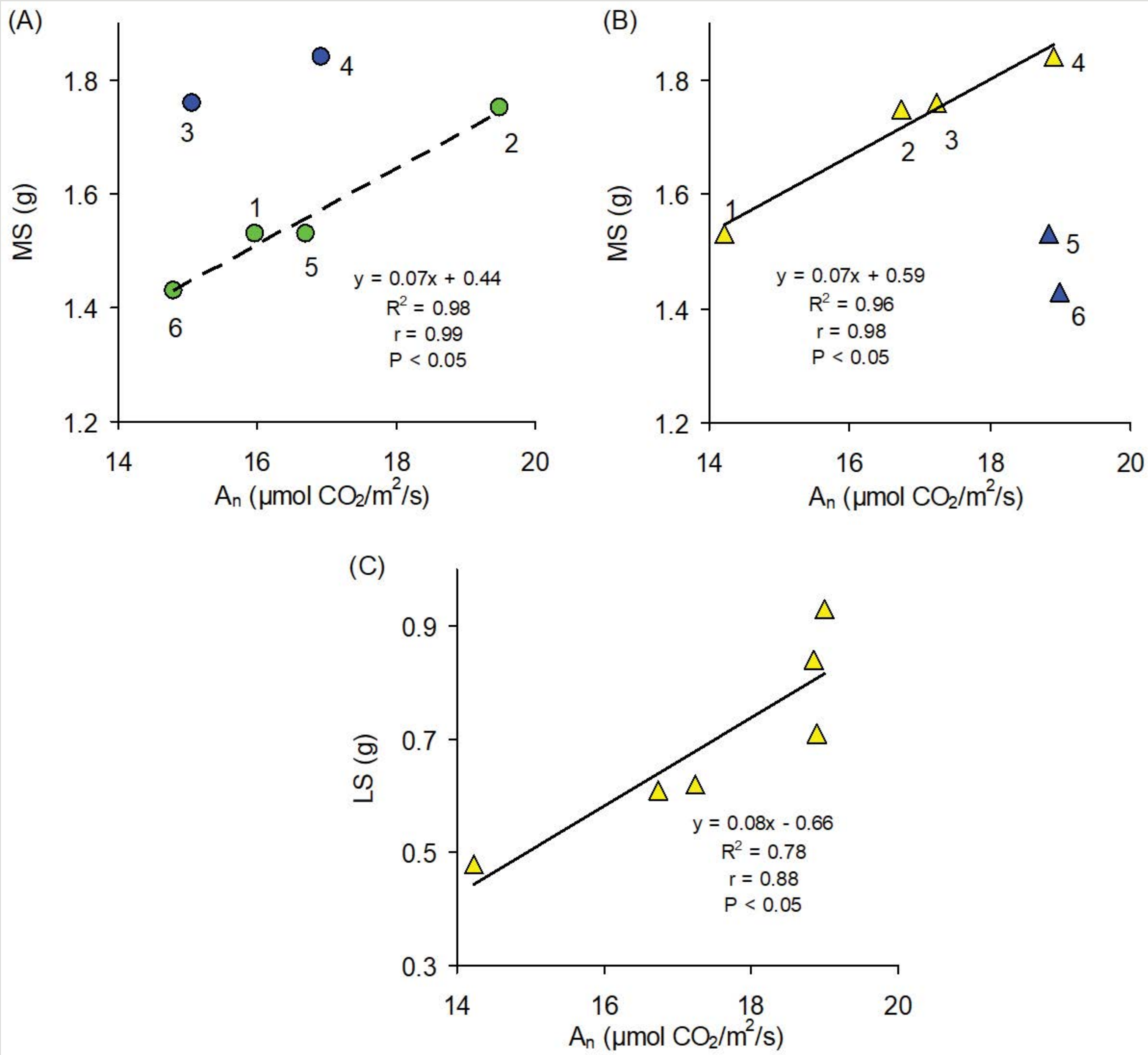

Figure 2. Relationship between the CO_2 net assimilation rate (A_n) in wheat leaves and grain weight – (A) for the main shoot (MS) and A_n at anthesis stage, (B) for the main shoot (MS) and A_n at ripening stage, (C) for the lateral shoots (LS) and A_n at ripening stage under seeds inoculation with rhizospheric diazotrophs and treatment with trace elements solution. The figures near the markers are the number of treatments

However, correlations were found between the A_n at the ripening stage and grain productivity of the whole plant (Figure 3A), and A_n at the ripening stage and the number of productive shoots (Figure 3B).

Therefore, the TES seeds treatment was more effective in increasing the main shoot productivity, while the TES treatment at the heading stage was more effective for the productivity of the whole plant. In the second case, this happened predominantly due to an increase in the productive tillering and grain weight from lateral shoots.

Although this technique had intensified the A_n in flag leaves at the ripening stage, however, at that time the demand for assimilates from the ear of main shoot had already begun to decrease, and they were redistributed in the source-sink system of plant towards the lateral shoots.

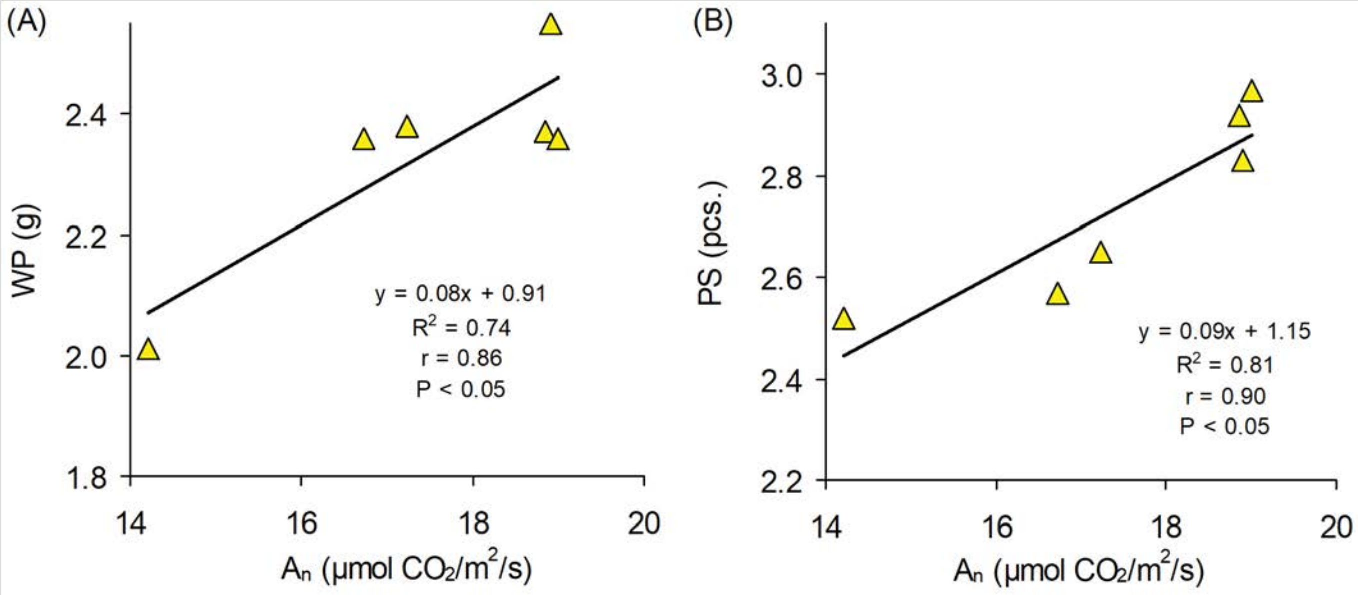

Figure 3. Relationship between the CO_2 net assimilation rate (A_n) in wheat leaves at the ripening stage and (A) – grain weight for the whole plant (WP), (B) – the number of productive shoots (PS), under seeds inoculation with rhizospheric diazotrophs and treatment with trace elements solution

It is interesting to note that an increase in the main shoot productivity under the seeds treatment with TES (both with and without inoculation by CRD) (Table 1) was accompanied by a slight increase in A_n at the anthesis stage (treatment 4 – CRD seeds inoculation + TES seeds treatment), or this index did not differ significantly from the control (treatment 3 – TES seeds treatment) (Figure 1).

At the same time, at the ripening stage, all treatments with TES (both seeds and plants at the heading stage) showed a significant increase in A_n compared to the control. Though in treatment 2 (only CRD inoculation), A_n at the ripening stage compared to the anthesis stage decreased, it remained higher than in control plants due to a decrease in this index in the last ones (Figure 1). This indicated a certain specific stimulating effect of TES on plants (Kabata-Pendias, 2010).

The relationships between stomatal conductance, CO_2 net assimilation and transpiration rates

In addition to A_n, we also analyzed such important parameters of leaf gas exchange as transpiration (T) rate and stomatal conductance. The latter determines the transfer speed of two oppositely directed gas flows – the water vapour from the leaf into the atmosphere and CO_2 from the atmosphere into the leaf (Matthews et al., 2017). Under normal water supply conditions, an increase in the CO_2 assimilation rate in mesophyll cells (for example, with an increase in illumination or under the influence of other stimulants) results in a decrease of intercellular CO_2 concentration, for what stomata respond by increasing in their conductance (Engineer et al., 2016). In the current experiment, the intercellular CO_2 concentration for all treatments (including the control) varied between 335–338 ppm (in the air around the leaf – 400 ppm), which indicated that their work was well coordinated with CO_2 net assimilation rate in the range of physiological optimum.

It was found that the stomatal conductances for CO_2 and H_2O correlated with A_n and T, respectively, with a tendency to reach saturation at high conductances (Figure 4). This pattern of dependence is known in the literature (Endres et al., 2010). In the case of CO_2 net assimilation rate a saturation at high conductances is explained by an increase in the significance of non-stomatal limitation of photosynthesis, the main components of which are the rate of biochemical processes directly related to CO_2 fixation, and, to a lesser extent, the conductance for CO_2

membranes and cytoplasm of mesophyll cells (Faralli and Lawson, 2020).

For transpiration, it explained by a decrease in the role of the so-called "edge effect" with an increase in the stomata aperture, as well as a decrease in the difference in water vapour pressure between the atmosphere and the intercellular space below the stomata (Yin et al., 2020).

A positive correlation also has been found between T and A_n, which was expressed by a linear function (Figure 5A). Calculations of the water use efficiency (WUE = A_n / T) showed that at the ripening stage, this index for all treatments significantly exceeded the control (Figure 5B).

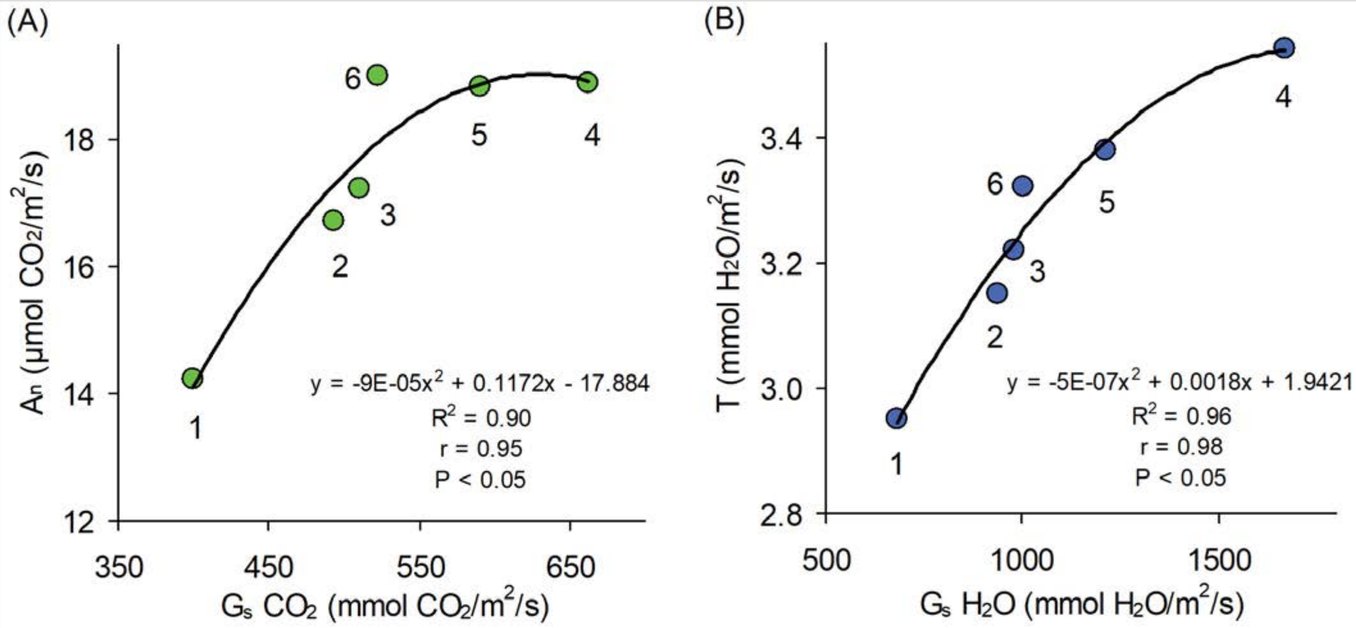

Figure 4. Relationships between the stomatal conductance for CO_2 (Gs CO_2) and the CO_2 net assimilation rate (A_n) of wheat leaves (A), and between the conductance for water vapour (Gs H_2O) and transpiration (T) (B) under seeds inoculation with rhizospheric diazotrophs and treatment with trace elements solution. The figures near the markers are the number of treatments.

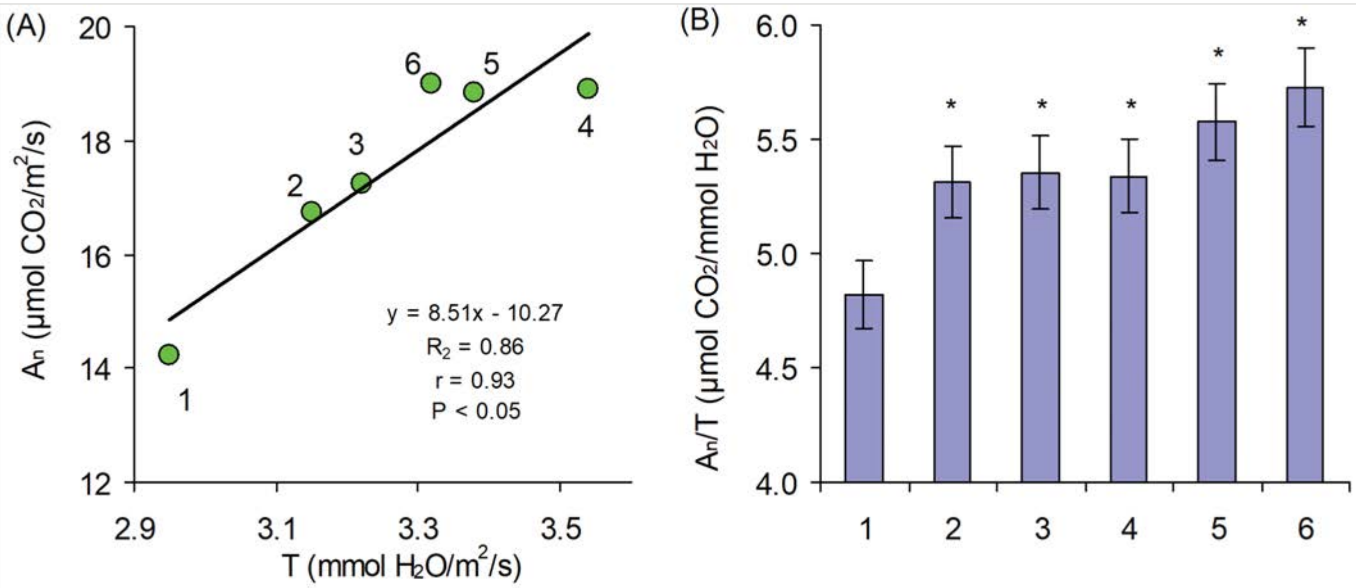

Figure 5. (A) – Relationship between the transpiration (T) and CO_2 net assimilation (A_n) rates, (B) – WUE (A_n/T) values in wheat leaves under seeds inoculation with rhizospheric diazotrophs and treatment with trace elements solution. The figures near markers (A) and on the x-axis (B) are numbers of treatments; *significant difference compared to control at P < 0.05

At the same time, treatments 5 (TES plants treatment at the heading stage) and 6 (CRD seeds inoculation + TES plants treatment at the heading stage) showed a tendency to exceed the other variants. Obviously, this happened due to a stronger effect of treatments directly on the mesophyll photosynthetic apparatus than the stomatal one. As a result, the CO_2 assimilation rate increased more than the transpiration, which led to an increase in WUE. This was especially promoted by the treatment of plants with TES at the heading stage.

CONCLUSION

In general, the results obtained indicated that inoculation of seeds with CRD and treatment with TES increased the leaf CO_2 net assimilation rate and WUE at the ripening stage, which had a positive effect on the grain productivity of wheat plants. The optimal combination of products used in the current experiments was the CRD seeds inoculation simultaneously with TES (treatment 4), where the highest main shoot and the whole plant grain productivity were observed. It was found that the seeds treatment with TES was more effective in increasing the main shoot productivity, while the treatment at the heading stage was more effective for the whole plant. These data can form the basis for the development of new technological elements for growing one of the leading food crops – wheat.

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