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Mitigation of the negative effect of auxinic herbicide by bacterial suspension of *Pseudomonas protegens* DA1.2 in wheat plants under drought conditions

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Abstract: Effect of auxin-producing bacterial strain (*Pseudomonas protegens* DA1.2) was investigated under conditions of drought and herbicide treatment in wheat plants. Positive effect of the bacterial suspension on wheat plants treated with auxinic herbicide under drought conditions was manifested in reducing the content of malondialdehyde and proline, preventing inhibition of plant growth and normalizing chlorophyll content. Under combined stress, changes in concentrations and redistribution of phytohormones in plants were detected. An imbalance in auxin distribution between shoots and roots could be the reason for the decrease in plant resistance to drought in combination with the herbicide. Treatment of plants with the bacterial suspension restored normal shoot-to-root ratio of auxins in plants. Thus, this bacterial strain showed the properties of synthetic auxin antidotes and can be recommended for optimizing the technology of herbicide application under drought conditions.

Key words: bacterial strain; water deficiency; auxinic herbicide; plant hormones; MDA; proline

Blaženje negativnih učinkov herbicidov na osnovi auksina s suspenzijo bakterije *Pseudomonas protegens* DA1.2 pri pšenici v razmerah suše

Izvleček: Učinek sevov bakterije, ki tvorijo auksin (*Pseudomonas protegens* DA1.2) je bil preučevan pri pšenici v razmerah suše in obravnavanja s herbicidi. Pozitivni učinki suspenzije bakterij na pšenici, ki je bila tretirana s herbicidi na osnovi auksina so se pokazali v zmanjšanju vsebnosti malondialdehida in prolina, kar je preprečilo zaviranje rasti rastlin in normaliziralo vsebnost klorofila. V razmerah kombiniranega stresa so se pojavile spremembe v koncentraciji in redistribuciji rastlinskih hormonov v tretiranih rastlinah. Neravnovesje v porazdelitvi auksina med poganjki in koreninami bi lahko bilo vzrok za zmanjšanje odpornosti rastlin na sušo v kombinaciji s herbicidi. Obravnavanje rastlin s suspenzijo bakterij je ohranilo normalno razmerje v vsebnosti auksinov med poganjki in koreninami. Zaključimo lahko, da je ta bakterijski sev pokazal lastnosti antidotov sintetičnih auksinov in bi ga lahko priporočili za optimiziranje tehnologije uporabe herbicidov v razmerah suše.

Ključne besede: bakterijski sev; pomankanje vode; auksinski herbicidi; rastlinski hormoni; MDA; prolin

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1 INTRODUCTION

Drought is a serious stress that reduces the yield of cultivated plants below their genetic potential (Shao et al., 2008; Asseng et al., 2020). Weeds are serious problem in modern agriculture, causing an average decrease in yield by 35 % (Oerke, 2006). The effectiveness of herbicides in weed control has allowed them to be widely used to prevent crop losses (Varhney et al., 2015). However, the use of preparations for chemical weeding of plants has a negative effect on cultivated plants, causing "herbicidal stress", which becomes most notable under drought conditions (Varshney et al., 2015).

It is known that under herbicidal stresses, photosynthesis is the most sensitive process contributing to the plant biomass production (Kutilkin et al., 2018). There are also evidences of the effects of herbicides on the antioxidant system of plants (Ahemad and Khan, 2010).

The role of plant growth regulators, such as hormones, in stress tolerance allows plants to choose the necessary strategy to counteract the adverse effects of abiotic stress and to develop stress resistance responses (Han et al., 2015). Hormones are necessary for the normal functioning of growth processes throughout the plant life cycle; as a result they influence the yield and quality of agricultural crops.

Recently, bacterial treatments are being given more attention in reducing plant abiotic stress. In the scientific literature, there are two areas of research aimed on reduction of the damage caused by currently used herbicides. The goal of the first of them is to accelerate the process of herbicide destruction thereby reducing the negative impact of herbicides on cultivated plants. Microbiological transformation and detoxification of pesticides is rather fully presented in the literature (Silva et al., 2007; Martins et al., 2011; Kanissery and Sims, 2011). However, the potential of bacteria in mitigating pesticide stress in plants (the other area of research) has not been sufficiently studied. There are few publications addressing this question (Ahemad and Khan, 2010; Bourahla et al., 2018; Chennappa et al., 2018). And we have not found any work devoted to the use of growth-stimulating bacteria under conditions of combined stress caused by drought and herbicide treatment.

The previously isolated strain of *Pseudomonas protegens* DA1.2 have shown to possess useful properties, namely resistance to herbicides, capacity of auxin synthesis, mobilization of inorganic phosphates, antagonism to phytopathogenic fungi, growth in the absence of a nitrogen source in the medium, growth-stimulating and antistress effects on wheat plants (Chetverikov et al., 2021).

The aim of the present work was to show the prospects of using the bacterial suspension of *Pseudomonas protegens* DA1.2 as an anti-stress agent when applied to wheat plants against the background of combined "herbicide + drought" stress.

2 MATERIALS AND METHODS

The studies were carried out in laboratory conditions on plants of bread spring wheat (*Triticum aestivum* L.) of a drought-resistant variety, Ekada 109. The bacterial strain *P. protegens* DA1.2 from the collection of microorganisms of the Ufa Institute of Biology, Ufa Federal Research Center, Russian Academy of Sciences and the herbicide (against dicotyledons) based on auxin-like active substances 2,4-D (2-ethylhexyl ester) and dicamba (sodium salts) – "Chistalan" extra (LLC "AHK-AGRO", Ufa), were used in the work. Drought conditions were achieved by reducing irrigation to 30 % of the total water capacity of the soil (this was 60 % in the control) and maintaining it throughout the experiment. Vessels with plants were watered daily with distilled water to the required weight every day.

Wheat seeds were sterilized and germinated for 3 days in a pallet on wet filter paper. Then they were planted in vessels with a volume of 0.5 l filled with a soil-sand mixture in the ratio of 9 : 1 of 6 plants each, the plants were watered only with distilled water. Seedlings were grown at 14 - h photoperiod, day/night temperature regimes of 26 / 22 °C and irradiance of 400 µmol m^{-2} s^{-1} from mercury-arc and sodium vapor lamps. Seven days after seed germination, 1 ml of an aqueous solution of "Chistalan" (the working concentration of the herbicide was 0.5 ml l^{-1}) and / or bacterial suspension of *P. protegens* DA1.2 (108 CFU ml^{-1}) grown in the King B was sprayed on the soil and directly on the plants, using a manual sprayer with a graduated capacity of 20 ml with a division price of 0.1 ml.

Three days after the treatment, shoots and roots of plants were sampled for analysis for hormones (3-indolylacetic acid (IAA), abscisic acid (ABA), and cytokinins), proline, malondialdehyde (MDA), and chlorophylls a, b while growth parameters were evaluated 2 weeks later.

Determination of hormones content was performed using an enzyme immunoassay method. The plant material (shoots and roots) was cut and ground in a porcelain mortar to a homogeneous state, then extracted with 80 % ethanol. Alcohol extract was evaporated to an aqueous residue, centrifuged and the aliquots of the supernatant were taken for analysis. Purification and concentration of ABA and IAA was carried out according to a modified scheme with a decrease in volume (Veselov et al., 1992; Vysotskaya et al., 2009). Cytokinins were concentrated on the C - 18 column and separated using thin-layer chromatography (Kudoyarova et al., 2014).

To determine proline content in wheat shoots, the suspension was homogenized in 10 ml of 3 % sulfosalicylic acid for protein precipitation. Then the homogenate was filtered, 1 ml of filtrate was taken into a clean test tube and 1 ml of acidic ninhydrin reagent (30 ml of glacial acetic acid + 20 ml of 6 M phosphoric acid + 1.25 g of ninhydrin) and 1 ml of glacial acetic acid were added. The mixture was incubated for 1 hour in a water bath at 100 °C, after which it was quickly cooled in ice. Two ml of toluene was added to the mixture and shaken. The colored organic phase was separated and its optical density measured against pure toluene at a wavelength of 520 nm on a spectrophotometer. The proline content was calculated using a calibration curve, using crystal proline for its construction, with further conversion to dry and fresh mass of the sample (Bates et al., 1973).

Lipid peroxidation was determined by the content of malondialdehyde. To achieve this, a sample of plant material was homogenized in 3 ml of distilled water and 3 ml of trichloroacetic acid. The homogenate was centrifuged at 10000 × g for 10 minutes. Two ml of 0.5 % thiobarbituric acid was added to 2 ml of the supernatant and incubated in a water bath for 30 minutes at 95 °C. The tubes were centrifuged and optical density was measured at wavelengths of 532 nm and 600 nm. The concentration of MDA was calculated using an extinction coefficient of 155 mM^{-1} cm^{-1} (Costa et al., 2002).

To determine the content of chlorophyll in wheat plants, 100 mg of leaves was taken. The samples were cut with scissors, placed in test tubes and filled with 6 ml of 96 % ethanol. The test tubes were tightly closed with lids and placed in a dark place for 24 - 48 hours. After the time elapsed, the tubes were shaken and the optical density was measured at wavelengths of 665 nm and 649 nm. The content of chlorophylls "a", "b" was calculated using the formulas:

Chl a = 13.7 *D665* – 5.76 *D649;*

Chl b = 25.8 *D649* – 7.6 *D665*

where Chl a and Chl b are the concentration of chlorophyll "a" and "b", and D665, D649 are the values of extinction at the corresponding wavelength. Concentration of chlorophyll was recalculated per gram of fresh or dry mass of the sample (Lichtenthaler and Buschmann, 2001).

Data were expressed as means ± S.E., which were calculated in all treatments using MS Excel. Significant differences between means were analyzed by one-way analysis of variance ANOVA test to discriminate means. The data were processed using the Statistica version 10 software (Statsoft, Moscow, Russia).

3 RESULTS AND DISCUSSION

Simultaneous exposure to drought and the application of auxinic herbicide resulted in a decrease in the fresh mass of both roots and shoots of wheat plants (by 24 % and 32 %, respectively). However, plants treatment with *bacterial suspension* under these conditions contributed to an increase in the mass of the shoot (by 13 % compared to stressed plants without bacteria), but without an increment in the mass of roots.

It is known that under water and / or mineral deficiency, relative activation of root growth is a typical adaptive reaction in plants (Davies et al., 2005; Vysotskaya et al., 2009). In our experiments, the root / shoot mass ratio of plants grown under herbicide treatment combined with water scarcity, did not change compared to the control (Figure 1 B), since the fresh mass of both roots and shoots was reduced to the same extent (Figure 1 A), while the addition of growth-stimulating bacteria to the herbicide solution led to a decrease in this ratio due to the relative activation of shoot growth.

Accumulation of low-molecular-weight compounds is one of the early adaptive reactions of plants to the action of stressors of various nature, amino acid proline being one of such stress metabolites. An increase in the

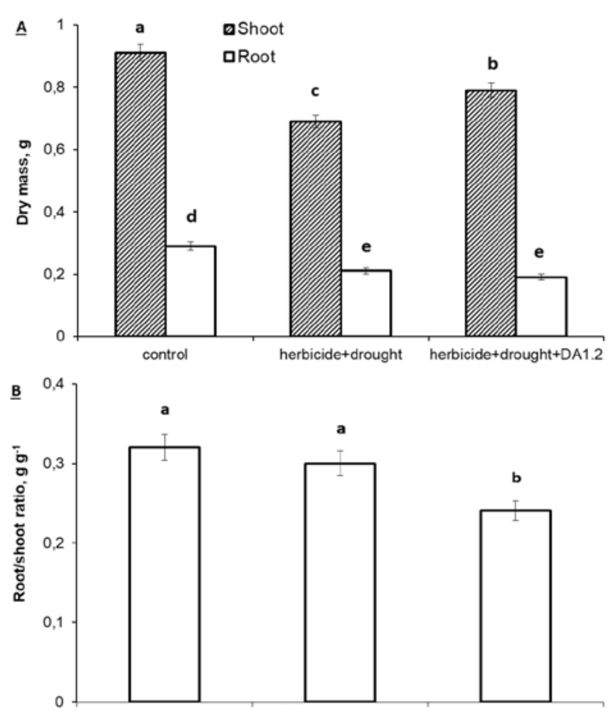

Figure 1: The dry mass of shoots and roots (A) and mass root/shoot ratio (B) of wheat plants 14 days after treatment. Significantly different means (n = 20) are labelled with different letters (ANOVA, LSD, $p \leq 0.05$)

concentration of proline in wheat leaves was observed in response to contamination with toxic metals, osmotic and temperature stress (Fatima et al., 2006; Yang et al., 2011; Mwadzingeni et al., 2016). Indeed, in our work, concentration of proline in plant shoots sharply increased (by more than 100 %) under combined action of water deficit and herbicide treatment, while introduction of the studied bacteria led to a decrease in proline content to the control level (Figure 2 A).

MDA is one of the metabolites of lipid peroxidation and its accumulation in plants shows that they are under severe oxidative stress (Reddy et al., 2004; Masoumi et al., 2011). A decrease in the MDA level indicates a decrease in the damaging effect of reactive oxygen species in leaves of wheat plants inoculated with *bacterial suspension* under conditions of combined stress, which contributed to maintaining their growth and indicated a favorable anti-stress effect of DA1.2 bacteria. Thus, the treatment of plants with herbicide during drought led to accumulation of MDA (15 % more than in the control), while introduction of bacterial strain *P. protegens* DA1.2 leveled the stress by reducing the growth of MDA concentration (Figure 2 B).

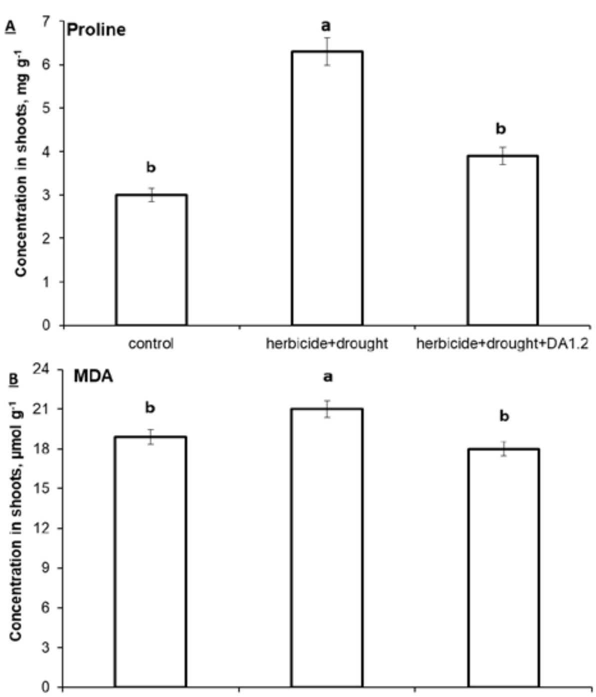

Figure 2: The content of proline (A) and MDA (B) in the shoots of wheat plants 3 days after treatment. Significantly different means (n = 9) are labelled with different letters (ANOVA, LSD, $p \leq 0.05$)

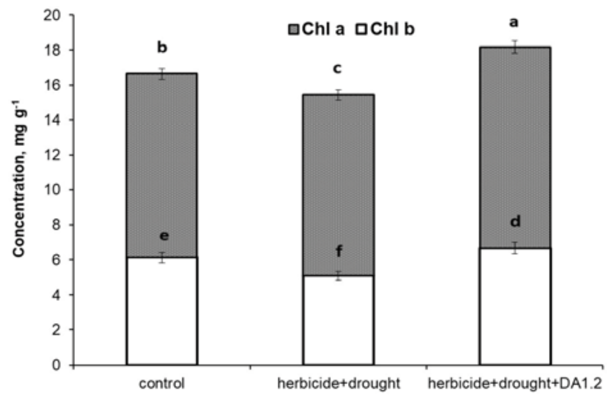

Figure 3: The content of chlorophylls a (Chl a) and b (Chl b) in the shoots of wheat plants 3 days after treatment. Significantly different means (n = 9) are labelled with different letters (ANOVA, LSD, $p \leq 0.05$)

Another important indicator of the plant status under stress conditions is the content of chlorophylls a, b (Nayyar, Gupta, 2006; Ashraf, Harris, 2013). The content of chlorophylls in wheat leaves was shown to decrease by 8 % (Figure 3) under combined "herbicide + drought" stress. The positive effect of bacteria was manifested in mitigating adverse action of the herbicide on photosynthetic apparatus. This was reflected in the quantitative content of pigments in plants: inoculation with bacteria led to an increase in the total content of chlorophylls under the combined stress by 17 %. High concentration of chlorophylls is a characteristic of healthy plants, as it is associated with greater efficiency of photosynthesis. Thus, the interaction of wheat with growth-stimulating bacteria of the strain DA1.2 improved plant status compared to plants untreated with bacteria under stress conditions.

Taking into account that under combined stress root mass of plant of both variants (treated and untreated with bacteria) remained equally low compared to the control, we evaluated possible involvement of hormonal regulation in the detected growth responses. The herbicide treatment (herbicide from the class of synthetic auxins) led to the accumulation of IAA in shoots by more than 2.5 times, while bacterial treatment significantly increased root IAA content (Figure 4 A). We suggested that this could be due to the ability of plants to absorb exogenous auxins. Otherwise, the herbicide could influence the content of endogenous IAA (Kudoyarova et al., 2017). This assumption does not contradict the existing reports (Sung, 1979). The observed imbalance in the auxin distribution between the shoot and root caused by the herbicide may be the reason of the decrease in resistance of plants to drought. And accumulation of aux-

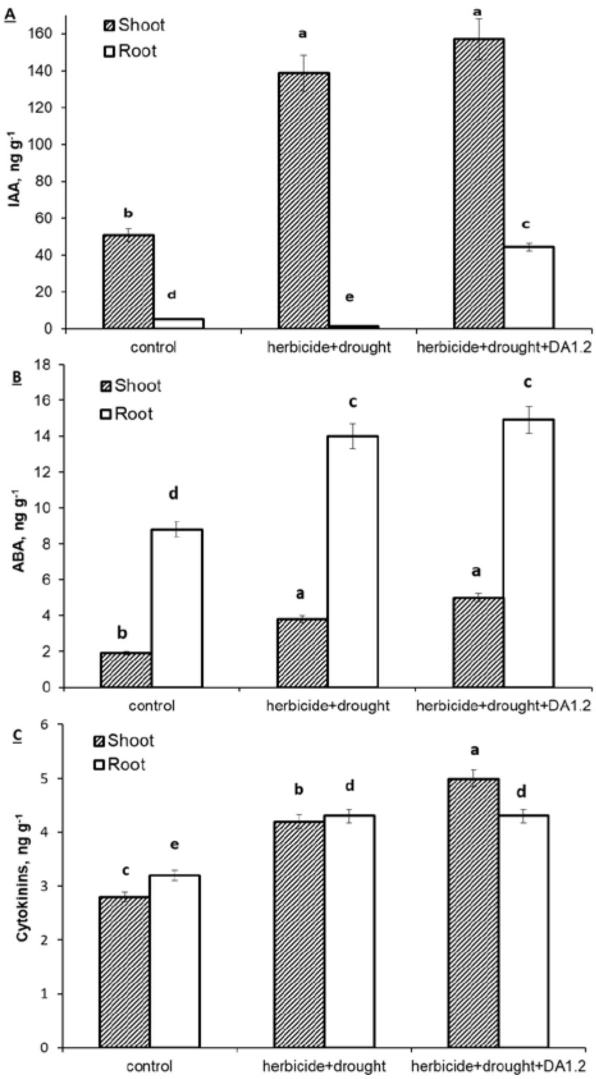

Figure 4: The content of IAA (A), ABA (B) and cytokinins (C) (calculated per g fresh mass) in shoots and roots wheat plants 3 days after treatment. Significantly different means (n = 9) are labelled with different letters (ANOVA, LSD, $p \leq 0.05$)

ins under the influence of bacteria in the roots serves as one of the mechanisms responsible for improving plant growth, plant status when treated with an herbicide under drought conditions.

Accumulation of ABA causes stomatal closure under conditions of water scarcity (Zhang et al., 2006; Leung et al., 2012). The decrease in cell turgor during dehydration dramatically activates synthesis of ABA, which accumulates mainly in the chloroplasts of leaf cells (Zhou et al., 2006; Venzhik et al., 2016). Accumulation of ABA in plants under stress was revealed. It was most strongly manifested in the roots, which could be the result of inhibition of their growth (Figure 4 B). As for cytokinins, stress led to an increase in their content (Figure 4 C), and with bacterial introduction, we observed the greatest accumulation of cytokinins in the shoot. Cytokinins are known to be essential for shoot growth (Martin et al., 2000), inhibit root growth (Werner et al., 2010), and are ABA antagonists (Dodd, 2005). Accumulation of cytokinins under the studied stress could compensate for the growth-inhibiting effects of the increased level of ABA, which were most pronounced in plants treated with bacteria.

4 CONCLUSIONS

It was shown that the herbicide-induced imbalance in the auxin distribution between shoot and root in plants may be the cause of a decrease in drought resistance. The bacterial suspension had a positive effect on the wheat plants treated with the auxinic herbicide under drought conditions, it was expressed in a decrease in the content of biochemical stress markers MDA and proline, preventing plant growth inhibition and normalizing the content of chlorophylls. These positive effects of growth-stimulating bacteria on wheat plants under combined stress caused by drought and herbicide treatment have been described for the first time. Thus, bacterial suspension of the strain *Pseudomonas protegens* DA1.2 can be considered as the basis of biological product for increasing plant tolerance.

5 ACKNOWLEDGMENTS

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