The Influence of Inoculation with *Streptomyces Albogriseolus*-89 on Durum Wheat (*T. Durum* Desf.) Growth and Development

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Received:- 07 June 2023/ Revised:- 13 June 2023/ Accepted:- 20 June 2023/ Published: 30-06-2023

Abstract— *The influence of actinomycete strain Streptomyces albogriseolus-89 (isolated from soils of Khevi region, Stepantsminda, Georgia) on growth and development, as well as the content of metabolites (chlorophylls, carotenoids, anthocyanins, ascorbic acid, proline, soluble phenols, total proteins, soluble carbohydrates) and peroxidase activity in leaves of durum wheat (T. durum Desf.) has been studied. It was established that inoculation of wheat seeds with the strain has stimulated stem and ear growth, positively affected protein content (raised by 12%) and peroxidase activity (increased by 1.3 times) as well as enhanced the yield and resistance of plant to biotic and abiotic stresses*.

Keywords— ***antioxidants, durum wheat, inoculation, Streptomyces albogriseolus-89.***

I. INTRODUCTION

Food supply of the world population is an ecological problem of an international scale. Raising the yield of food crops is one of the ways of the problem's salvation. For this purpose, environmentally safe alternative to chemical fertilizers - the biofertilizers, which are biopreparations made from living microorganisms, are successfully used in agriculture today. After the treatment of seeds or underground parts of the plant with such biofertilizer, the microorganisms of the biopreparation settle on the rhizosphere or inside of the plant and reveal a visible effect on its growth and development; this allows obtaining ecologically safe harvest along with the increase in productivity. In addition to direct effect, microorganisms reveal indirect influence on plant, protecting it from damage caused by various pathogens or abiotic stressors (Miliute et al., 2015). Accordingly, the demand for microbial inoculants and biofertilizers has increased significantly in recent decades. (Thomas, Singh, 2019; Umesha et al., 2018).

Among the promising microorganisms applied in agriculture are actinomycetes, which are producers of many commercially important metabolites, like enzymes, hormones, or antibiotics (Nagendran et al., 2021). Both the rhizosphere and tissues of plants are abundantly populated with actinomycetes. The secondary metabolites produced by these microorganisms improve soil fertility and plant mineral supply, as well as protect the plant from phytopathogens and increase its stress resistance (Silva et al., 2022).

Despite the great biotechnological potential of actinomycetes, the share of products based on these microorganisms on the world market is very small (Silva et al., 2022).

The purpose of the presented study was to investigate the effect of inoculation of durum wheat (*T. durum* Desf.) seeds with the local actinomycete strain - *Streptomyces albogriseolus-89* on the growth and development of the plant, and on the content of metabolites (chlorophylls, carotenoids, anthocyanins, ascorbic acid, proline, soluble phenols, total proteins, soluble carbohydrates) as well as peroxidase activity in leaves.

The strain *Streptomyces albogriseolus-89* was chosen as a test object because of its positive characteristics: high activity of prostaglandin synthetase and the active synthesis of polyunsaturated fatty acids. In addition, its antagonistic activity against a number of microorganisms, including plant pathogens such as: *Elitrosporangium brasiliense, agrobacterium tumefaciens, Aspergillus niger, Pectobacterium aroideae, Xanthomonas campestris,* has been established (Mamulashvili, 1995).

II. MATERIALS AND METHODS

2.1 Research area

The field trial was conducted on the experimental plot of the National Botanical Garden of Georgia, which is located at 700-800m above sea level; soils here are dark-brown, dry, loose, fine-grained, loamy (Urushadze, Blum, 2013). The weather is moderately warm, transitioning from steppe to moderately humid subtropical; according to general features - subtropical-semiarid (with elements of sub-Mediterranean climate). Winter is moderately cold, summer is hot. The average annual temperature is 12.7°C, the average temperature in January - +0.9°C, and in July - +24.4°C; The absolute minimum temperature -23°C, the absolute maximum - +40°C. The average annual amount of precipitations is 560 mm (Elizbarashvili, 2017).

Durum wheat (*T. durum* Desf.) seeds were provided by the Genetic Resources Laboratory of the Institute of Botany, of Ilia State University.

The actinomycete strain *Streptomyces albogriseolus-89* was taken from the collection of the Department of Microbiology of the Institute of Botany of Ilia State University. The strain was isolated from the soils of Stepantsminda district - Khevi.

Cultivation of *Streptomyces albogriseolus-89* was carried out on solid synthetic area of Krasilnikov (CP-I), for 7 days at 28°C in a thermostat (Zvyagintsev, 1991; Pandey et al., 2011). The microbial biomass obtained by cultivation was scraped off the surface of the nutrient medium and dissolved in water at the ratio: 1 g of biomass: 100 ml of distillate. Seeds, for inoculation, were kept in this suspension for 24 hours before sowing.

Both control (untreated) and experimental (inoculated) seeds were sown in triplicate on $4m^2$ (2mx2m) plots (1000 seeds per plot). During the vegetation period, the height and ear length of the test plants were measured; at the end of vegetation the weight of one thousand grains was recorded. The study of metabolites in wheat leaves during the stem elongation phase was carried out by methods described below. For experiments were taken mature, fully expanded leaves of the same age. Each index was determined in triplicate.

2.2 Biochemical assays

2.2.1 Plastid pigments

Chlorophyls and carotenoids were determined spectrophotometricaly. Fresh leaves (100-200mg) were mashed with sand and $CaCO_3$ and washed with ethanol. Optical density of the filtrate was measured (spectrophotometer SPEKOL 11, KARL ZEISS, Germany). Concentration of chlorophylls a and b, also carotenoides was calculated by the formula of Wintermanns (Gavrilenko et al., 1975).

2.2.2 Anthocyanins

100mg of grinded leaves were added with 20 ml of 96% acidified (with 1% HCl) ethanol (99:1). After 24h retention in dark the optical density at 540nm was measured (spectrophotometer SPEKOL 11, KARL ZEISS, Germany) (Ermakov, 1987).

2.2.3 Ascorbic acid

A titration method was used to measure the content of ascorbic acid in plant material. 2 g of fresh leaves were mashed in 15 ml of 2% hydrochloric acid and 10 ml of 2% metaphosphoric acid, and filtered. One ml of the filtrate was added to 25 ml of distilled water and titrated with a 0.001 M solution of dichlorphenolindophenole (Ermakov, 1987).

2.2.4 Proline

0.5 g of dry leaves were mashed in 10ml of 3% sulphosalicylic acid and filtered. 2 ml of the filtrate was added to 2 ml of acid ninhydrin and 2 ml of ice acetic acid. After 1 h exposition on a water bath the extract was cooled and added with 4 ml of toluene and divided in a separating funnel. Optical density of upper layer was measured on a spectrophotometer (SPEKOL 11, KARL ZEISS, Germany) at 520 nm (Bates et al., 1973).

2.2.5 Total phenols

A 0.5 g of fresh leaves was boiled in 80% ethanol for 15 min. After centrifugation the supernatant was saved, and residues of leaves were mashed in 60% ethanol and boiled for 10 min. Obtained extract was added to the first supernatant and evaporated.

The sediment was dissolved in distilled water. One ml of the received solution was added with the Folin-Ciocalteu reagent and optical density was measured at 765 nm. The chlorogenic acid served as control (Ferraris et al., 1987).

2.2.6 Total protein assay

Content of proteins was determined after Lowry (1951).

2.2.7 Soluble carbohydrates

Content of soluble carbohydrates was tested with anthrone reagent (Turkina and Sokolova, 1971). To 100mg of air-dry leaf material was added 96° alcohol for extraction (3-fold). The total amount of the obtained extract was evaporated on a water bath and dissolved in 5ml of distilled water. To 0.5ml of the tested water extract was added 2ml of anthrone reagent and heated in a water bath for 10min. After this procedure the test-tubes were placed in a cold water bath and 15min later the optical density of the solution was measured at 620nm with a spectrophotometer (SPECOL 11, KARL ZEISS, Germany).

2.2.8 Enzyme assay

Peroxidase activity was determined spectrophotometrically: optical density of the products of guaiacol oxidation was measured at the wave length of 470nm by the spectrophotometer (SPEKOL 11, KARL ZEISS, Germany) (Ermakov, 1987).

2.2.9 Statistical processing of data

One way ANOVA and Tukey's multiple comparison tests were used to test differences between the means. All calculations were performed using statistical software Sigma Plot 14.5.

III. RESULTS AND DISCUSSION

Actinomycete inoculation of wheat seeds stimulated both stem and ear growth in test plants (p=0.01) (Fig. 1). The weight of one thousand grains in the experimental variant was also statistically higher, compared to the control (p=0.04); i.e. Seed inoculation with *Streptomyces albogriseolus-89* has a stimulating effect on plant growth and yield (Fig. 1).

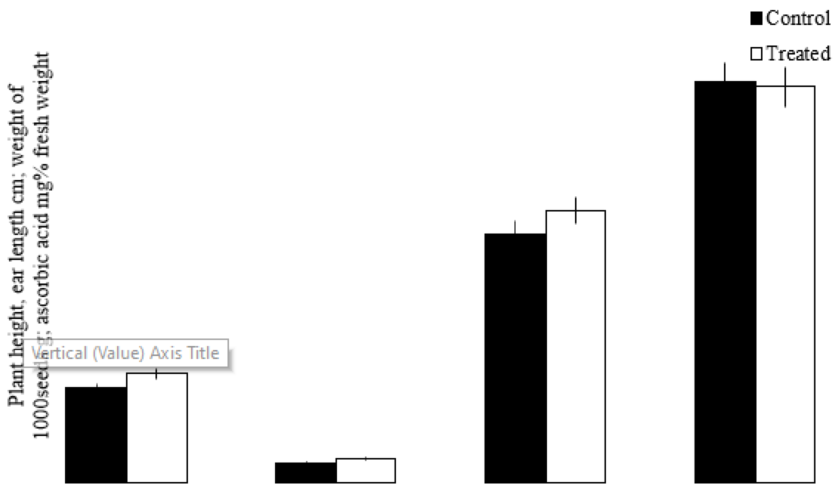

FIGURE 1: Influence of durum wheat (*T. durum* Desf.) seeds inoculation with Streptomyces *albogriseolus-89* on stem and ear length, weight of 1000seeds and leaf ascorbic acid content

It is generally known that the positive effect of plant growth-stimulating actinomycetes on the host plant is due to two main reasons: they supply the plant with growth-stimulating hormones and improve mineral uptake, be it nitrogen fixation, phosphorus solubilization, or siderophores formation (Silva et al., 2022). The stimulating effect of the used strain on the growth and development of test plants may be explained by the similar reason. Particularly which hormone or siderophore is produced by *Streptomyces albogriseolus-89* is a matter for future research.

A powerful photosynthetic apparatus is one of the principal conditions for high plant productivity (Stasik et al., 2016). The efficiency of the photosynthetic apparatus is evaluated according to the amount of chlorophylls and carotenoids (Lichtenthaller, Buschmann, 2001). The study of plastid pigments in leaves of tested plants revealed the statistical similarity (p=0.1) between the experimental and control variants; while the content of carotenoids in the actinomycete-treated plants decreased by 18% (p=0.01) (Fig. 2).

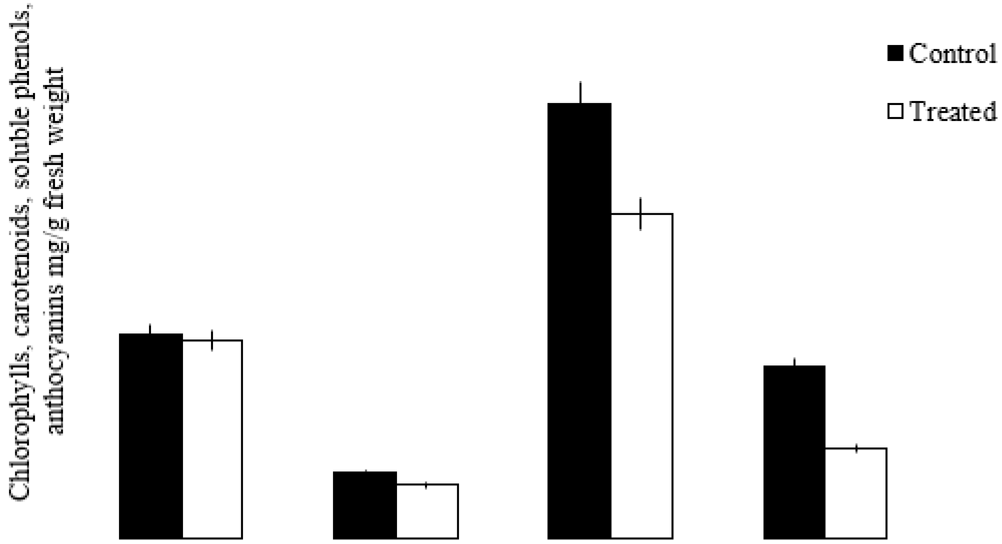

FIGURE 2: Influence of durum wheat (*T. durum* Desf.) seeds inoculation with Streptomyces *albogriseolus-89* on leaf chlorophylls, carotenoids, anthocyanins and soluble phenols content

By the content of carotenoids as antioxidant compounds, it is possible to judge the degree of stress, affecting the plant as well as the degree of plant resistance to this stress (Strzalka et al., 2003). Decrease in carotenoids in inoculated plants can be considered as an expression of the positive effect of inoculation. In hot summer, when the temperature reaches 40°C, which is combined with strong insolation and water deficit, the plants appear under severe stress. If under such conditions, inoculation with actinomycete leads to the reduction of carotenoids as protective antioxidant compounds, it should be assumed that inoculation caused alleviation of the stress negative effect on the expense of microorganism-produced metabolites.

It is known that plants have evolved physiological and biochemical mechanisms of stress resistance. Among them the antioxidant system represented by enzymes and low-molecular antioxidant compounds is particularly important (Li, and Liu, 2016; Laxa et al., 2019). Most of the metabolites studied in experimental plants are antioxidants. We thought that their quantitative study would give some idea about the influence of microbial origin compounds on the physiological state of the plant under the stressful summer conditions (high temperature, intensive insolation, lack of water).

It is established that ascorbic acid plays one of the main roles in protecting the photosynthetic apparatus from stress (Venkatesh, et al., 2014). Under stress conditions its content increases in plants (Yang et al., 2008). From the obtained results it is clear that the content of ascorbate in leaves of inoculated plants was statistically similar to the control variants ($p>0.05$) (Fig. 1).

Phenolic compounds play an important stress-protecting role in cell (Winkel-Shirley, 2002; Cesar, Fraga, 2010). A number of studies have shown the accumulation of phenols in plants under stress conditions (Sharma et al., 2019). A 33% reduction of these compounds was mentioned in the inoculated plants compared to the control ($p=0.02$) (Fig. 2).

One group of phenolic compounds - anthocyanins, acts as a light screen and protects chlorophyll from damage by excess light (Gould et al., 2018). It has been established that under various stress conditions, the accumulation of anthocyanins in vegetative tissue increases (Gould et al., 2018; Kamjad, et al, 2021). In our experiments the content of anthocyanins in the inoculated variants decreased by 1.9 times compared to the control ($p=0.04$) (Fig. 2). It should be assumed that the reduction of both soluble phenols and anthocyanins in actinomycete-treated plants may be due to the positive effect of actinomycete's metabolites.

It is well known that so-called osmoprotectants are also involved in plant protection against stress. Among them are free amino acids, soluble carbohydrates and proteins. They protect the membrane protein-lipid components from various stresses (Iqbal et al., 2020).

Amino acid proline and soluble carbohydrates are osmoprotectants that retain water in the cell and keep its turgor. Their positive role in plant resistance to water deficit has been emphasized in many works. (Ashraf et al., 2018; Laxa et al., 2019). At the same time, soluble carbohydrates reliably protect proteins from denaturation and provide membrane stability (Mohammadkhani and Heidari, 2008).

Obtained results clearly demonstrate that the content of both proline and soluble sugars in leaves of inoculated plants was statistically identical to the control variants (p=0.2). It can be assumed that the compounds produced by the inoculum do not significantly affect the metabolism of osmolytes (Fig. 3).

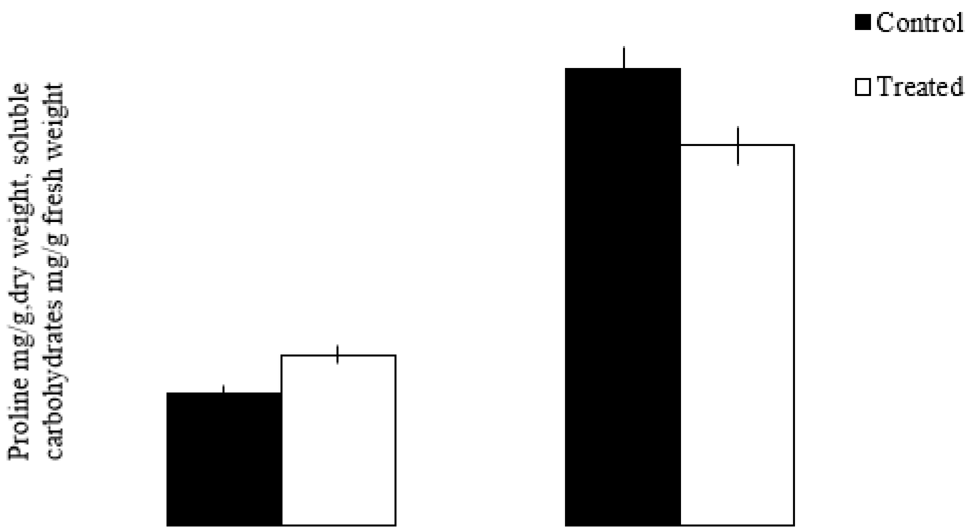

FIGURE 3: Influence of durum wheat (*T. durum* Desf.) seeds inoculation with Streptomyces *albogriseolus-89* on leaf proline and soluble carbohydrates content

Inoculation of wheat seeds with *Streptomyces albogriseolus-89* positively affected the protein content in leaves of experimental plants - this index increased by 12% (p=0.04) (Fig. 4).

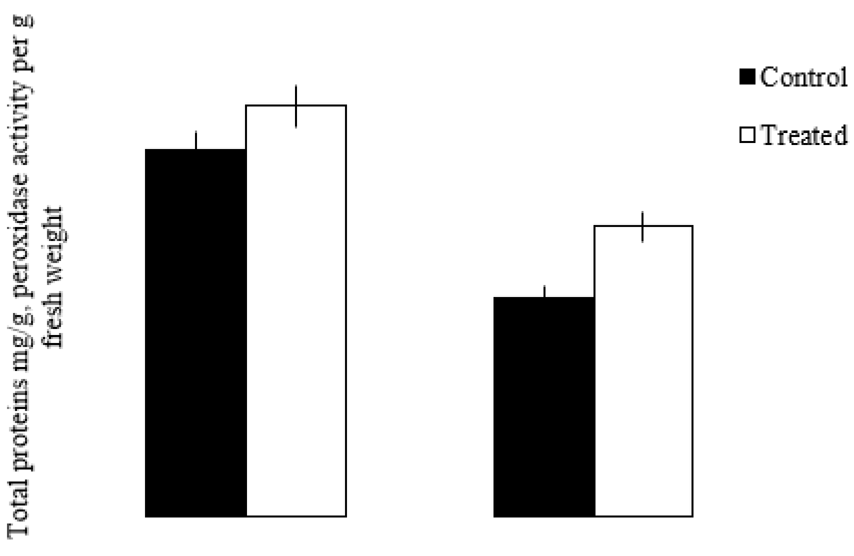

FIGURE 4: Influence of durum wheat (*T. durum* Desf.) seeds inoculation with Streptomyces *albogriseolus-89* on leaf peroxidase activity and total proteins content

It is known that the qualitative and quantitative composition of plant proteins changes under unfavorable conditions; The synthesis of stress proteins - dehydrins is activated, which act like osmolytes and participate in the stabilization of membrane proteins and osmotic regulation (Iqbal et al., 2020; Mohammadkhani and Heidari, 2008). Experimental results demonstrate a positive effect of microbial compounds on protein synthesis, which contributes to the plant's resistance to adverse environmental conditions. A 1.3-fold activation of antioxidant enzyme - peroxidase in inoculated leaves (p=0.003) can be considered as proof to this (Fig. 4).

Peroxidases (EC 1.11.1) are a large group of enzymes, ubiquitous in the cell and have various functions in plant metabolism (Passardi et al., 2005). In particular, they take an active part in plant growth and development, regulation of auxins and phenolic compounds amount, adaptation to unfavorable conditions, etc. (Graskova et al., 2010). It has been established that the activity of antioxidant enzymes, including peroxidases, is high in drought-resistant plants (Laxa et al., 2019; Kapoor et al., 2020).

The activation of peroxidase in leaves of inoculated plants should be considered as an expression of positive effect of *Streptomyces albogriseolus-89*, supporting plant resistance to stress.

IV. CONCLUSIONS

Obtained results clearly demonstrate that the inoculation of wheat seeds with *Streptomyces albogriseolus-89* strain has a stimulating effect on plant growth and development, increases its productivity and resistance to biotic and abiotic stresses. Accordingly, the potential of the strain may be used to make a plant growth stimulating preparation; for this purpose additional research is planned in future.

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