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EFFECT OF FOLIAR BIOACTIVE TREATMENTS ON THE OXIDATIVE STRESS TOLERANCE IN TOMATO SEEDLINGS

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Abstract

Under the current climate changes, enhancing temperature is now considered to be one of the major abiotic stresses inducing negative effects on plant growth as results of increased production of toxic reactive oxygen species. The tolerance of stress is correlated with higher activities of antioxidant defense enzymes which are activated to prevent oxidative damage. On the other hand, the use of biofertilizers appears to be involved in increase of tolerance to various abiotic stresses, besides their important role in regulating plant growth, development and fruiting. Influence of environmental conditions and of some growth regulators treatments (Spraygard 1%, Razormin 0.1%, BAC Foliar spray 0.3% and BIO Roots 0.2%) on the chlorophylls and carotenoids content and on the activity of peroxidase and catalase have been investigated in leaves of tomato seedlings. Analysis of the obtained data emphasize the potential of growth regulators in enhancing the resistance to abiotic stresses by protecting the photosynthetic apparatus and improving the activity of antioxidant enzymes system.

Key words*: carotenoids, catalase, chlorophylls, heat stress, peroxidase.*

INTRODUCTION

Tomato (*Solanum lycopersicum*) is considered one of the most important vegetable whose production is constantly growing since its fruits are widely consumed either fresh or processed. Beside the high nutritional value, the ripe tomato fruits are a valuable source of vitamin C, lycopene, carotenoids and minerals such as iron and phosphorous that are daily required for a healthy diet (Nour et al., 2013). At present, the tomatoes acquired great popularity among consumers, being considered a protective food since the discovery that lycopene has anti-oxidative and anti-cancer properties (Gajowik, 2014; Raiola et al., 2014).

Although tomato plants can grow under a wide range of climatic conditions, they are extremely sensitive to growing conditions: high temperature (both day and night), humidity, rainfall and light intensity are the limiting factors of tomato production (Ahmad, 2002).

Temperature stress induces negative effects on plant growth and metabolism, so high temperature is now considered to be one of the major abiotic stresses causing yield reduction in crops (Hasanuzzaman et al., 2012).

Recently some countries practice tomato growing even at high temperature through application of plant growth regulators. Several authors reported that application of substances like auxin, gibberellic acid, synthetic auxin 4-CPA (4-chloro phenoxy acetic acid) have resulted in good tomato production under adverse environmental conditions (Sasaki et al., 2005; Gemici et al., 2006; Khan et al., 2006; Poliquit et al., 2007; Gelmesa et al., 2012). Also good results in improving freezing resistance by growth regulators as Ruter AA, Terra Sorb and Razormin were noticed in experiment with winter wheat cultures (Gaveliene et al., 2016).

Besides their important role in improvement of nutritional quality of food crops and their efficiency to regulate plant growth, development, fruiting and senescence (El-Rokiek et al., 2012), it seems to be involved in induction of tolerance to various abiotic stresses (Salehi et al., 2011; Gaveliene et al., 2016).

Plants exposed to extreme temperatures activated the self-defense mechanisms including several non-enzymatic and enzymatic antioxidants (α-tocopherol, carotenoids, chlorophylls, glutathione, ascorbic acid, oxidases such as catalase, peroxidase, polyphenol peroxidase, superoxide dismutase) to prevent oxidative damage (Vranova et al., 2002; Torres-Barceló et al., 2013). Some scientific works reported that higher activities of antioxidant defense enzymes are correlated with higher stress tolerance (Almeselmani et al., 2006; Babu et al., 2008; Almeselmani et al., 2009).

This study was aimed to investigate the effect of growth regulators containing free amino acids, macro- and micro-elements (Razormin, Spraygard, BAC Foliar spray, Bio Roots) used as foliar treatments on tomato seedlings and the comparative results were studied. The effect of the bioregulators on antioxidant enzymes and photosynthetic pigments in tomato plant is less known, so that the influence of environmental conditions and of some foliar treatments on biochemical parameters (chlorophylls and carotenoids content, activity of peroxidase and catalase) has been investigated in leaves of tomato seedlings.

MATERIALS AND METHODS

The experiment was installed into an experimental greenhouse of the Hortinvest Research Centre - USAMV Bucharest. The main objective was testing the effects of applications of simple growth regulators Razormin 0.1%, Spraygard 1%, BAC Foliar spray 0.3% and BIO Roots 0.2% on heat stressed and unstressed tomato seedlings in two distinct stages: one week, respectively two weeks after transplant operation.

Razormin is a mixture of growth factors (amino acids, polysaccharides, macro and micro-nutrients), which induces development of the root system, stimulate the nutrients absorption, increases vegetative mass and quality of production.

Spraygard is a complex product that acts as safener, penetrant, dispersant, creates adhesion of the treatment solutions on the leaves.

BAC Foliar is a foliar organic nutrient which stimulates chlorophyll production in the leaves.

Bio Roots is a natural root growth supplement which contains vitamins, enzymes, organic and humic acids that helps plants establish healthy and vibrant roots.

Experimental variants were noted:

T0 - unstressed control;

T1 - tomato seedlings exposed at heat stress in absence of growth regulators treatment;

T2 - tomato seedlings exposed at heat stress and treated with Spraygard 1%;

T3 - tomato seedlings exposed at heat stress and treated with Razormin 0.1%;

T4 - tomato seedlings exposed at heat stress and treated with BAC Foliar spray 0.3%;

T5 - tomato seedlings exposed at heat stress and treated with BIO Roots 0.2%.

Specific agrotechnics for transplant nursery was applied during the growth period: daily ventilation, watering, weeding. The unstressed control variant was maintained at the temperature of 22-26°C at day and 18-20°C at night. For the heat stressed variants, the temperature was not controlled and registered the following variations:

- the maximum effective temperature average for April in the greenhouse was 31.8°C and the minimum effective temperature average at 24.6°C;
- the maximum effective temperature average for May in the greenhouse was 37.4°C and the minimum effective temperature average at 25.2°C.

The biochemical determinations in the active leaves were performed at the end of the experiment (after 27 days), when most of seedlings have reached the optimum for a succesfull planting.

In order to estimate the oxidative stress occurred on cell level, characteristic parameters were analyzed, such as proteins content, specific activities of catalase as well as peroxidase, assimilatory pigments content using appropriate methods of analysis.

- ***The proteins content*** was determined by Lowry method, which is based on the reactivity of the peptide nitrogen with the copper [II] ions under alkaline conditions and the subsequent reduction of the Folin-Ciocalteu phosphomolybdic-phosphotungstic acid to heteropoly-molybdenum blue by the copper-catalyzed oxidation of aromatic aminoacids (Lowry et al., 1951). The results were expressed in g/100 g fresh weight.
- ***The activity of peroxidase*** was determined by spectrophotometric measuring of the speed of colour achievement at 436 nm

and 25°C in the decomposition reaction of hydrogen peroxide with guaiacol as hydrogen donor, which is catalyzed by peroxidase (Bergmayer, 1974).

$$4 \text{ guaiacol} + 4\, H_2O_2 \xrightarrow{\text{peroxidase}} \text{tetraguaiacol} + 8H_2O$$

The enzymatic unit: the amount of enzyme which catalyzed the transformation of one micromole of hydrogen peroxide/minute at 25°C.

- ***The activity of catalase*** was determined with method essentially described by Beers and Sizer (1952), in which the disappearance of peroxide is followed spectrophotometrically at 240 nm.

The enzymatic unit: one unit decomposes one micromole of H_2O_2 per minute at 25°C and pH 7.0 under the specified conditions.

- ***Determinations of the assimilatory pigments content*** in the active leaves**:** *chlorophyll* and *carotenoid pigments* were extracted in 80% acetone and the absorbance of the extract was measured at three wavelengths (663 nm, 647 nm and 480 nm) with an UV/Visible ThermoSpectronic Helios spectrophotometer. The results were calculated using the extinction coefficients and equations described by Schopfer (1989) and were expressed in mg/100 g fresh weight (FW).

RESULTS AND DISCUSSIONS

Temperature is a major factor affecting the rate of plant development. Warmer temperatures expected with climate change will affect the physiological processes in plants, therefore the plant productivity. Some adaptation strategies are available to manage with temperature extremes depending on the plant species, being genetically determinate. Beside this, application of some bioregulators is expected to increase tolerance to high temperature stress. So analyze of assimilatory pigments and peroxidase and catalase activity as oxidative stress markers was performed.

Determination of assimilatory pigments content

***Chlorophylls* a *and* b** represent the major photosynthetic pigments in plants, playing an important role in the photochemical reactions involved in photosynthesis (Taiz and Zeiger, 2009), while carotenoids are considered as accessory components in the photosynthetic complex by providing photoprotection and stability of proteins present in the photosystem (Torres-Netto et al., 2005; Simkin et al. 2008).

Photosynthesis, one of the most heat sensitive processes, can be completely inhibited by high temperature possibly as a result of structural and functional disruptions of chloroplasts and reduction of chlorophyll accumulation under high temperature stress (Camejo et al., 2001; Dekov et al., 2000).

The determinations performed on the tomato seedlings showed that high temperature affected both chlorophylls *a* and *b*, therefore the total chlorophyll content. A decrease of about 40% was registered in the untreated plants exposed to heat stress (T1) compared to the unexposed control plants (T0) (Figure 1).

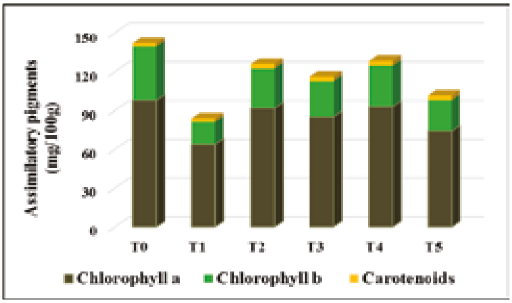

Figure 1. Assimilatory pigments content in the experimental variants

The effect of high temperature exposure on chlorophyll content has been extensively studied and similar results were reported at some tomato cultivars (Berova et al., 2009) and also in other plants as *Triticum aestivum* (Tewari et al., 1998; Almeselmani et al., 2012), *Festuca arudinacea* (Cui et al., 2006), *Solanum* ssp. (Aien et al., 2011). Other authors have found an increase in chlorophyll *a* content in lemon stressed plants with high temperatures (Martin et al., 1995).

A higher reducing in chlorophyll *b* content compared to chlorophyll *a* content (Table 1) was registered, so the chlorophyll *a*/*b* ratio registered an increase in the stressed plants compared with control plants, in according to the data reported by other authors (Cui et al., 2006; Zhu et al., 2011).

Table 1. Values and ratio of assimilatory pigments in the experimental variants

Variants	Chlorophyll (a+b) (mg/100 g FW)	Carotenoids (mg/100 g FW)	Chlorophyll (a+b)/ carotenoids	Chlorophyll a/b
T0	139.66	2.52	55.42	2.34
T1	81.25	2.66	30.55	3.70
T2	122.67	3.45	35.56	2.96
T3	112.68	3.57	31.56	3.08
T4	124.67	3.77	33.07	2.92
T5	98.06	3.71	26.43	3.12

Scientific studies reported that a 10-15°C increase over normal growth temperature results in degradation of chlorophyll and thus affecting photosynthesis process. The reasons for decreasing in photosynthetic pigments under high temperature may be attributed to the inhibition of biosynthesis, changes in ultrastructure of chloroplast and photo-deterioration (Tewari et al., 1998; Reda et al., 2011).

Observations and measurements performed on the experimental variants under growth regulators treatment showed a smaller diminution of total chlorophyll content in stressed plants: about 11% in the plants treated with BAC Foliar (T4) and Spraygard (T2) compared to the unexposed control plants (T0). Generally, it seems that the growth regulators treatment induced a better accumulation of chlorophylls in the tomato leaves.

Carotenoids are an important class of antioxidants which play a major role in the protection of plants against photo-oxidative processes (Stahl et al., 2003; Gramza-Michalowska et al., 2010). Therefore, maintaining a higher or invariable level of total carotenoids during stressful conditions may induces some stress tolerance of the plants (Loggini, 1999; Logan et al., 1996; Ruban, 1999). Carotenoids destruction through oxidation may reduce efficiency of the antioxidant defense system (Chedea et al., 2013).

A slight increase of the carotenoids content was registered as result of heat exposure of the tomato seedlings (Table 1): 2.66 mg/100 g FW carotenoids determined in stressed control in absence of treatment (T1) compared to 2.52 mg/100 g FW carotenoids in unstressed control. Also a decrease of *chlorophyll (a+b)/carotenoids* ratio was registered in the stressed plants (Table 1). Previous studies reported lower *chlorophyll (a+b)/carotenoid* ratio in two heat stressed cultivars of *Festuca arudinacea* in relation to the control plants (Cui et al., 2006) and carotenoids amounts increased in *Populus cathayana* cuttings exposed under moderate stress conditions (Xiao et al., 2008).

However, the increase of carotenoids amounts was more pronounced in the stressed tomato plants under growth regulators treatment (between 3.45 mg/100 g FW in Spraygard treated variant and 3.77 mg/100 g FW in BAC Foliar treated variant) compared with untreated control (2.66 mg/100 g FW at T1). Similar observations of bioregulators treatment increasing carotenoids content were reported in some species of pepper plants (*Capsicum annuum* var. *grossum*, *Capsicum annuum* var. *accuminatum)*, in eggplant seedlings (Balan et al., 2018), while higher bioregulator concentrations impacted negatively on carotenoid content in *Capsicum chinense* plants (Olaiya et al., 2013), also in *Triticum aestivum* plants (Sahu et al., 2011). Total carotenoid content did not change with stress conditions in tomato cv. Amalia, while increased in Nagcarlang cv. (Camejo, 2001). This results suggests that the response to treatments with the bioregulators is probably genetically determined.

It is well documented that carotenoids act as antioxidant compounds involved in protection of photosynthetic systems, therefore a higher level of total carotenoids support the plant to tolerate the stressful condition. These results are in agreement with previous studies related to plant acclimation to stress (Loggini et al., 1999; Ruban et al., 1999).

Determination of content in proteins

Proteins content analyzed in the leaves of tomato seedlings showed that heat exposure induced an increase, so that higher values of this parameter were registered in the stressed plants (1.37 g %) in absence of bioregulators treatment compared with the unstressed control (1.25 g %) (Figure 2). However, the increase of protein content was more pronounced in the leaves of the tomato treated with growth regulators (between 1.57-2.15 g %) in comparison to untreated stressed control (1.37 g %), BAC Foliar treated variant (T4) reaching the highest value of proteins amount (2.15 g %). Previous studies noticed that an increase in protein content might suggest a change in the gene expression that would be associated with a possible thermotolerance and acclimatization to stress condition (Camejo et al., 2001).

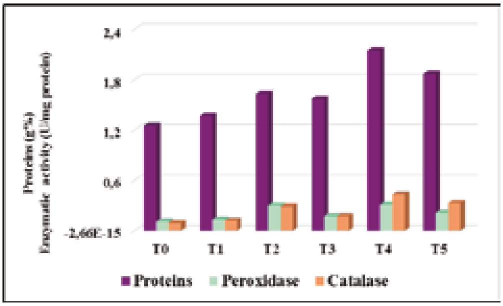

Figure 2. Enzymatic activity and proteins content in the experimental variants

Determination of enzymatic activity in the seedlings leaves

There are numerous previous studies which indicate that the tolerance to temperature stress in plants may be positively correlated with an increase in antioxidants content (Babu et al., 2008; Almeselmani et al., 2009; Hasanuzzaman et al., 2013).

A slight increased activity of ***peroxidase*** was registered in the untreated tomato seedlings under heat stress (0.13 U/mg protein) compared with control (0.11 U/mg protein), but response to heat stress was amplified in the tomato seedlings under growth regulators treatment by enhancing the peroxidase activity (between 0.17 U/mg protein in Razormin variant and 0.31 U/mg in BAC Foliar variant).

Catalase activity follows the same dynamics as peroxidase: 0.09 U/mg protein in control tomato seedlings, which increased at 0.12 U/mg protein in absence of bioregulators. Also the catalasic activity increased in the stressed plants treated with growth regulators. Higher catalase activities were registered in the tomato under treatment with roots stimulator BIO Roots (0.33 U/mg protein) and with BAC Foliar (0.43 U/mg protein).

Other authors also reported that some treatments of plants with plant growth regulators showed positive effects on oxidases activities. For example, methyl jasmonate-treated raspberries, strawberries and blueberries showed higher activities of peroxidase and superoxide dismutase (Chanjirakul et al., 2006).

Previous studies documented that the activation of protein synthesis in plants in combination with increase of oxidases activity under stress conditions may be the result of metabolism conversion in order to obtaining a good tolerance of plant to stressful conditions. (Tucic et al., 2007; Chkhubianishvili et al., 2011; Wu et al., 2014).

CONCLUSIONS

The researches performed on the tomato seedlings showed that high temperature affected both chlorophylls *a* and *b*, therefore the total chlorophyll content. At the same time it seems that the growth regulators induced a better accumulation of chlorophylls in the tomato leaves since a smaller diminution of total chlorophyll content in stressed tomato plant was noticed under growth regulators treatment.

A slight increase of the carotenoids content in the control plant occured as result of heat exposure but higher values of this parameter were determined in the stressed tomato plants under influence of growth regulators treatment.

Also the protein content and the oxidases activity were enhanced under high temperature conditions, mostly in the tomato seedlings treated with growth regulators.

An overview of the researches performed on the tomato seedlings allows us to conclude that growth regulators treatments determined an increased plant capacity to face the effects of heat stress by protecting the photosynthetic apparatus and enhancing antioxidant enzyme systems. Good results in this sense were obtained mainly with BAC Foliar spray and Razormin, which proved the best ameliorative effect under heat stress conditions.

However, further studies should be considered in order to asses possible combined treatments with several growth regulators to achieve optimum effects in improving the plants growth and productivity under environmental stress in the conditions of global climate change.

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