

BIOSTIMULANT EFFECTS OF REGLALG ON BIOCHEMICAL PARAMETERS OF HORTICULTURE PLANTS

EPECTELE BIOSTIMULANTE ALE REGLALGULUI ASUPRA PARAMETRILOR BIOCHIMICI AI PLANTELOR HORTICOLE

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***Abstract.** In this study, data are reported on the biostimulant pre-germination treatment effects of plant growth regulator (PGR) Reglalg on cucumber seeds germination, plant growth parameters, and biochemical indices, including catalase and peroxidase activity, on the total polyphenols content and total antioxidant activity in the roots. Spraying with Reglalg tomato plants under field conditions during the season also demonstrated a beneficial effect on plant growth and development, leaf chitinase activity, for both early and late repining cultivars. Soaking cucumber seeds in Reglalg and spraying with it tomato plants during vegetation represents a promising method of applying Reglalg to horticulture crops.*

Key words: PGR Reglalg, *Cucumis sativus* L., *Solanum lycopersicum* L., biochemical parameters

***Rezumat.** În acest studiu se aduc date despre efectele biostimulante ale regulatorului de creștere a plantelor (RCP) Reglalg, utilizat la tratarea semințelor de castravete înainte de germinare, asupra germinării semințelor, parametrilor de creștere a plantulelor, precum și asupra indicilor biochimici, inclusiv activitatea catalazei, peroxidazei, conținutului total de polifenoli și a activității antioxidante totale din rădăcini. De asemenea, este demonstrat efectul benefic al RCP Reglalg, utilizat la stropirea plantelor de roșii în condiții de câmp pe parcursul vegetației, asupra creșterii și dezvoltării plantelor, activității chitinazelor din frunze, atât soiului mediu-timpurii, cât și tardiv. Îmbibarea semințelor de castravete în soluțiile RCP Reglalg și stropirea plantelor de tomate în timpul vegetației cu acest preparat reprezintă o metodă promițătoare de aplicare a Reglalgului pentru culturile horticole.*

Cuvinte cheie: RCP Reglalg, *Cucumis sativus* L., *Solanum lycopersicum* L., parametrii biochimici

INTRODUCTION

In recent years, due to the global warming, agricultural crops, including horticultures, are exposed to abiotic and biotic stress factors (Bedsworth and Hanak, 2010). In response to stress factors, excessive production of reactive oxygen species (ROS) are formed in plant tissues, which affect the most physiological processes, including plant growth and yield (Gill and Tuteja, 2010). Plants possess

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enzymatic and non enzymatic mechanisms to annihilate the excess of the ROS, produced under unfavorable conditions (Gill and Tuteja, 2010). An important role in providing resistance of plants to stresses factors plays the enzymatic activity of peroxidase, catalase, chitinases, and a number of metabolic compounds, including polyphenols compounds (Takenaka *et al.*, 2009; Gomes and Garcia, 2013).

In agricultural technologies for cultivation of vegetable crops, including cucumber and tomato, plant growth regulators (PGR) are widely applied for seed treatments before sowing, as well as for plant treatments during vegetation to increase plant resistance to unfavorable growth conditions, ensuring the quality and productivity of plants (Cauș *et al.*, 2016; Dascaliuc *et al.*, 2018).

The purpose of this study was to investigate the effects of PGR *Regalg*, when applied for the treatment of cucumber seeds and for spraying of field grown tomato plants during the season, on the plant growth parameters and some antioxidant indices.

MATERIAL AND METHODS

Seeds of *Cucumis sativus* L. cv. Concurrent, used in the study, were immersed during of 24 hours in the dark at 6°C in water (control) or in water solutions containing $5 \cdot 10^{-4}$, $2,5 \cdot 10^{-4}$ and $1,25 \cdot 10^{-4}$ % active substance of *Regalg*. Then treated seeds were transferred for germinations in the dark at 25°C and 80 % relative humidity of the air. Seedlings of 3 days old were collected and used to assess their growth parameters, the enzymatic activity of peroxidase and catalase, the total polyphenols content and total antioxidant capacity in their roots.

Plants of tomato *Solanum lycopersicum* L., cultivars Julihirsutian (early ripening) and Anatolie (late ripening) grown under field conditions were sprayed during the vegetation period with water (control) or an aqueous solution containing $2.5 \cdot 10^{-4}$ % active substance *Regalg*.

The activities of peroxidase (PO) and catalase (Cat) in soluble protein fractions of cucumber seedling roots, the total polyphenols content (TPC) and total antioxidant capacity (TAC) in hydro ethanol extracts of cucumber seedling roots were determined by the methods described earlier (Caus *et al.*, 2016). Chitinase activity in tomato leaves extracts was determined according to (Neale *et al.*, 1990). Each experiment was performed three times in triplicate. The arithmetic means and their standard deviations (SD) were calculated.

RESULTS AND DISCUSSIONS

The results showed that soaking cucumber seeds in *Regalg* influenced germination and seedling growth parameters. As can be seen from table 1, the stimulation of biomass accumulation and stem height depended on the *Regalg*'s active substance concentrations in the aqueous solutions used for seed treatments. And also that *Regalg* concentrations of $2.5 \cdot 10^{-4}$ and $1.25 \cdot 10^{-4}$ % had stimulatory effects both on the increase of the root and air biomass and on the growth of stems in height.

The influence of various concentrations of the *Regalg's* active substance in the aqueous solutions, applied to seeds prior to germination on the height of stems, fresh weight of roots and aerial part of 3 days old cucumber seedlings

Treatments applied to seeds prior to germination	Concentration of the <i>Regalg's</i> active substance in the aqueous solution, %	Biomass accumulation, % against control		Height of stems, % against control
		Roots	The aerial part of the plants	
Control (H ₂ O)	-----	100	100	100
<i>Regalg</i> (R)	$5 \cdot 10^{-4}$	100.2 ± 1.24	110.0 ± 1.76	122.6 ± 4.92
	$2.5 \cdot 10^{-4}$	123.3 ± 1.70	119.0 ± 0.82	125.2 ± 5.66
	$1.25 \cdot 10^{-4}$	122.0 ± 1.49	113.2 ± 0.36	121.0 ± 9.05

At the 3rd day the control biomass of roots and aerial parts reached 0.05 g/plant and respectively 0.09 g/plant, and the height of stems – 2.7 cm.

Utilization of aqueous solution containing $2.5 \cdot 10^{-4}$ % *Regalg's* active substance showed the tendency to more strongly influence the growth of plant biomass and the height of the plantlets (tab. 1). The literature data demonstrates that germination processes and subsequent growth of plants are accompanied by the formation of ROS in plant tissues and the activation of the antioxidant system, including antioxidant enzymes, involved in the detoxification of free radicals excess (Gomes and Garcia, 2013). In this context we intended to determine whether the application of aqueous solution containing different concentrations of the *Regalg* active substance for seeds treatments subsequently can modify the activity of peroxidase (PO) and catalase (Cat), involved in the annihilation of excessive H₂O₂, which is one of the more stable and harmful ROS in plant cells.

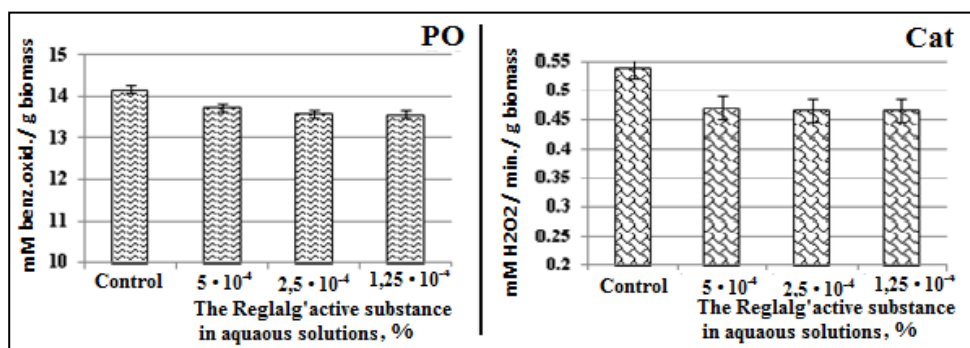


Fig. 1 The effect of cucumber seeds treatment with aqueous solutions containing different concentrations of the *Regalg's* active substance on the activity of peroxidase (PO) and catalase (Cat) of soluble protein fractions separated from roots of 3-days plantlets

Figure 1 presents the data on the changes of PO and Cat activity in cucumber seedling roots under the influence of different concentrations of *Regalg* active substance in aqueous solutions used for seed treatments. It can be

observed, that compared to the control, PO and Cat activity decreased significantly in roots of cucumber seedling, grown from seeds treated with different concentrations of *Regalg* (fig. 1). This suggests that during the growth and formation of cucumber root system there is no excess production of H_2O_2 in root seedlings grown from seeds treated with *Regalg*.

Considering that the application of different concentrations of *Regalg* has influenced the PO and Cat activities, we have proposed to determine whether seed treatment with solutions of *Regalg* caused induction of changes in the total polyphenols content and total antioxidant capacity, which together with the enzymes system, play an important role in determining the processes of SRO cleavage in plants cells. The analysis of total polyphenols contents (TPC) and their total antioxidant capacity (TAC) in the extracts from cucumber root seedlings, grown from seeds treated with *Regalg* demonstrated an increase of the TPC as well as their TAC at all concentrations of *Regalg* (fig. 2). The highest level of TPC and TAC was observed in cucumber roots of seedlings grown from seeds treated with aqueous solution containing $2.5 \cdot 10^{-4}\%$ *Regalg*'s active substance (fig. 2). So, these results support our hypothesis that the *Regalg*, applied to the seeds in various concentrations, especially of $2.5 \cdot 10^{-4} \%$, favours the growth and development of cucumber plants as well as the TPC and TAC increase of seedling roots.

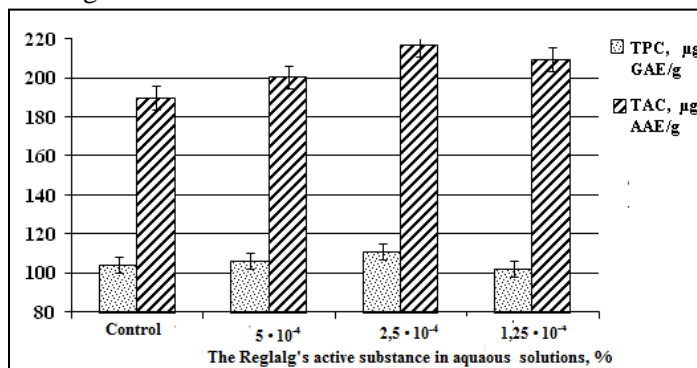


Fig. 2 The effect of cucumber seeds treatment with aqueous solutions containing different concentrations of the *Regalg*'s active substance on the total polyphenol content (TPC) and total antioxidant capacity (TAC) of 3 day old cucumber plant root extracts

Another purpose of this study was to investigate the effect of applying the aqueous solution containing $2.5 \cdot 10^{-4} \%$ *Regalg*'s active substance by spraying field grown tomato plants during their vegetation season. The research focussed on establishing the influence of *Regalg* on plant growth and development as well as on the possible changes of chitinase activity in leaves of early and late ripening tomato cultivars.

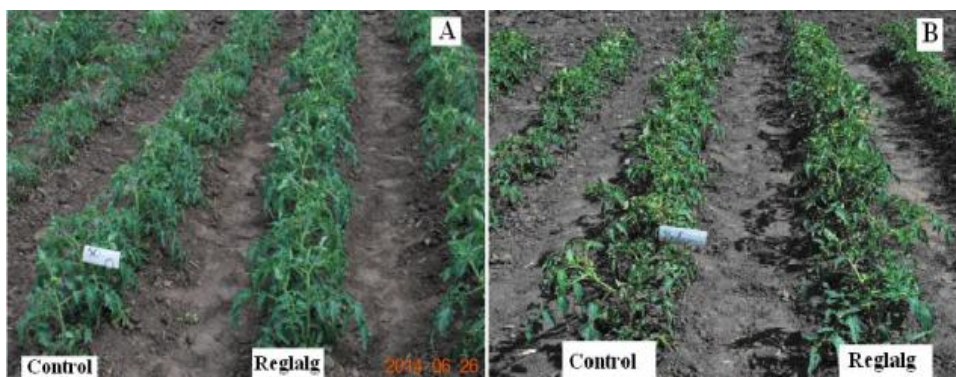


Fig. 3 Foto of tomato plants *Solanum lycopersicum* L., early (A) and late (B) ripening cultivars under field conditions treated with water (control) and an aqueous solution containing $2.5 \cdot 10^{-4}$ % active substance of the Reglalg during the vegetation period.

The field grown tomato plants presented in figure 3A and 3B demonstrate the beneficial effect of *Reglalg* on the growth of both early and late ripening cultivars of tomato, which is already manifest before the plants flowering period (June, 10). It should be noted that the treatment of tomato plants with *Reglalg* induced an enhancement of the vegetative growth, flowering and percentage of fruit binding, stimulated fructification and increased plant productivity (data not shown).

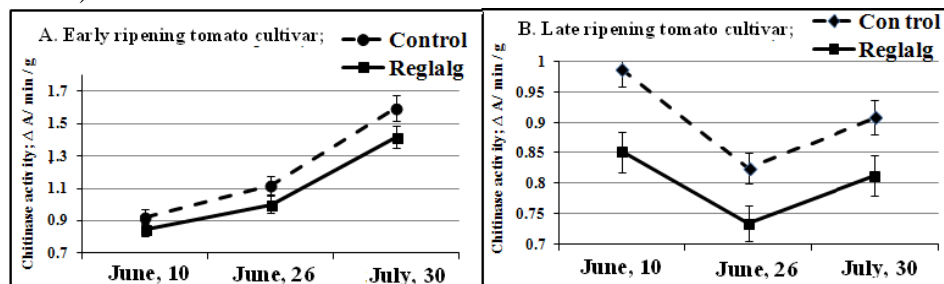


Fig. 4 Effect of foliar application of an aqueous solution containing $2.5 \cdot 10^{-4}$ % active substance of the *Reglalg* during the vegetation period, every 10 days, on the leaf chitinase activity of early (A) and late (B) ripening tomato cultivars.

The effects of *Reglalg* application on the enzyme activity of chitinase, extracted from the leaves of tomatoes plants are shown in figure 4. Analyzing the data (fig. 4), we can see that the chitinase activity in leaf extracts of both cultivars treated with *Reglalg* during their vegetation was at the lower level then in leaves of control plants, treated with water. But, the extracts from early ripening cv. Juhirsutian tomatoes leaves (fig. 4A) were characterized by a higher level of chitinase activity, compared to the enzyme activity of late ripening Anatoli cultivar (fig. 2B). Aging of plants is associated with increased activity of chitinases in both cultivars, but in the late ripening cultivar it occurs later, after

the initial decrease in chitinase activity towards the end of June (fig. 4B). Chitinases play an important role in the defensive response of plants to the action of biotic factors (Takenaka *et al.*, 2009). In accordance with the reaction of plants to the respective factors, an induction of the genes responsible for the biosynthesis and activation of the chitinases takes place. On the other hand, in addition to the apparent role of chitinases in the defensive reaction of plants to biotic agents, it is assumed that these enzymes are involved in plant development processes, including floral apparatus formation, leaf senescence and embryogenesis (Neale *et al.*, 1990; Zhi-Ping Xie *et al.*, 1996). Our results demonstrated that the aging of plants is associated with increased chitinase activity. They also support the vision of the "rejuvenation" effect of *Reglalg* on tomato plants. Decreasing level of chitinases activity in plants treated with *Reglalg* supports this view.

CONCLUSION

The results demonstrated that PGR *Reglalg* can be effectively used in agriculture practice for pre-germination treatments of cucumber seeds and for foliar application in spraying tomato plants during vegetation.

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