INFLUENCE OF RHIZOBACTERIA INOCULATION AND EXOGENOUSLY APPLIED SALICYLIC ACID ON THE CONTENTS OF HYDROGEN PEROXIDE AND WATER IN SOYBEAN (*GLYCINE MAX* L.) UNDER DROUGHT CONDITION

INFLUENȚA INOCULĂRII CU RIZOBACTERII ȘI APLICĂRII ACIDULULUI SALICILIC ASUPRA CONȚINUTULUI DE PEROXID DE HIDROGEN ȘI APĂ LA SOIA (*GLYCINE MAX*. L) ÎN CONDIȚII DE SECETĂ

ROTARU V.[1]*

[*]Corresponding author e-mail: rotaruvlad@yahoo.com

Abstract. *Rhizobacteria and salicylic acid (SA) have an important role in the promotion plant growth and enhancement of crops resilience to abiotic stresses. The aim of the study was to evaluate the effect of rhizobacteria (Bradyrhizobium japonicum) inoculation alone and in combination with salicylic acid on soybean (Glycine max L.) plants under moderate drought stress condition. Therefore, a pot culture experiment was conducted to test whether SA application at concentration of 0.5 mM through foliar spray could protect the soybean cultivar Horboveanca to short drought stress based on growth and alterations in hydrogen peroxide and relative water contents (RWC). The control treatment received 70% water holding capacity (WHC) of soil, whereas moderate drought stress corresponded to 35% WHC. Results showed that water deficit decreased RWC and increased hydrogen peroxide contents in leaves. The efficiency of Bradyrhizobium japonicum in the presence of salicylic acid was higher compare to plants treated with rhizobacteria alone. Thus, experimental findings demonstrated that integrated use of rhizobacteria and SA alleviates the adverse effects of water deficit on growth and contributed to drought tolerance of soybean through increased relative water contents and decreasing accumulation of reactive oxygen species in plant tissues.*

Key words: *Bradyrhizobium japonicum*, drought, hydrogen peroxide, salicylic acid, soybean, water content

Rezumat. *Rizobacteriile și acidul salicilic (AS) au rol important în promovarea creșterii plantelor și majorarea rezistenței culturilor la stresul abiotic. Scopul studiului a fost de a evalua efectul inoculării cu rizobacterii (Bradyrhizobium japonicum) separat sau în combinare cu aplicarea acidului salicilic la plantele de soia în condiții de stres moderat al secetei. Prin urmare, s-a organizat un experiment în vase de vegetație pentru a testa dacă aplicarea AS în concentrație de 0,5 mM prin tratarea foliară poate asigura protejarea mai bună a soii (cultivar Horboveanca) de secetă de scurtă durată bazată pe creștere și modificări în conținutul de peroxid de hidrogen și conținutul relativ de apă. Plantele control au fost irigate la 70% din capacitatea totală pentru apă (CTA)*

[1]Institute of Genetics, Physiology and Plant Protection, Republic of Moldova

a solului, iar stresul hidric moderat a fost instalat la nivelul de 35% CTA. Rezultatele cercetării au arătat că deficitul de apă a redus conținutul relativ de apă și a sporit conținutul de peroxid de hidrogen în frunze. Eficiența tratamentelor cu tulpina de Bradyrhizobium japonicum în prezența acidului salicilic a fost mai înaltă comparativ cu plantele tratate doar cu rizobacterii. Așadar, rezultatele experimentale au demonstrat că folosirea integrată a rizobacteriilor și AS atenuează efectele adverse ale deficitului de apă asupra creșterii și contribuie la o toleranță mai bună a soii datorită majorării conținutului de apă și diminuării conținutului de specii reactive de oxigen în țesuturile vegetale ale plantei.

Cuvinte cheie: *Bradyrhizobium japonicum*, soia, acid salicilic, peroxid de hidrogen, conţinut de apă, secetă

INTRODUCTION

Low phosphorus in soil and water deficit conditions are largely spread in many agricultural regions and they have destructive effects on the physiological and metabolic processes in plants. The disturbances provoked by abiotic stress factors lead to poor growth and low tolerance of crops to unfavourable environmental conditions.

Therefore, it is necessary to find out approaches in order to develop stress tolerant crops including genetic engineering (McKerise *et al.*, 1996), breeding (Vettakkorumakankav *et al.*, 1999), plant-growth promoting rhizobacteria (Sachs *et al.*, 2011) and the use of plant growth regulators (Khan *et al.*, 2019; Baninasab and Ghobadi, 2011; Stevens *et al.*, 2006).

Among plant growth regulators, salicylic acid (SA) plays a significant role in plant physiological processes and metabolic changes such as growth and development (Vicente and Plasencia, 2011; Jakhar and Sheokand, 2015), photosynthesis (Hayat *et al.*, 2012; Jakhar and Sheokand, 2015), ions absorption and transport, seed germination, glycolysis (Rivas-San and Plasencia, 2011).

It has been demonstrated that salicylic acid, as a pivotal signalling molecule in plants, is involved in defence mechanisms by improving physiological and biochemical functions and has beneficial effects on tolerance to biotic and abiotic stress factors (Gunes *et al.*, 2007; Nazar et al., 2011). Several studies have demonstrated that SA contributes to plant resistance to salinity (Nazar *et al.*, 2011; Khan *et al.*, 2014), drought (Yazdanpanah *et al.*, 2011) and high temperature (Wang *et al.*, 2010).

Likewise, several investigations have shown that SA plays an important function in modulating the redox balance across membranes, thereby counteracting the negative effects of reactive oxygen species (ROS) generated by oxidative stress (Yang *et al.*, 2004).

Hydrogen peroxide is a major reactive oxygen species (ROS) in plants and misbalance of ROS in cells is related to biochemical changes (Ahmad *et al.*, 2014). The decrease of peroxide concentration in plant tissues due to application of elicitors such as plant growth regulators and PGPR contribute to diminish oxidative stress and improve plant protective system to abiotic stresses.

Soybean plants are sensitive to drought compared to cereals. Drought tolerant plants of soybean could avoid the negative impact of water stress by maintaining higher relative water content (RWC) and diminishing oxidative stress (Hossain *et al.*, 2014).

It was established that SA application improves RWC and grain yield of common bean in water stress condition (Sadeghipour and Aghaei, 2012). Nevertheless, it is well known that application of rhizobacteria *Bradyrhizobium japonicum* displays many activities that promote plant growth and productivity of soybean (Patra *et al.*, 2012).

However, the efficiency of this species is dependent on its survival in soil as well as the environmental factors (Sachs *et al.*, 2011). Rhizobacteria efficiency is low under unfavourable environmental conditions, particularly under drought conditions.

There are many studies regarding the effect of rhizobacteria applied alone as well as SA application separately on physiological changes and plant growth.

However, the interactive effect of rhizobacteria and SA on physiological and metabolic alterations and their role in alleviation of drought stress in soybean plants was not investigated enough. In this study, the effect of rhizobacteria (*Bradyrhizobium japonicum*) inoculation alone and in combination with salicylic acid (SA) plant spray on changes of hydrogen peroxide and relative water contents (RWC) in soybean plants under water deficit condition was investigated.

MATERIAL AND METHOD

Seeds of soybean (cv. Horboveanca) were inoculated with rhizobacteria *Bradyrhizobium japonicum* before sowing. Some of the washed seeds were sown without any treatment (control).

The seeds were sown in pots filled with carbonated chernoziom soil (mixed with sand 3:1, v/v). Two plants per pot were cultivated and were normal irrigated until flowering stage of plants.

All pots were well watered at 70% water holding capacity (WHC) until drought stress was imposed. Drought stress was imposed on plants at the flowering stage for 12 days. SA was applied on the foliage at the concentrations of 0.5 mM on plants subjected to no stress (70% WHC) or drought stress (35% WHC) with a hand sprayer. Treatments were performed at the V1 stage (branching) and at the R1 stage (beginning bloom).

The concentration of SA was selected based on earlier findings (Nazar *et al.*, 2011). Soybean plants were sampled after 12 days of water deficit and analysed. Plant productivity was evaluated at full maturity stage. Leaf RWC was determined gravimetrically following the method of Galmes *et al.* (2007).

The content of H_2O_2 in leaves was measured according to the method of Velikova and Loreto (2005). The experiment followed a randomized complete block design and the number of replicates for each treatment was four.

Data were statistically analysed using analysis of variance (ANOVA) by Statistic program 7 and presented as treatment mean ± SE of three measurements. Least significant difference (LSD) was calculated for the significant data at $P \leq 0.05$.

RESULTS AND DISCUSSIONS

Plant growth regulators and PGPRs have a large range of activities to attenuate drought impact on plants, including reduction of oxidative stress and maintaining better the water balance in plants (Khan *et al.*, 2019).

H_2O_2 is a strong oxidizing agent in plant tissues which is accumulated under different unfavourable conditions.

To study the interactive influence of SA and rhizobacteria inoculation on oxidative stress under moderate drought, we estimated the hydrogen peroxide concentration in leaves of soybean under no stress and drought stress.

The treatments effects on changes of hydrogen peroxide in plants under normal soil moisture condition are presented in figure 1.

Experimental results revealed that application of rhizobacteria had no influence on this parameter under well-irrigated condition (70% WHC).

However, in such conditions the combined application of SA and rhizobacteria decreased the hydrogen peroxide concentration in leaves by 7% compared to plants inoculated with rhizobacteria alone.

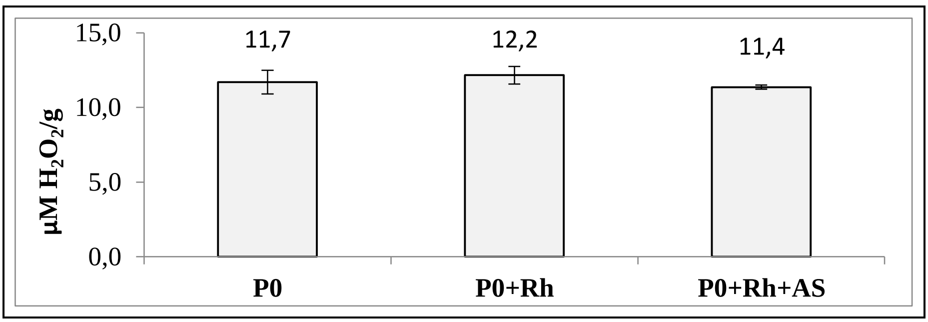

Fig. 1 Hydrogen peroxide content in soybean leaves under normal water conditions (70% WHC) and treated with rhizobacteria (*Bradyrhizobium japonicum*) and foliar 0.5 mM SA. Data are presented as treatments mean ± SE (n = 3), P<0.05.

The plants treated with rhizobacteria and SA and subjected to drought stress exhibited changes in hydrogen peroxide concentrations.

The contents of hydrogen peroxide in plants subjected to water insufficiency in relation to rhizobacteria and SA application are shown in figure 2.

The results demonstrated that influence of rhizobacteria and SA display the same trend as in normal soil moisture of soil. Under water deficit, the application of rhizobacteria insignificantly increased the content of hydrogen peroxide in leaves.

However, the combined application of rhizobacteria and SA significantly decreased (by 39%) this parameter compared to plants treated only with rhizobacteria.

This suggests that SA may play an important role in inducing tolerance to oxidative stress conditions in soybean plants.

Similar influence of SA was revealed in wheat genotypes under drought as reported by Agarwal *et al.* (2005).

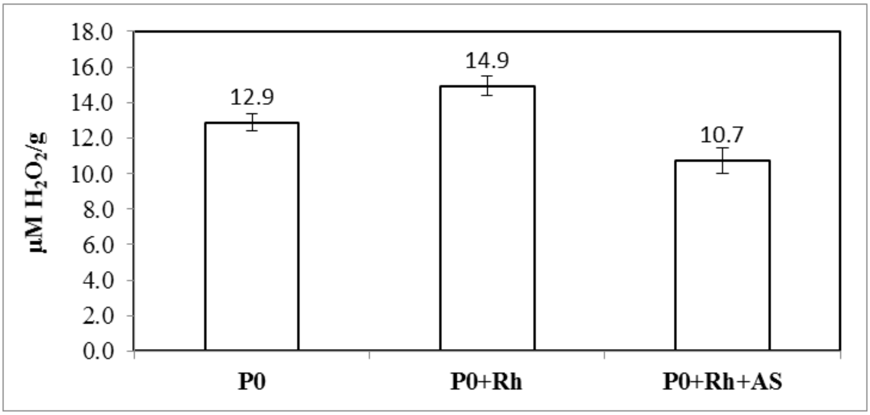

Fig. 2 Hydrogen peroxide content in soybean plants grown under drought condition (35% WHC) and treated with rhizobacteria (*Bradyrhizobium japonicum*) and foliar 0.5 mM SA. Data are presented as treatments mean ± SE (n = 3), $P<0.05$.

Thus, experimental data demonstrated that the decrease of reactive oxygen species accumulation due to integrated use of plant growth regulator and rhizobacteria contributes to provide a better protective system of plant against drought, diminishing oxidative stress in plants.

So, the application of 0.5 mM SA in conjunction with rhizobia inoculation under drought stress condition significantly reduced H_2O_2 compared to control (fig. 2).

Similarly, Hashem *et al.* (2016) reported that rhizobacteria *Bacillus subtilis* reduced the concentration of hydrogen peroxide in plants *Acacia gerradii* under saline stress.

However, in contrast with the results of this study Razmi *et al.* (2017) showed that exogenous application of 0.4 mM SA alone increased hydrogen peroxide concentration in soybean subjected to water deficit condition.

Hence, according to our experimental results it can be concluded that application of *Bradyrhizobium japonicum* strain in conjunction with foliage treatment with SA provide a synergic effect of induction protective mechanisms of plant tissues against low P and temporary water deficit condition.

According to literature data, the use of rhizobacteria as well as plant growth regulators of phenolic nature, the plant tolerance is associated with better absorption and utilization of water (Khan *et al.*, 2018; Khan *et al.*, 2019).

The relative water content (RWC) in leaves is a physiological marker to evaluate water balance in plants (Lat *et al.*, 2011).

Experimental results of this study revealed changes in water status of plants in relation to rhizobacteria and SA treatments (fig. 3 and fig. 4).

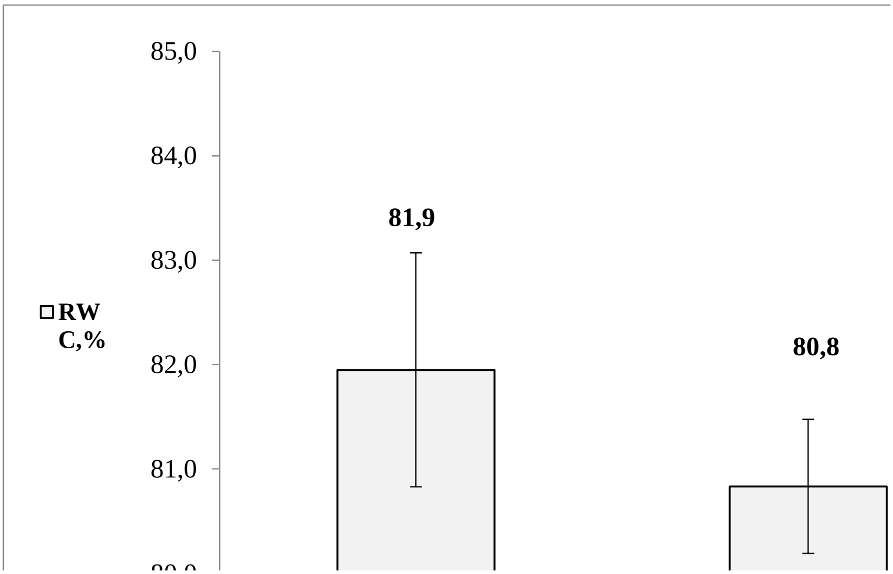

Fig. 3 The effect of rhizobacteria (*Bradyrhizobium japonicum)* and SA on RWC in soybean leaves of plants cultivated in normal soil moisture (70% WHC. Data are presented as treatments mean ± SE (n = 3), P<0.05.

In general, water deficit in soil reduced the relative water contents in soybean plants irrespective of treatments.

In contrast to the negative effect of water deficit, salicylic acid application improved water status of plants.

Experimental results show that integrated use of bacteria strain and SA improved water status of plants cultivated on soil with low P under good irrigation conditions.

Likewise, the beneficial effect of these elicitors was registered in plants subjected to temporary water deficit compared to control treatment or to treatment with bacteria strain alone (fig. 4).

Experimental results suggested that rhizobacteria enabled the leaf to maintain a high level of RWC, increased the plant performance and reduced adverse effect of water deficit in soybean plants.

The positive effect of rhizobacteria could be caused by the hydraulic nature of roots, which were better developed, and more fasciculate roots alleviate radial transport of water as demonstrated by Kothari *et al*. (2011).

The RWC changed less under combined application of SA and rhizobacteria in plants grown in water deficit condition compared to plants inoculated only with *Bradyrhizobium japonicum* strain.

These results could be explaining by better leaves development in comparison with leaves of plants inoculated with rhizobacteria strain alone.

Hence, the integrated use of rhizobacteria and SA enhanced water status of plants compared to control plants.

The changes induced by application of rhizobacteria and SA positively affected plant growth of soybean.

Plant dry mass accumulation significantly reduced in drought-treated plants in comparison to their respective normal irrigation plants (data not shown).

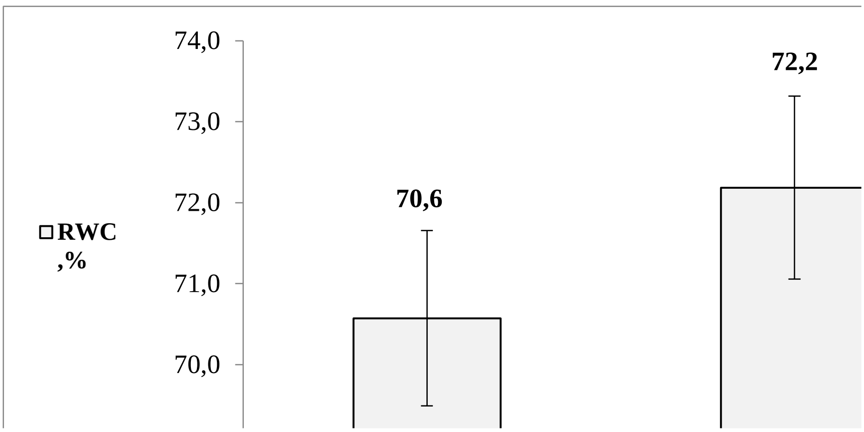

Fig. 4 The effect of rhizobacteria (*Bradyrhizobium japonicum)* and SA on RWC in soybean leaves of plants under drought condition (35% WHC). Data are presented as treatments mean ± SE (n = 3), P<0.05.

The combined use of SA and rhizobacteria assisted the plant to maintain growth by accumulating higher biomass under normal irrigation as well as under drought condition.

The increase of grain yield due to combined application of SA and rhizobacteria correlated with the improvement of RWC and reduced concentrations of hydrogen peroxide in plants (data not shown).

In summary, the experimental results revealed the beneficial role of integrated application of rhizobacteria and salicylic acid in regulating the drought response of plants and such approach could be useful in the adaptation of soybean plants to unfavorable environments.

CONCLUSION

The present study indicates that water deficit condition has adverse effects on soybean plants, including decreases in RWC and increases of hydrogen peroxide content and lowering plant growth.

The application of *Bradyrhizobium japonicum* in conjunction with foliar treatment of 0.5 mM SA improved soybean tolerance to water deficiency by limiting hydrogen peroxide accumulation and improving water status of plants.

Thus, the effect of salicylic acid treatment is efficient in soybean plants treated with rhizobacteria *Bradyrhizobium japonicum* inoculation and provide better tolerance to drought condition.

REFERENCES

1. **Agarwal S.K., Srivastavaa A., Tyagib G.C., Meena R.C., 2005 -** *Role of ABA, salicylic acid, calcium and hydrogen peroxide on antioxidant enzymes induction in wheat seedlings.* Plant Science, 169 (3), 559-570.
2. **Ahmad P., Ashraf M., Hakeem K.R., Azooz M.M., Rasool S., Chandna R., Akram N.A., 2014 -** *Potassium starvation‐induced oxidative stress and antioxidant defense responses in Brassica juncea.* J. Plant Inter., 9 (1), 1–9.
3. **Baninasab B., Ghobadi C., 2011 -** *Influence of paclobutrazol and application methods on high temperature stress injury in cucumber seedling.* J. Plant Growth Reg., 30, 213–219.
4. **Galmes J., Flexas J., Robert S., 2007 -** *Water relations and stomatal characteristics of Mediterranean plants with different growth forms and leaf habits: responses to water stress and recovery.* Plant Soil, 290,139–155.
5. **Gunes A., Inal A., Alpaslan M., Eraslan F., Bagci E.G., Cicek N., 2007 -** *Salicylic acid induced changes on some physiological parameters symptomatic for oxidative stress and mineral nutrition in maize (Zea mays L.) grown under salinity.* J. Plant Physiol., 164, 728–736.
6. **Hashem A., Abd-Allah E.F., Alqarawi A., Al-Huqail A.A., Wirt S., Egamberdieva D., 2016 -** *The interaction between arbuscular mycorrhizal fungi and endophytic bacteria enhances plant growth of Acacia gerrardii under salt stress.* Front. Plant Sci., 7:1089.
7. **Hayat Q., Hayat S., Ifran M., Ahmad A., 2010 -** *Effect of exogenous salicylic acid under changing environment: a review.* Environ. Exp. Bot., 68, 14–25.
8. **Hossain M.M., Liu X., Qi X., Lam H.M., Zhang J., 2014 -** *Differences between soybean genotypes in physiological response to sequential soil drying and rewetting.* Crop Journal, 2, 366–380.
9. **Jakhar S., Sheokand M., 2015 -** *Effect of foliar application of salicylic acid on photosynthetic pigments and antioxidative enzymes of soybean plant.* IJAPSA, 1, 7–15.
10. **Kang H.M., Saltveit M.E., 2002 -** *Chilling tolerance of maize and cucumber and rice seedling leaves and roots are differentially affected by salicylic acid.* Physiol. Plant., 115, 571 – 576.
11. **Khan N., Asghari B., Peiman Z., 2018 -** *Effects of exogenously applied plant growth regulators in combination with PGPR on the physiology and root growth of chickpea (Cicer arietinum) and their role in drought tolerance.* Journal of Plant Interactions, 13 (1), 239–247.
12. **Khan N., Bano A., Babar M.A., 2019 -** *Metabolic and physiological changes induced by plant growth regulators and plant growth promoting rhizobacteria and their impact on drought tolerance in Cicer arietinum L.* PLoS ONE 14(3): e0213040. https://doi.org/10.1371/journal. pone.0213040.

13. **Khan M.I.R., Asgher M., Khan N.A., 2014 -** *Alleviation of salt induced photosynthesis and growth inhibition by salicylic acid involves glycine betaine and ethylene in mung bean (Vigna radiata L.).* Plant Physiol. Biochem., 80, 67–74.
14. **Kothari S.K., Marchner H., George E., 1990 -** *Effect of VA mycorrhizal fungi and rhizosphere microorganisms on root and shoot morphology, growth and water relations in maize.* New Phytol. 116, 303–311.
15. **Kuchlan P., Kuchlan M.K., Husain S.M., 2017 -** *Effect of foliar application of growth activator, promoter and antioxidant on seed quality of soybean.* Legume Res., 40 (2), 313–318.
16. **Lat C., Jha S., Dixit V., Sreenivasulu N., Prasad M., 2011 -** *Differential antioxidative responses to dehydration-induced oxidative stress in core set of foxtail millet cultivars (Setaria italica L.).* Protoplasma, 248, 817-828. http://dx.doi.org/10.1007/s00709-010-0257-y.
17. **McKersie B.D., Bowley S.R., Harjanto E., Leprince O., 1996 -** *Water-deficit tolerance and field performance of transgenic alfalfa overexpressing superoxide dismutase*. Plant Physiol., 111, 1177–1181. 10.1104/pp.111.4.1177.
18. **Nazar R., Iqbal N., Syeed S., Khan N.A., 2011 -** *Salicylic acid alleviates decreases in photosynthesis under salt stress by enhancing nitrogen and sulfur assimilation and antioxidant metabolism differentially in two mungbean cultivars.* J. Plant Physiol., 168, 807–815.
19. **Patra R.K., Pant L.M., Pradhan K., 2012 -** *Response of soybean to inoculation with rhizobial strains: effect on growth, yield, N uptake and soil N status.* World Journal of Agricultural Sciences, 8 (1), 51-54.
20. **Razmia N. Ebadia A., Daneshianb J., Jahanbakhsh S., 2017 -** *Salicylic acid induced changes on antioxidant capacity, pigments and grain yield of soybean genotypes in water deficit condition.* J. of Plant Interactions, 12 (1), 457–464.
21. **Rivas-San Vicente M., Plasencia J., 2011 -** *Salicylic acid beyond defence: its role in plant growth and development.* J. Exp. Bot., 62, 3321–3338.
22. **Ruppenthal V., Zoz T., Steiner F., Lana M.C., Castagnara D.D., 2016 -** *Silicon does not alleviate the adverse effects of drought stress in soybean plants.* Semin. Cienc. Agrar., 37, 3941–3954.
23. **Sachs J.L., Russell J.E., Hollowell A.C., 2011 -** *Evolutionary instability of symbiotic function in Bradyrhizobium japonicum.* PLoS ONE, 6 (11), e26370 (doi:10.1371/journal.pone.0026370).
24. **Sadeghipour O., Aghaei P., 2012 -** *Biochemical changes of common bean (Phaseolus vulgaris L.) to pretreatment with salicylic acid (SA) under water stress conditions.* Adv. Environ. Biol., 2, 14–22.
25. **Sorkheha K., Shiran B., Khodambashi M., Rouhi V., Mosavei S., Sofo A., 2012 -** *Exogenous proline alleviates the effects of H_2O_2 - induced oxidative stress in wild almond species.* Russ. J. Plant Physiol., 59, 788–798.
26. **Stevens J., Seneratna T., Sivasithamparam K., 2006 -** *Salicylic acid induces salinity tolerance in tomato (Lycopersico cv Roma) associated changes in gas exchange, water relations and membrane stabilisation*. Plant Growth Regulation, 49 (1), 77-83.
27. **Velikova V., Loreto F., 2005 -** *On the relationship between isoprene emission and thermo tolerance in Phragmites ausrralis leaves exposed to high temperatures and during the recovery from a heat stress.* Plant Cell Environ., 28, 318–327.
28. **Vettakkorumakankav N.H., Falk D., Saxena P., Fletcher R.A., 1999 -** *A crucial role for gibberellins in stress protection of plants*. Plant Cell Physiol., 40, 542–548, doi: 10.1093/oxfordjournals.pcp.a029575.
29. **Vurukonda S.S., Vardharajula S., Shrivastava M., 2016 -** *Enhancement of drought stress tolerance in crops by plant growth promoting rhizobacteria.* Microbiol. Res., 184, 13–24.

30. **Wang L.J., Fan L., Loescher W., Duan W., Liu G.J., Cheng J.S., 2010 -** *Salicylic acid alleviates decreases in photosynthesis under heat stress and accelerates recovery in grapevine leaves.* BMC Plant Biol., 10, 34–40.
31. **Yang Y.N., Qi M., Mei C.S., 2004 -** *Endogenous salicylic acid protects rice plants fromoxidative damage caused by aging as well as biotic and abiotic stress.* Plant Journal, 40, 909–919.
32. **Yazdanpanah S., Baghizadeh A., Abbassi F., 2011 -** *The interaction between drought stress and salicylic and ascorbic acids on some biochemical characteristics of Satureja hortensis.* Afr. J. Agr. Res., 6 (4), 798-807.