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Effects of salicylic acid foliar application on growth and antioxidant potential of basil (*Ocimum basilicum* L.)

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***Abstract*:**

Karalija, E., Parić, A.: ***Effects of salicylic acid foliar application on growth and antioxidant potential of basil (Ocimum basilicum L.). Biologica Nyssana, 8 (2), December, 2017: 145-150.***

Salicylic acid is one of endogenous plant growth regulators that plays a key role in many physiological processes. The present study analysed the effect of different concentrations (0, 0.01, 0.1, ad 1.0 mM) of salicylic acid on morphological parameters, photosynthetic pigments, protein, proline, total carbohydrates, and secondary metabolites content as well as peroxidase activity. One month after sowing seedlings were replanted in new pots, and salicylic acid was applied in form of a foliar spray. Plants were harvested 60 days after salicylic acid application. Results showed that foliar application of salicylic acid induces long-term changes in plant growth and metabolism. We recorded increase in leaf area, secondary metabolites and peroxidase activity. Reduction in total sugar and proline content is also recorded. Decrease in proline content is probably result of degradation of proline in stress induced conditions.

Key words: chlorophylls; salicylic acid; secondary metabolites; proline; peroxidases; total carbohydrates

***Apstrakt*:**

Karalija, E., Parić, A.: ***Efekat folijarne primene salicilne kiseline na rast i antioksidativni potencijal bosiljka (Ocimum basilicum L.). Biologica Nyssana, 8 (2), Decembar, 2017: 145-150.***

Salicilna kiselina je jedan od endogenih biljnih regulatora rasta sa ključnom ulogom u mnogim fiziološkim procesima. Prezentirani rad analizira efekte različitih koncentracija (0.01, 0.1, ad 1.0 mM) salicilne kiseline na morfološke parametre, fotosintetske pigmente, proteine, ukupne ugljikohidrate i sadržaj sekundarnih metabolite kao i peroksidaznu aktivnost. Mesec dana nakon setve biljke su presađene u nove posude i salicilna kiselina je aplicirana u formi folijarnog spreja. Biljke su prikupljene za analizu 60 dana nakon aplikacije salicilne kiseline. Rezultati su pokazali da folijarna aplikacija salicilne kiseline indukuje dugoročne promene u biljnom rastu i metabolizmu. Zabeženo je povećanje površine lista, sadržaja sekundarnih metabolita i peroksidazne aktivnosti. Također, zabeležena je redukcija u sadržaju ukupnih šećera i sadržaja prolina. Redukcija sadržaja prolina verovatno je rezultat degradacije prolina indukovane stresnim uslovima.

Ključne reči: hlorofili; salicilna kiselina; sekundarni metaboliti; prolin; peroksidaze; ukupni ugljikohidrati

Introduction

Physiological efficiency of a plant can be improved by plant growth regulators through their effect on photosynthesis, flower and fruit formation and overall productivity of the plant (Asghari & Aghdam, 2010). Foliar application of plant feeding agents usually influences more plant characteristics and yield then those applied in the soil (Kazemi, 2013). Positive effects of salicylic acid (SA) have been recorded in wheat with recorded enhancement of germination rate and seedling growth (Shakirova, 2007). Also activation of antioxidant enzymes such as peroxidases have been reported after plant spraying with salicylic acid, especially in drought stressed plants (Hayat et al., 2008). Although numerous studies have proven positive effects of salicylic acid it is still a subject of interest due to dependence of the salicylic acid effects upon species, developmental stage, application mode as well as concentration of SA used (Vanacker et al., 2001, Horváth et al., 2007). Lower concentrations of salicylic acid have been beneficial for plant growth (Vicente & Plasencia, 2011), while different results for metabolite content have been reported (Wang et al., 2007, D'Onofrio et al., 2009).

Sweet basil (*Ocimum basilicum* L.), member of Lamiaceae family, is used as a fresh vegetable for many traditional foods, dressings and salads. Its medicinal value and organoleptic quality is a result of basil aroma and essential oil composition. Aim of this study was to investigate the long-term effect of short exposure to foliar application of salicylic acid on growth performances and quality of basil plants.

Material and methods

This study was conducted in Laboratory for Plant Physiology at Faculty of Natural Sciences and Mathematics, University of Sarajevo. Plants were grown in controlled growth chambers with artificial light system and main daily temperature of 22 °C and 75% humidity.

Plant material and treatments

The experiment was conducted in a factorial arranged (2 x 2 x 2) randomised block design with five replications with SA (Salicylic acid) as main factor in growth chamber of Laboratory for plant physiology at Department for Biology, Faculty of Natural Sciences and Mathematics, University of Sarajevo during 2014 and 2015. Commercial obtained sweet basil (Green Paradise S.R.L) seeds were planted in trays. One month old seedlings with two developed leaves similar in size were selected and transferred into 10 cm in diameter plastic containers containing about 3 kg of commercial soil. When 4-6 leaves were developed SA was sprayed in ratio 0.01, 0.1 and 1.0 mM until both sides of leaves were wet.

Two months after salicylic acid application plant material was collected (six plants from each replication) for further analysis.

Plant mass and leaf area analysis

All plants were carefully removed from soil, roots were washed to remove soil residues and fresh and dry weight (dried at 65 °C for 72 hours) was recorded. Analysis of water content was calculated according to the formula:

$$\%WC = \frac{A - B}{A}$$

where: %WC - percentage of water content; A - fresh mass (g); B - dry mass (g)

Leaves were photographed on millimetre paper and leaf area was determined using ImageJ software.

Photosynthetic pigments content

Photosynthetic pigments were determined from 80% acetone extract by absorbance reading at 663, 646 and 440 nm according to the method of to Arnon (1949). Calculations were made according to the equations proposed by Porra et al. (1989) and Holm (1954):

Chlorophyll *a* = $12.25*A_{663} - 2.55 * A_{646}$ (µg/ml)
Chlorophyll *b* = $20.31*A_{646}$-$4.91*A_{663}$ (µg/ml)
Total Chlorophylls = $17.76*A_{646}$-$7.34*A_{663}$ (µg/ml)
Carotenoids = $4.69*A_{440}$-$0.267\times(A_{663}*A_{640})$ (µg/ml)

Proline content

Proline content was determined according to the method described by Carillo et al. (2008). Dried leaf material (50 mg) was homogenized in 5 mL of ethanol : water mixture (60:40) and homogenate was incubated for 24 h at +4 °C, then centrifuged at 10 000 rpm to obtain supernatant. 500 µL of supernatant was mixed with 1000 mL of 1% acid ninhydrin. The reaction mixture was placed in water bath for 20 minutes at 95°C, after which was cooled to room temperature and the absorbance was measured at 520 nm. Proline standard was used for calibration curve and calculation of proline content in samples and expressed as mg proline per g of dry weight (mg pro/gDW).

Secondary metabolite analysis

Phenolics were extracted from aerial parts of plants by maceration in 80% methanol (HPLC grade) and

incubation for 24h at 4 °C. Extracts were centrifuged at 2000 rpm for 15 min, and supernatants were collected for further analysis. Total phenolic content was analysed according to Wolfe et al. (2003) using Folin-Ciocalteu reagent and Gallic acid as standard and it was expressed as mg of gallic acid equivalent per g of dry weight (mgGAE/gDW). Total flavonoid content was done using aluminium chloride method (Ordonez et al., 2006) with catechin as standard. Results were expressed as mg of catechin equivalent per g of dry weight (mgCE/gDW). Total flavanol content was analysed according to modified method of Gadzovska et al. (2007) using 1% DMACA reagent (*p*-dimethyl aminocinnamaldehyde in HCl:CH_3OH, 8:92) and catechin as standard. Results were expressed as mg of catechin equivalent per g of dry weight (mgCE/gDW).

Determination of soluble sugar content

Dry samples (0.1 g) were grinded and extracted with 2.5 ml of 80% (v/v) ethanol at 90 °C for 60 min, followed by centrifugation at 10 000 *g* at 4 °C for 10 minutes. The process was repeated for complete extraction. Total soluble sugar content was determined using anthrone reagent and glucose as standard (Roe, 1955). Results were expressed as mg soluble sugar/g DW.

Total protein content

Total protein content was determined according to the method of Bradford (1976) grinding 50 mg of fresh leaf sample in phosphate buffer (pH 7.0). The extract was centrifuged at 10 000 rpm for 30 min at +4 °C. Absorbance of reaction mixture containing supernatant and diluted Bradford reagent was done at 765 nm against blank.

Peroxidase activity

Extract obtained for protein analysis was used for total peroxidase activity according to Gonzales et al. (1984). The reaction mixture was prepared by mixing 20 μL of extract; phosphate buffer (pH 7.0); guaiacol (20 mM) and H_2O_2 (10 mM). The absorbance of reaction mixture was recorded continuously during 120 s against blank.

Statistical analysis

All obtained data were analysed for significant differences using factorial analysis of variance. Statistical analysis was performed using Statistica 10 software and the means were compared using Newman-Keuls Test at $p<0.05$.

Results ad discussion

Effect of SA on growth parameters

The statistical analysis showed that application of salicylic acid increased leaf area of basil seedlings in concentration dependent manner (**Tab. 1**). Dry mass and water content did not differ between different treatments. Statistically significant reduction in photosynthetic pigment contents was recorded dependent upon applied concentration indicating reducing effect of salicylic acid on photosynthesis. Reduction in photosynthetic pigment content was probably the reason for increase in leaf area as a response to reduced photosynthetic activity. Pancheva et al. (1996) showed that long-term treatment (7 days) of barley seedlings with SA decreased the rate of photosynthesis (chlorophyll content) and our results are in accordance with these findings indicating that this negative effect of salicylic acid on photosynthetic pigments is present also after 2 months.

Table 1. Effect of salicylic acid on growth parameters of basil

Salicylic acid, mM	Leaf area, mm^2	Dry mass, g	Water content, %	Chla, mg/gDW	Chlb, mg/gDW	Chla+b, mg/gDW	Car, mg/gDW
0	65.825 ± 4.651^d	0.095 ± 0.005^a	0.919 ± 0.010^a	5.404 ± 0.188^a	2.341 ± 0.109^a	7.827 ± 0.298^a	1.068 ± 0.025^a
0.01	83.739 ± 13.972^c	0.092 ± 0.005^a	0.931 ± 0.004^a	5.280 ± 0.033^b	2.235 ± 0.022^b	7.596 ± 0.055^b	1.059 ± 0.002^b
0.1	86.749 ± 25.654^b	0.107 ± 0.008^a	0.925 ± 0.004^a	4.650 ± 0.049^c	2.026 ± 0.024^c	6.746 ± 0.074^c	0.935 ± 0.008^c
1.0	90.650 ± 15.782^a	0.081 ± 0.001^a	0.943 ± 0.006^a	1.560 ± 0.037^d	0.783 ± 0.022^d	2.367 ± 0.060^d	0.413 ±0.008^d

Values within one parameter not sharing the same letter are significantly different at $p<0.05$

Table 2. Effect of salicylic acid on total protein, secondary metabolites and total carbohydrates content in basil

Treatment	Total Proteins, mg/gDW)	Proline, mg/gDW	Total Flavonoids, mg/gDW	Total Phenols, mg/gDW	Total Flavanols, mg/gDW	Total Carbohydrates, mg/gDW
K	0.508 ±0.019[a]	0.069 ± 0.009[b]	9.504 ± 0.169[c]	2.672 ± 0.431[cd]	3.663 ± 0.146[c]	41.651 ± 0.774[a]
001SA	0.351 ± 0.001[b]	0.028 ± 0.004[c]	14.909 ± 0.866[b]	2.981 ± 0.327[b]	4.383 ± 0.478[b]	38.776 ± 0.887[b]
01SA	0.391 ± 0.031[b]	0.033 ± 0.002[c]	15.708 ± 0.867[b]	2.803 ± 0.532[cb]	4.658 ± 0.014[b]	38.460 ± 0.742[b]
1SA	0.551 ± 0.019[a]	0.107 ± 0.003[a]	17.609 ± 0.361[a]	3.528 ± 0.371[a]	6.249 ± 0.057[a]	29.370 ± 0.700[c]

Values within one parameter not sharing the same letter are significantly different at $p<0.05$

Reduction of chlorophyll content is usual plant response to stress conditions and since SA plays role in stress induced signalling application of SA can result in reduction of chlorophylls (Farooq et al., 2009). Physiological and biological processes in plants are strongly regulated and SA plays significant role in this regulation. As such SA can be used to improve plant growth under environmental conditions, but the efficiency of exogenous SA can vary dependent upon the species, developmental stage, application method and SA concentration (Borsani et al., 2001).

Effect of SA on proline content

The analysis of variance for proline content showed that lower concentrations of SA induced decrease in proline content while the highest used concentration of SA (1.0 mM) induced statistically significant increase in proline content (**Tab. 2**). In many cases it was recorded that proline content increases under stress conditions (Nayyar & Walia, 2003). Also considering that analysis was 2 months after SA application, low proline content in plants sprayed with 0.01 and 0.1 mM SA could be explained by the breakdown of proline upon relief of stress providing sufficient reducing agents for mitochondrial oxidative phosphorylation and ATP generation for recovery from stress and alleviating stress-induced damages (Hare & Cress, 1998, Hare et al., 1998). Proline can be considered as one of the protective mechanisms induced by SA application in basil plants, but no long term accumulation is achieved in this experiment.

Effect of SA on secondary metabolites

Foliar application of SA induced statistically significant (**Tab. 2**) long-term increase in secondary metabolite content. Kabiri et al. (2014) recorded no changes in polyphenol content after SA application in control plants presenting only short term effects of SA on polyphenols. In our experiment long-term effects of salicylic acid result in significant increase in polyphenol content dependent upon applied SA concentration similar results of increase in metabolite production was recorded by Gharib (2006). Increase of secondary metabolite production in basil under stress condition has been previously noted (Karalija et al., 2016).

Soluble sugar content

The results showed that sugar content decreased dependent upon applied SA concentration (**Tab. 2**). Similar SA induced reduction of soluble sugar content in plants that are not under stress conditions was previously recorded in *Nigella sativa* plants (Kabiri et al., 2014). Our results show that this adverse effect of salicylic acid on sugar content is present also after 2 months.

Protein content and peroxidase activity

Application of SA induced significant increase in protein content dependent upon applied SA concentration (**Tab. 2**). Considering two month period from the SA application, this increase in protein content could be considered as a permanent effect of SA application. Considering that protein content is usually decreased when plants are exposed to stress (Kabiri et al., 2014) plants with higher protein content are primed for upcoming stress conditions.

Peroxidase activity was significantly higher in plants sprayed with 0.01 and 0.1 mM SA as compared with control plants and plants sprayed with 1.0 mM, even after 2 months (**Fig. 1**). These differences between peroxidase activities are probably result of activation of defence mechanisms.

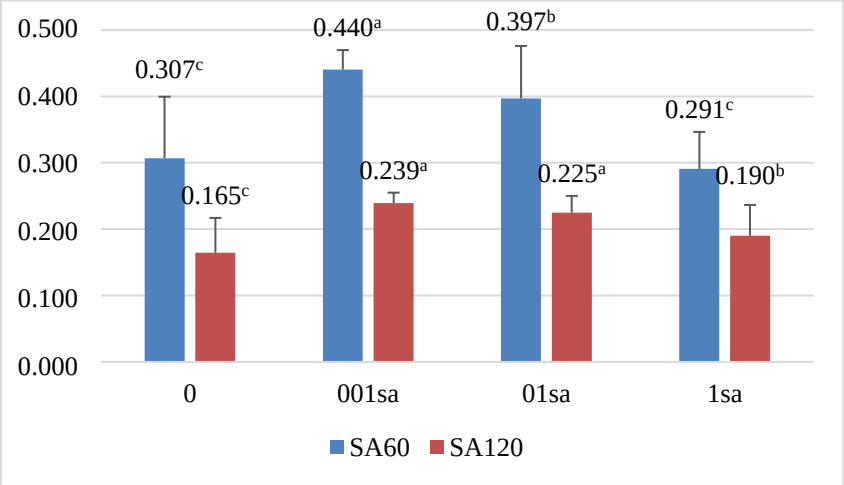

Fig. 1. Effect of salicylic acid on peroxidase activity in basil two months after treatment

Foliar pre-treatment of plants with abiotic elicitors induce plant resistance for different stressors. One of chemicals responsible for defence induction are phytohormones (Vicente & Plasencia, 2011; Maffei et al., 2007, War et al., 2011). Induction of plant defence is usually mediated through physiological, biochemical and molecular mechanisms (Vicente & Plasencia, 2011). Salicylic acid is an important endogenous plant growth regulator that induces different metabolic and physiological responses in plants involved in plant defence as well as in plant growth and development (Chen et al., 2009, Hayat et al., 2009, Zhao et al., 2009).

Conclusion

Foliar application of SA induces growth as well as biochemical changes in basil plants and these changes are evident also after 2 months. Having in mind presented results we can conclude that SA induces not only immediate plant defence response but also has long-term effect on plant growth and metabolism. Such effect primes the plant also for future upcoming stress conditions.

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