STUDIES REGARDING THE INFLUENCE OF ACETYLSALICILIC ACID ON SOME PHYSIOLOGICAL PROCESSES IN SUNFLOWER (*Helianthus* sp.) SEEDLING

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Abstract. In this paper we studied the influence of exogenous acetylsalicylic acid (ASA) solutions administrated in different concentrations (0.01 mM, 0.1 mM, 0.5 mM, 1.0 mM and 5.0 mM) to sunflower seeds – by presoaking it for 6 hour before germination -on some physiological and biochemical processes including: plant growth and development of plants, total absorption capacity of roots and assimilatory pigments content of the first leaves of sunflower seedlings, in comparison with the same parameters of the control lots which were treated with tape water. The results showed that exogenous 0.1mM ASA solutions, administrated to the sunflower seeds significantly increased the length of the sunflower seedlings, and the total absorption capacity of roots. The 5.0 mM ASA solution determined a very significant inhibition of the physiological processes. Spraying the primary leaves of sunflower with 0.1 mM ASA solution significantly increased the *total chlorophyllian* and *carotenoid pigments* content, and more concentrated solutions significantly decreased this parameter.

Keywords: sunflower, acetylsalicylic acid, root, growth, total absorption capacity, primary leaves, chlorophyllian pigments, carotenoid pigments.

INTRODUCTION

One of the new plant growth regulators is salicylic acid (SA) and its derivates like acetylsalicylic acid (ASA). They have a significant impact on the various aspects of the plant life. In this paper we study the action of ASA treatments under laboratory conditions, on some of physiological processes like plant growth and development of plants, total absorption capacity of roots and assimilatory pigments content of the first leaves of sunflower seedlings, in comparison with the same parameters of the control lot which were treated with tape water.

Aspirin, a trade name for acetylsalicylic acid (ASA), a close analog of salicylic acid, was introduced by the Bayer Company in 1898 and rapidly became one of the most popular pharmaceutical preparations in the world. During the 19th century many compounds belonging to the group of salicylates were isolated from a variety of plants. The story of salicylates has been summarized by Weissmann [15]. Aspirin undergoes spontaneous hydrolysis to SA. Exogenously applied it is rapidly converted to SA. Despite the fact that aspirin was not identified as a natural product, it is widely used by many plant scientists in their experiments. The reason is the similarity in their physiological effects.

The application of salicylic acid, or acetylsalicylic acid or other analogues of SA, to leaves of maize and soybean accelerated the growth of their leaf area, and the dry mass production, but plant height and root length remained unaffected [8]. Out of the various concentrations of SA solutions used, Fariduddin et al. [2], observed maximum increase in dry matter accumulation at a concentration of 10^{-5} M, supplemented to the leaves of the standing plants of *Brassica juncea,* but any concentration higher than this proved to have an inhibitory effect.

Gutiérrez-Coronado et al. [4] found that SA sprayed on leaves increases significantly the root growth in soybean plants, and Gutiérrez-Rodríguez et al. [5] found that SA stimulated root growth in carrot, radish, and beet plants. It's important to know if SA stimulated root growth in ligneous species such as *Pinus patula* Schl. Et Cham, one specie extensively planted in parks, gardens and forests of México [14].

The metabolic aspect of plants, supplied with SA solution or its derivatives shifted to a varied degree depending on the plant type and the mode of application of SA solution. The application of SA solution (20 mg/ml) to the foliage of the plants of *Brassica napus*, improved the chlorophyll contents [3]. Similarly, soaking the grains of wheat in 10^{-5}m of SA solution resulted in higher pigment contents in the plants which declined as the concentration of SA was increased above that concentration [6]. Moreover, 30 days old plants of *Brassica juncea* sprayed with 10^{-5}M of SA solution possessed chlorophyll 20% higher than those sprayed by water only, however the maximum concentration (10^{-3}) decreased the chlorophyll contents and the values were below that the water sprayed control at 60 days stage [2].

Xue Jianping et al. [16] studied the effects of different concentrations of SA solution on the growth of *Pinellia ternate*. When the height of the plant was about 10cm, we sprayed them with different concentrations of SA solution and measured height, total chlorophyll content, activity of SOD, mad content, photosynthesis speed, intercellular CO_2 concentration, the transpiration speed and the leaf temperature. The results indicated that intercellular CO_2 concentration increased, leaf temperature decreased and photosynthesis speed was well in 0.5 mM SA solution. In conclusion, the concentration of 0.5 mM SA solution was suitable for the growth of *P. ternate.*

Purcărea and Cachiţă [12], studying the influence of salicylic acid (SA) and acetylsalycilic acid (ASA), on the growth of sunflower (*Helianthus sp*.), seedling roots, on their total absorption capacity and on content of assimilatory pigments in the primary leaves, observed that diluted ASA solution, with 0.01 mM the 0.1 mM concentration determine an increase in the total chlorophyllian and carotenoid pigments content in

the primary leaves of sunflower plantlets especially for 0.01 mM the 0.1 mM concentration. Higher concentrations than 0.5 mM decreased the same parameter, the greatest inhibitions being obtained for the SA or ASA solutions of a 5.0 mM concentration. The same authors [13], comparing the effects of the two solutions was observed that on the 6th day of germination the diluted ASA solutions, with concentrations of 0.01, 0.1 and 0.5 mM had greater effects, the highest increase of the total absorption capacity of sunflower root system, with 159.6% compared to the control lot being recorded in case of the treatment with 0.1 mM ASA solution. In that case the sunflower seeds were germinated on a filter paper moistened with 0.01 mM, 0.1 mM, 0.5 mM, 1.0 mM or 5.0 mM ASA solutions, and with water, for the control lot.

The aim of this work was to study the influence of the exogenous ASA solution (applied by soaking the seeds for 6 hour in the solutions) on some physiological processes like plant growth and development of plants, total absorption capacity of roots and assimilatory pigments content of the first leaves of sunflower *(Helianthus sp.)* seedlings, in comparison with the same parameters of the control lots which were treated with water.

MATERIALS AND METHODS

For study the action of ASA treatments under laboratory conditions, the sunflower seeds were soaked for 6 hours in 0.01 mM (V_1), 0.1 mM (V_2), 0.5 mM (V_3), 1.0 mM (V_4) or 5.0 mM (V_5) ASA solutions and in tap water for the control lot (V_0). Then the seeds were germinated for 7 days in plastic boxes.

The germination was made on filter paper moistened with tape water, at 20±3 °C in a Sanyo MLR 351H phytotron, day/night, and relative humidity 65-85%, under natural photon flux density. Every day, the quantity of solution from the recipients was brought to the level of 20 ml.

Growth - After 6 days we measured the length of the embryonic roots, shoots and the total length of the sunflower seedlings obtained from the seeds germination under laboratory conditions and – in parallel – we studied the total absorption capacity of the sunflower seedling roots. For this, the sunflower seedlings were sunk for 1 hour in a 1/10000 concentration of a neutral red (a vital stain) solution. The dilution was realized with tap water. Neutral red (toluidine red) can be used as a vital stain, to stain living cells. It is used to stain cell cultures for plaque titration of viruses. Neutral Red is added to some growth media for bacteria and cell cultures. It usually comes as a chloride salt. These vital stains act as a pH indicator, changing from red to yellow between the pH 6.8-8.0.

After 1 hour, the seedlings were put out and then were washed with water. The neutral red was extracted from the roots with ethylic alcohol 70% mixed with acetic acid 1% solution, in equal parts [9]. The neutral red absorption, in the wheat seedling roots, treated or not treated with ASA solution, was determined spectrophotorimetric with UV-visible mini–1240 Shimadzu spectrophotometer, at 530 nm wavelengths. The total absorption (TA) was calculated after the neutral red concentration from the extract was determined. For each sample were made 3 determinations, and in order to calculate we used the following relations [1]:

$$TA = \frac{CxV}{n} mg / seedling / h$$

C = the concentration read on the etalon curve of the neutral red

V = the volume of the colorant solution extracted from the roots

n = number of seedlings

Assimilatory pigments - after 7 days of germination we planted the plantlets in sand, leaving them there for an additional 7 days, and sprayed their primary leaves each day with 1 ml of water.

On the 14th day we determined the content of chlorophyllian pigments of the sunflower plantlets primary leaves, using N,N-dimethylformamide, 99.9%, [10] for the extraction. The extraction of assimilatory pigments in higher plant tissue using N,N-dimethylformamide (DMF), expedites the process and enables the determination of small samples with low pigment level [11]. There is a vast array of solvents used for the extraction and determination of the chlorophyllian pigments, but most of them necessitate grinding and centrifuging of material with or without heating. The use of DMF renders the process simpler and faster, since the pigments can be extracted from intact tissue. For extraction, 50 mg fresh weight of primary leaves, were collected separately from each sample, and were blended with 5ml DMF and then cooled at 4˚C for 72 hours. The supernatant was separated and the content of the pigment was determined using a UV-visible mini–1240 Shimadzu spectrophotometer, at 664nm wave length for chlorophyll a, 647 nm for chlorophyll b and 480 nm for carotenoids. For each sample we made 3 determinations.

The data obtained after the spectrophotometrycal determination, was mathematically processed using formulae proposed by Moran and Porath [10]. For the determination of the specific extinction coefficients (SEC), they made pure chlorophyll a, and chlorophyll b pigments solution similarly prepared from DMF extracts. The SEC was determined by the equation:

$$A_\lambda = \varepsilon\, cl$$

Where A_λ is the absorbance at a given wavelength, ε is the SEC of the solution at wavelength λ, c is the concentration g/l, and l is the beam-path (1cm) in the measuring cuvette. Solving the equation for A_{664}, A_{647} and A_{480} wavelength, Moran (1982), obtained the formulae for determination of chlorophyll a, chlorophyll b contents.

Chlorophyll a (mg/g sp) = (11.65 a_{664} – 2.69 a_{647}) • V/sp

Chlorophyll b (mg/g sp) = (20.81 a_{647} – 4.53 a_{664}) • V/sp

Carotenoids (mg/g sp) = (1000 A_{480} – 1.28 chloroph.a – 56.7 chloroph.b)/ 245 • V/sp

when the spectrophotometer resolution was 1-4 nm.

The results obtained after the content of assimilatory pigments determination are averages of 3 determinations and was statistically processed using the "t- test" using Prisma 5 for Windows. The values of the probabilities were determined from tables using the values of the "t" distribution and the freedom degrees based on which the variance of the empiric series was calculated.

RESULTS

Studying the growth of the embryonic roots of the sunflower seedlings obtained from the seeds germination under laboratory conditions, after 6 days of germination, we observed that the influence of the exogenous ASA treatments was dependent on the concentration which was used (Table 1).

In comparison with the embryonic roots of control lot considered 100% (Table 1, Fig. 1) a very significant increase of the roots length was observed in the first 6 days of germination, when it was used 0.1; 0.5; 1.0 mM ASA solution (the increases were between 46% and 80%). We find a distinctly significant increase of it, with 34%, from the control lot after 6 hours of sunflower presoaking in 0.01 mM ASA solution. For the treatment with 5.0 mM ASA solution it was registered a very significant decrease of roots length, with 56.0% from control lot considered 100%.

For the shoots lengths it was observed a significant increase (between 11.2% and 15%) after presoaking the sunflower seeds in 0.1; 0.5 and 1.0 mM ASA solutions. At the variant of a 0.01 mM or 5.0 mM ASA solution treatment, after 6 day of germination the shoots length decreased distinctly significant, with 18.7%. The 5.0 mM ASA treatment determined a very significantly decrease of shoot length in comparison with the control lot.

Table 1. Estimative mean values for the length of the sunflower seedling roots, shoots and total lengths observed in the 6-th days of seeds germination on a filter paper moistened with tap water after 6 hour soaking for the control lot in water (V_0) and in ASA solutions of different concentrations

Biometrics / Statistic evaluation	Roots lenght (mm)	Shoots lenght (mm)	Plants lenght (mm)
Type V_0 (water)			
M ± sd	50±2,21	80±2,2	130±3,2
VC	4.42	2.77	2.46
Type V_1 (0.01 mM ASA)			
M ± sd	67±2.67	65±1.75	132±2.9
VC	3.98	2.69	0.21
Statistical signification	**	**	ns
Type V_2 (0.1 mM ASA)			
M ± sd	90±3.15	92±3.65	182±4.5
VC	3.5	3.96	2.47
Statistical signification	***	*	***
Type V_3 (0.5 mM ASA)			
M ± sd	82±2.85	90±3.61	172±3.9
VC	3.47	4.01	2.26
Statistical signification	***	*	***
Type V_4 (1.0 mM ASA)			
M ± sd	73±2.98	89±4.5	162±3.8
VC	4.08	5.05	2.34
Statistical signification	***	*	***
Type V_5 (5.0 mM ASA)			
M ± sd	22±0.98	18±0.656	40±1.2
VC	4.45	3.64	3.0
Statistical signification	***	***	***

M =mean value; st = standard deviation; VC= variability
p>0.05= no significant; p<0.05 * significant; p<0.01=** distinctly significant; p<0.001=*** very significant in comparison with control lot.

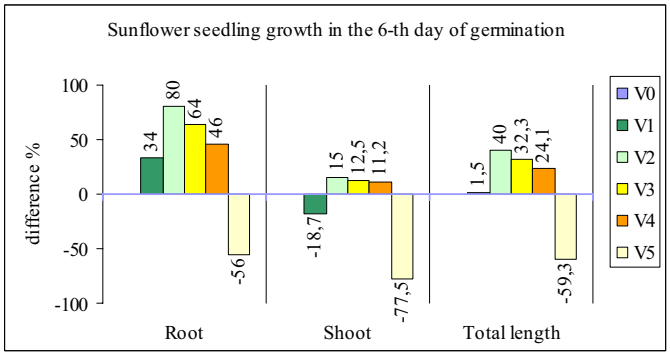

Figure 1. Percentage differences of the root, shoot, total length of sunflower seedlings obtained from seeds germination after soaking it in 0.01 mM, 0.1 mM; 0.5 mM; 1.0 mM and 5.0 mM concentration ASA solution, in comparison with the same parameter measured in seedling from the control lot soaked in water. The value for the control lot was considered 100%.

For the total length of the sunflower seedlings after 6-th day of germination we registered a very significant increase in the case of treatment with 0.1; 0.5 or 1.0 mM ASA solutions; a no significantly decrease for treatment with 0.01 mM ASA solution and a very significantly decrease for treatment with 5.0 mM ASA solution.

Table 2. Estimative mean values and statistical significance for the total absorption capacity of the sunflower seedling roots, in the 6-th days of seeds germination on a filter paper moistened with tap water, after 6 hour soaking for the control lot in water (V_0) and in ASA solutions of different concentrations

Parameters	V_0	Acetylsalicylic acid solutions				
		V_1	V_2	V_3	V_4	V_5
	Average ± standard deviation					
Total absorption (TA) mg/seedling/h	1.847±0,1	2.155±0,03 *	2.44±0.03 ***	1.91±0,02 ns	1.61±0.1 *	1.03±0.02 ***

p>0.05= not significant; p<0.05 * significant; p<0.01=** distinctly significant; p<0.001=*** very significant in comparison with control lot.

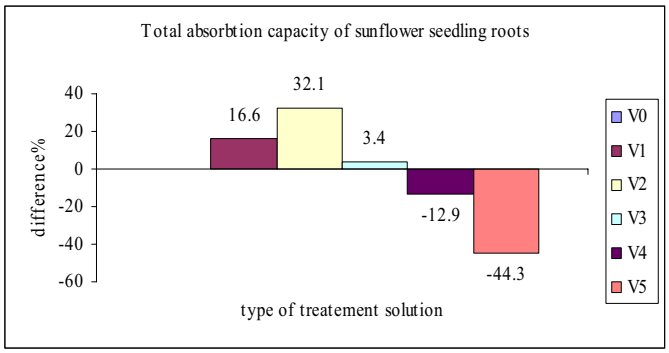

Figure 2. Percentage differences of the total absorption capacity of sunflower seedlings roots obtained from seeds germination after soaking it in 0.01 mM, 0.1 mM; 0.5 mM; 1.0 mM and 5.0 mM concentration ASA solution, in comparison with the same parameter measured in seedling from the control lot soaked in water. The value for the control lot was considered 100%.

Table 3. Estimative mean values for the assimilatory pigments content of the sunflower seedling leaves after treatment with ASA solutions of different concentrations.

Parameters	V_0	Acetylsalicylic acid solutions				
		V_1	V_2	V_3	V_4	V_5
	Average ± standard deviation					
Total chlorophyllian pigments mg/g	0.629±0.011	0.692±0.005 *	0.72±0.003 ***	0.557±0.008 ***	0.468±0.006 ***	0.304±0.003 ***
Chlorophyll a mg/g	0.376±0.04	0.396±0.01 ns	0.423±0.003 *	0.334±0.006 *	0.281±0.009 **	0.145±0.002 ***
Chlorophyll b mg/g	0.253±0.002	0.266±0.001 ns	0.297±0.003 ***	0.223±0.01 *	0.187±0.003 ***	0.159±0.005 ***
Carotenoid pigments mg/g	0.128±0.005	0.168±0.004 ***	0.142±0.002 *	0.118±0.006 ns	0.100±0.006 **	0.07±0.001 ***

P>0.05= non-significant; p<0.05= * significant; p<0.01=** distinctly significant; p<0.001=*** very significant in comparison with the control lot

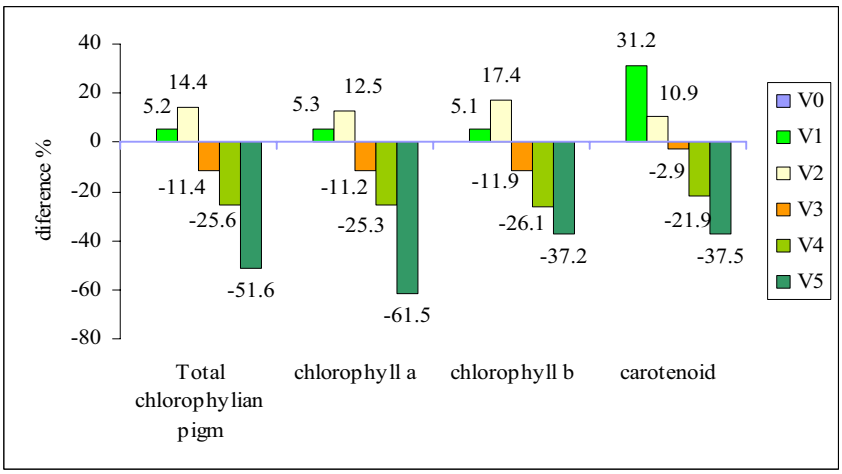

Figure 3. Percentage differences of the content of assimilatory pigments in the primary leaves of sunflower (*Helianthus sp.)* seedlings obtained from seeds germinated on filter paper moistened with water, at 20±3°C. The sunflower seedlings were planted for an additional 7 days in sand and their primary leaves were sprayed with 0.01 mM, 0.1 mM, 0.5 mM, 1.0 mM or 5.0 mM concentration ASA solution in comparison with the same parameter measured in the leaves of sunflower plantlets from the control lot sprayed with water. The value for the control lot was considered 100% (marked with 0 on the graphic).

The results obtained for the *total absorption (TA)* of the neutral red in the sunflower seedling roots were presented in Table 2, and the percentage differences were graphically represented in the Fig. 2.

In comparison with the control lot, after 6 days of germination, the total absorption (TA) of neutral red in the sunflower seedling roots presented a significant increase. Total absorption increased significantly with 16.6% for 0.01 mM ASA solution treatment, very significantly with 32.1% for 0.1 mM ASA solution, and no significantly with 3.4% in the case of treatment with 0.5 mM ASA solution. In the case of 1.0 mM SA solution the TA decreased significantly, with 12.9%, and very significantly, with 44.3%, for 5.0 mM ASA solution.

Studying the content of chlorophyllian pigment (chlorophyll a and b) and carotenoids on the primary leaves of the sunflower seedling obtained from each experimental variant, we observed that the influence of the exogenous ASA solutions treatment was dependent on the concentration which was used. The results obtained were presented in Table 3 and graphically represented in Fig. 3.

The content of chlorophyll a increased no significantly (with 5.3% from control lot considered 100%) after seeds presoaking in 0.01 mM ASA solution. A significant increase of chlorophyll a contents, with 12.5% from the control lot, was observed in the case of treatment with 0.1 mM ASA solution. For higher concentrations than 0.1 mM the chlorophyll a content decreased significantly and very significantly from the control lot.

In the case of the chlorophyll b contents a no significant increase could be observed, with 5.1% from control lot when using a 0.01 mM ASA solution, and a very significant increase, with 17.4 % from the control lot, in the case of treatment with 0.1 mM ASA solution. 0.5 mM, 1.0 mM or 5.0 mM ASA solutions significantly or very significantly decreased the chlorophyll b contents in primary leaves of the sunflower plantlets.

Studying the carotenoids pigments content in the case of treatment with 0.01 mM concentrations ASA solution, the results show that the accumulation of these pigments in the leaves of sunflower seedling on the 14^{th} day of germination, increased very significantly, with 31.2%, in comparison with the same parameter determined from the control lot. The treatment with 0.1 mM ASA solution significantly increased this pigment contents, with 10.9%, from control lot. After treatment with 0.5 mM, 1.0 mM and 5.0 mM ASA solution significantly, distinct significantly or very significantly decreased the carotenoid pigment contents, with values between 2.9% and 37.5% from the control lot.

DISCUSSIONS

The exogenous 0.01 mM, 0.1 mM, 0.5 mM and 1.0 mM ASA solutions enhanced the growth of the sunflower seedling but any concentrations above these values proved to have an inhibitory effect Similar results was obtained by Fariduddin et al.[2], when they are studying the effect of exogenous SA and its derivatives on growth of *Brassica junceea* observed maximum increase in dry matter accumulation at a concentration of 0,01 mM SA, supplemented to the leaves of the standing plants of *Brassica juncea,* but any concentration higher than this proved to have an inhibitory effect.

Hussein et al. [7], spraying maize plants with salicylic acid in the rate of 200 ppm improved all growth characters i.e. plant height, number and area of green leaves, stem diameter and dry weight of stem, leaves and whole plant.

Soaking sunflower seeds for 6 hours in 0.01mM, 0.1mM or 0.5 mM, ASA solutions and after 6 day germination, on a filter paper moistened with water, the total absorption capacity of the sunflower root system increased significantly. This moment coincided with the first leaf formation, and its intense growth. For higher concentration the total absorption capacity of the sunflower root system decreased significantly.

Diluted ASA solutions, with 0.01 mM the 0.1 mM concentration, determined an increase in the total chlorophyllian and carotenoid pigments content in the primary leaves of sunflower plantlets. Higher concentrations than 0.5 mM decreased the same parameter, the greatest inhibitions being obtained for the ASA solutions of a 5.0 mM concentration. Hayat et al. [6] soaking the grains of wheat in 10^{-5}m of SA solution resulted in higher pigment contents in the plants which declined as the concentration of SA was increased above that concentration. Moreover, 30 days old plants of *Brassica juncea* sprayed with 10^{-5}M of SA solution possessed chlorophyll 20% higher than those sprayed by water only.

Comparing the effects of different concentration ASA solutions it was observed that on the 6^{th} day of germination the diluted ASA solutions, with 0.1 and 0.5 mM concentrations had greater effects, the highest increase was registered for 0.1 mM ASA solution, for the majority of the parameters.

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