THE INFLUENCE OF SALICYLIC ACID AND ACETYLSALICYLIC ACID ON THE GROWTH OF SUNFLOWER (*HELIANTHUS SP.*) SEEDLING ROOTS AND ON THEIR TOTAL ABSORPTION CAPACITY

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ABSTRACT. In this paper we studied the influence of exogenous Salicylic Acid (SA) and 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 the sunflower seeds on the growth of the embrionary roots and on the total absorption capacity of the roots, in comparison with these parameters registered in the control lot, which was germinated in tap water. The results showed that, in comparison to the control lot germinated in tape water, the exogenous 0.01mM, 0.1mM and 0.5 mM SA or ASA solutions administrated to the sunflower seeds, increased the length of the seedling roots, the 1.0 mM SA or ASA solutions significantly decreased this parameter, and the 5.0 mM SA or ASA solution inhibited the germination of sunflower seeds.The total absorption capacity of sunflower roots was determined by the uptake in the roots of a neutral red solution (a vital stain). The results showed that, in comparison with the same parameters determined on the control lot germinated on a filter paper moistened with tap water, this absorption was significantly modified when the sunflower seeds were germinated on a filter paper moistened in SA or ASA solution.

Keywords: sunflower, salicylic acid, acetylsalicylic acid, root, growth, total absorption capacity, neutral red

INTRODUCTION

Salicylic acid (SA) or ortho-hydroxybenzoic acid and related compounds belong to a diverse group of plant phenolics. Salicylates from plant sources have been used in medicines since antiquity. In 1828 in Munich was isolated for the first time a small amount of salicin, the glucoside of salicyl alcohol, from willow bar. Ten years latter Raffaele Piria named it SA, from the Latin word Salix for willow tree. The first commercial production of synthetic SA began in Germany in 1874 (Raskin, 1992). Aspirin, 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 (1991). Aspirin, a trade name for acetylsalicylic acid (ASA), 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 salicylic acid (SA) or o-hydroxibenzoic acid, nowadays considered to be an important signal molecule, involved in the development process of plants (Ryan and Farmer, 1992). Actually, SA is considered a plant growth regulator, which influences many of the physiological, and biochemical processes, including photosynthesis (Hayat and Ahmad, 2005), ion uptake, membrane permeability (Barkosky and Einhellig, 1993), enzymes activities, flowering (Cleland and Ajami, 1974), growth and development of plants (Hayat and Ahmad, 2007), and may function as a plant growth regulator (Arberg, 1981).

Picomolar levels of SA enhance cell growth and the embryogenesis in the cell suspension cultures of *Coffea arabica*, as compared to entreated control cultures (Quiroz-Figueroa *et al.*, 2001). The authors suggested that it is possible as these phenolic compounds act as a signal, which induce the differentiation processes. An alternative explanation is the possibility that, due to the chelating properties of these compounds, some inhibitors present in the embryogenic cultures are inactivated.

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 (Khan *et al.*, 2003). Out of the various concentrations of SA solutions used, Fariduddin *et al.*, (2003) 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.* (1998) found that SA sprayed on leaves increases significantly the root growth in soybean plants, and Gutiérrez-Rodríguez et

al., 1999, found that SA stimulated root growth in carrot, radish, and beet plants. Its important to know if SA stimulated root growth in ligneous species such as *Pinus patula* Schl. Et Cham, one specie extensivily planted in parks, gardens and forests of México (Perry, 1991).

The aim of this work was to study the influence of the exogenous SA or ASA solution on the total absorption capacity of the sunflower (*Helianthus sp.*) roots system.

MATERIALS AND METHODS

Sample preparation: SA or ASA treatments were applied to lots of 150 sunflower seeds/sample, germinated in 3 plastic recipients. The sunflower seeds used in this study have a 95% germination faculty.

To study the action of SA or ASA treatments under laboratory conditions, 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 SA or ASA solutions, and with water, for the control lot. Then, the seeds were germinated for 6 days in plastic boxes. The germination was made at 22±2ºC. Every day, the quantity of solution from the recipients was brought to the level of 20 ml. After 6 days we measured the length of the embrionary roots 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 neutral red (a vital stain) solution. The dilution was realised with tap water. Neutral red (toluylene 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. This vital stains acts 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 (Marki and Cachiţă–Cosma, 1968). The neutral red absorption, in the sunflower seedling roots, treated or not treated with SA or ASA solution, was determined spectrophotorimetric with Spekol 11 Carl Zeiss Jena, at 530 nm wavelengths. The total absorption (TA) were 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 (Apostol and Cachiţă-Cosma, 1969):

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

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

The results obtained after biometrical measurement and total absorption capacity determination were statistically processed using the "t- test" in 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 AND DISCUSSION

Studying the growth of the embrionary 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 SA or ASA treatments was dependent on the concentration which was used (table 1).

In comparison with the embrionary roots of control lot considered 100% (table 1, figure 1) a very significant increase of the roots length was observed in the first 6 days of germination, when it was used 0.01 mM SA or ASA solution (with 45% for SA solution and with 32.2% for ASA solution). In the case of germination on a filter paper moistened with 0.1 mM SA or ASA solution, we observed a very significant increase of the roots lenght, with 100% from control lot, for the SA solution and with 112.9% when we used ASA. We find a nonsignificant increase of it, with 3.3%, from the control lot after 6th day of germination on a filter paper moistened with 0.5 mM SA solution and a significant increase of roots length, with 29% from control lot considered 100%, when we used 0.5 mM ASA solution.

At the variant of a 1.0 mM SA or ASA solution treatment, after 6 day of germination the roots length decreased very significantly, with 33.4% from control lot, in case of treatement with SA solution and it increased significantly, with 9.7% from control lot when we used 1.0 mM ASA solution.

The 5.0 mM SA or ASA solution completly inhibited the germination of sunflower seeds.

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 figure 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 58.1% for 0.01 mM SA solution, with 68.8% for 0.1 mM SA solution, with 24.5%% in the case of treatment with 0.5 mM SA solution and with 23.9% in the case of 1.0 mM SA solution.

In the 6th day of germination a significant increase for total absorption (TA) was observed, with 73.6% for 0.01 mM ASA solution, a very significant increase with 159.6% for 0.1 mM ASA solution, with 95.6% from control lot for 0.5 mM ASA solution, and with the TA were insignificantly decreased with 0.2% in the case of treatment with 1.0 mM ASA solution.

Table 1

Estimative mean values for the length of the sunflower seedling roots and hypocotiles observed in the first 6 days of seeds germination on a filter paper moistened with tap water for the control lot and with SA or ASA solutions of different concentrations

The type of solutions	Parameters	Control	Solutions concentration (mM conc)			
			0.01mM	0.1mM	0.5mM	1.0mM
			Average ± standard deviation			
Salicylic acid SA	Embryonary root length (mm)	60±2.3	87±1.92 ***	120±4.5 ***	62±2.4 ns	40±1.67 ***
Acetilsalicylic acid ASA	Embryonary root length (mm)	62±1.11	82±4.3 ***	132±2.6 ***	80±2.8 ***	56±1.87 *

*p>0.05= nonsignificant; p<0.05 * significant; p<0.01=** distinctly significant; p<0.001=*** very significant in comparison with control lot*

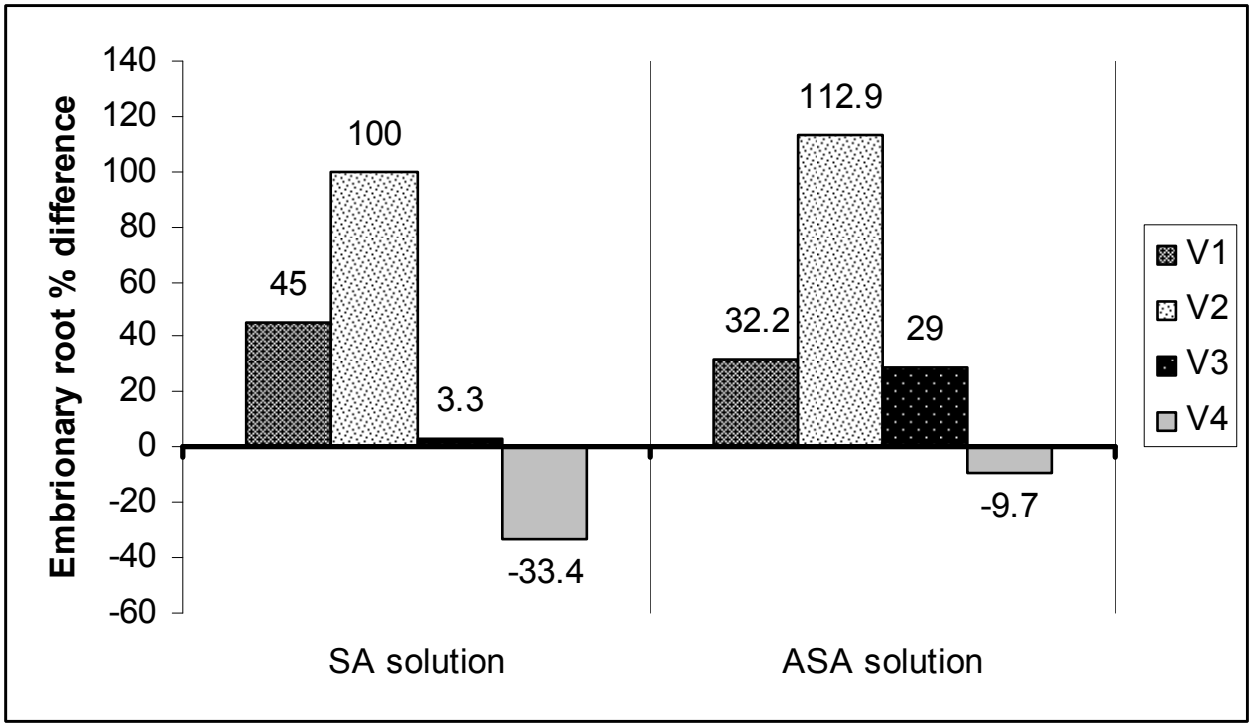

Fig. 1 Percentage differences of the embryonary root length of sunflower seedlings obtained from seeds germinated in 0.01 mM, 0.1 mM; 0.5 mM; 1.0 mM concentration SA or ASA solution, in comparison with the same parameter measured in seedling from the control lot germinated in water. The value for the control lot was considered 100%

Table 2

Estimative mean values for the *total absorption* (TA) of sunflower seedling roots observed after 6 days of germination on a filter paper moistened with SA or ASA solutions of different concentrations or tap water for control lot

Parameters	Control	Salicylic acid or Acetylsalicylic acid solutions (mM conc)			
		0.01mM	0.1mM	0.5mM	1.0mM
		Average ± standard deviation			
Total absorption (TA) mg/seedling/h SA treatment	1,67±0,02	2,64±0,03 ***	2,82±0,05 ***	2,48±0,2 **	2,27±0,15 **
Total absorption (TA) mg/seedling/h ASA treatment	1,67±0,02	2,9±0,1 ***	4,33±0,21 ***	3,26±0,18 ***	1,651±0,1 ns

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

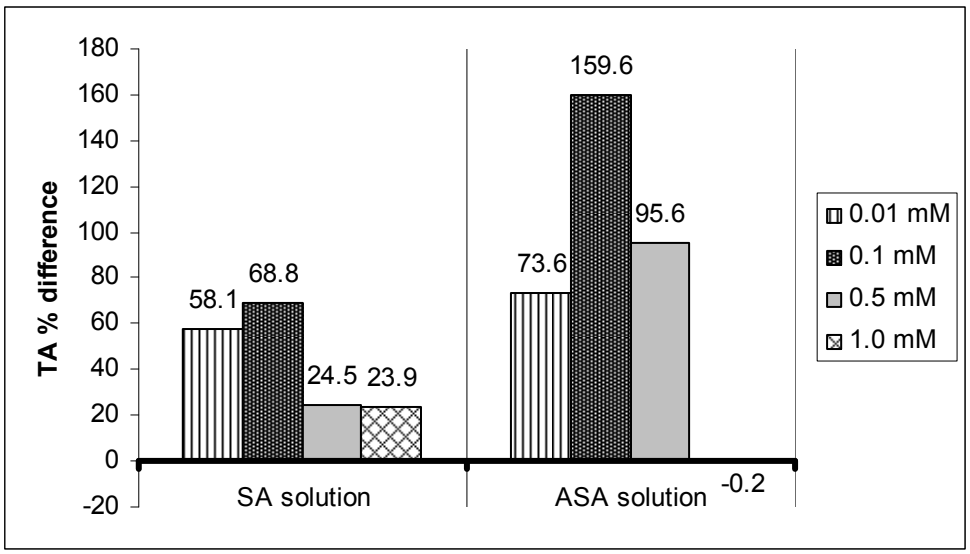

Fig. 2 Percentage difference of (TA) of the sunflower seedling roots after 6 days of germination obtained from seeds germinated on filter paper moistened with SA or ASA solutions of 0.01 mM, 0.1 mM, 0.5 mM, or 1,0 mM concentration in comparison with the same parameter measured in seedlings from the control lot germinated on filter paper moistened with water. The value for the control lot was considered 100%

CONCLUSIONS

The exogenous 0.01 mM, 0.1 mM and 0.5 mM SA or ASA solutions enhanced the growth of the sunflower seedling roots, but any concentrations above these values proved to have an inhibitory effect.

The 5.0 mM SA or ASA solution completly inhibited the germination of sunflower seeds.

The exogenous 0.01 mM, 0.1 mM and 0.5 mM ASA solutions treatments determine more significant increases on the growth of sunflower seedling roots than treatments with SA solutions in the same concentrations do, the highest increase with 112.9% from control lot being recorded in case of treatement with 0,1 ASA solution.

After 6 days of germination, on a filter paper moistened with 0.01mM, 0.1mM, 0.5 mM or 1.0 mM SA solutions, the total absorption of the sunflower root system increased significantly. This moment coincided with the first leaf formation, and its intense growth.

The 0.01mM, 0.1mM or 0.5 mM ASA solutions significantly increased the total absorption of the sunflower root system.

Comparing the effects of the two solutions it was observed that on the 6th day of germination the diluted ASA solutions, with concentrations of 0.01, 0.1 si 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.

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