COMPARATIVE STUDIES ABOUT THE INFLUENCE OF SALICYLIC AND ACETYLSALICILIC ACID ON CONTENT OF ASSIMILATORY PIGMENTS IN THE PRIMARY LEAVES OF SUNFLOWER (*HELIANTHUS SP.*) PLANTLETS

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ABSTRACT. Salicylic acid (SA) and acetylsalicylic acid (ASA) are phenolic compounds recently recognized as plant growth regulators involved in many physiological processes including photosynthesis. The aim of this paper is to study the influence of exogenous salicylic and acetylsalicylic acid in different concentrations on the assimilatory pigments contents of the leaves of sunflower seedlings in comparison with the same parameters of the control lots which were treated with water. The sunflower seedlings primary leaves were sprayed for an additional 7 days with 1 ml salicylic acid (SA) or acetylsalicylic acid (ASA) 0.01mM; 0.1mM; 0.5 mM, 1.0mM, 5.0 mM and with water for the control lots. In the 14th day of germination we determined the content of assimilatory pigments extracted with *N,N-dimethylformamide* (DMF). The results showed that exogenous 0.01mM; 0.1mM SA and ASA solutions, increased the *total chlorophyllian* and *carptenoid pigments* content, and more concentrated solutions decreased this parameter.

Keywords: sunflower, salicylic acid, acetylsalicylic acid, primary leaves, total clorohyllian pigments carotenoid pigments

INTRODUCTION

In addition to the classical plant hormones, new natural growth substances with regulatory roles in tissue culture have been discovered in the last few years (Gross and Parthier, 1994). One of these substances is salicylic acid and its derivatives. Salicylic acid could be raised to the status of the above phytohormones because it has significant impact on the various aspects of the plant life (Hayat and Ahmad, 2007).

Salicylic acid or ortho-hidroxibenzoic acid belongs to a diverse group of plant phenolics. These are compounds with an aromatic ring bearing a hydroxyl group or its functional derivative (Raskin, 1992). Salicylic acid is a natural signaling molecule involved in the regulation of different physiological processes including photosynthesis.

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 (Ghai et al., 2002). Similarly, soaking the grains of wheat in 10-5m of SA solution resulted in higher pigment contents in the plants which declined as the concentration of SA was increased above that concentration (Hayat et al., 2005). Moreover, 30 days old plants of *Brassica Juncea* sprayed with 10-5m 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 (Fariduddin et al., 2003).

Soaking the seeds of *Vigna mungo* in aqueous solutions of SA (10-150μm) lead to a decrease in the content of chlorophyll and carotenoid in the leaves of subsequent plants, but supplementing SA through irrigant did not prove as severe as seed treatment (Anandhi and Ramanujam, 1997).

Salicylic acid activated the synthesis of carotenoids, xanthophylls and the rate of de-epoxidation but decreased the level of chlorophyll pigments, both in wheat and moong plants also the ratio of chlorophyll a/b, in wheat plantlets (Moharekar et al., 2003).

The application of salicylic acid, acetylsalicylic acid or other analogues of SA, to leaves of corn and soybean accelerated their leaf area and dry mass production, but plant height and root length remained unaffected. However the leaves of corn and soybean treated with acetylsalicylic acid (ASA) or gentisic acid (GTA) exhibited no change in their chlorophyll contents (Khan et al, 2003).

Jianping Xue et al., 2006, 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*.

MATERIALS AND METHODS

For the study the action of SA and ASA treatments under laboratory conditions, the sunflower seeds were soaked for 6 hours in tap water. Then the seeds were germinated for 7 days in plastic boxes. The germination was made on moistened filter paper with tape water, at 20±3ºC.

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 0.01 mM, 0.1 mM, 0.5 mM, 1.0 mM or 5.0 mM SA or ASA solutions or with water for the control lot.

On the 14th day we determined the content of chlorophyllian pigments of the sunflower plantlets primary leaves, using *N,N-dimethylformamide*, 99.9%, (Moran and Porath, 1980) 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 (Moran, 1982). 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 Spekol 11 (Carl Zeiss Jena) 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 (1982). 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:

$$A_\lambda = \varepsilon\, c\, l$$

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 obtained the formulae for determination of chlorophyll a , chlorophyll b contents.

$$\text{Chlorophyll a (mg/g sp)} = (11.65\, a_{664} - 2.69\, a_{647}) \times V/sp$$
$$\text{Chlorophyll b (mg/g sp)} = (20.81\, a_{647} - 4.53\, a_{664}) \times V/sp$$
$$\text{Carotenoids (mg/g sp)} = (1000\, A_{480} - 1.28\ \text{chloroph.a} - 56.7\ \text{chloroph.b})/\ 245 \times V/sp$$

When the spectrophotometer resolution was 1-4 nm, or

$$\text{Chlorophyll a (µg/ml)} = 12A_{663.8} - 3.11A_{646.8}$$
$$\text{Chlorophyll b (µg/ml)} = 20.78A_{646.8} - 4.88A_{663.8}$$
$$\text{Carotenoids (µg/ml)} = (1000\, A_{480} - 1.12\ \text{chloroph.a} - 34.07\ \text{chloroph.}_b)/245$$

when the spectrophotometer resolution was 0.1-0.5m

Where:
A_{480} – the value read with a 480 nm filter
A_{647} – the value read with a 647 nm filter
A_{664} – the value read with a 664 nm filter
V – ml of solvent used
sp – mg of material used for one extraction/sample
chlorophyll a and b – quantity in mg calculated in the first two formulas

The results obtained after the content of assimilatory pigments determination are averages of 3 determinations and were 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 AND DISCUSSION

Studying the content of chlorophyllian pigment (chlorophyll a and b) and carotenoids on the primary leaves of the sunflower plantlets obtained from each experimental variant, we observed that the influence of the exogenous SA or ASA solutions treatment was dependent on the concentration which was used. The results obtained were presented in table 1 and 2 and graphically represented in figure 1.

The content of chlorophyll a (table 1 and 2 figure 1) increased insignificantly (with 11.1% respectively with 5.3% from control lot considered 100%) after treatment with 0.01 mM SA or ASA solution. A significant increase of chlorophyll a contents, with 14.3% and with 12.5% from the control lot, was observed in the case of treatment with 0.1 mM SA or ASA solution. For higher concentrations than 0.5 mM the chlorophyll a content decreased significantly and very significantly from the control lot.

In the case of the chlorophyll b contents (table 1 and 2, figure 1) an insignificant increase could be observed, with 4.3% and 5.1% from control lot when using a 0.01 mM SA or ASA solution, and a significant increase, with 15.4 % or 17.4 % from the control lot, in the case of treatment with 0.1 mM SA or ASA solution. 0.5 mM, 1.0 mM or 5.0 mM SA or ASA solutions significantly decreased the chlorophyll b contents in primary leaves of the sunflower plantlets.

Studying the carotenoids pigments content (table 1 and 2, figure 1), in the case of treatment with 0.01 mM concentrations ASA solution, the results show that the

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accumulation of these pigments in the leaves of sunflower seedling on the 14th day of germination, increased very significantly, with 31.2%, in comparison with the same parameter determined from the control lot,. Same concentration of SA solution nonsignificantly increased the carotenoid pigments content (with 1,6%). 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 SA or ASA solution significantly, distinct significantly or very significantly decreased the carotenoid pigment contents, with values between 6.1% and 45.3% from the control lot.

CONCLUSIONS

Comparing the effects of the two solutions it was observed that the treatments with low concentration of ASA solutions had greater effects than SA solution for same concentration. 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.

Table 1

Estimative mean values for the assimilatory pigments content of the sunflower seedling leaves after treatment with SA solutions of different concentrations

Parameters	Control lot	Salycilic acid				
		0.01 mm	0.1 mm	0.5 mm	1.0 mm	5.0 mm
		average ± standard deviation				
Total chlorophyllian Pigments mg/g	0.802±0.009	0.805±0.006 Ns	0.812±0.011 *	0.73±0.008 *	0.562±0,008 ***	0.513±0.002 ***
Clorofila a mg/g	0.442±0.01	0.452±0.02 Ns	0.450±0.003 *	0.437±0.01 Ns	0.290±0.01 ***	0.263±0.01 ***
Clorofila b mg/g	0.360±0.004	0.353±0.01 Ns	0.362±0.02 *	0.293±0.003 ***	0.272±0.002 ***	0.250±0.003 ***
Carotenoid pigments Mg/g	0.182±0.003	0.185±0.002 Ns	0.180±0,005 Ns	0.171±0.003 *	0.149±0.004 ***	0.116±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*

Table 2

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

Parameters	Control lot	Acetylsalicylic acid				
		0.01 mM	0.1 mM	0.5 mM	1.0 mM	5.0 mM
		average ± standard deviation				
Total chlorophyllian pigments mg/g	0.629±0.011	0.622±0.005 Ns	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*

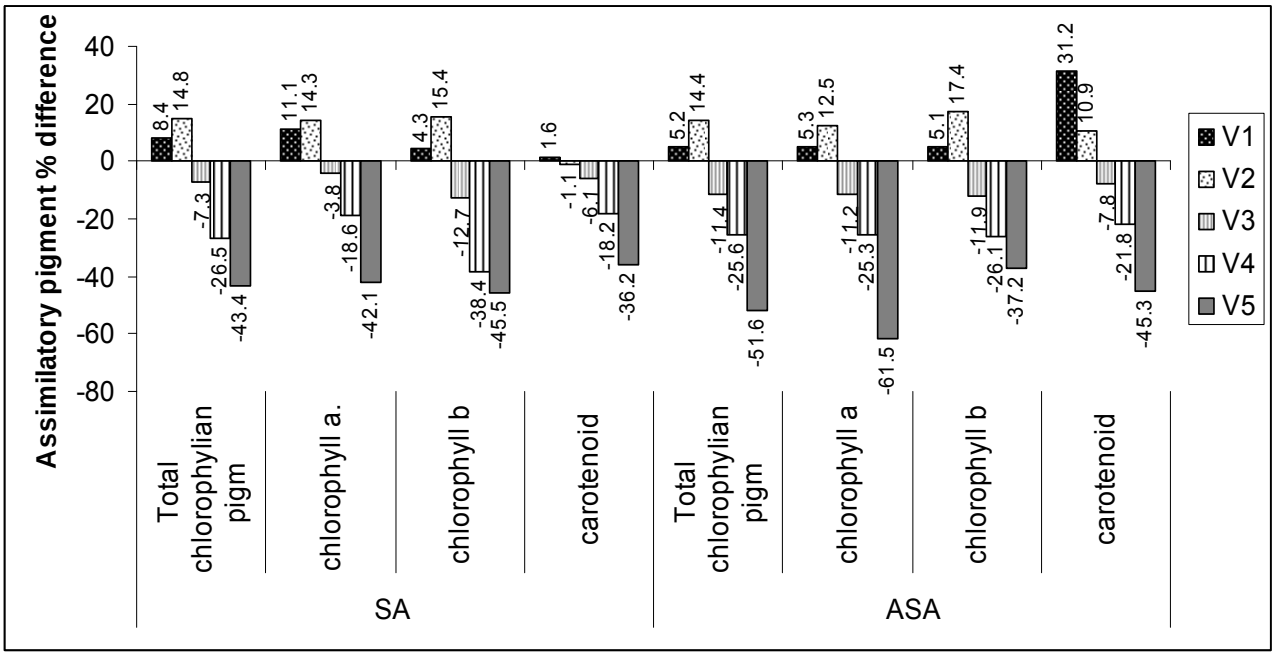

Fig. 1 Percentage differences of the content of assimilatory pigments in the primary leaves of sunflower (*Helianthus sp.)* seedlings obtained from seeds germinated on filter paper moistened with water, at 20±3 °C. The sunflower seedlings were planted for an additional 7 days in sand and their primary leaves were sprayed with 0.01 mM, 0.1 mM, 0.5 mM, 1.0 mM or 5.0 mM concentration SA or 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)

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