COMPARATIVE STUDIES ABOUT THE INFLUENCE OF SALICYLIC AND ACETYLSALICILIC ACID ON CONTENT OF ASSIMILATORY PIGMENTS IN THE PRIMARY LEAVES OF WHEAT (*Triticum aestivum)* PLANTLETS

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Summary: Salicylic acid (SA) and some of its derivates are phenolic compounds recently recognized as plant growth regulators involved in many physiological processes including photosynthesis. One of the important derivates of Salicylic Acid is the Acetylsalicylic Acid. In the present investigation we studied the influence of exogenous Acetylsalicylic and Salicylic acid with different concentrations on the assimilatory pigments contents of the primary leaves of wheat seedlings in comparison with the same parameters of the control lots which were treated with water. The wheat seedlings were soaked for 6 hours in 0.01mM; 0.1mM; 0.5mM and 1 mM SA or ASA solutions and in water for the control lot, germinated for 7 days on filter paper moistened with water. After that, we planted the plantlets in sand and sprayed their coleoptiles and primary leaves, each day for an additional 7 days, with water. In the 14th days of germination we determined the content of assimilatory pigments extracted with *N,N-dimethylformamide* (DMF). The results showed that exogenous 0.01 mM, 0.1mM, 0.5 mM or 1.0 mM SA solution treatments cause more significant increases in the assimilatory pigments contents in leaves of wheat plantlets than treatments with ASA solutions of the same concentrations do.

Keywords: wheat, salicylic acid, acetylsalicylic acid, primary leaves, clorohyll a, clorophyll b, 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, soakink the grains of wheat in 10^{-5}M of SA solution determinated in the plants a higher pigment contents 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^{-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 (Fariduddin *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).

Soaking the seeds of *Vigna mungo* in aqueous solutions of SA (10-150µm) lead 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).

Jianping Xue et al., 2006, studied the effects of different concentration of SA solution on the growth of *Pinellia ternate*. When the height of plant was about 10cm, sprayed them with different concentration 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 was 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

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

To study the action of SA or ASA treatments under laboratory conditions, the seeds were soaked for 6 hours in 0.01mM; 0.1mM; 0.5mM and 1 mM SA or ASA solutions and in water for the control lot. For every concentration of the solutions we made 3 repetitions.

The germination was made at room temperature on filter paper moistened with water. Every day, the quantity of solution from the recipients was brought to the level of 20 ml. The germination temperature was around 20-23˚C. After 6 days of germination we planted the seedlings in sand or dirt leaving them there for an additional 7 days spraying them with water. After 7 days we determine the chlorophyllian pigment contents of the maize seedling leaves, using N,N-dimethylformamide (Moran and Porath, 1980).

The extraction of the pigments was realised with N,N-dimethylformamide (DMF, 99.9%): 50 mg for each sample separately were collected from each variant, and were blended with 5ml DMF and then cooled at 4˚C for 72 hours. Supernatants were separated and the pigment contents was determined using a 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 read at the spectrophotometer was mathematically processed using formulae proposed by Moran and Porath (1982). The results obtained after assimilatory pigments content determination, were statistically processed using the "t- test".

Chlorophyll a (mg/g SP) =

$$11.65\ A664 - 2.69\ A647 \bullet v/SP$$

Chlorophyll b (mg/g SP) =

$$20.8\ A647 - 3.14\ A664 \bullet v/SP$$

Carotenoids (mg/g SP) =

$$(1000\ A_{480} - 1.28\ \text{chloroph.a} - 56.7\ \text{chloroph.b})/\ 245 \bullet v/SP$$

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

RESULTS AND DISCUSSIONS

Studying the content of chlorophyllian pigment (chlorophyll a and b) and carotenoids on the primary leaves of the wheat seedlings obtained from each experimental variant, we observed that the influence of the exogenous SA or ASA solutions treatment was dependent on the type of solution, on the concentration which was used and the type of pigment which was analysed. The results obtained were presented in table 1(a) and 1(b) and graphically represented in figure1.

Table 1. Estimative mean values for the assimilatory pigments content of the wheat seedling leaves after treatment with SA (a) or ASA (b) solutions of different concentrations.

a.

Paramenters	Control lot V_0	Salicylic acid			
		0.01 mM V_1	0.1 mM V_2	0.5 mM V_3	1.0 mM V_4
		Average ± standard deviation			
Chlorophyll a mg/g	0,65±0,005	0,77±0,004 ***	0,91±0,008 ***	0,87±0,003 ***	0,80±0,006 ***
Chlorophyll b mg/g	0,53±0,006	0,64±0,005 ***	0,73±0,004 ***	0,70±0,005 ***	0,65±0,004 ***
Carotenoid pigments mg/g	0,26±0,004	0,34±0,005 ***	0,55±0,004 ***	0,32±0,005 ***	0,35±0,001 ***

b.

Paramenters	Control lot V_0	Acetylsalicylic acid			
		0.01 mM V_1	0.1 mM V_2	0.5 mM V_3	1.0 mM V_4
		Average ± standard deviation			
Chlorophyll a mg/g	0,64±0,08	0,66±0,05 ns	0,82±0,02 *	0,72±0,05 ns	0,42±0,02 *
Chlorophyll b mg/g	0,44±0,03	0,47±0,03 ns	0,77±0,05 ***	0,62±0,06 **	0,48±0,005 ns
Carotenoid pigments mg/g	0,28±0,02	0,31±0,008 ns	0,43±0,03 **	0,36±0,03 *	0,20±0,02 **

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

The content of chlorophyll a (table 1, figure 1.) increased very significantly (with values between 18.5% and 40%, from control considered 100%) after treatment with 0.01 mM; 0.1 mM; 0,5 mM and 1.0 mM SA solution, and non-significantly (with 3.1% and 12.5%) or just significantly increased (with 28.1%) after treatment with ASA solutions of the same concentrations. The treatment with 1.0 mM ASA solution significantly decrease the chlorophyll a content, with -34.4%

In the case of the chlorophyll b contents (table 1, figure 1), a very significant increase of chlorophyll b contents was observed, with values between 20.7% and 37.7%, from the control lot in the case of treatment

with 0.01 mM; 0.1 mM; 0,5 mM and 1.0 mM SA The treatment made with 0.1 mM and 1.0 mM ASA solution non-significantly increased the content of this pigment, with 6.8% or 9.1%, from control lot considered 100%. In the case of the chlorophyll b contents a significant increase could be observed, with 75% and 40.9%, from control lot when using a 0.1 mM or 0.5 mM ASA solution.

Studying the carotenoids pigments content (table 1, figure 1), the results show that the accumulation of these pigments in the leaves of wheat seedling on the 14th day of germination, increased very significantly in comparison with the same parameter determined from the control lot, in the case of treatment with all concentrations of SA solution used (with values between 23% and 111.5% from the control lot). The treatment with 0.01 mM ASA solution non-significantly increased this pigment contents, with 10.7%. ASA solution with 0.1mM and 0.5 mM concentration, significantly or distinctly significantly increased the carotenoid pigment contents (with 53.6% and 28.6%), whereas after treatment with 1.0mM SA solution a distinctly significant decrease was observed (with -28.6%).

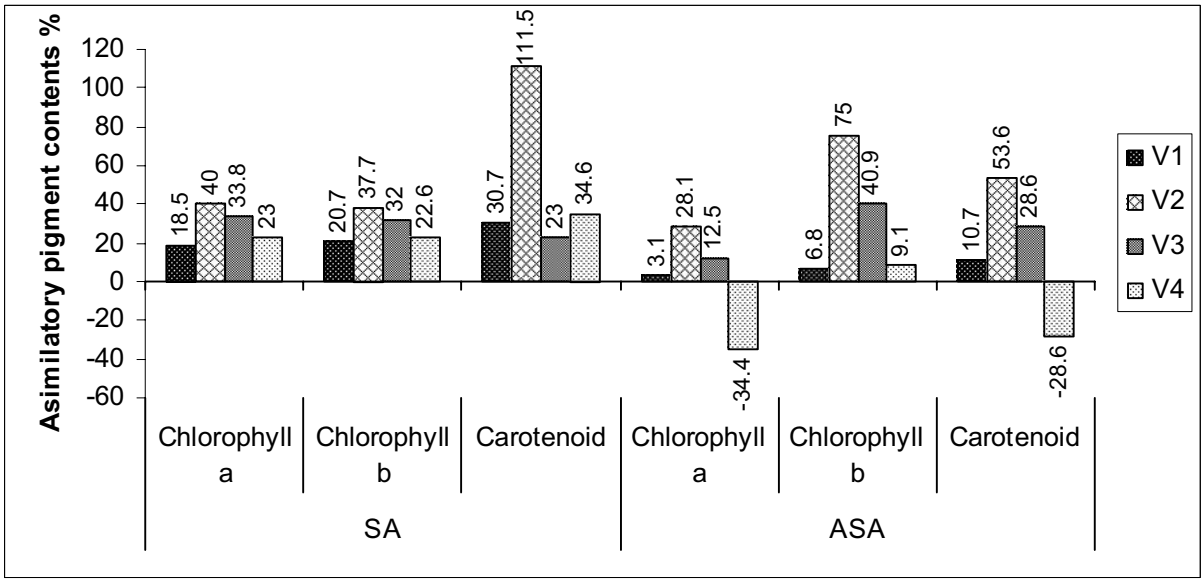

Figure 1. Percentage differences of the content of assimilatory pigments in the primary leaves of wheat (*Triticum aestivum* L.*)* seedlings obtained from caryopsis germinated on filter paper moistened with water, at 22±2 °C, after 6 a hour presoaking in 0.01 mM, 0.1 mM, 0.5 mM or 1.0 mM concentration SA or ASA solution, in comparison with the same parameter measured in the leaves of wheat seedling from the control lot germinated in water. The wheat seedling were planted for an additional 7 days in sand and their primary leaves were sprayed with water. The value for the control lot was considered 100% (marked with 0 on the graphic).

CONCLUSION

The exogenous 0.01 mM, 0.1 mM, 0.5 mM and 1.0 mM SA solutions treatments determine more significant increases in the assimilatory pigments content in the primary leaves of wheat plantlets, especially for the 0.01 mM concentration than treatments with ASA solutions in the same concentrations do.

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