Bulg. J. Plant Physiol., Special Issue 2003, 314–319

SALICYLIC ACID PREVENTS THE DAMAGING ACTION OF STRESS FACTORS ON WHEAT PLANTS

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Summary. We investigated the effect of salicylic acid (SA) on plant resistance to environmental stress factors. Treatment of wheat plants with 0.05 mM SA increased the level of cell division within the apical meristem of seedling roots which caused an increase in plant growth. Phytohormones are known to play a key role in plant growth regulation. It was found that the SA treatment caused accumulation of both ABA and IAA in wheat seedlings. However, the SA treatment did not influence cytokinin content. We suppose, that the protective and growth promoting effects of SA are due to the phenomenon described above. The SA treatment reduced the damaging action of salinity and water deficit on seedling growth and accelerated a restoration of growth processes.Treatment with SA essentially diminished the alteration of phytohormones levels in wheat seedlings under salinity and water deficit. The SA treatment prevented the decrease in IAA and cytokinin content completely which reduced stress-induced inhibition of plant growth. Also, high ABA levels were maintained in SA treated wheat seedlings which provided the development of antistress reactions, for example, maintenance of proline accumulation. Thus protective SA action includes the development of antistress programs and acceleration of normalization of growth processes after removal stress factors.

Keywords: Salicylic acid, salinity, water deficit, abscisic acid, indoleacetic acid, cytokinins

Abbreviations: SA – salicylic acid; ABA – abscisic acid; IAA – indoleacetic acid; PEG – polyethylene glycol

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Introduction

Salicylic acid is (SA) is an endogenous growth regulator of phenolic nature, which participates in the regulation of physiological processes in plants. SA, for example, plays a role of natural inductor of thermogenesis in *Arum* lily, induces flowering in a range of plants, controls ion uptake by roots and stomatal conductivity (Raskin, 1992). There are experimental data indicating participation of SA in signal regulation of gene expression in the course of leaf senescence in *Arabidopsis* (Morris et al., 2000) Moreover SA might serve as a regulator of gravitropism (Medvedev and Markova, 1991), inhibition of fruit ripening (Srivastava and Dwivedi, 2000) and of other processes.

During the last 20 years this substance drew the attention of researchers due to its ability to induce systemic acquired resistance (SAR) in plants to different pathogens, which is manifested in the appearance of pathogenesis related proteins (PR), while SA is considered to serve as a signal in the induction of expression of these genes (Metraux, 2001)

At the same time at present considerable interest has been aroused by the ability of SA to produce a protective effect on plants under the action of stress factors of different abiotic nature. Thus convincing data have been obtained concerning the SA-induced increase in the resistance of wheat seedlings to salinity (Shakirova and Bezrukova, 1997), and water deficit (Bezrukova et al., 2001), of tomato and bean plants to low and high temperature (Senaratna et al., 2000), as well as the injurious action of heavy metals on rice plants (Mishra and Choudhuri, 1999).

The important role of SA in protecting is probably played by its ability to induce expression of genes coding not only for PR-proteins but also for example the extensin gene in *Arabidopsis* plants (Merkouropoulos et al., 1999). There are data about SA induced synthesis of heat shock proteins in tobacco plants (Burkhanova et al., 1999) and accumulation of wheat lectins (Shakirova and Bezrukova, 1997), fast activation of 48-kD protein kinase in suspension cell culture of tobacco at osmotic stress (Mikolajczyk et al., 2000). This suggests the involvement of SA in realization of different antistress programs. However, the way of signal regulation of plant resistance to unfavorable factors of environment induced by SA are still not clear.

The aim of the present work was to study the character of changes in hormonal systems induced by SA in wheat plants under stress conditions.

Materials and methods

The object of investigation were 4-d-old plants of *Triticum aestivum* L. cv. Saratovskaya 29. Treatment of wheat seedlings with a concentration of SA optimal for seedling growth (0.05 mM) have been used. For this purpose after washing of seeds with tap water they were soaked in 0.05 mM SA solution for 3 h and then dried in a desiccator over $CaCl_2$. Then, after excision of endosperm 3-d-old seedlings were transferred into glasses, containing 2% sucrose, incubated for 24 h for withdrawal of wounding stress (Shakirova et al., 1993), and then 4-d-old seedlings were transferred to a mixture of 2% sucrose and NaCl or PEG at different concentration. Plants incubated on 2% sucrose solution served as a control in all these experiments.

Growth was estimated on the basis of changes in fresh weight of 30–50 wheat seedlings.

Assay of free ABA, IAA and cytokinin was carried out with the help of rabbit antibodies specific to each class of phytohormone and secondary antirabbit antibodies labeled with peroxidase. The procedure of succession of phytohormone extraction and immunoassay has been described earlier (Kudoyarova et al., 1990; Shakirova et al., 1994). The data on cytokinin measurement presents three samples of zeatin derivatives immunoreactive to antiserum raised against zeatin riboside (Kudoyarova et al., 1990). Proline content was determined according to the method of Bates (Bates et al., 1973). The experiments were repeated at least three times.

Results and discussion

Dynamics of phytohormone level in plants under salinity and water deficit

Unfavorable environmental factors lead to sharp changes in the balance of phytohormones associated with not only accumulation of ABA, but also with a decline in the level of growth activating hormones such as IAA and cytokinins (Zholkevich and Pustovoytova, 1993; Jackson, 1997) As can be seen from Fig. 1a and 1b, incubation of seedlings on the medium containing 2% NaCl or 18% PEG resulted in transitory ac-

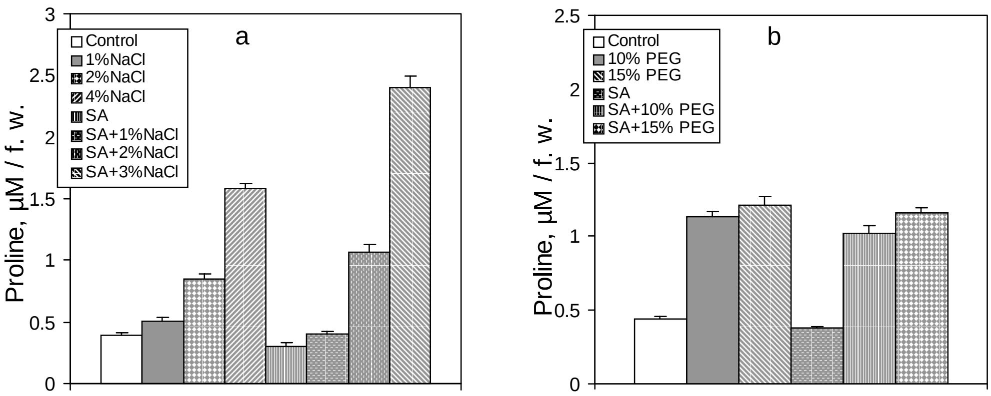

Fig. 1. The effects of seed presowing treatment with SA and salinity (a), water deficit (b) on proline content in 4-d-old wheat seedlings. 4-d-old seedlings were subjected to the influence of NaCl and PEG for 24 h

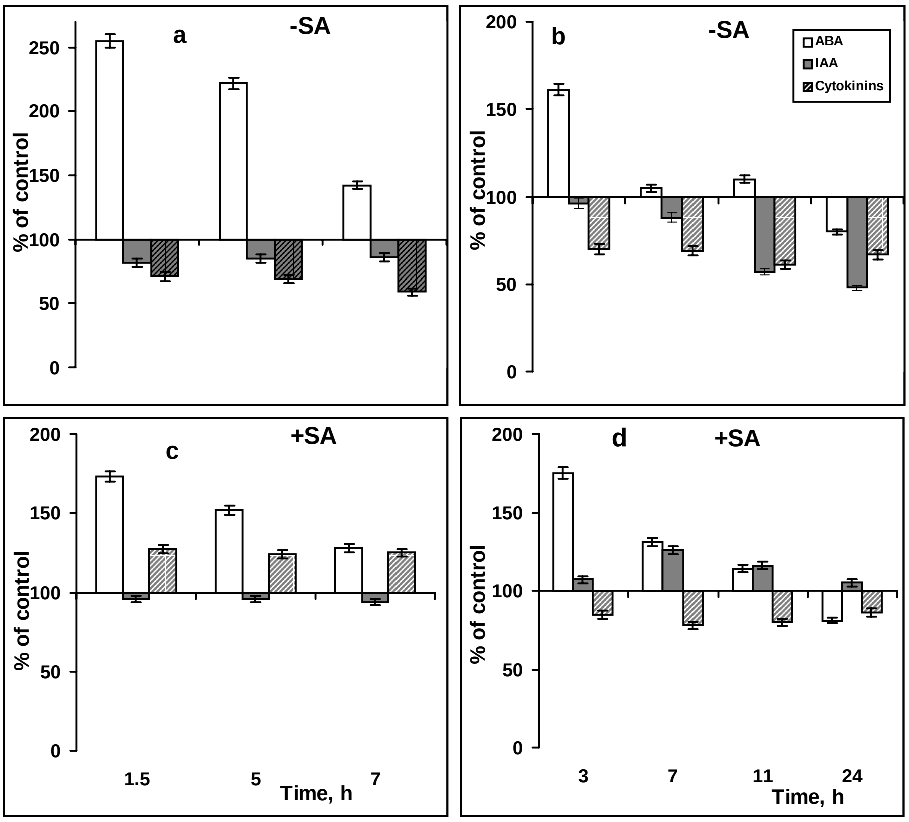

Fig. 2. The effects of salinity (a, c) or water deficit (b, d) on phytohormonal balance of wheat seedlings pretreated with 0.05 mM SA. 4-d-old seedlings were subjected to the influence of 2%NaCl and 18%PEG for 7 h.

cumulation of ABA and a progressive decline in cytokinins as well as small decrease in the level of IAA.

Presowing treatment with SA (Fig. 2c and 2d) completely prevented salinity-induced and water deficit-induced declines in the concentration of IAA and cytokinins in seedlings and reduced accumulation of ABA, which might be a prerequisite for acceleration of growth resumption of wheat seedlings after withdrawal of stressor from the medium. Alongside this, maintaining a comparatively high level of ABA under stress conditions in plants pretreated with SA is of primary importance from our point of view since ABA might serve an important regulating factor in SA-induced unspecific plant resistance. Since proline is one of the important components of defence reactions of plants to salinity (Kuznetsov and Shevyakova, 1999), it might be expected that pretreatment with SA contributes to accumulation of this amino acid under stress through maintaining an enhanced level of ABA in seedlings.

The effect of SA on concentration of proline under salinity and water deficit

Salinity and water deficit induces accumulation of proline in seedlings (Fig. 1). These data suggests that proline is an important component in the spectra of SA-induced ABA-mediated protective reactions of wheat plants in response to salinity and water deficit, which contribute to a reduction of injurious effects of stress factors and acceleration of restoration processes during the period after action of stress, which might be a manifestation of the protective action of SA on wheat plants.

The effect of presowing treatment with SA on growth of seedlings subjected to salinity and water deficit

Salinity and water deficit result in a decline in metabolic activity of plant cells, which should be inevitably reflected in inhibition of their growth. Action of 2% NaCl or 18% PEG on 4-d-old wheat seedlings led to noticeable and almost equal extent of inhibition of growth of plants both treated and not treated with SA, however, since by the moment of the start of experiment seedlings pretreated with SA had greater biomass (72 mg per seedling) compared to control (64 mg per seedling), even after the action of 2% NaCl or 18% PEG this characteristic was still higher (68 mg per seedling) than in control not treated with salt and PEG plants (66 mg per seedling).

Consequently, although presowing treatment with SA does not prevent negative effects of 2% NaCl and water deficit on growth of plants, it nevertheless, in general, noticeably reduces its injurious effect as compared to control plants.

The data presnted in total indicate overall that presowing treatment of wheat seeds with SA contributes to the increase in the resistance of plants to stress factors of environment and ABA serves as a rmediator in the manifestation of the protective action of SA. SA-treatment induces a sharp accumulation of ABA, which in turn is an inducer of a wide spectra of antistress reactions in plants, which is why it is likely that the effect of SA on the increase of ABA lies at the root of the preadaptive action of SA to possible stress situations. Maintaining a high level of ABA in SA-treated plants under stress contributes to protective reactions aimed to decrease its injurious effect on growth and acceleration of growth resumption.

Acknowledgements: This work was supported by RFBR-Agidel, Grant No 02-04-97919 and Integracia, No Ya 0108.

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