Chapter 4

ROLE OF HORMONAL SYSTEM IN THE MANIFESTATION OF GROWTH PROMOTING AND ANTISTRESS ACTION OF SALICYLIC ACID

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Abstract: Salicylic acid (SA) is an endogenous plant growth regulator. When applied to wheat plants in concentration similar to that used in case of exogenous hormones (0.05 mM), SA causes growth promoting and protective effects against an abiotic stresses. SA was shown to cause changes in hormonal system associated with transitory parallel accumulation of IAA and ABA with no change in cytokinins, which took place in case of treatment of seeds before sowing as well as seedling treatment. SA-induced accumulation of ABA lead to no detrimental effects, evidenced by clearcut stimulation of growth of root cells both by division and expansion, accumulation of raw and dry mass of seedlings and productivity of wheat treated with SA. This indicated an important role to IAA in the expression of growth stimulating action of SA. ABA is likely to be intermediator in manifestation of antistress action of SA. This is evidenced by the data showing that SA-induced accumulation of ABA was followed by enhanced expression of genes of dehydrins and accumulation of proline, i.e. substances having a relation with osmoprotection of cells. Moreover, SA causes activation of superoxide dismutase and peroxidase, including anionic peroxidase, phenylalanin-ammonia-lyase, favouring accelerated lignification of cell walls of seedlings roots. This is likely to contribute to a decline in the extent of injurious effects of salinity and water deficit on plants, pretreated with SA, evidenced by a decline in the level of lipid peroxidation and leakage of electrolytes from plant tissues as well as by more intensive growth processes as compared to control plants. It is important to underline that pretreatment with SA prevents a sharp decline in IAA and cytokinin content observed under stress and maintains a high level of ABA. Such a character of SA effect on the state of hormonal system may well contribute to protective reactions of plants and acceleration of reparative processes during a post-stress period.

1.   INTRODUCTION

Salicylic acid (SA) is an endogenous growth regulator of phenolic nature, which participates in the regulation of different physiological processes in plants. SA, for example, might play a role of natural inductor of thermogenesis in *Arum* lily, induce flowering in a range of plants, controls ions uptake by roots and stomatal conductivity (Raskin, 1992). There are experimental data indicating participation of SA in signalling and regulation of gene expression in the course of leaf senescence in *Arabidopsis* (Morris *et al*., 2000). Moreover SA might serve as a regulator of biogenesis of chloroplasts (Uzunova and Popova, 2000) and activity of photosynhtesis (Fariduddin *et al*., 2003), gravitropism (Medvedev and Markova, 1991), inhibition of fruit ripening (Srivastava and Dwivedi, 2000) and of other processes.

However, recently this substance has drawn attention of researchers because of its ability to induce system acquired resistance (SAR) in plants to different pathogens, which is manifested as appearance of pathogenesis related proteins (PR), while SA is considered to serve as a signal in the induction of expression of these genes (Ward *et al*., 1991; Metraux, 2002).

Moreover, a considerable interest has been generated by the ability of SA to produce protective effects in plants under the action of abiotic stress factors. Thus convincing data have accumulated about the SA-induced increase in the resistance of wheat seedlings to salinity (Shakirova and Bezrukova, 1997; Shakirova *et al*., 2003; Sakhabutdinova *et al*., 2004), and water deficit (Bezrukova *et al*., 2001; Singh and Usha, 2003), maize (Janda *et al*., 1999), cucumber and rice to low temperature (Kang and Saltveit, 2002), tomato and bean plants to low and high temperature (Senaratna *et al*., 2000), as well as about injurious action of heavy metals on rice plants (Mishra and Choudhuri, 1999) and freezing on winter wheat leaves (Tasgin *et al*., 2003).

The important role of SA in protective action is probably played by its ability to induce the expression of genes coding not only for PR-proteins but also, for gene of extensin in *Arabidopsis* plants (Merkouropoulos *et al*., 1999). There are reports about SA induced synthesis of heat shock proteins in tobacco plants (Burkhanova *et al*., 1999) and accumulation of wheat lectin (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 shows the participation of SA in realization of different

antistress programs. However the routes of signal regulation in plant resistance to abiotic factors of environment are still not clear enough. Progress in understanding this problem can, however, be achieved by a complex study of the mechanisms of SA action in plants under normal and stressful conditions of growing.

2.   INFLUENCE OF SA ON GROWTH AND HORMONAL STATUS OF WHEAT PLANTS

Since salicylic acid is an endogenous growth regulator, it was important to study the influence of exogenous SA on intensity of growth processes in wheat plants. In order to reach this goal two types of treatments on wheat (*Triticum aestivum* L., *Saratovskaya 29*) with a concentration of SA (0.05 mM), optimal for seedling growth, have been planned (a) soaking of seeds (Shakirova and Bezrukova, 1997; Shakirova *et al*., 2003) and (b) treatment of intact seedlings (Sakhabutdinova *et al*., 2004).

2.1   Soaking of seeds in SA

In order to study the action of SA, under laboratory conditions, the seeds were soaked in 0.05 mM SA solution for 3 h and then were germinated for 5 d on moist filter paper in cuvetters (24-h light/dark photoperiod with the light phase duration of 16 h; light intensity, 15 klx; temperature; 22-24^{0}C). Estimation of the influence of presowing seed treatment with 0.05 mM SA on hormonal status of leaves and milky grains and various characteristics in wheat were carried out in microplots (2 m^2) in the field of Chishminsky Crop Production, Bashkortostan, Russia.

Presowing seed treatment with SA leads to an activation of germination and seedling growth (Shakirova *et al*., 2003), while the enhancement of the division of root apical cells is an important contribution to the growth stimulating effect of SA (Table 1). This effect was also revealed in field experiments when elements of yield structure were analysed. As evident from table 2, plants pretreated with SA were characterized with increased size of ears, mass of 1000 seeds and grain yield, indicating prolonged effect of presowing treatment of seeds, which produced stimulative effect on the productivity of wheat, at harvest.

Phytohormones are known to play a decisive role in the regulation of plant growth and development and thus it was important to study the effect of the treatment with SA on hormonal system of seedlings. Data presented on figure 1 demonstrates fast transitory shifts in hormonal balance of wheat seedlings in the course of germination under the influence of SA, associated

with simultaneous sharp accumulation of IAA and ABA with maximum on the second day, while noticeable changes in cytokinin level were not revealed. Thus growth promoting effect of SA on wheat seedlings was associated with the increase in the level of IAA, but it is necessary to underline that accumulation of ABA did not produce inhibitory effect on the growth of seedlings. Moreover, the intensity of growth processes of plants treated with SA was noticeably higher than in control (Table 1).

Table 1. Mitotic index of root meristem cells of wheat seedlings (Data are the mean $\pm$ S.E. of three assays)

Age of seedlings	2-day		3-day		4-day	
	Control	SA	Control	SA	Control	SA
	3.0$\pm$0.07	4.0$\pm$0.10	3.2$\pm$0.10	5.2$\pm$0.13	3.5$\pm$0.06	5.3$\pm$0.11

Table 2. The effect of presowing treatment with salicylic acid on wheat productivity (Data are the mean $\pm$ S.E. of three assays

Variant	Quantity of seeds in main ear	Seeds weight in ear, g	Weight of 1000 seeds (g)	Yield, g m^{-2}
Control	18.7	0.67	35.5	199
SA	23.7 (127%)	0.93 (139%)	38.2 (115%)	272 (137%)

Analysis of hormone content in wheat plants in the course of ontogenesis of wheat plants revealed a parallel accumulation of IAA and ABA in leaves in the phase of tillering albeit this study as well as the previous one concerning seedlings revealed no significant change in cytokinin concentration (Figure 2a). Thus observed changes in the concentration of IAA under the impact of SA are important in the regulation of growth and development of young wheat plants, playing a significant role in the activation of growth processes and the accumulation of ABA probably enhancing unspecific resistance of wheat plants.

In milky grains (14 days, after flowering) changes in hormone balance are mainly manifested to two fold increases in the level of cytokinins (Figure 2b) known to play an important role in the control of grain development, in the course of its maturation. This is likely to make an important contribution in the control of grain size and mass during the period of its filling, since by the time the crop is harvested the mass of grains obtained from experimental plants significantly exceeded those of the control ones (Table 2).

An important contribution, in the control of grain development, is attributed to ABA, which is due not only to its involvement in the assimilate

attraction (alongside with cytokinins and auxins) but also to induced synthesis of storage proteins, control of germ dormancy and inhibition of germination (Rademacher and Grabe, 1984; Bewley, 1997).

Thus shift in the balance of phytohormones induced by preseeding treatment with SA are of great importance for the growth stimulating action of SA on wheat plants.
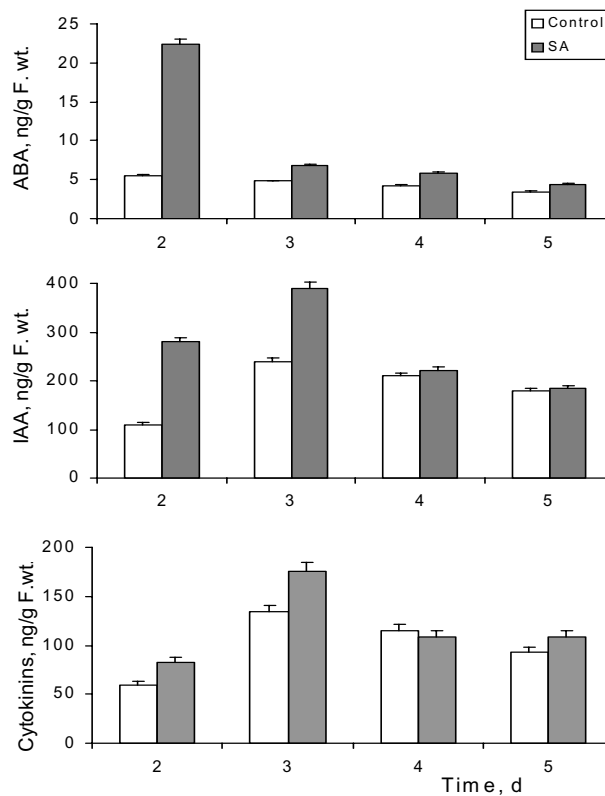

Figure 1. The effect of presowing seed treatment with 0.05mM SA on phytohormone content in wheat seedlings. Data are mean $\pm$ S.E. of three assays.

2.2   Treatment of seedlings with SA

The 0.05 мM SA has prominent growth stimulating effects when applied to seedlings. Incubation of seedlings on a solution of SA during a day leads to noticeable increase in their linear size and weight (Figure 3). Clearly expressed influence of SA on mitotic activity of cells of apical meristem contributes significantly to its growth stimulating effect. Thus mitotic index (MI) of cells of root apical meristem was incresed by 70 %, 24 hours after the start of SA treatment.

As in case of presowing treatment parallel transitory accumulation of IAA and ABA is induced by SA treatment of seedlings (Figure 4), that is the way to achieve its growth promoting effect on wheat plants.Alongside with this, SA-induced an increase in ABA content that might have contributed to preadaptation of plants to different stressful influences not only of abiotic but also of biotic nature, since ABA is ascribed to have a key role in triggering protective reactions of plants, in induction of the synthesis of a range of stress proteins (Rock, 2000) and a range of PR-proteins (Moons *et al*., 1997) in particular.
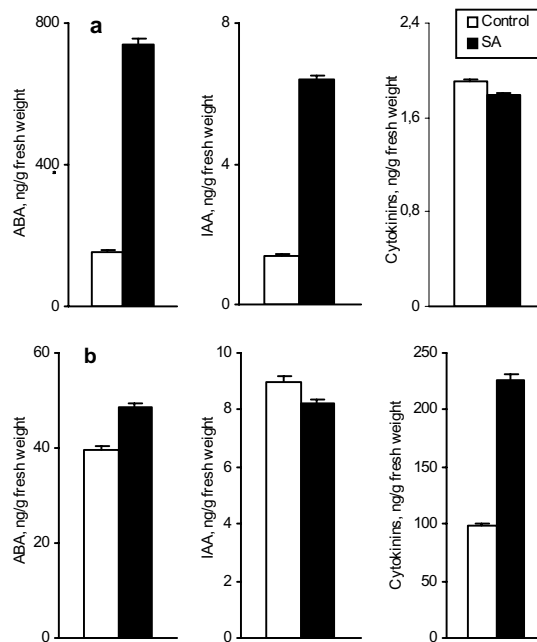

Figure 2. The influence of presowing seed treatment with 0.05 mM SA on hormonal status during wheat ontogenesis. Mean data of three independent replicates and their S.E. are presented.

Accumulation of proline, which is a well-known effective cell osmoprotectant (Pesci, 1987; Kuznetsov and Shevyakova, 1999), might serve as an example of such reactions. Consequently, it is reasonable to suggest a significant role of ABA-controlled changes in the concentration of proline in SA-induced preadaptation of wheat plants to stress factors, which disturb water relations. The treatment of seedlings with SA caused a significant increase in the concentration of proline in seedlings, which reached a level about 3-4 times, within 7 hours, compared with the control (Figure 5a). Increase in the concentration of proline was preceded by a

transitory accumulation of ABA, induced by SA treatment that indicates the involvement of endogenous ABA in the control of accumulation of proline in cells (Figure 5a). The data obtained is an evidence for the implication of proline in the spectrum of the mechanism of SA action in wheat plants, which is achieved through its influence on the accumulation of ABA.
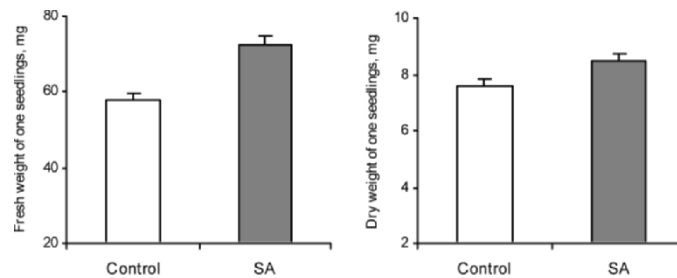

Figure 3. The effect of 1-day treatment with 0.05 mM SA on fresh and dry weight of 5-days-old wheat seedlings. S.E.s of three assays are indicated.
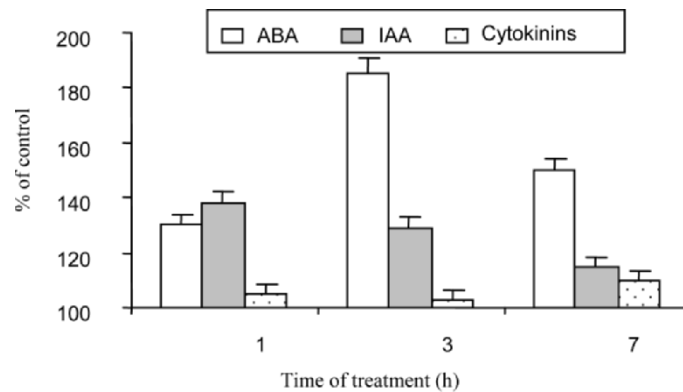

Figure 4. The effect of treatment of 4-day-old seedlings with 0.05 mM SA on phytohormone content. Data are mean $\pm$ S.E. of three assays.

Another example illustrating the key role of ABA in the expression of protective action of SA on wheat plants is presented by the results of experiments showing the influence of SA on the dymamics of ABA accumulation and level of transcription activity of wheat dehydrin gene *TADHN*. Dehydrins are related to rab-(ABA-responsible) proteins. They belong to LEA-(late embriogenesis abundant) proteins, whose synthesis is dated to the stage of grain maturation accompanied by dehydration (Close, 1996; Rock, 2000). Alongside with these protective functions of dehydrins manifested in their ability to retain water and to prevent denaturation of cell biopolymers under dehydration turned out to be useful for vegetating plants

under conditions leading to disturbance in water relations (Allagulova *et al.*, 2003). It was revealed by us earlier that ABA treatment of wheat seedlings results in an increase in the expression of dehydrin gene *TADHN* (Shakirova *et al.*, 2005). Data presented in figure 5b, demonstrates the ability of SA to increase the level of dehydrin mRNA, which is preceded by SA-induced accumulation of ABA. These data indicate the involvement of dehydrins in the spectrum of ABA-mediated protective action of SA on wheat plants.
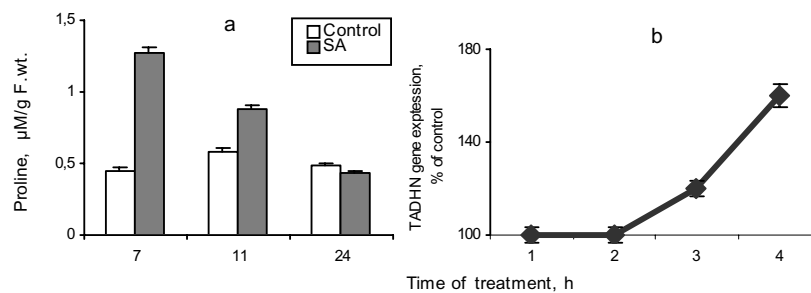

Figure 5. The effect of treatment of 4-day-old seedlings with 0.05 mM SA on proline content (a) and level of wheat dehydrin gene TADHN expression (b). Mean data of three independent replicates and their S.E. are presented.

Taking into account the fact of the observed participation of ABA in the control of osmoprotectors, we may make a conclusion concerning the important role of stress hormone ABA in the expression of preadaptive action of SA on wheat plants. Consequently ABA might have served as an intermediate in the development of SA-induced mechanisms of preadaptation of wheat plants to possible stress factors, leading to the disturbance of water relations.

It is important to underline that SA caused similar changes in hormonal balance of wheat plants with both variants of SA-treatment, which is likely to explain its growth promoting and protective action.

3.   MECHANISMS OF PROTECTIVE EFFECTS OF SA ON WHEAT PLANTS, UNDER SALINITY

3.1   Effect of pretreatment with SA on growth and balance of phytohormones of wheat seedlings

Salinity results in a decline in metabolic activity of plant cells (Ramagopal, 1987), which should be inevitably reflected as inhibition of

their growth. Action of 2% NaCl on 4-d-old wheat seedlings led to a noticeable and almost equal extent of inhibition of growth of plants both with or without SA treatment, however, by the start of experiment seedlings pretreated with SA had greater biomass compared to control (Figure 6) and even after the action of 2% NaCl this characteristic was still higher than in control (not exposed to NaCl).
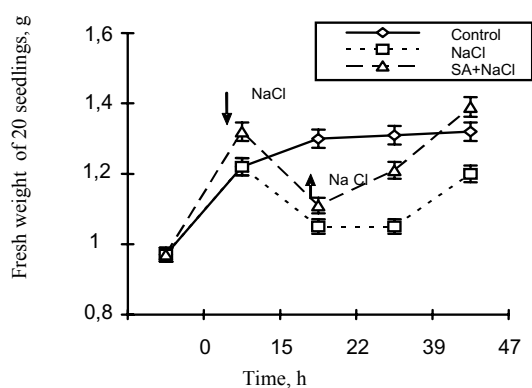

Figure 6. The effect of pretreatment of wheat seedlings with 0.05 mM SA on their fresh mass under salinity. 4-d-old seedlings after treatment with SA were subjected to 2% NaCl for 7 h. Data are mean $\pm$ S.E. of three assays.

Moreover, the treatment with SA accelerated reparation of growth processes in seedlings after this stress as evidenced by the results of analysis of mitotic activity of root meristem cells of 4-5-d-old seedlings (Table 3).

Table 3. Mitotic index (%) of root meistem cells of wheat seedlings, under salinity (Data are the mean $\pm$ S.E. of three assays

Variant	Before influence 2%NaCl	2%NaCl (7 ч)	One day after 2%NaCl
Control	3.52$\pm$0.06	2.90$\pm$0.05	2.92$\pm$0.07
SA	5.31$\pm$0.11	3.51$\pm$0.08	5.50$\pm$0.1

Incubation in 2% NaCl for 7 h led to an inhibition of cell division of not only roots of untreated seedlings but also that of pretreated with SA. However, it is necessary to underline the observations that initially cells of root meristem of these seedlings were characterized by significantly higher MI (mitotic index) but salinity decreased MI of cells down to the level that

of the control plants, grown under normal conditions. Moreover, root meristem cells of seedlings pretreated with SA completely restored their high level of mitotic activity after a short-term effect of salinity, the untreated plants did not recover (Shakirova *et al.*, 2003). Thus, the results of these experiments evidence an SA-induced activation of division at root meristem cells, which contributed to SA-induced growth of wheat seedlings, and also maintains high intensity of mitotic activity in seedlings under salinity. Such an effect of SA on cell division is likely to be an important mechanism, which enables not only a decline in the extent of injurious effect of this stress factor on growth in general, but also accelerates the resumption of growth processes in seedlings during recovery after being exposed to salinity.

Pretreatment of wheat with SA contributes to the development of resistance in plants to salinity. It may be assumed that SA-induced changes at the level of transcription of dehydrin gene *TADHN* and the increased concentration of proline in seedlings, in the course of pretreatment with this growth regulator, might have served as an important contributor to protect the plants from salinity-induced dehydration.

In order to understand the mechanisms of the protective effects of SA on growth, we carried out analysis of the state of hormonal system in plants pretreated and untreated with SA grown under salinity. Unfavourable environmental conditions generate to sharp changes in the balance of phytohormones, associated not only with the accumulation of ABA, but also with the decline in the level of growth activating hormones, IAA and cytokinins (Jackson, 1993; Zholkevich and Pustovoytova, 1993).

In fact, as seen from figure 7, incubation of seedlings on the medium containing 2% NaCl results in transitory accumulation of ABA and decline in cytokinins as well as a limited decrease in the level of IAA. The pretreatment with SA (Figure 7) completely prevented salinity-induced decline in the concentration of IAA and cytokinins in the seedlings and reduced accumulation of ABA, which could be a prerequisite for the resumption of accelerated growth of wheat seedlings, after the withdrawal of stressor from the medium.

Along with this, maintenance of comparatively higher level of ABA, under stress conditions, in plants pretreated with SA is of primary importance from our point of view because ABA might serve as an important regulating factor in SA-induced unspecific plant resistance.

3.2 Influence of SA on the state of prooxidant-antioxidant system in wheat plants

Stress-factors are well-known to cause a shift in the prooxidant-antioxidant balance in plant cells. This shift is due to an increase in the rate of generation of reactive oxygen species (ROS), which induce lipid peroxidation (LPO) in the membrane structures of the cells (Zenkov *et al.*, 2001; Tarchevskii, 2002). Antioxidant system plays an important role in the process of neutralization of the after effects of oxidative stress. The literature contains evidences of SA-induced effects on the rate of generation of O_2^- and H_2O_2 (Chen *et al.*, 1993; Minibayeva *et al.*, 2001) and the activity of
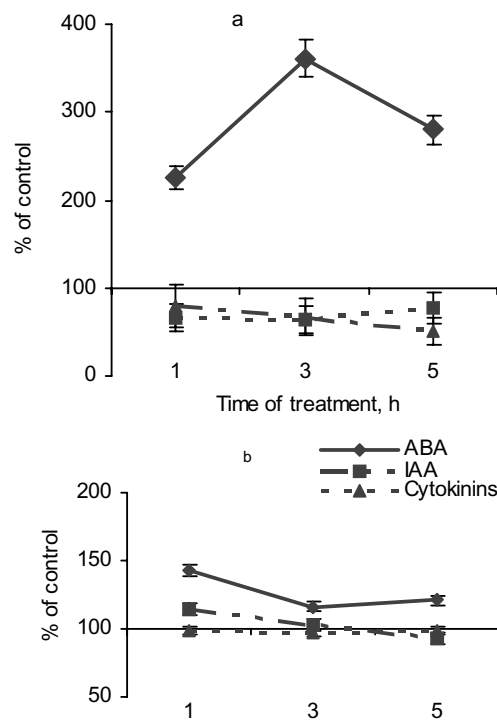

Figure 7. The influence of salinity on hormonal status in wheat seedlings, untreated (a) and pretreated with SA (b). Data are mean $\pm$ S.E. of three assays.

superoxide dismutase (SOD), peroxidase, and catalase, i.e., the enzymes involved in the utilization of ROS (Janda *et al.*, 1999; Senaratna *et al.*, 2000; Kang and Saltveit, 2002). However, at the time when this work was planned, there were no reports in the literature on the effects on the level of ROS and

changes in the activity of the ROS-detoxifying system in wheat plants exposed to stress-factors. On the other hand, this information is important for understanding the mechanisms of the antistress effects of SA in plants, which is a prerequisite to justify the use of this natural growth regulator for increasing wheat resistance to salinity.

3.2.1   Normal conditions of growing

Treatment of seedlings with SA caused a transitory enhancement of O_2^- and H_2O_2 production by plants and simultaneous increase in the activity of SOD (Figure 8), which is one of key enzymes of antioxidant system (Wu *et al.*, 1999; Alscher *et al.*, 2002). Dismutation of O_2^- with the help of SOD results in the accumulattion of H_2O_2 in the control tissues, where the important role is attributed to peroxidase. Consequently it is not surprising that enhancement of the generation of H_2O_2 in SA treated seedlings is accompanied by the activation of peroxidase (Figure 8). These results confirm the fact of enhancement of production of O_2^- and H_2O_2 under the influence of SA (Minibayeva *et al.*, 2001; Kawano *et al.*, 2003), and activity of SOD (Vuletic *et al.*, 2003) and peroxidase (Janda *et al.*, 1999; Kang and Salveit, 2002) in plants. Along with this it is also known that a low level of ROS generation may play an important role in the preadaptive activity of SA in seedlings with respect to extended stress situations, because it is well known that ROS acts as signaling molecules that triggers the cascade of protective reactions in plants (Tarchevskii, 2002), including activation of antioxidant enzymes, favouring a decrease in the level of stress-induced generation of ROS, under subsequent conditions of stressful environment.

The data showing SA-induced SOD activation in wheat plants was consistent with the results obtained with other various plant species: maize (*Zea mays* L.), cucumber (*Cucumis sativus* L.), and rice (*Oriza sativa* L.). These plants demonstrated an increase in the activity of SOD and, therefore, SA-induced development of resistance to further hypothermia (Kang and Salveit, 2002). The effect of SA on the activation of SOD may facilitate the integrity of membrane structures of the cell, because SOD is involved in the processes of LPO deactivation (Zenkov *et al.*, 2001). Elevation of total cell SOD may be associated both with changes in the activity of latent isozymes of SOD and with de novo biosynthesis of this enzyme. It is well known that ABA is involved in the process of induction of the expression of genes, encoding proteins involved in plant stress responses (Rock, 2000), including the genes of SOD (Sakamoto *et al.*, 1995). Moreover there are data that water stress-induced accumulation of ABA in maize leaves resulted in the enhancement in the activity of SOD (Jiang and Zhang, 2002). Therefore, we assume that SA-induced activation of SOD is due to its effect on ABA, but

this suggestion should be tested experimentally. Our data show that SA is involved in the regulation of peroxidase activity, which controls intracellular concentration of H_2O_2. Therefore, it is safe to suggest that this enzyme contributes to the preadaptation effect of SA on wheat plants. Indeed, there is evidence in the literature that pretreatment with SA for 1 day increased peroxidase activity in maize plants. This fact offered an explanation for SA-induced resistance of plants to low temperature (Janda *et al*., 1999). The ability of SA to stimulate H_2O_2 production and activation of peroxidase system may play an important role in biochemical processes associated with the biosynthesis of lignin and suberin, which are involved in the strengthening of barrier properties of cell walls (Kolattukudy *et al*., 1995). On the other hand, the effect of SA on the activation of SOD may facilitate the integrity of membrane structures of the cell, SOD being involved in the deactivation of LPO processes (Zenkov *et al*., 2001).
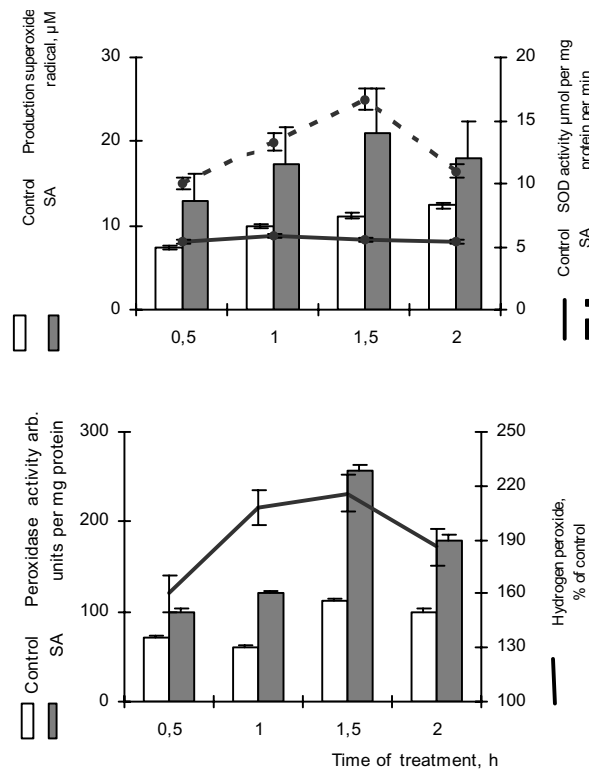

Figure 8. The effects of the treatment of 4-day-old wheat seedlings with 0.05 mM SA on the production of ROS and activity of SOD and peroxidase in plants. Mean data of three independent replicates and their S.E. are presented.

Thus, the data obtained by us demonstrate the ability of SA to cause simultaneous and reversible changes in the level of the production of ROS and activity of antioxidant enzymes in wheat seedlings and as a result of this the prooxidant-antioxidant balance is not disturbed, which is illustrated by the absence of noticeable changes in the concentration of the final LPO product, malonic dialdehyde (MDA) (Figure 10) and by the clearly expressed growth promoting effect of SA on plants. At the same time, activation of antioxidant enzymes induced by the treatment with SA may contribute to its antistress effects in plants.

3.2.2   Effect of SA on the pro-antioxidant balance in wheat seedlings under salinity stress

It is known that stress-factors of various origins induce the generation of ROS in cells. Therefore, it was not surprising that incubation of four-day-old seedlings in a medium containing 2% NaCl caused a significant increase in the concentration of O_2^- and H_2O_2 in roots (Sakhabutdinova *et al*., 2004), which was fairly consistent with the data on the enhancement of ROS generation in plants exposed to salinity (Mittova *et al*., 2004). Concentrations of O_2^- and H_2O_2 in seedlings pretreated with SA and incubated with 2% NaCl were substantially less than in untreated seedlings (about a 50%). Similar picture was observed when activity of SOD and peroxidase was estimated in roots of seedlings pretreated with SA. A sharp stress-induced increase in the concentration of ROS may have severe destructive consequences, which require ROS neutralization by antioxidant enzymes. Therefore, our subsequent experiments involved measurement of SOD and peroxidase activities in roots of seedlings under salinity.

Figure 9 shows that salinity causes a significant increase in the activity of SOD and peroxidase in roots of seedlings, which may be associated with a significant increase in the rate of generation of O_2^-. and H_2O_2. On the other hand, this parameter in seedlings pretreated with SA and incubated with 2% NaCl was substantially lower than in untreated seedlings.

The balance between antioxidant system and LPO is an important prerequisite for normal functioning of cells, because of the direct correlation between the stress-factor intensity and the rate of ROS generation (i.e., LPO development). Concentration of MDA can serve as an indicator of the rate of oxidative processes in cell. In untreated seedlings, the addition of 2% NaCl caused only insignificant changes in the concentration of MDA, the effect remained at a low level even after 7 h of incubation (concentration changes did not exceed 30% of the control level). This parameter in the seedlings pretreated with SA was indistinguishable from that in control plants. However, following 1-day exposure to salinity, there was a twofold increase

in the level of MDA in untreated seedlings, whereas pretreatment with SA brought about a 50% decrease in the concentration of MDA (Figure 10a). This could be the consequence of the preadaptation effect of SA on wheat plants, in which SA-induced events leading to activation of the antioxidant system were initiated before the exposure of the plants to 2% NaCl. This may facilitate the strengthening of cell walls and effectively neutralize excessive (damaging) increase in the level of ROS during further exposure to the stress-factor, thereby preventing the damage of cellular membrane structures and changes in their permeability under stress conditions. The results of the measurement of leakage of electrolytes may provide information on cell membrane permeability changes: incubation in 2% NaCl for 3 h exerted a strong damaging effect on cells, judging by the two-fold increase in the rate of release of electrolytes, compared to the control (Figure 10b). Pretreatment with SA for 24 h protected wheat plants against the salinity-induced damage, which was manifested as a substantial decrease in the rate of the stress-induced leakage of electrolytes. The SA pre-treatment for 1 day did not cause any substantial increase in the rate of release of electrolytes itself, which indicated that SA-induced effect was favorable for the plants. This conclusion is supported by the facts that pretreatment with SA stimulated cell growth in roots, and increased their fresh and dry mass.
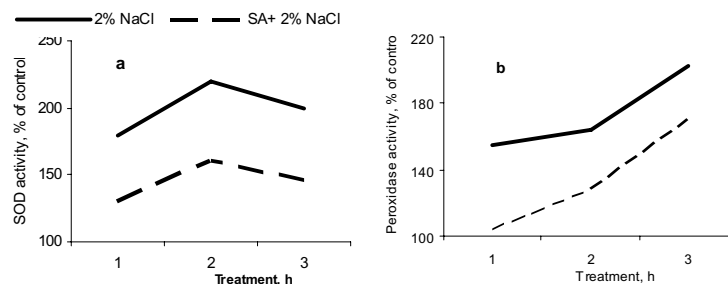

Figure 9. The effect of pretreatment with 0.05 mM SA on the activity of SOD and peroxidase in wheat seedlings, under salinity.

While discussing these results it is of interest to present the data showing that overproduction of dehydrins in Citrus unshiu Markov (CuCOR19) increases resistance of transgenic plants to hypothermy by decreasing cold-induced POL probably at the expense of direct neutralization of ROS (Hara *et al*., 2004). Consequently we may suggest that dehydrins of wheat, which enhanced the observed expression under the effect of SA, might contribute to normalize pro/antioxidant balance of SA-treated seedlings.

As mentioned above the SA-treatment itself leads to the enhancement of H_2O_2 production and an increase in the total activity of peroxidase, involved

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in lignin synthesis in roots of seedlings. Phenylalanin-ammonia-lyase (PAL) is known to play a key role in the synthesis of phenolic substances necessary for lignification (Dixon and Palva, 1995; Shadle *et al.*, 2003). There are data indicating the ability of SA to induce expression of the genes of PAL as well as anionic peroxidase (*POX*) in the cell culture of parsley (Thulke and Conrath, 1998). Our observation showed that SA increased the activity of PAL in the roots of wheat seedlings 12 hours after the start the treatment, with a maximum at 24 h. This allows us to suggest the involvement of SA in the acceleration of lignification of cell walls which in turn might have contributed to an increase in salt resistance of wheat plants.

   Table 4 does show that in 2 days SA accelerated lignification of the cell walls of vasculatures as compared to the control. The treatment with 2% NaCl also lead to the acceleration of this process, however it was observed one day later than under SA-treatment alone. Alongside with this, pretreatment with SA contributed to the additional enhancement of lignification of cell walls of roots of seedlings under salinity. Thus, this set of data evidences that the increase in PAL activity, enhancement of ROS generation and activation of antioxidant enzymes in the course of SA treatment promoted additional strengthening of barrier properties of cell walls of roots under salinity, which might contribute to the increase in resistance of seedlings to this stress.
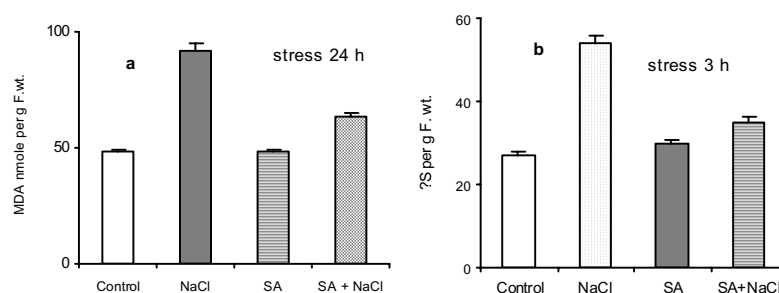

Figure 10. Effect of pretreatment with 0.05 mM SA on the concentration of MDA and leakage of electrolytes induces by the incubation of wheat seedlings in 2% NaCl.

   Consequently, the changes in the state of prooxidant-antioxidant system presented by balanced activation of ROS generation and antioxidant enzymes, the increase in activity of PAL, acceleration of lignification of cell walls in roots of wheat seedlings, observed in the course of pretreatment with SA make an important contribution to the increase in resistance of wheat plants to subsequent action of salinity. This is expressed in a decline in the level of stress-induced POL, release of electrolytes and is reflected in

growth parameters of SA-treated plants and acceleration of reparation of growth processes after withdrawal of stress.

Table 4. Dynamics of lignin accumulation in basal part of roots of wheat seedlings pretreated and not treated with SA and after exposure to 2% NaCl for 24 h

Variants	5 days	6 days	7 days
Control	-	-	+
CK	+	+	++
2 % NaCl	-	+	++
CK + 2% NaCl	++	+++	++++

*4-days-old seedlings pretreated with SA were transferred to the solution of NaCl for 24 h; mark «-» indicates absence of colouring by phloroglucinol, mark «+» reflects the extent of colour intensity (Sedlarova and Lebeda, 2001).

4. CONCLUSION

The set of data obtained by the authors evidences the growth promotive and clearly pronounced protective action of SA on wheat plants under salinity. In expressing these properties a decisive role may be attributed to hormonal system responding sensitively to changes in the environment. The SA treatment induces a shift in hormonal balance coupled with transitory accumulation of IAA and ABA. We link growth promoting action of SA with accumulation of auxin, while its protective action may be explained by that of ABA, osmoprotectants contributing to the latter effect in a great extent. We revealed an increase in the concentration of proline and expression of wheat dehydrin gene in SA-treated seedlings preceded by SA-induced accumulation of ABA, which might promote an increase in their resistance to stress factors.

The effect of salicylic acid on the state of pro- and antioxidant system plays an important role in its protective action on wheat plants. Balanced, elevated generation of O_2^- and H_2O_2 and activity of SOD and peroxidase influenced by SA is likely to prepare plants for forthcoming oxidative stress caused by unfavorable factors decreasing its detrimental effect. It is not excluded that SA controls the activity of antioxidant enzymes through transit accumulation of ABA in wheat seedlings, which is evidenced by the data that indicates implication of ABA in the enhancement of the expression of SOD genes and activation of the enzyme. It is of interest to note that antioxidant activity was revealed in the spectrum of functions attributed to dehydrin, ABA participating in the control of dehydrin synthesis. Ability of SA to regulate production of ROS, activity of peroxidase and PAL correlates with the acceleration of lignification of cell walls under its influence, which

contributes in the protection of wheat plants to forthcoming stressful influences.

Present data clearly indicates that treatment of wheat seeds or that of seedlings with SA contributes to increase the resistance of plants to stress factors of the environment and ABA might serve as an intermediate in the protective action of SA.

Preadapting wheat plants by SA to salinity is illustrated by the data. This shows that SA treatment prevents a decline in the content of IAA and cytokinins and maintains increased level of ABA, over the control and reduces stress-induced generation of ROS and activation of antioxidant enzymes. In general, this is manifested as a decline in the level of injurious effect of salinity on the integrity of cell membrane in SA-treated plants, evidenced by a decline in POL and leakage of electrolytes. This is also indicated by the growth of seedlings and growth recovery during the period following stress withdrawal. It is important to underline that in our experiments we used 0.05 mM concentration of SA, which is comparable with that of the classical hormones, used for the treatment of plants in the study of the mechanism of their action. Data presented in this chapter allows us to consider endogenous growth regulator SA as an effective inductor of unspecific resistance of wheat plants, in whose regulation important role is assigned to the hormonal status and pro- antioxidant system.

5. ACKNOWLEDGEMENT

6. REFERENCES

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