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WHEAT GERMAGGLUTININ RESTORES CELL DIVISION AND GROWTH OF WHEAT SEEDLINGS UNDER SALINITY

A. M. Avalbaev, M. V. Bezrukova, A. R. Kildibekova, R. A. Fatkhutdinova, F. M. Shakirova

Institute of Biochemistry and Genetics, Ufa Scientific Centre, Russian Academy of Sciences, 69 pr. Octyabrya, 450054 Ufa, Russia

Summary. We investigated the effect of wheat germ agglutinin (WGA) on plant resistance to salinity. We showed that treatment of wheat seedlings with exogenous WGA resulted in an increase in cell division and elongation. Incubation of 4-d-old seedlings in 2% NaCl solution for 24 h resulted in appreciable decrease in mitotic activity of meristem cells in roots and growth of whole seedlings. At the same time, pretreatment of seedlings with WGA during 24 h completely removed the detrimental effect of salinity on these parameters. Moreover, the treatment of seedlings, subjected to short-term (7 h) salt stress, with WGA for 24 h resulted in restoration of mitotic activity of root cells while the control plants did not restore. These data show the defense effect of WGA on growth processes of wheat plants.

Keywords: mitotic index, salinity, *Triticum aestivum*, wheat germ agglutinin

Abbreviation: WGA – wheat germagglutinin, conA – concanavalin A, PHA – phytohaemagglutinin

Introduction

Wheat germ agglutinin (WGA) is a typical representative of cereal lectins whose structure, physico-chemical and biological properties are well-known (Raikhel et al., 1993; Peumans, Van Damme, 1995). Nevertheless its physiological importance in wheat plants is still a matter of conjecture (Raikhel et al., 1993; Rudiger, 1997). Due to its specificity for N-acetyl-D-glucosamine a protective function against chitin-containing

* Corresponding author, e-mail: shakirova@anrb.ru

pathogens is most probable (Mirelman et al., 1975). In fact, when wheat plants were infected and treated with elicitors, the level of the WGA substantially increased that might indicate the involvement of lectin in wheat defensive responses during pathogenesis (Cammue et al., 1990; Ciopraga et al., 1999). Meanwhile, evidence about ABA being essential for the sharp reversible accumulation of wheat lectin in response to drought (Cammue et al., 1989; Singh et al., 2000), osmotic shock (Cammue et al., 1989), salinity (Shakirova, Bezrukova, 1998), heat-shock (Shakirova et al., 1996) and infection (Khairullin et al., 1993) suggests that accumulation of this protein is a common ABA-controlled response of wheat plants to different stresses (Shakirova, 2001).

At the same time the importance of such essential rise in WGA level under unfavorable conditions for wheat plants is not clear nor the role this lectin plays in antistress reactions of plants. With the purpose of finding out the importance of the fast reversible increase in WGA content for plants we investigated the influence of exogenous WGA on wheat resistance. As a stress we used salinity, which was simulated by introducing 2% NaCl into the nutrient media. In this connection the aim of the present work was to study the protective action of this protein on seedling growth under salinity.

Materials and Methods

Wheat seeds (*Triticum aestivum* L.) c.v. Saratovskaya 29, obtained from Chishminsky Crop Production, Bashkortostan, Russia, after sterilization with 70% ethanol were grown at 22–24ºC and 16-h photoperiod (15 klux) for 3 d. After endosperm excision, the seedlings were incubated for 24 h on a 2% sucrose solution as a source of carbon nutrition of seedlings (Shakirova, Bezrukova, 1998). With the purpose to check the effect of WGA and other plant lectins on cell division and elongation of root meristem cells the 3-d-old seedlings devoid of the endosperm were incubated for 24 h on a 2% sucrose solution, containing WGA, concanavalin A (ConA) or phytohaemagglutinin (PHA) which were used at concentrations 1 mg/l. In some experiments, 3-d-old seedlings isolated from endosperm after incubation for 24 h on a 2% sucrose solution, containing 1 mg/l WGA, were transferred to a mixture of 2% sucrose and 2% NaCl for 24 h. In an other experiment 4-d-old seedlings (incubated after excision of endosperm on 2% sucrose solution) were transferred to a mixture of 2% sucrose and 2% NaCl for 7 h. Then seedlings were incubated in a medium containing 2% sucrose and 1 mg/l WGA for 24 h. Plants incubated on 2% sucrose solution served as a control in all these experiments.

Growth parameters were estimated on the basis of changes in fresh weight of 3050 wheat seedlings and intensity of cell division and elongation in cells of seedling roots. The relative growth rate of 30–50 seedlings was calculated by subtracting initial fresh weight from the final one accumulated during two days and relating the difference to

initial fresh weight. Mitotic index of root meristem cells of seedlings was determined using about 3000 cells on variant (Alexeeva, Pausheva, 1988; Danilova et al., 1990). The area of cells in the zone of root elongation was calculated on the base of measurement of length and width of 500 cells by means of ocularmicrometre (MOB-1x15). Each variant of experiment included not less than 40 seedlings.

The experiments were repeated at least three times.

Results and Discussion

WGA is known to be involved in defensive wheat plant responses to salinity stress (Shakirova, Bezrukova, 1998). On the other hand for a long time the mitogenic activity was revealed for some phytolectins, for example ConA and PHA not only *in vitro*, but also in itact systems (Markov, Khavkin, 1983). Participation of WGA in regulation of cell division was considered alongside with its other probable functions (Peumans, 1984). Indeed, the pretreatment of 3-d-old seedlings with 1mg/l WGA during 24 h resulted in an essential strengthening of mitotic activity of root meristem cells and an increase of cell area in the zone of root elongation (Fig. 1). Probably, such WGA effect makes an important contribution to the observed acceleration of root elongation, their fresh and dry weight, and also percentage of root mass in seedlings compared to control (data not shown). It is interesting, that phytolectins, having a clearly pronounced mitogenic effect, conA and PHA, to a smaller extent stimulated cell division in comparison with WGA and did not change the area of cells in the zone of elongation throughout 24 h of treatment (Fig. 1).

At the same time WGA is a excretory protein and which is why its high accumulation under adverse environmental conditions allow us to assume its participation in the defense of seedling growth under stress conditions, in particular, salinity. In this connection, we studied the effect of exogenous WGA on mitotic activity of meristem cells in roots and other growth parameters of seedlings under salinity.

Figure 2 demonstrates that incubation of non-treated with WGA 4-d-old seedlings on 2% NaCl resulted in a significant decline in relative elongation growth rate of 5-d-old seedlings and also inhibition of cell division. Pretreatment of 3-d-old seedlings for 24 h with 1 mg/l WGA prevented salinity-induced change of mitotic activity of root meristem cells (Fig. 2a) and relative growth rate of seedlings (Fig. 2b).

Another experiment was carried out in order to confirm our assumption about the protective action of WGA on wheat plants under stress conditions. In this case we have performed a treatment with WGA not prior to but immediately after 7 h of action of salinity on 4-d-old seedlings. A day after the short-term influence of 2% NaCl 5-d-old seedlings were characterized by a lower intensity of cell division and elongation in roots in comparison with the control, whereas seedling transfer to a solu-

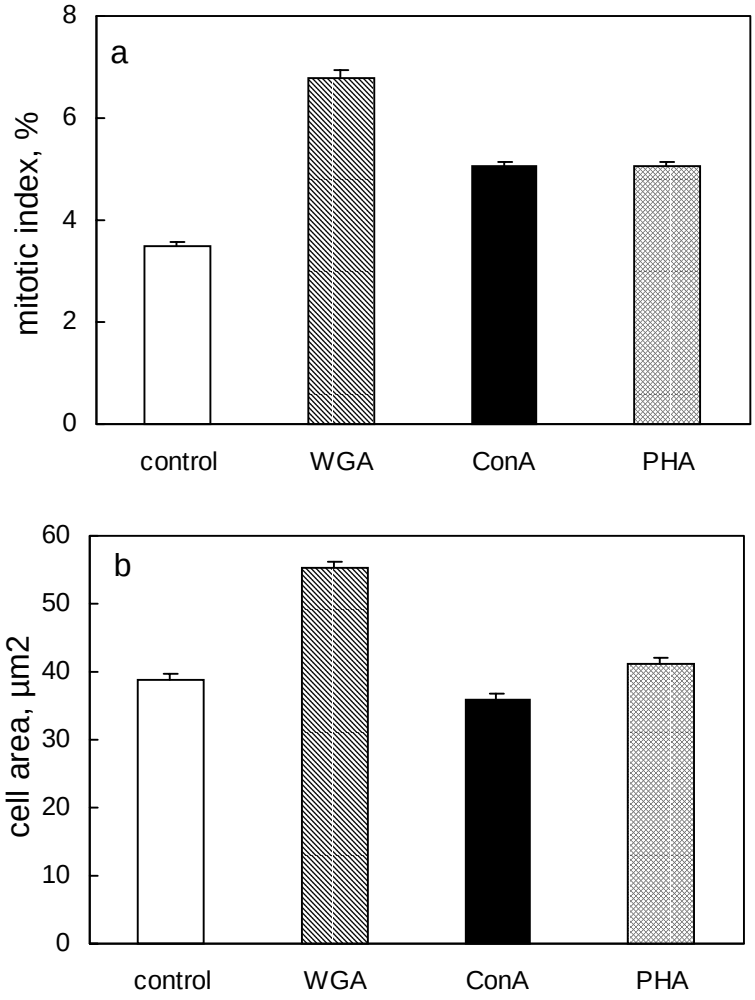

Fig. 1. The influence of wheat germ agglutinin, concanavalin A and phytohaemagglutinin, used at concentrations of 1 mg/l, on mitotic index of root meristem cells (a) and area of cells in a zone of root elongation (b) of 4-d-old wheat seedlings. 3-d-old seedlings were incubated in mixture of 2% sucrose with different proteins for 24 h.

tion of 1 mg/l WGA after 2% NaCl within one day completely removed the inhibitory effect of salinity on mitotic activity and cell growth (Fig. 3).

There are many reports about considerable WGA accumulation under conditions of different kinds of stress. The mechanisms of the protective function of wheat lectin in response to chitin-containing phytopathogens is quite clear because of its specificity for N-acetyl-D-glucosamine (Raikhel et al., 1993; Ciopraga et al., 1999), but in the

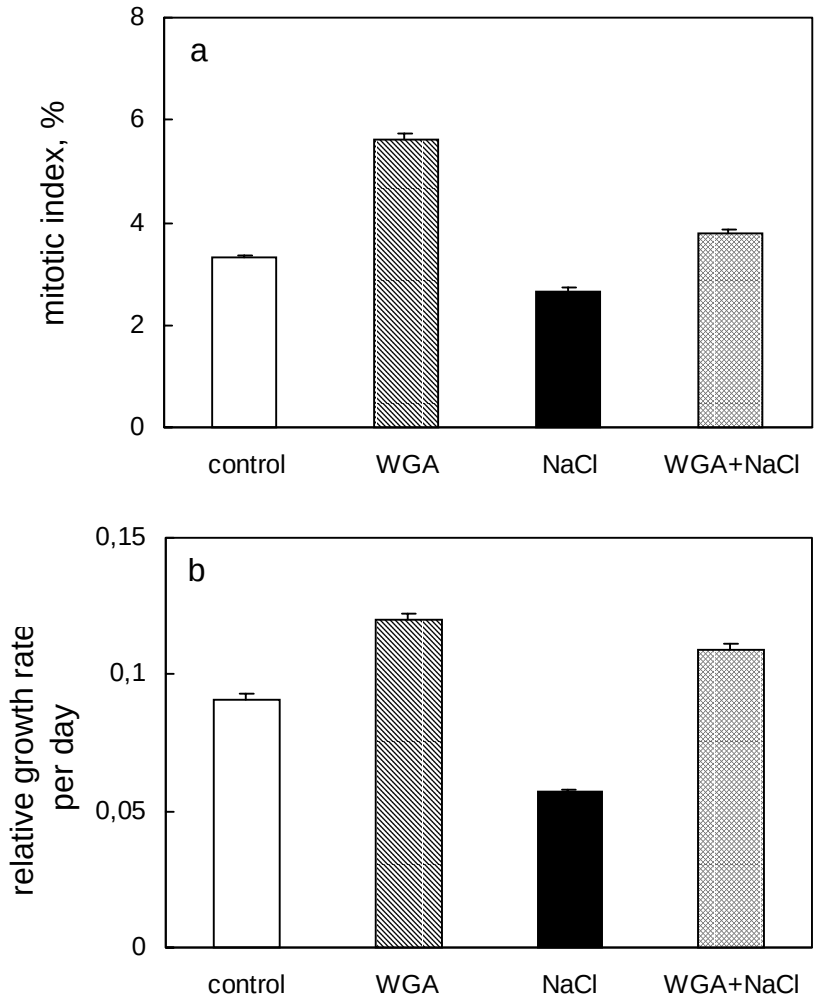

Fig. 2. The effect of pretreatment of 3-d-old seedlings with 1 mg/l WGA on mitotic index of root meristem cells (a) and relative growth rate (b) of 5-d-old seedlings under salinity. 4-d-old seedlings have been subjected to the influence of 2% NaCl for 24 h.

case of influence of abiotic stress factors the serious arguments in favour of protective properties of this protein has not been received yet. Our data indicate on the ability of WGA to prevent stress-induced inhibition of cell division and elongation and to restore these after removal of the stressor from the medium that evidences participation of lectin in the regeneration of meristematic tissue after abiotic stress action. At the same time, WGA, being a protein excretable to the external medium, might protect rhizosphere of plants weakened by stress influences from a possible soil infection.

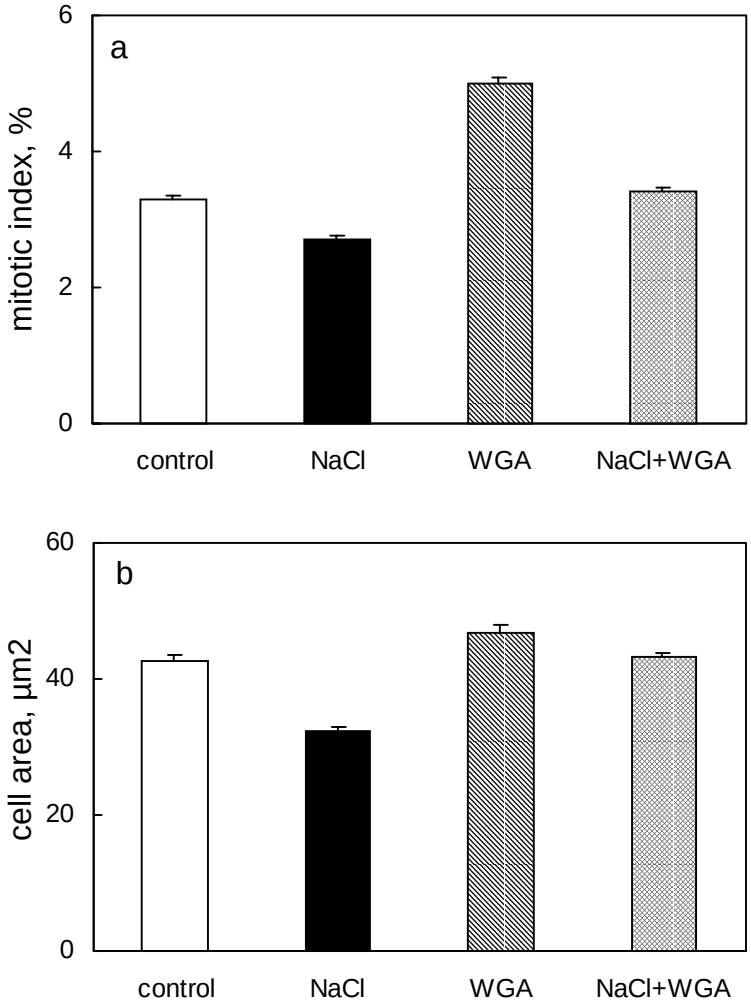

Fig. 3. The effect of WGA on mitotic index of root meristem cells (a) and area of cells in a zone of root elongation (b) of 5-d-old seedlings. 4-d-old seedlings have been subjected to the influence of 2% NaCl for 7 h, after that they were incubated on 1 mg/l WGA solution for 24 h.

These data show the defense effect of exogenous WGA on growth processes of seedlings and may indicate the ability of WGA to render protective action on wheat plants under the influence of abiotic stress factors.

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