INFLUENCE OF ULTRAVIOLET IRRADIATION AND ACID PRECIPITATIONS ON THE CONTENT OF ANTIOXIDANTS IN WHEAT LEAVES

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Abstract. Combined effect of pre-sowing treatment with UV and post-emergence spraying with acid solution on antioxidant system of wheat species (*Triticum aestivum* L.- soft wheat, *T. durum* Dest. – durum wheat, *T. macha* Dek. Et Men. – macha wheat (endemic of Georgia, partly wild, relict species), and three varieties of *T. persicum* Zhuk. - dika: *T. persicum var. stramineum, T. persicum var. fuliginosum* and *T. persicum var. rubiginosum* (white, black and red dika respectively) has been studied. The combination of two stresses induced full stimulation of the enzymatic system (catalase, peroxidase), caused increase of phenolic substances content (16-58%) and anthocyanins synthesis in leaves of tested species. Impact of both stresses increased content of proteins in leaves of Macha wheat, soft wheat and white and red varieties of dika, while in rest species content of proteins diminished. Influence of two stresses diminished the activity of the protective ascorbate system in leaves of all tested species, except the durum wheat. Normal termination of vegetation cycle and grain harvest of all tested species points to their resistance to the applied stresses.
Keywords: *UV irradiation of seeds, artificial acid precipitations, antioxidants, wheat.*

Introduction

Under natural conditions plants are usually attacked by several stress factors simultaneously. According to investigations the plant's response to the combined effect of several stressors differs from its reaction on the same factors separately (Craufurd and Peacock, 1993; Jiang and Huang, 2001; Pnueli et al., 2002). Plants resistance to simultaneous effect of different stresses is of particular interest in selection. This problem is very popular today, though little is known about the molecular mechanisms responsible for plant adaptation to combined effect of different stressors (Rizhsky et al., 2004).

Mostly data on simultaneous influence of drought and high temperature on agricultural plants are given in literature (Craufurd and Peacock, 1993; Jiang and Huang, 2001). It is established that the combination of these two stressors comparatively negatively influences plant growth and development, than each of them separately (Jagtap et al., 1998; Wang and Huang, 2004). The ultraviolet irradiation (UV) and acid precipitations (AR) are among those abiotic factors, which significantly influence plant. Effect of each of these stressors separately on different plant species was investigated by a number of scientists. Though, there is no data on the combined influence of UV and AR on plants.

The combined effect of pre-sowing treatment with UV and acid spraying on antioxidant system of some wheat species has been studied in the given work. The

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purpose of the investigation was: 1. To establish the reaction of the antioxidant system of plants on the successive impact of two stressors. In particular, how the effect of one stressor (UV irradiation) stimulates or diminishes the tolerance of plant towards another stress (acid precipitation). 2. To reveal comparatively stress-resistant species of wheat. We supposed that combination of UV irradiation and acid precipitations may have positive influence on physiological and biochemical indices of plant connected with stress resistance. In case of positive results they could be taken into account in the optimization of agriculture.

Review of literature

Generally it is known that influence of any stress induces formation of reactive oxygen species (ROS) in plant. Therefore the mechanism of stress-resistance to different stressors seems to be similar in plant (Kreps et al., 2002; Cheong et al., 2002). Although, investigations have revealed activation of various genes in response to ROS formed as a result of influence of different stressors in Arabidobsis (Mittler et al., 2004). According to these data we must suppose that adaptation to separate stress-factor is unique in plant and is realized following the plant's demand at a particular moment. Thus, combined effect of two or more stressors seems to induce diverse response in plant (Mittler, 2006). Moreover, these responses may be antagonistic (Mittler, 2002; Barcelo and Poschenrieder, 1990). In literature we meet examples on synergetic and antagonistic relations between the responses to different stresses (Walter, 1989; Sandermann, 2004).

Thus, the appropriate respond is needed for plant adaptation to combinated affect of different stress-factors, which would fit each of stressors and at the same time must compensate the antagonistic aspects of stress-factors combination.

UV irradiation is one of the stresses attacking plants during the whole life. It is known that high doses of UV-B and UV-C irradiation negatively influence plant growth and development, photosynthesis and other vital processes. UV irradiation stimulates formation of ROS and oxidative stress, which have undesirable effect on macromolecules and may lead to the cell death (Toncheva-Panova et al., 2010; Zu et al., 2010; Pradedova et al., 2011; Schreiner et al., 2012). Though, low doses of UV-B and UV-C may increase the stress-adaptive reactions in plant, which considers activation of the enzymatic and non-enzymatic defense systems (Lavola et al., 2003; Katerova and Todorova, 2011; Rai et al., 2011).

A number of investigations deal with the positive role of pre-sowing treatment of seeds with different type of electromagnetic irradiation (among them is UV irradiation) on yield amount and quality, also in protection against different diseases; because treatment of seeds with chemicals against diseases is inconvenient both ecologically and economically (Dubrov, 1977; Ghallab and Omar, 1998; Delibaltova and Ivanova, 2006; Aladjadjiyan, 2007).

In our early experiments irradiation of some vegetable seeds with full spectrum of UV affected the metabolism of plants developed from these seeds, and caused activation of stress-adaptive mechanisms, by stimulating the synthesis of antioxidants in leaves. The results depended on irradiation intensity, plant species and the type of antioxidant system (Kacharava et al., 2013).

Recently acidic precipitation ("acid rain") has been considered to be one of the natural stressors endangering the ecological balance in the biosphere. In spite of the

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efforts of the international community, acidification of the environment still remains a significant ecological problem (Rogizhin et al., 2000; Dukhovskiy et al., 2003; Chupakhina and Maslennikov, 2004). Acidic precipitations induce the formation of free radicals and active oxygen species—deleterious to plants and limiting their tolerance against different environmental factors (Merzliak, 1999; Munzuroglu et al, 2004).
According to our previous observations spraying leaves of different species of wheat with simulated acid precipitations (pH2.5 and pH1.5) caused changes in the qualitative characteristics of the elements of antioxidative system and in many cases had stimulative effect. Though, revealed changes were not equal and depended on plant species (Chkhubianishvili et al., 2008; Rapava et al., 2010).

Materials and methods

Plant material

Following species of wheat were selected for testing: winter wheats - *Triticum aestivum* L.- soft wheat (as widely cultivated and popular species of wheat), *T. durum* Dest. – durum wheat (valuable species of high nutritional quality), *T. macha* Dek. Et Men. – macha wheat (endemic of Georgia, partly wild, relict species), and spring wheats – three varieties of *T. carthlicum* Nevski: *T. carthlicum var. stramineum, T. carthlicum var. fuliginosum*, and *T. carthlicum var. rubiginosum* (white, black and red dika respectively, endemics of Georgia). Seed material was received from the laboratory of genetic resources of the Institute of Botany of Ilia State Uniuversity, Georgia.

UV irradiation and acid spraying of the experimental material

Dry seeds of experimental plants were irradiated with full spectrum of UV rays during 3h from an artificial source (lamp ДРТ-400, Russia) which was situated at 30cm from seeds. The intensity of irradiation was measured with a UVP radiometer (UVP Inc. USA) and exposure was 1.19mW/cm^2, 1.3mW/cm^2 and 1.84mW/cm^2 for A, B and C sections of UV spectrum respectively. Irradiated seeds were sowed on the experimental plot of the Institute of Botany of Ilia State University, Georgia, under field conditions. Plants were sowed in rows. Three row for each variant. Control, UV-irradiated and UV irradiation+acid sprayed variants were situated in separate blocks. The tested plants were sprayed with a water solution of a mixture of sulfuric and nitric acids 2:1, pH1.5, since the stem rising phase (Z30 by Zadok scale), at intervals of 5 days, till the end of vegetation (Z77 by Zadok scale) (totally 9 sprayings). Plants sprayed with distilled water served as controls for each studied species or cultivar. Analyses were made in the flowering phase (Z60-65 by Zadok scale), one week after the last spraying. Material for analysis was picked randomly for all three variants. Analyses were made in five biological replicates.

Antioxidant enzyme assay

Activity of peroxidase was determined spectrophotometricaly, using guaiacole. Optical density of guaiacole oxidized products was measured at wavelength of 470 nm over a period of 2 min (Ermakov et al., 1987). Results are given in conditional units per one gram of fresh weight.

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Catalase activity was measured gasometrically: the volume of the oxygen released after adding H_2O_2 (30%) to water extract of experimental leaves was determined (Pleshkov, 1985).

Anthocyanins

One g of fresh leaves was placed in a mix of 20 ml of 96% ethanol and 2 ml of 1% HCL for 24 h. The extinction of the obtained extract was measured at 529 nm (Ermakov et al., 1987).

Ascorbic acid

A titration method was used to measure the content of ascorbic acid. 2 g of fresh leaf material was mashed in 15 ml of 2% hydrochloric acid and 10 ml of 2% metaphosphoric acid, and filtered. One ml of the filtrate was added to 25 ml of distilled water and titrated with a 0.001 M solution of dichlorphenolindophenole (Ermakov et al., 1987).

Proline

0.5 g of dry leaves were mashed in 10ml of 3% sulphosalicylic acid and filtered. 2 ml of the filtrate was added to 2 ml of acid ninhydrin and 2 ml of ice acetic acid. After 1 h exposition on a water bath the extract was cooled and added with 4 ml of toluene and divided in a separating funnel. Optical density of upper layer was measured on a spectrophotometer at 520 nm (Bates et al., 1973).

Total phenols

A 0.5 g of fresh leaves was boiled in 80% ethanol for 15 min. After centrifugation the supernatant was saved, and residues of leaves were mashed in 60% ethanol and boiled for 10 min. Obtained extract was added to the first supernatant and evaporated. The sediment was dissolved in distilled water. One ml of the received solution was added with the Folin-Ciocalteu reagent and optical density was measured at 765 nm. The chlorogenic acid served as control (Ferraris et al., 1987).

Total protein assay

Content of proteins was determined after Lowry (1951).

Soluble carbohydrates

Anthrone reagent was used for determining the content of soluble carbohydrates (Turkina and Sokolova, 1971). To 100 mg of air-dry leaf material was added 96° alcohol for extraction (3-fold extraction). The total amount of the obtained extract was evaporated on a water bath and dissolved in 5 ml of distilled water. To 0.5 ml of the tested water extract was added 2ml of anthrone reagent and heated in a water bath for 10 min. After this procedure the test-tubes were placed in a cold water bath and 15 min later the optical density of the solution was measured at 620 nm with a spectrophotometer (SPECOL 11, KARL ZEISS, Germany).

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Statistical analysis

One way ANOVA and Tukey's multiple comparison tests were used to test differences between the means. All calculations were performed using statistical software Sigma Plot 12.5.

Results

Catalase and peroxidase

High activity of peroxidase was characteristic for soft wheat among the tested species (*Figure 1*). Seed irradiation caused diminishing of the enzyme activity in leaves of macha wheat and durum wheat, while in soft wheat enzyme's activity significantly increased ($p < 0.05$). UV irradiation appeared to be stimulative for peroxidase activity in leaves of spring wheats as well. Here white dika distinguished with high peroxidase activity in control variants. Irradiation of seeds with UV for 3 h increased the enzyme activity in all varieties of dika ($p < 0.05$).

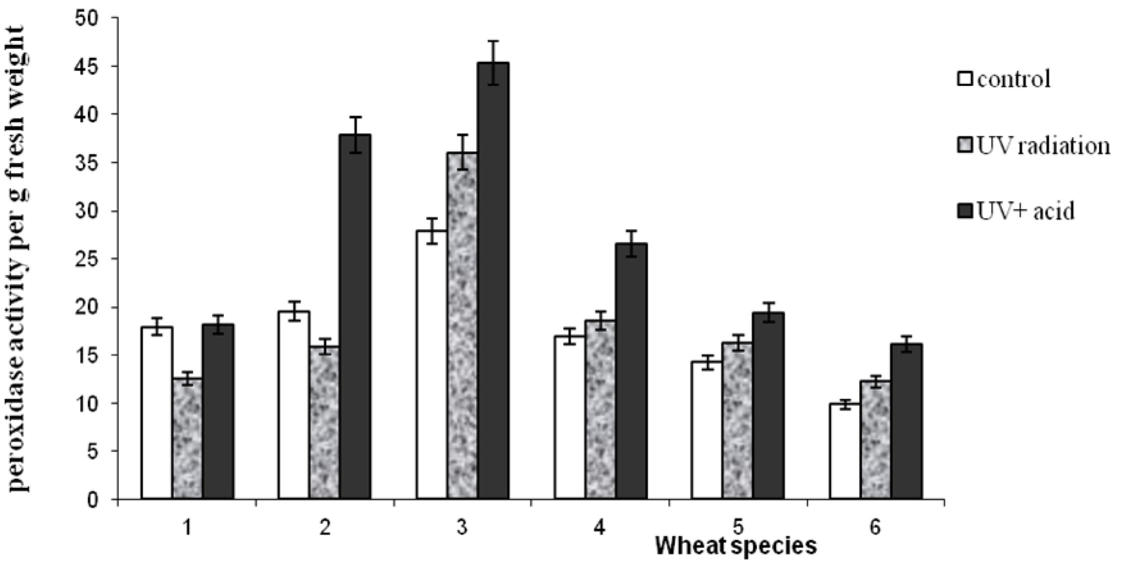

Figure 1. *Combined effect of seed UV treatment and acid spraying of leaves on the activity of peroxidase in leaves of wheat species. 1. Triticum macha Dek. et Men. (p < 0.001) 2. T. durum Desf. (p < 0.001) 3. T. aestivum L. (p < 0.001). 4. T. persicum var. stramineum Zhuk. (p < 0.001) 5. T. persicum var. fuliginosum Zhuk. (p < 0.001) 6. T. persicum var. rubiginosum Zhuk. (p < 0.001)*

Effect of both stressors has stimulated activity of peroxidase in leaves of all experimental species. This time stimulation was higher, than in case of seed irradiation.

Observations have revealed that catalase activity in leaves of all tested plants was similar (*Figure 2*). Both treatments – seed pre-sowing irradiation with UV and acid spraying of plants, developed from the irradiated seeds, have stimulated catalase activity in leaves of experimental plants. Though, combination of UV and acid spraying appeared to have more stimulative effect on enzyme's activity, than only seed UV treatment.

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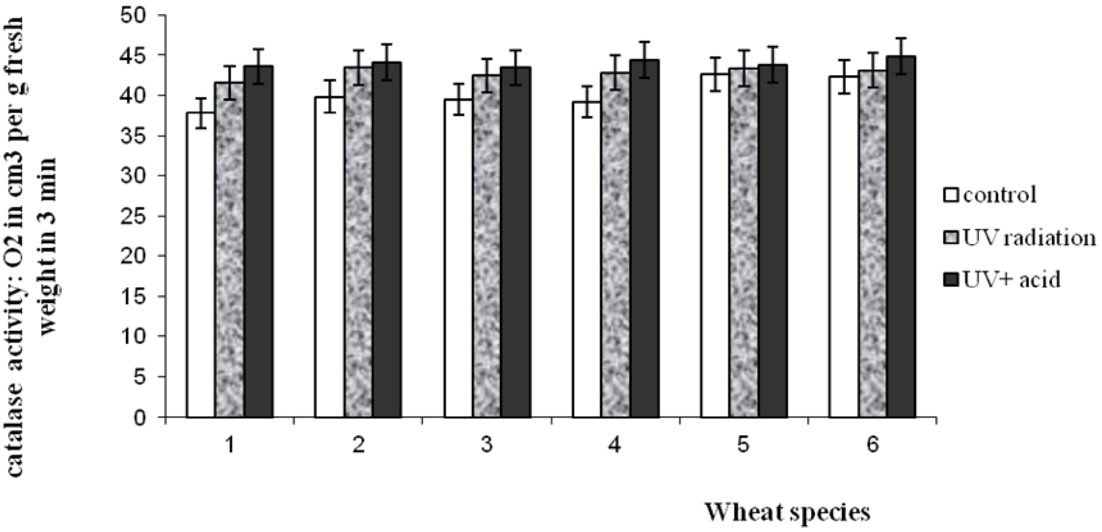

***Figure 2**. Combined effect of seed UV treatment and acid spraying of leaves on the activity of catalase in leaves of wheat species. 1. Triticum macha Dek. et Men. ($p < 0.001$) 2. T. durum Desf. ($p < 0.001$) 3. T. aestivum L. ($p < 0.001$). 4. T. persicum var. stramineum Zhuk. ($p < 0.001$) 5. T. persicum var. fuliginosum Zhuk. ($p = 0.001$) 6. T. persicum var. rubiginosum Zhuk. ($p < 0.001$)*

Ascorbic acid

Experimental results demonstrate between-specious and intra-specious differences in reactions to one and the same stressors among the experimental plants.

With high content of leaf ascorbate were distinguished black and red varieties of dika (*Figure 3*). Seed irradiation has changed amount of the vitamin in all tested plants but at different levels. Content of ascorbic acid increased in leaves of macha wheat, durum wheat and red dika, but decreased in leaves of soft wheat and black dika. Combinations of two stressors (seed irradiation and spraying with acid of plants developed from the irradiated seeds) inhibited ascorbic acid synthesis in all species, except the durum wheat. In the last in contrary, increase of the index was mentioned (*Figure 3*). Moreover, in irradiated and later sprayed with acid variants the content of the ascorbate diminished with higher extent than in irradiated variants.

Proline

High content of proline was established in species of winter wheat (*T. macha, T. durum, T. aestivum*). Pre-sowing irradiation of seeds decreased content of the substance in leaves of all experimental plants (macha wheat was exception) (*Figure 4*). Spraying leaves with acid appeared to be more depressing for proline content in all spring species, compared with control and irradiated variants; while in winter species increase of proline took place, compared with irradiated variants. Macha wheat and durum wheat made exception: here the index was lower than in control, while in soft wheat content of proline exceeded the control.

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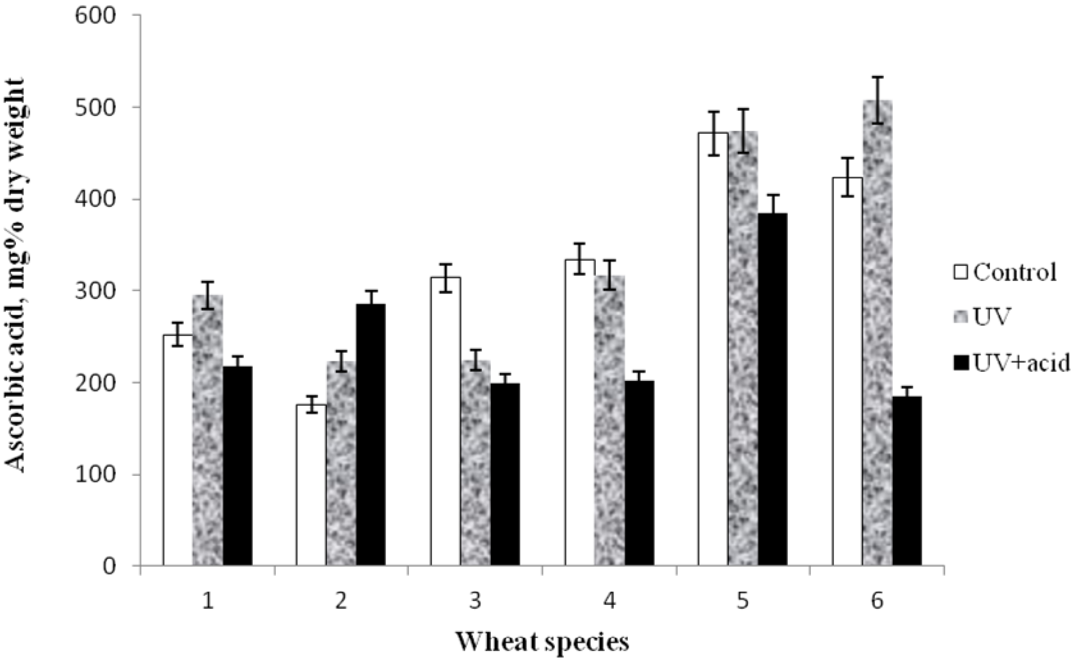

__Figure 3.__ Combined effect of seed UV treatment and acid spraying of leaves on the content of ascorbic acid in leaves of wheat species. 1. Triticum macha Dek. et Men. ($p < 0.001$) 2. T. durum Desf. ($p < 0.001$) 3. T. aestivum L. ($p < 0.001$). 4. T. persicum var. stramineum Zhuk. ($p < 0.001$) 5. T. persicum var. fuliginosum Zhuk. ($p = 0.001$) 6. T. persicum var. rubiginosum Zhuk. ($p < 0.001$)

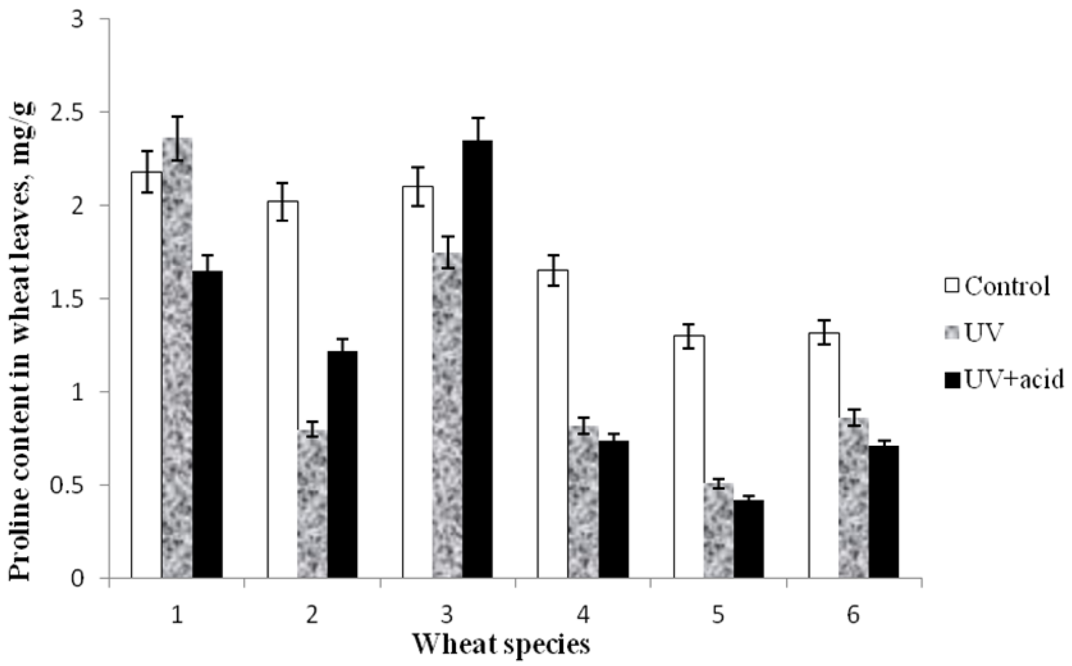

__Figure 4.__ Combined effect of seed UV treatment and acid spraying of leaves on the content of proline in leaves of wheat species. 1. Triticum macha Dek. et Men. ($p = 0.003$) 2. T. durum Desf. ($p < 0.001$) 3. T. aestivum L. ($p = 0.022$). 4. T. persicum var. stramineum Zhuk. ($p < 0.001$) 5. T. persicum var. fuliginosum Zhuk. ($p < 0.001$) 6. T. persicum var. rubiginosum Zhuk. ($p < 0.001$)

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Soluble phenols

Investigation of the content of soluble phenols has revealed the high extent of accumulation of these substances in macha wheat, and white and black dika. Comparatively low was this index in soft wheat and red dika (*Figure 5*). Both, seed irradiation and spraying with acid precipitations appeared to be stimulative for synthesis of phenols in leaves of all studied species. Moreover, the percentage of stimulation was higher in winter wheats (29-33%) compared with varieties of dika (11-16%). Combination of two stresses was more stimulative for phenol synthesis, than only UV irradiation (*Figure 3*). The percentage of the increment of phenolic substances in case of double stress varied between 16-58%.

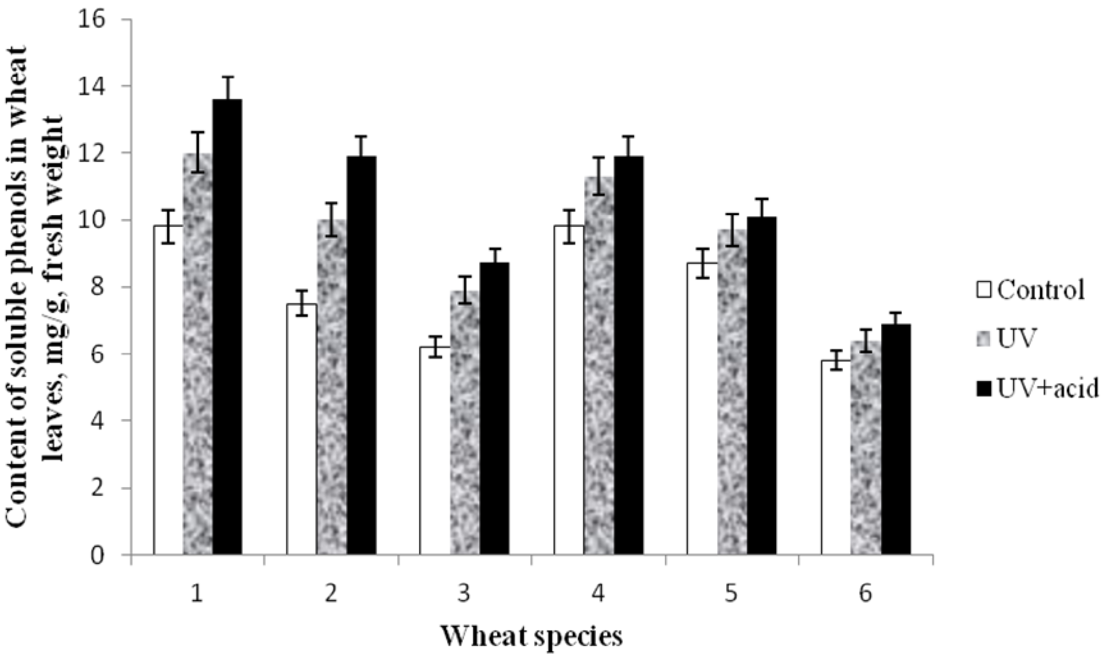

Figure 5. *Combined effect of seed UV treatment and acid spraying of leaves on the content of soluble phenols in leaves of wheat species. 1. Triticum macha Dek. et Men. ($p < 0.001$) 2. T. durum Desf. ($p < 0.001$) 3. T. aestivum L. ($p = 0.002$). 4. T. persicum var. stramineum Zhuk. ($p = 0.009$) 5. T. persicum var. fuliginosum Zhuk. ($p = 0.01$) 6. T. persicum var. rubiginosum Zhuk. ($p = 0.01$)*

Anthocyanins

According to obtained results high content of antocyanins among control variants was mentioned in leaves of durum wheat and black and red varieties of dika. In other species data were alike. Pre-treatment of seeds with UV inhibited anthocyanins synthesis in leaves of macha wheat, soft wheat and white dika. Stronger was the effect in case of spraying these species with acid solution (*Figure 6*). In durum wheat and black and red varieties of dika incontrary, UV treatment and spraying with acid as well, stimulated the process of accumulation of anthocyanins in leaves. Though, the index of double stressed (UV treated+acid sprayed) variants was lower, compared with UV treated ones, but prevailed the control variants.

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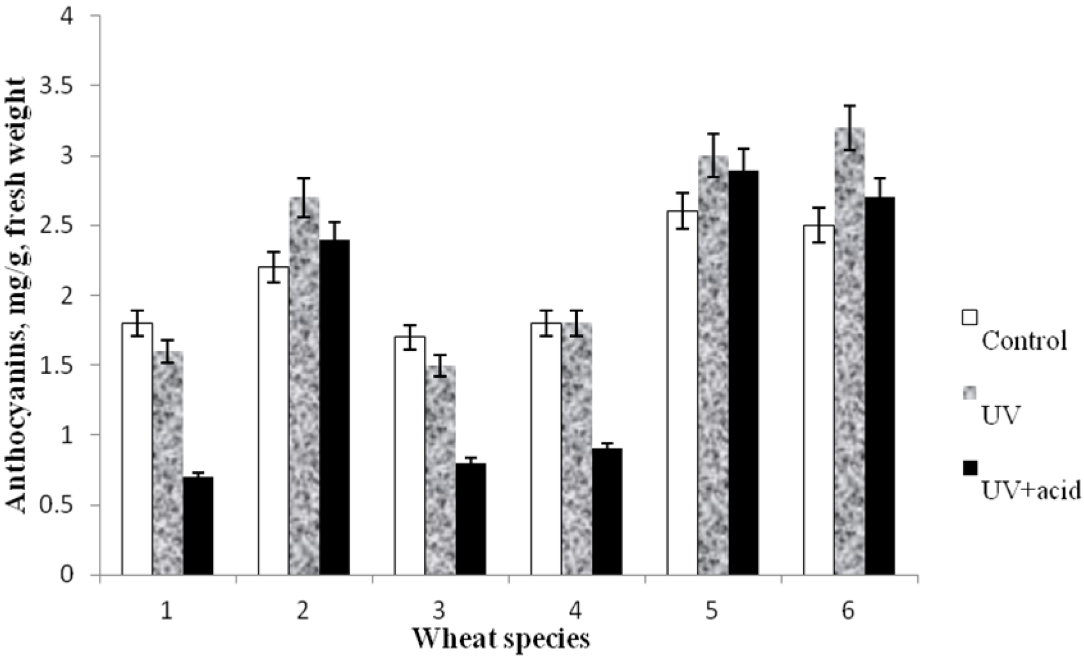

Figure 6. *Combined effect of seed UV treatment and acid spraying of leaves on the content of anthocyanins in leaves of wheat species. 1. Triticum macha Dek. et Men. ($p = 0.004$) 2. T. durum Desf. ($p = 0.01$) 3. T. aestivum L. ($p < 0.001$). 4. T. persicum var. stramineum Zhuk. ($p < 0.001$) 5. T. persicum var. fuliginosum Zhuk. ($p = 0.01$) 6. T. persicum var. rubiginosum Zhuk. ($p = 0.006$)*

Total proteins

Macha wheat and durum wheat appeared to have comparatively high level of proteins in leaves (*Figure 7*). Pre-treatment with UV diminished the content of total proteins in the named species. Irradiation stimulated synthesis of proteins in soft wheat and all varieties of spring wheat. Impact of both stressors increased content of proteins in leaves of macha wheat, soft wheat and white and red varieties of dika, while in rest species content of proteins diminished.

Soluble carbohydrates

Among the tested wheats macha wheat and white and red varieties of dika were distinguished with high content of soluble carbohydrates in leaves (*Figure 8*). UV treatment of seeds and impact of two stress combination as well, appeared to be suppressing for soluble carbohydrates synthesis in leaves; especially the double stress did. The sharp decrease of the soluble carbohydrates under stress conditions was mentioned in those species, which were distinguished with high content of these substances in leaves (macha wheat, white dika. 63% and 50% respectively).

Discussion

Pre-sowing treatment of seeds with UV irradiation supplies the dormant seeds with extra energy, which is stress for them. Free radicals produced during this process, changes in cell membrane permeability and electric potential, presumably initiate

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diverse metabolic responses including biosynthesis of antioxidants. This, from its side, must be reflected on further development of plant and formation of adaptive mechanisms to unfavorable environmental conditions (Dubrov, 1977).

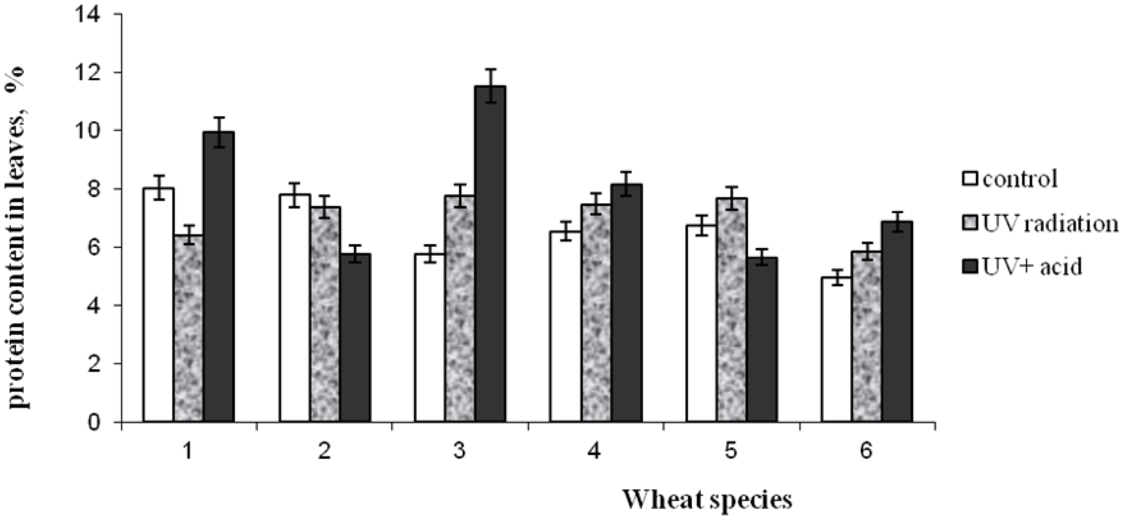

Figure 7. *Combined effect of seed UV treatment and acid spraying of leaves on the content of total proteins in leaves of wheat species. 1. Triticum macha Dek. et Men. (p < 0.001) 2. T. durum Desf. (p < 0.001) 3. T. aestivum L. (p < 0.001). 4. T. persicum var. stramineum Zhuk. (p < 0.001) 5. T. persicum var. fuliginosum Zhuk. (p < 0.001) 6. T. persicum var. rubiginosum Zhuk. (p = 0.01)*

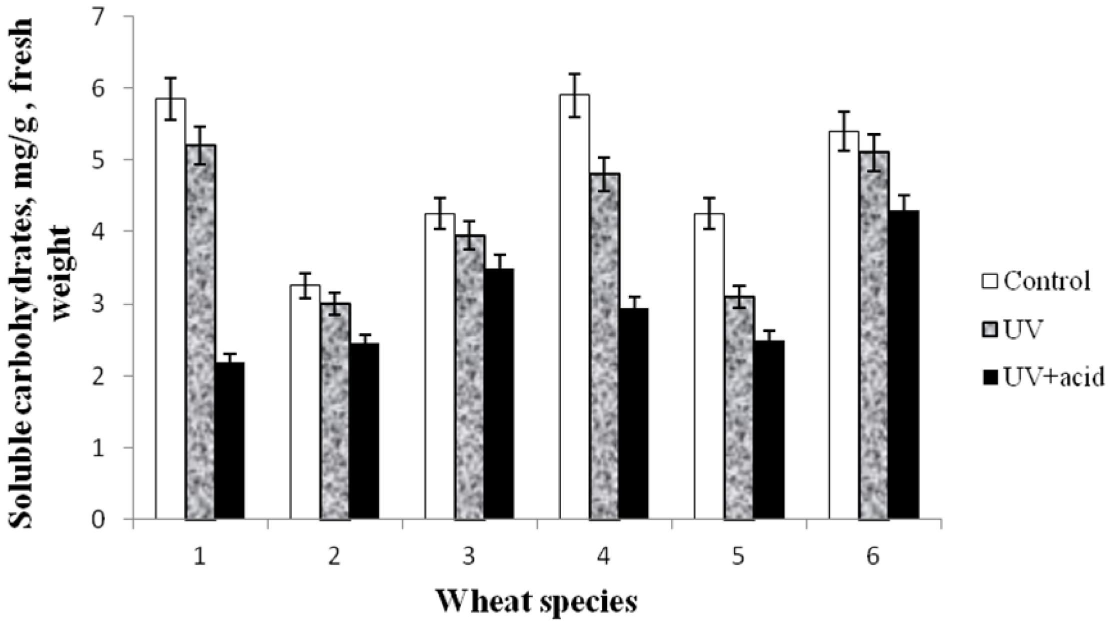

Figure 8. *Combined effect of seed UV treatment and acid spraying of leaves on the content of soluble carbohydrates in leaves of wheat species. 1. Triticum macha Dek. et Men. (p < 0.001) 2. T. durum Desf. (p = 0.04) 3. T. aestivum L. (p = 0.047). 4. T. persicum var. stramineum Zhuk. (p = 0.001) 5. T. persicum var. fuliginosum Zhuk. (p < 0.001) 6. T. persicum var. rubiginosum Zhuk. (p = 0.05)*

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It is established that the concentration of ROS serves as a signal for switching on of different genes and signal systems, controlling the responses to stress (Dalton et al., 1999; Foyer and Noctor, 2003). Evidently UV-treatment of seeds must have stimulated such type of signaling systems in seeds; besides, this stimulation was so strong, that clearly influenced plant's vital processes during a long period after emergence. In particular, leaves of plants, emerged from the irradiated seeds were tested in flowering phase, while the effect of irradiation on the antioxidant system still was evident.

Catalase and peroxidase

Enzyme catalase (CAT, EC 1.11.1.6) accumulates in peroxisomes and is responsible for destruction of hydrogen peroxide. During stress conditions the number of these organelles increases in the cell. This plays an important role in detoxication of hydrogen peroxide, diffused in peroxisome from other parts of the cell (Mittler, 2002).

According to the classical view on peroxidase, its main function is to protect the organism from damaging effect of peroxide. Peroxidases (EC 1.11.1) are a big group of enzymes, met everywhere in the cell and fulfilling multilateral function in plant metabolism (Passardi et al., 2005). In particular, they neutralize the hydrogen peroxide, formed during stress and take an active part in adaptation of plant to unfavorable environmental conditions; peroxidases effect processes of plants growth and development, regulate content of auxins and phenolic substances, etc (Andreeva, 1988; Cevahir et al., 2004; Graskova et al., 2010). Moreover, cell wall peroxidases take part in formation of ROS, which play a protective role against abiotic stresses and have signaling function in case of some stresses, manifested in activation of stress-defensive mechanisms of plants (Mika et al., 2004).

Increased activity of catalase in all irradiated variants of our tested plants once again proves the fact that the activation of catalase-synthesizing genes takes place in case of different, among them radiation stresses (Scandalios et al.,1997). If we suppose that UV treatment of seeds caused intensification of respiration in mitochondria and generally increased the content of ROS in plant, activation of catalase for neutralizing of stressors would be a logical result (*Figure 2*).

In case of peroxidase experimental results were rather different (*Figure 1*). By the opinion of scientists decrease of peroxidase activity in plant leaves may be caused by the low energetic potential of metabolic reactions, while activation of the enzyme is the result of essential shifts in metabolic, in particular the respiration system, aiming adaptation of the plant and retention of cell homeostasis (Sergeichik and Sergeichik, 1988; Tucic et al., 2007). Accordingly, we may suppose that seed irradiation changed the metabolic processes of macha wheat and durum wheat to rather different direction, by diminishing the activity of the enzyme, than in the rest species, where increase of peroxidase activity took place; i.e. from the point of view of peroxidase activity macha wheat and durum wheat may be considered as comparatively sensitive to irradiation, than other species.

Spaying of irradiated plants with acid precipitations revealed a synergistic effect with UV-irradiation and caused further activation of catalase in leaves of experimental plants (*Figure 2*).

Spraying with acid appeared to be stimulative for peroxidase as well. Activity of this enzyme increased in leaves of all tested species (*Figure 1*). Presumably, combination of two stresses fully activates the enzymatic system of the given species, demonstrating the adaptivity of their enzymatic systems.

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It must be mentioned that results of combined effect of irradiation and acid spraying in some cases differ from our early experimental data, obtained with artificial acid rains (Kacharava, 2013). In particular, spraying of leaves with pH1.5 acid solution inhibited activity of catalase in red dika, and of peroxidase – both, in red and black dika (Kacharava, 2013). We may suppose that pre-treatment of seeds with UV has stimulative effect on these antioxidant enzymes and increases adaptivity of plants to acid stress.

It must be mentioned that in spite of significant role in ROS detoxication the enzymatic antioxidative system is not able to provide full protection of the cell, conditioned by a number of factors. That is why the opinion on the effective role of low molecular antioxidant substances (ascorbic acid, proline, anthocyanins, etc.) in protection of cell metabolism from ROS has appeared (Blokhina et al., 2003).

Ascorbic acid

L-ascorbic acid or vitamin C is an important metabolite both in plants and animals. Its antioxidative activity stipulates stress resistance and life span in plants. According to great number of recent investigations besides signaling function, vitamin C takes an active part in plant protection against environmental stresses (heavy metals, salinity, temperature, UV-B radiation, etc.) by activation of corresponding genes (Shalata and Neumann, 2001; Vwioko et al., 2008). Moreover, ascorbic acid directly neutralizes ROS, and plays a role of secondary antioxidant in the reduction cycle of oxidized form of α-tocopherol and some carotenoids (Noctor and Foyer, 1998; Potters et al., 2002). At the same time, ascorbic acid is a co-factor of many enzymes (Arrigioni and De Tullio, 2000). The response of ascorbic acid to stresses is regulated by a complex chain of successive biochemical reactions, comprising activation or inhibition of key enzymatic reactions, synthesis of stress-responsible proteins and other protective substances (Khan et al., 2011). As the concentration of ascorbic acid prevails content of other antioxidants in plant, it is regarded as the main antioxidant in plant (Gallie, 2013). Thus, the content of vitamin C in leaves may be regarded as the main indicator for stress resistance and adaptation.

Literary data exist on ascorbate concentration increase in plants under stress conditions (Zengin and Munzuroglu, 2005; Mogren et al., 2012; Priyanka et al., 2014). According to our experimental data it may be concluded that the increase or maintenance of ascorbate at a control level in response to applied UV radiation in tested species (macha wheat, durum wheat, black and red varieties of dika) is indication to their resistance to the used doses of radiation (*Figure 3*).

Our early experiments on the influence of simulated acid rains on different plant species demonstrate that in most experimental plants spraying with acid solution stimulated synthesis of ascorbic acid in leaves, though in some cases opposite effect was observed (Chkhubianishvili et al., 2008).

Spraying leaves of experimental plants with acid solution has revealed the synergistic effect with UV-radiation stress in tested species. Exclusions were macha wheat, black and red varieties of dika. In these cases, in contrary, antagonistic effect was observed. Thus, acid stress diminished content of ascorbic acid in leaves of tested plants (*Figure 3*). Only in durum wheat combination of two stresses appeared to be stimulative for ascorbate synthesis; but discussion about stress-tolerance of experimental species only by ascorbate content would not be reasonable.

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Proline

From literary data it is known that proline protects plants aginst stress as osmolite, by regulation of water retention in the cell (Saradhi et al., 1995). Besides, it plays a role of proteins stabilizer (Anjum et al., 2002) and regulates NAD/NADH ratio of the cell (Alia and Saradhi, 1993). Recently a multilateral function of proline in stress adaptation of plants and signal transduction has been established. Stimulation of proline synthesis in chloroplasts presumably supports stabilization of the redox balance by dissipation of extra energy and retains cell homeostasis. Moreover, by means of modulation of responses to biotic or abiotic stresses proline plays a role of metabolic signal, which regulates the metabolic pull and expression of great number of genes, effecting this way plant's growth and development (Szabados and Savoure, 2009).

According to literary data, amount of endogenous proline increased under stress conditions in different plants. This is the indication to stress resistance (Szabados and Savoure, 2010; Kaur et al., 2011). Diminishing of proline content in irradiated variants of the tested plants in our experiments may be expression of the proline pull sensitivity to the applied doses of UV irradiation (*Figure 4*).

It is known that the reductive equivalent must be formed as a result of fast catabolism of proline, which promotes oxidative phosphorilation in mitochondria and synthesis of ATP to recover stress-induced lesion (Hare et al., 1998). Presumably, mitochondria, as the main functioning organelles of dormant seeds, are most of all affected by UV stress, in pre-sowing treatment experiments. Pre-sowing UV-irradiation of experimental seeds must have switched on the stress-protective mechanisms, presumably with the assistance of proline, which would defend the stressed mitochondria. Apparently it was the reason of proline decline in UV-treated variants.

Combination of two stresses diversely changed proline content in leaves of experimental plants (*Figure 4*). Spraying with acid solution stimulated proline synthesis in durum and soft wheats. Apparently, under the influence of acid stress additionally were activated those genes, which are associated with proline synthesis.

Results of our early experiments on acid spraying of wheat plants, once more prove the great influence of UV pre-treatment of seeds on vital processes of the developed plants. In particular, spraying of leaves with pH1.5 acid solution activated proline synthesis in all three varieties of dika (Kacharava et al., 2013). Apparently, the effect of UV-treatment on proline synthesis in tested plants was so intensive, that spraying leaves with the same acidity (pH1.5) solution could not change the effect of irradiation.

Soluble phenols

Phenols are the most active secondary metabolites in plant. The antioxidant properties most of them prevails that of such antioxidants, as ascorbic acid and tocipherol (Hernandez et al., 2009). Phenols neutralize the ROS till they manage to damage the cell (Lovdal et al., 2010). Activation of many phenol-synthesizing genes takes place under stress conditions. Significant increase of the content of phenolic substances under different biotic or abiotic stresses has been established (Winkel-Shirley, 2002).

Activation of the synthesis of phenolic substances under UV-stress in all tested species of wheat may be regarded as a confirmation of resistance of the experimental plants to the applied doses of radiation (*Figure 5*). They are surely protected by the phenilpropanoid pathway of synthesis of phenolic substances (Lapshin and Zagoskina,

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2004). Spraying leaves with acid solution has increased the effect of irradiation and intensified the synthesis of phenolic substances in all tested plants.

Obtained results support the idea, which explains the promotion of phenolic metabolisms in wheats under stress conditions by the activation of synthesis of those enzymes, which support convertion of the primary products of photosynthesis into necessary for phenolics synthesis ones (Chumachenko, 1965; Dubrov, 1968).

In early, acid spraying experiments the stimulative effect of acid on the synthesis of phenolic substances in varieties of dika has been established (Kacharava et al., 2013). Thus, in case of phenolic substances the combination of two stressors revealed synergism and increase stress-resistance of studied species (*Figure 5*).

Anthocyanins

They belong to the group of phlavonoids, which are concentrated mainly in vacuole and possess strong antioxidant properties (Kahkonen and Heinonen, 2003). The antioxidant properties of anthocyanins are stipulated by transport of electrons, belonging to hydroxyl groups, or hydrogen atoms to ROS (Blouin and Peynaud, 2005). Anthocyanins are able to join metal ions as well, which take an active part in oxidation process (Tita, 2004). Accumulation of anthocyanins in vacuole prevents to their direct contact with the sites of ROS formation. In spite of it, increase of anthocyanins content under metal or other type of stresses has been established (Mobin and Khan, 2007).

As it is known, ascorbic acid is the co-factor of those enzymes, which support anthocyanins synthesis (Gallie, 2013). Though, according to our experimental results, the evident relation between the content of ascorbic acid and synthesis of anthocianins was not revealed. Increase in ascorbate content did not always coincide with stimulation of anthocyanins synthesis. Evidently, the specific peculiarities of stress-protective strategy are being revealed here too.

Pre-sowing UV-treatment of seeds has activated anthocyanins synthesis in most tested species (exceptions were macha wheat and soft wheat) (*Figure 6*). The same effect was observed in case of double stress, compared with the control variants. Thus, the phlavonoid pathway of stress-protection was activated in these species, together with the phenilpropanoid pathway, indicating to more adaptability to unfavorable conditions.

Total proteins

Recently, bioactive peptides from enzymatic hydrolysis of various food proteins such as soy protein, casein, whey protein, gelatin and wheat gluten have been shown to possess antioxidative activity (Elias et al., 2008). So called heat shock proteins are also synthesized in plant. This is a general name of protein substances, formed in plant during different stresses (cold, heat, drought, light, heavy metals, salinity, etc.) (Timperio et al., 2008; Kochhar and Kochhar, 2005). Shock proteins protect other proteins from stress damage.

According to literary data the content of total proteins in case of different stresses increases in stress tolerant and adapted plants (Ali and Basha, 1998; De Britto et al., 2011; Kosakivska et al., 2008; Kamal et al., 2010). Thus, increase of total protein amount in leaves of dika varieties and soft wheat in our experiments may indicate to more adaptivity of these plants compared with macha wheat and durum wheat (*Figure 7*). In the last ones the index decreased.

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Stimulation of proteins synthesis in macha wheat by the acid spraying may be the result of activation of any shock protein synthesis, increasing plant's resistance to stress.

Soluble carbohydrates

Though the soluble carbohydrates are related to the metabolic ways of ROS formation, they play important role in their neutralization as well. Increase of glucose may cause intensification of NADPH synthesis (by the pentoso-phosphate cycle), which is a significant intermediate product of ascorbate-glutathione cycle (Couee et al., 2006). It was demonstrated that in some species of plants abiotic stress increased the level of glucose and sucrose as well. Though their role in protective mechanisms is not confirmed (Couee et al., 2006). Van den Ende and Valluru (2009) suppose that sucrose may protect membrane against drought and cold stress. Accumulation of soluble carbohydrates in response to different stresses in various parts of a plant has been demonstrated in a number of works (Prado et al., 2000; Finkelstein and Gibson, 2001; Nayer and Reza, 2008). Generally it is established that the metabolism of soluble carbohydrates under stress conditions is a dynamic process and comprises reactions of catabolism and synthesis simultaneously (Hilal et al., 2004).

In all experimental variants of wheat, both irradiated and irradiated and then sprayed with acid, amount of soluble carbohydrates in leaves decreased (*Figure 8*), which may be explained by the intensification of their respiration (activation of catalase may also serve as confirmation to it). Besides, soluble carbohydrates may take part in intensive synthesis of phenolic compounds, since the link of fructose with lignin and phenolics synthesis has been demonstrated (Hilal et al., 2004).

In our early experiments artificial acid precipitations caused activation of the synthesis of soluble carbohydrates in wheat leaves (Rapava et al., 2010). Presumably, inhibitory effect of the UV-irradiation on seeds is so significant, that spraying with acid solution could not decline it, or, obtained results demonstrate the effect of the double stress.

Summarizing it may be concluded:

1. The protective system of catalase is resistant to UV stress in all studied species. According to peroxidase activity macha wheat and durum wheat may be regarded as more sensitive to UV stress, compared with other species.

   The combination of two stresses induced full stimulation of the enzymatic system in tested species and adaptivity of the latter to the applied stresses may be concluded.

2. Increase or the same level of the ascorbic acid content in response to the applied dose of UV in some studied species (macha wheat, durum wheat, black and red varieties of dika) may indicate to their resistance to radiation stress. Combination of two stresses diminished the activity of the protective ascorbic acid system in leaves of the tested plants, except the durum wheat. In the latter double stress appeared to be stimulative for ascorbate content and presumably enhanced its stress tolerance.

3. The protective proline system of the tested species seems to be sensitive towards UV irradiation and UV-acid spraying stress. Acid stress presumably caused activation of genes associated with proline synthesis in durum wheat and soft wheat, stipulating increase of their resistance to double stress.

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4. Activation of the synthesis of phenolic substances in response to applied stresses in all tested plants may be regarded as a confirmation of their resistance and well protection against stress with the phenilpropanpid way of phenolics synthesis.
5. Increase of anthocyanins synthesis in response to seed irradiation and irradiation-acid spraying treatments in most tested species is demonstration of stress-protective phlavonoid pathway activation as well.
6. Gain of total protein content in response to UV stress in dika varieties and mild wheat may serve as indication to more adaptability of these species to UV radiation. Acid stress caused stimulation of protein synthesis in macha wheat, which may be linked with the activation of stress-protective shock-proteins.
7. Decrease of soluble carbohydrates content in UV-irradiated variants of the tested species may caused by the intensification of leaves respiration (catalase activation also indicates to it). Moreover, soluble carbohydrates may take part in intensified synthesis of phenolic substances, as the relation between fructose and synthesis of lignin and phenolic substances has been demonstrated.

Thus, if we discuss about stress-tolerance of plants by the amount of activated parameters of the antioxidant system, relative resistance of the studied wheat species to UV and acid stresses may be supposed. According to our scheme, high sensitivity to pre-sowing treatment with UV demonstrates macha wheat, and mild wheat (four parameters were activated or retained at control level under the stress). Dika varieties seem to be less sensitive to the same stress (especially red and black varieties. Six indices were activated).

Sensitivity of macha wheat to combined influence of UV-acid stresses remained similar to radiation stress; while in other species it was not so. In particular, white and black varieties of dika were sensitive to double stress (four parameters were activated), while in durum wheat in contrary – stress-tolerance increased (six indices were activated).

Finally we can say that normal termination of vegetation cycle and grain harvest of all tested wheat species point to their resistance to the applied types of stresses.

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