Influence of Presowing Irradiation and High Concentrations of Salts on Wheat

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Abstract— *To investigate the salt resistance of Georgian endemic wheat species – Makha (Triticum aestivum subsp. macha (Dekapr. & Menabde) McKey) and Zanduri (Triticum timopheevii subsp. zhukovskyi (Menabde & Ericzjan) L. B. Cai) on the one hand, and the effect of pre-sowing irradiation on growth and development of the same species, on the other, experiments with 1.5% solutions of NaCl and Na2SO4 and pre-sowing treatment with ultraviolet irradiation (UV) (C section of the ultraviolet, distance 30cm from the source, irradiation for 1h) have been carried out. Wheat species were affected with stressors separately and in combination. The percentage of seed germination and length of 5-6 week seedlings was studied. Obtained results demonstrate that:*

1. *Zanduri seeds are equally resistant to both chloride and sulfate salinization, while Makha seeds revealed more resistance to chloride salinization.*
2. *Irradiation of Zanduri seeds with C section of UV-radiation inhibited germination, while treatment with the same spectrum of Makha seeds in contrary, led to significant activation of the process. This effect of seeds pre-sowing irradiation was maintained during the growth and development stages as well.*
3. *Pre-sowing UV treatment of Makha seeds has canceled the inhibitory effect of NaCl on seed germination; while in variants with pre-sowing UV treatment and further processing with Na2SO4 and Na2SO4+NaCl even the stimulation of seed germination was mentioned.*
4. *In order to increase the seed resistance to chloride and sulfate salinity, we consider it advisable to irradiate Makha seeds with UV before sowing; however, the optimal dose of radiation should be selected.*

Keywords— chloride salinity, sulphate salinity, ultraviolet irradiation, wheat.

I. INTRODUCTION

Under natural conditions plants usually experience impact of several unfavorable factors. That is why the resistance to the simultaneous exposure to several stressors is of great importance in selection. Moreover, studies have shown that a plant's response to the combined effect of several stressors differs from the response to a single stress exposure (Craufurd and Peacock, 1993; Jiang and Huang, 2001; Pnueli, *et al.*, 2002); e.g. it was established that activation of diverse genes took place in response to different stresses in *Arabidobsis* the (Mittler *et al.* 2004). Moreover, literary data prove that between the responses to different stresses synergic or antagonistic relations may exist (Walter, 1989; Sandermann, 2004).

More common are data on the simultaneous effects of drought and high temperature stresses on agricultural crops (Craufurd and Peacock, 1993; Jiang and Huang 2001). It has been established that the combined effects of these two stressors have a far more detrimental effect on plant growth and development than the effect of each individually (Jagtap *et al.*, 1998; Wang and Huang, 2004).

Natural ultraviolet (UV) radiation and soil salinization are the stressors that often cause problems for agricultural crops.

Soil salinization is one of the most acute environmental problems in today's agriculture. According to the Food and Agriculture Organization of the United Nations (FAO), more than 20% of the world's sown area and half of its irrigated land is salinized due to secondary salinization and alkalination and became unsuitable for agriculture. Over the next 25 years, 30%

of irrigated land is expected to be salinized, and by 2050 this number will increase to 50% (Gogue, 2014). Georgia is no exception in terms of soil salinization - the total area of saline soils is 1.6% (Urushadze and Blum, 2014).

One of the approaches to solve this problem is identification of existing salt resistant species, or breeding of new, resistant forms.

Investigation of the effect of UV irradiation on plants is very popular as well. B and C sections of UV radiation are known to affect negatively plant growth, photosynthesis, and other vital processes, due to the production of active forms of oxygen. It is well known that these forms of oxygen damage cell macromolecules and can even cause its death (Toncheva-Panova *et al*., 2010; Zu *et al.*, 2010; Schreiner *et al.*, 2012). However, on the other hand, it is established that low doses of UV-B or UV-C may enhance stress adaptive responses in the plant, due to activation of enzymatic and non-enzymatic defense systems (Lavola *et al*., 2003; Katerova and Todorova, 2011).

To study the effect of UV radiation on plants is interesting from another point of view, as well. In particular, today there is a growing interest in pre-sowing treatment of seeds by physical, environmentally safe methods. Numerous data are available on the positive influence of pre-sowing treatment of seeds with various types of electromagnetic radiation, including UV, on both yield quantity and quality, as well as on plant protection against various diseases (Dubrov, 1963; Ghallab and Omar, 1998; Delibaltova and Ivanova, 2006

Investigation of the effect of high concentrations of salts and pre-sowing treatment with UV on economically important crop - wheat was the aim of the study. It included several aspects:

1. Testing of the salt resistance of experimental wheat species;
2. Investigation of the impact of direct UV irradiation on the growth and development of the studied species;
3. Studying of the effect of seeds pre-sowing irradiation on the salt resistance of plants, developed from these seeds.

According to the purpose of the study, the impact of each stress factor on research objects was studied separately and in combination. The results obtained allow judging how pre-sowing irradiation affects the salt resistance of the studied wheat species.

II. MATERIALS AND METHODS

2.1 Experimental plants

Georgian endemic species of wheat Makha - *Triticum aestivum* subsp. *macha* (Dekapr. & Menabde) McKey and Zanduri - *Triticum timopheevii* subsp. zhukovskyi (Menabde & Ericzjan) L. B. Cai were selected as test objects. According to recent classification they are regarded as sub-species. Experiments were made on a widely spread species of mild wheat - *Tr. aestivum* L. as well. Experimental seeds were kindly provided by the department of plant genetic resources of the Institute of Botany of Ilia State University (Georgia).

Selection of Makha and Zanduri for experiments was stipulated by the data on their salt resistance (Badridze *et al*., 2009). Mild wheat was taken for comparison, as widely spread dominant, as well as relatively salt resistant culture (Wang and Xia, 2018).

Experiments were performed using solutions of sodium chloride (NaCl) and Glauber' salt (Na2SO4). The salts solutions were prepared from chemically pure Chimex LTD reagents. An ultraviolet bactericidal lamp ZW40S 19W-21199 (Biobase biodustry (Shandong) Co. LTD), that emits 253.7 nm waves (corresponding to UV-C section), was used for seed irradiation.

2.2 Description of the experiment

At the first phase of the experiment, observations were made on seeds sown on Petri dishes. Experimental seeds were immersed in a solution of potassium permanganate for several minutes, for disinfection (the part of the seeds which were irradiated before sowing did not need disinfection), then washed under running water and dried on a filter paper.

Part of the tested seeds before sowing, was irradiated with UV bulb (ZW40S 19W-21199 Biobase biodustry (Shandong) Co. LTD) for 1 hour. A distance of the irradiated material from the bulb - 30 cm. Prepared for sowing seeds was placed on Petri dishes in the amount of 50 pieces per one dish. According to the objectives of the work, 8 experimental variants were provided:

1. **Control, without any impact:** seeds were placed on filter paper soaked in distilled water.

2. **Impact with sodium chloride:** seeds were placed on filter paper soaked in 1.5% solution of sodium chloride.
3. **Impact with Glauber's salt:** seeds were placed on filter paper soaked in 1.5% solution of Glauber's salt.
4. **Joint exposure of seeds with table and Glauber' salts:** seeds were sown on filter paper soaked in a solution of both salt mixtures at a total concentration of 1.5%.
5. **Seed UV treatment:** irradiated seeds were placed on filter paper soaked in distilled water.
6. **Combined exposure to UV irradiation and sodium chloride:** irradiated seeds were sown on filter paper soaked in 1.5% sodium chloride solution
7. **Combined exposure to UV irradiation and Glauber's salt:** irradiated seeds were sown on filter paper soaked in 1.5% solution of Glauber's salt
8. **Joint exposure with irradiation and both salt solutions:** irradiated seeds were sown on filter paper soaked in 1.5% solutions of both salts.

Petri dishes with experimental seeds were placed at room temperature (24^o-26^oC) under natural day/night illumination. The filter paper of the test-variants periodically was wetted with distilled water to keep the paper from drying out and to maintain the concentration of active salts at the same level. The germination of seeds on Petri dishes and further growth and development of sprouts up to the three-leaf phase was observed. The growth and development of seeds was assessed by Mano's 9-point scale (Badridze *et al*., 2009).

Two weeks after sowing, the germination percentage of seeds (germinated seeds amount/number of total seeds× 100%) was calculated (Fig. 1).

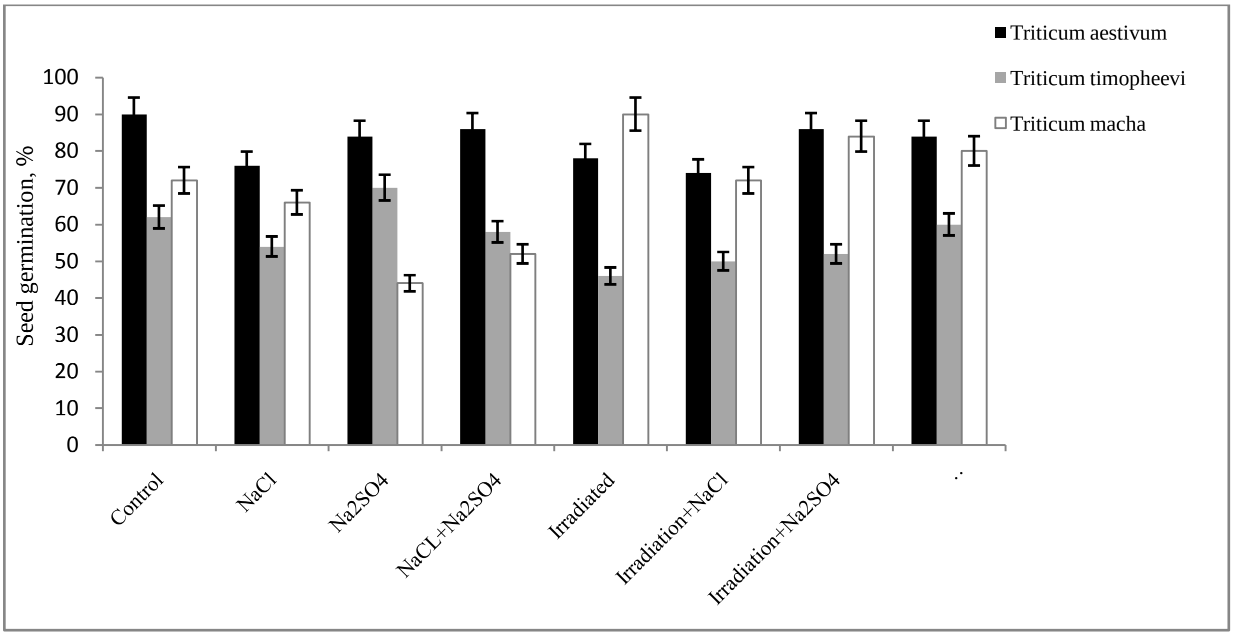

FIGURE 1: Germination percent of soft wheat (*Tr. aestivum*), Zanduri (*Tr. timopheevi*) and Makha (*Tr. macha*) seeds under the influence of sodium chloride (NaCl), Glauber's salt (Na2SO4) and UV-C irradiation

At the second step of experiments the three-leaf phase sprouts were transferred in soil, in plastic containers. Experimental plants were watered with distillate as needed. At this stage of the experiment observation of plants growth and development was continued. The height of plants was measured periodically. The data taken in stem elongation phase, in the 5th-6th weeks of development were used for calculations. The experiment was stopped in stem elongation phase when plants had at least 5 leaves.

2.3 Statistical processing of data

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.

III. RESULTS AND DISCUSSION

3.1 Percent of germination

Seed germination is one of the major and important stages in the life cycle of a plant, on which the crop is highly dependent. It has been established that soil salinization has negative impact on seed germination of many plants, including crops. Moreover, this effect is multilateral. In particular, seed swelling is inhibited, due to the low osmotic potential of the environment; the activity of many enzymes is changed, as well as hormonal balance, nutrient supply is inhibited, etc. (Parihar *et al*., 2015).

The stressful effect of salinization is mainly attributed to sodium ions. A number of papers have shown that the coexistence of anions may increase the toxicity of Na^+. Moreover, in many species sulfate salinity has more toxic effect than chloride (Renault *et al*., 2001; Martin *et al.*, 2015). However, according to some authors, chloride salinity in some cases is more toxic than sulfate (Munns *et al*., 2002).

Two weeks after sowing, the number of germinated seeds which were likely to continue development was evident. That is why the percentage of seed germination was calculated at this stage of development (Fig. 1).

From the obtained results it is clear that the exposure to sodium chloride reduced germination rate of Zanduri seeds (by 8%, $p < 0.05$) compared to the control; while Glauber's salt, in contrary had a stimulating effect - the index increased by 8% ($p < 0.05$).

The degree of Makha seeds germination under the influence of salts, especially Glauber's salt, reduced, compared to the control (by 6% in the case of sodium chloride application, by 28% in the Glauber salt variant, $p<0.05$) (Fig. 1).

The percentage of seed germination under the influence of salts solutions was reduced in soft wheat as well. However, in contrast to Makha, this species was found to be more sensitive to sodium chloride (the emergence rate decreased by 14% when exposed to sodium chloride and by 6% when exposed to Glauber's salt, $p<0.05$) (Fig. 1).

Seed germination percentage decreased in the variant of combined application of both salts (compared to the control). The combined action of both salts on Zanduri seeds partially preserved the effect of sodium chloride, i.e. the studied index was slightly lower than the control (by 4%, $p < 0.05$) (Fig. 1).

In the case of Makha, the result was averaged between the sodium chloride and Glauber' salt data; while in soft wheat it was closer to the result obtained in the variant with Glauber's salt (Fig. 1).

Thus, at early stages of germination and growth, chloride salinity had more negative effect on both Zanduri and soft wheat than sulfate one; In Makha, the result was opposite. In addition, an antagonistic relationship between the anions of the used salts was found, which was evident in the variant of exposure to both salts. In the case of Macha, this should be due to the attenuation of the inhibitory effect of sulphate anions in the mixed solution of both salts, compared to the Glauber salt variant; In the case of soft wheat, sulfate anions seemed to "remove" the negative effect of chloride ions and the result was similar to the data obtained with sulfate anion (Fig. 1,2).

Seed irradiation means that the dormant seed is supplied with excess energy which is stressful for it. Free radicals (active forms of oxygen) generated in seeds during irradiation, as well as change in the conductivity and electrical potential of the seed and cell membrane are likely to be the starting point for the activation of various metabolic systems, including the antioxidant one. This, in turn, should be reflected on plant further development and formation of adaptation mechanisms to unfavorable environmental conditions (Dubrov, 1963).

From the obtained results, it is clear that the used dose of UV radiation had a depressing effect on the germination of Zanduri seeds and reduced it by 16% ($p<0.05$) compared to the control variant. The inhibitory effect of irradiation on seed germination was maintained in the salt exposure variants, albeit to a lesser degree: the germination of Zanduri irradiated seeds under the influence of sodium chloride reduced by 12% ($p < 0.05$), compared to the control; in the case of Glauber's salt - by 10%. In the case of combined action of both salts the inhibitory effect of irradiation was eliminated by the stimulating effect of sulfate-anion on the seed germination ability and the result was closer to control (Fig. 1).

Irradiation had a positive effect on Makha seeds germination ability and increased it by 18%, compared to the control variant ($p < 0.05$) (Fig. 1). Seeds UV irradiation seemed to eliminate the negative effect of salts in Makha: the percentage of seed germination in "irradiation+salt" variants was either equal to the control (when exposed to sodium chloride) or exceeded it (when exposed to Glauber' salt). The stimulating effect of irradiation was maintained in both salts exposure variant as well (Fig. 1).

As for the mild wheat, presowing UV irradiation diminished its seeds germination by 12% ($p<0.05$). The inhibitory effect of irradiation did not increase in "irradiation+salt" variants and results were similar to those of salts impact variants ($p<0.05$).

Different abiotic stresses (drought, radiation, salinization, high or low temperatures, heavy metals, etc.) usually affect the plant in combination. The plant has several molecular mechanisms to cope with stress. These are general or specific mechanisms of resistance that are complex and depend on the strength and duration of stress; as well as the tissue or organ on which it acts (Vahdati and Leslie, 2013).

In the conducted experiments, tested species of wheat were exposed to two stressors - high concentrations of salts and UV radiation. Thus, the impact of stressors would lead to the "switch-on" of appropriate protection mechanisms in the plant, which may be discussed by the comparison of the results of experimental and control variants.

Obtained results demonstrate that the applied dose of UV radiation was a stronger stress for the experimental species than the high concentration of salts (especially this is said for Zanduri). However, irradiation of seeds in experimental species induced various reactions. In Makha it presumably activated the general mechanisms of protection against stress, which were also directed against salinity. This may explain the partial neutralization of the negative effects of salts by irradiation.

Opposite - inhibitory effect of pre-sowing irradiation on seed germination was revealed in soft wheat. Presumably, irradiation and exposure to salts resulted in the activation of the same stress protection mechanism in this species; thus, no amplification of the negative effect occurred in the joint exposure variants of the two stressors (Fig. 1).

Moreover, at the seed germination stage of experimental plants, an antagonistic relationship between the seeds pre-sowing irradiation and exposure to salts was found. This was especially evident in the exposure variants of both salts: the inhibitory impact of radiation on seed germination in both Zanduri and soft wheat was overleped by salts effect (irradiation+salt variants), manifested in a reduction of radiation effect. Presumably, the protective response to salinity stress in the early stages of development was faster than the response to radiation. As this anti-stress reaction should be directed generally against stress, abation of the inhibitory effect of radiation in "radiation + salts" variants was evident.

3.2 Height of seedlings

Growth inhibition is one of the manifestations of the negative effect of salinity stress on plant. This is due to two reasons: a plant has difficulty of absorbing water from the soil due to the low water potential in the latter, on the one hand. Moreover, after salts penetrate into the transpiration current, they cause leaves damaging. It has been established that sodium ions negatively affect the development of leaves more than roots. They accumulate in leaves and cause necrosis of aged leaves, which starts from the tip and edge and spreads throughout the total leaf (Tester and Davenport, 2003; Parihar *et al*., 2015). Thus, the height of the shoot, which is related to the growth and development of the leaves, provides some information about the effect of stressors on the plant.

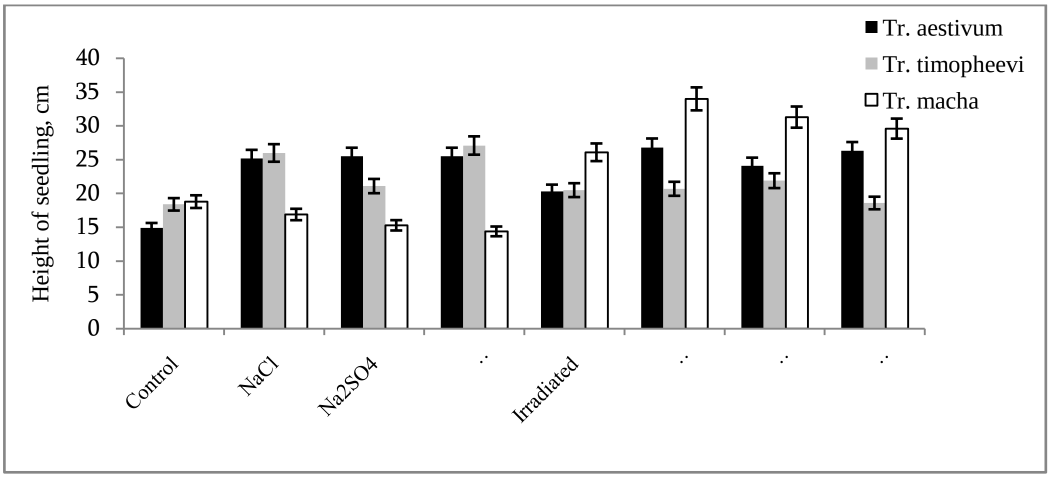

FIGURE 2: Influence of seeds treatment with sodium chloride (NaCl), Glauber's salt (Na2SO4) and UV-C irradiation on the height of seedlings of soft wheat (*Tr. aestivum*), Zanduri (*Tr. timopheevi*) and Makha (*Tr. macha*)

Comparison of heights of 5-6 week experimental seedlings cleared, that the negative effect of exposure to 1.5% sodium chloride solution, which was revealed at the germination stage in Zanduri and soft wheat, no longer was evident at seedling’s growth phase, and even stimulation of growth was mentioned, compared to control ($p<0.05$) (Fig. 2). The stimulating effect was also observed in Glauber’ and both salts-exposure variants (compared to the control, $p <0.05$). The reason for such results probably was the transplantation of seedlings into the soil. Since the experimental plants were transferred into the soil, they were irrigated with tap water. Evidently, the high concentration of salts which affected the plants till the three-leaf phase (before being transferred in the soil) were washed out and diluted into the soil. The plants no longer suffered from the inhibitory impact of high concentrations of salts, and the stress removal stimulated their growth. Such an effect may be the result of the activation of stress protection systems at the germination phase, which was caused by the salt stress and retained even after its removal.

In the case of Makha, the inhibitory effect of salts on the growth of 5-6 weeks old seedlings was well expressed, and completely eliminated in the "irradiation + salt” exposure variants. It may be assumed that UV radiation caused such a strong stimulation of stress protection systems in Makha seeds that this effect prolonged in the plant for a long time after germination and led to the complete cancellation of the inhibitory effects of applied salts (Fig. 2). It should be noted, however, that the combined effect of radiation and salt was stronger than only radiation.

The inhibitory effect of UV irradiation, which was observed in Zanduri during the seed germination phase, was no longer observed in the stem elongation phase and the results were statistically close to the control variant ($p<0.05$). The negative effect of irradiation was also "removed" in combined exposure variants of irradiation and salts (“irradiation+NaCl” and “irradiation+NaCl+Na2SO4” variants. $p<0.05$), and the results were statistically similar to the control variant, or even a slight growth stimulation was mentioned (“radiation+Na2SO4”, $p<0.05$), compared to the control (Fig. 2). Presumably, the used dose of UV radiation was found to be more stressful for Zanduri than the applied concentrations of salts. As it was mentioned, the protective response to salinity stress in early stages of development was faster than to radiation, although it was generally directed against stress. Therefore, in the seed germination stage in “irradiation +salts” exposure variants the inhibitory effect of irradiation abated. Later irradiation in Zanduri also led to the activation of certain defense systems. As UV radiation appeared to be the strongest stress for Zanduri wheat, the activity of radiation defence system surpassed the salt protection system. This may explain the cancellation of the growth-stimulating effect of the salts exposure variants (non-irradiated) in the “radiation+salt” exposure variants (Fig. 2).

IV. CONCLUSIONS

1. Zanduri seeds are equally resistant to both chloride and sulfate salinization. However, in terms of resistance to chloride salinity, it even surpasses soft wheat. Makha seeds are more resistant to chloride salinity than sulfate.
2. Irradiation of Zanduri seeds with UV-C radiation caused inhibition of germination, whereas irradiation with the same spectrum of Makha seeds on the contrary led to significant activation of germination quality. This effect of radiation was maintained during the growth and development stage.
3. The inhibitory effect of sodium chloride on the germination of Makha seed was cancelled by pre-sowing UV treatment; while pre-sowing UV irradiation of seeds with further application of Glauber’s salt, or joint exposure to Glauber's and sodium chlorides even had a stimulating effect on their germination ability, compared to control.
4. In order to increase the seed resistance to chloride and sulphate salinities, we consider it advisable to irradiate Makha seeds before sowing; however, the optimal dose of radiation should be selected.

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