IRSTI 34.49.19 https://doi.org/10.26577/ijbch.2020.v13.i2.12

E.S. Jafarov*, M.Z. Velijanova

Institute of Radiation Problems, NAS of Azerbaijan, Baku, Azerbaijan
*e-mail: elimkhan.jafarov@gmail.com

Study of the adaptive reaction to salt stress of *Cicer arietinum* L., grown from seeds exposed to presowing γ – irradiation

Abstract. It was found that γ-irradiation under certain conditions accelerates the growth and development of plants, improves their productivity and quality characteristics. Based on this, in the presented article, we tried to determine the range of the seed irradiation dose, which helps to reduce the negative effect of salt. For this, a fairly wide range of both the radiation dose and the salt concentration was used. The response of the plant to salt stress was determined on the basis of changes both in a biometric parameters, and in the content of malondialdehyde (MDA), proline, and total protein. According to the results, the main biometric indices increased markedly in chickpea upon pre-sowing irradiation at doses of 1-5 Gy and germination under normal conditions. The maximum increase in individual indices was found at a dose of 5 Gy. In the case of non-irradiated seeds grown under salt conditions, even a low concentration (1 mmol) of salt led to inhibition of the plant development. Seedlings, germinated from seeds irradiated in the dose range from 5 to 100 Gy, were shown to grow normally even at high salt concentrations. At a stimulating dose of 5 Gy, an increase in salt concentration from 1 to 50 mmol led to a gradual increase in MDA levels in the leaves. An increase in salt concentrations in the range of 1-200 mmol also led to a marked increase in the proline content. Under high concentrations (from 10 to 200 mmol) of salt, seed irradiation led to a sharp decrease in the total protein content. Irradiation of seeds at doses of 5- 100 Gy is assumed to reduce the effects of salt stress to some extent. At high salt concentrations, proline plays a significant role in protecting the plant from salt stress.

Key words: *Cicer arietinum* L., presowing γ-irradiation, salt stress, biometric indices, malondialdehyde, proline, total protein.

Introduction

It is generally known that seeds during their germination are quite susceptible to the action of various environmental factors [1-3]. This attribute facilitated the use of various physical or chemical agents prior to sowing, primarily focused on increase in productivity of agricultural plants [4].

At the present time, due to its relative simplicity and cheapness, pre-sowing γ-irradiation of seeds has become the most used seed treatment method. The results of previous studies have shown that pre-sowing γ-irradiation of seeds allows increasing the economic efficiency of crop cultivation, which is reflected in acceleration of plant productivity, reduction of the vegetation period and cost price of production.

For example, in their studies, Singh et al. [5] showed that an irradiation treatment, in general, causes an improvement in plant growth and yield characteristics, such as shoot and root mass, root length and surface area, leaf area and chlorophyll Soil Plant Analysis Development (SPAD) index, tiller number and grain yield. The study concludes that γ-irradiation at a low dose (25 Gy or lower) stimulates, while a high dose (100 Gy and above) inhibits plant growth and development of wheat. The adverse effect at 100 Gy and more are explained by the author by the low efficiency of carbon and nitrogen assimilation and plant assimilation of mineral nutrients, which are a determining factor in plant health.

Jae-Sung Kim et al. investigated the effect of small doses of γ-rays on the germination rate and the germination physiology of the seeds of Welsh onion (*Allicm fistulosum* L.) and spinach (*Spinacia oleracea* L.). The authors showed that the germination rate of the irradiated group was significantly higher than that of control; most notably in the irradiation groups of 1 or 2 Gy prior to sowing of spinach seeds. On the Welsh bulb, the germination rate of the 1 Gy irradiation group increased by 17% as compared to control [6].

Subhan et al. investigated the effect of γ- radiation at the rate of 10, 20 and 30 krads on growth and

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yield of barley. Irradiation had positive effects on grain yield with maximum production at the rate of 10 krads of γ- rays [7].

The influence of γ- radiation in doses of 100, 200, 300 and 400 Gy on the germination and physiological characteristics of wheat seedlings was studied by Borzouei et al. [8]. The authors showed that the mean germination time, the length of the roots and shoots, as well as the dry weight of the seedling decreased with increasing radiation dose. However, the proline content in a dose of 100 Gy increased approximately 2 times in comparison with the control. According to the authors, the positive regulation of some physiological characteristics and the growth of wheat seedlings after treatment with γ- radiation can be used to combat diseases such as drought and salt stress.

Brazilian scientists Ramabulana et al. have found that ionizing radiation causes metabolites, glucomorin and its derivatives in *Moringa oleifera* with various biological activities in plants. In their opinion, these molecules can be considered as components of the inducible defense mechanism of a plant from oxidative stress [9].

Another study examined the effect of low dose γ- radiation on improving the drought resistance of a local Iranian rice variety. The results showed that under stressful conditions, preliminary treatment of seeds with γ-irradiation cause a significant increase in callus growth compared to those of controls [10].

Pre-sowing irradiation of seeds is not only an agricultural method for increasing yield, but it is also used to improve the quality characteristics of agricultural plants [11]. For example, there was an increased sugar content in sugar beet, protein in cereal plants, starch in potato, useful alkaloids in medicinal plants, and vitamins in fruit and vegetable crops [12,13]. There was also an increase in the content of proline and chlorophyll in wheat seedlings grown from γ-irradiated seeds at a dose of 100 Gy [4], an increase in the concentration of soluble sugars, proteins and proline in soybeans, grown from seeds subjected to pre-sowing γ-irradiation at a dose of 20 Gy [14].

Ionizing radiation is suggested to activate intracellular defense systems, thereby leading to stimulation of physiological processes through a complex chain of signaling pathways [15].

There is another opinion, according to which the stimulating effect of γ-irradiation of seeds at small doses is the result of a phytohormonal balance change [16].

It was established that pre-sowing treatment of seeds with γ- irradiation was not inferior in results (sometimes are even superior) to the chemical treatment of seeds [17] and human lymphocytes exposed to low doses of ionizing radiation become insensitive to both high doses of radiation and chemical mutagens that cause double-stranded DNA break [18].

Another interesting fact is that it was found that high concentrations of NaCl cause a decrease in plant growth, the content of photosynthetic pigments, the total content of soluble protein, the content of nucleic acids, and yield characteristics. At the same time, lipid peroxidation and the content of non-enzymatic antioxidants such as anthocyanin, ascorbic acid and α-tocopherol are also increased [19-21]. However, irradiation of seeds with γ- rays mitigates the adverse effect of salt stress compared to non-irradiated seeds [19].

It should be noted that, despite the complete suppression of metabolism, the genetic program of plant development is preserved in the seed [22]. Therefore, when seeds are in the aquatic environment, it contributes to the proceeding of metabolic reactions. However, energy is consumed in the course of these reactions. The energy of ionizing radiation absorbed by seeds was shown to be able to accelerate the transition of the deeply repressed genome of embryonic cells to active state and, thereby, reduce the harmful effects of the stressor [23-25].

The radiation energy in stimulating doses may be sufficient to accelerate the implementation of the genetic program of plant development without changing it, which will result in the reduction of the maturation time, an increase in yield and an improvement in its qualitative characteristics.

Hanafy Ahmed et al. [26] show an increase in the resistance of *Ambrosia maritima* L. to salt stress, the seeds of which were subjected to pre-sowing γ- irradiation. The authors found that irradiation of plant seeds with 40 or 80 Gy increased plant tolerance to salinity comparing to control, concerning plant height, fresh and dry weights, photosynthetic pigments. It was noticed that radiation alleviates the adverse effect of salinity by increasing total sugar and total soluble phenols in shoots of damsissa plants.

Kumar et al. [27] carried out a more detailed study of the effect of pre-sowing γ- radiation of seeds on salt tolerance. The authors tried to establish the role of pre-sowing γ- radiation of seeds at 2.5, 5, 10, 20, 50, and 100 Gy on the growth of pigeon peas, seed yield and seed quality under salt stress at 0, 80 and 100 mM NaCl. Irradiated plants showed better results than non-irradiated plants, even with increasing salinity. Seed yield and protein and iron content were also positively influenced by low-dose γ- radiation under NaCl stress.

Kumar et al. [28] also showed that a low dose of γ-radiation leads to accelerated growth and a number of other physiological signs in non-leguminous and leguminous crops. As a result, plants become more salt tolerant. The relationship between γ- radiation of seeds and the response to salinization stress may be associated with the favorable maintenance of gas exchange characteristics, antioxidant enzyme activity, membrane stability, and proline and glycine betaine contents. According to the authors, one or more of these mechanisms can simultaneously contribute to the salt tolerance of agricultural plants.

In the present work, by studying the defense reactions, namely, we tried to find out the role of pre-sowing γ-irradiation of seeds in the development of chickpea under high salinity conditions. The reaction of the plant was assessed by estimating changes in both biometric indices and in the content of MDA, proline and total protein.

A quite wide range of irradiation doses (from 1 to 200 Gy) and salt concentrations (from 1 to 200 mmol) was used. The effects of irradiation and salt were studied both individually and in combination.

Materials and methods

*Object of the study***.** Chickpea (*Cicer arietinum* L.) is an annual plant, which belongs to *Fabaceae* family. The variety of chickpea Uqu nene was chosen. The choice of peas as an object of research is because the local variety of peas "Uqu nene" has been released in Azerbaijan. The variety is patented by the Ministry of Agriculture of the Republic of Azerbaijan (number and time of patent filing – No. 00278, 02/14/2019), and the patent holder of the variety is the Azerbaijan Scientific Research Institute of Agriculture [29]. A characteristic feature of this variety is that it grows normally in the soil and climatic conditions of the Republic. In addition, peas are one of the main elements of the human diet. The increase in the size of saline lands all over the world (including in Azerbaijan) prompted the study of the influence of salt on the development and quality indicators of this variety.

Equipment – RUXUND installation with a ^{60}Co γ-radiation source, spectrophotometer JENWEY – 6_7 Series (UK), centrifuge HIMAC-CT 15 RE (UK), dielectric separator SDL-1 (Kropotkin Plant MiSSP-SOVPLAST, Inc., Russia), grain moisture meter Fauna-M (LLC firm Lepta, Russia), thermostat (Lasers and Equipment Co., Russia), chamber – phytotron – FED 53 (Binder, Germany) for growing seedlings.

Plant growth conditions. Considering that the development of plants significantly depends on seed moisture, seeds with the moisture content of 16-17% were selected for experiments. Seed samples were separated by an electric separator SDL-1 (Kropotkin Plant MiSSP-SOVPLAST, Inc., Russia) and the moisture content was measured by the dielcometer Fauna-M (LLC firm Lepta, Russia). Using a ^{60}Co irradiation source (Rosenergoatom Concern, Russia), seeds were exposed to pre-sowing γ-irradiation (the irradiation dose in all cases was 0.048 Gy/s). For this, the seeds were placed in paper packets (30 seeds into each packet) with a surface area of 20 cm^2. To study the dose-response relationship, doses of 1, 5, 10, 50, 100 and 200 Gy were applied.

Both irradiated at doses of 1, 5, 10, 50, 100 and 200 Gy, and non-irradiated seeds were grown in the dark (thermostat, 300 K) in a 9 cm-diameter plastic Petri dishes (15 seeds per Petri dish). 4 days later, seedlings, both test and control, were placed in dishes filled with tap water and placed in a growth chamber (temperature, humidity presented below) in conditions of hydroponics. In order to create salt stress, NaCl in concentrations of 1, 5, 10, 50, 100, and 200 mM was added to dishes with water before planting seedlings.

The experiments were carried out in three different variants:

1 – seeds irradiated at different doses were grown in an aqueous medium without salt;

2 – non-irradiated seeds were grown in saline solutions with different concentrations of NaCl;

3 – seeds irradiated at different doses were grown in different concentrations of salts.

In all cases, conditions were created that were similar to the natural ones. While grown in the thermostat, seeds were exposed to 12/12 day-night period (by wrapping the Petri dishes in dark paper). The temperature and humidity in the growth chamber where seedlings were grown during the day was 23 ± 1°C and 55 %, and at night 15 ± 1°C and 70 %, respectively. To provide the necessary lighting conditions, a fluorescent lamp (37.6 W/m^2) was used.

*Estimation of malondialdehyde content***.** The malondialdehyde (MDA) content, as a product of lipid peroxidation, was determined by thiobarbituric acid reaction [30]. After recentrifugation at 12,000 g for 10 min, the absorbance of supernatant was recorded at 532 and 600 nm. The value for non-specific absorption at 600 nm was subtracted. The concentration of MDA was calculated using the formula:

$$C_{MDA} = (D_1 - D_2) \cdot V_2 / \varepsilon \cdot I \cdot V_1,$$

where: D_1 and D_2 – optical densities at 532 and 600 nm, respectively; ε – coefficient of absorbance (155 mM^{-1} cm^{-1}); V_1 – the total and V_2 – the final volume of the ditch in cm^{-3}; I – the length of the ditch in cm.

MDA concentration was estimated in mmol/L per 1 g of dry weight.

Estimation of proline content. The content of free proline in fresh plant material was determined using the method of Bates et al. [31]. The plant material was homogenized in 3% sulfosalicylic acid. The homogenate was filtered and centrifuged for 15 min at 1,000 g. The ninhydrin reagent prepared without heating (1.25 g of ninhydrin, 30 mL of glacial acetic acid, 20 mL of 6 M H_3PO_4 solution) and 2.0 mL of glacial acetic acid were added to the filtrate. The reaction mixture was incubated for 1 h on a water bath at 100^{0}C and then it was quickly cooled to the room temperature. After cooling, 4 mL of toluene was added to each tube, shaken for 30 s and settled. After 15 min, the upper toluene layer, into which all the dye passed, was separated from the aqueous phase. The color intensity was measured using a spectrophotometer JENWEY – 6_7 Series (UK), at a wavelength of 520 nm against toluene.

The proline content was determined from a calibration curve constructed using a set of standard solutions in 3% sulfosalicylic acid. The data obtained were expressed in μmol of proline per 1 g of fresh weight.

Estimation of the total protein content. The method developed by Sedmak and Grossberg [32] was used to determine the protein content in leaf samples. For this purpose, a 0.12% Sedmak solution (0.6 g of Coomassie brilliant blue G-250 + 500 mL of $HClO_4$) and a diluted leaf extract solution (10 mL of leaf extract diluted with 90 μL of dH_2O) were prepared. The Sedmak solution (750 μL), glycerol-water solution (1:1) (750 μL) and homogenization buffer (40 μL) were added to the leaf extract solution (10 μL). The optical density of the resulting mixture was determined at 610 nm, and the protein concentration was established using a calibration curve constructed on the basis of known optical densities. To build the calibration curve, bovine serum albumin (Reanal, Hungary) was used as a standard.

The total protein content was determined by the formula:

$$m = A \cdot E / H,$$

where: A – the protein concentration determined by the calibration curve; E – the dilution coefficient; H – the plant material mass.

Study of biometric parameters and germination. For 4-day-old seedlings the number of seeds germinated at a certain time was determined by conventional method (manual counting). For 2-week-old seedlings some biometric parameters (sprout length, main root length, the number of rootlets, number and size of leaves, number, and average length of internodes) along with MDA level, proline and total protein content in the leaves were estimated.

The experiments on biometric parameters were carried out in three biological replicates. In all cases, the results were almost the same (results of one of them are presented in Table 1 and shown on Figure 1). The experiments were also carried out in three analytical replicates. To evaluate the experimental data, parametric statistical methods were used, and to assess the reliability of the difference between the experimental data and the control, the Student's criterion was used [33]. In this case, the average statistical error of analytical replication was approximately 15 – 20%, and the differences between the experimental data and the control became significant at $| t | > 2$ ($p < 0.05$).

Results and discussion

Biometric indices of chickpea seedlings germinated from seeds subjected to pre-sowing γ – irradiation. It should be noted that the method of pre-sowing irradiation of seeds, leading to radiation-induced stimulation of plant growth and development, was actively developed at the end of the last century [12]. However, researchers continue to obtain guaranteed improvement in product quality. This is attributed to the fact that over the past years the set of regionalized varieties and crops has almost completely changed. A need arises to determine the optimal dose range for each crop and regionalized variety, since the economic effect of pre-sowing irradiation depends on it [34]. Considering this, we compared biometric parameters of the plant, such as the length of the sprout, length of the main root, number of rootlets, number and size of leaves, number, and average length of internodes for assessment of the reaction of chickpea seeds to irradiation in the dose range of 1-200 Gy.

The general view of chickpea seedlings grown from irradiated seeds under normal conditions is shown in Figure 1a and the data determined for its biometric indices is presented in Table 1.

Biometric observations have shown that plants grown from irradiated seeds in the dose range of 5-10 Gy significantly exceeded control in growth intensity. In this case, there was an increase in both length of stems and root. In this dose range, the number and average length of internodes also increased. The maximum increase in individual indices (about 20%, compared to control) was observed at a dose of 10 Gy, while relatively larger doses inhibited plant growth and development.

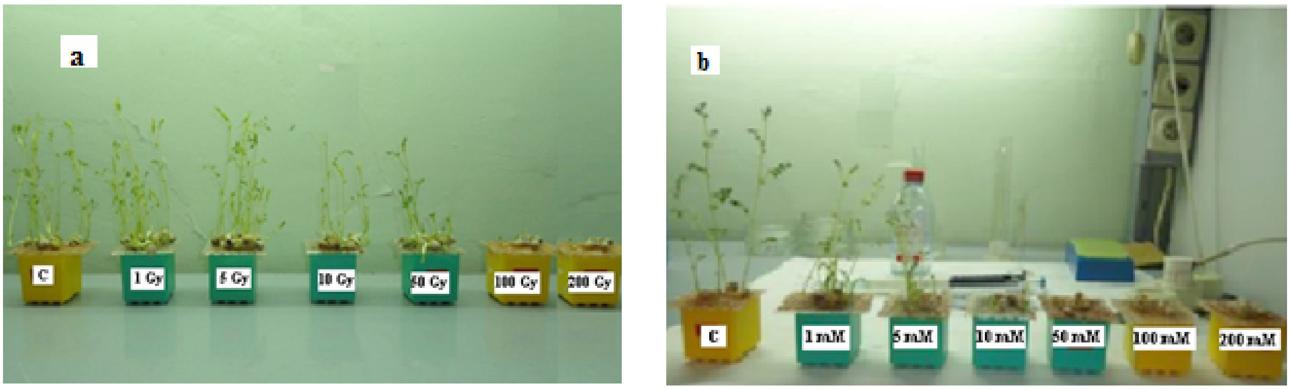

Figure 1 – Visibility of chickpea seedlings growing from:
a – irradiated seeds under normal conditions; b – non-irradiated under salt conditions.

Table 1 – Dependence of the biometric indices of chickpea on the irradiation doses of seeds

Irradiation doses, Gy	Sprout length, см	Number of leaves	Number of internodes	Average length of internodes, cm	Main root length	Number of rootlets	Leaf surface area, cm^2
C	18-19	13	8	2.2 – 2.3	7-8	10	48
1	19-20	14	9	2.2 – 2.3	7-8	12	46
5	20-21	15	9	2.3 – 2.4	9-10	10	50
10	20-21	15	8	2.2 – 2.3	8-9	11	46
50	17-18	10	6	2.1 – 2.2	5-6	10	44
100	13-14	8	6	2.1 – 2.2	3-4	10	42
200	13-14	7	6	2.0 – 2.1	3-4	9	42

Approximately same results were obtained for wheat. It was shown that low doses (25 Gy and lower) of γ- radiation contribute to the development of the plant, which manifests itself in an increase in the basic biometric parameters [5]. Moreover, high doses inhibit the growth and development of wheat [8].

The irradiated seeds of Welsh onion (*Allicm fistulosum* L.) and spinach (Spinacia oleracea L.) at low doses (1-2 Gy) also showed high germination rate compared to control [6]. Kumar et al. [28] also showed that a low dose of γ-radiation leads to increased growth and several other physiological attributes in non-legume and legume crops.

The studied seeds of the chickpea were characterized by high germination (87-92%). Interestingly, the studied range of this parameter was practically independent of the irradiation dose.

The number of rootlets was also practically independent of the irradiation dose.

A different picture was observed for the length of the roots. Irradiation in the dose range of 1 to 10 Gy stimulated root development.

Based on the results of studying the growth and development of the chickpea plant, we can conclude that the stimulating dose for seeds was in the range of 5 – 10 Gy. Wherein, irradiation doses of more than 10 Gy were found to negatively affect the growth and development of this plant.

Biometric indices of chickpea seedlings grown in salt solutions at various concentrations of NaCl. High salinity is known to be one of the main abiotic factors in some places leading to the destruction of the ionic balance of the soil. In this case, plants are exposed to even greater salt concentrations, which inevitably leads to violation of the metabolism of plant cells. In particular, it was found that salt stress leads to the destruction of the biological structure of membranes, chloroplasts, and mitochondria [35]. As a result, their biological functions are disrupted, and the electron transfer rate decreases leading to the decreased productivity of cultivated plants and reduced biodiversity of wild plants [35].

According to the results of our experiments, germination of chickpea seeds strongly depends on salt concentration (Figure 1b). Its significant decrease was noted with increasing NaCl concentrations. For instance, about 90 % germination rate was observed for the control variant, whereas at 1 mmol, 5 mmol, 10 mmol and 50 mmol concentrations of NaCl the approximate germination was, respectively, 80%, 70%, 50%, and 20%. The seeds did not grow at higher concentrations of NaCl, namely 100 and 200 mmol. Biometric indices of chickpea grown at various concentrations of NaCl are presented in Table 2.

According to the results, salt stress has a more dramatic effect on the plant development. Even low salt concentration (1 mmol) leads to the inhibition of the plant development. Further increase in the concentration of salt from 1 mmol to 10 mmol has more inhibitory effects: plant growth practically stopped at 10 mmol concentration.

Table 2 – Dependence of the biometric indices of chickpea on the concentration of NaCl

Concentrations of NaCl, mmol	Sprout length, см	Number of leaves	Number of internodes	Average length of internodes, cm	Main root length	Number of rootlets	Leaf surface area, cm^2
C	18	13	8	2.2 – 2.3	7-8	11	47
1	15	9	6	1.8 – 1.9	7-8	12	26
5	11	5	5	1.3 – 1.4	5-6	11	11
10	5	2	2	0.4 – 0.5	3-4	10	0,7
50	2	-	-	-	2-3	8	-
100	-	-	-	-	1-2	8	-
200	-	-	-	-	-	-	-

At 1-10 mmol concentrations of NaCl, biometric indices, such as the leaf size, the length of the main root, the number and the average length of internodes also decrease in addition to the plant growth. The number of rootlets remains practically unchanged.

It is known that salt stress is one of the main environmental factors that reduce plant productivity. Plants under stress survive due to the functioning of protective mechanisms [36]. Zhani et al. [37], using five Tunisian varieties of chili peppers (*Capsicum frutescens* L.) as an example, showed that increased salt stress for all varieties negatively affects basic biometric parameters, such as length, fresh and dry root mass, amount, and surface area of leaves. The authors note that in response to salt stress in the leaves of the studied plants, proline biosynthesis is activated.

A significant slowdown in growth and development with an increase in the concentration of NaCl was also demonstrated for seedlings of *Jatropha curcas* L. [21].

Salt stress resulted in the decrease of total fresh and dry weight by 41.75% and 53.62%, respectively [38].

NaCl induced significant differences in quantities of proteins and enhanced activities of superoxide dismutase (SOD), catalase (CAT) and peroxidase (POX) [39]. The authors suggest that an increase in antioxidant enzyme activity may be a response to cellular damage caused by NaCl. According to them, an increase in the activity of these enzymes could not stop the harmful effect of NaCl, but it reduced the severity of stress and, thus, allowed *Excoecaria agallocha* to grow in the salty habitat of mangroves.

Change in biometric indices of chickpea seedlings grown from irradiated seeds at various concentrations of NaCl. The effects of salt stress on the development of chickpea grown from seeds subjected to pre-sowing γ-irradiation are presented on Figure 2.

According to the results, when 1 Gy dose pre-irradiated seeds germinated under salinity, the normal development of the seedlings was delayed. This was more pronounced at NaCl concentrations of 50 mmol or more.

It is interesting that when seeds were irradiated in doses of 5-100 Gy, seedlings grew normally even at high concentrations of salt.

These results attract the most attention. Phenomenon called cross-adaptation has been confirmed by some researchers [40-42]. According to this phenomenon, the adaptation of a plant to the effects of any environmental factor increases its tolerance to the effects of another environmental factor. We assume that our results are completely consistent with these conclusions.

Considering that in real conditions, plants are simultaneously exposed to the combined effects of several environmental factors, and these actions can be antagonistic, additive, or synergistic, our results on the combined effects of irradiation and salt stress on plants are explainable from this point of view.

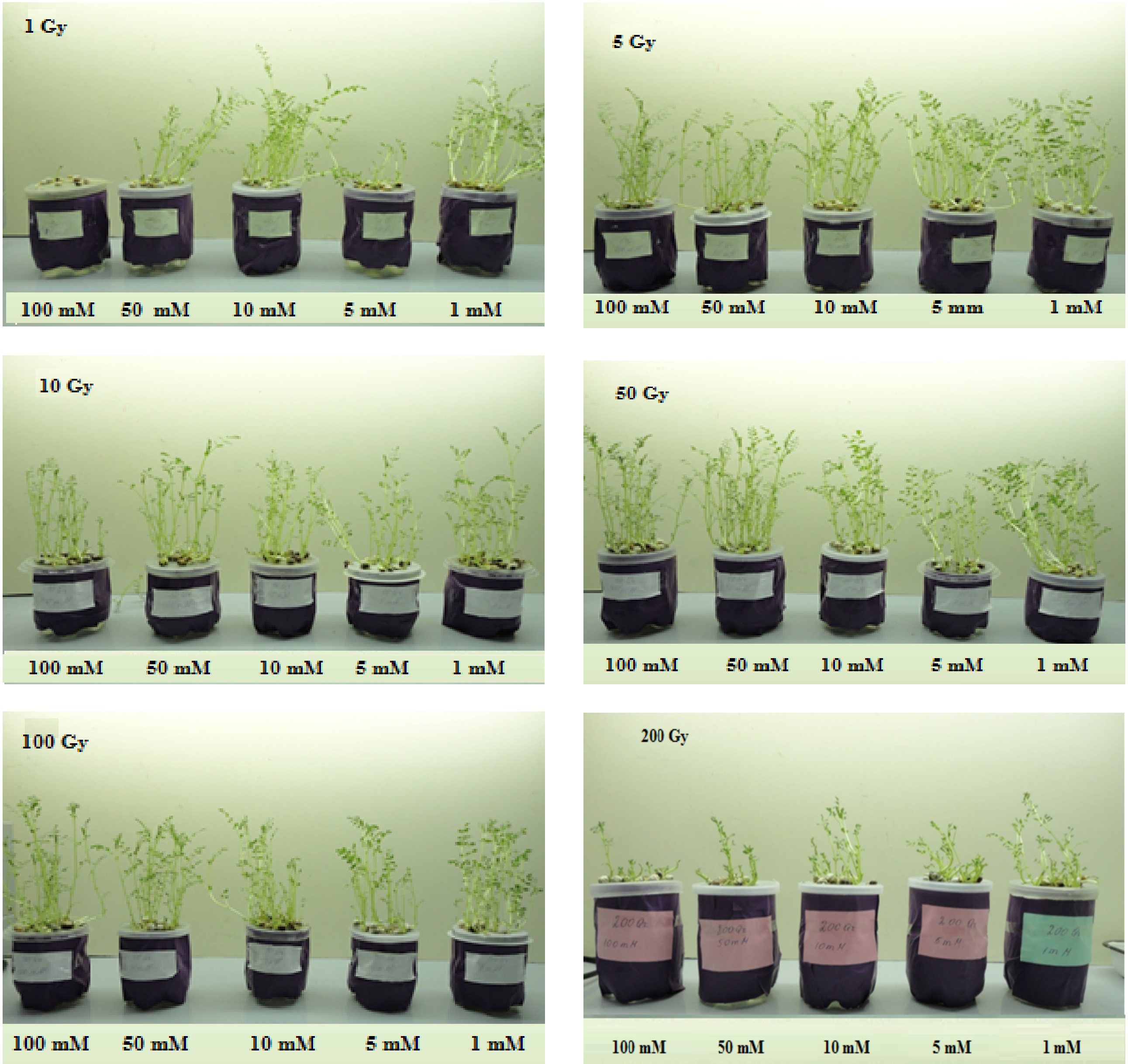

Figure 2 – Biometric indices for chickpea grown from seeds exposed to pre-sowing γ –irradiation under salt stress

Increasing the resistance of plants to salt stress, the seeds of which were subjected to pre-sowing γ-radiation, is shown in many works. For example, in the case of damssisa plants (*Ambrosia maritima* L.) it was shown that irradiation of seeds at doses of 40 (or 80 Gy) significantly increases the resistance of plants to salinization compared to control [26].

It is interesting to note that pre-sowing seed irradiation positively affects the biometric parameters in drought conditions [43]. This suggests the identity of the role of pre-sowing irradiation of seeds under stressful conditions of various kinds.

Kumar et al. [27] showed that irradiation of pea seeds at relatively low doses leads to an increase in the salt tolerance of the plant. At the same time, irradiated plants show better results than non-irradiated ones, even with an increase in salinity.

Wang et al. [44] showed that γ-irradiation with a dose of 50 Gy has a beneficial effect on seedlings of highland barley under stress conditions with lead/ cadmium. Moreover, seedlings irradiated at a dose of 50 Gy have a lower content of hydrogen peroxide and MDA under stress compared with seedlings without prior irradiation. Moreover, proline levels in γ-irradiated seedlings at 50 Gy were significantly higher than in non-irradiated seedlings under stress conditions with lead/ cadmium. The authors suggest that γ- radiation, to some extent, reduces the toxic effects of heavy metals on crops.

From the large amount of data available on transcript-profiling studies in plants subjected to drought and salt it is becoming apparent that plants perceive and respond to these stresses by quickly altering gene expression in parallel with physiological and biochemical alterations [35]

Brazilian scientists Ramabulana et al. have found that ionizing radiation causes production of glucomoringin and its derivatives in *Moringa oleifera*. These molecules can in turn be regarded as components of the inducible defense mechanism of the plant against the effects of oxidative stress [9].

Effect of salt stress on the content of MDA in chickpea grown from the seeds exposed to pre-sowing γ-irradiation. The mechanisms of the effects of irradiation on biological objects are known to be associated either with chemical transformations in the cells (indirect effect) [45], or with direct effects on DNA (target theory) [46]. The mechanism of indirect action is based on the interaction of ionizing radiation with water molecules and the reactions caused by various ions and free radicals, which are formed as a result of such effects. Mechanisms of direct exposure are associated with the direct effect of ionizing radiation on DNA and RNA, which play the role of a target in the cell [46]. It should be noted that the interaction of free radicals with lipids of the cell membranes takes place. As a result of this interaction, the oxidation of lipids by the radical chain mechanism occurs [47]. The oxidation and damage of membranes were shown to cause the formation of several final products including MDA [47,48] and the degree of structural damage was determined by the level of this product.

The results of our studies regarding the effect of salt stress (at various NaCl concentrations) on the dynamics of changes in the MDA content in leaves of chickpea, grown from the seeds exposed to pre-sowing γ – irradiation are presented in Figure 3.

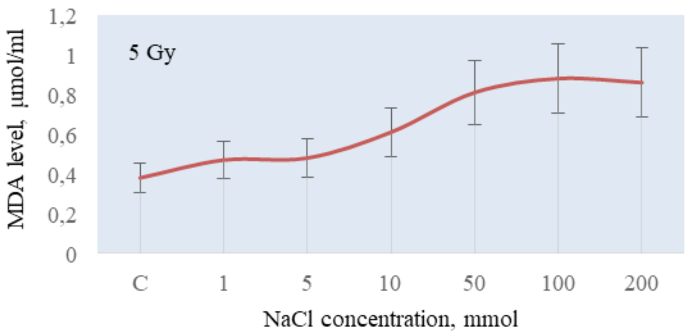

Figure 3 – The dynamics of changes in the MDA content under the combined conditions of radiation and salt stresses.

In this case, the dose of seed irradiation was 5 Gy. This dose was selected for the following reason: firstly, it is a stimulating dose for chickpea growing under normal conditions, and secondly, the biometric indices at irradiation doses of 10, 50, and 100 Gy under all concentrations of salt are almost identical to those obtained at 5 Gy (Figure 2).

According to the results, with increasing salt concentrations from 1 to 50 mmol, the MDA content gradually increased in chickpea seedlings, germinated from the seeds exposed to pre-sowing irradiation at a dose of 5 Gy. However, a further increase in the salt concentration from 50 to 200 mM did not lead to a noticeable increase in the MDA content.

Salt stress can be assumed to manifest itself at 1 mmol concentration of NaCl. A further increase in salt concentration, i.e., an increase in the intensity of salt stress, causes an increase in the structural dismantling of membrane lipids, which is accompanied by a noticeable increase in the content of MDA. The increase in the content of MDA, i.e., structural dismantling of membrane lipids continued till 50 mmol concentration of salt (MDA content in the 50 mmol NaCl solution is approximately 1.5 times higher compared to control). However, at higher concentrations of NaCl (greater than 50 mmol), further oxidation and damage to lipid membranes did not occur.

At salt concentrations of 1 to 10 mM, the MDA content does not increase significantly. A marked increase occurs at concentrations from 10 to 50 mM NaCl.

Similar results were obtained for barley under the action of heavy metals [44], where γ- irradiation with a dose of 50 Gy demonstrated a beneficial effect on seedlings of highland barley under stress conditions with lead/ cadmium. Moreover, seedlings irradiated at a dose of 50 Gy have a lower content of H_2O_2 and MDA under stress compared with seedlings without irradiation.

Our previous research showed that for non-irradiated chickpea grown at 50 mmol NaCl, the MDA content exceeded the control level by about 2.1 times [49].

Based on these data, it can be assumed that irradiation of seeds in stimulating doses to some extent prevents the lipid peroxidation of cell membranes of chickpea, grown at low concentrations of NaCl (1-50 mmol NaCl). Therefore, salt stress does not significantly damage the membranes, and as a result, a large amount of MDA does not form.

However, at higher concentrations of NaCl, the nature of the action of salt stress, i.e., the dynamics of changes in the content of MDA changes. In this case, pre-sowing irradiation of seeds does not allow the further development of the reaction of lipid oxidation, and the content of MDA remains almost constant.

Effect of salt stress on the proline content in leaves of chickpea, grown from the seeds exposed to pre-sowing γ-irradiation. The response of plants to the damaging effects of the stressor is known to lead mainly to increased development of reactive oxygen species, which is usually accompanied by an increase in the activity of antioxidant enzymes. Low molecular weight antioxidants, including proline, have protective effect on the plant [50]. An understanding of the molecular mechanisms of plant responses to various abiotic stresses gives hope that genetically modified crops will cope better with these stresses.

Kadhimi et al. [43] showed that pre-sowing γ-irradiation, having a positive effect on the growth and development of rice under drought conditions, contributes to an increase in proline content. Zhani et al. [37] also demonstrated the activation of proline biosynthesis for *Capsicum frutescens* L. under conditions of salt stress. Borzouei et al. [8] showed that with γ-irradiation the proline content for wheat can increase up to two times.

Concentration-dependent changes in the proline content in freshly picked chickpea leaves are presented in Figure 4.

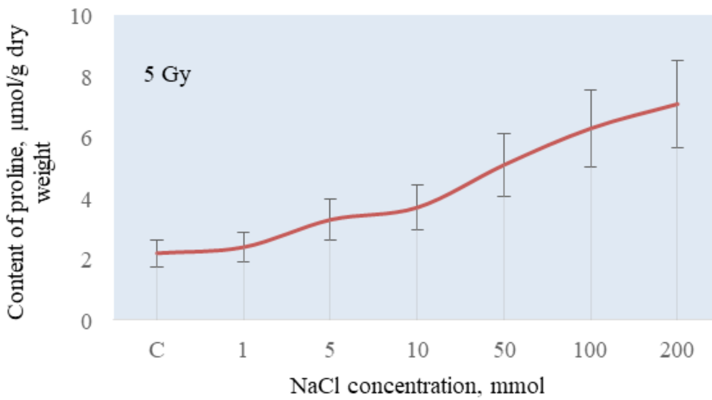

Figure 4 – The dynamics of changes in the proline content under the combined conditions of radiation and salt stresses

According to the results of our studies, there is a concentration-dependent dynamic of changes in the content of proline. In other words, an increase in the salt concentration in the studied range (1-200 mmol) leads to a marked increase in the proline content. However, the levels of these changes at various concentrations are not the same. More precisely, while at low concentrations (1-10 mmol) of salt, the dynamics of changes is more monotonous and the scale of changes is not large, then at high concentrations (50-200 mmol), changes in the proline content are larger.

A significant increase in proline content under stressful conditions (including salt stress) is indicated for many plants. An increased proline content under stress conditions was reported previously by Kishor et al. [51] and Kolupaev et al. [52]. Proline accumulation under abiotic stress, depending on the type and degree of stress amounts to several millimolar concentrations [53]. Proline accumulation is believed to be caused by *de novo* synthesis or a decrease in its degradation, or both [54].

It has been established that for many plants under conditions of salt stress and drought, due to an increase in synthesis and/ or a decrease in degradation, the proline content reaches up to 80% of the amino acid pool (under normal conditions, it is 5%) [55,56].

It should be noted that genes encoding the majority of enzymes associated with proline synthesis and degradation were cloned and partially characterized. However, factors regulating the expression of these enzymes have not been identified [51].

Several attempts have been made to increase the level of proline accumulation in plants by transferring genes that activate its biosynthesis pathways. For example, tolerance to abiotic (in particular, salt) stress, as well as improved growth and development were observed in various transgenic plants, which were characterized by an increased content of proline [51].

A significant increase in proline content under stressful conditions (including salt stress) is indicated for many plants. Proline accumulation is associated with both an increase in synthesis and a decrease in its degradation [37,51]. Similarly, a decrease in the level of accumulated proline in the rehydrated plants is due to both down regulation of proline biosynthetic pathway enzymes and upregulation of proline degrading enzymes [51].

Effect of salt stress on the total protein content in leaves of chickpea, grown from the seeds exposed to pre-sowing γ – irradiation. The main goal of cultivating cereals and leguminous crops is, first of all, to increase the content of proteins, carbohydrates, lipids, vitamins, etc. [57]. Therefore, researches on the effect of various factors on the qualitative and quantitative composition of agricultural plants are still relevant. Ionizing radiation has a special place among these factors. Although the chemical composition of plants, which determines their quality, is formed in the process of evolution, variability is also inherent in it. Under the influence of external factors, both quantitative and qualitative changes in the chemical composition of plants can occur. The creation of new, more tolerant to environmental conditions and more productive plant genotypes is conditioned by this circumstance. Moreover, the ability of plant cells to respond to adverse effects ensures their existence also in severe environmental conditions [58].

There are facts according to which the nature of protein biosynthesis also changes, metabolic processes and physiological functions of the organism as a whole are disrupted [59,60].

In the leaves of seedlings germinated from seeds exposed to pre-sowing irradiation and grown at 1-10 mmol NaCl concentrations, the total protein content was approximately the same as in the control sample (Figure 5). However, under high salt concentrations (10-200 mmol), seed irradiation led to a sharp decrease in the total protein content.

It should be noted that different dependence is characteristic of non-irradiated chickpea seeds grown under salinity. At low NaCl concentrations (up to 1 mmol NaCl), changes in the protein content were not observed. A significant change in the protein content occurred at NaCl concentrations higher than 1mmol. At these concentrations, salt stress was accompanied by a significant decrease in the total protein content [61].

The comparison of the results of our current and previous researches shows that the irradiation of seeds in stimulating doses relative to the total protein content, to some extent expands the field of plant tolerance to salt stress. In other words, irradiation of seeds at a dose of 5 Gy prevents the destruction of proteins in the range of NaCl concentrations from 1 to 10 mmol. Because, under such severe conditions, the total protein content remains almost constant and does not differ from the content of the control sample. It is seen that pre-sowing irradiation of seeds at a dose of 5 Gy, to some extent, facilitates the effect of salt at its low concentrations. Since, in contrast to high concentrations, in this case, the total protein content does not undergo a large-scale decrease.

It should be noted that our results on the total protein content for chickpeas do not differ from the results obtained for pigeon peas. For two genetically diverse varieties of pigeon peas (Pusa-991 and Pusa-992), pre-sowing γ- radiation at low radiation doses (<0.01 kGy) gave a positive effect. In other words, low-dose γ- radiation in this case also had a positive effect on protein content under stress NaCl [27].

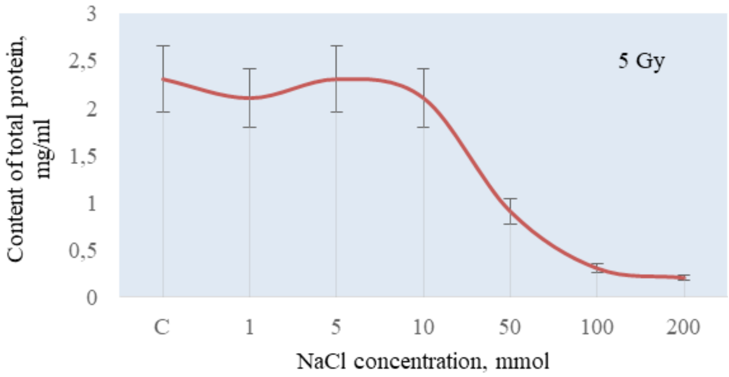

Figure 5 – The dynamics of changes in the total protein content under the combined conditions of radiation and salt stresses.

Conclusion

The results of our research have shown that:

- a significant increase in the main biometric indices is observed in chickpea seeds irradiated at doses of 5-10 Gy and grown in normal conditions. The maximum increase in individual indices (about 20% compared to control) is detected at a dose of 10 Gy. Negatively affecting, irradiation doses higher than 10 Gy, inhibit plant growth and development;

- for non-irradiated chickpea seeds grown under salinity conditions, even a small concentration (1 mmol) of salt leads to an inhibition of the development of this plant (salt stress is manifested at 1 mmol NaCl). A further increase in the concentration from 1 to 10 mmol impedes the development even more, and at concentrations above 10 mmol, plant development practically stops. At salt concentrations in the range of 1-10 mmol, in addition to growth, biometric indices of plants, such as number and size of leaves, length of the main root, number and average length of internodes also decrease;

- seedlings germinated from seeds irradiated in doses of 5-10 Gy grow normally even at high concentrations of NaCl;

- at stimulating doses increase in salt concentration from 1 to 50 mmol leads to a gradual increase in the MDA content in the leaves. However, further increase in salt stress does not lead to a marked increase in the content of the product of membrane lipid peroxidation;

- an increase in the NaCl concentration in the range of 1-200 mmol, at stimulating doses leads to a noticeable increase in the proline content. The scale of the changes at different salt concentrations is not the same. More precisely, while at low salt concentrations (1-10 mmol) the dynamics of changes is more monotonous and the changes are not large, at high concentrations (50-200 mmol), changes in the proline content are more significant, which proves its positive role in protecting plants from salt stress;

- irradiation of seeds at stimulating doses relative to the total protein content, to some extent expands the degree of plant tolerance to salt stress. In other words, when NaCl concentrations range from 1 to 10 mmol, irradiation of seeds at a stimulating dose prevents the destruction of proteins, the total protein content remains almost constant and does not differ from its content in control. However, at high salt concentrations (from 10 to 200 mmol), seed irradiation leads to a sharp decrease in the total protein content.

In general, the results showed that irradiation of seeds in doses from 5 to 10 Gy, to some extent, reducing the effects of salt stress, can partially balance the destructive consequences of excess salt.

We suggest that pre-sowing treatment of seeds with low doses of γ- irradiation (from 5 to 10 Gy) can be used to increase tolerance to salt stress and minimize yield loss caused by excess salt. This perspective method can serve as a useful tool for promotion of agriculture in areas with low salinity.

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