

UDC 582:539.1.047

INVESTIGATION OF THE EFFECT OF PRESOWING GAMMA IRRADIATION OF SEEDS ON THE ANTIOXIDANT ACTIVITY OF ZEA MAYS, GROWING UNDER CONDITIONS OF SALT STRESS

M.Z.Veljanova

(Presented by Academician of ANAS A.A.Garibov)

The paper presents the results on the effect of pre-sowing γ -irradiation of seeds on the antioxidant activity of corn growing under conditions of salt stress. Antioxidant activity was studied based on changes in the activity of antioxidant enzymes superoxide dismutase (SOD), ascorbate peroxidase (APO) and catalase (CAT). It was shown that CAT and APO are highly active in the salt concentration range of (1–10) mM and (5–50) mM, respectively. It is assumed that an increase in the activity of antioxidant enzymes helps to reduce the negative effects of salt at these NaCl concentrations. It was shown that a decrease in the activity of APO and CAT in the range of salt concentration from 50 to 100 mM occurs against the background of an increase in SOD activity. The results obtained allow us to express the opinion that “antioxidant enzymes under conditions of relatively high stress function, to some extent, in an interconnected, coordinated manner”

Keywords: pre-sowing γ -irradiation of seeds, salt stress, corn (*Zea mays* L.), antioxidant activity, superoxide dismutase, ascorbate peroxidase, catalase

Introduction

Given this, the seeds during growth are very susceptible to various external influences, for a long time they are treated with different physical or chemical agents before sowing.

Currently due to its simplicity and low cost, pre-sowing γ -irradiation of seeds has become the most common seed treatment method [1]. The results of a number of studies have shown that pre-sowing γ -irradiation of seeds can increase the economic efficiency of growing crops, which is reflected in accelerating plant growth and development, shortening the growing season, increasing yields and lowering production costs [2].

Today, pre-sowing γ -irradiation of seeds is not only an agricultural method for increasing plant productivity, it has also become a way that improves the qualitative characteristics of agricultural plants. For example, γ -irradiation of seeds contributed to an increase in sugar content in sugar beets, protein in cereal plants, starch in potatoes, useful alkaloids in medicinal plants and vitamins in fruit and vegetable crops [3]. There was also an increase in

the content of proline and chlorophyll in wheat seedlings grown from γ -irradiated seeds [4], an increase in the concentration of soluble sugars, proteins and proline in soybeans grown from γ -irradiated seeds [5].

It is believed that ionizing radiation activates intracellular defense systems, which, in turn, through a complex chain of signaling pathways leads to stimulation of physiological processes [6].

There is another opinion, according to which the stimulating effect of γ -irradiation of seeds in small doses is the result of changes in the phytohormonal balance [7].

The use of low-dose γ -irradiation of seeds to increase the resistance of plants to abiotic stresses has recently attracted great attention of researchers due to the constant environmental degradation. In separate examples, it was shown that γ -irradiation in low doses can improve the resistance of plants to various stress conditions [8, 9].

In the present work, given that salt stress is one of the main factors that reduce the production of agricultural plants, we attempted to

clarify the role of pre-sowing γ - irradiation of seeds in the salt tolerance of *Zea mays*. The reaction of irradiated seeds to salt stress was evaluated by the activity of antioxidant enzymes such as superoxide dismutase, catalase, and ascorbate peroxidase.

Material and methods

The objects of the research was corn (*Zea mays*).

Equipment - source of γ - radiation - Co^{60} , centrifuge - type HIMAC CT 15 RE (United Kingdom), spectrophotometer – type JENWAY – 67 Series (United Kingdom).

Ascorbate peroxidase (APX - EC1.11.1.11) activity was determined by the method of Nakano and Asada [10]. The optical density was recorded in a spectrophotometer at 290 nm against a control without enzyme extract. Enzyme activity (in $\text{mmol} / (\text{g} \cdot \text{min})$) was calculated based on a molar extinction coefficient ($\epsilon = 2.8 \text{ mM}^{-1} \cdot \text{cm}^{-1}$).

For determination of **Catalase (CAT - EC 1.11.1.6) activity** was used the method developed in the work of Kumar and Knowles [11]. Decline of optical density was measured on a spectrophotometer at 240 nm per 1 min. Enzyme activity (in $\text{mmol} / (\text{g} \cdot \text{min})$) was calculated based on a molar extinction coefficient ($\epsilon = 39.4 \text{ mM}^{-1} \cdot \text{cm}^{-1}$).

The activity of **Superoxide dismutase (SOD - (EC 1.15.1.1))** also were determined by the method developed by the Kumar and Knowles [11]. The reaction was started by adding the riboflavin, followed by incubation for 20 min on a white light (4000 lux). Optical density was measured spectrophotometrically at 560 nm. The unit of SOD activity was taken 50% inhibition of formazan formation.

Statistical processing was performed by standard methods of variation statistics. The significance of differences of control and experimental results was assessed by Student's t – criterion [12]. The differences were significant at $|t| > 2$ ($p < 0.05$).

Results and discussion

The results of our previous studies showed

that pre-sowing γ -irradiation of seeds in certain doses could stimulate the growth and development of corn under conditions of salt stress. It was found that irradiation of seeds at a dose of 50 Gy stimulates the development of this plant in relatively low (5 - 50 mM) salt concentrations [13]. The main purpose of the present work was to elucidate the mechanism of the stimulating action. In other words, an attempt is made in this work to clarify the reaction of corn to the negative influence of an adverse condition.

As you know, when deactivating reactive oxygen species (including free radicals) that form under adverse stress conditions and are a real source of danger to cells, the antioxidant system plays a significant role. It has been established that this system consists of high molecular weight antioxidant enzymes and low molecular weight non-enzymatic components [14]. The paper presents the results on the functioning of the antioxidant defense system under conditions of salt stress for corn, the seeds of which were subjected to pre-sowing γ -radiation at a dose of 50 Gy. At the same time, the functioning of the protective system was evaluated on the base of changes in the activities of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APO), which constitute the “fore front” of plant protection from adverse conditions.

Our results on changes in the activity of antioxidant enzymes depending on the concentration of NaCl are presented in the figure (A - SOD, B - APO. C - CAT). The results show that pre-sowing γ -irradiation of seeds at a dose of 50 Gy promotes a change in the activity of antioxidant enzymes at certain salt concentrations. The results on the activity of SOD, which plays the role of dismutation of superoxide anion radicals that are formed under unfavorable conditions for plant development, show that at relatively low concentrations (1 - 10 mM) of NaCl in the case of irradiated and unirradiated seeds, there are no significant differences between the activities of this enzyme. In addition, at high salt concentrations, seed irradiation leads to a significant increase in SOD activity (Fig. A).

Based on these data, it can be assumed that at low salt concentrations there is no great need for the functioning of SOD, however, at high salt concentrations, plants develop due to the active functioning of this enzyme. In all likelihood, this is due to the fact that increased salt stress leads to increased formation of superoxide anion radicals. And the increase in SOD activity ultimately determines, to some extent, the weakening of the negative influence of salt.

The change in the activity of APO and CAT depending on the salt concentration differs from the change in the activity of SOD (Fig. B and Fig. C).

It can be seen from the data presented that a similar form of change dynamics characterizes the CAT and APO activities. In other words, in the case of a salt concentration of 1 mM in both plants growing from irradiated and unirradiated seeds, the activity of CAT and APO is almost the same. In addition, an increase in the salt concentration in the ranges of (1–10) mM and (5–50) mM (for CAT and APO, respectively) leads to a large-scale increase in the activity of these enzymes. Moreover, small changes in their activities accompany a further increase in salt concentration for both enzymes.

It is known that both CAT and APO play the role of catalysts in the decomposition of hydrogen peroxide (H_2O_2) into water and molecular oxygen. Hydrogen peroxide is one of the more active and more “aggressive” (from the point of view of cell damage) forms of oxygen. In addition, it is known that if CAT performs this function at high concentrations of H_2O_2 , then at low concentrations APX goes to the “leading front” [15].

Our results show that at relatively low salt concentrations (1 - 5 mM), the need for CAT is greater than for APO. In all likelihood, it is in this concentration range that more H_2O_2 is formed. Because of this, the activity of CAT increases and at the same time there is no need for APO.

It can be assumed that an increase in the activity of CAT and APO in the salt concentration ranges of (1–10) mM and (5–50) mM, respectively,

helps to weaken the negative effects of salt.

A decrease in the activity of APO and CAT against the background of an increase in the activity of SOD at relatively high (50 - 100 mM) salt concentrations can be considered the result of the interconnected and coordinated functioning of antioxidant enzymes under conditions of severe salt stress.

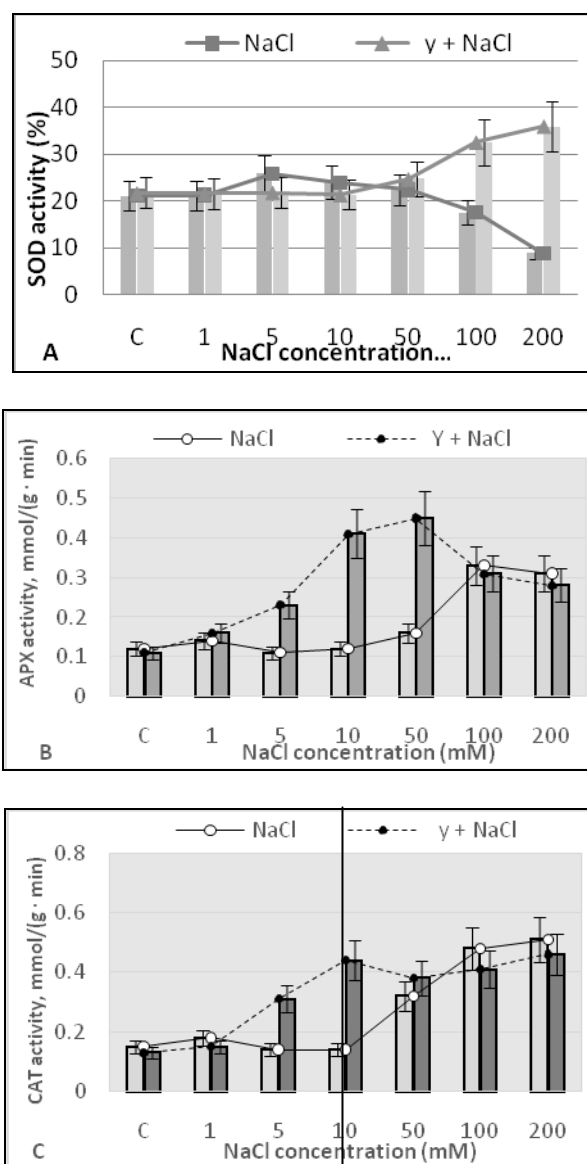


Fig. Changes of antioxidant enzymes activities under salt conditions at different concentrations of NaCl in corn, the seeds of which were subjected to pre-sowing gamma irradiation at a dose of 50 Gy

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Institute of Radiation Problems of ANAS
elimkhan.jafarov@gmail.com

TOXUMLARIN SƏPİNDƏN ƏVVƏL QAMMA ŞÜALARLA İŞLƏNMƏSİNİN DUZ STRESİ ŞƏRAİTİNDƏ YETİŞƏN QARĞIDALI BİTKİSİNİN ANTIOKSIDANT AKTİVLİYİNƏ TƏSİRİNİN TƏDQIQI

M.Z.Vəlicanova

İşdə toxumların səpindən əvvəl γ -şüalarla işlənməsinin duz stressi şəraitində yetişən qarğıdalı bitkisinin antioksidant aktivliyinə təsirinə dair nəticələr öz əksini tapmışdır. Antioksidant aktivlik antioksidant müdafiə sisteminin superoksiddismutaza (SOD), askorbatperoksidaza (APX) və katalaza (CAT) kimi antioksidant fermentlərinin aktivliklərinin dəyişməsi əsasında öyrənilmişdir. Müəyyən edilmişdir ki, 1–10 mM duz konsentrasiyası intervalında CAT, 5–50 mM duz konsentrasiyası intervalında isə APX fermenti yüksək aktivlik nümayiş etdirir. Hesab olunur ki, antioksidant fermentlərin aktivliklərinin artması şüalanmanın həmin intervalda uzun neqativ təsirinin zəifləməsinə səbəb olur. Müəyyən edilmişdir ki, 50–100 mM duz konsentrasiyası intervalında APX və CAT-in aktivliklərinin azalması SOD-un aktivliyinin artması fonunda baş verir. Alınmış nəticələr “nisbətən güclü duz stressi şəraitində antioksidant fermentlər müəyyən mənada uzlaşmış, koordinasiya fəaliyyət göstərir” fikrini söyləməyə əsas verir.

Açar sözlər: toxumların səpindən əvvəl γ -şüalarla işlənməsi, duz stressi, qarğıdalı (*Zea mays* L.), antioksidant aktivlik, superoksiddismutaza, katalaza, askorbatperoksidaza

**ИССЛЕДОВАНИЕ ВЛИЯНИЯ ПРЕПОСЕВНОГО ГАММА ОБЛУЧЕНИЯ СЕМЯН
НА АНТИОКСИДАНТНУЮ АКТИВНОСТЬ *ZEА МАУS*, ПРОИЗРАСТАЮЩЕЙ В УСЛОВИЯХ
СОЛЕВОГО СТРЕССА**

М. З. Велиджанова

В работе представлены результаты влияния предпосевного γ -облучения семян на антиоксидантную активность кукурузы, произрастающей в условиях солевого стресса. Антиоксидантная активность изучена на основе изменения активностей антиоксидантных ферментов супероксиддисмутазы (СОД), аскорбатпероксидазы (АПО) и каталазы (КАТ). Показано, что КАТ и АПО проявляют высокую активность в интервале концентрации соли (1 – 10) мМ (5 – 50) мМ, соответственно. Предположено, что увеличение активностей антиоксидантных ферментов способствует снижению негативного воздействия соли в этих концентрациях NaCl. Показано, что снижение активностей АПО и КАТ в интервале концентрации соли от 50 до 100 мМ происходит на фоне увеличения активности СОД. Полученные результаты позволяют высказать мнение, согласно которому «антиоксидантные ферменты в условиях относительно сильного солевого стресса функционируют, в какой-то мере, взаимосвязанно, координированно».

Ключевые слова: *предпосевное γ -облучение семян, солевой стресс, кукуруза (*Zea mays L.*), антиоксидантная активность, супероксиддисмутаза, аскорбатпероксидаза, каталаза*