



Effect of gamma irradiation and salt stress on germination, callus, protein and proline in rice (*Oryza sativa* L.)

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Abstract

This study was carried out to determine the pretreatment effect of gamma irradiation and salt stress on improvement of germination and physiological factors, protein and proline contents in rice cultivar Taroom Hashemi. Mature and healthy seeds irradiated with 4 doses (0, 100, 200, 300 Gy) and under 3 salinity doses (0, 5, 15 and 25 mmol/L NaCl) were cultured. After one week, radicle length was assayed in control and treatment of MS culture. After 1 month proline and protein contents were measured. The obtained data were analyzed by SPSS using ANOVA and Duncan test. The results showed that the lowest percentage of germination and shoot length was observed in treatments under 300 Gy gamma irradiation and salinity, 15 mmol/L. Also minimum length of radicle was observed in treatments under 300 Gy gamma irradiation in all salinity treatments. Moreover, the lowest percentage of callus regeneration was recorded in the treatments of various doses of gamma radiation in the salt concentration 25 mmol/L. The callus length of 100 Gy seedlings in 5, 15 and 25 mmol/L salinity was highest compared to other group. With increasing irradiation and salt concentration proline content was increased. The protein content on the other hand, decreased with increasing irradiation and salinity concentration. These results show that the up-regulation of some physiological characteristics and seedling growth of rice following gamma radiation treatment may be used to control abiotic stresses such as drought and salt.

Keywords: gamma radiation; rice; salt stress; germination; proline; protein

Introduction

Rice (*Oryza sativa* L.) is a staple food source for more than thirty percent of the world population.

However, its productivity like that of other crops is severely affected by salt stress. Salinity is an important abiotic stress which affects all stages of rice growth and yield. Plant growth and productivity is severely affected by salt stress conditions. Chemical and radiation mediated *in vitro* mutagenesis and selection has been

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successfully used to improve agronomic traits such as salinity and drought tolerance in different crop plants. Gamma radiation can be useful for the alteration of physiological characters (Kiong et al., 2008). The biological effect of gamma-rays is based on the interaction with atoms or molecules in the cell, particularly water, to produce free radicals (Kovács and Keresztes, 2002). These radicals can damage or modify important components of plant cells and have been reported to affect differentially the morphology, anatomy, biochemistry and physiology of plants depending on the radiation dose (Ashraf et al., 2003). The irradiation of seeds with high doses of gamma rays disturbs the synthesis of protein, hormone balance, leaf gas-exchange, water exchange and enzyme activity (Hammed et al., 2008). Therefore, cell and tissue culture selection for salt tolerance calluses and regeneration of tolerant plants is useful to select stress – tolerant clones.

Considering the effects of radiation on plants, the present study was conducted to determine the effects of radiation on rice germination and some key physiological characteristics of rice seedlings.

Materials and Methods

Rice seeds (*Oryza sativa* L.) cv. Tarrom Hashemi (obtained from Mazandaran Agricultural Research Center) was used in this study. Mature and healthy seeds were irradiated with 4 doses of gamma radiation (0, 100, 200 and 300 Gy) and with 4 different levels of salt NaCl (0, 5, 15 and 25 mmol/L). Fully mature seeds of these varieties were surface sterilized in ethanol (70 %) for 1.5 minutes followed by 4 - 5 rinses in an autoclave. Distilled water was used to remove traces of ethanol. Sterilized seeds of control and treatment were then cultured in MS (Murashige and Skoog, 1962) medium, basal salts supplemented with 100 mg L⁻¹ casein hydrolysate, 5% coconut water, 2% gelrite and 0, 1.04, 3.16 and 5.27 NaCl g L⁻¹. The cultures were incubated in darkness at 25± 2 °C. Calluses were developed after one week of inoculation. The percentage of germination was determined after 5 days of culture in a salt selection medium using the following equation:

$$\text{Percentage of Germination} = \frac{\text{number of germinated seeds}}{\text{total number of seeds}} \times 100$$

Seed culture

Pure and healthy seeds were sterilized for 15-20 minutes in the sodium hypochlorite 10% and then for 30 seconds in 2% fungicides Benomyl. The seeds were then put in distilled water for a period of 8 - 10 hours for disinfection. An incubator was used for the germination of the seeds at 20-24 °C for 3 days. Afterwards, the seedlings were transferred to hydroponics medium for 2 weeks. After one month proline (Bates 1973) and protein (Lowry, et al., 1951) contents of rice seedlings were measured.

Estimation of total protein

Proteins were determined by the method of Lowry et al. (1951), using bovine serum albumin as a standard. In the extraction procedures was used the nitrogen content multiplied by the 6.25 factor. Absorbance at 280 nm was also used to monitor protein in the column eluates.

Determination of proline content

The method suggested by Bates et al. (1973) was used to measure the proline content. In brief, 100 mg of frozen plant material was homogenized in 1.5 ml of 3% sulphosalicylic acid and the residue was removed by centrifugation. Two ml glacial acetic acid and 2 ml acid ninhydrin (1.25 g ninhydrin warmed in 30 ml glacial acetic acid and 20 ml 6 M phosphoric acid until dissolved) were added to 100 µl of the extract for 1 h at 100 °C and the reaction was then completed in an ice bath. The reaction mixture was added 1 ml toluene. The mixture was warmed to room temperature and its optical density was measured at 520 nm. The amount of proline was determined from a standard curve in the range of 20 –100 µg.

Statistical analysis

The experimental design was completely randomized blocks. The analysis of variance

(ANOVA) was used to determine the differences in average of all tested parameters between irradiated and non-irradiated plantlets.

Results

Seed germination test after gamma radiation (0, 100, 200 and 300 Gy) revealed that maximum percentage of germination was observed in control plantlets, treatments receiving 100 and 200 Gy doses of gamma and in all salinity treatments. Gamma radiation had no significant effect on final germination percentage (Fig. I). As illustrated in Fig. I, irradiated rice seeds kept their germination capacity compared to the control. Maximum decrease in germination percentage was observed with 300 Gy. Gamma ray imposed a significant impact on the shoot length.

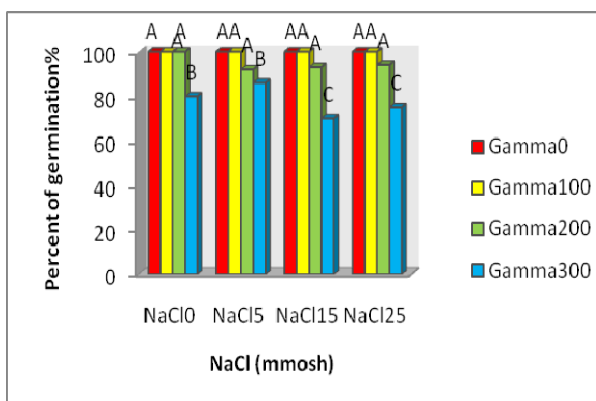


Fig. I. Effects of gamma irradiation and salinity on percent of germination in rice seed. Same letters show that the difference between treatments is not significant at $p < 0.05$.

The highest length of shoot (3.8cm) was observed at control plants (0 Gy and 0 mmosh/l NaCl). Data analysis is expressed between salinity and concentration of gamma radiation in the $p < 0.05$ significant differences exists (Fig. II). By increasing radiation dose to 300 Gy and salinity to 25 mmosh/L, shoot length declined compared to the control (Fig. II). Shoot length decreased in both 300 Gy and 25 mmosh/L of salinity. The maximum decrease in shoot length was observed, when rice genotypes were exposed by gamma ray dose higher than 200 Gy (Fig. II).

Results showed that radiation and salinity have a significant effect on radicle length

of this cultivar of rice (Fig. III). Maximum radicle length was measured in control, while radicle length of rice seedlings in treatments with 100 Gy and 15 and 25 mmosh/l salinity was highest compared to the other group. a minimum length of the radicle was found in 300 Gy and in all salinity treatments. In both treatment salinity and gamma irradiation a significant effect ($p < 0.01$) of on the radicle length was observed. Maximum reduction in radicle length was observed after 300 Gy dose in all plants. With increasing salinity concentration, callus produce percent declined.

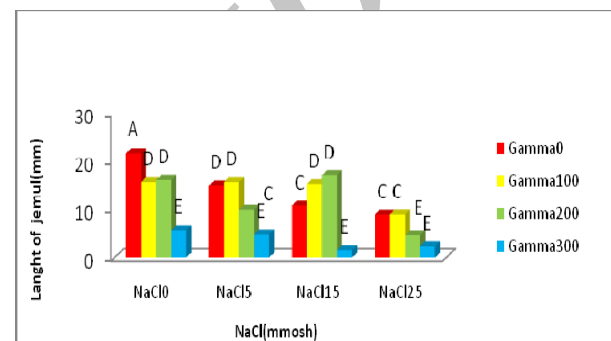


Fig. II. Effect of gamma irradiation and salinity on rice seedlings shoot Lengths (mm). Same letters show that the difference between treatments is not significant at $p < 0.05$.

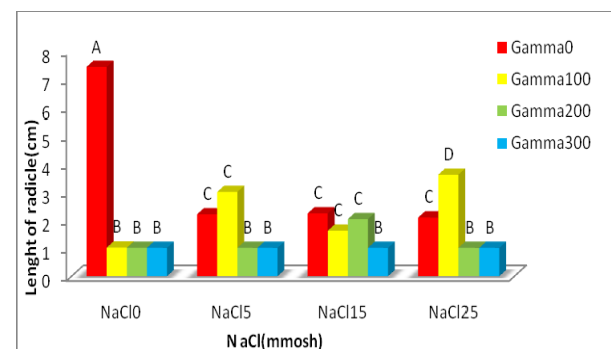


Fig. III. Effect of gamma irradiation and salinity on rice seedling root Lengths (mm). Same letters show that the difference between treatments is not significant at $p < 0.05$.

Biochemical differentiation based on protein and proline content revealed that seedlings irradiated at 100, 200 and 300 Gy. The proline contents were increased after imposing different levels of gamma radiation of seeds as compared with non-irradiated control (Fig. IV). However, with increasing irradiation and salt concentration proline content were increased. The highest proline content observed in 300 Gy and salinity

25 mmsh/lit concentration as compared to control plants (Fig. IV).

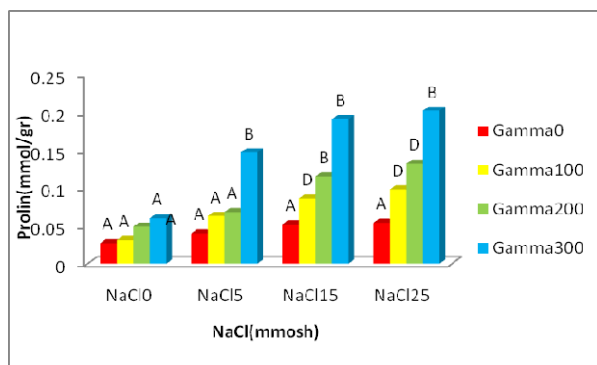


Fig. IV. Effect of gamma irradiation and salinity on proline contents of treated rice seedlings (mg/g FW). Same letters show that the difference between treatments is not significant at $p < 0.05$.

The protein content decreased with increasing irradiation and salinity concentration. Maximum content of protein (mg/g FW) was observed in control plants. Data analysis shown that there is significantly differences between protein content of treated rice seedlings that treated by radiation and salinity (Fig V).

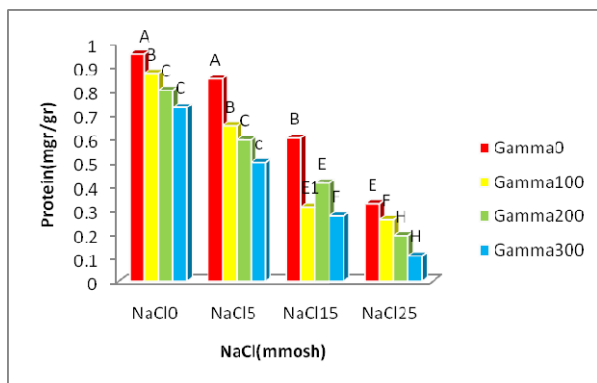


Fig.V Effect of gamma irradiation and salinity on protein content (mg/g FW). Same letters show that the difference between treatments is not significant at $p < 0.05$.

Discussion

Maximum decrease in germination percentage was observed with 300 Gy. These results were in accordance with the germination test done by Melki and Marouani (2009) whereby there was no significant difference in germination and survival percentage of irradiated and non-

irradiated seedlings of hard wheat. The results of Kiong et al., (2008) have shown that survival of plants to maturity depends on the nature and extent of chromosomal damage. Increasing frequency of chromosomal damage with increasing radiation dose may be responsible for less germinability and reduction in plant growth and survival. Changes in the germination percentage were found to attribute to gamma rays treatments. The stimulating causes of gamma ray on germination may be certified to the activation of RNA or protein synthesis, which occurred during the early stage of germination after seeds irradiated (Abdel-Hady et al., 2008). Chaudhuri (2002) reported that in higher radiation dose, germination percentage reduced in addition to root and shoot length, while, in lower dose i.e., 0.1 kGy the germination percentage was not significantly different from control. In another study by Kiong et al., (2008), it was found that radiation increases plant sensitivity to gamma rays and this may be caused by the reduced amount of endogenous growth regulators, especially the cytokines, as a result of breakdown, or lack of synthesis, due to radiation. These results are in agreement with the findings of Chaomei and Yanlin (1993) on wheat (*Triticum aestivum* L.), who noticed that treating seeds with high rates of gamma radiation reduced germination with a corresponding decline in growth of plants. The symptoms frequently observed in the low-or high-dose-irradiated plants are enhancement or inhibition of germination, seedling growth, and other biological responses (Kim et al., 2000; Wi et al., 2007). Although, no certain explanations for the stimulatory effects of low-dose gamma radiation are available until now, in accordance to the results obtained by Wi et al., (2007), there is a hypothesis that the low dose irradiation will induce the growth stimulation by changing the hormonal signaling network in plant cells or by increasing the anti oxidative capacity of the cells to easily overcome daily stress factors such as fluctuations of light intensity and temperature in the growth condition (Wi et al., 2007). In contrast, the high-dose irradiation that caused growth inhibition has been ascribed to the cell cycle arrest at G2/M phase during somatic cell division and/or various damages in the entire

genome (Preussa and Britta, 2003). In a study of the gamma radiation effects on chickpea seeds by Toker et al., (2005) seedlings irradiated at 200 Gy may have some significant increase in their shoot length, but at 400 Gy an obvious depression in shoot length was observed. Melki and Marouani (2009) also reported an improvement of 18 and 32% in root number and root length of hard wheat at the 20 Gy dose, respectively. In the present study, the variability as measured by mean values of the root/shoot lengths decreased with increase in the radiation dose. Chaudhuri (2002) reported that when radiation is sufficient to reduce the rooting percentages, then the root lengths do not exceed a few millimeters in length. Due to metabolic disorders in the seeds after gamma irradiation, the seeds are unable to germinate. Low dose gamma stimulates cell division and high doses inhibiting cell division is due to the creation of free radicals damage DNA system is. Potential loss of water environment on germination salinity increased toxicity is the most important reactions in plant salinity conditions delay the emergence of embryonic tissue and reduce the speed and germination is (Fares 2001). High doses of gamma rays cause DNA damage to callus regeneration genes are (Patade et al., 2008). Some enzymes, including Phosphates acid and protein patterns that are involved in under salinity structure and gene expression changes related to changes in salinity that callus growth is salinity concentrations (Redy, 2009). Effect of gamma radiation on protein can cause DNA damage caused losses see and breaking hydrogen bonds in proteins are strings (Maity, 2008). Reduce the effect of increasing protein concentration due to salinity to prevent bio-synthesis of proteins under salinity stress is performed because a noticeable change in the concentration of total nitrogen in plant leaves can be observed. Gamma radiation was reported to induce oxidative stress with overproduction of reactive oxygen species (ROS) such as superoxide radicals, hydroxyl radicals and hydrogen peroxide, which react rapidly with almost all structural and functional organic molecules, including proteins, lipids and nucleic acids causing disturbance of cellular metabolism (Al-Rumaih and Al-Rumaih, 2008; Ashraf, 2009;

Noreen and Ashraf, 2009). To avoid oxidative damage, plants have evolved various protective mechanisms to counteract the effects of reactive oxygen species in cellular compartments (Kiong et al., 2008). This defense was brought about by alteration in the pattern of gene expression. This led to modulation of certain metabolic and defensive pathways. One of the protective mechanisms in the synthesis of osmolytes which is essential to plant growth was proline synthesis (Esfandiari et al., 2008). The results of this study revealed that increase in proline content was observed in irradiated and salt stressed plants. There was a convincing evidence which showed that the osmolyte synthesis such as proline involved in protective mechanisms were altered with several environmental stresses, including gamma irradiation (Al-Rumaih and Al-Rumaih, 2008). Proline is a compatible osmolyte and it may interact with enzymes to preserve enzyme structure and activities. Indeed, proline has been shown *In vitro* to reduce enzyme denaturations caused due to heat, NaCl stress, gamma stress, etc. (Kavi Kishor et al., 2005; Ashraf and Foolad, 2007). The present increase in proline content was reported to cope with the problem of oxidative reagents (Falahati et al., 2007). In this study the proline contents of gamma irradiated seedlings showed a increase as the gamma doses increased. However, Falahti et al. (2007) contradicted this statement by proposing that the radiation may have promoted the level of antioxidants and consequently there would be no need for extra amount of proline to cope with the same problem of oxidative reagents.

The results of this research showed that different doses of gamma radiation has different effects on biochemical plant characteristics, such as increasing of proline and protein content, stimulation of germination and seedling growth. It is clear that this technique can be used for production of a mutant with ability for environmental stress tolerance.

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