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Full Length Research Paper

Evaluation of drought tolerance of γ-irradiated mutants of *Hordeum vulgare*

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The various physio- and biochemical parameters including photosynthetic rate, stomatal conductance, transpiration rate, relative average growth, chlorophyll content, soluble sugar and proline content were investigated on mutant *Hordeum vulgare* obtained from first generation upon two doses of γ-rays, viz., 300 and 350 Gy, respectively. The level of stress marker "proline" was accumulated higher whereas physiological parameters were decreased at these doses as compared to control ones. Thus, γ-rays irradiations have strong mutagenic impact which led to generation of reactive oxygen species (ROX) on barley and its mutagenecity could be interesting to understand this mechanism of mutation.

Key words: *Hordeum vulgare*, field capacity, γ-rays, mutation, stomatal conductance.

INTRODUCTION

Hordeum vulgare L., (Barley, family Poaceae) is extensively grown in the arid and semiarid regions of the Mediterranean for forage purposes and as a grain crop (Al-Karaki, 2001; Mass, 1984; Francois and Mass, 1999). The barley is the second strategic agricultural crop subsequent of wheat that is utilized for feeding and brewery purpose. It is the main cereal grown in these areas because of its conservative strategy in water use when compared to other species (Acevedo, 1987). However, its productivity is limited by terminal water stress and high temperatures during grain filling. In these conditions, the plant breeders are looking for novel genotypes which have good potential yield, and phenological and physiological characteristics which favor drought tolerance (Blum et al., 1983; Acevedo, 1991; Annicchiarico and Pecetti, 1995; Cantero-MartoAnez et al., 1995).

Plant breeding techniques can be used to introduce favorable traits in new cultivars. However, the action of a single trait may have little effect and several traits may be required to obtain tolerance. Two traits, which have potential role to cope the drought tolerance, are osmotic adjustment capacity (OA) and stomatal leaf conductance. It can be an effective means of obtaining drought tolerance (Ackerson et al., 1980; Morgan, 1984; Blum, 1989). In barley, OA studies have been conducted in controlled conditions. Thus, Blum (1989) found a negative correlation between OA and growth reduction by drought stress in barley plants, whereas Gunasekera et al. (1994) studied the influence of OA on the acclimation of wild barley plants to water-stress conditions. They found that the genotypes that maintained higher photosynthesis at low leaf water potential were better acclimated when exposed to water deficits and underwent greater OA under stress.

The biological effect of gamma-rays is based on the interaction with atoms or molecules in the cell, particularly water, to produce free radicals, which can damage different important compounds of plant cells. However, gamma rays accelerate the softening of fruits, causing the breakdown of middle lamella in cell wall. They also influence the plastid development and their function, such as starch-sugar inter-conversion. Although, gamma radiation is a mutational tool with diverse applications in agriculture, industry and medicine, its potential exploitation in agriculture is limited mainly because of lack of information awareness on optimal dose of irradiation which differs from one crop to another crop and from one application to another application. Radiation mediated morphological, structural and/or

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functional changes in a plant are governed by the intensity and duration of the gamma irradiation. These irradiations influence the grain quality development. Mashev et al. (1995) used high irradiation dose of 5000 to 15000 R to achieve a reduced plant height and an increased in yield, and suggested that even higher irradiation dose could be used to develop yield efficient wheat plant types. Wheat grains from irradiated plants were also rich in proteins and essential amino acids (Mashev et al., 1995). Din et al. (2003a) studied the effect of gamma irradiation on different wheat varieties at seed irradiation dose of 10, 20, 30 and 35 krad. A higher dose of 30 and 35 krad created some abnormalities in plant types for instance, a tiller having two ears attached with each and/ or prevalence of sterile ears etc. Gamma rays with ionization molecules specially the water around of DNA cause to make free radicals then these free radicals attacked to DNA molecule and cause to make differentiation on one alkali but at most of cases it cause to breaking one or two chains of DNA (Hagberg and Persson, 1968; Jyoti et al., 2009). Almost 89% of mutant varieties were developed by using of physical mutagens and about 60% of them were created by applying gamma rays mutants in barley caused to produce high yield, resistant to mildew, strong stem, high protein and skinless seeds (Ananthaswamy et al., 1971). The high yield and dwarf barley mutant such as, Diamant and Golden Promise cultivars had positive effect on beer brewing industry in Europe (Lundqvist and Franckowiak., 1997; Selim and Banna, 2001). The low dosage of gamma rays such as 20 Gy, resulted to increase in plant height in barley and furthermore high dosages such as 40, 80 and 120 Gy decreased plant height (Hagberg and Persson, 1968; Bilge and Ersoy, 1972).

Drought is one of the main environmental factors limiting the plant growth and the productivity of many crops (Araus et al., 2002; Chaves, 2002). However, plants are able to adapt to water deficiency by shortening their growth cycle or have the capacity of avoiding drought stress by augmenting root growth, thus increasing their water uptake (Molnar et al., 2004). Impacts of terminal water stress on cereals have been thoroughly investigated, while studies of early season drought are lacking. An early season drought may affect considerably yields through the limitation of tiller survival rate and number of kernels produced in wheat (Baldy, 1986; Hafid et al., 1998). Selection of tolerant cultivars has been considered as an economic and efficient means to improve drought tolerance (Chloupek and Rod, 1992; Turner, 1997). A better understanding of mechanisms of adaptation to water deficit and maintain of growth, development and productivity during stress periods would help the drought-tolerance breeding (Turner, 1997). Nevertheless, drought tolerance is a complex trait resulting from the contribution of numerous factors. Therefore, in this project, we studied on mutant *H. vulgare* and investigated physio-biochemical parameters under various field capacities.

MATERIALS AND METHODS

Plant material

This study was conducted in Arid and Oasis cropping laboratory at the arid land Institute of Medenine in Tunisia in 2006 to 2007, and focused on barley seeds (cultivar named “Ardahoui”) which obtained upon gamma irradiation at doses 300 and 350 Gray. The mutant seeds of first generations were sowed in pots have sandy soil and investigated the physiological and biochemical parameters after a time intervals. Before sowing, pots were irrigated with tap water to determine their field capacities. The parameters selected in this study were concerned to water stress at different field capacity level.

The relative average growth (RAG)

The relative average growth (RAG) was observed in shoot and root after 60 days of sowing. Plants were harvested before and after water stress. The roots and shoots were chosen to measure RAG. The samples were dried in oven at 70°C for 48 h and RAG was calculated using the formula (Hunt, 1990).

$$RAG=(DW_{T1}-DW_{T2})/DW(T_2-T_1). \quad (1)$$

$$\text{Where } DW=(DW_{T2}-DW_{T1})/\ln(DW_{T2})-\ln(DW_{T1}). \quad (2)$$

DW_{T1}: Dry matter (mg) before water stress, DW_{T2}: Dry matter (mg) under water stress

Substituting formula (2) from (1)

$$RAG=\ln(DW_{T2})-\ln(DW_{T1})/T_2-T_1$$

Measurement of photosynthetic rate, stomatal conductance and evapo-transpiration

The photosynthetic rate, stomatal conductance (gs) and evapo-transpiration (E) were measured using LCi portable photosynthesis system. Measurements were taken between 10 and 11 AM and data were transferred on computer for analysis.

The chlorophyll content

The chlorophyll content was measured using a chlorophyll meter (SPAD meter) and measurements were taken at above mentioned time in a day.

Estimation of proline

The proline was estimated after 60 days of sowing according to the method (Troll and Lindsleg, 1955). The fresh plant material (100 mg each) with 5 ml of 40% methanol was incubated on a water bath at 80°C for 30 min. After cooling of samples, 1 ml of the extract was taken into a fresh tube and added 2 ml of acetic acid, 1 ml of ninhydrin solution (25 mg / ml) and 2 ml from a mixture containing 120 ml of distilled water 300 ml of acetic acid and 80 ml of ortho-phosphoric acid. The sample mixture was incubated on a water bath at 100°C for 30 min. After cooling of the sample mixture, 3 ml of toluene was added and stirred vigorously. The upper phase was taken into a fresh tube and dehydrated by adding a pinch of

anhydrous Na_2SO_4. The absorbance of above sample was taken with spectrophotometer at 525 nm and values were plotted on the standard curve ranging from 0.01 to 0.2 mg/ml and proline was investigated in those mutant plants produced upon gamma irradiation and compared from control ones.

Estimation of soluble sugar

The total soluble sugar was determined using the method Dubois et al. (1956). The 100 mg of fresh plant material was taken into a test tube and added 3 ml of 80% ethanol to the tube and incubated at room temperature for 48 h. Subsequently, the tubes were placed in oven at 80°C to evaporate the alcohol. The 20 ml of distilled water was added in each tube. The 2 ml of sample solution was taken into a fresh tube and added 1 ml of 5% phenol followed by 5 ml of 96 % concentrated sulphuric acid and the tubes were vertexed and left for 20 min at 30°C. The absorbance was taken with spectrophotometer at 640 nm.

RESULTS AND DISCUSSION

Barley (*H. vulgare* L.) is an important cereal crop species, ranking fourth in the world after rice, wheat and maize (Bengtsson, 1992). It is also an established model species for genetic and physiological studies (Koornneef et al., 1997). Gamma rays have strong mutagenic effects and create mutation in plants through generation of reactive oxygen species. Mashev et al. (1995), observed a significant decline in grain yield of wheat at doses above 0.10 kGy, however, lower doses of 0.01 and 0.025 kGy enhanced grain yields. Spielmeyer et al. (2007) had used a high vigour breeding line "Vigour 18" to identify a QTL on chromosome 6A that accounted for up to 8% of the variation for coleoptile length, 14% of seedling leaf width and was associated with increased in plant height. Singh and Datta (2010) provided concrete evidence for usefulness of low dose gamma irradiation at 0.03 to 0.07 kGy in improving plant vigour and grain productivity of wheat. The implementation of gamma ray doses such as 200, 700 and 1200 Gy on barley, the highest doses had negative and hazardous effects on barley morphology and growth as compared to control ones (Sarduie-Nasab et al., 2010). The impact of γ- irradiation on barley was studied for observation of physiological disturbances caused by reactive oxygen species taking different physio-biochemical parameters. The proline content was enhanced as compared to control ones under two mutagenic doses of gamma irradiation. The more proline accumulation was observed in those plants which produced at irradiation of 350 Gy followed by 300 Gy and it was 6 and 3 times higher as compared to control plant. Environmental factors such as water, temperature and nutritional status affect the biochemical responses of plants and resulted in loss of yield and several quality traits. However, plants have genetically controlled mechanisms that allow them to live and grow under various types of stresses (Boyer, 1982). Increasing evidence indicates that stress results in the oxidative deterioration of biological macromolecules, and therefore, at least in part, in the oxidative tissue destruction (Gallego et al., 1996). Stress conditions can be detected with physiological methods which measure anti-oxidative enzymes and membrane destruction (Halliwell, 1982; Nakano and Asada, 1981).

Effect of water stress on assimilation rate

The rate of assimilation was investigated after 15, 30, 45 and 60 days after sowing (DAS) (Figures 1a, b and c) under various field capacities such as (100% fc), (70% fc) and (30% fc) and variation was more after 60 days of sowing in barley plants produced from mutants at 300 and 350 Gy, respectively (Figure 1d). All mutant plants were compared with control ones. At normal conditions, the photosynthetic rate was 7.51 to 8.647 µmol CO_2 m^{-2} s^{-1}, 8.25 to 8.52 µmol CO_2 m^{-2} s^{-1} and 8.261 to 9.278 µmol CO_2 m^{-2} s^{-1}, respectively after 15 days of sowing for the three batches of barley including control and mutant plants at 300 and 350 Gy. The rate of assimilation was declined after 30 days of sowing and it was 7.91 and 7.44 µmol CO_2 m^{-2} s^{-1} in mutant plants produced at 300 and 350 Gy. At irrigation of 70 and 100% fc, the rate of assimilation was slightly varied among mutant plants and control ones. The rate of assimilation was increased initially for 30 days as it was 7.5, 7.7 and 8 µmol CO_2 m^{-2} s^{-1}, in control ones and mutant plants produced at 300 and 350 Gy. After 60 days of sowing, it was decreased more and found 6, 6.72 and 5.6 µmol CO_2 m^{-2} s^{-1} in control ones and mutant plants. The rate of assimilation was immensely influenced at 30% fc in control plants and mutant plants.

Effect of water stress on stomatal conductance (gs)

The stomata control the entry of CO_2 for photosynthesis and release water vapor which resulted from the movement due to the potential difference between the turgor pressure of a cell and guard cells of stomata. Indeed, results from first 15 days of sowing, the stomatal conductance was increased in control ones and mutant plants at 300 and 350 Gy which was 0.068 to 0.091 mol $m^{-2}s^{-1}$, 0.07 to 0.08 mol $m^{-2}s^{-1}$ and 0.077 to 0.088 mol $m^{-2}s^{-1}$ (Figures 2a, b and c). After 60 days of sowing, the stomatal conductance was 0.066, 0.056 and 0.06 mol $m^{-2}s^{-1}$ for control ones and followed mutant plants obtained at 300 and 350 Gy. The data obtained at 70 and100% fc, the stomatal conductance were almost same, whereas variation was more at 30% fc (Figure 2d). At 70% of field capacity, the stomatal conductance increased for first 30 days which was 30.23, 24.69 and 20.54% for control ones, followed mutant plants at 300 and 350 Gy, thereafter it was declined. The stomatal conductance was decreased at 30% fc in control ones and mutant plants at

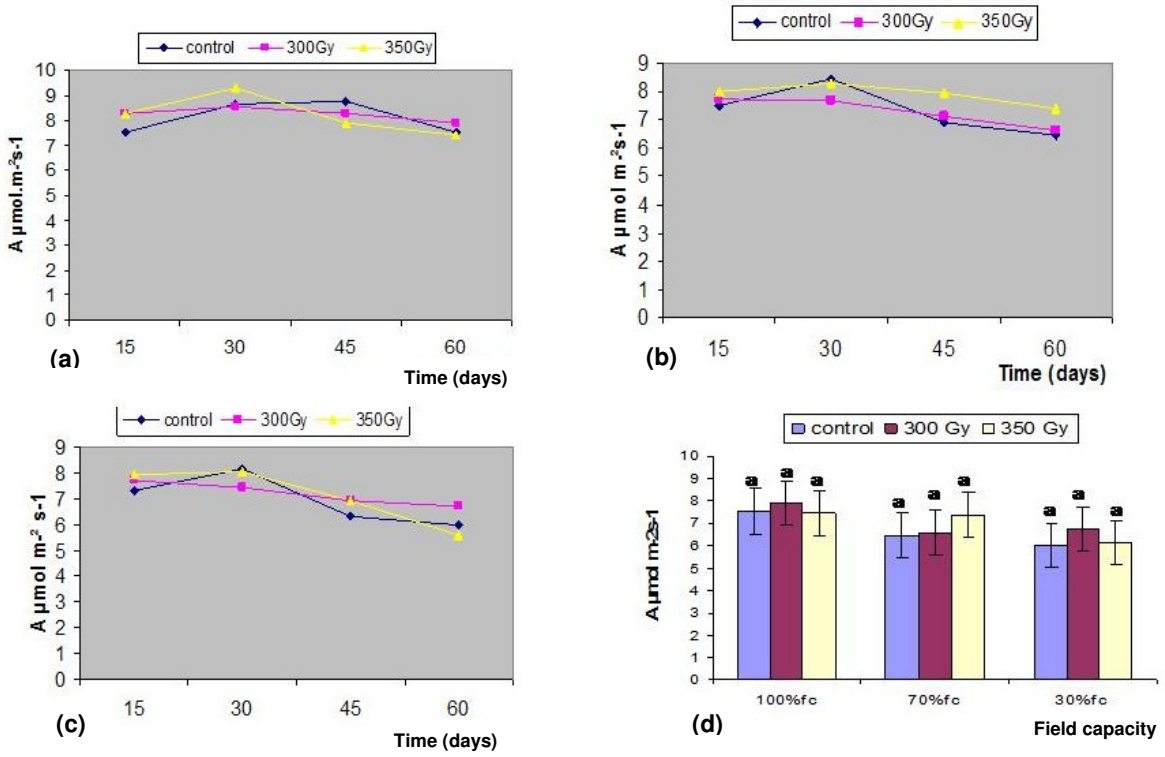

Figure 1. Rate of photosynthesis (control and barley) (a)100% fc, (b) 70%fc, (c) 30% fc, (d) Average at 100, 70 and 30% fc.

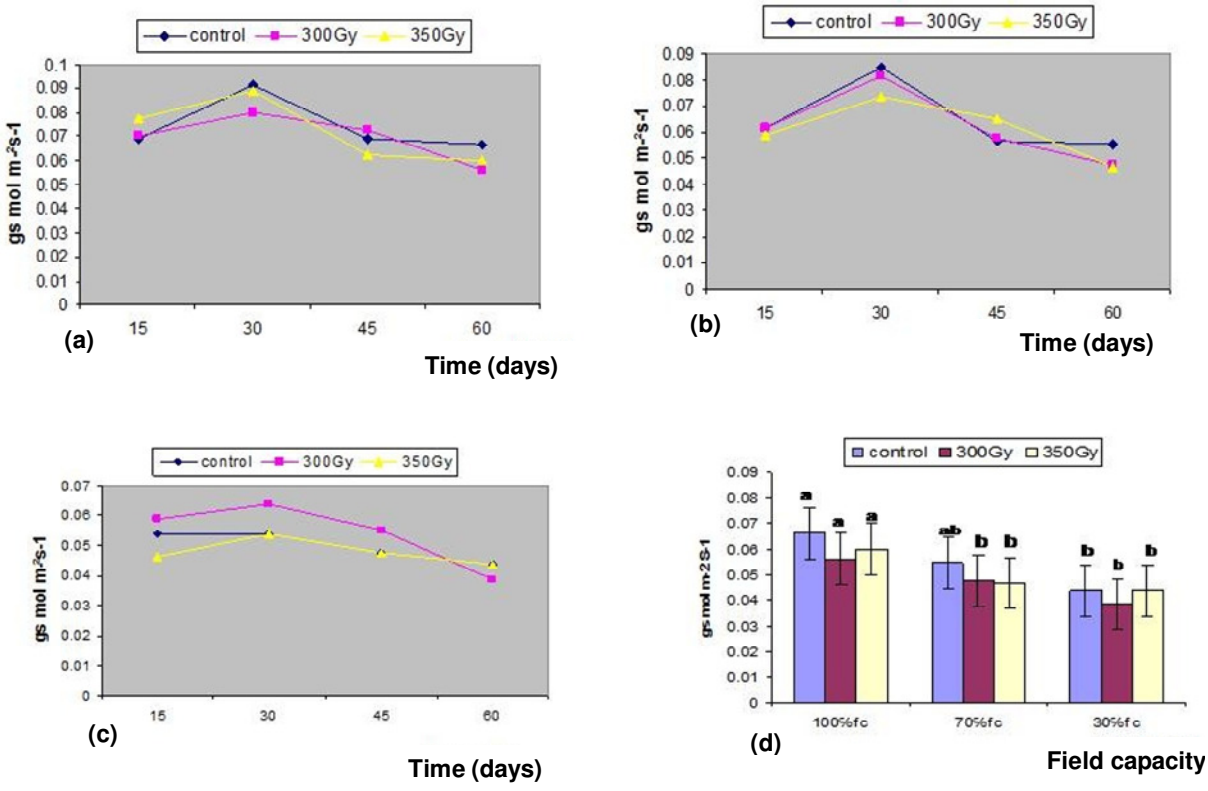

Figure 2. Stomatal conductance (control mutant barley) (a) 100% fc, (b) 70% fc, (c) 30% fc, (d) Average at 100, 70 and 30% fc.

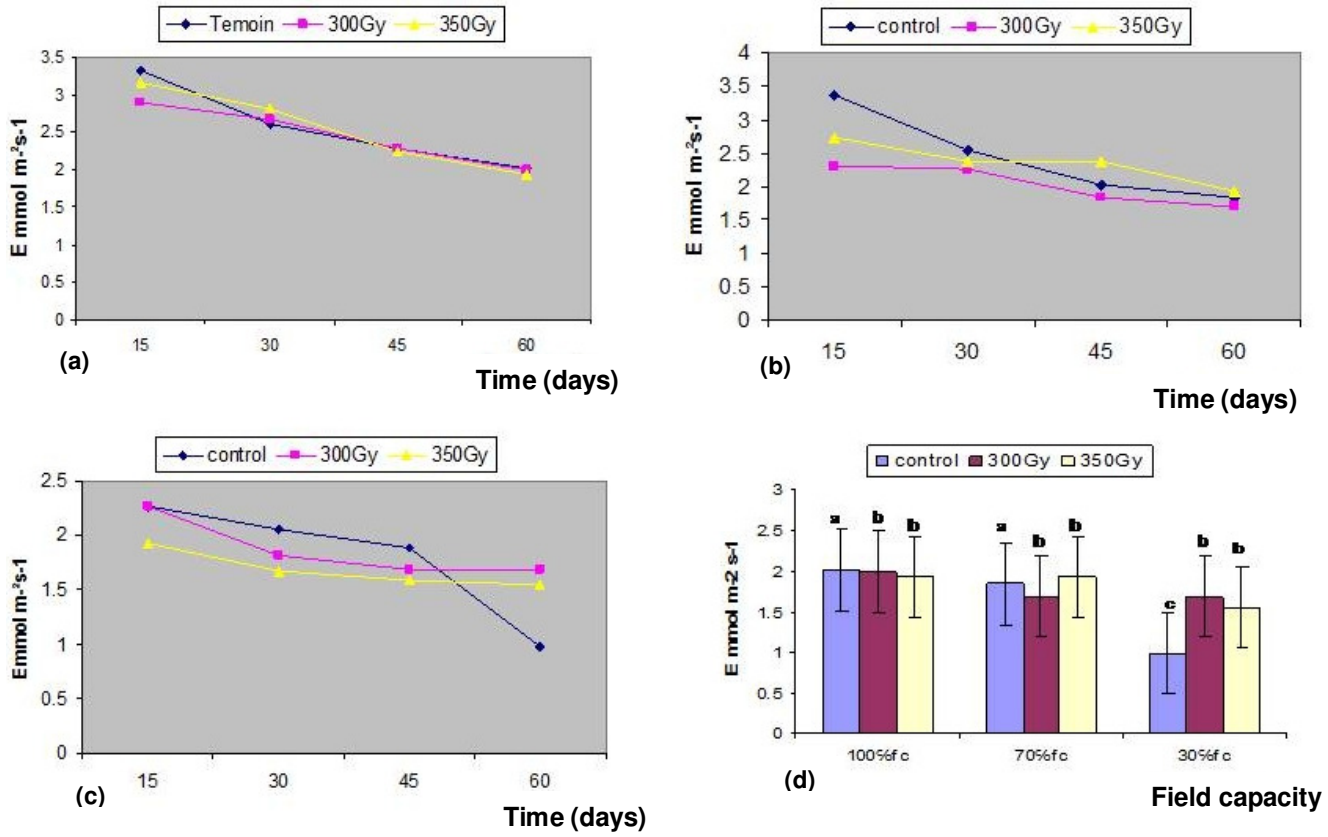

Figure 3. Changes in transpiration rate as a function of time (control mutant barley) (a)100% fc, (b) 70% fc, (c) 30% fc, (d) Average at 100, 70 and 30% fc.

300 and 350 Gy, which were found 0.053, 0.058 and 0.046 mol $m^{-2}s^{-1}$ and 0.043, 0.038 and 0.043 mol mol $m^{-2}s^{-1}$, respectively.

Effect of water stress on transpiration rates (E)

At 100% fc, the transpiration rate was increased as compared to 70 and 30% fc. After 30 days of sowing, the transpiration rate was 3.3, 2.89 and 3.14 mM H_2O m^{-2} s^{-1} for control ones and barley mutants at 300 and 350 Gy (Figures 3a, b and c). After 60 days of sowing, there was significant declined in transpiration rate which was 39.08, 31.28 and 38.65% for control ones and barley mutants at 300 and 350Gy. At 70% fc, the rate of transpiration was decreased from starting of experiment and it was lesser than those irrigated at 100% fc which was 45.23, 27.05 and 29.04% in control ones and barley mutants at 300 and 350 Gy. Likewise, at 30% fc, the transpiration rate is significantly affected by water stress and maximum values were recorded at the beginning such as, 2.26, 2.27 and 1.93 mM H_2O m^{-2} s^{-1}, respectively for control ones and barley mutants at 300 and 350 Gy. After 60 days of sowing, the rate of transpiration was 0.98, 1.685 and 1.55 mM H_2O m^{-2} s^{-1} for control ones and barley mutants at 300 and 350 Gy (Figure 3d).

Chlorophyll content

The chlorophyll content was measured using chlorophyll meter (Minolta SPAD-502) and variation was found more between control ones and mutant plants (Figure 4a, b and c). There was a slight reduction in synthesis at 100% fc as compared to 70% fc which was found 5.76, 12.53 and 12.72% for the control ones and mutants plants, respectively. At 30% fc there was a significant reduction of chlorophyll synthesis which was 13.15, 9.76 and 5.25%, respectively for the control ones and mutant obtained at 300 and 350 Gy (Figure 4d). Likewise, the total chlorophyll content decreased with elevated dose of γ-rays and net reduction in photosynthetic rate was found in *Fagopyrum dibotrys* Hara (Jia and Li, 2008) and this reduction in photosynthetic rate was resulted from both stomatal and non-stomatal limitations.

Proline content

Drought is a serious problem, constraining crop production and quality globally. It is a complex physico-chemical process, in which many biological macromolecules and small molecules are involved, such as nucleic acids (DNA, RNA, micro RNA), proteins,

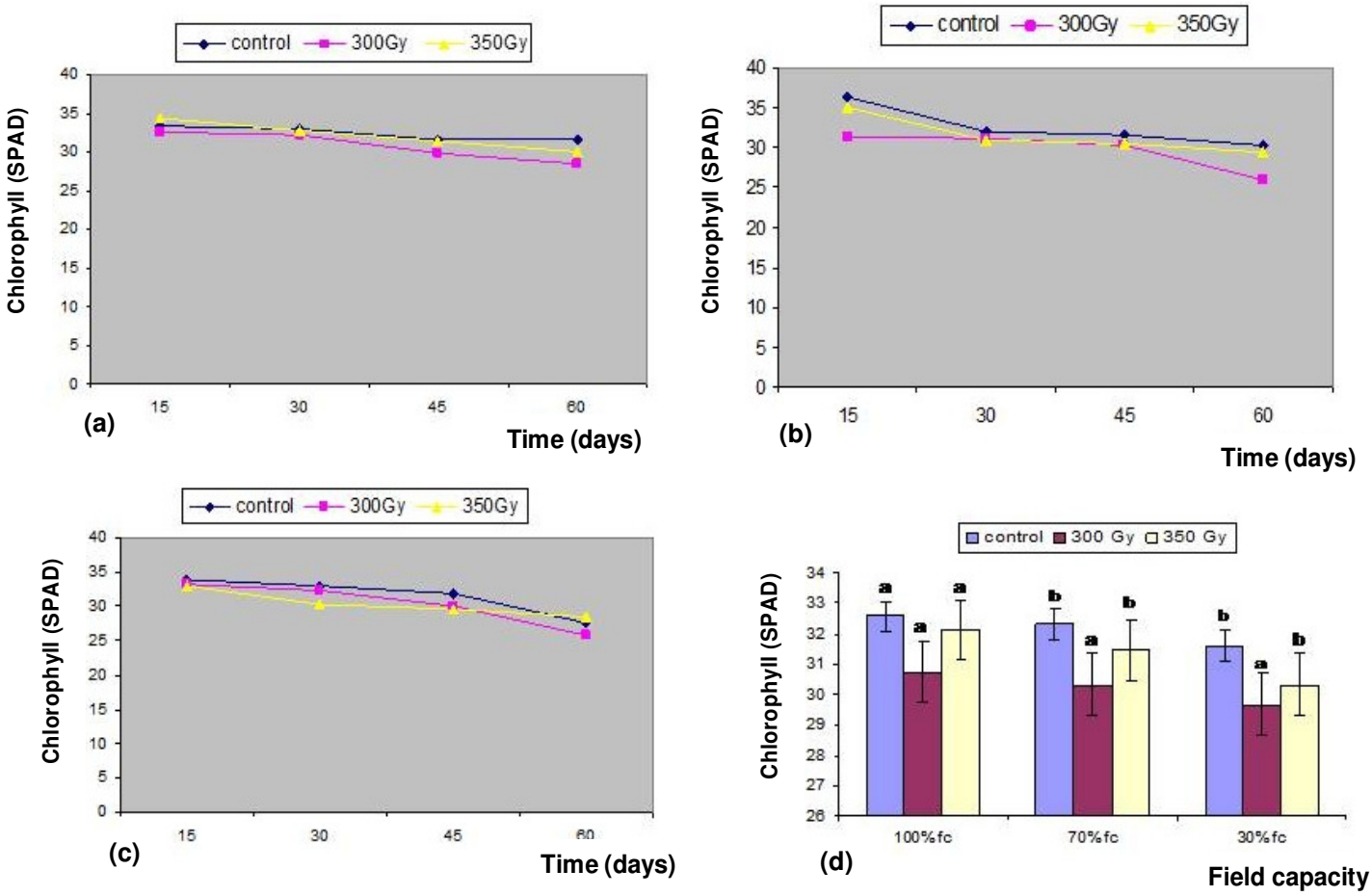

Figure 4. Variation in chlorophyll content with (SPAD) at (a)100% fc, (b) 70% fc, (c) 30% fc, (d) Mean content of chlorophyll barley at 100, 70 and 30% fc.

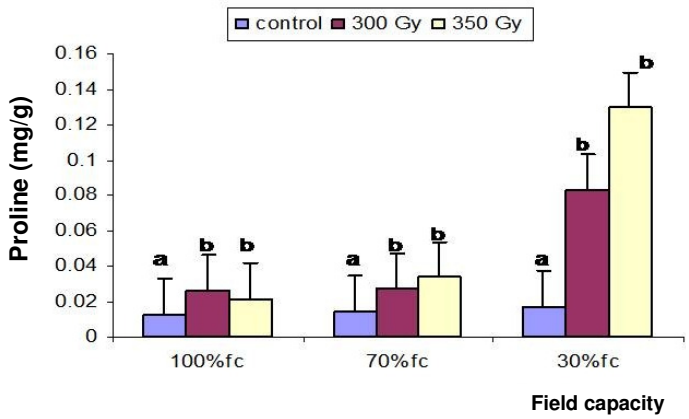

Figure 5. Accumulation of proline content according to the capacity in barley.

carbohydrates, lipids, hormones, mineral elements, ions and free radicals. In addition, drought is also related to other stresses such as salt, cold, high temperature, acid, alkaline, pathological reactions, senescence, growth, development, cell circle, UV-B damage, wounding, embryogenesis, flowering, signal transduction and so on (Erdei et al., 2002; Fricke et al., 2004; Fiehn, 2002; Glombitza et al., 2004; Gao, 2000; Hernandez et al., 2004; Hiral et al., 2004; Harding et al., 2003; Li et al., 2003; Philippe et al., 2005; Munns, 2005). Therefore, drought is concerned with almost all aspects of biology. Much advancement in relation to this hot topic, including molecular mechanism of anti-drought and corresponding biotechnological breeding has taken place (Shou et al., 2004; Liu and Bush, 2006). Proline (content) is closely linked with plant anti-drought, especially under soil water deficits and many reports from crops and other plants have proved its anti-drought property. The proline content was increased with decreasing irrigation regime in those barley plants obtained from γ-irradiation as compared to control ones. After 60 days of sowing, the mutant plants obtained at 300 and 350 Gy, the proline content accumulation was higher as compared to control ones and it was 3 and 6 times higher on mutant plants at 300 and 350 Gy at 70 and 30% fc as compared to control ones (Figure 5). Thus, these doses of γ-irradiation on

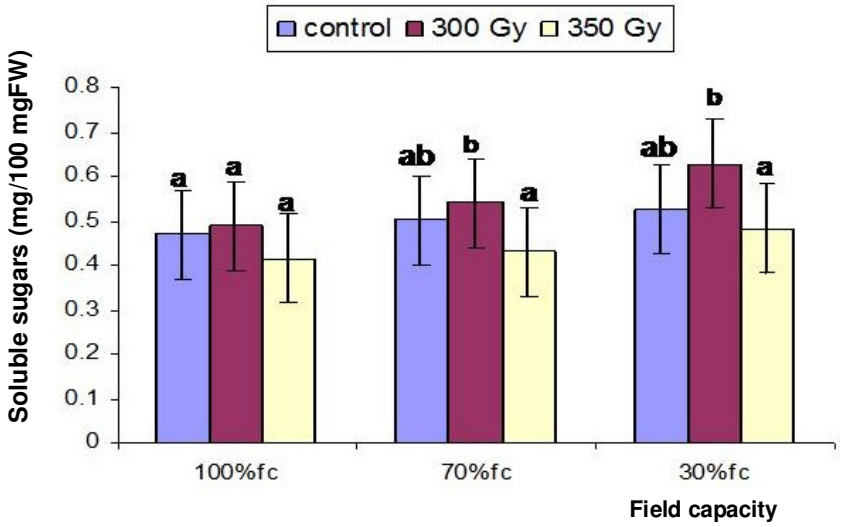

Figure 6. Effect of irradiation and water stress on accumulation of soluble sugars.

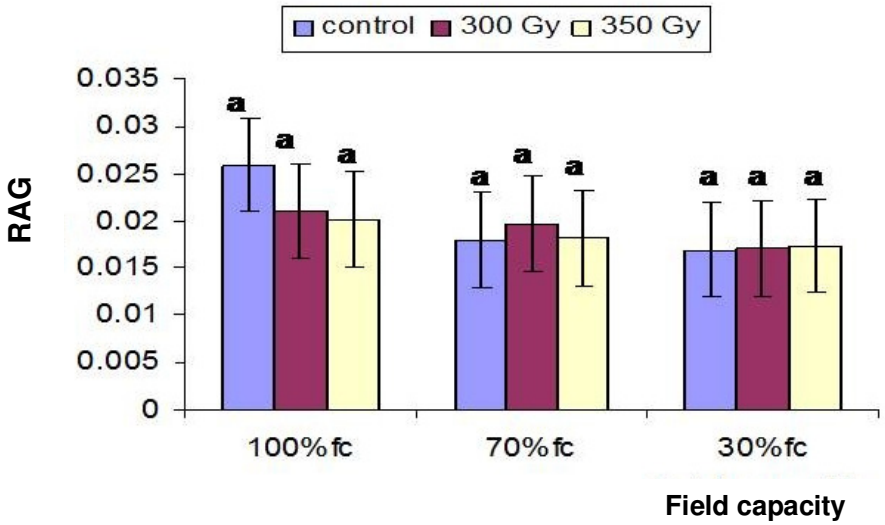

Figure 7. Effect of irradiation on relative average growth of shoot after 60 days of sowing at 100, 70 and 30% fc.

seeds have high damaging impact, which implying by accumulation of stress marker “proline”.

The total soluble sugar content

The plant produced from mutant seeds at 350 Gy, had soluble sugar 0.0145, 0.0162, and 0.0182 mg /g fresh weight at 100% fc, 70 and 30% fc (Figure 6). In contrast, non-irradiated barley had lower level of soluble sugar which was 0.0124, 0.0129 and 0.0144 mg/g fresh weight of plant at 100, 70 and 30% fc, respectively. The barley plant, obtained at 300 Gy irradiation the soluble sugars was 0.0141, 0.0150, and 0.0157 mg/g fresh weight at 100, 70 and 30% fc, respectively.

The average relative growth of shoot and root

The average relative growth (RAG) was investigated under various field capacities on the plant parts of barley. The application of water stresses at 70 and 30% fc caused a reduction in growth activity of the shoots (Figure 7), thereby biosynthetic activity was reduced and was found 30.69, 6.28 and 9.40% at 70% fc with control ones and mutant plants at 300 and 350 Gy. The application

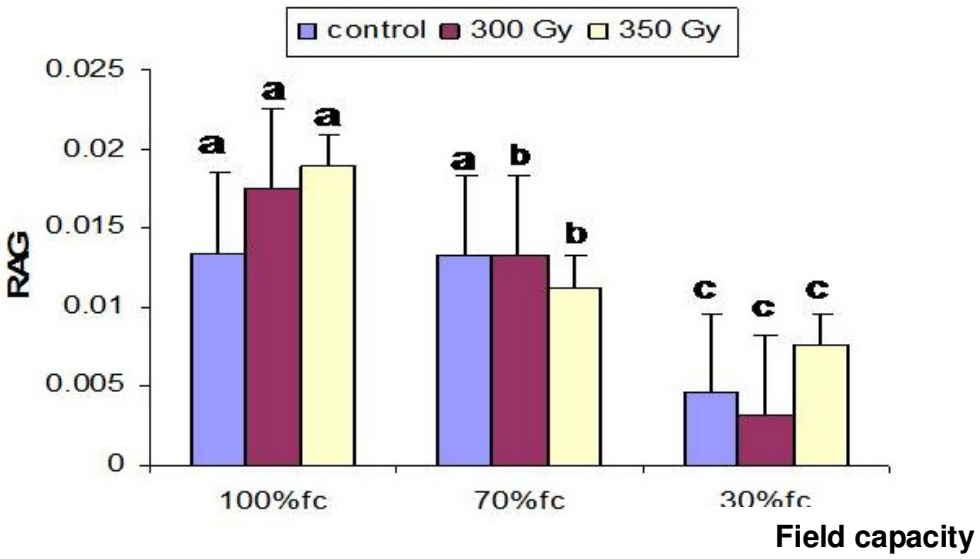

Figure 8. Effect of irradiation on relative average growth of root after 60 days of sowing at 100, 70 and 30% fc.

of a severe water stress, that is 30% fc caused a reduction in biosynthetic activities which were 34.55, 18.52 and 13.63% with control ones and mutant plants obtained at 300 and 350 Gy. The application of moderate stress that is 70% fc, caused a reduction in biosynthetic activities which was recorded 1.4, 24.04 and 40.68%, respectively for control ones and mutant plants at 300 and 350 Gy, respectively. The severe water stress caused a more reduction in the biosynthetic activity of the root and it was found 66.19, 81.89 and 59.89% for control ones and in mutant plants produced at 300 and 350 Gy (Figure 8).

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