



Full Length Article

Alterations of DNA, Ions and Photosynthetic Pigments Content in Date Palm Seedlings Induced by X-Irradiation

NORAH A. AL-ENEZI AND JAMEEL M. AL-KHAYRI^{1†}

Department of Biology, Science College, Dammam University, Dammam, Saudi Arabia

[†]Department of Agricultural Biotechnology, College of Agricultural and Food Sciences, King Faisal University, Al-Hassa, Saudi Arabia

¹Corresponding author's e-mail: jkhayri@kfu.edu.sa

ABSTRACT

This study determined the effect of X-irradiation on physiological and biochemical aspects in date palm (*Phoenix dactylifera* L.) seedlings. Using a medical X-ray device, 15-days-old seedlings were exposed to various X-rays doses (0, 5, 10, 25, 50, 100, 250, 500, 750, 1250 & 1500 rad). After growing the irradiated seedlings in soil for 2 months, chemical analysis was conducted. The data indicated that DNA content was dose dependent showing a significant increase with 5 rad, the lowest radiation dose tested and reaching the highest level at 25 rad. Similarly, the highest ions content was observed at 25 rad. A dose of 5 rad caused a significant increased sodium, potassium and phosphorus ions but a minimum dose of 10 rad was necessary to significantly enhance the content of calcium and magnesium ions. Conversely, total photosynthetic pigments started to diminish at 5 rad and continued to decrease with dose increases reaching the lowest level at 1500 rad. Significant reductions of chlorophyll a and carotenoids content started at 5 rad, whereas reduction of chlorophyll b occurred at 10 rad. This suggests that chlorophyll a and carotenoids are more sensitive to X-irradiation than chlorophyll b. © 2012 Friends Science Publishers

Key Words: DNA content; Ionizing radiation; Ions; *Phoenix dactylifera*; Photosynthetic pigments; X-rays

INTRODUCTION

Ionizing radiations (IR), mainly X-rays and gamma-rays, have been used in plant research to achieve improved genetic traits and enhanced crop production (Mortazavi *et al.*, 1999). Plant responses to IR vary greatly depending upon a number of factors including irradiation type, dose, and plant genotype (Yoshida *et al.*, 1999; Din *et al.*, 2002; Esnault *et al.*, 2010). The biological effects of IR range from stimulation by low doses i.e., hormesis, to inhibition by high doses (Sheppard *et al.*, 1987; Sheppard & Chubey, 1990; Charbaji & Nabulsi, 1999; Chakravarty & Sen, 2001; Ling *et al.*, 2008).

Exposure to IR causes the ionization of water, an integral component of living tissues, produces free radicals in addition to secondary reactive oxygen species (ROS), which triggers the activity of detoxifying enzymes to resist oxidative stress (Montelone, 1998; Gudkov *et al.*, 2010; Esnault *et al.*, 2010; Legue & Chanal, 2010). These ions are highly mutagenic and react randomly with macromolecules including lipids, proteins, carbohydrates and nucleic acids causing oxidative stress, which may lead to cellular damages or even death (Hutchinson, 1963; Shirazi *et al.*, 2007; Roldan-Arjona & Ariza, 2009; Pizzimenti *et al.*, 2010).

The impact of IR on the genetic material is well documented in numerous plant species (Esnault *et al.*, 2010). In several plant species, exposure to X-rays resulted in gene alterations (Evans & Paddock, 1977; Kranz *et al.*, 1994; Ken *et al.*, 2005) as well as chromosomal changes (Ecochard, 1966; Jakob & Wolff, 1973; Berkofsky & Roy, 1977; Rizzoni *et al.*, 1987; Shirley *et al.*, 1992; El Araqi *et al.*, 1997; Kovalchuk *et al.*, 2007; Watanabe *et al.*, 2009). Moreover, X-irradiation was shown to negatively influence DNA synthesis (Evans & Scott, 1964) as well as RNA and protein synthesis (Roy *et al.*, 1972). Conversely, Joshi and Ledoux (1970) reported no significant modification of DNA, RNA and acid soluble content, while protein content was adversely affected by X-irradiation. Studies related to the effect of X-rays on date palm nucleic acids are not available.

Moreover, IR can influence cell membrane permeability as evident by changes in uptake and loss of ions (Higinbotham & Mika, 1954; Tanada, 1958; Srb, 1964). Using flow cytometry, Baisch (1978) has shown that X-irradiation increased the rate fluorescein efflux linearly with radiation dosage. Modified permeability alters plant nutritional status and affects ions accumulation in the cell. Studies related to the effect of X-rays on element composition of date palm are lacking.

Several studies showed that IR induces changes in photosynthetic pigments, mainly chlorophylls and carotenoids, in response to gamma-radiation (Strid *et al.*, 1990; Marwood & Greenberg, 1996). Increases in chlorophyll content in low doses of gamma-irradiated plants were observed but at higher doses an inhibition occurred (Abu *et al.*, 2005; Kiong *et al.*, 2008; Ling *et al.*, 2008; Borzouei *et al.*, 2010). The effects of IR on photosynthetic pigments not only vary among plant species but also differ among cultivars (Kim *et al.*, 2004). Furthermore, Kim *et al.* (2005) noted that carotenoid pigments were more sensitive to gamma rays than chlorophylls.

Studies related to the effects of X-radiation on photosynthesis pigments have indicated the involvement of dose and exposure duration. Shull and Mitchell (1933) observed chlorophyll increases in response to X-rays but decreased after prolonged exposures. Sprey (1972) reported dose-dependent changes in thylakoid number and chlorophyll content following exposure to X-radiation. Literatures describing the effect of ionizing radiations on photosynthetic pigments of date palm were not encountered.

Date palm (*Phoenix dactylifera* L.) is a monocotyledonous, dioecious perennial diploid species belonging to the Palmaceae (Arecaceae) family. To avoid heterozygosity associated with seed-derived progeny, it is traditionally propagated by offshoots and recently by tissue culture methodology (Al-Khayri, 2005 & 2007). Date palm trees provide the most sustainable agro-ecosystems in the dry hot the regions of the world (Zohary & Hopf, 2000). Its agriculture is concentrated mainly in Southern Asia and North Africa but has expanded to Australia, Southern Africa, South America, Mexico and the United States of America (Hodel & Johnson, 2007). Beside its high nutritional value date palm provides a major economical source for local farmers and associated industries (Zaid, 2002). The genetic improvement of date palm is hindered by numerous inherent limitations associated with traditional breeding methodologies (El-Hadrami & El-Hadrami, 2009; El-Hadrami *et al.*, 2011). Mutation induction technique, based on chemical or physical mutagens, is an indispensable tool in plant genetic improvement programs for its effectiveness in expanding the genetic pool available to breeders (Shima & Ichikawa, 1994; Ahloowalia & Maluszynski, 2001; Aslam *et al.*, 2004; Puchooa, 2005; Jain, 2010; Magori *et al.*, 2010). A case in point, gamma radiation was successfully used for inducing mutations and selecting date palm genotypes with improved resistance to Bayoud disease (Jain, 2005, 2007, 2011; Sedra & Lazrek, 2011).

The objective of this study was to evaluate the effects of various doses of X-rays on DNA content, element ions accumulation and photosynthetic pigments distribution in date palm seedlings. Information gained from this study may contribute to utilizing X-radiation for improving genetic traits, propagation and production of date palm.

MATERIALS AND METHODS

Plant material and growth conditions: Seeds collected from female date palm trees of cv. Khalas were surface sterilized with 1% sodium hypochlorite for 5 min, soaked in water for 24 h, then germinated on moist filter paper at 37°C. After irradiation as described below, the seedlings were grown individually in a 20-cm plastic pot containing potting mix (1 soil: 1 peat moss: 1 vermiculite) and maintained in a greenhouse under natural light at 30-40°C with a relative humidity of approximately 50%. The seedlings were watered as needed to ensure that they were not stressed for moisture.

Irradiation treatments: At the age of 15 days, date palm seedlings were placed in 9 cm Petri dishes and submerged in 10 mL of distilled water prior to exposure to X-rays. Using a therapeutic medical X-rays device (Clinac 23 EX Linear Accelerator, Varian Medical Systems, USA), samples were exposed to different treatments (0, 5, 10, 25, 50, 100, 250, 500, 750, 1250 & 1500 rad), which are equivalent to (0, 0.05, 0.1, 0.5, 1, 2.5, 5, 7.5, 12.5 & 15 Gy). Each Petri dish contained 10 seedlings and 7 dishes were treated with each dose.

DNA extraction and estimation: Total DNA was isolated from 0.1 g leaf samples excised from 2-months-old date palm seedlings. DNA isolation was performed using a commercial DNA isolation kit (DNeasy Plant Mini Kit, Qiagen, Germany) following the manufacturer's instructions. Total DNA was measured at 260 nm absorbance with UV/VIS spectrophotometer (Model V-530, Jasco Inc, USA) and the concentrations were calculated according to the following equation: DNA concentration ($\mu\text{g/mL}$) = $A_{260} \times (\text{dilution factor} \times 50)/1000$.

Analyzing and measuring elements: Date palm leaves were oven dried at 70°C for 24 h using a vacuum drying oven (Duo-Vac Oven 3620, Lab Line Instruments Inc., USA). A microwave assisted digestion system was used to extract elements from leaf samples in a closed microwave system (Zarcinas *et al.*, 1987; Ryan, 2005). The dried samples were digested by weighing 0.25 g of sample into a Teflon® PFA lined microwave digestion vessel and adding 3 mL of 10 M HNO₃ (Merck Tracepur) and 1 mL of H₂O. Microwave digestion was applied at a power of 600 Watt and a pressure of 350 (Pa) at 120°C for 3 min followed by 200°C for 10 min. Following digestion, solutions were cooled and transferred to 25 mL volumetric flasks and diluted to volume with deionized water. The elements composition was determined using Inductive Couple Plasma-Atomic Emission Spectroscopy (ICP-AES) (Varian-Liberty-ICP-OES series II, USA).

Estimation of photosynthetic pigments: Photosynthetic pigments were determined according to Lichtenthaler & Wellburn (1983) method. Briefly, fresh leaf samples, 0.5 g, were ground in a pre-chilled mortar in an ice bath mortar and pestle with 5 mL of 80% acetone and then filtered through No. 2 Whatman filter paper. Using UV/VIS

spectrophotometer (Model V530, Jasco Inc., USA), extract absorption was measured at 470, 646 and 663 nm. The amounts of chlorophyll a, chlorophyll b and carotenoids were calculated in $\mu\text{g/mL}$ according to the following equations: Chlorophyll a = $12.21 (A_{663}) - 2.81 (A_{646})$; Chlorophyll b = $20.13 (A_{646}) - 5.03 (A_{663})$; Carotenoids = $(1000A_{470} - 3.27 (\text{chlorophyll a}) - 104 (\text{chlorophyll b}))/227$; Total pigments=chlorophyll a+chlorophyll b+carotenoids.

Statistical analysis: The experiment was randomly designed with a single factor consisting of 11 levels with 7 replications. Data were subjected to analysis of variance (ANOVA) and the means were separated, where appropriate using the least significant difference (LSD) at 5%. Standard deviation for each treatment was also calculated.

RESULTS

Effect of X-rays on DNA content: The results indicated that DNA content increased at 5 rad, the lowest radiation dose tested, giving a 1.6-fold increase with respect to the control (Fig. 1). Similarly, increasing the dose to 10 rad and then to 25 rad resulted in significant increases in DNA content reaching 2.6 and 3.2 folds, respectively as compared to the control. The latter dose resulted in the maximum DNA content, $16.22 \mu\text{g/g}$. A steady decline in the DNA content was observed as the doses increased from 50 to 1500 rad; however, the DNA concentrations remained significantly higher than the non-treated seedlings.

Effect of X-rays on elements content: Ions content increased significantly in the date palm seedlings treated with X-rays (Fig. 2). Generally, ions concentrations (Ca^{2+} , Na^+ , K^+ , Mg^{2+} & P^{3-}) increased following the incremental increases of the X-rays dose, reaching maximum accumulations at 25 rad. At doses higher than 25 rad, ion concentrations began to decline reaching minimum levels at 1500 rad, the highest dose tested. On the other hand, the minimum doses eliciting significant changes in ions content varied depending upon the ion. Furthermore, at the doses higher than 25 rad, the effect of the incremental increases appeared to vary depending on the ion.

Calcium content was not affected by exposure to 5 rad (Fig. 2A). Significant changes in calcium concentration began at 10 rad. The optimum treatment of 25 rad, resulted in $63.7 \mu\text{g/g}$ calcium as compared to $42.57 \mu\text{g/g}$ found in the non-treated seedlings. As the X-rays dose increased, calcium content started to decline reaching the lowest value at 1500 rad.

Sodium concentration increased significantly with respect to control starting at 5 rad (Fig. 2B). It continued to increase as the concentration increased to 25 rad reaching a maximum of $24.57 \mu\text{g/g}$ as compared to $17.29 \mu\text{g/g}$ obtained from the control. Irradiation with doses between 50-1500 rad caused a reduction of sodium content as compared to the optimum treatment (25 rad); however, resultant sodium concentration was not significantly different than that of the control.

Fig. 1: The impact of X-rays doses on DNA content in leaves of date palm seedlings. The trend line represents a third order binomial regression

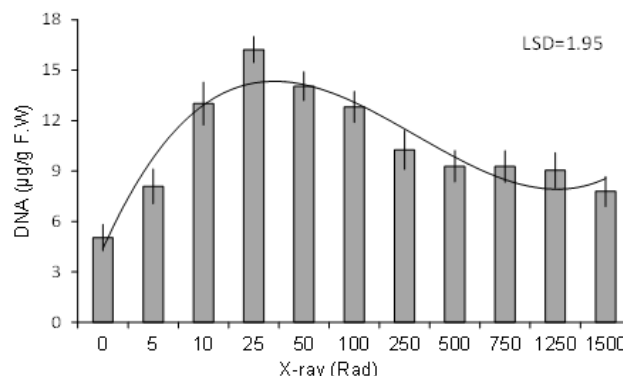
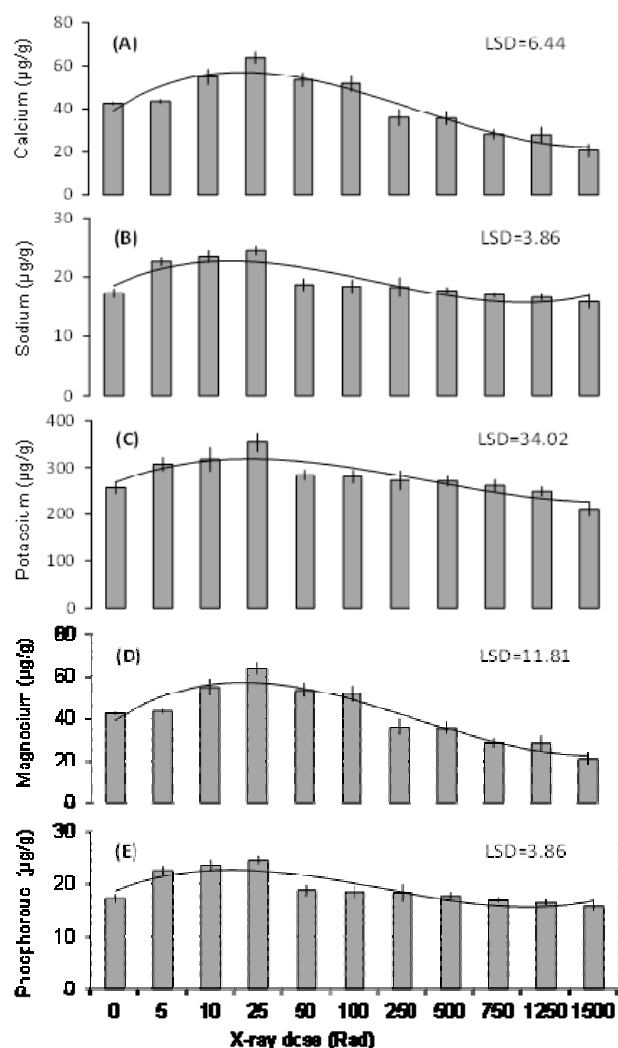


Fig. 2: The impact of X-rays doses on ions content in leaves of date palm seedlings: (A) Calcium, (B) Sodium, (C) Potassium, (D) Magnesium and (E) Phosphorus. The trend line represents a third order polynomial regression



Similar to the effect of X-rays dose on sodium content, 5 rad was sufficient to cause a significant increase in potassium content (Fig. 2C). As the dose increased to 25 rad, the highest potassium content of 353.72 $\mu\text{g/g}$ was reached as compared to 307.04 $\mu\text{g/g}$ associated with the control. Doses above 25 rad caused a steady reduction in Potassium content as compared to the optimum treatment. However, potassium concentrations associated with doses ranging between 50-1250 rad were not significantly different than each other and remained higher or equivalent to the control. At 1500 rad, potassium content was lower than the control.

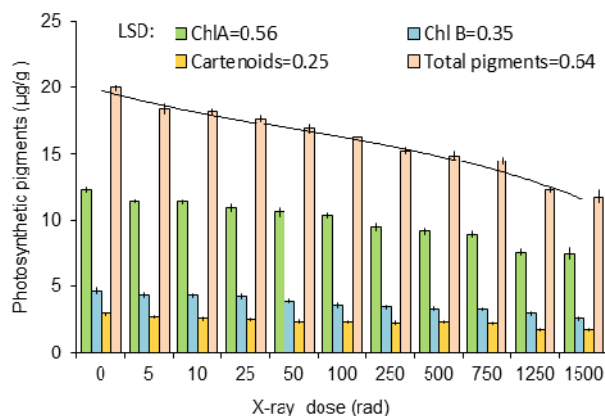
Similar to the effect of X-rays doses on calcium, magnesium content increased significantly in response to 10 rad, whereas 5 rad caused no significant changes with respect to the control (Fig. 2D). Magnesium content significantly increased as the dose further increased to 25 rad giving a maximum concentration of 63.71 $\mu\text{g/g}$ as compared to 42.57 recorded for the control. As the dose increased above 25 rad, a steady decrease in magnesium content was observed reaching its lowest level at 1500 rad. High-dose treatments ranging from 750 to 1500 rad significantly reduced magnesium content as compared to the control.

The content of phosphorus increased in response to 5 rad and continued to increase until the X-rays dose reached 25 rad, which recorded 23.8 $\mu\text{g/g}$ as compared to 17.28 $\mu\text{g/g}$ recorded for the control (Fig. 2E). Phosphorus content associated with the optimum treatment was not significantly different from that observed in response to 5 and 10 rad exposures. Doses ranging from 50 to 1500 rad decreased phosphorus content as compared to the optimum treatment but their effects were comparable to control treatment.

Effects of X-rays on photosynthetic pigments: Photosynthetic pigments (chlorophyll a, chlorophyll b, carotenoids & total pigments) concentrations were significantly affected by X-ray. As compared to the control, seedlings exposed to X-rays exhibited a significant reduction in the concentrations of the three photosynthetic pigments analyzed. An inverse relationship was observed between the X-rays dose and the content of the photosynthetic pigments.

In general, as the X-rays dose increased, the content of chlorophyll a, chlorophyll b, and carotenoids decreased (Fig. 3). The reduction for chlorophyll a started at 5 rad with 11.43 $\mu\text{g/g}$ and reached its lowest level of 7.49 $\mu\text{g/g}$ at 1500 rad as compared to 12.35 $\mu\text{g/g}$ obtained from the non-exposed seedlings. Whereas, chlorophyll b content started to decrease significantly at 10 rad with 4.31 $\mu\text{g/g}$ and reached its lowest level of 2.55 $\mu\text{g/g}$ at 1500 rad as compared to 4.68 $\mu\text{g/g}$ recorded for the control. Carotenoids reduction tended to be similar to that of chlorophyll a, where the significant reduction started at 5 rad with 2.66 $\mu\text{g/g}$ to reach its lowest level of 1.73 $\mu\text{g/g}$ at 1500 rad as compared to 2.94 $\mu\text{g/g}$ obtained from the control. Consequently, the total photosynthetic pigments was negatively affected.

Fig. 3: The impact of X-rays doses on content of photosynthetic pigments (chlorophyll a, chlorophyll b, & carotenoids) in leaves of date palm seedlings. The trend line represents a third order binomial regression



A steady reduction in the total photosynthetic pigments was observed as the X-rays dose increased reaching its lowest value of 14.79 $\mu\text{g/g}$ at 1500 as compared to 19.97 $\mu\text{g/g}$ associated with the non-treated seedlings.

DISCUSSION

The effect of radiation on living tissues is a function of various factors including tissue moisture. Ehrenberg *et al.* (1953) have shown that seed soaking influenced the effects of irradiation in barley. Radiation-induced changes become stronger in the presence of moisture. This was also demonstrated by Kamra *et al.* (1960) who reported that X-rays induced damages to barley seedling increased, in terms of chromosomal aberrations and mutation frequency, with increasing time of seed soaking before X-irradiation. Similarly, Joshi and Ledoux (1970) observed maximum radioresistance at intermediate moisture levels in a study testing the effect of X-rays on barley (*Hordeum vulgare*) seeds with varying moisture content ranging from 3 to 14%. Also, Amjad and Anjum (2002) found that radiosensitivity was affected by different moisture contents of onion (*Allium cepa* L.) seeds. Accordingly, the procedures used in the present study involved adding water to the date palm seedlings prior to X-irradiation. This is to assure an equal distribution of radiation and to maximize the radiosensitivity of date palm seedlings.

The current study has shown that the exposure to X-rays can influence biochemical and physiological processes in date palm seedlings. The extent of modifications of these biological processes appeared to be related to the radiation dose, another important factor controlling the response to irradiation. In addition the radiation dose, the effect of radiation may be stimulatory or inhibitory depending on the biological process being considered. Metabolic processes involved in DNA synthesis and/or degradation, as reflected by the total DNA content of date palm seedlings, were influenced by the X-rays dose. Similarly, the process

involved in elements accumulation such as absorption and/or transport were also affected by X-rays dose.

The DNA and elements contents were enhanced by low doses and reduced in response to high doses. This may be explained by the fact that the oxidative stress caused by ionizing radiation produces radical ions that interact directly with electrons causing changes in cellular functions which may be harmful (Mortazavi *et al.*, 2003; Slupphaug, 2003). The abundance of free radicals is related to the radiation dose. High doses of ionizing radiation elicit accumulation of free radicals, whereas this effect was noticed with low doses (Macklis & Bresford, 1991). On the contrary, processes involved in photosynthesis pigments synthesis and degradation, as revealed by the concentration of these pigments in date palm seedlings, were regulated even at low doses of X-rays. This indicates that sensitivity of the various cellular functions to low radiation doses vary.

It is pertinent to note that diverse results relevant to the effects of radiation on various cellular constituents and activities are reported in the literature (Foyer, 1994; Yoshida *et al.*, 1999; Kovalchuk *et al.*, 2007; Wi *et al.*, 2007). Although this is the first study addressing these responses in date palm, previous reports have shown similar effects in some plant species, whereas others plants exhibited differing responses, which may be due to genotypic-dependency. Dissimilarity in the results may also be attributed to the variable doses used in various studies. Difficulties to compare results are complicated by the fact that various reports expressed the radiation dose used differently.

The present study indicated that DNA content was significantly affected by X-rays dose. Low doses, up to 25 rad, stimulated DNA accumulation, while higher doses ranging from 50 to 1500 rad were inhibitory. With these high doses, however, the DNA content remained higher than the control. In a relevant study, Dhawi and Al-Khayri (2009a) observed that exposing date palm seedlings to magnetic fields, a form of non-ionizing radiation, caused a reduction in the DNA content.

Other studies reported declines or no changes in DNA, RNA and protein content under X-ray impact. Roy *et al.* (1972) has shown that exposure of pine (*Pinus pinea*) seedlings to 15 kR of X-rays increased chromosomal aberrations and caused a 40% reduction in protein content. Berkofsky and Roy (1977) demonstrated that exposure of pine seedlings to a lower X-ray dose of 5 kR resulted in no significant alterations in the relative amount of proteins as well as DNA and RNA.

Ledoux *et al.* (1962) noticed a reduction of RNA, DNA, protein, acid soluble and soluble N content in barley seedlings in response to the X-rays doses tested, which ranged from 500 to 82500 rad. However, Joshi and Ledoux (1970) reported no significant modification of DNA, RNA and acid soluble content, while protein content was adversely affected in barley exposure to X-radiation doses ranging from 10 to 15 kR. Others have demonstrated the

adverse affect of X-irradiation on the content of DNA. Imamura *et al.* (1987) found that X-irradiation significantly reduced the amount of DNA of the irradiated tobacco (*Nicotiana tabacum*) and henbane (*Hyoscyamus muticus*).

Modifications of the ions content after exposure to X-radiation has been attributed to changes in membrane permeability (Higinbotham & Mika, 1954; Tanada, 1958; Srb, 1964; Baisch, 1978). Osmotic properties of epidermal cells were also affected by ionizing radiation (Srb, 1965). Moreover, water flux of isolated roots was affected by X-irradiation (Lott & Wall, 1968). Furthermore, stomatal openings and transpiration were also found to be affected by X-irradiation (Roy, 1974). Although, the role of radiation on biological membranes remains unclear Benderitter *et al.* (2003) reported alterations of biochemical and biophysical properties of biological membranes caused by oxidative stress of ionizing radiations. Low doses of X-rays appear to induce membrane changes conducive of absorption and preventive of the loss of nutrients. High radiation doses appear to reverse this effect. Consequently, irradiation could impact ions content through modifications of mechanisms involved in water movement and nutrients uptake and transport. Enhanced ions content caused by low doses of X-radiation may be beneficial to improve nutritional status and consequently boost plant growth.

The current study has shown that increased ions content of calcium, sodium, potassium, magnesium and phosphorus occurred only with low X-rays doses, up to 25 rad, while high doses decreased the content of these elements in date palm seedlings. In previous studies, Dhawi and Al-Khayri (2009b) and Dhawi *et al.* (2009) have shown that increasing exposure of magnetic fields increased the concentration of Ca, Mg, Mn, Fe, Na and Zn ions, but decreased P content.

Sensitivity of ions accumulation mechanisms to X-irradiation differ among plant species. In a study on onion (*Allium cepa*), potassium and calcium decreased with high doses of X-rays ranging from 750 to 1000 rad, while 500 rad stimulated calcium accumulation (Lott & Wall, 1968). In another study, Ehandi *et al.* (1970) demonstrated that the loss of electrolytes from gamma-irradiated carrot (*Daucus carota*) tissues to an aqueous medium was dose dependent. Calcium was the most readily leached, followed by copper, zinc, sodium, phosphorus, boron, iron, aluminum and manganese. Abboud and Roy (1979) working with common bean (*Phaseolus vulgaris*) detected 30% inhibition of ions accumulation of calcium and strontium by isolated mitochondria after 50 krad X-irradiation. Lower doses, 10 and 25 krad, had no significant effect on ions accumulation. It has been suggested that the accumulation of ions in plants is involved in osmotic adjustment to stress factors (Patakas *et al.*, 2002).

The effect of X-rays dose on the content of photosynthetic pigments in date palm seedlings exhibited a differing trend as compared to DNA and ions concentrations. The content of chlorophyll a, chlorophyll b,

and carotenoids decreased in response to X-rays. Although this reduction was dose dependent, doses as low as 5 rad induced a significant inhibition of photosynthetic pigments. In a relevant study using magnetic fields, Dhawi and Al-Khayri (2008) demonstrated that low doses had a stimulative effect on photosynthetic pigments of date palm, whereas high doses had a negative effect. In addition, they observed that chlorophyll a and carotenoids were more sensitive to magnetic fields than chlorophyll b.

Chlorophyll has an effective role in light energy transformation to chemical energy. Its content is an indicative of the plant metabolisms efficiency and health status. Whereas, carotenoids plays a major role in photoprotection and defending plant cell from oxidative stress (Bartley & Scolnik, 1995; Demmig *et al.*, 1996; Lefsrud *et al.*, 2005; Kopsell *et al.*, 2007).

Reduction in photosynthetic pigments may be attributed to the detrimental effect of oxidative stress induced by the free radicals caused by ionizing irradiation. The impact of oxidative stress is counteracted by antioxidant cellular mechanisms that are destructed when stress reaches critical levels (Foyer, 1994). According to Al-Nimer Marwan and Abdul Lateef (2009), a low dose of X-rays, 1.9 Gy/min, significantly modified the activity of antioxidants in extracts of dry leaves of green tea (*Camellia sinensis*) and sage (*Salvia officinalis*), barks of cinnamon (*Cinnamomum verum*) and tuberos of ginger (*Zingiber officinale*). Pigments contribute to stress resistance and may be consumed as they participate in radical ions elimination and energy dissipation (Strzalka *et al.*, 2003; Wilson *et al.*, 2006). This may explain the reduction of photosynthetic pigments content after exposing date palm seedlings to X-rays. These pigments were found to be highly sensitive to radiation-induced stress. In response to gamma irradiation, chloroplasts were found extremely sensitive compared to other cell organelles, based on transmission electron microscopy (Wi *et al.*, 2007).

Others studies have demonstrated dose-dependent effect of X-rays on photosynthetic pigments. For instance, Rao and Rao (1978), reported 50% reduction in the total chlorophyll content at 8 kR, while an increased chlorophyll metabolism was observed at lower doses of 4 and 7 kR in okra (*Abelmoschus esculentus*). Unlike, inhibitory effect observed in the current study with date palm where photosynthetic pigments were inhibited within the dose range tested. Other studies involving gamma radiation have shown that modified plastid structures like thylakoids and functions like starch-sugar conversion (Kovács & Keresztes, 2002), altered photosynthetic pigments and antioxidant mechanisms (Kim *et al.*, 2004), affected photosynthesis (Strid *et al.*, 1990) and influenced chloroplast development (Marwood & Greenberg, 1996). Ling *et al.* (2008) obtained lower chlorophyll content from gamma-irradiated plantlets as compared to non-irradiated plantlets of sweet orange (*Citrus sinensis*); however, chlorophyll content was virtually insensitive to low doses of gamma irradiation. Conversely,

Abu *et al.* (2005) obtained an increase in chlorophyll a, b and total chlorophyll levels in gamma irradiated cowpea (*Vigna unguiculata*).

CONCLUSION

Date palm seedlings exposed to X-rays treatments showed significant responses. Irradiation affected the amount of DNA, accumulation of elements (Ca^{2+} , Na^+ , K^+ , Mg^{2+} & P^{3-}) and content of photosynthetic pigments (chlorophyll a, chlorophyll b & carotenoids). The observed effects of X-irradiation on date palm seedlings were dose dependent. The accumulation of DNA and ions reflect a positive effect of low doses of X-rays. However, photosynthetic pigments were negatively affected at low doses. This indicated that the sensitivities of cellular mechanisms to X-rays exposures differ. Changes in DNA content may be an indicator of mutations. Altered ions content may reflect X-rays influence on membrane permeability. Reduction in photosynthetic pigments may be caused by oxidative stress reactions, where pigments are consumed in scavenging of radical ions induced by irradiation. More advanced analysis is required to explore these possibilities. Investigations related to date palm stress physiology and mutation-assisted genetic improvement would be facilitated by the information presented in this study.

Acknowledgment: The authors are thankful to Maha Al-Adrisi and Mousa Al-Anazi at King Fahd Specialist Hospital, Dammam, Saudi Arabia, for the complimentary use of the analytical facilities and the x-ray device.

REFERENCES

- Abboud, S. and R.M. Roy, 1979. The effects of X-irradiation on respiration and cation accumulation by isolated mitochondria of *Phaseolus vulgaris*. *Environ. Exp. Bot.*, 19: 277–283
- Abu, J.O., K. Muller, K.G. Duodu and A. Minnar, 2005. Gamma irradiation of cowpea (*Vigna unguiculata* L. Walp) flours and pastes. *Food Chem.*, 95: 138–147
- Ahloowalia, B.S. and M. Maluszynski, 2001. Induced mutations - A new paradigm in plant breeding. *Euphytica*, 118: 167–173
- Al-Khayri, J.M., 2005. Date Palm *Phoenix dactylifera* L. In: Jain, S.M. and P.K. Gupta (eds.), *Protocols of Somatic Embryogenesis in Woody Plants*, pp: 309–318. Springer, Berlin, Germany
- Al-Khayri, J.M., 2007. Micropropagation of Date Palm *Phoenix dactylifera* L. In: Jain, S.M. and H. Haggman (eds.), *Protocols for Micropropagation of Woody Trees and Fruits*, pp: 509–526. Springer, Berlin, Germany
- Al-Nimer Marwan, S.M. and Z.W. Abdul Lateef, 2009. X-rays radiation directly produced favorable and harmful effects on the constituents of different medicinal plants. *Phcog. Res.*, 1: 331–335
- Amjad, M. and M.A. Anjum, 2002. Radiosensitivity of onion seeds with different moisture contents. *Int. J. Agric. Biol.*, 4: 468–473
- Aslam, M., N. Iqbal, A.A. Bandesha and M.A. Haq, 2004. Induction of mutations through crosses with gamma irradiated pollen in cotton. *Int. J. Agric. Biol.*, 6: 898–900
- Baisch, H., 1978. Effects of X-rays on cell membranes. *Radiat. Environ. Biophys.*, 15: 221–228
- Bartley, G.E. and P.A. Scolnik, 1995. Plant carotenoids: Pigments for photoprotection, visual attraction and human health. *Plant Cell*, 7: 1027–1038

- Benderitter, M., L. Vincent-Genod, J.P. Pouget and P. Voisin, 2003. The cell membrane as a biosensor of oxidative stress induced by radiation exposure: a multiparameter investigation. *Radiat. Res.*, 159: 471–483
- Berkofsky, J. and R.M. Roy, 1977. Effects of X-irradiation on soluble nucleohistone of *Pinus pinea*. *Environ. Exp. Bot.*, 17: 55–61
- Borzouei, A., M. Kafi, H. Khazaei, B. Naseriyan and A. Majdabadi, 2010. Effects of gamma radiation on germination and physiological aspects of wheat (*Triticum aestivum* L.) seedlings. *Pakistan J. Bot.*, 42: 2281–2290
- Chakravarty, B. and S. Sen, 2001. Enhancement of regeneration potential and variability by γ -irradiation in cultured cells of *Scilla indica*. *Biol. Plant.*, 44: 189–193
- Charbaji, T. and I. Nabulsi, 1999. Effect of low doses of gamma irradiation on in vitro growth of grapevine. *Plant Cell Tiss. Org. Cult.*, 57: 129–132
- Demmig, A.B., A.M. Gilmore and W. Adams, 1996. Carotenoids 3: *in vivo* function of carotenoids in higher plants. *Fed. American Soc. Exp. Biol.*, 4: 403–412
- Dhawi, F. and J.M. Al-Khayri, 2008. Magnetic fields induce changes in photosynthetic pigments content in date palm (*Phoenix dactylifera* L.) seedlings. *Open Agric. J.*, 2: 121–125
- Dhawi, F. and J.M. Al-Khayri, 2009a. Magnetic fields-induced modification of DNA content in date palm (*Phoenix dactylifera* L.). *J. Agric. Sci. Technol.*, 3: 5–9
- Dhawi, F. and J.M. Al-Khayri, 2009b. The effect of magnetic resonance imaging on date palm (*Phoenix dactylifera* L.) elemental composition. *Comm. Biometr. Crop Sci.*, 4: 11–18
- Dhawi, F., J.M. Al-Khayri and H. Essam, 2009. Static magnetic fields influence on elements composition in date palm (*Phoenix dactylifera* L.). *Res. J. Agric. Biol. Sci.*, 5: 161–166
- Din, R., M. Qasim and K. Ahmad, 2004. Radio sensitivity of various wheat genotypes in M_1 generation. *Int. J. Agric. Biol.*, 6: 898–900
- Echandi, R.J. and L.M., Jr. Massey, 1970. Loss of electrolytes and amino acids from gamma-irradiated carrots. *Radiat. Res.*, 43: 372–378
- Ecochard, R., 1966. Effects of X-irradiation on the meiosis of *Vicia faba*. *Mutat. Res./Fund. Molec. Mech. Mutagen.*, 3: 314–326
- Ehrenberg, L., A. Gustafsson, U. Lundqvist, N. Nybom, 1953. Irradiation effects, seed soaking and oxygen pressure in barley. *Hereditas*, 39: 493–504
- El Araqi, K.T., J. Gilot-Delhalle, I. Roelandts, G. Weber, R. Deltova and J. Moutschen, 1997. Modified fast rejoining of chromosomes after fractionated X-ray exposures with iron and copper sulfate pretreatments related to uptake detected by PIXE analysis in *Nigella damascena* seeds. *Environ. Exp. Bot.*, 38: 87–97
- El-Hadrami, I. and A. El-Hadrami, 2009. Breeding date palm. In: Jain, S.M. S.M. and P.M. Priyadarshan (eds.), *Breeding Plantation Tree Crops*, pp: 191–216. Springer, New York
- El-Hadrami, A., F. Daayf and I. El-Hadrami, 2011. Date palm genetics and breeding. In: Jain, S.M., J.M. Al-Khayri and D.V. Johnson (eds.), *Date Palm Biotechnology*, pp: 479–512. Springer Science+Business Media BV, Dordrecht
- Esnault, M.A., F. Legue and C. Chenal, 2010. Ionizing radiation: Advances in plant response. *Environ. Exp. Bot.*, 68: 231–237
- Evans, D.A. and E.F. Paddock, 1977. X-ray induced increase of mitotic crossovers in *Nicotiana tabacum*. *Environ. Exp. Bot.*, 17: 99–106
- Evans, H.J. and D. Scott, 1964. Influence of DNA synthesis on the production of chromatid aberrations by X rays and maleic hydrazide in *Vicia faba*. *Genetics*, 49: 17–38
- Foyer, C.H., 1994. Photo oxidative stress in plants. *Physiol. Plant*, 92: 696–717
- Gudkov, S.V., S.A. Garmash, I.N. Shtarkman, A.V. Chernikov, O.E. Karp and V.I. Bruskov, 2010. Long-lived protein radicals induced by X-ray irradiation are the source of reactive oxygen species in aqueous medium. *Dokl. Biochem. Biophys.*, 430: 1–4
- Higinbotham, N. and E.S. Mika, 1954. The Effect of X-rays on uptake and loss of ions by potato tuber tissue. *Plant Physiol.*, 29: 174–177
- Hodel, D.R. and D.V. Johnson, 2007. *Imported and American Varieties of Dates (Phoenix dactylifera) in the United States*. University of California Agriculture Resources, Publication 3498, Oakland, California 04608-1239, USA
- Hutchinson, F., 1963. Radiation effects on macromolecules of biological importance. *Ann. Rev. Nucl. Sci.*, 13: 535–564
- Imamura, J., M.W. Saul and I. Potrykus, 1987. X-ray irradiation promoted asymmetric somatic hybridisation and molecular analysis of the products. *Theor. Appl. Genet.*, 74: 445–450
- Jain, S.M., 2005. Major mutation-assisted plant breeding programmes supported by FAO/IAEA. *Plant Cell Tiss. Org. Cult.*, 82: 113–121
- Jain, S.M., 2007. Recent advances in date palm tissue culture and mutagenesis. *Acta Hort.*, 736: 205–211
- Jain, S.M., 2010. Mutagenesis in crop improvement under the climate change. *Romanian Biotechnol. Lett.*, 15: 88–106
- Jain, S.M., 2011. Radiation-induced mutations for date palm improvement. In: Jain, S.M., J.M. Al-Khayri and D.V. Johnson (eds.), *Date Palm Biotechnology*, pp: 271–286. Springer, Dordrecht, The Netherlands
- Jakob, K.M. and S. Wolff, 1973. The Effect of post-irradiation centrifugation on chromosome aberrations in *Vicia faba*. *Plant Genet.*, 14: 121–130
- Joshi, R.K. and L. Ledoux, 1970. Influence of X-irradiation and seed-moisture on nucleic-acid and protein metabolism in barley. *Radiat. Bot.*, 10: 437–443
- Kamra, O.M.P., S.K. Kamra, R.A. Nilan and C.F. Konzak, 1960. Radiation response of soaked barley seeds II. Relation of radiobiological damage to substances lost by leaching. *Hereditas*, 46: 261–273
- Ken, N., K. Makoto, S. Naoya, T. Toshiya and T. Atsushi, 2005. Transmissible and no transmissible mutations induced by irradiating *Arabidopsis thaliana* pollen with X-ray and carbon ions. *Genetics*, 169: 881–889
- Kim, J.H., M.H. Baek, B.Y. Chung, S.G. Wi and J.S. Kim, 2004. Alterations in the photosynthetic pigments and antioxidant machineries of red pepper (*Capsicum annuum* L.) seedlings from gamma-irradiated seeds. *J. Plant Biotechnol.*, 47: 314–321
- Kim, J.H., B.Y. Chung, J.S. Kim and S.G. Wi, 2005. Effects of in planta gamma-irradiation on growth, photosynthesis, and antioxidative capacity of red pepper. *J. Plant Biol.*, 48: 47–56
- Kiong, A.L.P., A.G. Lai, S. Hussein and A.R. Harun, 2008. Physiological responses of *Orthosiphon stamineus* plantlets to gamma irradiation. *American Eurasian J. Sustain. Agric.*, 2: 135–149
- Kopsell, D.A., D.E. Kopsell and M.G. Lefsrud, 2007. Pre-harvest cultural growing conditions can influence carotenoid phytochemical concentrations in vegetable crops. *Acta Hort.*, 841: 283–293
- Kovács, E. and A. Keresztes, 2002. Effect of gamma and UV-B/C radiation on plant cells. *Micron*, 33: 199–210
- Kovalchuk, L., J. Molinier, Y. Yao, A. Arkhipov and O. Kovalchuk, 2007. Transcriptome analysis reveals fundamental differences in plant response to acute and chronic exposure to ionizing radiation. *Mutat. Res./Fund. Molec. Mech. Mutagen.*, 624: 101–113
- Kranz, A.R., K.E. Gartenbach and M.W. Zimmermann, 1994. Initial approach to comparative studies on the evolutionary potentials of space radiation effects in a plant system. *Adv. Space Res.*, 14: 383–388
- Ledoux, L., P. Galand and R. Huart, 1962. Nucleic acid and protein metabolism of barley seedlings—III. Effect of X-rays. *Radiat. Bot.*, 2: 119–122
- Lefsrud, M.G., D.A. Kopsell, D.E. Kopsell and J. Curran-Celentano, 2005. Air temperature affects biomass and carotenoid pigment accumulation in kale and spinach grown in a controlled environment. *Hort. Sci.*, 40: 2026–2030
- Legue, B. and A. Chanal, 2010. Mitochondrial free radical generation, oxidative stress and aging. *Free Radical Biol. Med.*, 29: 222–230
- Lichtenthaler, H.K. and A.R. Wellburn, 1983. Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. *Biochem. Soc. Trans.*, 11: 591–592
- Ling, A.P.K., J.Y. Chia, S. Hussein and A. Harun, 2008. Physiological responses of *Citrus sinensis* to gamma irradiation. *World Appl. Sci. J.*, 5: 12–19
- Lott, J.R. and M.J. Wall, 1968. A potometric determination of the effects of X-irradiation on water, K and Ca flux in isolated roots. *Radiat. Bot.*, 8: 37–44

- Macklis, R.M. and B. Bresford, 1991. Radiation hormesis. *J. Nucl. Med.*, 32: 350–359
- Magori, S., A. Tanaka and M. Kawaguchi, 2010. Physically induced mutation: ion beam mutagenesis. In: Kahl, G. and K. Meksem (eds.), *The Handbook of Plant Mutation Screening*, pp: 3–16 WILEY-VCH Verlag GmbH and Co. KGaA, Weinheim
- Marwood, C.A. and B.M. Greenberg, 1996. Effect of supplementary gamma irradiation on chlorophyll synthesis and accumulation of photosystems during chloroplast development in *Spirodela oligorrhiza*. *Photochemistry*, 64: 664–670
- Montelone, B.A., 1998. Free radicals in biology: Oxidative stress and the effects of ionizing radiation. *Int. J. Radiat. Biol.*, 65: 27–33
- Mortazavi, S., P. Mehdi and M. Tanavardi, 2003. The biopositive effects of diagnostic doses of x-rays on growth of *Phaseolus vulgaris* plant: A possibility of new physical fertilizers. *Asian J. Exp. Sci.*, 20: 27–33
- Mortazavi, S.M.J., T. Ikushima, H. Mozdarani and A.A. Sharafi, 1999. Radiation hormesis and adaptive responses induced by low doses of ionizing radiation. *J. Kerman Univ. Med. Sci.*, 6: 50–60
- Patakas, A., N. Nikolaou, E. Zioziuo, K. Radogluo and B. Notisakis, 2002. The role of organic solute and ion accumulation in osmotic adjustment in drought-stressed grape veins. *Plant Sci.*, 163: 361–367
- Pizzimenti, S., C. Toaldo, P. Pettazoni, M.U. Dianzani and G. Barrera, 2010. The "two-faced" effects of reactive oxygen species and the lipid peroxidation product 4-hydroxynonenal in the hallmarks of cancer. *Cancers*, 2: 338–363
- Puchooa, D., 2005. *In vitro* mutation breeding of Anthurium by gamma radiation. *Int. J. Agric. Biol.*, 7: 11–20
- Rao, S. and D. Rao, 1978. Effect of X-irradiation on physiological and morphological variability in *Abelmoschus esculentus* (L.) Moench. *Proc. Indian Acad. Sci.*, 87: 129–133
- Rizzoni, M., E. Vitagliano, M.C. Marconi, A. Sottili and B. Gustavino, 1987. Micronucleus induction by low doses of X-rays in *Vicia faba* root tips. *Mutat. Res./Fund. Molec. Mech. Mutagen.*, 176: 205–209
- Roldan-Arjona, T. and R.R. Ariza, 2009. Repair and tolerance of oxidative DNA damage in plants. *Mutat. Res./Rev. Mutat. Res.*, 681: 169–179
- Roy, R.M., 1974. Transpiration and stomatal opening of X-irradiated broad bean seedlings. *Rad. Bot.*, 14: 179–184
- Roy, R.M., B. Donini and A. Brunori, 1972. Biochemical and cytological studies on developing cotyledons of *Pinus pinea* following X-irradiation of dry seeds. *Rad. Bot.*, 12: 249–260
- Ryan, A., 2005. *Rapid Measurement of Major, Minor and Trace Elements in Plant and Food Material using the Varian 730-ES*. ICP-OES Application Note No. 33, Varian, Inc., <http://www.varianinc.com/image/vimage/docs/applications/apps/io-033.pdf>
- Sedra, M.Y.H. and B.H. Lazrek, 2011. *Fusarium oxysporum* f. sp. *albedinis* toxin characterization and use for selection of resistant date palm to bayoud disease. In: Jain, S.M., J.M. Al-Khayri and D.V. Johnson (eds.), *Date Palm Biotechnology*, pp: 253–270. Springer Science+Business Media BV, Dordrecht, The Netherlands
- Sheppard, S.C. and B.B. Chubey, 1990. Radiation hormesis of field-seeded broccoli, parsnip and cauliflower. *Canadian J. Plant Sci.*, 70: 369–373
- Sheppard, S.C., C.L. Gibb, J.L. Hawkins and W.R. Remphrey, 1987. Investigation of hormetic stimulation in strawberry plants using ionizing radiation. *Canadian J. Plant Sci.*, 67: 1181–1192
- Shima, N. and S. Ichikawa, 1994. Synergisms detected among methyl methanesulfonate, ethyl methanesulfonate and X-rays in inducing somatic mutations in the stamen hairs of *Tradescantia* clone BNL 4430. *Environ. Exp. Bot.*, 34: 393–408
- Shirazi, A., G. Ghobadi and M. Ghazi-Khansari, 2007. A radiobiological review on melatonin: a novel radioprotector. *J. Radiat. Res.*, 48: 263–272
- Shirley, B.W., S. Hanley and H.M. Goodman, 1992. Effects of ionizing radiation on a plant genome: analysis of two arabidopsis transparent testa mutations. *Plant Cell*, 4: 333–347
- Shull, C.A. and J.W. Mitchell, 1933. Simulative effects of X rays on plant growth. *Plant Physiol.*, 8: 287–296
- Slupphaug, G., 2003. Human Uralic- DNA glycosylase and protein inhibitor: protein mimicry of DNA. *Cell*, 82: 701–708
- Sprey, B., 1972. Effect of X radiation on plastid differentiation in primary leaves of barley. *Radiat Bot.*, 12: 399–400
- Srb, V., 1964. Immediate and short-term changes in cell permeability after X-irradiation. *Radiat. Res.*, 21: 308–313
- Srb, V., 1965. Irreversible effects of ionizing RTG-radiation on the osmotic properties of the epidermal cells of *Allium cepa* L. bulbs. *Biol. Plant.*, 7: 270–284
- Strid, A., W.S. Chow and J.M. Anderson, 1990. Effects of supplementary gamma irradiation on photosynthesis in *Pisum sativum*. *Biochim. Biophys. Acta*, 1020: 260–268
- Strzalka, K., A. Kostecka-Guge and D. Latowsk, 2003. Carotenoids and environmental stress in plant: significance of carotenoid-mediated modulation of membrane physical properties. *Russian J. Plant Physiol.*, 50: 168–107
- Tanada, T., 1958. A short-lived effect of x-irradiation on rubidium absorption by excised mung bean roots. *Radiat. Res.*, 9: 552–559
- Watanabe, K., M. Pacher, S. Dukowic, V. Schubert, H. Puchta and I. Schubert, 2009. The structural maintenance of chromosomes 5/6-Complex promotes sister chromatid alignment and homologous recombination after DNA damage in *Arabidopsis thaliana*. *Plant Cell*, 21: 2688–2699
- Wi, S.G., B.Y. Chung, J.S. Kim, J.H. Kim, M.H. Baek, J.W. Lee and Y.S. Kim, 2007. Effects of gamma irradiation on morphological changes and biological responses in plants. *Micron*, 38: 553–564
- Wilson, A., G. Ajlani, J.M. Verbavatz, I. Vass, C.A. Kerfeld and D. Kirilovsky, 2006. A soluble carotenoid protein involved in phycobilisome-related energy dissipation in Cyanobacteria. *Plant Cell*, 18: 992–1007
- Yoshida, S., S. Kinoshita, I. Murata and H. Masui, 1999. Stimulatory of low ionizing radiation on plant. *Nucl. Sci. Technol.*, 49: 19–28
- Zaid, A., 2002. *Date Palm Cultivation*. FAO Plant Production and Protection, Paper No. 156. Food and Agriculture Organisation of the United Nations, Rome, Italy
- Zarcinas, B.A., B. Cartwright and L.R. Spouncer, 1987. Nitric acid digestion and multielement analysis of plant material by inductively coupled plasma spectrometry. *Commun. Soil Sci. Plant Anal.*, 18: 131–146
- Zohary, D. and M. Hopf, 2000. *Domestication of Plants in the Old World: the Origin and Spread of Cultivated Plants in West Asia, Europe and The Nile Valley*, 3rd edition. Oxford University Press, Oxford, UK

(Received 04 October 2011; Accepted 05 December 2011)