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REGULAR ARTICLE

Effect of X-irradiation on date palm seed germination and seedling growth

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Abstract

This study was conducted to examine the effect of low doses of X-rays on date palm (*Phoenix dactylifera* L., cv. Khalas) seed germination and seedling growth. A significant reduction in seed germination was observed in response to X-irradiation as compared to the non-irradiated control. A significant reduction in seed germination percentage (82.85%) began at 0.25 Gy of X-rays and persisted to decline to 61.42% at 15 Gy. Based on accumulative growth percent increase (AGPI), a significant enhancement of root growth was observed in response to increasing X-rays doses. The mean root length increase ranged between 3.7% at 0.05 Gy to 8.05% at 15 Gy after 1 week of X-rays exposure. Similarly after 2 weeks of exposure, a significant increase in root growth started at 0.1 Gy and root growth reaching the highest value (10.94%) at 15 Gy, while root growth reached 15.19% in week 4 at the same dose. A significant increase in leaf length of 0.05-0.25 Gy was observed but at higher doses reduced growth occurred. This study provides an insight into the potential use of X-rays in manipulating growth parameters of date palm and enhances understanding of the physiological responses inflicted by irradiation stress.

Key words: Date palm, Leaf, Radiation, Root, Seed germination, Growth, X-ray

Introduction

The date palm (*Phoenix dactylifera* L.) is one of the most economically important tree species in the arid regions (Alshuaibi, 2011; Al-Abbad et al., 2011). Propagation is normally carried out by offshoots; however, tissue culture techniques are currently used for commercial mass propagation of date palm (Al-Khayri, 2007; Zaid et al., 2011). There are more than 3,000 named cultivars worldwide concentrated in areas between latitudes 10° and 30° North, mostly in the Middle East and North Africa with the greatest production in Iraq, Saudi Arabia, Iran, Pakistan, and Egypt (Johnson, 2011). Worldwide date production has increased exponentially over the last three decades with an annual expansion of about 7%. The total number of date palms in the world has been estimated to be approximately 100 million trees with fruit production of 2.6 million metric tons (Rajmohan, 2011). Date palm production is challenged by numerous diseases and pests (Zaid et al., 2002) especially because of the incompatibility of traditional breeding methodologies with the long generation cycle and the dioecious, heterozygous nature of date palm (El-Hadrami et al., 2011). Modern biotechnological approaches, including radiation technology, are gaining interest to augment the efforts of date palm genetic improvement (Jain, 2011).

The application of nuclear technology for genetic improvement of date palm was demonstrated through inducing mutations by exposing callus to gamma radiation and subsequent *in vitro* selection of plants resistant to Bayoud disease (Jain, 2005; 2007). Studies related to the effect of radiation on date palm physiological and molecular process are limited. However, some physiological responses of date palm seedlings to static magnetic field (SMF) and alternating magnetic field (AMF) were recently elucidated (see review by Dhawi and Al-Khayri, 2011). Magnetic field inflicted modifications in proline content (Dhawi and Khayri, 2008a); chlorophyll (Dhawi and Al-Khayri, 2008b); DNA (Dhawi and Al-Khayri, 2009a); ion content including Mg, Ca, Na, P, K, Fe, Mn and Zn (Dhawi and Al-Khayri, 2009b; Dhawi et al., 2009); and water content as well as growth expressed in fresh weight (Dhawi and Khayri, 2009c). These parameters were also found to be modified by X-rays irradiation of date palm

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seedlings (Al-Enezi and Al-Khayri, 2012a,b). Other studies examined the effects of X-rays radiation on several physiological and biochemical processes in different plant species including cotton, *Gossypium barbadense* (Younis et al., 1962); barley, *Hordeum disticum* (Joshi and Ledoux, 1970); beans, *Vicia faba* (Roy, 1974); wheat, *Triticum aestivum* (Erickson et al., 1979); okra, *Abelmoschus esculentus* (Rao and Rao, 1978); corn, *Zea mays* (Romanova et al., 2000); lotus, *Nelumbo nucifera* (Arunyanart and Soontronyatara, 2002); rocketsalad, *Eruca vesicaria* (Atsushi and Miho, 2006); slender goldenweed, *Haplopappus gracilis* (Tage, 2006); tangerines, *Citrus reticulala* (Palou et al., 2007); as well as green tea, *Camellia sinensis*; sage, *Salvia officinalis*; cinnamon, *Cinnamomum verum* and ginger, *Zingiber officinale* (Al-Nimer and Abdual-lateef, 2009).

Exposure to ionizing radiations, such as X-rays and gamma rays, is suggested to inflict physicochemical stresses thus inducing growth and physiological modifications (Levitt, 1972; Ahloowalia and Maluszynski, 2001). The effects of ionizing radiation are largely damaging and at high doses the effect is detrimental (Ehrenberg, 1955). However, some reports provide evidence of a stimulating effect on growth when seeds or seedlings are exposed to low-dose ionizing radiation (Breslavets 1946; Younis et al., 1962; Zaka et al., 2004; Mortazavi et al., 2006). Growth stimulation in plants by very low doses of ionizing radiation became a widely recognized phenomenon known as *hormesis* (Sheppard and Regitnig, 1987; Sheppard and Chubey, 1990; Macklis and Bresford, 1991; Luckey, 2003). Moreover, the sensitivity to radiation is dependent upon several factors including radiation form and dose as well as plant physiological status, age and genotype (Yoshida et al., 1999; Din et al., 2004; Esnault et al., 2010; Borzouei et al., 2010; Tabasum et al., 2011).

Whereas previous date palm studies investigated the effect of exposure to X-rays at the seedling stage, the current investigation examined the influence of low doses of X-rays irradiation on seed germination and growth of both roots and leaves of the emerging seedlings. Based on the available literature, this is the first study addressing the effect of radiation on seed germination of palms in general.

Materials and Methods

Plant material

Seeds were collected from female date palm trees of cv. Khalas, a mid-season cultivar grown in the Al-Hassa oasis, Saudi Arabia. Seeds were surface sterilized with 1% sodium hypochlorite for 5 min and soaked in water for 24 h prior to irradiation.

Irradiation treatments

Soaked date palm seeds were placed in 9 cm Petri dishes (10 seeds per dish) containing 10 ml distilled water. Using a therapeutic medical X-rays device (Clinac 23EX Linear Accelerator, Varian Medical Systems, USA), seed samples were exposed to different X-rays doses (0, 0.05, 0.1, 0.5, 1, 2.5, 5, 7.5, 12.5, and 15 Gy) using 7 Petri dishes (70 seeds) per treatment.

Growth conditions

Following irradiation the seeds were germinated on moist filter paper at 37 °C. After 2 weeks, the germinated seeds were counted and the seedlings were planted individually in a 20-cm plastic pot containing potting mix (1 soil: 1 peat moss: 1 vermiculite). They were maintained in the greenhouse under natural light at 30-40 °C with a relative humidity of approximately 50% and watered as needed to ensure adequate moisture.

Determination of seed germination percentage

Germination percentage of seeds exposed to X-ray was calculated by using the equation:

Germination percentage = Number of germinating seeds / Total number of seeds × 100

Determination of the growth rate

Growth was calculated by measuring the root length of seedlings and leaves every week for a period of 4 weeks. Growth was calculated as accumulative growth percent increase (AGPI) according to the following equation:

Accumulative growth percent increase (AGPI) = (Final length - Initial length) / (Final length) x 100.

Seedling growth was assessed based on the differences in length between weeks 1 and 2 (AGPI 1), week 2 and 3 (AGPI 2), and week 3 and 4 (AGPI 3).

Statistical analysis

The experiment was randomly designed as a single factor (X-rays dose) with 11 levels and 7 replications (plates), N=77 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 and Discussion

Seed moisture at the time of irradiation plays an important role in the expression of radiation effects (Ohba, 1961; Bhattacharya and Joshi, 1977). Seeds are normally dry and require absorption of water before cellular metabolism and hydrolytic enzymes are activated (Bewley, 1997). To ensure proper moisture to support metabolic activities, the date palm seeds were soaked in water prior to irradiation. This procedure was recommended by Younis et al. (1962) working with cotton seeds. The date palm seeds were also kept in water during irradiation as a precaution to prevent desiccation.

Several studies conducted on different plants types have proven the ability of X-radiation to cause important physiological and morphological changes such as changes in the constituents of medicinal plants (Al-Nimer and Abdual-lateef, 2009), chromosomal aberrations (Moutschen et al., 1987), morphology of axillary buds (Langenauer et al., 1972), plastids variations (Palta and Mehra, 1968), gametophyte generation (Palta and Mehra, 1973) and mutations (Lesley and Lesley, 1965). This is due to high doses of X-radiation which form free radicals, known for their adverse effect on major macromolecules including DNA and proteins (Jade et al., 2010; Legue and Chanal, 2010). Moreover, X-rays were observed to affect root growth (Scandalios, 1964), percentage of seed germination (Sheppard and Chubey, 1990) and leaf growth (Sheppard et al., 1987).

According to the analysis of variance (Table 1), seed germination percentage was affected by X-rays in a significant one-way. The results show that the germination percentage of treated seeds significantly decreased by X-irradiation as compared to the control. The significant reduction in seed germination percentage (82.85%) began at 0.25 Gy of X-rays and continued to decline to up to 61.42% at 15 Gy (Figure 1). Similarly, Dhakshanamoorthy et al. (2010) found that germination of Barbados nut, *Jatropha curcas* L. decreased with the increase of gamma rays at doses ranging from 5 to 25 Kr. Similarly, Hameed et al. (2008) found that the percentage seed germination of chickpea *Cicer arietinum* was inversely related to the irradiation doses of gamma rays tested at 100-1000 Gy. Reduction of seed germination percentage was observed in other different plant including barley, *Hordeum vulgare* (Joshi and Ledoux, 1970); petunia, *Petunia hybrida* (Gilissen, 1978); jack pine, *Pinus banksiana* (Rudolph, 2003); Scots pine, *Pinus silvestris* (Zelles, 2003) and wheat, *Triticum aestivum* (Floris and Anguillesi, 2003). Unlike date palm seeds, germination of broccoli (*Brassica oleracea* L.) seeds was improved at 2 Gy of X-rays but higher doses, 8 and 16 Gy, caused reduction of the germination rate (Sheppard and Chubey, 1990). In contrast, Borzouei et al. (2010) working with wheat (*Triticum aestivum* L.) showed that mean germination time, root and shoot length, and seedling dry weight decreased with increasing radiation doses; whereas, final germination percentage was not significantly affected by gamma radiation doses ranging from 100 to 400 Gy. In a study of hard wheat (*Triticum durum* Desf.), low doses of gamma rays, 5 to 30 Gy, did not affect seed germination, shoot and epicotyl growth (Melki and Marouani, 2009).

Table 1. Analysis of variance for seed germination percentage of date palm after 4 weeks under the influence of different levels of X-rays; P values less than 0.05 are significant.

Factor	df	M S	F	p-value
X- Ray	10	906.0	9.689	0.0001
Error	66	93.5		

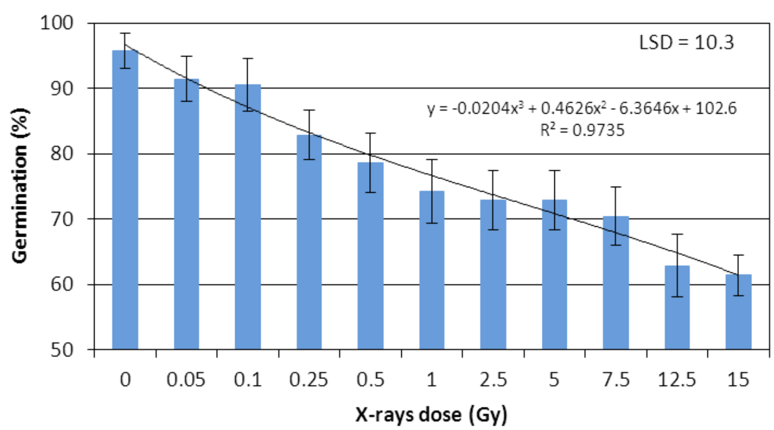

Figure 1. Effect of seed X-rays irradiation on the germination percentage of date palm seeds.

Table 2. Analysis of variance for the roots length of date palm plant under the influence of different levels of X-rays after 1, 2 and 3 weeks; P values less than 0.05 are significant.

Factor	df	MS	F	p-value
Accumulative root length growth increase after 1 week				
X-Ray	10	13.658	70.579	0.0001
Error	66	0.194		
Accumulative root length growth increase after 2 weeks				
X-Ray	10	16.218	16.885	0.0001
Error	66	0.961		
Accumulative root length growth increase after 3 weeks				
X-Ray	10	32.393	18.124	0.0001
Error	66	1.787		

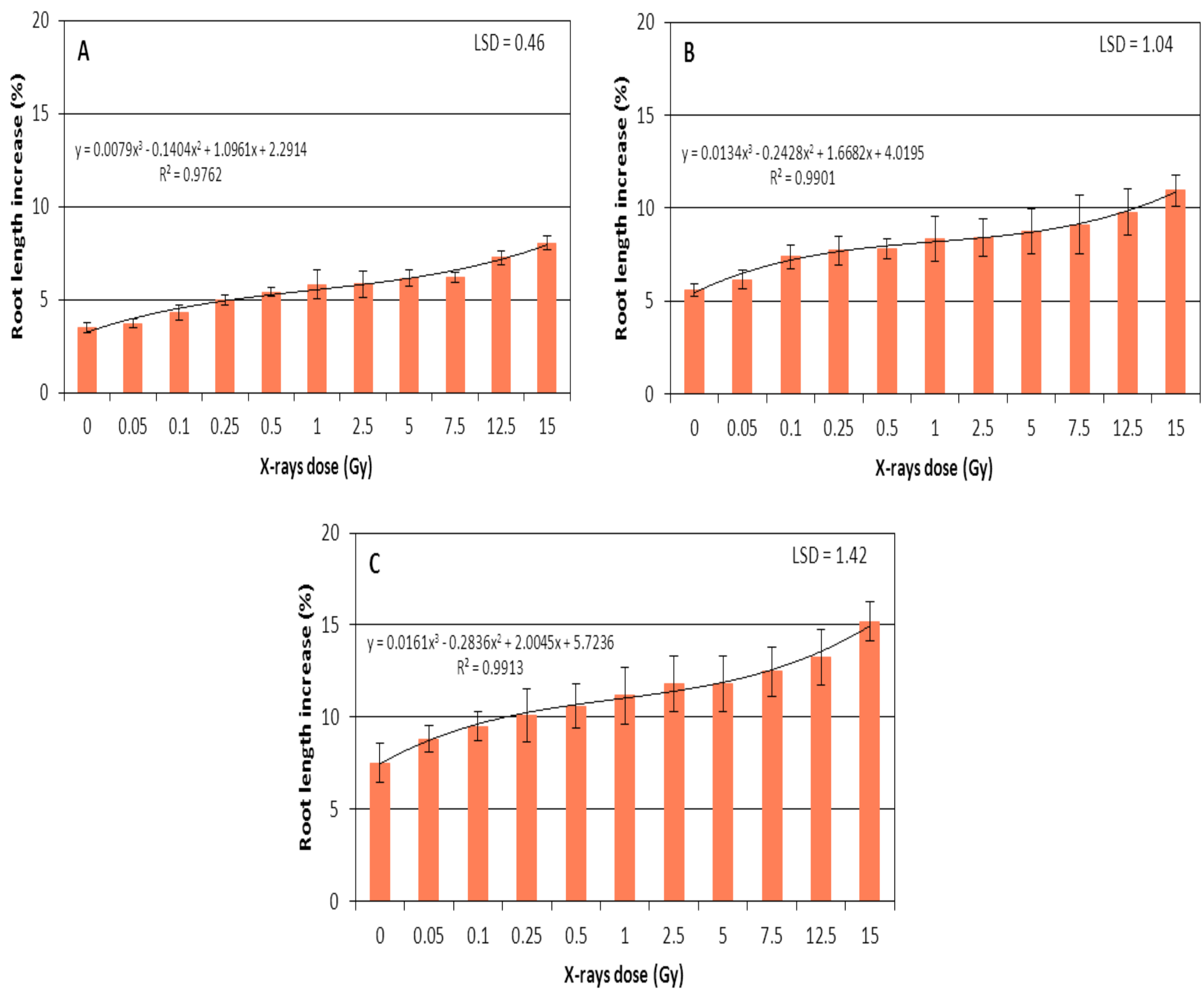

Figure 2. Accumulative growth percent increase (AGPI) of roots of date palm seedlings under the influence of different levels of X-rays measured after 1 week (A), 2 weeks (B), and 3 weeks (C).Table 3: Analysis of variance for leaves

growth of date palm under the influence of different levels of X-rays after 1, 2 and 3 weeks; P values less than 0.05 are significant.

Factors	df	MS	F	p-value
Accumulative leaf length growth increase after 1 week				
X-Ray	10	138.419	104.447	0.001
Error	66	1.325		
Accumulative leaf length growth increase after 2 weeks				
X-Ray	10	159.027	155.158	0.001
Error	66	1.025		
Accumulative leaf length growth increase after 3 weeks				
X-Ray	10	148.18	126.121	0.001
Error	66	1.175		

The analysis of variance for the cumulative roots growth during the 4 weeks indicates a significant increase under the influence of X-rays in one direction, a level of exposure at level of 5% (Table 2). Root growth increased significantly when exposed to different doses of X-rays, and this appears in the first week after exposure and the mean length of roots ranged between 3.7% at the dose of 0.05 Gy to 8.05% at 15 Gy (Figure 2). The significant increase began at 0.1 Gy of X-rays exposures. On the other hand, the high doses of 2.5-15 Gy of X-rays exposures showed apparent differences in the root length among them, with the exception of the doses ranging from 5-7.5 Gy compared to doses of less than 0.05-5 Gy which showed significant statistical differences. Similarly in the second week of exposure, the significant increase in root length began at dose 0.1 Gy and root growth reached the maximum value at 15 Gy (10.94%), while root growth reached 15.19% in the third week at the same dose. In chickpea, 15 Gy of gamma rays induced a significant improvement in root length, nearly 20% more than the control (Melki and Sallami, 2008). Similarly, Melki and Marouani (2009) obtained improvements of 18% to 32% in root number and root length of wheat (*Triticum durum* Desf.) seeds irradiated with 20-Gy gamma dose and concluded that the improvement of root growth might be useful in case of drought.

The results of analysis of variance ANOVA also showed the one-way significant interaction which had an effect on the proportion of the cumulative leaf growth of date palm seedlings exposed to X-ray at 5% level of significant (Table 3). One week after exposure to X-rays, leaf growth of seedlings was enhanced at 0.05 Gy reaching 24.56% more than the control. However, the maximum leaf length occurred at a dose of 0.25 Gy reaching a 30.7% increase as compared to the control. In contrast, at doses ranging from 0.5-15 Gy, significant decreases in leaf length was observed (Figure 3). After 2 weeks of exposure to X-ray radiation, total leaf length under the influence of doses of 0.05-0.5 Gy significantly increased as compared to the control experiment, while the average leaf length showed a significant reduction at doses of 2.5-15 Gy compared to experience control. After 3 weeks, a similar response was observed which clearly showed a significant increase of leaf length at 0.05-0.5 Gy as compared to the control. Whereas, a significant reduction in the leaf length occurred at 5-15 Gy. Although the X-rays led to a significant increase in leaf growth, this increase was only at the low doses, reaching maximum at 0.25 Gy, while high doses led to a significant decrease in the leaf growth. These results are consistent with previous studies which revealed increase leaf growth at low doses of X-rays exposures in other plants such as cotton (Younis et al., 1962) and okra, *Abelmoschus esculentus* (Rao and Rao, 1978). In a related study, Dhakshanamoorthy et al. (2010) found that a stimulatory effect on shoot length, seedling length and vigor index, plant height, petiole length and yield characters in Barbados nut, *Jatropha curcas* plants when seeds were exposed to 5 Kr of gamma radiation while at 25 Kr an inhibitory effect was observed.

Overall, date palm seed germination and root growth were significantly reduced with increasing doses of X-rays radiation; however, leaf growth was enhanced to an optimum level at 0.25 Gy, beyond which growth decreased. In a previous report, a significant increase in DNA content and ion content in date palm seedlings with as low dose as 0.05 Gy was noted, reaching the highest level at 0.25 Gy. This was accompanied by significant reductions of photosynthetic pigments content (chlorophyll a and carotenoids) starting at 0.05 Gy and chlorophyll b reduction at 0.1 Gy (Al-Enezi and Al-Khayri, 2012a). In another study, a direct relationship between the X-rays dose and proline accumulation along with increased fresh weight and water content were observed in irradiated date palm seedlings (Al-Enezi and Al-Khayri, 2012b).

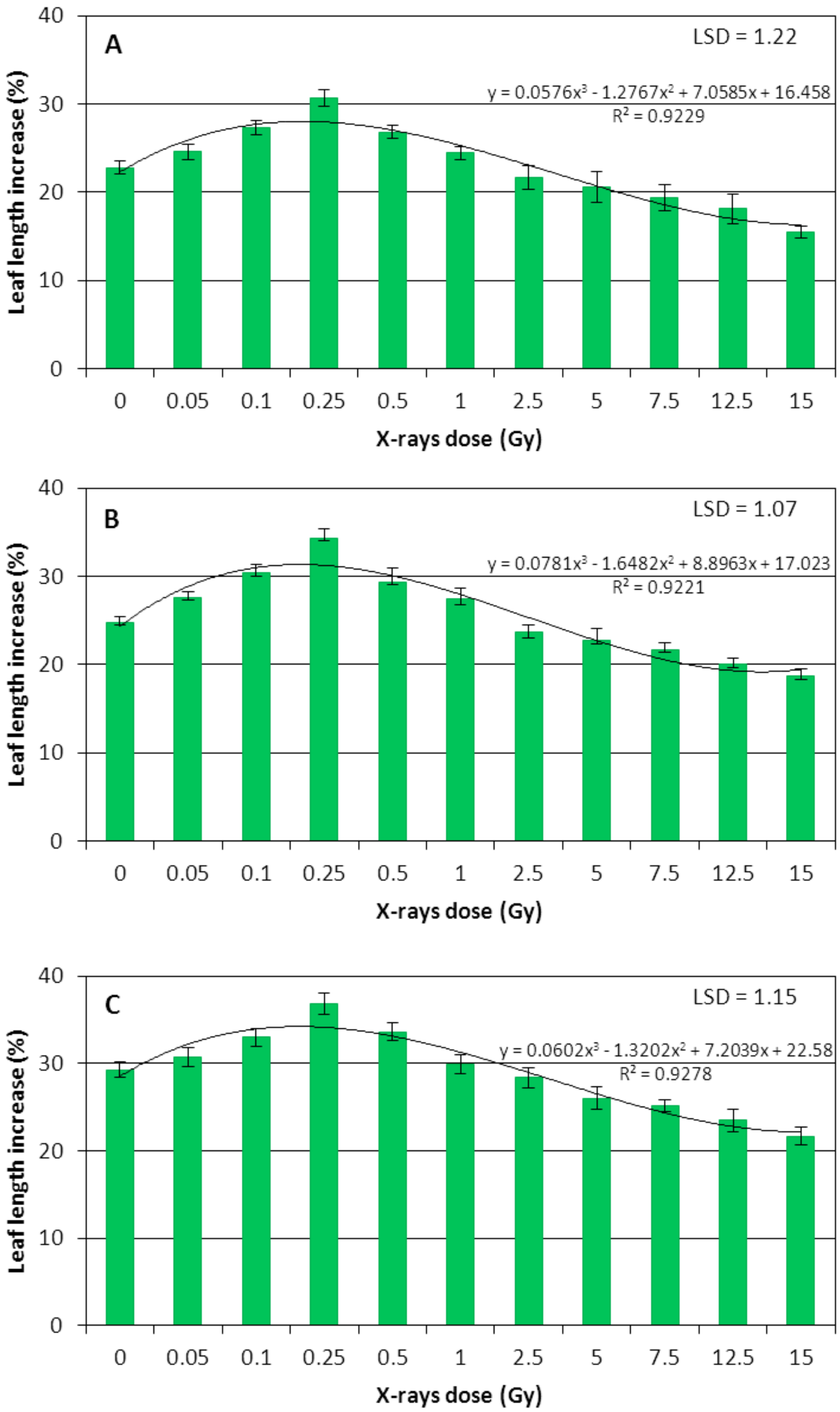

Figure 3. Accumulative growth percent increase (AGPI) of leaves of date palm seedlings under the influence of different levels of X-rays measured after 1 week (A), 2 weeks (B), and 3 weeks (C).

The observed modifications of growth in response to irradiation may be due to changes in membrane permeability, transpiration and the opening of stomata (Roy, 1974). Ionizing radiations can also cause changes in the activity of endogenous plant growth regulators and may in turn influence growth (Maherchandani, 1975). Changes of membrane permeability may reduce the absorption of some nutrients, especially calcium, which plays a major role in various growth and development processes (Sanders et al., 2002; Sze et al., 2000). Reduction in calcium leads to the appearance of yellowed leaves, reduced plant growth, breakdown of cell walls, and an increase of plant sensitivity to pathogens (Medveddev, 2005; White and Broadley, 2003). Ion content increased in response to low doses of X-rays in date palm seedlings; however, doses above 0.25 Gy caused reduction of calcium ions as well as sodium, potassium, phosphorus and magnesium ions in leaf tissue (Al-Enezi and Al-Khayri, 2012a). The current study has shown that treatment with doses above 0.25 Gy also inflected reduction in leaf growth.

In conclusion, the current study has shown that X-rays radiation exerts an inhibitory influence on germination of date palm seeds. Root growth was stimulated in response to increasing doses; however, leaf growth reached a peak at 0.25 Gy, above which growth started to decline. This suggests that root growth is more sensitive to irradiation than leaf growth. Information gained from this study may facilitate the development of strategies for the utilization of X-rays in date palm mutagenesis and physiological studies. Because of genetic variability controlling the sensitivity to irradiation, future studies with various cultivars are essential to further enhance our understanding of date palm responses to irradiation stress. Moreover, investigations involving higher doses are necessary to determine the lethal dose and the induction of mutagenesis to assist genetic improvement of date palm.

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