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Gamma Irradiation Effects on Durum Wheat (*Triticum durum* Desf.) under Various Conditions

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Abstract: The effects on morphological and physiological characters of durum wheat (*Triticum durum* Desf.) plants issued from seeds upon irradiation with low doses of cobalt γ rays (i.e., 0, 10, 20 and 30 Gy), were studied. The study is carried out in the Experimental Research Station of Ecole Supérieure d'Agriculture du Kef (North West Tunisia) in 2008/2009. In Petri dishes, the 20 Gy dose caused an increase of the speed and Germination Capacity (GC) of the seeds as compared to non irradiated ones. Plants from these treated seeds maintained on Knops' culture medium (culture medium used to study plant growth in test tubes), improved root system in terms of length, volume and weight when compared to the plants issued from the non treated seeds. This irradiation dose (20 Gy) also improved in a significant way the above ground system growth of the plants. Under glass house conditions with a water stress, the plants issued from seeds treated with 20 Gy, had higher water content and membrane stability as compared of those from the non irradiated ones. Furthermore, seed irradiation with this dose had a positive effect on the chlorophyll content and maximum quantum yield of the irradiated plants. These results suggest that ionizing irradiation may be considered as an alternative in improving root growth of the plant and therefore controlling drought.

Key words: Water stress, root length, root volume, membrane stability, chlorophyll content, germination capacity

INTRODUCTION

The use of the ionizing radiation technology may be considered as a revolution in agronomic research, especially in the plant protection, plant breeding and crop production (El-Bazza Zainab *et al*., 2000; Jamil and Khan, 2002; Khan *et al*., 2003). Gamma rays in particular, are well known with their effects on the plant growth and development by inducing cytological, physiological and morphological changes in cells and tissues (Thapa, 2004). In a similar study using corn (*Zea mays*) seeds, Singh (1971) showed that irradiation with gamma rays increased the chlorophyll content in the leaves. Several workers reported that when used at low doses, gamma rays have positive effects on the plants since there is an increase in the root depth and growth of these plants: *Oryza sativa* L., *Phaseolus mungo* L. and *Cicer arietinum* (Maity *et al*., 2005; Melki and Sallami, 2008). The same stimulative effects of gamma irradiation on morphological traits were also mentioned by Badr *et al.* (1997), Charbaji and Nabulsi (1999), Irfaq and Nawab (2001) and Klarizze (2005). Melki and Marouani (2009) showed that durum wheat seeds irradiated with 20 Gy generated plants with longer roots. Such improvement resulted in more important soil volumes to be explored by durum wheat plants. Then, they concluded that this phenomenon may help wheat plants to overcome water stress. In this study, the same experiment is performed but by using an artificially caused water stress in order to verify the hypothesis.

MATERIALS AND METHODS

The study is carried out in the Experimental Research Station of Ecole Supérieure d'Agriculture du Kef (North West Tunisia) in 2008/2009. Seeds of durum wheat (*Triticum durum*, Cv Khiar) are used. Seeds are subjected to irradiation in the National Center of Nuclear Technologies of Sidi Thabet (Tunisia) at doses of 0, 10, 20 and 30 Gy using a speed of 0.015 Gy sec^{-1}.

The irradiated and non irradiated seeds used in these studies, are disinfected with commercial Clorox bleach (12% sodium hypochlorite, w/v), rinsed 3 times with distilled water and then sowed in sterile Petri dishes containing moist filter paper (20 seeds $dish^{-1}$). These dishes, which the diameter is 120 mm, are placed in an incubator for 8 days at 20°C. The experiment is performed in a completely randomized design and is repeated 3 times (Petersen, 1985). The germinated seeds are scored in a daily basis and the germinated speed is determined according to the following formula (Chiapusio *et al*., 1997):

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$$S\ (\text{Seeds day}^{-1}) = (N_1 \times 1) + (N_2\text{-}N_1)\times\frac{1}{2} + (N_3\text{-}N_2)\times\frac{1}{3} + ...+ (N_n\text{-}N_{N\text{-}1})\times\frac{1}{n}$$

With N1, N_2, N_3 ...N_{-1} Numbers of germinated seeds observed after 1, 2, 3... n days.

The number of germinated seeds at 8 days is used to determine the GC (Chiapusio *et al*., 1997):

$$GC\ (\%) = \frac{\text{Total No. of germinated seeds}}{20}\times 100$$

The wheat seeds that germinated are placed in 180×17 mm sterile test tubes containing liquid Knops' culture medium (Table 1). Three test tubes are used for each irradiation dose. The test tubes are placed for 3 weeks in an incubator where the temperatures and photoperiod are 25°C in the day and 16°C at night (Hayek *et al*., 2000). Afterwards, the following parameters are measured: Root Volume (RV), Root Length (RL) and Root Dry Matter Weight (RDMW), Leaf Fresh Weight (LFW) and Leaf Dry Matter Weight (LDMW).

The durum wheat seeds irradiated with the different γ ray doses are sown in plastic pots containing greenhouse wet soil (4 kg pot^{-1}). Ten seeds are used in each pot and these pots are maintained in a glass house. The humidity (20%) of the pots is kept at the field capacity level which is obtained by weight adjustment of the pots every other day. When the plants issued from the irradiated seeds are at the 4 leaf stage, they are subjected to a 40% water stress for 10 days. The Relative Water Content (RWC) of the stressed plant is then determined according to Melki and Sallami (2008) according to the following formula:

$$RWC\ (\%) = (FW\text{-}DW)/(WS\text{-}DW)\times 100$$

Where:

FW = Fresh Weights

DW = Dry Weights

WS = Weights at saturation of discs.

The Membrane Integrity (MI) of their cells is calculated using the formula of Blum and Ebercon (1981):

$$MI\ (\%) = 1\text{-}\frac{IC}{FC}\times 100$$

Table 1: Composition of Knops' culture medium (El-Hamdouni *et al*., 2000)

Elements	Quantity (g L^{-1})
$Ca(NO_3)_2$	1.00
KNO_3	0.25
$MgSO_4$	0.25
KH_2PO_4	0.25
$FeCl_3$	0.001

where, IC = Initial Conductivity; IF = Final Conductivity (measured with a conductivity meter Type Hi8033). The maximum quantum yield of photosynthesis (Fv/Fm) of the wheat plant issued from the irradiated and non irradiated seeds is determined using a Fluorescence Indicator Monitor (FIM 1500). The chlorophyll content (CC) is then measured according to a method used by Arnon (1949).

The data collected are computed for statistical analysis using SAS.

The results of statistical analysis may be presented in a table of means, with their Least Significant Difference (LSD).

RESULTS

Table 2 shows the results of the experiments conducted in the laboratory and glass house using the seeds irradiated with different gamma rays doses.

The results of the Petri dish experiment showed that irradiating the seeds with 10 Gy has no effects on their germination capacity, whereas the irradiation with 20 and 30 Gy significantly improved the germination percentage of the seeds as compared to the non irradiated ones (Table 2). The study also showed irradiating the seeds with 10 Gy provoked a decrease in their germination speed as opposed to irradiation with 20 and 30 Gy which caused an increase in the germination speed of seeds by 67 and 54%, respectively (Table 2).

The results of the Knops' culture medium experiment indicates that seed irradiation with 20 Gy caused an important root elongation (14%) of the plants issued from these seeds as compared to those issued from the irradiated ones. However, root depth remains unaffected with the 10 and 30 Gy doses (Table 2). Root elongation of the plants is accompanied with a significant increase of

Table 2: Effects of irradiation with Gamma rays on the behavior of durum wheat in (a) Petri dishes, (b) on Knops' medium and (c) under glass house

	Irradiation doses (Gy)				
Parameters	0	10	20	30	LSD
In petri dishes					
GC (%)	69.7c	69.5c	96.6a	82.7b	9.4836
GS (Seeds day^{-1})	9.5b	7.11c	15.9a	14.7a	1.9549
On Knops' medium					
RV (mL $plant^{-1}$)	0.83c	1.03bc	1.43a	1.16b	0.2404
RL (cm $plant^{-1}$)	12.70ab	11.21b	14.54a	12.70ab	2.7072
RDMW (g $plant^{-1}$)	0.04b	0.04b	0.05a	0.04b	0.0047
LFW (g $plant^{-1}$)	0.64c	0.78b	0.91a	0.83ab	0.0911
LDMW (g $plant^{-1}$)	0.10b	0.12a	0.12a	0.13a	0.0140
Under glass house					
RWC (%)	52.60b	57.16ab	67.03a	60.34ab	10.451
M I (%)	12.31bc	16.56ab	18.40a	11.42c	5.0217
Fv/Fm	0.83b	0.79b	0.91a	0.77b	0.0587
CC (μg g^{-1} fresh weight)	22.08b	20.27b	28.28a	20.53b	4.3919

Same letters are not significantly different at 5% level root volume and fry matter (72 and 25%, respectively) of these plants. It is also important to notice that the irradiation of seeds with 10 and 30 Gy provoked an increase in the fresh and dry matter contents of the above ground parts of the plants issued from these seeds. Consequently, there is a reduction in the RDM/LDM ratios in the plants (Table 2).

The results of the glass house experiment showed seed irradiation with 20 Gy caused a 27% increase in the relative water content of the plants issued from the treated seeds as compared to non treated ones. Additionally, wheat plants issued from seeds irradiated with the different gamma ray doses, maintained more stable membrane structures at 20 Gy than the non treated plants following a severe water stress imposed on them for 10 days. The chlorophyll fluorescence as evaluated by the Fv/Fm ratio, may be used as a simple and rapid indication of the efficient (PS II) photochemical activity (Percival and Sheriffs, 2002). Measures taken at different leaf levels strongly support the efficiency of the 20 Gy dose in increasing the Fv/Fm ratio in the plants issued from the irradiated seeds as compared to those from the non irradiated ones. No variations were evident in the photochemical activities of the plants with the 10 and 30 Gy irradiations doses as compared to the control ones (Table 2). The better Fv/Fm ratio obtained following the durum wheat seed irradiation is an indicator of a better assimilation of the PS II energy at the exited state as well as of a normal functioning of the PS II reaction centers of the plants despite the severe water stress (Kasraoui *et al*., 2006; Ykhlef and Djekoun, 2000) with the 20 Gy dose, the plants generated from the irradiated seeds and subjected to water stress for 10 days, were able to maintain their chlorophyll content 28% higher than those issued from the non irradiated seeds. An equilibrium between the degraded and elaborated chlorophylls may have been reached in such this case.

DISCUSSION

The present study confirms the hormesis phenomenon (Szarek, 2005) and proposes seed treatment of durum wheat with low doses of such rays. This may be considered as a new way to control water stress for short periods. In fact, the 20 Gy irradiation dose with these rays improved the growth as well as the root length and volume of wheat plants issued from the irradiated seeds, thus enhancing the absorption of water and mineral salt that are needed for the plant survival as was reported by Brown *et al.* (1987). This irradiation dose also increased the GC and speed of the seeds due to the denaturation with gamma rays of the enzymes implicated in the inhibition of germination. This study shows also that some physiological characters are affected as a result of gamma rays irradiation in durum wheat seeds since a tolerance to water stress is evident in the plants irradiated seeds as compared to those from the irradiated ones. Furthermore, cells of the plants obtained from seeds irradiated with 20 Gy, have higher water contents (27%) than those from the control ones. This is in accordance to a report indicating that high water content in the plant tissues has protective effects on the protein structures and membranes of the cells (Zerrad *et al*., 2008). Several investigations have been conducted on this topic and showed that the conservation of the fluorescence extinction activity as well as the efficiency of the photochemical activity of the (PS II), are powerful indicators of the plant tolerance to water stress (Ernez and Lannoye, 1991; Kasraoui *et al*., 2006). The high chlorophyll content in the plants issued from irradiated seeds is another indisputable proof of the photosynthetic efficiency of the irradiated plant despite the intensive water stress. Some workers recently reported positive correlations between the photosynthetic activity and chlorophyll content in plants (Bettaieb *et al*., 2008).

The study of the behavior of durum wheat (*Triticum durum*, Cv Khiar) issued from seeds irradiated with 20 Gy (Gamma rays) and subjected to water stress, shows that some physiological and morphological characters of the treated plants are significantly affected: The changes contributed efficiently in improving durum wheat ability to overcome water shortage in semi arid areas.

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