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Studies the Effects of Low Dose of Gamma Rays on the Behaviour of Chickpea under Various Conditions

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Abstract: In this study, the irradiation effects spring chickpea seeds with low doses (0, 5, 10, 15, 20 and 25 Gy) of radioactive cobalt (^{60}Co) γ-rays, on the germination characteristics as well as on the root and shoot growth, are investigated. The effects of such irradiation doses on the relative water content and cell membrane stability following a water deficit, are also studied. The irradiated seeds kept their germination speed and capacity in Petri dishes. On Murashige and Skoog (MS) liquid medium, the dose of 15 Gy induced a significant improvement (nearly 20%) in root length as compared with the 0 Gy dose. Under glass house conditions, the root and shoot lengths and dry weights of plants grown from seeds irradiated with a dose of 15 Gy, are found to be improved at rates of 19 and 89%, respectively when compared with plants issued from non-irradiated seeds. The same irradiation dose allowed the plants subjected to a water deficit to maintain a better water level and a more stable cell membrane as compared to the control plants.

Key words: Irradiation, chickpea, roots, water deficit

INTRODUCTION

There are several usages of nuclear techniques in agriculture. In plant improvement, the irradiation of seeds may cause genetic variability that enable plant breeders to select new genotypes with improved characteristics such as precocity (Salado-Navaroo *et al*., 1986), salinity tolerance (Ibrahim, 1998), grain yield and quality (Sobieh and Mustapha, 1998; Neeraj *et al*., 1998).

Ionizing radiations are also used to sterilize some agricultural products in order to increase their conservation time (Kader, 1986) or to reduce pathogen propagation when trading these products within the same country or from country to country (Moy, 1983).

On the physiological level, several factors may be involved in the inhibition of germination and the growth of the plants issued from seeds following their exposure to high irradiation doses (Thapa, 2004; Sadhan, 2002; Cengiz *et al*., 2005), although the stimulated growth of plants issued from seeds that have been exposed to low irradiation doses was reported by Charbaji and Nabulsi (1999), Alarkon *et al*. (1987), Raghuvanshi (1992) and Moussa (2006).

In this study, radio-stimulation effects on the root and shoot growth of spring chickpea (*Cicer arietinum*) is investigated in order to explore new ways that would improve drought tolerance. Spring chickpea yields are often hindered by water deficiency and this is the main reason of using this species as a plant material.

MATERIALS AND METHODS

This study was carried out in the Experimental Station of the Higher School of Agriculture of Kef (North-Western of Tunisia) in 2007/2008.

Study of germination efficiency and speed: Spring chickpea seeds (cv. Amdoun 1) irradiated with different γ-ray doses (0, 5, 10, 15, 20 and 25 Gy) at the rate of 0.0151 Gy sec^{-1}, are disinfected before placing them in Petri dishes containing filter papers humidified with distilled water (10 mL $dish^{-1}$). Ten seeds are used for each irradiation dose and a completely randomized design with 6 replications (6 boxes) is observed in this experiment. These boxes are then placed in an incubator for 8 days at 25°C. The germinating seeds are scored on a daily basis to measure the germination speed (S) using the following formula (Chiapusio *et al*., 1997):

$$S\ (\text{seed day}^{-1}) = (N_1 \times 1)+(N_2-N_1)\times 1/2+(N_3-N_2)\times 1/3+\ldots+(N_n-N_{n-1})\times 1/n$$

with

$N_1, N_2, N_3 \ldots, N_{n-1}, N_n$ = Proportion of germinated seeds observed at 1, 2, 3…, n-1 and n days

The germination capacity is measured on the 8th day using the formula:

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$$\text{Germination} = \frac{\text{(Total No. of germinated seeds)}}{10} \times 100$$

Investigation of the root and shoot growth: The pre-germinated chickpea seeds are transplanted in 18×1.7 cm glass tubes (one seed/tube) containing 34 mL modified MS liquid medium (Table 1). Thirty tubes and 5 irradiation levels are used in this experiment which is repeated 3 times. These tubes are maintained in an incubator with temperatures of 25°C daily and 16°C nightly, a photoperiod of 12/24 h and a light intensity of 20,000 lux. Ten days of incubation under these conditions, the root lengths of the plants issued from the seeds are measured.

In parallel to this study, another one is carried out in 10×50 cm PVC tubes containing approximately 4.5 kg of dried soil and maintained in a glasshouse. Three seeds are placed in each tube and these tubes are distributed in 3 blocks using a completely randomized block design model (18 tubes block^{-1}). The tubes are divided into six batches corresponding to the irradiation doses in each bloc. No fertilizers are added since the experiment is of short time duration. The tubes are maintained to field capacity for one month by weighting them daily. The plants then are pulled out of the tubes, their roots rinsed to remove soil and their lengths measured. The upper and lower parts of each plant are dried separately at 105°C for 24 h and their dry matter contents determined.

Study of the relative water content and membrane integrity: Chick pea seeds irradiated with different γ-ray doses are placed in pots containing 3.6 kg of soil each and maintained at 3% relative humidity, in a completely randomized model with 3 repetitions. These pots are kept at field capacity for 20 days. Following germination, only 3 plants are saved in order to be subjected to a water deficit (30% of field capacity) for 5 days. The Relative Water Content (RWC) and membrane integrity of the plants were the determined.

Determination of the Relative Water Content (RWC): Following 5 days of water stress, 8 discs measuring 5 mm each, are excised from the 4th leaf of each plant and the Fresh Weights (FW) of these discs are immediately determined. The discs then are placed in vials containing 10 mL distilled water and kept in the dark at 5°C. Ten hours later, the discs are removed from the vials and placed between 2 layers of filter paper to remove water excess and their weights at saturation (WS) were determined. Finally, the discs are placed in an oven maintained at 100°C for 24 h and their Dry Weights (DW) are determined. The Relative Water Content (RWC) each disc is calculated using the following formula:

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

Table 1: MS liquid medium composition (Murashige and Skoog, 1962)

Ingredients	Concentration (mg L^{-1})
NH_4NO_3	1.600
H_3BO_3	6.200
$CoCl_2.6H_2O$	0.025
$MgSO_4.7H_2O$	370.000
$CuSO_4.5H_2O$	0.025
KH_2PO_4	170.000
KNO_3	1.900
$ZnSO_4.7H_2O$	8.600
EDTA	37.200
Sequesterene*	93.000
pH	5.700

*: Instead of $FeSO_4$ EDTA

Determination of the membrane integrity: Eight leaf discs are excised from the 6th leaf of each plant. These discs are washed 3 times with distilled water, placed in vials containing 10 mL distilled water and incubated at 10°C for 24 h.

Using a water bath and a conductimeter (Hi 8033), these samples are equilibrated at 25°C and their initial conductivity (CI) is measured. These same samples are autoclaved at 120°C for 20 min, equilibrated at 25°C in a water bath and their Final Conductivity (CF) measured. The membrane integrity is determined by using the following formula:

$$\text{Integrity (\%)} = 1-(\text{CI/CF})\times 100$$

RESULTS AND DISCUSSION

Investigation of the germination: Figure 1 shows that regardless of the irradiation dose, the germination speed and capacity of *Cicer arietinum* seeds were not affected

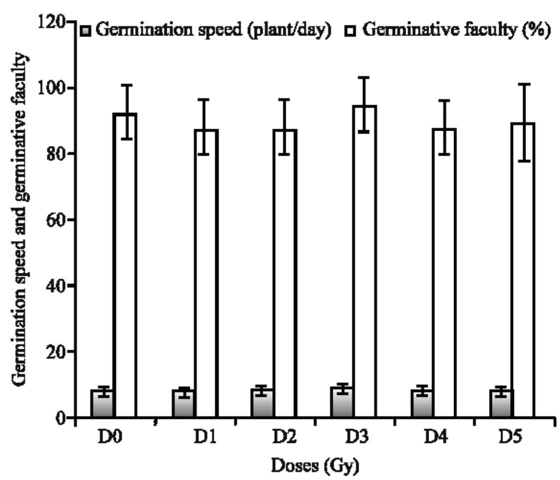

Fig. 1: Effect of irradiation on germination speed and germinative faculty (D0 = 0 Gy; D1 = 5 Gy; D2 = 10 Gy; D3 = 15 Gy; D4 = 20 Gy; D5 = 25 Gy). The bar chart represents the average±SE (n= 6)

when compared to the non-irradiated ones. Seed irradiation with γ-rays for agronomic purposes was applied to winter chickpea seeds prior to sowing, using 10 Gy in order to improve the symbiotic nitrogen fixation in soils with high mineral nitrogen content (Kurdali *et al*., 2000). This method may be recommended provided that it does not interfere with the germination characteristics of chickpea. The effects the irradiation with γ-ray doses ≤25 Gy on the chickpea seed germination seem to be reassuring if such a technique is to be adopted.

Investigation of the growth of roots and shoots: In Murashige and Skoog (1962) liquid medium (Fig. 2), a significant effect ($p<0.05$) of the irradiation dose on the root length is observed. The 15 Gy dose gave an improvement of nearly 20% in root length in comparison with that of the control (0 Gy). Similarly, the root length of chickpea plant issued from seeds exposed to 5, 10, 20 and 25 Gy, was nearly the same as that of the control plants (0 Gy).

The same phenomenon was almost reproduced with chickpea plants grown in soil containing tubes and maintained in a glass house. The root length of plants issued from seeds irradiated with 5, 20 and 25 Gy did not change when compared to that of the control ones. However, increases ($p<0.01$) ranging from 19 and 17%

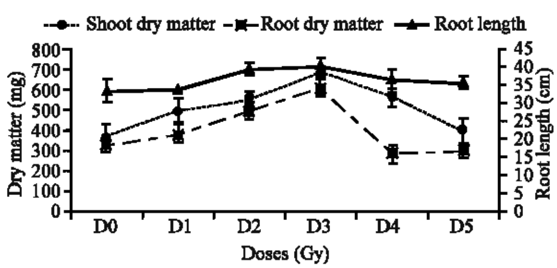

Fig. 2: Effect of irradiation on root length and on root and shoot dry matter under glass house (D0 = 0 Gy; D1 = 5 Gy; D2 = 10 Gy; D3 = 15 Gy; D4 = 20 Gy; D5 = 25 Gy)

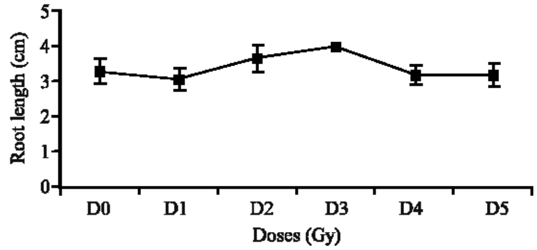

Fig. 3: Effect of irradiation on the root length in MS liquid environment (D0 = 0 Gy; D1 = 5 Gy; D2 = 10 Gy; D3 = 15 Gy; D4 = 20 Gy; D5 = 25 Gy)

are obtained in the plants issued from seeds exposed respectively to 15 and 10 Gy (Fig. 3). The stimulation and elongation of the roots are mainly caused by the 15 Gy dose and to a lesser degree by the 10 Gy. This is reflected by the significant improvement in the dry matter content of the roots as compared to the control ($p<0.01$). Nevertheless, it seems that the 20 and 25 Gy doses produced less dry matter content in the roots of the irradiated plants as compared to those issued from non-irradiated seeds, although the observed differences are not significant. Moreover, these results show that the shoot dry weight is significantly improved in comparison to the control in the interval of 5 to 20 Gy, with a maximum improvement reached at 15 Gy.

The radio-stimulation found in these investigations and brought about by low doses, is not a specific to chickpea phenomenon. Many researchers reported similar results following the exposure to low ionizing radiation doses of different species such as *Pinus* sp. (Thapa, 2004), *Anacardium occidentalis* (Klarizze, 2005), *Vitis vinifera* (Charbaji and Nabulsi, 1999), *Eruca vesicaria* (Moussa, 2006) and *Pisium sativum* (Zaka *et al*., 2004).

The hypothetical origins of this stimulation are reported to be the acceleration in cell division (Zaka *et al*., 2004) and the activation of the growth regulator auxin (Gunckel and Saparow, 1961). The results of this study may lead to inquire about the role if any, of the stimulation in chickpea root elongation (by exposure of the seeds to 15 Gy) that may play in water absorption under water deficiency conditions.

Effect of the irradiation on the relative water content: The plants issued from seeds irradiated with 15 Gy and subjected to a severe water stress (30% of field capacity) showed a RWC significantly higher (36.82%) than that of the non-irradiated ones but subjected to the same water stress. Irradiation with 10 Gy also, gave a 19.24% increase in the RWC. However, the treatment of chickpea seeds with 20 and 25 Gy caused important RWC decreases of 24.20 and 38.25%, respectively as compared to plants grown from the non-irradiated seeds (Table 2).

Table 2: Effect of the irradiation on the relative water content of chickpea plants (expressed as % of the non irradiated)

	Irradiation doses (Gy)					
	0	5	10	15	20	25
Percentage	100	101.25ns	119.24**	136.82**	75.80**	61.75**

**Significant at a level of 1% (as compared to the non irradiated) and ns: Non significant at a level of 1% (as compared to the non irradiated)

Table 3: Effect of the irradiation on the membrane integrity of chickpea plants (expressed as % of the non irradiated)

	Irradiation doses (Gy)					
	0	5	10	15	20	25
Percentage	100	100.62ns	126.17*	117.61ns	96.45 ns	78.37*

*Significant at a level of 5% (as compared to the non irradiated) and ns: Non significant at a level of 5% (as compared to the non irradiated)

Effects of the irradiation on the membrane integrity: A water stress provoked a destruction of cell walls and therefore the liberation of electrolytes. There is a relationship between the quantity of the liberated electrolytes and the membrane integrity. Plants that are more tolerant to drought are able to maintain their membranes intact following water deficit and fewer electrolytes will leak outside their cells.

Table 3 shows that in chickpea plants issued from seeds irradiated with 10 and 15 Gy and subjected to a severe water deficit, the membrane integrity is improved (26.17 and 17.61%, respectively) as compared to the plants issued from non-irradiated seeds and subjected to the same stress. However, seeds treated with doses higher than 15 Gy gave plants that exhibited cell membrane damages more and more important with increasing irradiation doses.

CONCLUSION

The most notable result in this study is indisputably the improvement of the root growth in the chickpea plants issued from seeds subjected to irradiation with ^{60}Co γ-rays at a dose of 15 Gy. The improvement of the root system with this radio-stimulation probably is due the availability of more soil volumes to the plants to explore and therefore support better water shortages by keeping more hydrated tissues and less damaged cell membranes at least during their first development stages.

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