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RESEARCH ARTICLE

Effect of Gamma irradiation on morphological, biochemical, physiological character and cytological studies, of durum wheat mutants

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Abstract

This investigation was carried to determine the effect of Gamma radiation on germination and physiological characteristic of 6 varieties of durum wheat, 3 of them (Gta/dur (v1), Djenah khotifa(v2), Benimestina(v3)) was irradiated with different doses (10, 25, 50Gy) and three others (Waha(v4), Goumgoumrkham(v5), Mridj(v6)) was mutated with high dose (100, 180, 220Gy), the percentage of germination increased in seedling irradiated with 25Gy and 100Gy as compared to non irradiated one, plants maintained on Knops culture medium, improved with this same dose the root and shoot length, volume and weight as compared to non irradiated one, and others doses. Chlorophyll a was higher than chlorophyll b in both irradiated and non irradiated seeds. The mitotic index was also studied; it has been decreased with increasing doses of gamma radiation. The protein study by SDS PAGE (sodium dodecyl sulfate Polyacrylamide gel electrophoresis) generated a proteic polymorphism with different profiles: absence of certain bands as compared to control and presence of new bands, after inducing water stress by PEG(*polyéthylène glycol)* 6000 at 15% in culture medium. This result showed that the regulation of certain physiological parameters and the growth of plants after their irradiation with gamma can be used for the abiotic control like drought, and the inhibitory effects of gamma rays on the mitotic index indicated that gamma rays have mutagenic effects on embryonic roots of wheat.

INTRODUCTION

Triticum durum, also known as durum wheat is the only tetraploid wheat commercially important which is widely cultivated today (Shamsi IH, *et al*.,2006), Durum wheat is mainly grown under rained conditions where the frequency of droughts combined with heat stress is the main factor limiting grain yield (Araus *et al.,* 2002, 2003a,b; Condon *et al*., 2004). Unfortunately the constraints abiotic as the drought decreases the growth of the wheat and its productivity, drought is a major constraint in the agricultural production in numerous developing countries who limits agricultural crops, In particular the wheat in the world, especially in Algeria, the direct and indirect economic losses in the farming sector because of the drought are enormous , besides, the recent climate change requires the development of cultures more tolerant to the drought (J A de Ronde, *et al.*, 2004) The improvement for the tolerance to water stress is considered, these last years, as one of the first objectives of the programs of agronomic selection (Damania, 1991,Damania A.B., 1991). A single way of fighting against the drought is to develop cultures of agricultural importance which are more tolerant in the water stress by combining the vegetable physiology and the biotechnological techniques; this will be by the induction of mutation.

The use of the mutagenesis during the last 50 years played a major role in the development of superior-quality varieties around the world (S.Mohan Jain, 2010) with the induction of mutation; it is possible to improve a single line without causing an important disturbance in the genome. The use of the mutagenesis in the improvement of the cultures proved to be an effective approach to improve the yield, the quality and the resistance in biotic and abiotic stress (Bibi *et al.*,2009, Nichterlain *et al.,* 2000). So, the mutation induction can play an essential role in the improvement of the complex quantitative characters, including the tolerance in abiotic stress.
In this study; the effects of the irradiation gamma on roots and leaves were study, with the aim of exploring new ways for the tolerance to the drought.

MATERIALS AND METHODS

Plant material

Seeds of 6 varieties are treated with different dose of Gamma 60Co three varieties with: 10Gy, 25Gy, 50Gy, three others with 100Gy, 180Gy, 220Gy; using a speed of 2.48Gy/sec, in COMMENA centre of Algiers.
The irradiated and non irradiated seeds used in this experimentation are disinfected with sodium hypochlorite 12%, and rinsed 3 times with distilled water.

Prégermination

The seeds are sowed in sterile Petri dishes containing moist filter paper (30 seeds $dish^{-1}$). These dishes are placed in an incubator for 5 days at 20°C. The experiment is performed in a completely randomized design and is repeated 3 times (Petersen, 1985); the Number of germinated seeds was recorded during 5 days, and the speed of germination was determined as follows: (Chiapusio *et al*.,1997)

$$S(\text{seeds day}^{-1}) = (N_1 \times 1) + (N_2 - N_1)x1/2 + (N_3-N_2)x1/3 + \ldots + (N_n-N_{n-1})x1/n$$

With N1,N2, N3… numbers of germinated seeds observed after 1,2,3… days
The numbers of germinated seeds at 5 days is used to determine the GC (Chiapusio *et al.,* 1997)

$$GC\ (\%) = \text{total No of germinated seeds}/30 * 100$$

Hydroponic culture

The germinated seeds are placed in sterile tube containing Knops culture medium(table1), for each treatment three repetition are used, they are placed in culture room were the temperatures and photoperiod are 25°C in the day and 16°C at night (Hayek *et al*., 2000)
This parameters are measured: root length, root volume, root dry matter, root fresh matter, leaf fresh matter, leaf dry matter, dosage of chlorophyll a , b and total

Determination of chlorophyll content

Chlorophyll content of irradiated and non-irradiated plantlets was determined, the irradiated and non irradiated one are homogenized in 80% acetone and centrifuged at 10000g for 10min, and the supernatant were subjected to spectrophotometric determination of chlorophyll at 646nm and 663nm. The chlorophyll *a* (C*a*) *and b* chlorophyll *b* (C*b*) content in milligram per liter was determined according to the formulae below and further expressed in milligram per gram fresh weight of plant material(kiong, 2008)
Chlorophyll *a*, Ca = 12.25 (OD663) – 2.79 (OD646)
Chlorophyll *b*, C b = 21.50 (OD646) – 5.10 (OD663)
Total chlorophyll, Ca + C b = 7.15 (OD663) + 18.71 (OD646)

Induction of water stress

A water stress is induce after the measurement of parameters with PEG6000 at 15% three days

Determination of total protein

The extraction of total protein was done homogenized a determined weight of each leaf sample using mortar and pestle. With tris-HCL0.1M tampon (ph7.2), containing Bmercaptoéthanol 1%(v/v) and glycerol 5%(v/v), the obtained ground material are centrifuged at 4500g at 4C for 15 min, the supernatant are conserved at 20C until their utilization.
SDS-PAGE was carried out on protein extracts of irradiated and non-irradiated plantlets to determine the molecular weight profile of proteins.

Table 1: composition of medium culture KNOPS(El-Hamdouni *et al*., 2000)

Elements	quantities (g/l)
Ca(NO3)2	**1.00**
KNO3	**0.25**
MgSO4	**0.25**
KH2PO4	**0.25**
FeCl3	**0.001**

Cytogenetic studies The mitotic index test

Irradiated and non irradiated seeds are placed in distilled water for an hour at room culture; then each group of seed is transferred on Petri dishes for 24h to 48h approximately 21C; seeds germinated are taken, and root tips were cut length of 1-1.5 cm to prepare for mitotic index.

Root are treated with glacial water 24h, then fixed in 3:1 ethanol/acetic acid, for 24h, they are after rinsed 3 times for 5min each, transferred to alcohol 70%, and stored in a refrigerator until they were used.

The roots are hydrolyzed in 1NHCL for 6min at 65C in water bath, placed them in carmin acetic solution1h, Chromosome spreads were made by using the squash technique (Dille, *et al.*,1983 Dille, *et al.*,1986) the mitosis frequency is determined in irradiated and non irradiated seeds by the counting the number of dividing cells in total of 100cels with 3 repetitions.

Statistical analysis

The statistical analysis consist on an analysis of variance, comparison of the results of statistical analysis may be presented in a table of means, with their Least Significant Difference (LSD

RESULTS

The results of Petri dishes show that dose 50Gy of seeds v1 have no effects on germination capacity, but those irradiated with 25Gy and 100Gy increase the GC as compared to control, for v2 all the doses don't have effect on germination capacity.

This study shows that dose 10Gy causes a decrease of speed germination for v1 v3, as opposed to v2 where the dose increase the speed germination, for high doses the results show that CG were different for the three variety, seeds germinated more than control with dose 180Gy and 220Gy for v4, thus for v6, while for v5 the three dose don't increase the CG, for v6, 100Gy increase the GC as compared to 220Gy witch a generate a lower rate of seeds germinated. For the speed of germination, the results were correlated with GC, where 180Gy were the best for v4, v5, while for the speed, it increase for the seeds irradiated with 100Gy.

Results of knops medium culture indicate that irradiated seeds with 25Gy for v1 v3 generate an important elongation of root, while for v2 it was 50Gy which causes an elongation of root, it was accompanied with volume of root, where v1 and v3 absorb more water when irradiated with 25Gy, as compared to v2 were the absorption were better with 50Gy. For v4 and v6, elongation of root was interrupted in sample treated with 100Gy, unlike the v5 where this same dose provoke a important elongation of root, which was confirmed with volume of root system where the seeds treated with this dose absorb more water, while for 220Gy the root have almost absorb water, anyway it was found in fresh and dry weight of root and leaf.

Effect of Gamma rays on weight of leaf and root showed an important difference between v3 v2 v1, while high dose have inhibited the growth, which results in the weight, thus a difference between 100Gy and 180Gy for three varieties. The weight of root and leaf were important in 180Gy compared to others dose, but they have small root compared to control. The volume of the root system reflects an extension and / or branching of the root system that promotes colonization of a larger volume of soil, making more accessible to the plant water reserves of the soil. Varieties BM and GTA irradiated with 25Gy are distinguished by an increase of more RV, The variety DK for the same dose showed a smaller increase.

For the cytogenetic studies, results of mitotic index of control and Gamma mutant (10, 25,50Gy) et (100, 180, 220Gy) are shown in table 2 and 3, the greater frequency of IM is found in the control of all varieties and mutants of 25Gy, unlike the higher dose where is recorded the lowest frequency (220Gy). The difference between control and mutants (p0.05) is significant. Mitotic index decreases every time the dose of gamma rays increases the statistical analysis of these results make to several groups.

Data presented in figure 5 and 6 of chlorophyll content, indicated that there was significant difference between plants from irradiated seeds and controls in chlorophyll contents, the results show that chlorophyll a and b content was significantly increased in v2 with dose d3, and in v3 with the same dose, with v1 there is no significant difference. With high dose, the chlorophyll content decreased in some dose and increase in others like in variety v5

with 10Gy the total chlorophyll increase, the same with chlorophyll a and b. the irradiation of seeds save to modify metabolism and photosynthetic capacity.
Chlorophyll a was higher than b in genotype and groups (irradiated and non irradiated one), seedling exposed to 25Gy in V2 and 100Gy in v5 recorded highly significant changes in chlorophyll a, b and total chlorophyll content.

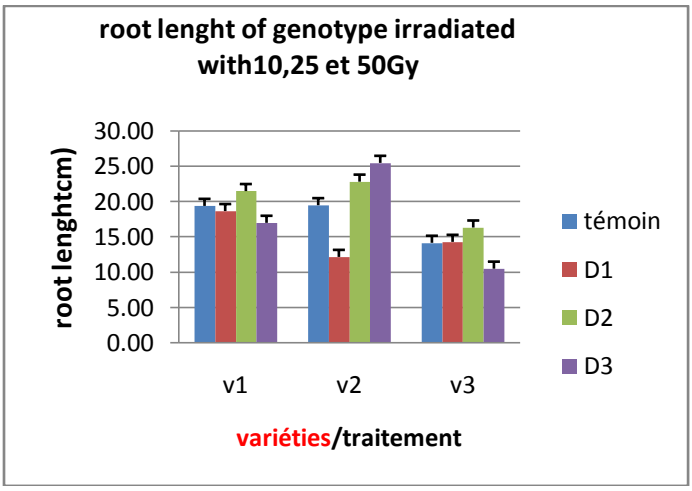

Fig 1 : root length scored for genotype v1 v2 v3 treated with Gamma

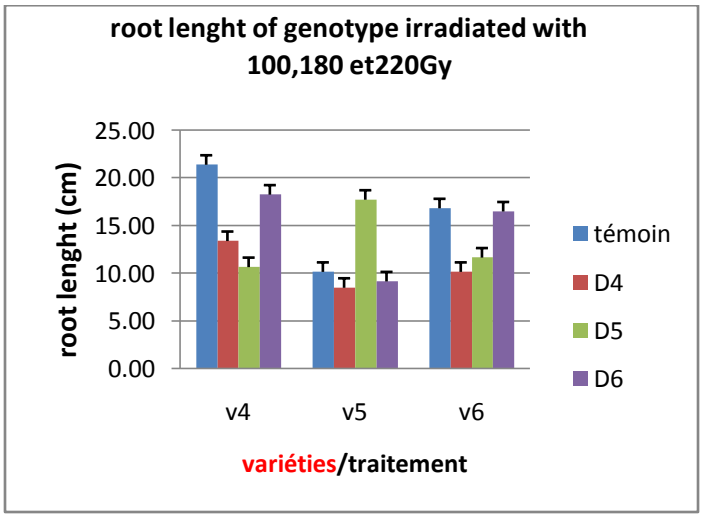

Fig 2 : root length scored for genotype v4 v5 v6 treated with Gamma

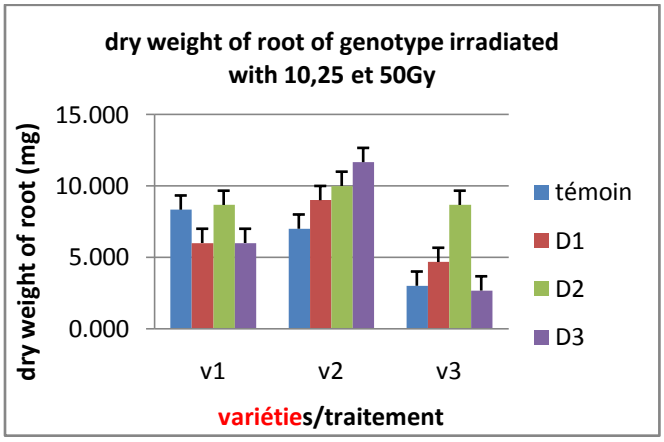

Fig 3 dry weight of root scored in v1 v2 v3 treated with Gamma rays

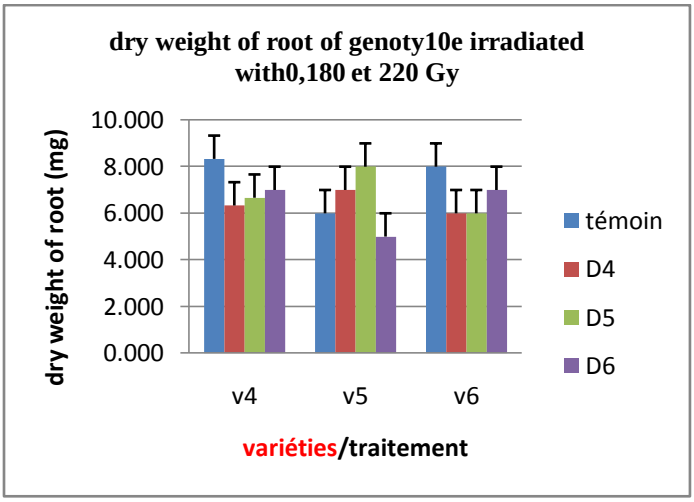

Fig 4: dry weight of root scored in v1 v2 v3 treated with Gamma rays

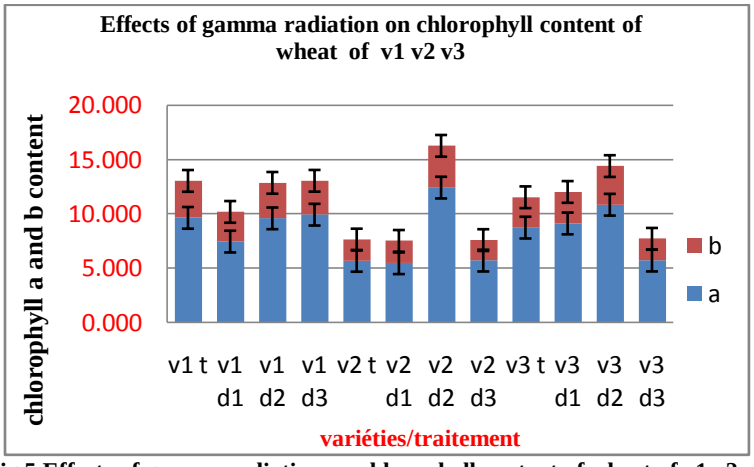

Fig 5 Effects of gamma radiation on chlorophyll content of wheat of v1 v2 v3

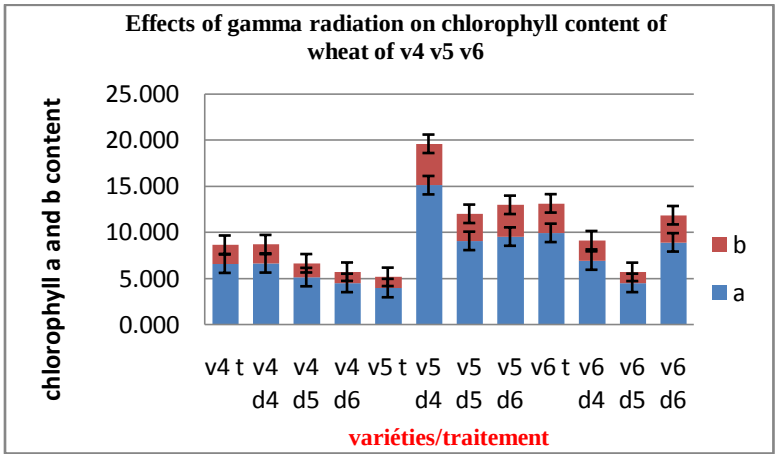

Fig 6 Effects of gamma radiation on chlorophyll content of wheat of v4 v5 v6

Electrophoretic profils

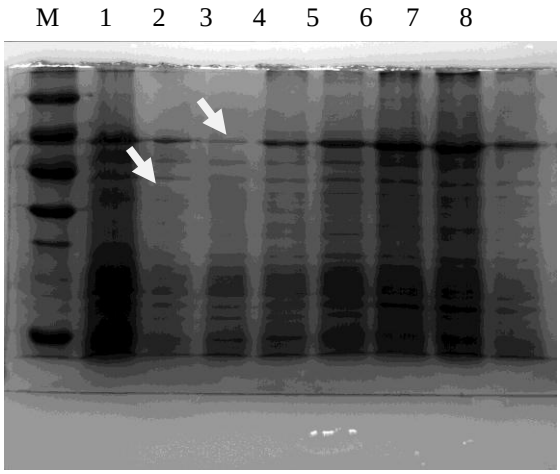

Fig.7 electrophoretic profils of foliaire proteins identified by SDS PAGE V1(1-T ;2-M100Gy, 3-M180Gy, 4-M220Gy) et V2 (5-T, 6-M10Gy, 7-M25Gy, 8-M50Gy)

Table 2 : effect of gamma rays (10, 25, 50 Gy), on germination rate (GR), root length (RT), dry and fresh weight of root and leaf(LFW, RFW, LDMW, RDMW), volume root system(RV) and mitotic index (MI) of three variéty GD; BM and DK

parameters	V1 GD				V2 BM				V3 DK			
	t	**D1**	**D2**	**D3**	**t**	**D1**	**D2**	**D3**	**t**	**D1**	**D2**	**D3**
GR												
GS	18.3	18.1	19.9	16.1	15.5	21.33	17.83	17.66	15.85	13.58	18.36	11.58
GC%	96.66	96.66	83.33	86.66	99	100	98.9	99.3	96.66	83.33	90	80
RL	19.40^{abcd}	19.5^{abcd}	14.17^{cde}	18.67^{abcd}	12.17^{de}	14.3^{cde}	21.5^{abc}	22.83^{ab}	16.33^{bcde}	17^{bcde}	25.5^{a}	10.50^{e}
LFW	126.6^{ef}	293.3^{b}	196.6^{cde}	126.6^{ef}	436.6^{a}	173.3^{cde}	253.3^{bc}	470.0^{a}	230.0^{bcd}	156.6^{def}	500.0^{a}	80.0^{f}
LDMW	23.6^{cde}	30.33^{c}	19.6^{de}	16.0^{e}	50.3^{ab}	17.33^{e}	41.67^{b}	52.3^{a}	28.0^{cd}	26.3^{cd}	57.3^{a}	29.0^{c}
RFW	356.6^{ab}	210.0^{cd}	76.6^{f}	166.6^{de}	280.0^{bc}	103.3^{ef}	285.0^{bc}	336.6^{ab}	90.33^{ef}	213.3^{cd}	373.3^{a}	46.6^{f}
RDMW	8.33^{ab}	7.0^{bc}	3.0^{cd}	6.0^{bcd}	7.0^{bc}	6.6^{bcd}	8.66^{ab}	10.0^{ab}	8.66^{ab}	6.0^{bcd}	11.6^{a}	2.6^{d}
RV	3.6^{Abc}	2.03^{de}	0.9^{f}	2.2^{d}	2.7^{cd}	1.1^{ef}	3.30^{bc}	3.80^{ab}	1.03^{f}	$2.6C^{d}$	4.367^{a}	0.7^{f}
MI	98^{a}	91.66^{abc}	95.66^{ab}	86.66^{cde}	91.66^{abc}	93.66^{ab}	92.66^{abc}	83.00^{e}	91.66^{abc}	89.66^{bcd}	90.66^{bc}	84.00^{de}

Values are mean at 95%

Table 3 : effect of gamma rays (100, 180, 220 Gy), on germination rate (GR), root length (RT), dry and fresh weight of root and leaf(LFW, RFW, LDMW, RDMW), volume root system(RV) and mitotic index (MI) of three variéty W, GGR and M

parameters	V4 W				V5 GRR				V6M			
	t	**D1**	**D2**	**D3**	**t**	**D1**	**D2**	**D3**	**t**	**D1**	**D2**	**D3**
GR GS GC%	 12.26 93.33	 15.43 93.33	 15.86 100	 15.23 100	 15.2 93.33	 14.2 83.33	 16.9 90	 12 73.33	 16 93.33	 19 96.66	 16 86.66	 15 73.33
RL	21.40^{a}	10.2^{e}	16.8^{abc}	13.4^{bcde}	8.5^{e}	10.2^{e}	10.6^{de}	17.73^{ab}	11.6^{cde}	18.26^{ab}	9.2^{e}	16.5^{abcd}
LFW	366.6^{ab}	273.3^{bcde}	303.3^{abcd}	340.0^{abc}	$246.6C^{def}$	320.0^{abcd}	286.6^{abcd}	156.6^{f}	180^{ef}	383.3^{a}	273.3^{bcde}	223.3^{def}
LDMW	37.0^{ab}	44.3^{a}	38.6^{ab}	38.3^{ab}	45.0^{a}	39.3^{ab}	30.6^{bc}	41.0^{ab}	19.0^{c}	37.6^{ab}	37.3^{ab}	36.3^{ab}
RFW	$203..3^{bc}$	226.6^{abc}	293.3a	213.0^{abc}	210.0^{abc}	210.0^{abc}	166.6^{c}	186.6^{bc}	163.3^{c}	223.3^{abc}	256.6ab	220.0^{abc}
RDMW	8.3^{a}	6.0^{a}	8.0^{a}	6.3^{a}	7.0^{a}	6.3^{a}	6.6^{a}	7.6^{a}	6.0^{a}	7.0^{a}	5.0^{a}	7.0^{a}
RV	2.8^{bcd}	2.8^{bcd}	3.5^{ab}	2.3^{cd}	3.4^{ab}	3.6^{ab}	2.3^{cd}	3.1^{abc}	2.1^{d}	3.4^{ab}	3.8^{a}	3.3^{ab}
MI	97.66a	97.33^{ab}	90.00^{c}	73.66^{f}	95.33^{abc}	94.66^{abc}	89.00^{cd}	79.00^{ef}	94.66^{abc}	95.00^{abc}	91.00^{bc}	83.66^{de}

Value are mean at 95%

DISCUSSION

results of this study may lead to more information on the role of exposure wheat to radiation on stimulation of root elongation, The 25Gy dose improves the growth ,the length and volume of the wheat root, thus enhancing the absorption of water and minerals which are necessary for the survival of the plant under drought stress, Radio-stimulation found in this study for low doses is not special for wheat, the presence of a low toxic dose triggers some self-repair mechanism in the cell, these mechanisms once activated are sufficient not only to neutralize the initial effect of radiation but also repair other defects that radiation did not cause, the origin of this stimulation was postponed to be an acceleration in cell differentiation (Zaka *et al.*, 2004), and activation of growth regulators Auxine (Gunckel and Sparrow, 1961), Which is the case for the 10 Gy dose where there is no differences, the plant is self-regulated following irradiation with this dose, similar results in exposure to low doses of different species: *pinus sp.* (Thapa, 2004),*snacardium accidentalis*(Klarizze,2005), *vitisvinfia*(Charbaji and Nabulsi, 1999),*eruca vesicaria*(Moussa, 2006) and chickpea(Mongui et Salami 2008).

The 25Gy dose increases the CG and the speed of germination which is due to the denaturation with gamma radiation of the enzymes involved in the inhibition of germination. For dose more than 100Gy irradiated seeds had no significant differences compared to the control, or this dose affected the growth of the roots, and leaves, the results of Kong *et al*.(2008) have shown that plants survived to maturity depends on the nature and extent of chromosomal damage. The increase of these lesions is enhanced by increasing doses of irradiation subsequently reducing the growth and survival of the plant. Chaudhuri (2008) reported that with high dose of irradiation the germination percentage decrease it was demonstrate in this study, with the elongation of leaf and root. Work of Chaomi and Yanlin (1993) on wheat(*Triticum aestivum*) mark that treatment of seeds with high doses decrease the germinate capacity, so irradiation increase the sensibility of plant to Gamma rays, who can be caused by the reduction of growth regulators especially the cytokinine(Kiong, 2008). The statistical analysis show several groups of each parameter.

The genetic information must be the same for the entire organism cell, but mutations can induce variations caused differences in cell level. The cytogenetic test of mitotic index is used to examine the effects of genotoxicity and effect on mutagenesis in different environment, this test is used to characterize the proliferating cell and identify the material who inhibit or induce progression of mitosis; in this study, the inhibition of mitotic activity following irradiation by different dose of Gamma on root of durum wheat, the differences of irradiated and non irradiated one was statistically significantly (0.05), increase in concentration of rays, decrease significantly the mitotic index as compared to control. This increase of dosage was showed in *Hordeum vulgare*, *Vicia faba*, *Zea mays*, *Secale cerale*, it was confirmed in this study, just for 25Gy dose, the mitotic index increase or stagnates, it was proved with the elongation and weight of root.

Inhibition of mitotic index by Gamma rays indicate that Gamma rays has a mutagen effect on root of durum wheat, it was showed for: *hordeum vulgare*(Yasemin Eroglu 2007), these results show that gamma rays are capable to create a new mutant with desired characters.

At increasing of dose of gamma rays, the pigment of chlorophyll decrease, but in some variety they increase, Wada *et al., (*1998) stated that high dose causes the inhibition of growth, chlorophyll degradation and morphological aberration, they modify metabolism, and photosynthetic capacity.

Other researchers that after gamma rays with high dos, plant at the late development stage of seed maturation suffer leaf senescence and lose cellular component (Craft Brandner 1987, Barcaccia 2001).

In this study, the chlorophyll content of gamma irradiated wheat displayed a gradual decrement as the gamma dosage increase. In addition to that, it can be observed that the concentration of chlorophyll a is higher than chlorophyll b in both irradiated and non irradiated plants, that was relatively in previous literatures, it has been reported that gamma rays resulted in greater reduction in the amount of chlorophyll b as opposed to a (Strid 1990), but other statement contradict our results were chlorophyll b is higher than a.

The reduction in chlorophyll b is due to a more selective destruction of chlorophyll b biosynthesis or degradation of chlorophyll b precursors (Marwood and Greenberg, 1996). Gamma radiation induces various physiological and biochemical alteration in plants.

The irradiation of plants with high dose of gamma rays disturbs the hormone balance, leaf gas-exchange, water exchange and enzyme activity (Kiong *et al*., 2008)

In the course of this study, we have conduct experimentation based on the analysis of total protein extract from leaves of 6 varieties and their mutants.

Total soluble protein of irradiated and non-irradiated one showed some differences, especially intensity, depending on the gamma dose irradiation.

We detect a polymorphism following profiles obtained by the extract of total protein, submitted to an electrophoresis on polyacrylamid gel; when used provided data by observation of presence or absence of bands, 168 bands were detected for the three genotype GTA/DUR, DK, BM, their molecular weight varied between 13.250 and 103.667 Kda, and 140 bands for others genotype irradiated with 100,180,220 Gy.

Certain bands present in controls grain, and absent in irradiated one, but others bands were expressed just in irradiated, example the band 13.603 kda was present in control of GTA/DUR and GTA/DUR irradiated with

10,25Gy, differences in intensity of bands was observed, intensity of band is directly attached with the concentration of proteins(JASSO *et al.;* 2002).
The mutation induces changement in proteic profiles, some proteins were inhibited, others were expressed, and others were appeared comparing to control.
Intensity of bands was different comparing between varieties, control and doses especially in high doses were the intensity is low with dose 220Gy.
It was apparent that there is bands relatively more intense comparing to those irradiated with 100 and 220Gy.
It could be deduced from SDS PAGE analysis that the proteins profiles of irradiated and non irradiated plantels varied slightly, major bands were observed in both irradiated and non irradiated one, and the intensities differ according to gamma's dosage.
Increasing Gamma dosage causes degradation of proteins. In our study, SDS PAGE showed no difference in the protein banding profiles between the irradiated and non irradiated one, there is some bands absent other extra bands, changes in intensity, but it's not significative, this results are similar to those of Kiong (2008).

CONCLUSION

Results of this research show that various doses of the irradiation Gamma have different effects on physiological and biochemical parameters, As the increase of the germination capacity, the presence of new proteic bands; It is clear that the mutagenesis can be used for production of mutants which are more tolerant to environmental stress. The induction of mutations is necessary to increase the genetic variability. Mutagenesis is able to isolate mutant with multiple characters, as compared to transgenese where only line can be introduced, it's the major advantage of induce mutations

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