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Gamma Irradiation Effects on Some Physiological Traits of Wheat (*Triticum aestivum* L.) under Control and Water Stress Conditions

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ABSTRACT

This study assessed the physiological changes in wheat plants exposed to gamma radiation and then subjected to water stress for 10 days. Seeds of wheat cultivar Hidhab and M4 mutant populations obtained previously by gamma rays treatment of cultivar Hidhab with 150 and 200 Gy were carried out in the green house at University of Bab-Ezzour (North Algeria) in 2012-2013. In water stress conditions, the 150 Gy dose caused a significant increase of total chlorophyll (30.13%) and proline content (31.48%) compared to non irradiated plants. Also the data show both treatments had a significant effect on Relative Water Content (RWC), where the high values of water content (48.57 and 54.10%) were recorded at 150 and 200 Gy, respectively under water stress. Overall, most of the physiological traits showed an improvement under gamma effect, now it is clear that the physical agents such as gamma rays can be used to enhance wheat cultivars in harsh conditions.

Key words: *Triticum aestivum*, gamma irradiation, water stress, physiological traits, induced mutation

INTRODUCTION

In Mediterranean areas especially in Algeria the climate is characterized by a low rainfall sometime these conditions cause a dramatic reduction in wheat yield, however, in Algeria wheat production is not sufficient to meet the demands of a growing population. The gap between production and demand is increasing. The average consumption of wheat is estimated at more than 190 kg/years per person, where a total requirement is around 7.3 million tons for a population more than 38.7 million. Moreover, improvement of drought tolerance is among objectives for breeders in these regions. Wheat as a strategic crop in Algeria, according to the statistics of the ONS (2009), total area in Algeria under bread wheat cultivation is around 587 thousand hectares with annual production of 9.521 million quintals and average yield of 16.3 q. ha^{-1}. To improve yield and other traits in wheat crop, many breeding techniques are being used successfully. Mutation breeding is one of the important techniques to induce variation. More than 3000 varieties of different crops have been officially released by mutation breeding technique (Laghari *et al*., 2012). Gamma irradiation is considered as physical mutagen imposing considerable effects on physiological and biochemical processes in plants (Begum and Dasgupta, 2011; Heidarieh *et al*., 2012). The irradiation of seeds may cause genetic variability that enable plant breeders to select new genotypes by improving characteristics such as precocity, tolerance to drought, grain yield and quality (Ashraf *et al*., 2003). The mutants developed in wheat had great potential for direct release and to include them in cross breeding program (Sakin *et al*., 2005).

Thus, the present study, aimed to assess whether gamma rays treatment would induce mutations that may improve some physiological characters in wheat plants and therefore to overcome water stress.

MATERIALS AND METHODS

Plant materials and growing conditions: Grains of wheat (*Triticum aestivum* L.) cultivar HD_{1220} known among the farmers as Hidhab were obtained from Biotechnology and Plant Breeding Laboratory of the National Agronomic Research Institute of Algeria (INRAA), Baraki. The experiment had been made on advanced mutants lines (M4) carried out in the green house at University of Bab-Ezzour (North Algeria) in 2012-2013. Ten seeds irradiated and non irradiated (control) were germinated in petri dishes, after germination seedlings transplanted into plastic pots. The water stress treatment was imposed when the plants were at 4 leaf stage. The control plants were irrigated normally, whereas those under stress, the irrigation were stopped for 10 days. The experiment had three replications in a Randomized Complete Blocks Design (RCBD).

Following the standard terminology used in induced mutation programs; seeds prior to mutagenic treatment are termed M_0 and after treatment are referred to as M_1. Seeds that develop on the M_1 plants are therefore the M_2 generation which develops into M_2 plants (Forster and Shu, 2012) up to obtained M_4 mutant plants that are developed from M_4 mutant generation seeds.

Irradiation treatments: The seeds of this cultivar were exposed to irradiation with 60 cobalt emitting gamma rays at doses of 150 and 200 Gy (Gray) using a speed of 13.01 Gy min^{-1} the time periods were 11.31 and 15.22 min for 150 and 200 Gy, respectively. The seeds were irradiated at the Algiers Nuclear Research Center (CRNA) located in center of Algeria.

Chlorophyll content: The chlorophyll content was determined as described by Lichtenthaler (1987). Leaf fresh weight (20 mg) was grinded into powder in the eppendorf tubes with 2 mL of 80% acetone and centrifuged at 1000×g for 10 min. The supernatant was subjected to spectrophotometer determination of chlorophyll content. Absorbance of chlorophyll a (Chl a) and chlorophyll b (Chl b) were recorded at 645, 652 and 663 nm.

The concentrations were determined according the following equations (Lichtenthaller, 1987):

$$\text{Chl a } (\mu g\ mL^{-1}) = 12.7\times(OD663)\text{-}2.7\times(OD647)$$

$$\text{Chl b } (\mu g\ mL^{-1}) = 22.9\times(OD647)\text{-}4.7\times(OD663)$$

$$\text{Chl a+Chl b} = \text{Chl total } (\mu g\ mL^{-1}) = 20.2\times(OD647)+8.02\times OD663) \text{ or}$$
$$\text{Chl total} = (OD\ 652)/34.5\ (\mu g\ mL^{-1})$$

Relative Water Content (RWC): The relative water contents of the stressed plants is determined according to the following equation (Clarke and McCaig, 1982):

$$RWC\ (\%) = \frac{[(FW - DW)}{(FW_S - DW)]} \times 100$$

Where:
FW = Fresh weight
DW = Dry weight
FW_S = Fresh weight at saturation

Proline determination: The proline amount in the leaves was determined according to the method of Troll and Lindsley (1955) improved by Magne and Laher (1992). Samples of 50 mg leaf Dried Weight (DW) were homogenized in 1 mL in distilled water in eppendorf tubes which are placed in a water bath at 100°C for 30 min. Then centrifuged at 13000×g for 15 min and supernatant was separated. The extraction was repeated for 2nd time and the second supernatant is added to the first one.

Two milliliter of acid ninhydrin reagent (1 g ninhydrin/100 mL of a solution containing 60 mL glacial acetic acid and 40 mL distilled water) were added to 1 mL of the extract. The closed test tubes were kept in a water bath for 30 min, after cooling the reaction mixture, 3 mL toluene was added and mixed thoroughly, the chromophore containing toluene was separated and absorption was read at 520 nm. The proline concentration was calculated on dry weight basis (mg g^{-1} DW).

Statistical analysis: The experimental design was analysed through randomized complete block design. The factor was gamma irradiation (3 levels) with three replications. The data statistically analyzed using GenStat Discovery ANOVA one-way (in randomized blocks) and the treatment means were compared with Least Significant Difference (LSD) at p = 0.05 significance level.

RESULTS

Chlorophyll content: As showed in Fig. 1 the amount of chlorophyll a was higher than chlorophyll b in both treatments (control and water stress). Under well watered conditions, chlorophyll b was significantly decreased 32.23 and 6.61%, respectively at 150 and 200 Gy compared to the plants issued from non irradiated seeds. However, this decreasing was not significant effect on total chlorophyll content (Table 1).

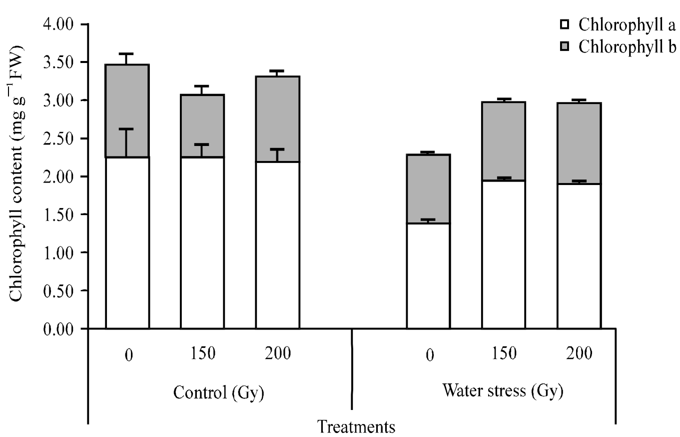

Fig. 1: Effects of gamma irradiation on chlorophyll content of wheat (*Triticum aestivum* L.) under well watered and water stress

Table 1: Impact of different gamma doses (0, 150 and 200 Gy) on chlorophyll a, b and total chlorophyll under normal and water stress conditions

	Chlorophyll a (mg g^{-1} FW)		Chlorophyll b (mg g^{-1} FW)		Total chlorophyll (mg g^{-1} FW)	
Dose (Gy)	Control	Water stress	Control	Water stress	Control	Water stress
0	2.26	1.39^{b}	1.21^{a}	0.89^{b}	3.48	2.29^{b}
150	2.24	1.94^{a}	0.82^{b}	1.040^{a}	3.07	2.98^{a}
200	2.18	1.90^{a}	1.13^{a}	1.06^{a}	3.31	2.97^{a}
F-test	0.973ns	<0.001***	0.04*	0.009**	0.67ns	<0.001***
LSD (0.05)	1.06	0.05	0.29	0.08	1.23	0.10
CV (%)	8.40	3.30	15.30	3.40	10.10	3.30
SE (±)	0.27	0.01	0.07	0.02	0.31	0.02

Mean values followed by different letters in same column, are significantly different according to the LSD test at *p<0.05; **p<0.01; ***p<0.001, SE: Standard error, ns: Not significant

Table 2: Effects of gamma treatments on relative water content (RWC), total chlorophyll content and proline content under well watered and water stress

	RWC (%)		Total chlorophyll content (mg g^{-1} FW)		Proline content (mg g^{-1} DW)	
Irradiation doses (Gy)	Control	Water stress	Control	Water stress	Control	Water stress
0	71.04	31.46^{b}	3.48	2.29	0.189^{a}	1.078^{b}
150	74.07	48.57^{a}	3.07	2.98	0.036^{b}	1.423^{a}
200	63.82	54.10^{a}	3.31	2.97	0.044^{b}	0.626^{c}
F-test	0.44	0.04	0.67	<0.001	0.008	0.002
LSD (0.05)	20.86ns	16.69*	1.23^{ns}	0.10***	0.070**	0.24**
CV (%)	8.40	7.20	10.10	3.30	17.100	7.40
SE (±)	5.31	4.25	0.31	0.02	0.010	0.06

*Mean values followed by different letters in same column, are significantly different according to the LSD test at *p<0.05; **p<0.01; ***p<0.001, SE: Standard error, ns: Not significant

Under water stress condition, plants germinated from irradiated seeds have recorded high change in chlorophyll a, b and the increasing was of 39.56 and 16.85%, respectively at 150 Gy compared to non irradiated ones.

Relative Water Content (RWC): Under well watered conditions (control), the highest RWC was observed in the plants germinated from irradiated seeds with 150 Gy (74.07%) but this result was not significantly different from non irradiated ones (Table 2). In contrast, the interaction of radiation and water stress condition has imposed a significant effect on RWC (Table 2). Maximum RWC was obtained for the plants irradiated seeds at 200 Gy (54.10%) while RWC of 150 Gy plants (48.57%) were in statistically similar group (Table 2).

Proline content: The results (Fig. 2) show an increase of the proline content which is even more pronounced in water stress conditions especially at the dose 150 Gy (31.48%) when compared to the non irradiated ones. But the plants germinated from irradiated seeds with 200 Gy exhibited the lowest rate of free proline (41.66%) during stress period compared to non irradiated seedlings.

Comparing the results of Relative Water Content (RWC) and proline content (Fig. 3). It was stated that the lowering of RWC is followed by osmolyte accumulation such as proline in response to stress.

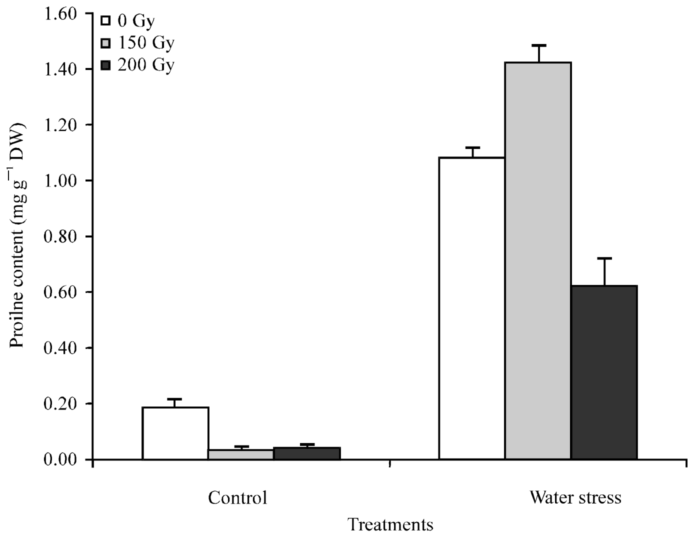

Fig. 2: Effects of gamma doses (0, 150 and 200 Gy) on proline content of wheat (*Triticum aestivum* L.) under well watered and water stress condition

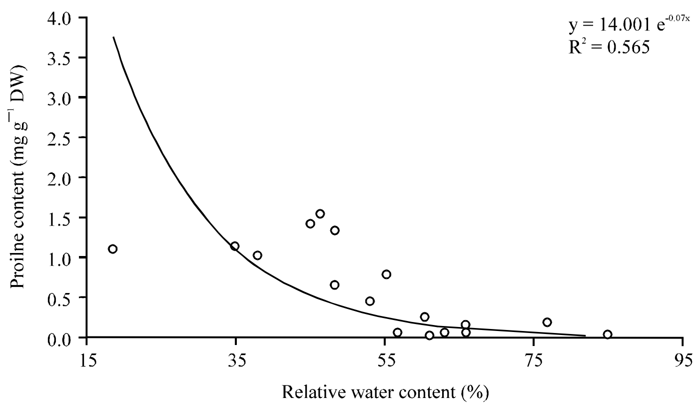

Fig. 3: Relation between Relative Water Content (RWC) and proline concentration in wheat (*Triticum aestivum* L.) under well watered and water stress

DISCUSSION

The plants generated from the irradiated seeds and subjected to water stress for 10 days, were able to maintain their total chlorophyll content higher (30.13% at 150 Gy and 29.69% at 200 Gy) than those germinated from the non-irradiated seeds. Furthermore, Borzouei *et al.* (2010) have evaluated the chlorophyll content on irradiated wheat seedling and they reported that, total chlorophyll increased 64.5% at 100 Gy, which can be correlated with stimulated growth. These results are in agreement with present findings.

According to the data showed in this study (Table 1) it can be concluded that gamma radiation rate at 150 and 200 Gy may improve the total chlorophyll content, it is another indisputable proof of the photosynthetic efficiency of irradiated plants despite the water stress.

The data in the Table 2 shows that some physiological traits are affected as result of gamma irradiation in bread wheat since a tolerance to water stress is evident in the plants irradiated seeds as compared to those from non-irradiated ones. This is in accordance to a report indicating that high water content in the plant tissues has protective effects on protein structures and membranes of cells (Zerrad *et al.*, 2008).

Proline accumulation has been demonstrated in many species and in different situations of stress (osmotic, water, heat) as osmolyte involved in protective mechanisms during abiotic stress including gamma irradiation (Al-Rumaih and Al-Rumaih, 2008). It is considered as compatible solute that have many properties such as osmoregulators, hydrophilicity, protection of macromolecules, active oxygen scavengers and the involvement in the system maintaining the cell pH-stat (Kuznetsove and Shevyakova, 2007). The hydrophilicity of proline and other compatible solutes play great role to placed water molecules around nucleic acid, proteins and membranes during drought period. Therefore, the interaction of proline and altered proteins causes increase in the stability of proteins (Irigoyen *et al.*, 1992).

When plants are subjected to water stress they increase their rate of proline, this enables to improve the capacity of the cell to maintain its turgor pressure at low water potential. This appears to be essential for physiological processes such as photosynthesis, enzyme activity and cell expansion (Tyree and Jarvi, 1982).

On the other hand, there are other authors reported that the increase in the level of proline can be a symptom of stress resulting from an imbalance in other metabolic pathways. However, group of plants irradiated at 200 Gy exhibited a low concentration of free proline under water stress compared to non-irradiated but they have highest RWC, it means this group was least sensitive to water stress that was imposed.

CONCLUSION

The results of this research showed that the rate of gamma irradiation has different effects on physiological processes in wheat plants and it is obvious that induced mutation via gamma rays can be used to have mutants with ability for drought tolerance.

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