

## Selection of High Yield M6 Wheat Mutant Lines Obtained from Stem Rust Resistant Parents

Mohammed Imad Eddin Arabi\*, Mohammed Jawhar and  
Eyad Al-Shehadah

<sup>1</sup> Department of Molecular Biology and Biotechnology, AECS, P. O. Box 6091 Damascus, Syria.

\*E-Mail: [ascientific@aec.org.sy](mailto:ascientific@aec.org.sy)

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Wheat is a major cereal crop grown worldwide. Mutation breeding with the objective to improving grain yield was performed for selection of mutant lines. Dry dormant seeds with approx. 11 % moisture of three Australian genotypes (Mendos, Coorong and Egret) were treated with 100, 160 and 200 Gy Co-60 gamma radiation and mutants were selected between the M1 and M5 generations for yield production under field experimental conditions. Decrease in survival rate (%) of plants was observed. Five seeds from each spike per plant of M1 plants were collected, bulked dose-wise and grown separately as M2. Fifteen promising mutant lines retested in a M5-trial for yield in comparison with the parent cultivars. Significant differences ( $P<0.05$ ) in 1000-grain weight and grain yield were detected among mutants, with values being consistently higher in promising mutants lines than in the test cultivars. Higher mean grain yield were found in the two promising mutants C-17-3 (1061 g/m<sup>2</sup>) and C-17-31 (976 g/m<sup>2</sup>) as compared with their mothers and the local control genotype Bouhouth10 (857 g/m<sup>2</sup>). The obtained results suggest that LD<sub>50</sub> (160 Gy) gamma radiation treatment can be useful from breeding point of view for selecting higher yielding wheat plants, and the results showed that the Australian parents had *Sr26* resistance gene against stem rust disease caused by *Puccinia graminis* f. sp. *tritici*, therefore, their utilization is suggested in wheat breeding programs to achieve resistant cultivars.

*Key words: Wheat, grain yield, cobalt-60 radiation-induced mutagenesis, genetic variability, mutation, stem rust*

Bread wheat (*Triticum aestivum* L.) is the main cereal crop of the world and is the staple food of millions of people worldwide. Its demand is increasing due to the growing global population together with the nutritional changes in countries with rapidly economic growth (Connor and Minguéz 2012; Enghiad *et al.*, 2017). In Syria, wheat production vary from season to season based on climatic conditions and cultivated cultivar. Therefore, development of high yielding wheat cultivars with acceptable quality has always been a main goal of breeding programs around the world (Laidig *et al.*, 2017).

Improvement of wheat depends on the level of genetic variation and the desirable characters (Kahrizi *et al.*, 2010). Mutagenesis is an important tool in crop improvement and is free of the regulatory restrictions imposed on genetically modified organisms (Ahloowalia *et al.*, 2004; Stacy *et al.*, 2021). Induced genetic diversity is a proven strategy in the improvement of all major food crops, and the use of mutagenesis to create novel variation is particularly valuable in those crops with restricted genetic variability (Begna 2021). The use of physical mutagen, like gamma radiation for inducing variation, is well established.

It is well known that gamma rays are short-wavelength electromagnetic irradiations with a high penetration depth that are produced when certain elements undergo radioactive disintegration. It has important effects on plant growth and development by changes of morphological, physiological, biochemical, genetic, and cytological in cells and tissues (Kiani *et al.*, 2022). These induced mutation help to develop many agronomical important traits such as grain yield and improvement tolerance or resistance to abiotic and biotic stresses use in major crops (Zakir 2018).

Improving grain yield is a main challenge in wheat production for meeting nutritional demands. However, due to the limited genetic diversity among existing wheat genotypes, the development of superior varieties is hampered greatly (Dwivedi *et al.*, 2017). Most of the bread wheat cultivars were developed from local landraces as Syria is the centre of diversity of these

species (Al Darvish *et al.*, 2022). In addition, wheat stem rust caused by *Puccinia graminis f. sp. Tritici*, is a devastating fungal disease of wheat in Syria and worldwide. Thus, the current work aimed at screening wheat superior mutant lines with a high potential grain yield under Syrian field conditions which are typical of Mediterranean environments. In addition, the presence of stem rust resistance Sr26 gene was checked in the used parents .

## MATERIALS AND METHODS

### Plant materials and irradiation treatment

Dried seeds (approx. 11% moisture content) of the three bread wheat genotypes from Australia (Mendos, Coorong and Egret) were irradiated by doses of 100, 160 and 200 Gy from a Co<sup>60</sup> source at the Atomic Energy Commission of Syria. Grains were planted immediately after irradiation in order to obtain M<sub>1</sub> plants. Data on seed germination and surviving plants were recorded considering whole plots of M<sub>1</sub> population. The local check genotype Bouhouth10 was introduced in the experiments.

### Field experiments

Field experiments were performed under natural disease infection in Syria, at a site of 970 m altitude (550-mm rainfall average). The M<sub>1</sub> generation was grown according to the standard agricultural practice in a bulk. Soil fertilizers; 50 kg/ha of nitrogen in the form of Urea (46%) were drilled in equal portions before sowing and after tillering, and 27 kg/ha superphosphate (33% P<sub>2</sub>O<sub>5</sub>) was drilled before sowing. . The untreated seeds (0 Gy) from all the wheat genotypes were also planted after every five rows as control for comparison with the M<sub>1</sub> population.

### Plant selection from M<sub>1</sub> to M<sub>5</sub> generations

Single spikes were harvested from each plant in order to develop the M<sub>2</sub> generation. The plants of M<sub>3</sub> and M<sub>4</sub> generations were planted in randomized blocks in three replications. Selection of the best lines from M<sub>1</sub> to M<sub>5</sub> was carried out based on individual plants. The best lines were tested with their parent variety in order to select advanced mutants. Grains of the best mutants were individually selected in each generation. After

harvesting the M<sub>5</sub> plants, the high-yielding potential mutant lines were selected.

#### 1000-grain weight and yield estimation

The three central rows of each plot of each mutant line were harvested for yield measurements. After threshing 1000 seeds of each genotype were weighed using an electrical balance and mean values were used for data analysis.

#### DNA extraction from wheat parents

Genomic DNA was isolated from young leaves the three Australian parents (Mendos, Coorong and Egret) using the CTAB extraction method described by Doyle and Doyle (1987). DNA concentration was determined by the use of BioSpecNanoDNA spectrophotometer. Quantified DNA samples were diluted to 25 ng/μL.

#### Sr26Analysis

The reaction consisted of 1x PCR buffer, 0.2 mM of each dNTP, 40 nM forward Sr26#43-F primer (5'- AAT CGT CCA CAT TGG CTT CT -3), 300 nM reverse primer, 300 nM Sr26#43-R primer (5'- CGC AAC AAA ATC ATG CAC TA -3') (Mago et al., 2005), 0.18 L Taq polymerase (MBI Fermentas, York, UK), and 40 to 80 ng genomic DNA in a final volume of 12 L. The PCR cycling conditions were 95C for 2 min, 35 cycles of 94 C for 60 sec, 60C for 60 sec, and 72C for 60 sec with a final extension at 72C for 10 min. Amplifications were performed a Gene Amp 9700 Thermocycler (Applied Biosystems, USA).

#### Statistical analysis

Data was subjected to analysis of variance using the STAT-ITCF statistical programme (2<sup>nd</sup> Version). Means were compared using Newman-Keuls test at 5% probability level (Anonymous 1988). The LD<sub>50</sub> was calculated using the survival percentage of irradiated vs nonirradiated seedlings, in which a larger genetic variability is expected to be found in advanced wheat mutant lines.

## RESULTS AND DISCUSSION

The results on survival rate of plants at maturity derived from irradiated seeds are graphically illustrated (Fig. 1). Survival of plants decreased to a considerable level as compared to non-irradiated seeds (Fig. 1). In case of 160 Gy, it was 50% while it was 95% in the non-

irradiated population. Different effects of gamma irradiation on survival (%) of wheat have been documented (Borzouei et al., 2010; Ahumada-Flores et al., 2021).

Significant differences ( $P < 0.05$ ) in 1000-grain weight and grain yield were detected among mutants, with values being consistently higher in promising mutants lines than in the parents. Fifteen mutants were selected and evaluated according to the results of the grain yield conducted up to M<sub>5</sub>. Table 1 shows the statistical procedure of grain yield of wheat mutants and parents. Higher mean grain yield were found in the two promising mutants C-17-3 (1061 g/m<sup>2</sup>) and C-17-31 (976 g/m<sup>2</sup>) as compared with their mothers and the local control genotype Bouhouth10 (857 g/m<sup>2</sup>). (Table 1; Fig 2).

On the other hand, statistical analysis showed highly significant difference for 1000-grain weight among wheat lines shown in Table 1. The maximum 1000 grains weight was found in mutant line C-17-3 (34.33g), while the minimum 1000 grains weight was recorded in line E-26-3 (29.11g). The result indicates that mutant line C-17-3 performed better in soil and climatic condition of Syria.

The increase in wheat grain yield may be attributed to the mutagen induced enhanced mitotic division, alteration in physiological, biochemical and metabolic pathways and interaction of mutagens with yield governing genes (Louali et al., 2015; Ahmed et al., 2018; Hong et al., 2022). Al-Salhi et al. (2004) and Hameed et al. (2008) reported that seeds irradiation with gamma rays effects protein synthesis, leaf gas exchange, Leaf water potential, hormonal balance and enzyme activity which might be the reasonable explanations of the different effects of gamma radiations on the grain yield in the current investigation. However, the present results suggest that gamma rays in general LD<sub>50</sub> (160 Gy) can be useful from breeding point of view for selecting higher yielding wheat plants in early generations.

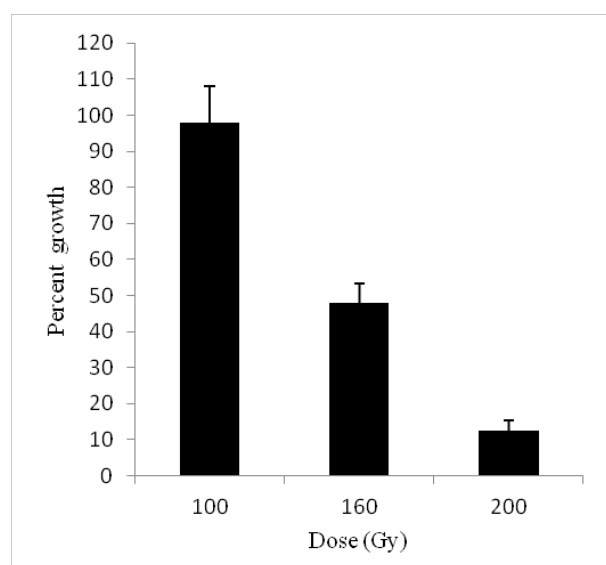
On the other hand, Gamma rays are efficient in broadening genetic variation and increasing grain yield of wheat cultivars, helping plant breeders to perform an efficient selection in the mutated generations (Al-Naggar et al., 2007; Bano et al., 2017). Frey (1969) reported that

mutagen derived variability for quantitative characters in agricultural crops is heritable and response to selection is suitable. We assumed that the causes of the higher yields of our 15 selected mutants must have been phenotypical traits rather than physiological traits under the growing reasons. Firstly, the differences in traits between higher yield mutants and their originals were consistent over the years. Secondly, we used phenotypic traits for our selection of the mutants. In general, gamma irradiation might have different effects on traits through the production of free radicals including plant yield (Kiani *et al.*, 2022).

A dominant marker Sr26, was identified in the used Australian parents (Mendos, Coorong and Egret) (Fig. 3), therefore, their utilization is suggested in wheat breeding programs to achieve resistant cultivars. Moreover, Our results are in agreement with previous works (Jamil and Khan 2002; Bano *et al.*, 2017) reported that higher yield were observed from wheat mutants. Mutant populations have also been used widely to improve yield of many cereal crops, including rice (Viana *et al.*, 2019), durum wheat (Louali *et al.*, 2015) and the cowpea (Raina *et al.*, 2022).

**Table 1.** Grain yield and 1000-grain weight of wheat mutants as compared with the local genotype.

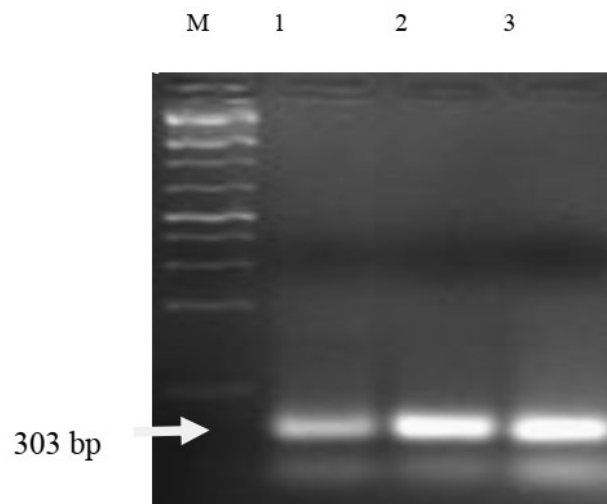
No.	Lines	grain yield (g/m <sup>2</sup> )	1000-grain weight (g)
1	C-17-3	1061	34.33
2	C-17-31	976	33.12
3	C-17-10	884	32.85
4	C-17-33	867	32.5
5	C-17-20	722	31.15
6	C-17-11	718	31.1
7	E-26-4	689	29.55
8	C-17-6	685	29.85
9	C-17-4	676	29.38
10	E-26-14	662	29.55
11	C-17-22	610	29.44
12	M-27-8	579	31.2
13	E-26-3	520	29.35
14	M-27-5	443	31.2
15	M-27-6	324	31.85
16	Bouhouth10	739	33.8
17	Mendos (M-27)	348	33.125
18	Coorong (C-17)	862	29.11
19	Egret (E-26)	394	29.88
LSD		101	2.1



**Figure 1.** Effects of different doses of gamma irradiation on plant survival of wheat genotypes



**Figure 2.** Some wheat mutants obtained from the three Australian genotypes used in this study



**Figure 3.** Agarose gel electrophoresis showing the amplification product of marker Sr26 in Australian parents; Mendos (1), Coorong (2) and Egret (3). M, molecular weight marker (Hinfl; MBI Fermentas, York, UK)

## CONCLUSIONS

This study aimed to develop  $M_5$  mutant lines of spring wheat by using gamma irradiation treatment and identify genetic variability in grain yield. We have successfully selected higher yield 15 mutants retested in a  $M_5$ -trial for yield in comparison with the parent genotypes. Significant differences ( $P < 0.05$ ) in grain yield and 1000-grain weight were detected among mutants, with values being consistently higher promising mutants lines than in the parents. The two promising mutants C-17-3 and C-17-31 showed the best performance by producing highest 1000-grain weight and hence highest

grain yield. However, data suggest that 160 Gy of gamma radiation can be useful from breeding point of view for selecting higher yielding wheat plants in early generations, and the Australian parents having Sr26 resistance gene against stem rust disease can be suggested in wheat breeding programs.

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## CONFLICTS OF INTEREST

The authors declare that they have no potential conflicts of interest.

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