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# Resistance to white rot disease and enhancement of yield and its components by selection in mutants of two garlic cultivars

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## Abstract

The study was conducted during the winter seasons of 2015 to 2019 to select garlic clones superior in yield, quality and tolerant or resistant to white rot disease. Fourteen mutants from Balady and five mutants from Egassed-1 garlic cvs. were isolated in stable form and selected after 4 successive generations (M1V4) from previous breeding program using mutagens *viz.*, di-ethyel sulphate (DES), di (2-chloro ethyel) amine (DEA) and gamma ray. Cloves of two local garlic cultivars (Balady and Egassed-1) were irradiated with gamma ray doses *i.e.* 1, 3, 5,10 and 15 Gy or treated with previous mutagens. Out of Balady selected clones, Mut 6-1 and Mut 6 had the heaviest cloves compared to the original cultivar with values of 3.8 and 3.6 g/cloves, in 2015/2016 and 2016/2017 seasons, respectively. Likewise, Mut 6-2 and Mut 7 which resulted from Eggaseed-1 *cv.* had the heaviest cloves compared to the original cultivar with values of 7.6 and 7.2 g/cloves, in the first and second seasons, respectively. Significant positive correlations were found between the most of the desirable traits. Artificial infestation with *Sclerotium cepivorum* was carried out under greenhouse conditions during two successive seasons of 2017/2018 and 2018/2019 and under natural infestation in the open field to evaluate the resistance in the two cultivars and their mutants. In case of Balady cv., Mut 6-1 (10 GY) and Mut 2 (0.1 % DEA) were highly tolerant in the two tested seasons with infection percentage of 11.33 and 12.33 % in the first season and 13.33 and 15.33 % in the second one with efficacy 80.4, 78.74 and 78.15, 74.87 %, respectively, compared to Balady (58 and 61 % infection). Regarding, Eggaseed-1 cv., Mut 6-2 and Mut 6-1 resulted from *γ* irridiation (10 GY) were highly tolerant mutants in the two tested seasons with infection percentage of 8.33 and 10.67 % in the first season and 9 and 10.67 % in the second one with efficacy 81.21, 75.93 % and 81.38, 79.99 %, respectively as compared to original cultivar (44.33 and 48.33 % infection). Results of employed QRT-PCR technique showed that Mut 6-1 (10 GY) which had significantly high tolerance to garlic white rot disease showed great regulation-up of detective defense genes (*PR1, PR5, PAL and HQT*) with high relative expression values compared to original cultivar, Balady. Similar results were obtained in case of Eggaseed-1 *cv.* Results showed that respective encoded PR and polyphenol synthase genes might have played important role in defense reaction of obtained mutants of two tested garlic cultivars against *S. cepivorum* infection. Mutants with high tolerance to garlic white rot disease could be used by growers in the infested areas.

**Key words:** Garlic, *Allium sativum*, mutations, gamma rays, DES, DEA, white rot, *Sclerotium cepivorum*, induced resistance, defense system, PR genes, polyphenol synthetic genes, QRT-PCR

## Introduction

Garlic (*Allium sativum* L.) is a widely grown vegetable crop for use as a flavoring component in foods and medicinal purpose. In productivity, Egypt tops the others (30.55 t/ha) followed by USA (20.14t/ha) whereas the productivity in India is 5.07 t/ha (Aney, 2013). White rot disease, caused by the soil borne fungus *Sclerotium cepivorum* Berk. is a rigorous disease of the *Allium* spp which is the second host for this disease. This disease is reported to be a limiting factor for commercial production of *Allium* species in several parts all over the world (Entwistle, 1990 and Melero-vara *et al.,* 2000). For example, between 1965 and 1982, white rot disease caused a 65 % reduction in the production area of winter onions in Egypt, and a 90 % fall in exports. The pathogen survives in the soil as sclerotia for 20 years (Coley-Smith, 1990).

Mutagens, especially gamma rays and ethyl methan sulfonate (EMS) may be considered as dependable alternative breeding methods (Batchvarov, 1993). In Egypt, Shalaby *et al.* (1983a and b) found that in the M2 generation, gamma radiation and EMS treatments increased the variability of garlic plants with value to some characters, such as bulb weight, clove weight and date of maturity. Alvarez *et al.* (1996) reported that different doses from gamma-ray induced different phenotypic variations in the second mutant (M2) garlic population. Metwally and Abou Shousha (2002) showed that original cultivar "Balady" had the highest values of most vegetative characters as compared with the nine mutants under Egypt conditions. In both seasons the differences were highly significant among mutants means for bulb yield and its components. Six out of the nine mutants Mut 17 and Mut 21 produced the highest bulb yield. Number of cloves/bulb ranged from 61.4 for Mut 18 to 39.4 for Mut 21. The heaviest cloves were produced from Mut 17 and Mut 21 in both seasons, while original cultivar had the lightest cloves in both seasons. Shashidhar *et al.*

(2005) showed that garlic survival percentage decreased with increasing the dose of M1V2. However, it is very important to determine suitable mutagen dose for improving crops by mutation breeding (Joshi *et al.,* 2011). Hemada *et al.* (2012) found that higher doses of gamma-irradiation decreased the growth, yield and its components when compared with the untreated plants in two tested cultivars. The high concentrations of di-ethyel sulphate (DES) and di (2-chloro ethyel) amine (DEA) mutagens increased most of the studied characteristics in the M1V1 and M1V2 in both generations. Also, low concentration of DES and DEA increased most of the studied characters in the M1V1 generation in both cultivars. Chenta *et al.* (2016) and Chenta (2017) showed that frequency of viable mutants was, in general, higher in treatments with ethyl methane sulphonate (EMS) than with gamma rays in the garlic M1 generation.

Mutations induced by chemical mutagens are point mutations and are less damaging than large rearrangements (Sri Devi and Mullainathan, 2012). Among the chemical mutagens, ethyl methane sulfonate (EMS) is the most commonly used in plants (Hajra, 1979). Ethyl methane sulfonate (EMS) is a mono functional ethylating agent that has been found to be mutagenic in a wide variety of genetic test systems. EMS may produce both GC to AT and AT to GC transition. Alkylating agents such as (EMS) induce chemical modification of nucleotides, which results in mis-pairing and base changes. Strong, biased alkylation of guanine (G) residues results, forming O6-ethylguanine, which can pair with thymine (T) but not with cytosine (C). Through subsequent DNA repair, the original G/C pair can then be replaced with A (adenine)/T (Greene *et al.,* 2003). The functions and mechanisms of action of other monofunctional alkylating agents such as diethyl sulfate (DES) and dimethyl sulfate have been extensively reviewed by Hoffmann (1980). He reported that comparisons of DES and EMS have shown that they share similar alkylating activities and genetic effects. Piron *et al.* (2010) demonstrated that EMS mutagenesis has been used to develop resistance against potyvirus, a widespread destructive virus for tomato.

The induction of PR genes is a general response to Fusarium head blight, but it has been observed that few PR proteins are up-regulated, earlier, faster and/or more in resistant genotypes than in susceptible genotypes (Steiner *et al.*, 2008). In lettuce infected with *V. dahliae,* PR-3, PR-5 and putative cysteine protease (LsCP2) genes were identified as expressed only in symptomatic leaves harvested 3 weeks after infection, using suppression subtractive hybridization (Klosterman *et al.,* 2011).

The phenylalanine ammonia lyase (PAL) enzyme is very important for plant defense and is one of the most studied enzymes in all of secondary metabolism (Buchanan *et al.*, 2000). The *tPAL5* gene has been shown to have two initiation sites giving rise to a long transcript that appears to be constitutive and a shorter transcript that is induced by both biotic and abiotic stimuli (Lee *et al.*, 1994).

Among various quantification methods of measuring gene expression, quantitative real-time QRT-PCR is the most sensitive and flexible and can be used to compare the levels of mRNAs in different sample populations, characterize patterns of mRNA expression, discriminate between closely related mRNAs and analyze RNA structure. Several investigators used QRT-PCR to measure the expression of some candidate resistance and defense-related genes expressed during the disease process (Obembe *et al.*, 2009 and Djami-Tchatchou *et al.,* 2013).

The cultivars generally used by Egyptian garlic farmers are soft neck (white) Balady, Eggaseed-1 and Sids-40 hard neck (purple) and no new cultivars were developed since more than ten years, because garlic is sterile, and propagated only vegetatively. Therefore, there is a requirement for variety development, to improve its productivity, quality and disease resistance characters. So, in contemporary study the QRT-PCR technique for investigation of expression pattern of *PR1, PR5,PAL* and *HQT* genes of garlic mutants in response to garlic white rot disease was studied. However, the major objective of this work was to select garlic clones superior in yield, quality and more tolerant or resistant to white rot disease than its parent Balady and Eggaseed-1 cultivars.

## Materials and methods

The study was conducted during the period from 2015 to 2019 winter seasons. Fourteen mutants from Balady and five mutants from Egassed-1 garlic cvs. have been isolated in stable form and selected after 4 successive generations (M1V4) from previous breeding program using mutagenesis *viz.*, di-ethyel sulphate (DES), di (2-chloro ethyel) amine (DEA) and gamma ray. Cloves of two local garlic cultivars (Balady and Egassed-1) were irradiated with gamma ray doses *i.e.* 1, 3, 5,10 and 15 Gy or treated with previous mutagenesis. The previous breeding program using mutagenesis was carried out as follows:

**Production of vegetative mutagenic generation:** The experiments were carried out at the Experimental Farm of Sids Horticulture Research, Agriculture Research Center, Beni-Suef Governorate, Egypt, during the 6 successive winter seasons of 2011 to 2017. For raising the first mutant generation for this vegetative reproduction crop; large clove seeds of two commercial cultivars of garlic *Allium sativum* namely; Balady and Egaseed-1 were exposed to five different doses of gamma-rays *i.e.* 1, 3, 5, 10 and 15 Gy. Gamma-radiation source was cobalt-60 and the time of exposure was 20 mints (Gy= 100 rad). These treatments were done at the National Center of Research, Dokki, Giza, Egypt. In addition, large clove seeds of the two tested garlic cultivars were treated with freshly prepared aqueous solution of di-ethyel sulphate (DES) and di (2-chloro ethyel) amine (DEA). The concentrations of the two chemical mutagens were 0.05, 0.1, 0.2 and 0.3 for 12 hours at room temperature followed by washing under running tap water for one hour. Untreated cloves of the two tested cultivars were planted as control.

**The first vegetative mutagenic generation (M1V1):** On October 1st 2011/2012, the treated cloves (13 treatments from the two cultivars, Balady and Eggassed-1) and the untreated cloves (control) were planted in the field. A randomized complete-blocks design with 3 replicates was used. Each plot (4.2 $m^2$) consisted of 2 rows of 3.5 m long and 60 cm width. Cloves were spaced 10 cm apart on the Northern side of the row. Normal cultural practices were applied until maturity. At harvest on 5th April, 10 bulbs from each plot were selected, sampled and stored for next plantation.

**The second vegetative mutagenic generation (M1V2):** Cloves from each individual M1V1 bulbs from each dose were planted as a family, On October 1st 2012/2013, giving 10 families from each dose. One row of untreated cloves was used as a control for

each family of treated cloves. At maturity, 50 plants were selected from Balady *cv.* and 25 plants were selected from Eggaseed-1 based on the field and laboratory tests. These mutants had one or more of the following traits; *i.e.* vigorous or weak growth, early maturity, big bulbs and low number of cloves/bulb.

**The third vegetative mutagenic generation (M1V3):** The selected plants from Balady and Eggaseed-1 cvs. and control were planted separately, each in one row, on October 1st 2013/2014. At maturity, 20 different plants were selected out of the fifty selected ones of Balady *cv.* and 10 different plants were selected out of the 25 selections of Eggaseed-1 *cv.* based on the field and laboratory tested.

**The fourth vegetative mutagenic generation (M1V4):** After 6 months of storage one bulb was selected for each (20 plants from Balady *cv.* and 10 plants from Egseed-1cv.). On October 1st 2014/2015, all cloves of the selected bulbs were planted in one row as M1V4 plants. At maturity, 14 different plants from Balady *cv.* were verified from the 20 ones and 5 different plants Eggaseed-1 *cv.* were verified from the 10 ones based on the field and laboratory tests. In M1V5 ten bulbs from each clone from Balady and Eggaseed-1 were selected after 6 months of storage.

In the present study, the pedigree of the fourteen selected clones from Balady were. *i.e.* Mut 6 and Mut 14 (resulted from 1 Gy of gamma rays), Mut *1* (resulted from 5 Gy of gamma rays), Mut 6-1 and Mut 6-2 (resulted from 10 Gy of gamma rays), Mut1, Mut 2 and Mut3 (resulted from 15 Gy of gamma rays), Mut 6, Mut 2 and Mut 7 (resulted from DES in concentration of 0.1 %), Mut 10 (resulted from DES in concentration of 0.3 %) and Mut 2 and Mut 2-1 (derived from DEA in concentration of 0.1 %). The five selected mutants from Eggaseed-1 *i.e.* Mut 2 (resulted from 1 Gy of gamma rays), Mut 6-1 and Mut 6-2 (resulted from 10 Gy of gamma rays), Mut 1 (resulted from 15 Gy of gamma rays), Mut 7 (resulted from DES in concentration of 0.3 %) as well as the original two cultivars from Balady and Eggaseed-1 cvs. were evaluated during the two successive seasons of 2015/2016 and 2016/2017. Sowing was done on the 10th of October in both seasons. A randomized Complete-Blocks design with three replicates was used. The soil was divided to experimental units. The area of each experimental unit was 10.5 $m^2$ and consisted of 5 rows with 60 cm width and 3.5 m length. Cloves were planted on both sides of each row at 10 cm apart. All agricultural practices were done as are performed in the commercial field.

**Vegetative growth parameters:** Ten plants were taken randomly from each selection at 165 days from planting date to measure: plant height (cm), number of leaves/plant and fresh weight of vegetative portion (g/plant).

**Yield and its component:** Garlic plants were harvested at the 14th and 20th of April in the first and second season, respectively and the fresh yield (kg/plot) at harvesting time was recorded and converted to ton/fed. The harvested garlic plants were left in the open field to be cured for 21 days. Ten plants from each experimental plot were randomly taken to determine; cured bulb weight (g), cured bulb diameter (cm), clove weight (g), number of cloves/bulb.

**Genotypic correlation coefficient:** The correlation coefficient was done following the method of Draper and Smith (1966).

**Statistical analysis:** Mean values of each trait were subjected to the analysis of variance to test the significance as described by Gomez and Gomez (1984). Duncan's multiple range test and correlations were calculated using MSTAT C Ver. 4 software (MSTAT C, 1985).

**The fungal pathogen:** The isolate of *Sclerotium cepivorum* used in this study was obtained from Onion, Garlic and Oil Crops Diseases Research Department, Plant Pathology Research Institute, Agriculture Research Center, Giza, Egypt and characterized for its high virulence.

**Greenhouse experiment for pathogenic study:** Pot experiment was carried out in a randomized complete block design at Integrated Protection Lab., Plant Protection Research Station (Sabahia, Alexandria, Egypt). Pots (35 cm-dia.) were sterilized by dipping in 5.0 % formalin solution for 15 min, left to dry for two days to get free of formalin residues, then filled with soil previously sterilized by autoclaving. Fungal inoculation of *S. cepivorum* was prepared using sorghum-coarse sand water (2:1:2 v/v) medium. The ingredients were mixed, bottled and autoclaved for one hour at 1.5 air pressure. The sterilized media in glass bottles were inoculated separately using agar discs obtained from the periphery of 5 days old colony of the tested fungus and incubated at (20±2 °C) for two weeks and used for soil infestation. Fungal propagules of *S. cepivorum* were added to the potted natural soils (7 kg soil/pot) at the rate of 2 % (w/w), mixed thoroughly with the soil surface of each pot then irrigated with water and left for one week for the inoculum establishment.

On October 1st 2017, apparently healthy garlic cloves from each of the fourteen selected clones in the M1V4 Balady and the five selected mutants from Eggaseed-1 were planted in potted soil infested with *S. cepivorum*. As well as apparently healthy garlic cloves of each original cultivar, Balady and Eggaseed-1 were planted separately in the infested soil. Five replicates (pots) for each particular mutant or original cultivar were used in this experiment.

The number of plants having specific white rot disease symptoms was counted after four months from planting and their percentage were calculated according to Hovius and Goldman (2004) as follows:

$$\text{Infection (\%)} = \frac{\text{No. of infected plants with white rot}}{\text{Total No. of planted cloves}} \times 100$$

**Field experiments for pathogenic study:** Under open field and natural infection conditions, vigorous garlic cloves from the fourteen selected clones of Balady and the five selected ones from Eggaseed-1 as well as the two original cultivars Balady and Eggaseed-1 were planted and evaluated for their susceptibility and resistance to white rot disease. The experiments were carried out at the Experimental Farm of Sids Horticulture Research Station, Agriculture Research Center, Beni-Suef Governorate, Egypt, during two successive seasons of 2017/2018 and 2018/2019. Sowing was done on the 10th of October in both seasons for each selected clones and original cultivar as mentioned in greenhouse experiment. A Randomized Complete Block design with three replicates was used. The natural infested soil was divided to experimental units with 10.5 $m^2$ area and consisted of 5 rows with 60 cm width and 3.5 m length. Cloves were planted on both

sides of each row at 10 cm apart. All recommended agricultural practices were followed.

At the end of each tested season, disease incidence and infection percentage were calculated as mentioned before and also efficacy of treatments to reduce the infection was evaluated and the obtained data were statistically analysed using MSTAT C program to calculate the LSD at 5 % according to Snedecor and Cochran (1972).

***Molecular study***

**RNA extraction and cDNA synthesis:** Fresh samples of garlic leaves for each treatment were collected from greenhouse experiment. Leaves sample (1 g) of each clone and original cultivar was washed. Total RNA was extracted from garlic leaves using Tri Azol reagent (Bio Flux), and then RNA pellet was resuspended in DEPC-treated water. The RNA quantity and quality were determined by using spectrophotometer and gel electrophoresis. The isolated RNA was subjected to cDNA using the cDNA synthesis system kit (Fermentas, Germany) in the existence of oligo-dT primer (5`-TTTTTTTTTTTTTTT-3`). Total 25 µL reaction mixture contained 2.5 µL (5X) buffer with MgCl2, 2.5 µL (2.5 mM) dNTPs, 1 µL (10 pmol) oligo (dT) primer, 2.5 µL from plant RNA (2 mg/mL), and 0.5 µL /5U reverse transcriptase enzyme. The thermal cycler was programmed at 42 °C for 1 h, 72 °C for 10 min, and a soak at 4 °C (Chin *et al.*, 2000). The amplified cDNA was used as a template for quantitative real-time PCR (qPCR).

**qPCR assay and data analysis:** Quantitative real-time PCR (QRT-PCR) and the expression level of polyphenol and PR genes were conducted for the two original cultivars Balady and Egassed-1 and also the clones resulted from them in M1V4. Four primers set specific for *PR1* and *PR5* and polyphenol biosynthetic genes (PAL, HQT) were synthesized according to previous studies (Table 1). The housekeeping gene β-actin (Table 1) was used as a reference gene in order to normalization of the transcript expression levels. Each sample on all reactions run in triplicate on a Rotor-Gene 6000 (QIAGEN, ABI System, USA) using the SYBR Green PCR Master Mix (Fermentas, USA). The 25 µL reaction consist of 12.5 µL of 29Quantitech SYBR_ Green RT Mix, 1 µL of each primer (25 pm/ µL), 1 µL of the cDNA (50 ng), and 9.25 µL of RNase-free water. Samples were spun before loading into the rotor's wells. The amplification program of thermal cycling included an initial denaturation step at 95 °C for 10 min, followed by 40 cycles consisting of: denaturation at 95 °C for 15s, annealing at 60 °C for 30s and extension at 72 °C for 30s. The relative expression ratio was accurately quantified and calculated according to Livak and Schmittgen (2001).

Therefore, for each biological samples, the difference (**Δ**) in quantification cycle value ($C_T$) between the target ($C_{T\ (target)}$ averaged from three technical repeats) and the reference ($C_{T(reference)}$, a fixed $C_T$ value was used for all samples) was first transformed into relative quantity (RQ) using the exponential function with the efficiency (*E*) of the PCR. The $C_T$ (threshold of cycle) value of each detected gene was determined by automated threshold analysis on ABI system. The $C_T$ value of each target gene was normalized to $C_{T(reference)}$ to obtain $\Delta C_{T\ (target)}$ where:
$\Delta C_{T\ (control)} = (C_{T\ (control)} - C_{T(reference)})$.

Table 1. List of primer sequences of PR protein genes, polyphenol biosynthetic genes and the housekeeping gene (β-actin) used in quantitative real-time PCR (qPCR)

| Tested Gene | Direction | Primer sequences 5'…….....……3' | References |
|---|---|---|---|
| PR1 | Forward | TTCTTCCCTCGAAAGCTCAA | ElMorsi *et al.* (2015) |
| | Reverse | CGCTACCCCAGGCTAAGTTT | |
| PR5 | Forward | ATGGGCTACTTGACATCTTCTT | |
| | Reverse | TTATGGGCAAAAAACAACCCT | |
| PAL | Forward | ACGGGTTGCCATCTAATCTGACA | Andre *et al.*, (2009) |
| | Reverse | CGAGCAATAAGAAGCCATCGCAAT | |
| HQT | Forward | CCAATGGCTGGAAGATTAGCTA | |
| | Reverse | CATGAATCACTTTCAGCCTCAACAA | |
| β-actin | Forward | CTCGCCTTTGCCGATCC | Livak and Schmittgen, (2001) |
| | Reverse | GATCTTCATGAGGTAGTCAGTC | |

The relative expression quantity of the target gene was indicated with
$\Delta\Delta C_T = (\Delta C_{T\ (target)} - \Delta C_{T\ (control)})$ according to $2^{-\Delta\Delta CT}$ algorithm.

The relative expression values of three replicates for each set levels were analysed by one-way ANOVA with $P \leq 0.05$, using MSTAT C Ver. 4 software. The significant differences of the relative expression levels were plotted, and standard error (± SE) is shown as a column bar. Relative expression levels more than 1 demonstrate an increase of accumulation (up-regulation) and values lower than 1 means a decrease in expression (down-regulation).

## Results and discussion

Both, physical and chemical mutagens are used in inducing mutations in seeds and other planting resources. Subsequently, selection for agronomic characters is done in the first generation; many a times most selections may be redundant. The agronomic traits are confirmed in the second and third generations through evident phenotypic steadiness, while other evaluations are carried out in the subsequent seasons. Finally, only the clones with advantageous traits are preferred as a new variety. Mutagenesis is the process where unexpected genetic changes occur in the genetic information of an organism not caused by genetic segregation or genetic recombination, but induced by chemical, physical or biological gents (Kodym and Afza, 2003; Roychowdhury and Tah, 2013).

**Vegetative growth:** Data presented in Tables 2 and 3 showed that, a significant difference was found for plant height (cm) and fresh weight of vegetative portion (g/plant). However, the differences among genotypes were significant in first season only for plant height for Balady clones.

About number of leaves/plant, the differences among genotypes were not significant in both the seasons. Similar results were recorded for Eggaseed-1 genotypes. In general, garlic plants from the original cultivar had the highest values of plant height, number of leaves/plant and fresh weight of vegetative portion. In this respect, Metwally and Abou Shousha (2002) showed that the original cultivar "Balady" had the highest values of most vegetative traits when compared with the new nine mutants clones. Also, Hemada *et al.* (2012) found that higher doses of gamma-irradiation decreased the growth, yield and its

Table 2. Vegetative traits of garlic genotypes (14 selected mutants and Balady cv.) grown during 2015/2016 and 2016/2017 seasons

| | Genotypes | Plant height (cm) | | Number of leaves/plant | | Fresh weight of vegetative portion (g/plant) | |
|---|---|---|---|---|---|---|---|
| | | 1st | 2nd | 1st | 2nd | 1st | 2nd |
| | γ rays (1 Gy) | | | | | | |
| 1 | MUT 6 | 105.7 ab | 101.5 | 11.6 | 11.7 | 81.83 bcd | 77.43 cd |
| 2 | MUT 14 | 104.2 ab | 100.0 | 11.5 | 11.6 | 79.10 cde | 74.60 de |
| | γ rays (5 GY) | | | | | | |
| 3 | MUT 1 | 101.8 bc | 99.4 | 11.5 | 11.6 | 73.57 def | 69.10 efgh |
| | γ rays (10 GY) | | | | | | |
| 4 | MUT 6-1 | 114.5 a | 110.8 | 12.5 | 12.0 | 92.17 a | 92.00 a |
| 5 | MUT 6-2 | 102.0 bc | 106.7 | 11.9 | 11.7 | 90.33 ab | 87.00 ab |
| | γ rays (15 GY) | | | | | | |
| 6 | MUT 1 | 100.9 c | 98.6 | 11.1 | 11.5 | 71.10 ef | 65.50 feh |
| 7 | MUT 2 | 100.5 c | 98.3 | 11.0 | 11.4 | 67.97 fg | 65.00 gh |
| 8 | MUT 3 | 99.7 c | 98.0 | 11.0 | 11.3 | 72.80 def | 64.80 h |
| | DES (0.1 %) | | | | | | |
| 9 | MUT 6 | 109.5 ab | 108.3 | 12.0 | 11.8 | 91.11 ab | 89.97 a |
| 10 | MUT 6-1 | 107.2 ab | 106.4 | 11.7 | 11.7 | 84.77 abc | 82.23 bc |
| 11 | MUT 7 | 105.0 b | 103.0 | 11.7 | 11.6 | 84.27 abc | 75.33 de |
| | DES (0.3 %) | | | | | | |
| 12 | MUT 10 | 105.0 b | 100.0 | 11.6 | 11.4 | 79.17 cde | 71.33 defg |
| | DEA (0.1 %) | | | | | | |
| 13 | MUT 2 | 103.0 bc | 99.6 | 11.5 | 11.3 | 77.90 cde | 71.90 def |
| 14 | MUT 2-1 | 101.8 bc | 99.0 | 11.2 | 11.2 | 72.60 def | 66.33 fgh |
| | Original cultivar | | | | | | |
| | Balady | 116.8 a | 113.6 | 11.2 | 11.0 | 90.33 a | 89.50 a |

Means within each column followed by the same letter are not statistically different at $P$=0.05 (Duncan's range test).

Table 3. Vegetative traits of garlic genotypes (5 selected mutants and Eggesed-1 cv.) grown during 2015/2016 and 2016/2017 seasons

| | Genotypes | Plant height (cm) | | Number of leaves/plant | | Fresh weight of vegetative portion (g/plant) | |
|---|---|---|---|---|---|---|---|
| | | 1st | 2nd | 1st | 2nd | 1st | 2nd |
| | γ rays (1 GY ) | | | | | | |
| 1 | Mut 2 | 70.70 bc | 77.50 | 11.6 | 11.80 | 59.43 ab | 62.00 ab |
| | γ rays (10 GY ) | | | | | | |
| 2 | Mut 6-1 | 69.50 bc | 73.10 | 11.5 | 11.53 | 59.90 ab | 62.10 ab |
| 3 | Mut 6-2 | 78.90 a | 80.70 | 11.8 | 12.10 | 62.67 a | 65.43 a |
| | γ rays (15 GY ) | | | | | | |
| 4 | Mut 1 | 69.10 bc | 72. 20 | 11.4 | 11.50 | 51.17 ce | 53.27 bc |
| | DES 0.3 % | | | | | | |
| 5 | Mut 7 | 75.80 ab | 78.10 | 11.7 | 12.00 | 60.00 ab | 60.77 abc |
| | Original cultivar | | | | | | |
| | Eggaseed-1 | 80.13 a | 81.50 | 11.1 | 11.40 | 65.37 a | 66.7 a |

Means within each column followed by the same letter are not statistically different at $P$=0.05 (Duncan's range test).

components when compared with the untreated plants in the two cultivars. The high concentrations of DES and DEA mutagens increased most of the studied characters in the M1V1 and M1V2 in both generations. In the mutants, low concentration of DES and DEA increased most of the studied characters in the M1V1 generation in both cultivars.

Mutation breeding employs types of mutagenesis: irradiation (gamma rays, X-rays, ion beam, etc.) or treatment with chemical mutagens; site-directed mutagenesis, which is the process of creating a mutation at a defined site in a DNA molecule; and insertion mutagenesis, which is due to DNA insertions, either through genetic transformation and insertion of T-DNA or activation of exchangeable elements (Kharkwal and Shu, 2009; Forster and Shu 2012).

**Yield and its components:** Data in Tables 4 and 5 showed that all mutant clones produced higher total fresh yield than the original cultivar (Balady and Eggaseed-1). Mutant clones of Balady type, Mut 6-1 and Mut 6 produced the highest total fresh yield in the first and second seasons with values of 14.05 and 13.70 ton/fed. respectively. While, mutant clones of Eggaseed-1 type , Mut 6-2 and Mut 7 produced the highest total fresh yield in the first and second seasons with values 14.30 and 13.97 ton/fed., respectively. However, the original Balady *cv.* produced 11.10 and 11.30 ton/fed. in the first and second seasons, respectively. Even as the original Eggaseed-1 *cv.* produced 12.50 and 12.17 ton/fed. in the first and second season, respectively. The differences among clone means for total fresh yield were highly significant in both cultivars and seasons. Yield components *i.e.*, average bulb weight and diameter, number of cloves/bulb and average clove weight are presented in Tables 4 and 5.

Data showed that, on Balady types in the first season, average bulb weight ranged from 81.8 g for original cultivar to 95.5 g for clone Mut 6-1. In second season, it ranged from 75.8 g for original cultivar to 90.7 g for mutant line Mut 6. While for Eggaseed-1 type, in the first the season, average bulb weight ranged from 91.40 g for original cultivar to106.3 g for mutant line Mut 6-2. In the second season, it ranged from 90.70 g for original cultivar to 102.8 g for mutant line Mut 7. About average bulb diameter for Balady cv., mutant line 6-2 produced the large bulb size, while, the original cultivar produced the smallest bulb size in both the seasons. As average bulb diameter for Eggaseed-1, mutant line 7 produced the large bulb size, while, the original cultivar produced the smallest bulb size in both seasons. The differences among treatment means were highly significant in both types and seasons. For the number of cloves/bulb, for Balady type, the data showed that original cultivar had the highest number of cloves/bulb while Mut 6-1 had the lowest number of cloves/bulb. Concerning the number of cloves/bulb for Eggaseed-1 type. the data showed that original cultivar had the highest number of cloves/bulb, while Mut 6-2 had the lowest number of cloves/bulb. The differences among treatment means were highly significant in both types and seasons. The data presented large variations in clove size among the different mutant clones in Balady type. The results indicated that clone Mut 6-1 and Mut 6 had the heaviest cloves with values of 3.8 and 3.6 g/cloves, in first and second seasons, respectively. The original cultivar produced 1.6 to 1.4 in the first and the second years, respectively.

Regarding Eggaseed-1 type, the data showed large variations in clove size among the differences mutants. The Mut 6-2 and Mut 7 had the heaviest cloves with values of 7.6 and 7.2 g/cloves in the first and second seasons, respectively. The original cultivar produced

Table 4. Yield and its components for 14 selected mutants of garlic and their original cultivar Balady grown during 2015/2016 and 2016/2017 seasons

| | Genotypes | Total fresh yield (tons/fed.) | | Average cured bulb weight (g) | | Average bulb diameter (cm) | | Number of cloves / bulb | | Average clove weight (g) | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1st | 2nd | 1st | 2nd | 1st | 2nd | 1st | 2nd | 1st | 2nd |
| | γ rays (1 Gy) | | | | | | | | | | |
| 1 | MUT 6 | 13.10 cd | 11.95 def | 86.7 ab | 80.7 cde | 6.30 a-e | 6.64 a-d | 28.8 gh | 30.0 ef | 2.8 a-e | 2.7 e |
| 2 | MUT 14 | 12.34 e | 11.69 ef | 85.5 b | 78.2 de | 5.83 cde | 6.55 bcd | 32.2 ef | 31.3 de | 2.5 c-f | 2.3 g |
| | γ rays (5 GY) | | | | | | | | | | |
| 3 | MUT 1 | 11.71 fg | 11.60 ef | 84.4 b | 77.8 de | 5.70 cde | 5.93 cde | 33.3 c-f | 33.9 cd | 2.3 efg | 2.1 h |
| | γ rays (10 GY) | | | | | | | | | | |
| 4 | MUT 6-1 | 14.05 a | 13.22 ab | 95.5 a | 87.7 ab | 7.30 abc | 6.86ab | 23.8 i | 22.9 h | 3.8 a | 3.4 ab |
| 5 | MUT 6-2 | 13.49 bc | 12.89 abc | 88.7 ab | 85.6 abc | 7.60 a | 7.45 a | 27.1 h | 26.4 g | 3.0abc | 2.9 bc |
| | γ rays (15 GY) | | | | | | | | | | |
| 6 | MUT 1 | 11.52 f-g | 11.53 ef | 83.1b | 76.8 e | 5.50 de | 5.69 e | 35.5 bcd | 36.3 bc | 2.2 h | 1.9 ij |
| 7 | MUT 2 | 11.37 f-g | 11.42 ef | 82.2 b | 76.4e | 5.40 de | 5.63 e | 35.9 bc | 37.6 b | 2.1 gh | 1.8 j |
| 8 | MUT 3 | 11.27 gh | 11.34 f | 82.0 b | 76.2e | 5.30 e | 5.63 e | 36.7 b | 37.9 b | 2.0 gh | 1.7 j |
| | DES (0.1 %) | | | | | | | | | | |
| 9 | MUT 6 | 13.74 ab | 13.70 a | 89.9 ab | 94.7 a | 7.00 ab | 6. 89 ab | 26.5 hi | 25.5 gh | 3.1 ab | 3.6 a |
| 10 | MUT 6-1 | 13.70 ab | 12.56 bcd | 85.0 b | 83.7 ab | 6.80 a-d | 6.78 abc | 27.7 h | 27.9 fg | 2.9abc | 2.8 cd |
| 11 | MUT 7 | 13.19 cd | 12.23 cde | 84.5 b | 81.47 bcd | 6.50 a-e | 6.71 abc | 28.4 gh | 29.7 ef | 2.8a-d | 2.4 d |
| | DES (0.3 %) | | | | | | | | | | |
| 12 | MUT 10 | 12.93 d | 11.75 def | 83.1 b | 79.9 b-e | 6.07 b-e | 6.60 bcd | 31.7 fg | 30.8 def | 2.5b-e | 2.3g |
| | DEA (0.1 %) | | | | | | | | | | |
| 13 | MUT 2 | 11.82 f | 11.61 ef | 83.0 b | 78.1 cde | 5.80 cde | 6.20 b-e | 32.4 def | 31.8 de | 2.4 d-f | 2.3g |
| 14 | MUT 2-1 | 11.60 fg | 11.59 ef | 83.5 b | 77.2 e | 5.60 cde | 5.80 de | 34.5 b-e | 36.0 bc | 2.3fgh | 1.8 hi |
| | Original cultivar | | | | | | | | | | |
| | Balady | 11.10 h | 11.30 f | 76.8 b | 70.8 e | 5.20 e | 5.61 e | 43.7 a | 45.4 a | 1.6 h | 1.4 k |

Means within each column followed by the same letter are not statistically different at 0.05 level (Duncan's range test).

Table 5. Yield and its components for 6 selected mutants of garlic and their original cultivar Eggaseed-1 grown during 2015/2016 and 2016/2017 seasons

| | Genotypes | Total fresh yield (tons/fed.) | | Average cured bulb weight (g) | | Average bulb diameter (cm) | | Number of cloves / bulb | | Average clove weight (g) | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1st | 2nd | 1st | 2nd | 1st | 2nd | 1st | 2nd | 1st | 2nd |
| | γ rays (1 gy) | | | | | | | | | | |
| 1 | Mut 2 | 13.75 ab | 13.53 ab | 93.3 ab | 92.80 ab | 6.80ab | 7.1 ab | 12.2 abc | 12.40 bc | 7.10ab | 7.0 ab |
| | γ rays (10 Gy) | | | | | | | | | | |
| 2 | Mut 6-1 | 13.58 ab | 13.40 ab | 92.7 ab | 90.60 ab | 6.50 abc | 6.80cd | 12.8 abc | 13.00bc | 6.80ab | 6.50ab |
| 3 | Mut 6-2 | 14.30 a | 13.75 ab | 97.30a | 94.80 ab | 7.30 a | 7.20a | 11.9 d | 11.80d | 7.60a | 7.50a |
| | γ rays (15 gy ) | | | | | | | | | | |
| 4 | Mut 1 | 13.28 ab | 12.73 bc | 91.70 ab | 87.30 b | 6.30cd | 6.40cd | 13.80b | 13.50b | 6.20ab | 5.9 ab |
| | Des 0.3 % | | | | | | | | | | |
| 5 | Mut 7 | 14.00 ab | 13.97 a | 95.30a | 93.80a | 7.80a | 7.50a | 12.10c | 12.00c | 7.30a | 7.20a |
| | Original cultivars | | | | | | | | | | |
| | Eggaseed-1 | 12.50 c | 12.17 c | 85.40 c | 83.70 c | 6.10d | 6.00d | 16.20a | 16.70a | 4.90c | 4.7 c |

Means within each column followed by the same letter are not statistically different at 0.05 level (Duncan's range test).

cloves weighted 4.9 to 4.7 g in the first and the second years, respectively.

In Egypt, developing new garlic clones requires special consideration due to the importance of this crop to the Egyptian farmers and the national income. However, induced mutation and clonal selection proved as an effective way for improving garlic crop. Mettwally and Abou Shousha (2002), Hemada *et al.* (2012) and Chenta (2017) found that the higher magnitudes of the range values in most of the economic garlic characteristics such as number of cloves per bulb, bulb diameter, clove size and fresh and cured yield were induced by mutagen.

**Genotypic correlation coefficients:** Concerning the genotypic correlation coefficients, there was significant and desirable positive correlations between plant height with number of leaves/plant, fresh weight of vegetative portion (g/plant), total yield (tons/fed.), average bulb weight (g) while negative correlations with average bulb diameter (cm) and number of cloves/bulb (Table 6). Also, significant positive correlations were detected between total yield (tons/fed.), average bulb weight (g), number of cloves/bulb, average clove weight (g) but negative correlations with average bulb diameter (cm) in Balady type.

Results in Table 7 showed that significant positive correlations between total yield (tons/fed.), number of cloves/bulb and average clove weight (g). Negative correlations between average bulb diameter (cm) and number of leaves/plant were detected. Number of cloves/bulb had significant positive correlations with plant height/cm, number of leaves/plant, fresh weight of vegetative portion (g/plant) and total yield (tons/fed.). Average clove weight (g) had significant positive correlations with plant height/cm, number of leaves/plant, fresh weight of vegetative portion (g/plant) and total yield (tons/fed.) and average bulb weight (g) but negative correlations with average bulb diameter (cm) and number of cloves/bulb in Eggaseed-1 type. The obtained results indicated the importance of the studied characters in yield

Table 6. Genotypic correlation coefficients among eight traits in garlic genotypes of Balady type

| Characters | Plant height (cm) | Number of leaves/ plant | Fresh weight of vegetative portion (g/plant) | Total yield (tons/fed.) | Average bulb weight (g) | Average bulb diameter (cm) | Number of cloves / bulb | Average clove weight (g) |
|---|---|---|---|---|---|---|---|---|
| Plant height (cm) | 1.00 | -0.140 | -0.179 | 0.215* | -0.161 | -0.037 | 0.809** | 0.130 |
| Number of leaves/plant | | 1.00 | 0.956** | -0.307 | 0.991** | 0.940** | 0.966** | 0.055 |
| Fresh weight of vegetative portion (g/plant) | | | 1.00 | -0.098 | 0.011 | 0.163* | -0.127 | 0.012 |
| Total yield (tons/fed.) | | | | 1.00 | 0.990** | -0.341 | 0.962** | 0.987** |
| Average bulb weight (g) | | | | | 1.00 | 0.992** | -0.345 | 0.924** |
| Average bulb diameter (cm) | | | | | | 1.00 | 0.935** | -0.362 |
| Number of cloves/bulb | | | | | | | 1.00 | -0.263 |
| Average clove weight (g) | | | | | | | | 1.00 |

*, ** = Significant at 0.05 and 0.01 level of probability respectively.

Table 7. Genotypic correlation coefficients among eight traits in garlic genotypes (mutant lines and Eggaseed-1 cv.)

| Characters | Plant height (cm) | Number of leaves/ plant | Fresh weight of vegetative portion (g/plant) | Total yield (tons/fed.) | Average bulb weight (g) | Average bulb diameter (cm) | Number of cloves / bulb | Average clove weight (g) |
|---|---|---|---|---|---|---|---|---|
| Plant height (cm) | 1.00 | 0.405 | -0.179 | 0.502* | 0.340 | 0.506* | 0.902** | 0.479 |
| Number of leaves/plant | | 1.00 | 0.968** | -0.921 | 0.963** | 0.952** | 0.923** | 0.686* |
| Fresh weight of vegetative portion (g/plant) | | | 1.00 | 0.640* | -0.473 | 0.732** | 0.565* | 0.715* |
| Total yield (tons/fed.) | | | | 1.00 | 0.953** | -0.917 | 0.978** | 0.906** |
| Average bulb weight (g) | | | | | 1.00 | 0.978** | -0.946 | 0.937** |
| Average bulb diameter (cm) | | | | | | 1.00 | 0.978** | -0.940 |
| Number of cloves/bulb | | | | | | | 1.00 | -0.961 |
| Average clove weight (g) | | | | | | | | 1.00 |

*, ** = Significant at 0.05 and 0.01 level of probability respectively.

improvement and could be considered in the selection program. Significant correlation between fresh yield and plant height was reported by Singh *et al.* (2011); Singh *et al.* (2013) and Satesh *et al.* (2015), and with clove polar diameter and bulb yield by Sharma *et al.* (2016). Consequently, these characters *viz.*, average clove weight (g)**,** number of cloves/bulb and average bulb weight (g**)** turned out to be the major components of total yield and useful in direct selection for yield improvement.

Improvement of crop resistance to various pests is one of the main goals in all agricultural breeding techniqes. Phytopathogens cause huge yield losses in the agriculture every year with large economic losses and damage to ecosystems. The term mutagenesis applies to methods used for the induction of random or site directed mutations in plant DNA to create novel valuable traits in well-adapted cultivars. Mutagenesis could be one of the answer to challenges faced in agriculture. Breeding for mutation has been important area of research in several countries, due to economics and conservation of diversity by stopping gene erosion. According to the FAO/IAEA database, there are 320 cultivars with improved disease resistance using mutagenic agents that were obtained as direct mutant or derived from hybridization with mutant or from progeny (for example by self-fertilization). Induced mutations have been used to improve economically important crops such as wheat, barley, rice, cotton, peanut, banana etc.

**Greenhouse test for pathogenic study:** Data presented in Table 8 showed the effect of percentage white rot infection with *S. cepivorum* under the greenhouse conditions on different clones of Balady type. All mutants were more resistant to white rot infection

Table 8. Evaluation of fourteen mutants of garlic and their original cultivar Balady to artificial infestation with *S. cepivorum*in under greenhouse conditions

| | Treatment/Mutants | White rot infection (%) | Efficacy (%) |
|---|---|---|---|
| | γ rays (1 Gy) | | |
| 1 | MUT 6 | 26.4±1.82 fg | 66.67 |
| 2 | MUT 14 | 29.6±3.21ef | 62.63 |
| | γ rays (5 GY) | | |
| 3 | MUT 1 | 52.8±3.56 c | 33.33 |
| | γ rays (10 GY) | | |
| 4 | MUT 6-1 | 12.8±1.64 i | 83.84 |
| 5 | MUT 6-2 | 73.2±2.80 b | 07.58 |
| | γ rays (15 GY) | | |
| 6 | MUT 1 | 29.0±2.74 ef | 63.38 |
| 7 | MUT 2 | 30.0±4.18 e | 62.12 |
| 8 | MUT 3 | 74.2±2.77 b | 06.31 |
| | DES (0.1 %) | | |
| 9 | MUT 6 | 49.0±2.24 d | 38.13 |
| 10 | MUT 6-1 | 21.0±2.24 h | 73.48 |
| 11 | MUT 7 | 30.6±2.41 e | 61.36 |
| | DES (0.3 %) | | |
| 12 | MUT 10 | 72.0±2.35 b | 09.09 |
| | DEA (0.1 %) | | |
| 13 | MUT 2 | 14.4±2.61i | 81.82 |
| 14 | MUT 2-1 | 23.4±2.07 gh | 70.45 |
| | Original cultivar Balady (Control) | 79.2±3.11a | 00.00 |
| | LSD (*P*=0.05) | 3.4103 | - |

All data are averages of five replications ± standard deviation (SD). Means followed by the same letter(s) are not significantly different (*P* = 0.05).

compared to the original cultivar Balady and had a significant difference with it. Mut 6-1 which resulted from treatment with γ rays (10 GY), Mut 2 which resulted from treatment with DEA (0.1 %) and Mut 6-1 that resulted from treatment with DES (0.1 %) were significantly highly resistant clones to the disease with infection percentage 12.8, 14.4 and 21.0 % and efficacy 83.84, 81.82 and 73.48 %, respectively compared with original cultivar Balady (79.2 % infection). At the same time, Mut 3 that resulted from γ rays (15 GY) and Mut 6-2 which resulted from γ rays (10 GY) were more infected with the disease with infection percentage 74.2 and 73.2 % with efficacy 6.31 and 7.58 %, respectively compared with original cultivar Balady.

Results illustrated in Table 9 showed that, Mut 6-2 and Mut 6-1 which resulted from treatment with γ rays (10 GY) were significantly highly resistant to the disease with infection percentage 10.4 and 13.8 % and efficacy 85.87 and 81.25 %, respectively compared with original cultivar Eggaseed-1 (73.6 %). Mut 1 that resulted from γ rays (15 GY) was the highly infected one with the disease infection percentage of 65.8 % and efficacy 10.6 %, respectively compared with original cultivar Eggaseed-1.

Table 9. Evaluation of five mutants of garlic and their original cultivar Eggaseed-1 to artificial infestation with *S. cepivorum* under greenhouse conditions

| | Mutants | White rot infection (%) | Efficacy (%) |
|---|---|---|---|
| | γ rays (1 Gy) | | |
| 1 | Mut 2 | 25.4±2.07c | 65.49 |
| | γ rays (10 Gy) | | |
| 2 | Mut 6-1 | 13.8±2.59d | 81.25 |
| 3 | Mut 6-2 | 10.4±1.52d | 85.87 |
| | γ rays (15 Gy) | | |
| 4 | Mut 1 | 65.8±3.19b | 10.60 |
| | Des 0.3 % | | |
| 5 | Mut 7 | 25.6±3.05c | 65.22 |
| | Original cultivars | | |
| | Eggaseed-1(Control) | 73.6±4.10a | 00.00 |
| | LSD (*P*=0.05) | 3.6355 | - |

All data are averages of five replications ± standard deviation (SD). Means followed by the same letter(s) are not significantly different (*P* = 0.05).

**Field test for pathogenic study:** Throughout two successive seasons of 2017/2018 and 2018/2019, the reaction of new mutants which resulted from the two original cultivars (Balady and Eggaseed-1) to infection with white rot disease was evaluated under infested field conditions. Data presented in Table 10 showed the effect of white rot disease under natural infestation with *S. cepivorum*in conditions on different mutants which resulted from original garlic cultivar Balady. Similar to of the results in greenhouse test, most of obtained mutants scored less infection to white rot infection comparing with their original cultivar Balady. Mut 6-1 that resulted from treatment with γ rays (10 GY) and Mut 2 from treatment with DEA (0.1 %) were significantly highly resistant mutants to the disease in the two tested seasons with infection percentage of 11.33 and 12.33 % in the first season and 13.33 and 15.33 % in the second one with efficacy 80.47, 78.74 and 78.15, 74.87 %, respectively. The original cultivar, Balady scored 58 and 61 % infection, respectively in two seasons. On the other hand, mutant Mut 3 resulted from γ rays (15 GY) and Mut 10 resulted from DES (0.3 %) were more infected with the disease in the two tested seasons with infection percentage of 53.33 % in the first season and 58.00 and 55.00 % in the second one with efficacy 8.05 % in the first season and 4.92, 9.84 % in the second one, respectively compared with their original cultivar, Balady. Correspondingly, Mut 6-2 resulted from treatment with γ rays (10 GY) was more susceptible than the original cultivar, Balady in the two tested seasons with infection percentage 61.67 and 66 % by efficacy -6.33 and -8.2 %, respectively.

Results explained in Table 11 showed that Mut 6-2 and Mut 6-1 resulted from treatment with γ rays (10 GY) had significantly less infection percentage 8.33 and 10.67 % in the first season and 9 and 10.67 % in the second one with efficacy 81.21, 75.93 and 81.38, 79.99 %, respectively, compared to original cultivar, Eggaseed-1 (44.33 and 48.33 % infection). On the other hand, mutant line Mut 1 which resulted from γ rays (15 GY) was highly infected with the disease and more susceptible than the original cultivar, Eggaseed-1 in the two tested seasons.

As yet, a number of mutants that display improved disease reaction to phytopathogens have been recognized, many of them constitutively display defense response phenotypes, including natural lesions and defense gene expression. Several of these are also smaller in size, indicating a negative correlation between defense induction and growth (Dietrich *et al.,* 1995; Rate *et al.,* 1999). For the enhancement of disease resistance, the induction of forced mutations is applied by different mutagenesis approaches: virus induced gene silencing, RNA-mediated interference, *Agrobacterium*-mediated insert mutagenesis, radiation and chemical mutagenesis and with combined approaches such as Targeting Induced Local Lesions in Genome (TILLING). Using radiation breeding programs for improving yield, quality, taste and disease and pest resistance have been reported in garlic, onion, cereals, legumes, cotton, peppermint, sunflowers, peanut, grapefruit, sesame, banana and cassava by Kozjak and Meglič (2012).

Peiris *et al.* (2008) defined a tomato cultivar resistant to bacterial wilt (*Ralstonia solanacearum*). It was obtained by irradiation with 320 Gy gamma-rays in Sri Lanka. In the same way, numerous cultivars of rice, maize, wheat, cotton, chickpea, rapeseed, sesame, apple and durum wheat have been released which are resistant to different bacteria, viruses and pathogens (Kiruki *et al.,* 2006).

Our results are in agreement with Pérez-Moreno *et al.* (1991) who recorded an increase of garlic resistance to *S. cepivorum* by gamma induced mutations. They performed selection, only in M1V2 and without a real selection pressure. Al-Safadi *et al.* (2000) conducted a mutation breeding programme to improve garlic (*Allium sativum* L.) resistance to white rot (*S. cepivorum*) and to enhance its stability under natural conditions. Twelve clones from *cv.* Kisswany had only a 3 % infection percentage. Cloves of two local garlic cultivars (Kissvany and Yabroudy) were irradiated with gamma ray doses 4, 5, 6, and 7 Gray (Gy). The cloves were planted in the field and the plants were advanced for 4 generations to isolate mutations in established form. Twelve lines from Yabroudy *cv.,* had less than 5 % infection percentage as compared to 20 % in the control. Also, stability under natural conditions has enhanced. Weight loss during storage decreased from 8 % in the control to only 4 % in some Kisswany clones and from 10 to 3 % in some Yabroudy clones.

Table 10. Evaluation of fourteen mutants of garlic and their original cultivar Balady on under natural infestation with *S. cepivorumin* under open field conditions during 2017/2018 and 2018/2019 seasons.

| | Treatment /Mutants | White rot infection (%) | | Efficacy (%) | |
|---|---|---|---|---|---|
| | | 2017/2018 | 2018/2019 | 2017/2018 | 2018/2019 |
| | γ rays (1 Gy) | | | | |
| 1 | MUT 6 | 19.33±1.53 fg | 22.00±1.00 ij | 66.67 | 63.93 |
| 2 | MUT 14 | 19.67±1.35 fg | 23.33±1.53 hi | 66.08 | 61.75 |
| | γ rays (5 GY) | | | | |
| 3 | MUT 1 | 44.33±2.52 c | 44.67±2.89 e | 23.57 | 26.77 |
| | γ rays (10 GY) | | | | |
| 4 | MUT 6-1 | 11.33±0.58 h | 13.33±1.53 k | 80.47 | 78.15 |
| 5 | MUT 6-2 | 61.67±2.08 a | 66.00±2.00 a | -06.33 | -08.20 |
| | γ rays (15 GY) | | | | |
| 6 | MUT 1 | 18.67±1.53 g | 19.33±0.58 j | 67.81 | 68.31 |
| 7 | MUT 2 | 23.00±2.65 f | 26.00±2.00 h | 60.34 | 57.38 |
| 8 | MUT 3 | 53.33±1.53 b | 58.00±1.00 c | 08.05 | 04.92 |
| | DES (0.1 %) | | | | |
| 9 | MUT 6 | 36.33±3.79 d | 39.33±1.53 f | 37.36 | 35.52 |
| 10 | MUT 6-1 | 18.33±153 g | 22.67±0.58 i | 68.40 | 62.84 |
| 11 | MUT 7 | 28..33±2.5 e | 30.67±3.06 g | 51.16 | 49.72 |
| | DES (0.3 %) | | | | |
| 12 | MUT 10 | 53.33±3.10 b | 55.00±1.00 d | 08.05 | 09.84 |
| | DEA (0.1 %) | | | | |
| 13 | MUT 2 | 12.33±1.53 h | 15.33±1.53 k | 78.74 | 74.87 |
| 14 | MUT 2-1 | 21.67±3.51 fg | 24.00±1.73 hi | 62.64 | 60.66 |
| | Original cultivar | | | | |
| | Balady (Control) | 58.00±2.00 a | 61.00±1.00 b | 00.00 | 00.00 |
| | LSD (*P*=0.05) | 3.9354 | 2.8451 | - | - |

All data are averages of three replications ± standard deviation (SD). Means followed by the same letter(s) are not significantly different ($P = 0.05$) in the same season.

Table 11. Evaluation of five mutants of garlic and their original cultivar Eggaseed-1 under natural infestation with *S. cepivorum*in under open field conditions during 2017/2018 and 2018/2019 seasons

| | Treatment/Mutants | White rot infection (%) | | Efficacy (%) | |
|---|---|---|---|---|---|
| | | 2017/2018 | 2018/2019 | 2017/2018 | 2018/2019 |
| | γ rays (1 gy) | | | | |
| 1 | Mut 2 | 26.67±1.53 c | 26.00±3.46 b | 39.84 | 46.20 |
| | γ rays (10 Gy) | | | | |
| 2 | Mut 6-1 | 10.67±0.58 e | 09.67±0.58 d | 75.93 | 79.99 |
| 3 | Mut 6-2 | 08.33±0.58 e | 09.00±1.00 d | 81.21 | 81.38 |
| | γ rays (15 gy ) | | | | |
| 4 | Mut 1 | 48.67±2.08 a | 51.00±1.00 a | -09.79 | -05.52 |
| | Des 0.3 % | | | | |
| 5 | Mut 7 | 15.33±3.06 d | 13.33±1.15 c | 65.42 | 72.42 |
| | Original cultivars | | | | |
| | Eggaseed-1(Control) | 44.33±2.52 b | 48.33±1.16 a | 00.00 | 00.00 |
| | LSD (*P*=0.05) | 3.7136 | 3.2146 | - | - |

All data are averages of three replications ± standard deviation (SD). Means followed by the same letter(s) are not significantly different ($P = 0.05$) in the same season.

Shah *et al.* (2009) used gamma irradiation and ethyl methane sulphonate (EMS) for resistance in chickpea to Fusarium wilt in natural wilt sick plot during 2003-2004 seasons. All the four parent genotypes showed a highly susceptible reaction to the disease. Out of a total of 249 morphological mutants, 75 mutants demonstrated a highly resistant reaction (less than 10 %) followed by 31 mutants resistant (11 to 20 %), 34 mutants moderately resistant/tolerant (21 to 30 %), 35 mutants susceptible (31 to 50 %) and 75 mutants were highly susceptible (50 to 100 %). The mutagenic treatments proved to be mutable in producing morphological mutations along with improved tolerance to Fusarium wilt. These mutants with resistant to tolerant reaction for Fusarium wilt could be used in a hybridization program for transferring resistance genes into high yielding elite cultivars. Arici *et al.* (2017) showed that there was a significant difference between the EMS concentrations ($P < 0.05$) in potato mutant's resistance to Fusarium dry root (*Fusarium avenaceum*). The most effective level of EMS for disease resistance was 20 mM EMS, 20 min (22 % strength plant). The highest mortality rate (96 %)was observed in plants treated with 100 mM EMS, 20 min. No statistically significant difference was observed in plant length of all applications. Plants showed normal development as a result of mutation application. Furthermore, variety produced a different response with addition of EMS concentration, and different levels of resistance were observed in this variety.

**Molecular pathology study:** Real-time PCR differs from classical PCR by the measurement of the amplified PCR product at each cycle throughout the PCR reaction. In practice, a video camera records the light radiated by a fluorochrome unified into the newly synthesized PCR product. Therefore, real-time PCR allows the amplification to be followed in real-time during the exponential phase of the run, and thus allows the amount of starting material to be determined exactly. In current study, we employed QRT-PCR technique for investigation the expression pattern of some defense genes (*PR1, PR5, PAL* and *HQT*) which regulated response towards *Sclerotium cepivorum* infection in garlic mutant lines and their original cultivars. Upon elicitor, fungal, and bacterial infections, plants pledge a large spectrum of defense responses and transcript of several genes involving several pathways (Liu *et al.,* 1994; Newman *et al.,* 1994). For instance, phenylpropanoid biosynthesis pathway is controlled by different genes such as phenylalanine ammonia-lyase (PAL) and chalcone synthase (CHS) which code for key enzymes involved in the synthesis of lignin precursors and the synthesis of flavonoids and isoflavonoid-derived phytoalexins (Saunders and O'neill, 2004). Plants yielded a group of *PR1* proteins that display differential toxicities to numerous plant pathogens (Niderman *et al.*, 1995). The PR proteins such as *PR5* and *PR2* proteins have generally resulted in enhancing disease resistance in some plant species. For example, potato osmotin (PR5) improves resistance to *Phytophthora infestans*, the causal pathogen of potato late blight and induced b-1,3- glucanase (BGL) in turnip leaves in the incompatible interactions than in the compatible interaction after inoculation with pushovers of *Xanthomonas campestris* (El-Komy *et al.,* 2010). In another study on six defense-related genes, *PR1, PR2, PR4, PR9, PR10*, and phenylalanine ammonia-lyase (PAL), in wheat plants infected with *Barley stripe mosaic virus* (BSMV), Tufan *et al.* (2011) concluded that the defense-related genes, except PR2, were significantly induced in BSMV–wheat infected plants. The *PR5* family comprises thaumatin-like proteins (Wang *et al.,* 1996).

Data illustrated in Fig. 1 recorded that all tested genes regulated-up or down in different mutants in comparing with their original cultivar (Balady). *PR1* gene showed high significant expression and regulated-up in the mutant named Mut 6 (1 Gy), Mut 6-2 and Mut 6-1 resulted from treatment with γ rays (10 GY), Mut 1 (15 GY), Mut 6 (0.1 % DES) and Mut 2 (0.1 % DEA) with relative expression values 1.5996, 1.6348, 1.5430, 1.4161,1.1462 and 1.6172, respectively. *PR1* gene expression was regulated-down and showed highly significant reduction in the mutant lines Mut 3 (15 GY) with relative expression value 0.8771 in comparision with the original cultivar (Balady). While, *PR5* gene showed high significant expression and regulated-up in the mutant lines Mut 6-1 (10 GY), Mut 1 (15 GY) and Mut 6 (0.1 % DES) with relative expression values of 1.4502, 1.1846 and 1.1527, respectively. *PR5* gene expression was regulated-down and showed highly significant reduction in the mutant lines Mut 6 (1 Gy), Mut 6-2 (10 GY), Mut 3 (15 GY) and Mut 10 (0.3 % DES) with relative expression value of 0.8659, 0.9111, 0.8539and 0.7491, respectively. *PAL* gene regulated-down and showed highly significant reduction in all obtained mutant lines except Mut 6 (1 Gy), Mut 14 (1 Gy) and Mut 6-1 (10 GY) which showed highly significant expression and regulated-up with relative expression values of 1.2791, 1.3109 and 1.3006, correspondingly. *HQT* gene was regulated-up in most obtained mutant lines with highly relative expression value 2.1822 for each one, Mut 6-1(10 GY) and Mut 2 (0.1 % DEA) but regulated-down with highly significant reduction and relative expression values of 0.7288 and 0.7079 for Mut 3 (15 GY) and Mut 10 (0.3 % DES), correspondingly. We can extract that, Mut 6-1 resulted from treatment with γ rays (10 GY) which had significantly high resistance to garlic white rot disease showed great regulation-up of detective defense genes (*PR1, PR5, PAL* and *HQT*) with high relative expression values as compared to their original cultivar, Balady. On the other hand, mutant line named Mut 3 resulted from γ rays (15 GY) which was more infected one with white rot disease showed great regulation-down of detective defense genes (*PR1, PR5, PAL* and *HQT*) with low relative expression values as compared to their original cultivar, Balady. Our results support the role of mutation treatments in induction of these defense genes and their effect on resistance against garlic white rot disease.

Data demonstrated in Fig. 2 verified that tested defense genes (*PR1, PR5, PAL* and *HQT*) were up-regulated in more resistant mutant lines (Mut 6-2 and Mut 6-1) to garlic white rot disease. Both mutants resulted from treatment with γ rays (10 GY) and Mut 7 (0.3 % DES), respectively. They showed great relative expression values equated with original cultivar, Eggaseed-1. On the other hand, mutant named Mut 1 that resulted from γ rays (15 GY) and was more infected with white rot disease showed great regulation-down of detective defense genes (*PR1, PR5* and *HQT*) with low relative expression values and presented limited regulation-up of *PAL* gene expression comparing with its original cultivar, Eggaseed-1. Result showed that respective encoded PR genes and polyphenol synthetic genes might play important roles in defense reaction of obtained mutants of two tested garlic cultivars to *S. cepivorum* infection.

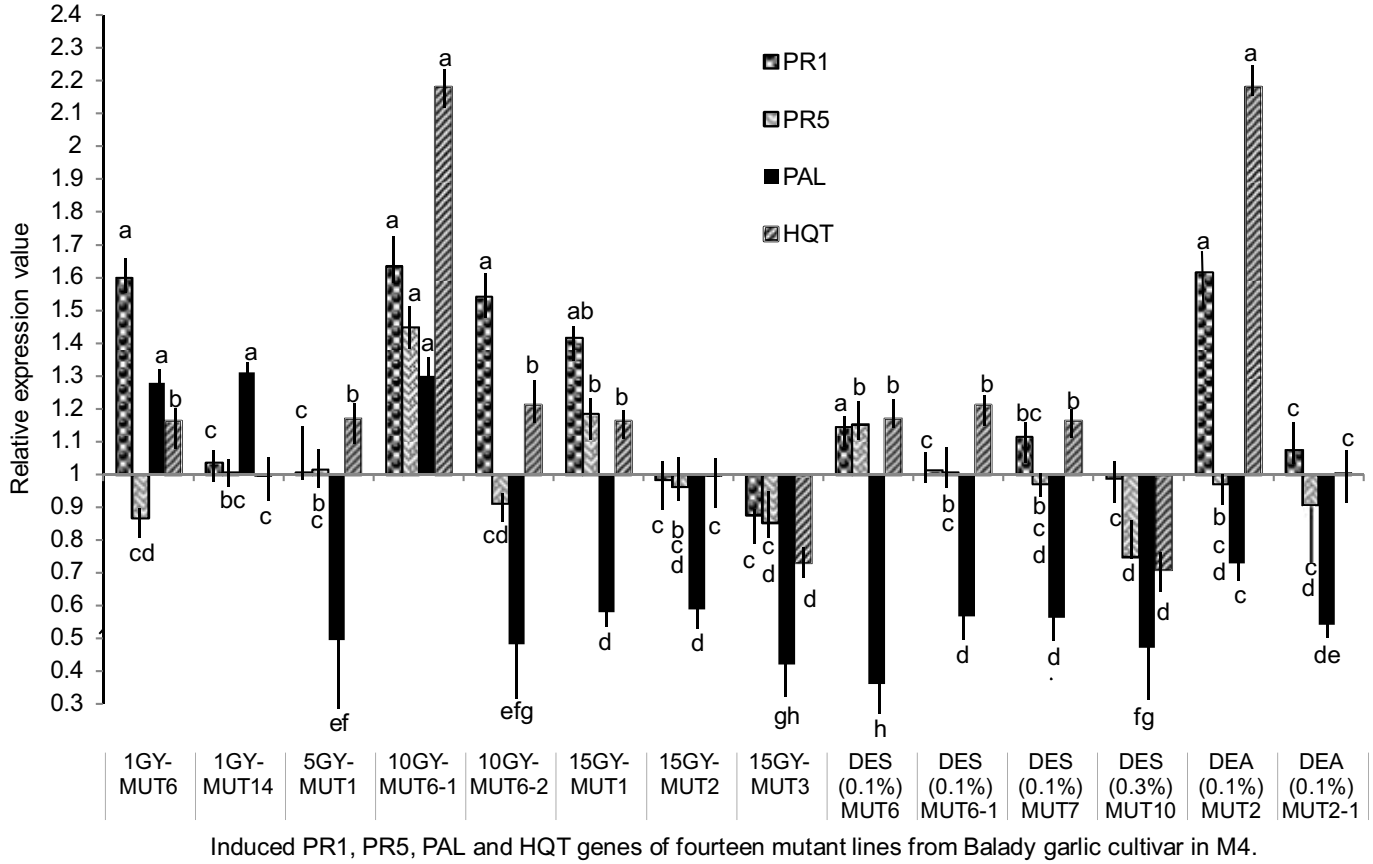


Fig 1. Relative expression value of four tested genes (*PR1, PR2, PAL* and *HQT*) regulated in fourteen mutants clones from Balady garlic cultivar in M1V4 against infection with *Sclerotium cepivorum,* the causal pathogen of white rot disease. Relative expression levels more than 1 revealed an increase of accumulation (up-regulation) and values lower than 1 means a decrease in expression (down-regulation) in matching of the original cultivar. Columns labeled by the same letter(s) are not significantly different ($P = 0.05$) in the same primer for each mutant line comparing with original cultivar (Balady) and the error bars indicate the standard errors of three biological replicates.

Our results were in harmony with that recorded by several investigators who demonstrated induction of PR genes and their role in plant diseases resistance mechanisms and using of real-time qPCR to determine that. Satoh *et al.* (2011) found that the Rice dwarf virus also leads to the activation of the *PR5* gene, suggesting that the *PR5* domain might bind a polypeptide ligand, and raising the possibility that mature *PR5* proteins may also interact with polypeptides. Hafez *et al.* (2013) studied the viral coat protein gene expression in leek infected by iris yellow spot virus (IYSV) using real-time PCR. El Morsi *et al.* (2015) suggested that the expression of the *PR1* gene is a response to viral infection and not due to the thrips feeding. The expression of *PR1* after 1 dpi and subsequent increased peak levels after 8 dpi suggested that up-regulation of this protein is among the first defense responses against IYSV infection in onion cells.

Behiry *et al.* (2017) demonstrated that through qPCR results, high levels of *PR5* transcripts expression in potato tissues infected with soft rot *Pectobacterium carotovorum* subsp. *carotovorum* were seen in the resistant cultivar, "Nicola" when compared to the susceptible cultivar Ladypal, but the transcript levels dropped immediately in the two cultivars, but it was earlier in case of ladypal for (after 6 h) when compared with Nicola, indicating a potential early role for this gene in soft rot resistance in potato. They proposed that the suppression of the two genes (*PR2* and *PR5*) was early in the susceptible cultivar compared with the resistant one due to the high effect of the *P. carotovorum* on the plant immune system. This effect may be kind of gene–gene interaction. It was observed that the gene expression of the *PR2* gene in the control plants was higher in case of Ladypalfor when compared with Nicola.

Mahmoud *et al.* (2019) demonstrated that the effect of gamma irradiation on the plant phenotypic variation was observed by estimating morphological features of plants. Nevertheless, the difference in morphological characteristics may be due to the environmental differences. Thus, the genetic diversity between radiation treatments and the control (untreated) was estimated based on RAPD and ISSR markers and by estimating GS. ISSR revealed a higher polymorphism among treatments than did RAPD. Furthermore, the genetic similarity results showed that treatments of Sids-40 with 4 and 6 Gy were vaguely related, and these doses had similar effect. This includes the high genetic diversity between treatments Sids-40 with 8 Gy and Balady with 6 Gy. Genetic makeup of Sids-40 genotype was different from all genotypes due to the effect of γ ray (8 Gy), and the high genetic vividness between treatments Sids-40 with 2, 4 and 6 Gy from the other treatments of radiation.

In conclusion, the findings of the present study revealed that application of some physical and chemical mutagens had been able to improve garlic. Fourteen mutants clones from Balady and five mutants clones from Egassed-1 garlic cvs. had been selected in M1V4. Mutants clones of both tested garlic cultivars had been superior in yield, quality and more resistant to white rot disease than its original Balady and Eggaseed-1 cultivars. Also, defense genes against *S. cepivorum* infection were stimulated in some resulted mutants in comparision with the original.

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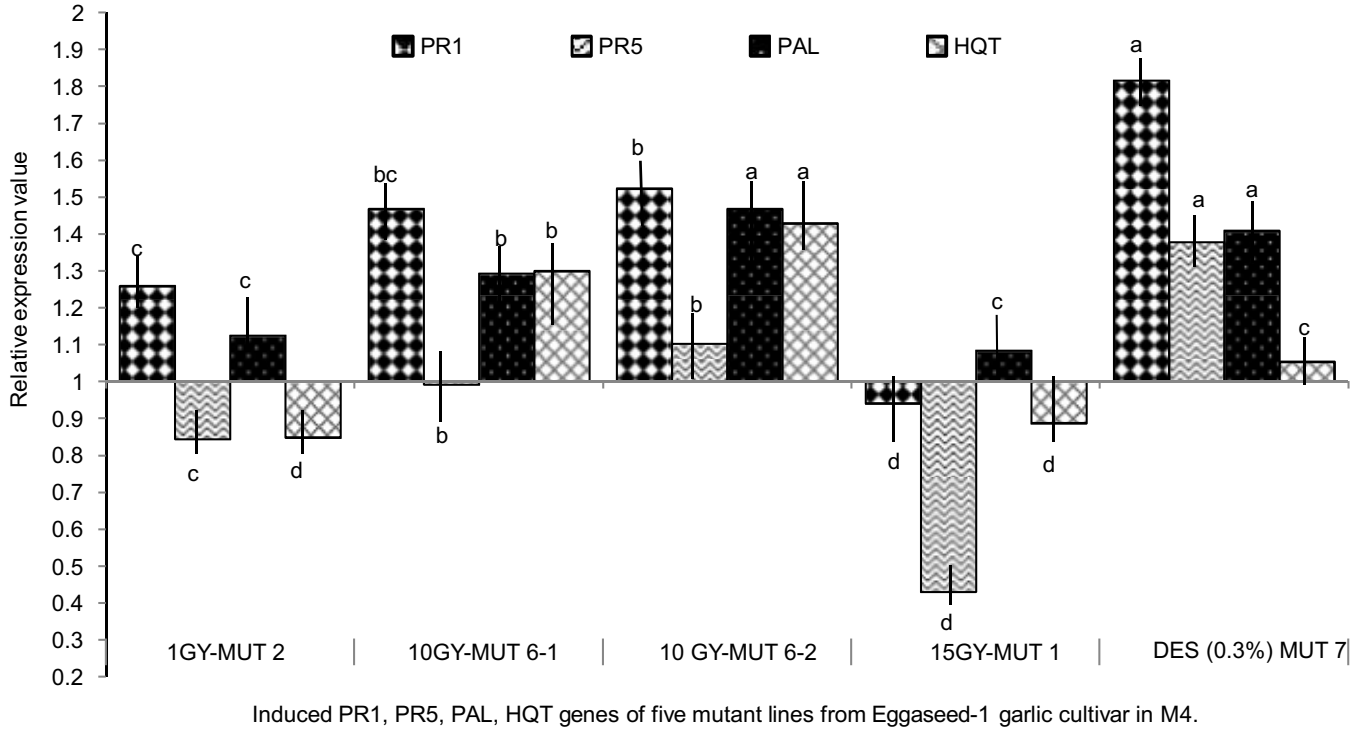


Fig 2. Relative expression value of four tested genes (*PR1, PR2, PAL* and *HQT*) regulated in induced five mutant clones from Eggassed-1 garlic cultivar in M1V4 against infection with *Sclerotium cepivorum,* the causal pathogen of white rot disease. Relative expression levels more than 1 revealed an increase of accumulation (up-regulation) and values lower than 1 means a decrease in expression (down-regulation) in matching of the original cultivar. Columns labeled by the same letter(s) are not significantly different ($P = 0.05$) in the same primer for each mutant line comparing with original cultivar (Eggaseed-1) and the error bars indicate the standard errors of three biological replicates.

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