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Research Article

Enhancing Growth and Physiological Traits of Faba Bean Plants through Inoculation with *Trichoderma Viride* and UV-Induced Mutants

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Abstract:

The experiment examined the effects of inoculating *Trichoderma viride* and its UV-induced mutations various physiological characteristics and growth indices in faba bean plants (*Vicia faba* L). The findings showed that, in comparison to the control group, inoculation with *Trichoderma viride* and its variants markedly improved the growth parameters of faba bean plants at the 21-day stage. UV-induced mutations, namely mutants 28 and 40, showed significant increases in fresh and dry weights of shoot and root, leaf area, shoot and root lengths, and pigment content, particularly levels of carotenoid and chlorophyll. Additionally, the mutations resulted in decreased synthesis of malondialdehyde (MDA) and increased content of proline. *Trichoderma viride* and its variants significantly increased the activity of antioxidant enzymes, such as polyphenol oxidase (PPO) and ascorbate peroxidase (APX), with the most notable benefits occurring in mutants 28 and 40. These results highlight how *Trichoderma viride* and its mutations can improve the physiological responses and vigor of faba bean plants, providing information about how they might be used in agricultural techniques to increase crop productivity and stress tolerance.

1. Introduction

Faba bean is regarded as the preeminent food legume crop in Egypt. The global economic significance of faba bean agriculture can be attributed to its substantial nutritional content, including vitamins, protein, carbs, and other substances (Barakat et al., 2014).

Trichoderma is a filamentous fungal genus inhabiting soil, possessing teleomorphs, and belonging to the order Hypocreales within the division Ascomycota. Trichoderma has significant genetic diversity, encompassing various capacities across distinct strains of agricultural and industrial importance. It has established a significant role in agriculture as an effective biocontrol agent, in addition to functioning as a plant growth booster and enhancing soil fertility through its disease suppression and composting capabilities (Ventorino et al., 2014; Colla et al., 2015; Du Jardin, 2015; Lorito and Woo, 2015; Rouphael et al., 2015; Viscardi et al., 2016). *Trichoderma*–plant association can remediate sites with multiple contaminants due to its metal-detoxifying properties and other physiological traits, such as deleting organic contaminants (Tripathi et al., 2013). Besides pesticide activity, some Trichoderma strains have bio stimulant activity, plant growth stimulation, better yield and nutritional quality, and abiotic stress mitigation (López-Bucio et al., 2015; Hermosa et al., 2012; Lorito et al., 2010).

Inducing mutation in *Trichoderma* spp. may improve agricultural yield and quality (Saued et al., 2013). Different gamma ray dosages can cause beneficial responses by causing physiological and metabolic changes. By genetically altering these microbes, the research improved their growth-stimulating effects. Inducing genome mutation with certain doses of gamma rays has increased *Trichoderma's* antagonistic power and biocontrol abilities. They boost Trichoderma species' function by altering their fungal spores (Rezalou et al., 2023).

UV light causes UV-signature and triplet mutations in cells and skin (Ikehata and Ono, 2011). Different kinds of DNA damage are caused by UV radiation at various wavelengths. UVB produces DNA photoproducts by directly exciting the DNA molecule, primarily pyrimidine, pyrimidone, and cyclobutane pyrimidine dimers (CPDs) (6,4-PPs). During replication, these photoproducts misincorporate adenine opposite cytosine, resulting in common mutations known as C→T transitions (Rünger and Kappes, 2008). The current study aims to 1- Induced new mutants of Trichoderma by UV. 2- Evaluate the effect of *Trichoderma viride* and its mutants on faba bean plants (*Vicia faba*) on the level of growth parameters, including (lengths, fresh and dry weights of the root and shoot and leaf area) and physiological response such as photosynthetic pigments, proline, malondialdehyde, and antioxidant enzymes.

2. Materials and Methods

Experiment location and plant material

This study was conducted during the 2022–2023 growing season at the Agricultural Experiment farm, Faculty of Agriculture, Tanta University, Egypt. The faba beans (*Vicia faba*) cultivar used in this investigation was Sakha 1 that obtained from the agricultural research center, Sakha, Kafr El-Sheikh, Egypt.

Fungus strains

The fungal strain *Trichoderma viride* was generously donated by Dr. Ibrahim Tolba of El-Azhar University's Faculty of Agriculture's Plant Pathology branch.

Induction mutation for *Trichoderma viride* by UV

Conidia from one-week-old PDA plates were suspended in 11 ml sterile water. One ml was taken as control, and 10 ml was treated with UV at 15 cm from the UV source (Philips TUV 30W G30T8 ultraviolet lamp) and taken at various times after 5, 15, 30, 45, and 60 min.. Samples of 1 ml conidial suspension from each treatment were diluted by serial dilution. Each dilation is plated on a PDA as complete media. The plates were incubated at 28 ^{o}C. After incubation for two days, colonies were counted for survival and tested for mutation after five days of incubation.

Experimental design

Seeds were planted in black plastic bags filled with soil (Bulk soil (0–30 cm) was obtained from agricultural land in El-Gharbia governorate, Egypt, air-dried, pulverized, sieved through a 2 mm screen, and stored in plastic bags until experimental usage with 6 kilograms per bag(Elbasiouny et al., 2023)); each treatment had four replicates (eight seeds per replicate). The faba bean seeds were sown on 15th November at the rate of 8 seed/ bags for broad beans at equal distances and depth; after 2 weeks from sowing, the plants were thinned to 6 seedlings and inoculated with a spore suspension of Trichoderma viride wild type and its mutants (10 ml of 5 ×10^{7} / ml) (Table 1). After 21 days, the samples were measured for physiological and biochemical parameters

Table 1. The code of Trichoderma and its mutants

No.	Treatment	No.	Treatment
1	Control	35	UV 30 min
2	Wild type	36	UV 30 min
28	UV 5 min	37	UV 30 min
29	UV 5 min	38	UV 30 min
30	UV 15 min	39	UV 60 min
31	UV 15 min	40	UV 60 min
32	UV 15 min	41	UV 60 min
33	UV 15 min	42	UV 60 min
34	UV 30 min	43	UV 60 min

No.: number of treatment, 1 and 2 refer to the control groups, from 28 to 43 refer to the mutants of *Trichoderma viride*; UV: ultraviolet; 5, 15, 30 and 60 minutes refer to the time of UV exposure.

Growth measurements:

Five seedlings of each replicate were taken for the following traits: plant length (shoot and root (cm), shoot and root fresh weights (g), shoot and root dry weights (g), and leaf area (cm^{2}).

Physiological traits:

Physiological and biochemical traits (chlorophyll contents, antioxidants enzymes, MDA, and proline content) were recorded on five fresh seedlings from each treatment for testing the effect of inoculation with *Trichoderma viride* and its mutants.

Photosynthetic pigments

Chlorophyll levels were estimated using the spectrophotometric (UV1901PC) method, according to (Abdelfattah et al., 2024; Nofal et al., 2024). Chlorophyll was expressed as mg g^{-1} FW by this equation:

Chlorophyll a (mg g^{-1} FW) = 15.65 A666 - 7.340 A653

Chlorophyll b (mg g^{-1} FW) = 27.05 A653 - 11.21 A666

Carotenoids (mg g^{-1} FW) = (1000*A470) - (2.86 – Ch a) – (129.2*Ch b /245)

Where A 666, A470 and A653 are absorbance at A653, A470 and A666 nm.

Antioxidants enzymes activity assay

Polyphenol oxidase activity (PPO)

PPO activity was determined according to the method of (Mayer and Harel, 1979) at 490 nm. The test solution was prepared by mixing 200 µl of enzyme solution with 0.01 mM catechol solution (prepared in 0.1 M Na phosphate buffer, pH 6.5). The blank was prepared with the same amount of catechol and 0.03 ml of 50 mM Na phosphate buffer without enzyme extract.

Ascorbate peroxidase enzyme activity (APX)

The following reaction mixture was used (Yoshimura et al., 2000): 0.2 ml of the enzymatic extract, 25 mM phosphate buffer (pH = 7), 1 mM hydrogen peroxide, 0.25 mM ascorbic acid, and 0.1 mM EDTA. For the estimation, H2O2 was added to the mixture to start the enzymatic process. After adding the enzyme extract, a spectrophotometer measured the light absorption for one minute at 290 nm.

Biochemical contents

Proline content

The following proline concentration was measured spectrophotometerically at 520 nm using the ninhydrin methodology (Bates et al., 1973). The homogenate was filtered through filter paper after homogenizing roughly 0.5 g of plant material in 10 mL of 3% aqueous sulfosalicylic acid (SSA). In a test, 2 milliliters of the filtrate were combined with 2 milliliters of glacial acetic acid and 2 milliliters of ninhydrin acid. The tubes were heated to 100 ^{o}C for an hour in a water bath, and then they were placed in a bath with ice to finish the reaction. Finally, Four milliliters of toluene were combined with the reaction mixture. For twenty to thirty seconds, it was vigorously mixed. After heating it to room temperature, toluene was used as the blank, and the absorbance was measured at 520 nm. Using a standard curve as a guide, the proline concentration was computed using fresh weight as follows: (mg proline x ml toluene) / 115.5]/[(g sample)/5] = moles of proline/g of fresh weight material. 115.5 is the molecular weight of proline

Malondialdehyde (MDA)

MDA content was measured according to **(Heath and Packer, 1968)**. Samples weighing approximately 0.5 g were dissolved in 10 mL of trichloroacetic acid (0.1%) (w/v). After centrifuging the homogenate at 1500 × g for 10 minutes, the liquid was filtered and used for the analysis. Two milliliters of the diluted extract and two milliliters of TBA (0.67%) made with TCA (20%) were mixed. It should be incubated in water (95–100°C) for 30 minutes. After that, after five minutes at room temperature, it should be put in an ice bath. In that order, the aqueous phase's absorbance was measured at 450, 532, and 600 nm.

The following formula was used to determine the MDA concentration in the aqueous phase: C (µmol/L) = 6.45 × (A532 − A600) − 0.56 × A450.

Statistical analysis

The data were presented as means ±SD. The statistical significance was calculated by two-way ANOVA (analysis of variance) using SPSS 20 software, and the individual comparisons between treatments were measured by the Turkey multiple range test at $P<0.05$.

3. Results and discussion

3.1. Total count of *Trichoderma viride* after treatment with UV at various exposure times

Figure (1) shows the total count of *Trichoderma viride* after treatment with UV at different exposure times. A reduction in total count was observed with increasing exposure time. The total count for the control group was 2,760 × 10^3. Significant differences were noted between exposure times, except between 30 and 60 minutes.

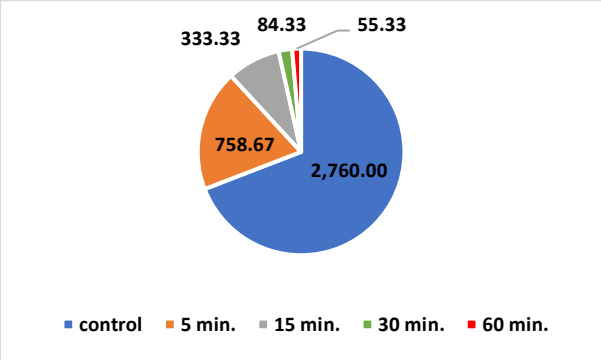

Figure 1. Total count of *Trichoderma viride* after treatment with UV at various exposure times

3.2. Effect of inoculation faba bean plants by *Trichoderma viride* and its mutants on the growth traits.

The experiment was conducted to investigate the effect of *Trichoderma viride* and its mutants induced by UV at different times on the growth traits (lengths, fresh and dry weights of the root and shoot and leaf area) of faba bean plants (*Vicia faba* L).

The results revealed that inoculation with *Trichoderma viride* (wild type and most of its mutants) produced a significant increase in length, fresh and dry weights of the shoot and root and leaf area of faba bean plants at 21 days compared with control. The mutants induced by UV, 28 and 40 recorded high percentage ratios by 154, 228, 266, 335, 388, 333, 156% and 151, 225, 225, 450, 411, 262, 140% lengths of shoot and root, fresh and dry weights of the shoot and root and leaf area respectively in relation to the negative control (Fig. 2-8)

These results agree with (El-Dabaa and Abd-El-Khair, 2020), who stated that when administered, *Trichoderma* spp. *(T. harzianum, T. viride, and T. vierns)* improved the experiment's faba bean plants' growth characteristics, including shoot length, shoot fresh weight, shoot dry weight, and leaf number. Also (López-Bucio et al., 2015) stated that certain strains of *Trichoderma* are distinctive for their prolonged use in horticulture because of their major biostimulant effect. *Trichoderma* releases auxins, small peptides, volatiles, and other active metabolites into the rhizosphere to increase plant growth and yield. This process of phytostimulation involves multilevel communication with the root and shoot systems. Several substances secreted by the fungal mycelium improve the root system's ability to branch, which enhances the uptake of nutrients and water.It may be concluded that increased availability and uptake of the elements led to a higher weight of full grains in rice plots treated with the fungus, as these plots had higher yields and less accessible soil P and Zn after harvest (Cuevas, 2006). According to (Ali et al., 2022), most *Trichoderma* strains release organic acids such as fumaric, citric, and gluconic acid into their environment. This mechanism aids in the dissolution of iron, manganese, magnesium, phosphate, and micronutrients, ultimately fostering plant growth. The results of this investigation indicate that the relationship between *Trichoderma viride* and other soil microbes significantly affects the faba bean's growth and yield (Kumar et al., 2022).

3.3. The effect of inoculation *Trichoderma viride* and its mutants on pigment contents

There was a significant increase in pigment content under inoculation of *Vicia faba* with *Trichoderma viride* and most of its mutants compared with the negative control. At 21 days, the mutants 28 and 40 induced by UV gave a high percentage compared to the other mutants. They gave 257, 338% and 280, 410% in total chlorophyll and caroteniods compared to the negative control (Fig. 9 , 10) (Rezalou et al., 2023) reported that bio-priming seeds with *Trichoderma* spores improved chlorophyll and carotenoid levels the highest. Additionally, *Trichoderma* species mutants had stronger biostimulant effects than wild types. Soil microbes produce metabolites, break down organic molecules, produce growth enhancers, and increase nutrient availability through cooperative partnerships, improving plant growth. Under varying irrigation levels, *Trichoderma harzianum* boosted proline, soluble carbohydrates, photosynthetic pigments, and antioxidant activity (Salahiostad et al., 2022). Additionally, all bio-inducer treatments raised the amount of chlorophyll in the treated faba bean plants according to (Ahmed, 2015). Additionally, plants inoculated with *Trichoderma* exhibited a more balanced synthesis of carotenoid and

chlorophyll, suggesting a less disrupted photosynthetic system (Guler et al., 2016).

3.4. The effect of inoculation *Vicia faba* with *Trichoderma viride* and its mutants on proline level.

Figure (11) illustrates the effect of inoculation *Vicia faba* with *Trichoderma viride* and its mutants induced by UV on the proline level in *Vicia faba*. The mutants induced by UV increase the proline level. The mutants 28 (366%) and 40 (325%), gave high values compared with the other treatments, respectively.

These findings concur with those of (Contreras-Cornejo et al., 2014),who found that the combination of salt and Trichoderma spp. boosted proline content in *Arabidopsis* compared to the salt-only treatment. In contrast to uninoculated controls and drought-stressed plants, tomatoes inoculated with *T. harzianum* exhibited higher root and shoot development and chlorophyll pigments (Mona et al., 2017). Under both normal and drought circumstances, plants inoculated with *T. harzianum* exhibited an increase in proline and total soluble protein content. Additionally, according to (Salahiostad et al., 2022), the plant's proline, soluble carbohydrates, photosynthetic pigments, and antioxidant activity all rose when *Trichoderma harzianum* was applied at varying irrigation levels.

3.5. The effect of inoculation *Vicia faba* with *Trichoderma viride* and its mutants on the level of MDA.

Figure (12) shows the inoculation of *Vicia faba* with *Trichoderma viride* and its mutants and their effects on MDA production. There is a decrease in MDA with inoculation by *Trichoderma* and its mutants. The mutants 28 (24%) and 40 (33%) gave the lowest percentage compared to the other treatments.

According to (Contreras-Cornejo et al., 2014) when *Arabidopsis* plants were treated with salt and *Trichoderma* spp., their proline content rose more than when treated with salt alone. Additionally, (Abd El-Baki and Mostafa, 2014) discovered that as the soil's salinity increased, the amounts of proline and MDA gradually increased. Treatments with *Trichoderma* significantly slowed the buildup of both parameters in roots and shoots.

According to (Guler et al., 2016) uninoculated seedlings accumulated much higher MDA than inoculated seedlings. Results from chickpeas that showed non-inoculated plants had a greater MDA level than *Trichoderma*-inoculated plants were comparable to our findings (Rawat et al., 2013).

3.6. The effect of inoculation *Vicia faba* with *Trichoderma viride* and its mutants on antioxidant enzymes

Trichoderma viride and its mutant's treatments significantly increase antioxidant enzymes compared to the control. The antioxidant enzymes increase with inoculation of *Trichoderma viride* and its mutants (Fig. 13, 14).

The activity of antioxidant enzymes (Ascorbate Peroxidase, and polyphenol oxidase) was studied to know the effect of *Trichoderma viride* inoculation and its mutants on the Faba-bean plant's vigors. Faba-bean plants treated with *Trichoderma viride* and its mutants significantly increased the activity of ascorbate peroxidase (APX) and polyphenol oxidase (PPO).

About APX the mutants 28 (643%) and 40 (393%) gave the highest values compared with the other treatments. About PPO, the mutants 28 (940%) and 40 (573%) gave the highest values compared with the other treatments.

The results demonstrate that *Trichoderma* and its mutants benefited plants, which is consistent with research by (Sorahinobar et al., 2024) who found that plants treated with *Trichoderma* exhibited noticeably higher levels of antioxidant enzymes, including superoxide dismutase (SOD), peroxidase (POX), and polyphenol oxidase (PPO).

The antioxidant defense system in melon plants was modified by applying compost and/or *Trichoderma* inoculation. *Trichoderma* and citrus compost together had a biostimulant effect that was associated with an increase in peroxidase and ascorbate recycling enzymes (monodehydroascorbate reductase, dehydroascorbate reductase). Additionally, the addition of Trichoderma to both composts enhanced the activity of antioxidant enzymes, particularly those involved in the recycling of ascorbate (Bernal-Vicente et al., 2015)

Funding: This research received no external funding

Conclusion: The findings indicate that *Trichoderma viride* and its mutations can substantially enhance the development and resilience of faba bean plants, underscoring their potential to improve agricultural output and stress tolerance.

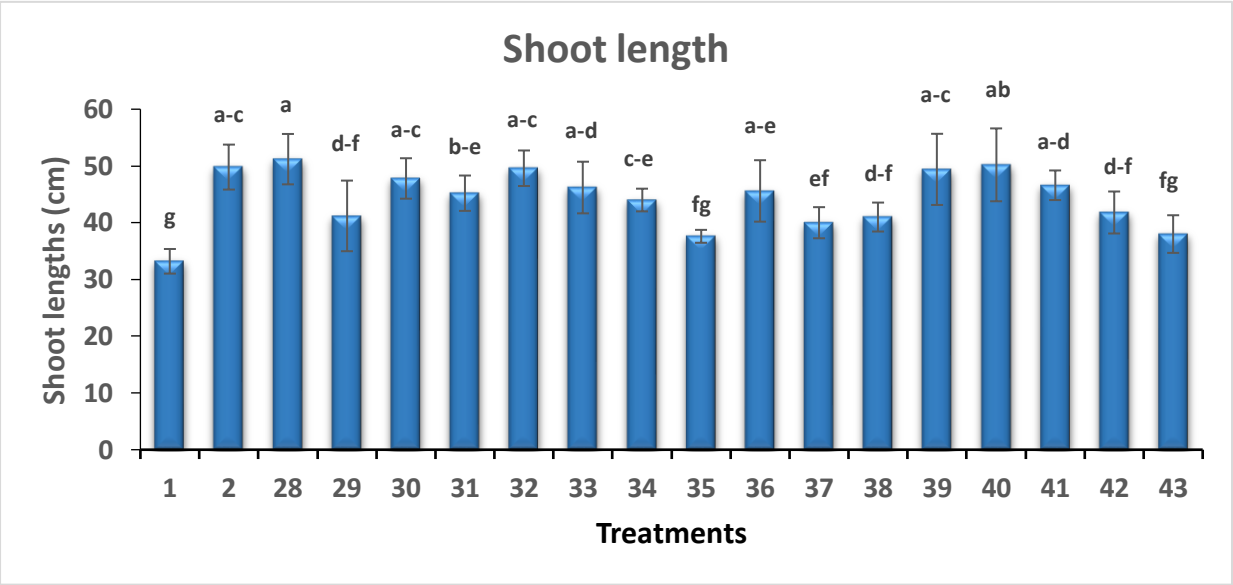

Figure 2. Effect of inoculation *Vicia faba* with *Trichoderma viride* (wild type and its mutants induced by UV) on the shoot length at 21 days. Duncan's multiple range test indicates significant columns with distinct letters at $P \leq 0.05$.

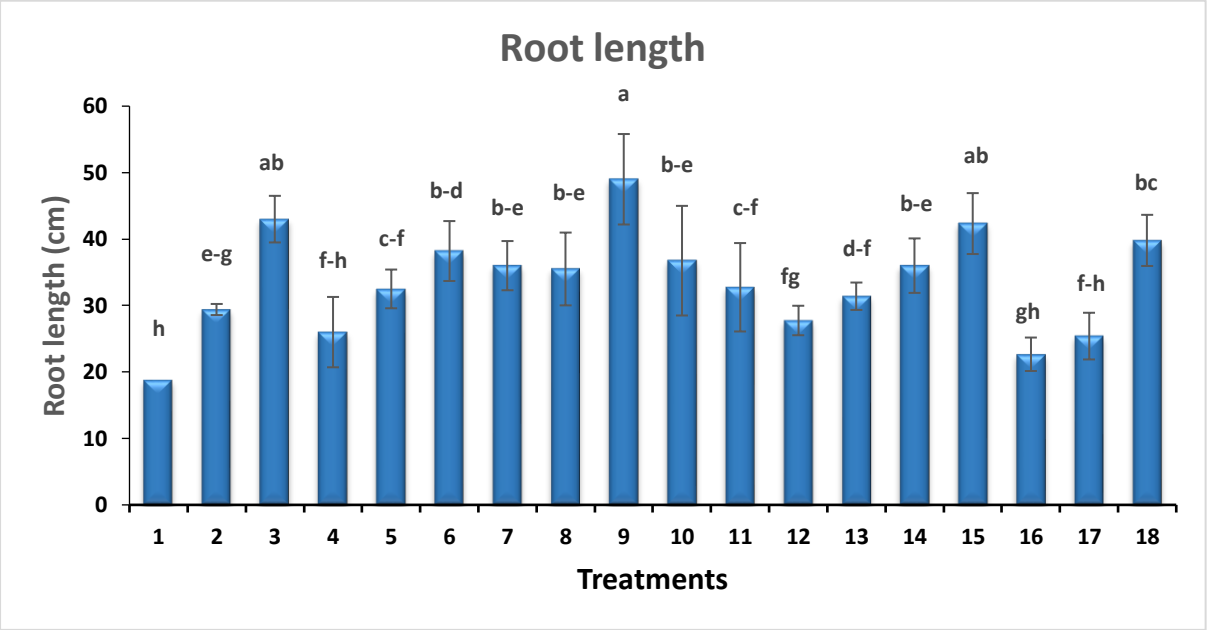

Figure 3. Effect of inoculation *Vicia faba* with *Trichoderma viride* (wild type and its mutants induced by UV) on the root length at 21 days. Duncan's multiple range test indicates significant columns with distinct letters at $P \leq 0.05$.

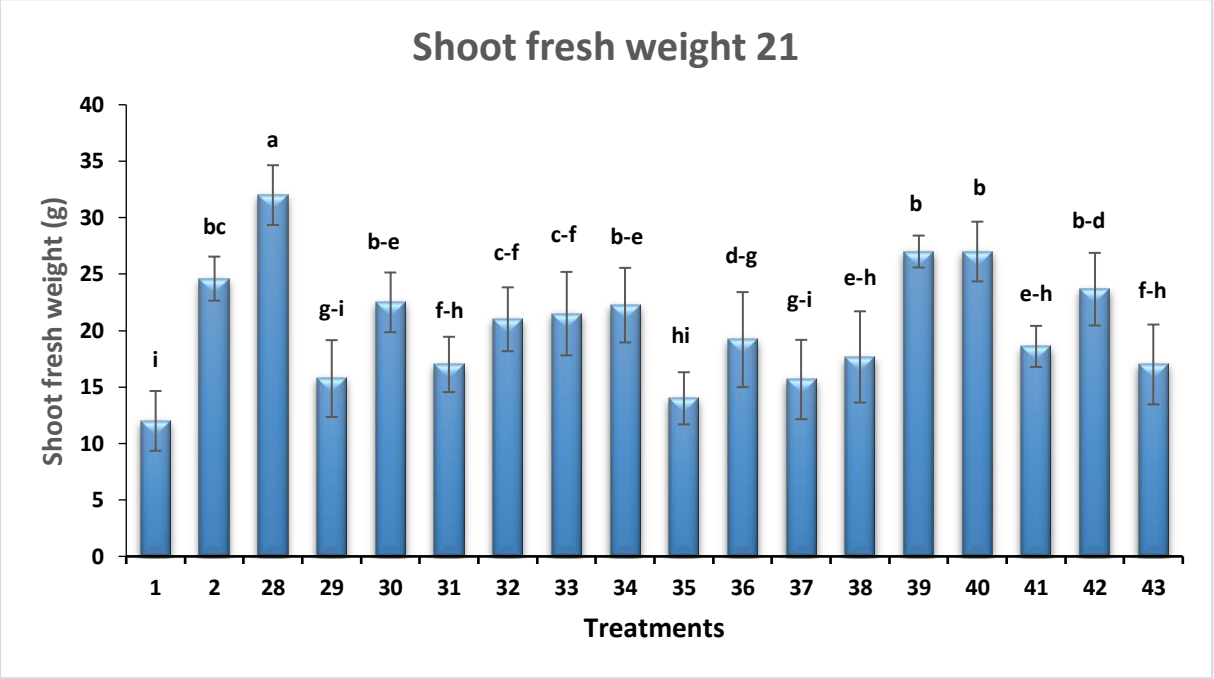

Figure 4. Effect of inoculation *Vicia faba* with *Trichoderma viride* (wild type and its mutants induced by UV) on the shoot fresh weight at 21 days. Duncan's multiple range test indicates significant columns with distinct letters at $P \leq 0.05$.

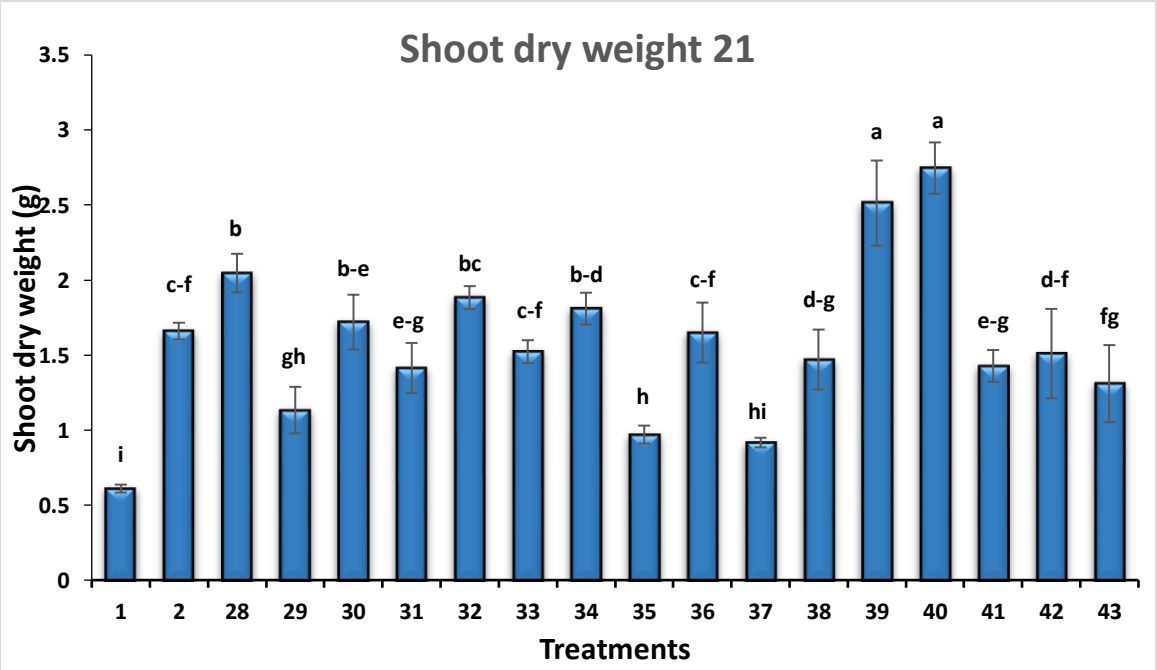

Figure 5. Effect of inoculation *Vicia faba* with *Trichoderma viride* (wild type and its mutants induced by UV) on the shoot dry weight at 21 days. Duncan's multiple range test indicates significant columns with distinct letters at $P \leq 0.05$.

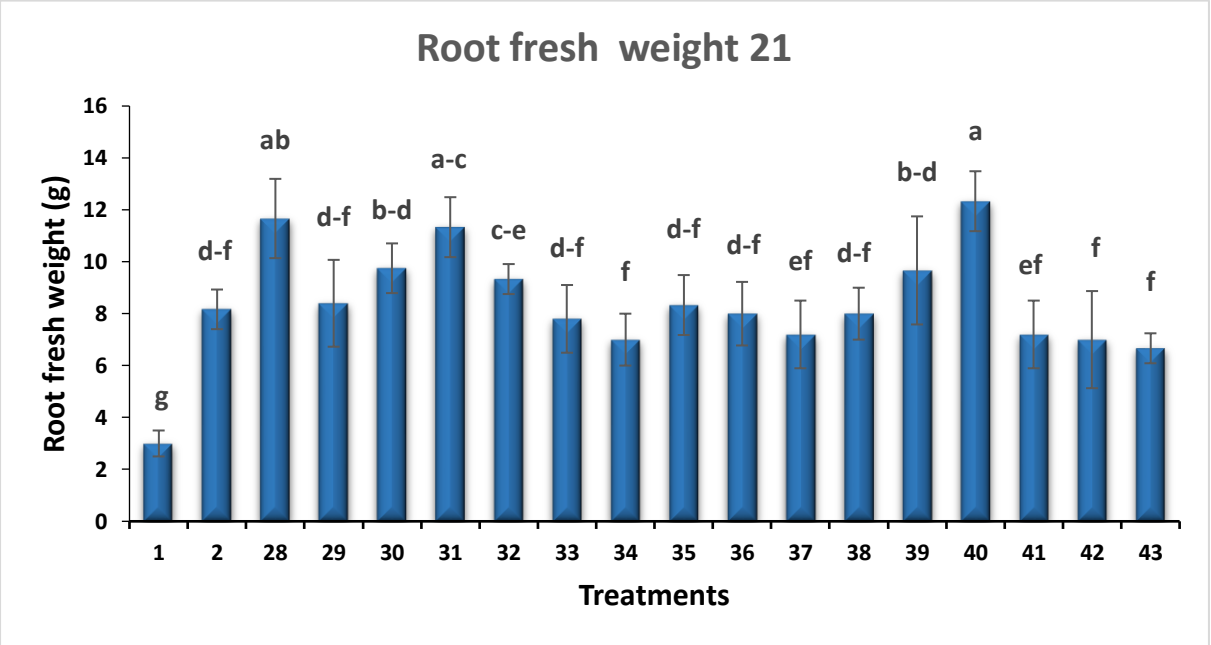

Figure 6. Effect of inoculation *Vicia faba* with *Trichoderma viride* (wild type and its mutants induced by UV) on the root fresh weight at 21 days. Duncan's multiple range test indicates significant columns with distinct letters at $P \leq 0.05$.

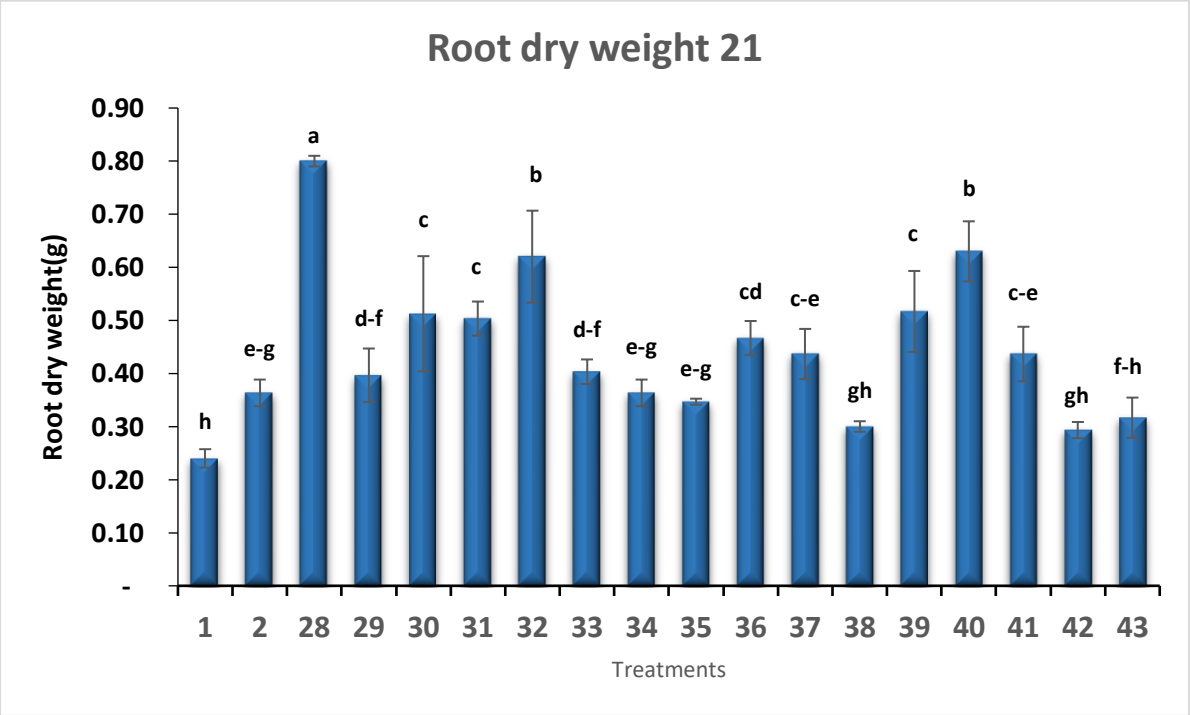

Figure 7. Effect of inoculation *Vicia faba* with *Trichoderma viride* (wild type and its mutants induced by UV) on the root dry weight at 21 days. Duncan's multiple range test indicates significant columns with distinct letters at $P \leq 0.05$.

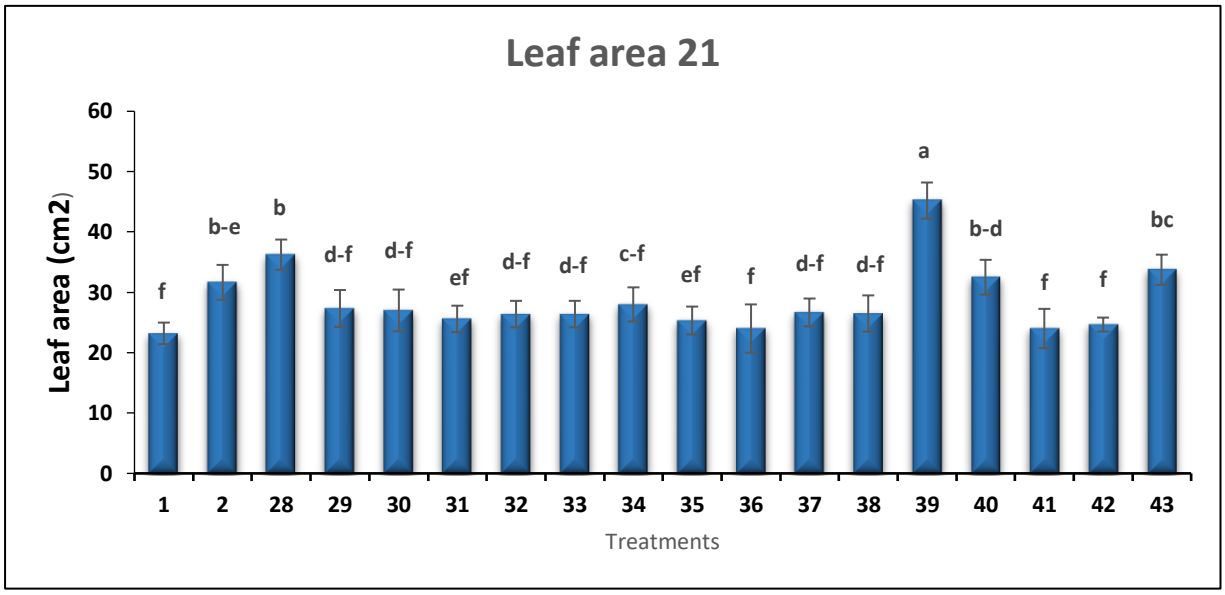

Figure 8. Effect of inoculation *Vicia faba* with *Trichoderma viride* (wild type and its mutants induced by UV) on the leaf area at 21 days. Duncan's multiple range test indicates significant columns with distinct letters at $P \leq 0.05$.

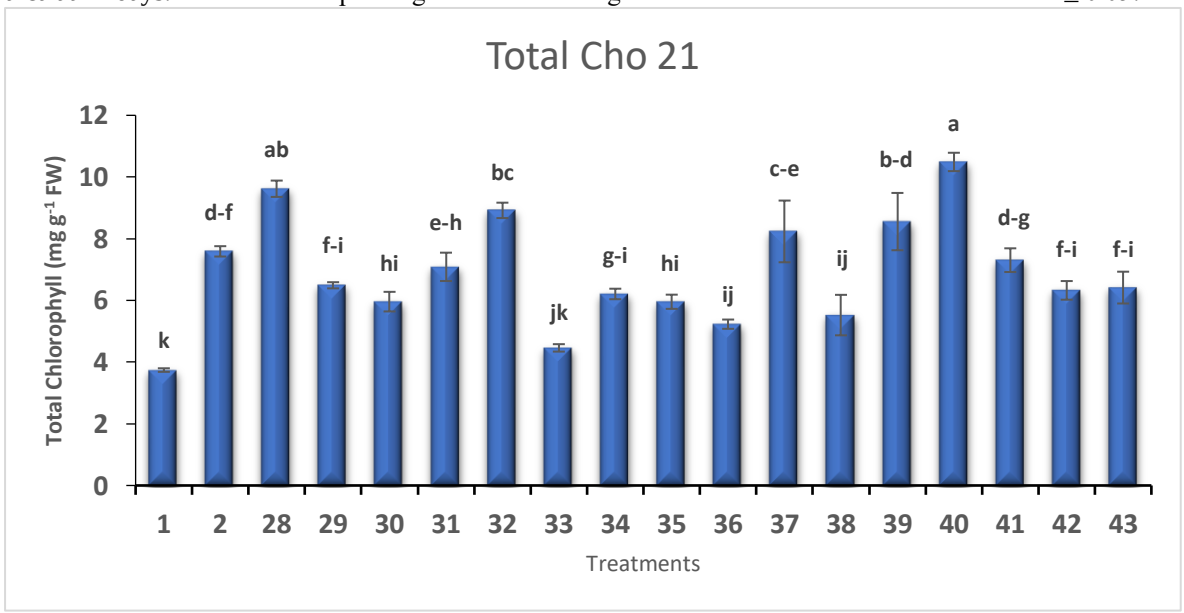

Figure 9. Effect of inoculation *Vicia faba* with *Trichoderma viride* (wild type and its mutants induced by UV) on total chlorophyll at 21 days. Duncan's multiple range test indicates significant columns with distinct letters at $P \leq 0.05$.

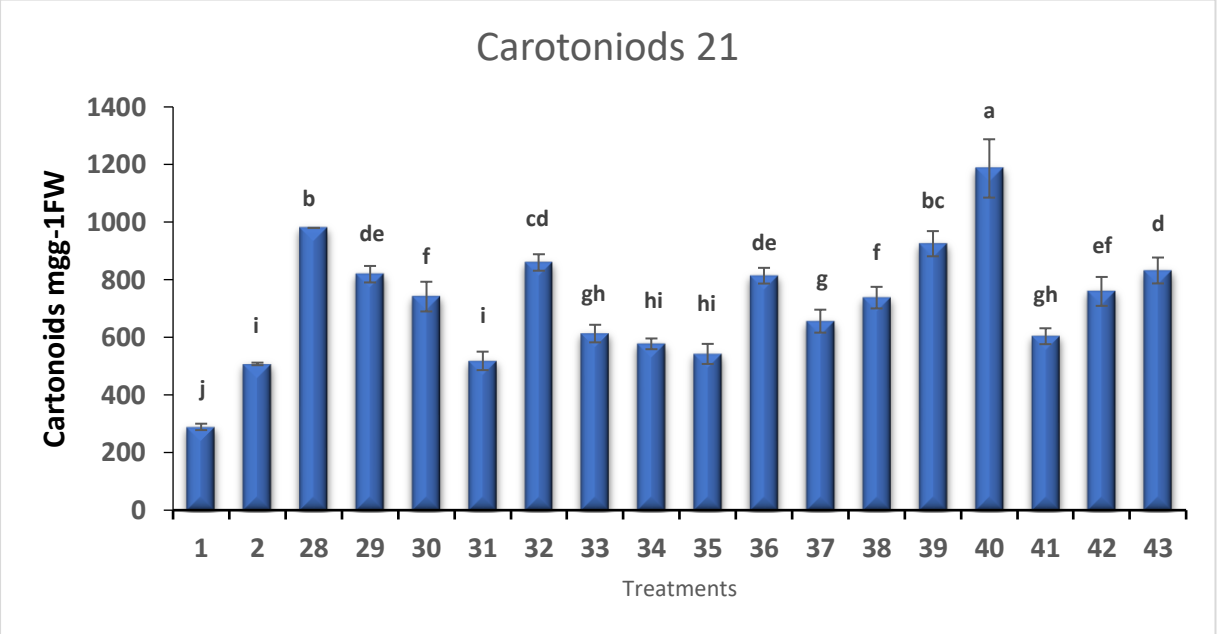

Figure 10. Effect of inoculation *Vicia faba* with *Trichoderma viride* (wild type and its mutants induced by UV) on carotenoids at 21 days. Duncan's multiple range test indicates significant columns with distinct letters at $P \leq 0.05$.

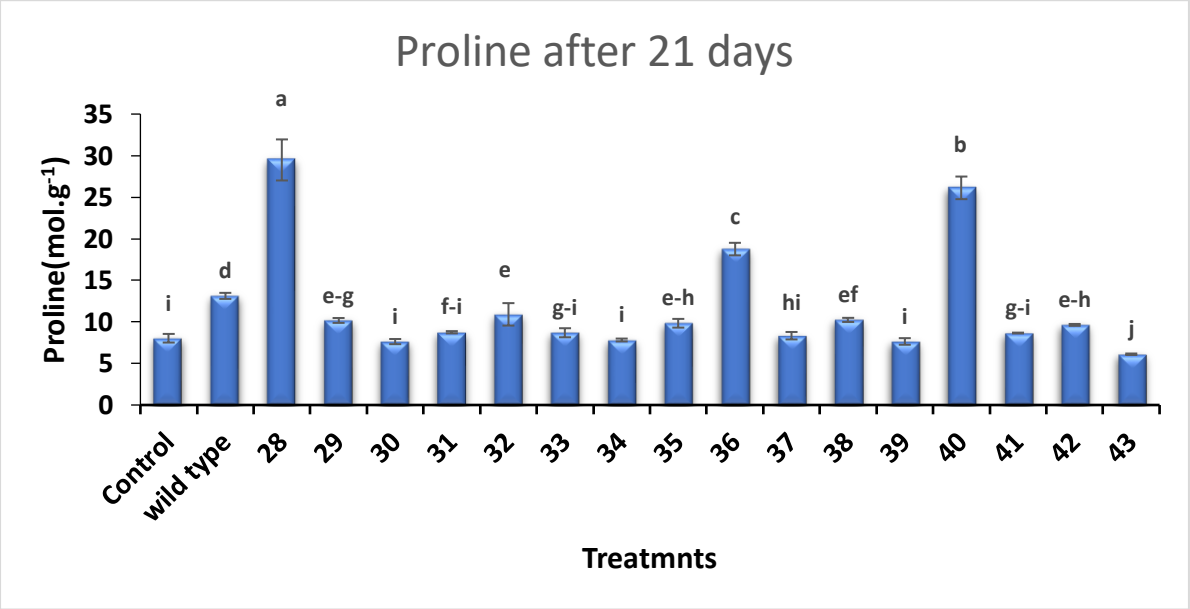

Figure 11. Effect of inoculation *Vicia faba* with *Trichoderma viride* (wild type and its mutants induced by UV) on proline at 21 days. Duncan's multiple range test indicates significant columns with distinct letters at $P \leq 0.05$

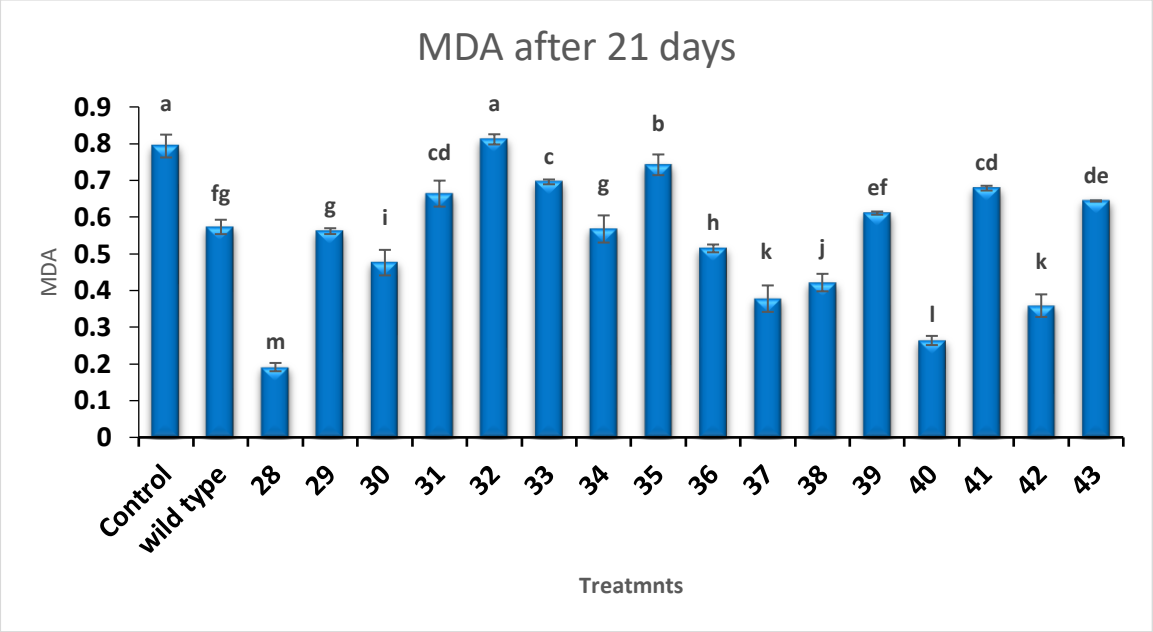

Figure 12. Effect of inoculation *Vicia faba* with *Trichoderma viride* (wild type and its mutants induced by UV) on MDA at 21 days. Duncan's multiple range test indicates significant columns with distinct letters at $P \leq 0.05$.

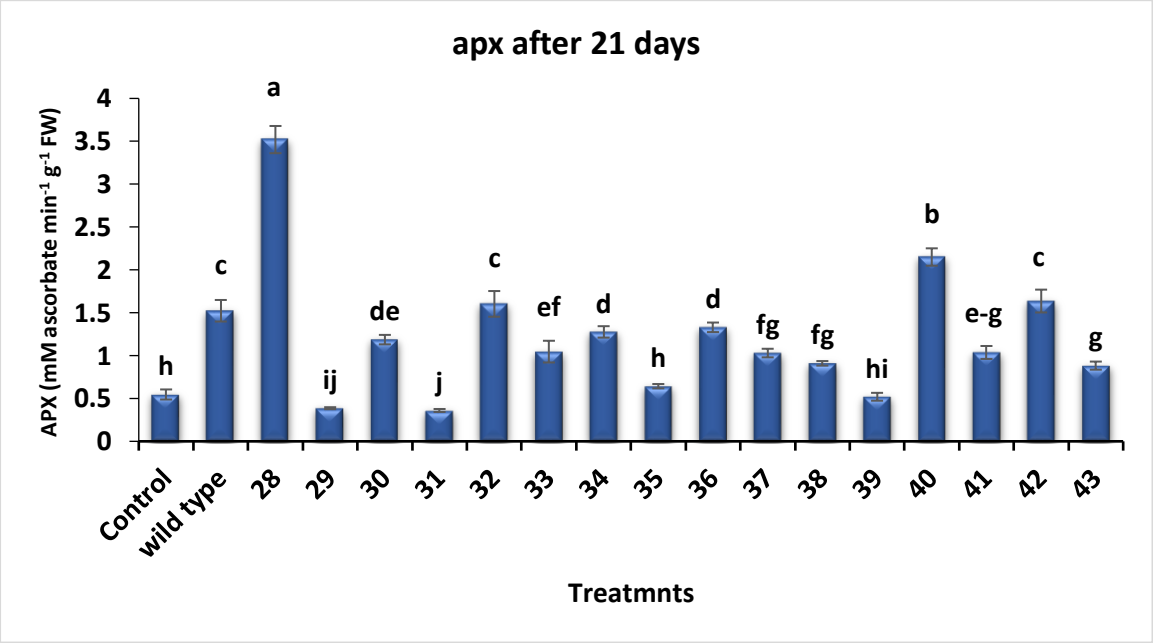

Figure 13. Effect of inoculation *Vicia faba* with *Trichoderma viride* (wild type and its mutants induced by UV) on APX at 21 days. Duncan's multiple range test indicates significant columns with distinct letters at $P \leq 0.05$.

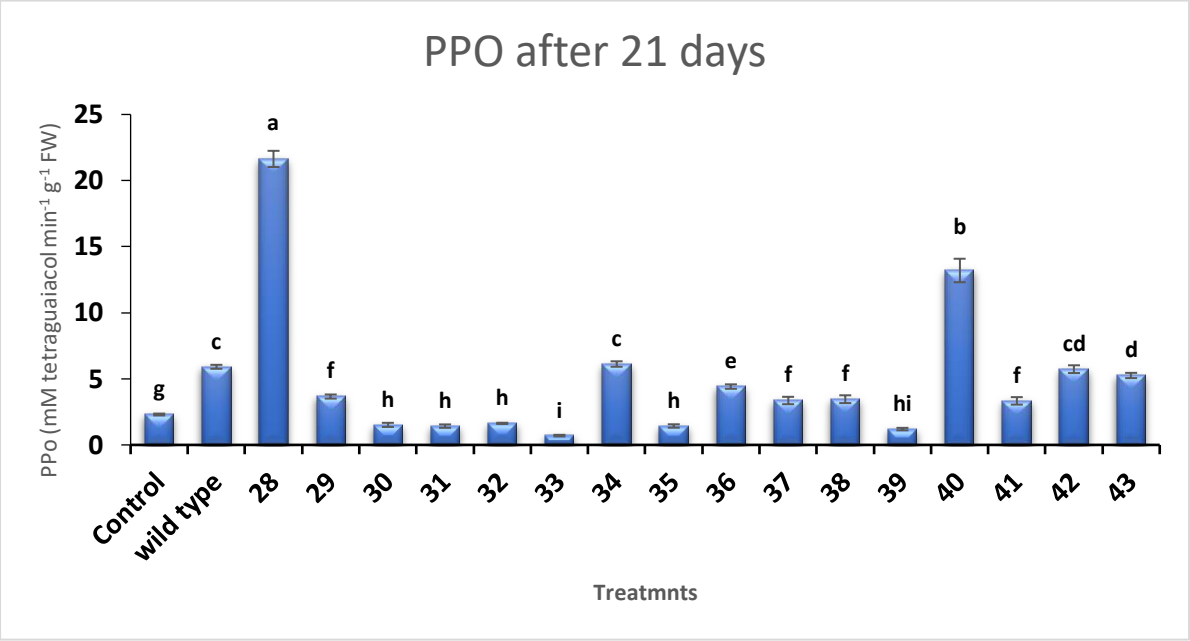

Figure 14. Effect of inoculation *Vicia faba* with *Trichoderma viride* (wild type and its mutants induced by UV) on PPO at 21 days. Duncan's multiple range test indicates significant columns with distinct letters at $P \leq 0.05$.

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