GRASAS Y ACEITES, 61 (3),
JULIO-SEPTIEMBRE, 288-294, 2010,
ISSN: 0017-3495
DOI: 10.3989/gya.111509

Influence of ionizing irradiation on the antioxidant enzymes of *Vicia faba* L.

By **Amina[1*] A. Aly** and **Hossam[2] E. S. El-Beltagi**

[1]Natural Product Dept., National Center for Irradiation Research and Technology.
P. Box 29, Nasr City, Cairo- Egypt.
[2]Biochemistry Dept., Faculty of Agriculture, Cairo University, P. Box 12613,
Gamma st, Giza, Cairo- Egypt.
[*](Corresponding author: aly_amina@yahoo.co.uk)

RESUMEN

Influencia de la radiación ionizante en enzimas antioxidantes de *Vicia faba* L.

Semillas de *Vicia faba* L. Giza 834 fueron expuestas a γ-irradiación a dosis de 2.5, 5.0, 10.0 y 20.0 kGy y después expuesta a una radiación laser de He–Ne (632.8 nm) o de diodo (650.0 nm) durante 5 minutos. Las actividades de las enzimas POD, APOX, CAT, SOD y GST fueron favorecidas significativamente, y éstas alcanzaron un máximo a una dosis de 5.0 kGy para las enzimas POD, APOX, CAT y SOD, y a 10.0 kGy para la enzima GST. Más aún, a diferentes dosis de γ-irradiación (2.5, 5.0, 10.0 and 20.0 kGy) con y sin laser de He-Ne, la inducción enzimática fue significativamente favorecida correlacionando positivamente con las dosis de γ-irradiación en combinación con tratamientos con laser de He-Ne. Hubo un incremento significativo en la concentración de MDA y este incremento fue más pronunciado a dosis de 20.0 kGy (38.2 µmol/g d.w) comparada con el control (3.9 µmol/g d.w). Mientras que el tratamiento con el laser de He-Ne o de diodo solamente causó un ligero incremento ($P<0.05$) en el contenido de MDA (4.4 y 5.08 µmol/g d.w, respectivamente) comparado con el control (3.9 µmol/g d.w). El contenido de H_2O_2 aumento significativamente con todos los tratamientos y este incremento alcanzó un máximo a una dosis de 20.0 kGy (36.3 µmol/g d.w) comparado con el control (2.3 µmol/g d.w.). Por otra parte, los tratamientos con laser de He-Ne o de diodo combinado con γ-irradiación lo decrecieron significativamente en comparación con los tratamientos con γ-irradiación únicamente. En el caso del contenido de glutatión, hubo un incremento significativo con γ-irradiación a dosis de 2.5, 5.0, 10.0 and 20.0 kGy. Es más la combinación de γ-irradiación con el laser de He-Ne y de diodo produjo un marcado incremento que fue más pronunciado que con γ-irradiación únicamente.

PALABRAS CLAVE: Enzimas antioxidantes – Gamma irradiación – Irradiación con laser – Vicia faba L.

SUMMARY

Influence of ionizing irradiation on the antioxidant enzymes of *Vicia faba* L.

The seeds of *Vicia faba* L. Giza 834 were exposed to γ-irradiation at dose levels of 2.5, 5.0, 10.0 and 20.0 kGy and after that exposed to He–Ne (632.8 nm) or diode (650.0 nm) laser irradiation for 5 min. The activities of POD, APOX, CAT, SOD and GST enzymes were significantly stimulated and this stimulation reached its maximum at a dose level of 5.0 kGy for enzymes POD, APOX, CAT and SOD, but for GST enzyme at a dose level 10.0 kGy. For He-Ne laser, with or without different doses (2.5, 5.0, 10.0 and 20.0 kGy) of γ-irradiation, enzyme induction was significantly stimulated and positively correlated with the dose levels of γ-irradiation in combination with the He-Ne treatment. There was a significant increase in the concentration of MDA and this increase was more pronounced at dose level 20.0 kGy (38.2 µmol/g d.w) compared to the control (3.9 µmol/g d.w). Laser treatment by He–Ne laser or diode laser only caused a slight increase ($P<0.05$) in MDA content (4.4 and 5.08 µmol/g d.w respectively) compared to the control (3.9 µmol/g d.w). H_2O_2 content significantly increased in all treatments and this increase reached its maximum at dose level 20.0 kGy (36.3 µmol/g d.w) compared to the control (2.3 µmol/g d.w.). On the other hand, He-Ne or diode laser treatments combined with γ-irradiation significantly decreased in comparison with γ-irradiation treatments alone. In the case of glutathione content, there were significant increases by γ-irradiation at dose levels 2.5, 5.0, 10.0 and 20.0 kGy. Furthermore, with a combination of γ-irradiation and He-Ne or diode laser, a marked increase in glutathione content was found and was more pronounced than that of gamma irradiation alone.

KEY-WORDS: Antioxidants enzyme – Gamma irradiation – Laser irirradiation – Vicia faba L.

1. INTRODUCTION

Under natural conditions of growth and development, plants are inevitably exposed to different types of stress, which may cause an increased production of active oxygen species (ROS) (Smirnoff, 1993). These include superoxide radicals ($O_2^{\bullet-}$), singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2) and hydroxyl radicals ($OH^{\bullet}$), which cause tissue injury**.** Plants have evolved various protective mechanisms to eliminate or reduce ROS. In plant cells, one such protective mechanism is an antioxidant system, composed of both non-enzymatic and enzymatic antioxidants (Foyer *et al*., 1994b). The capacity of the antioxidant defense system is often increased under stress conditions (Gressel and Galun, 1994)**,** but in most situations the response is moderate (Foyer *et al*., 1994a). ROS are highly reactive in the absence of any protective mechanism, they can seriously disrupt normal metabolism through oxidative damage to membrane lipids, proteins, pigments and nucleic acids. These ROS are detoxified by the sequential and

simultaneous action of a number of enzymes, including glutathione reductase (GR; EC 1.8.1.7) superoxide dismutase (SOD; EC 1.15.1.1), peroxidase (POD; EC 1.11.1.7), catalase (CAT; EC 1.11.1.6) and glutathione-S-transferase (GST; EC 2.5.1.18). SOD is located in various cell compartments, a major scavenger of superoxide (O^{2-}), and its enzymatic action results in the formation of H_2O_2 and O_2 (Smirnoff, 1993). The hydrogen peroxide produced is then scavenged by CAT and a variety of POD. Catalase, which is located in peroxisomes, glyoxysomes and mitochondria, and is apparently absent in the chloroplast, transforms mostly photorespiratory/respiratory H_2O_2 into water and molecular $O_2^{\cdot-}$ (Asada, 1992 and Willekens *et al*., 1997). In biological systems, reduced glutathione (GSH) appears to be one of the most important antioxidants (Noctor and Foyer, 1998). Application of irradiation has revolutionized current research in the field of agricultural science (Bari *et al*., 2003). There is compelling evidence which shows that the activities of enzymes involved in reactive oxygen species (ROS) scavenging were altered by several environmental stresses, including gamma irradiation. The activity and isozyme patterns of POD in *Nicotiana debneyi* and *Nicotiana tabacum,* SOD in *Nicotiana debneyi*, and CAT in *Nicotiana tabacum* increased in response to gamma irradiation treatment (Wada *et al*., 1998). Moreover, Chaomei and Yanlin (1993) reported an increase in the activity of POD and CAT with a corresponding decline in the growth of *Triticum aestivum* plants under higher irradiation doses. Singh *et al*. (1993) reported that ascorbate peroxidase (APOX; E.C. 1.11. 1. 11**)** activity in two sugar cane varieties grown under γ-irradiation was increased. Lasers are divided into pulsed and continuous wave lasers described by Markolf (1996). Previous studies showed that He–Ne laser and CO_2 laser (continuous wave) had a positive role in accelerating the plant growth and metabolism (Cai *et al*., 2000 & Qi *et al*., 2000). In agriculture, forestry and food technology, He-Ne lasers are used which emit red light with a wavelength of 630–650 nm. With these lasers it is possible to treat seeds, seedlings, plants and fields. Laser pretreatments have apparently induced the enzymatic activities, changed thermodynamic parameters, accelerated physiological and biochemistry metabolism and enhanced the growth of seedlings according to Chen *et al*. (2005). Laser irradiation significantly changed enzyme activities in faba bean seeds, particularly at the initial stage of their germination and led to a rise in the activity of amylolitic enzymes (Podlecœny, 2002). Broad bean (*Vicia faba* L.) is one of the important legumes in the Middle Eastern countries. It can be used as a dietary item alone or can serve as a potential supplement to cereal diets, especially for the preparation of inexpensive protein-rich food for children (Al-Kaisey *et al*., 2002). GSH, a disulfide reductant that protects thiols of enzymes, regenerates ascorbate (As) and reacts with singlet oxygen, hydrogen peroxide and hydroxyl radicals. Therefore, Kunert and Foyer (1994) found that, GSH plays a central role in protecting plants from the active oxygen species. Therefore, the objective of this study was to elucidate the relationship between gamma irradiation and laser irradiation on *Vicia faba* L. seeds concerning the behavior of antioxidant enzymes as well as lipid peroxidation, hydrogen peroxide and reduced glutathione.

2. MATERIALS AND METHODS

2.1. Irradiation treatments

Seed samples of *Vicia faba* L. Giza 843 were selected for uniform size, packed in polyethylene high density bags and radiated at different dose levels of γ-irradiation (2.5, 5.0, 10.0 and 20.0 kGy) at room temperature (25 ± 1ºC). γ-Irradiation was performed using a Gamma cell 200 apparatus equipped with a ^{60}Co γ source (dose rate, 6.5 kGy/h) at the National Center for Irradiation Research and Technology, Cairo, Egypt. Packed seed samples without irradiation served as the control.

2.2. Laser treatment

After γ-irradiation treatments at different dose levels, seeds were exposed to laser He-Ne (wavelength 632.8 nm, power density 30.0 mW, beam diameter 1.0 mm model Griat, U.S.A) and diode laser (wave length 650.0 nm, power density 30.0 mW, beam diameter 3.0 mm model NILES , A.R.E.), for 5 min, respectively. Laser applications were carried out at in the National Institute of Laser Enhanced Sciences (NILES), Laser Technology Center, Cairo Univ., Giza, Egypt.

2.3. Preparation of enzyme extracts

Ground samples, (1.0 g each) were homogenized in 3 ml of 50 mM phosphate buffer pH 7.0, 1% PVP (Sigma), 1 mM ascorbate (Sigma) at 4ºC. After centrifugation at 15,000×g for 15 min the supernatant was collected according to Vitória et al. (2001).

2.4. Enzymes activities

Peroxidase activity (POD; EC 1.11.1.7)

Peroxidase activity was assayed by monitoring the increase in absorbance at 430 nm due to the oxidation of pyrogallol (ε=2.6 mM^{-1} cm^{-1}), as described by Nakano and Asada (1981). The reaction mixture consisted of 50 mM potassium phosphate buffer (pH 7.0), 20 mM pyrogallol, 5 mM H_2O_2 and 20 µl of enzyme extract. POD activity was expressed as units mg^{-1} protein. One unit of enzyme was the amount necessary to decompose 1 µmol of substrate per minute at 25ºC.

Ascorbate peroxidase (APOX; E.C. 1.11. 1. 11)

Ascorbate peroxidase activity was estimated according to the method of Nakano and Asada (1981). Enzyme activity was determined by the decrease in absorbance of ascorbate at 290 nm. The

reaction mixture consisted of enzymatic extract, 50 mmol L^{-1} sodium phosphate buffer (cold), pH 7, 0.5 mmol L^{-1} ascorbate, 0.1 mmol L^{-1} hydrogen peroxide and 0.1 mmol L^{-1} EDTA, in a 0.3 mL final volume. The reaction started after the hydrogen peroxide addition. The molar extinction coefficient 2.8 $mmol^{-1}$ cm^{-1} was used to calculate ascorbate peroxidase activity. Enzyme activity was expressed as unit's mg^{-1} protein. One unit of enzyme was the amount necessary to decompose 1 µmol of substrate per minute at 25°C.

Catalase (CAT; EC 1.11.1.6)

Catalase activity was determined as H_2O_2 consumption measured as the decrease in absorbance at 240 nm according to the method of Aebi (1983). The assay contained 50 mM KH_2PO_4/K_2HPO_4 (pH 7.0), 10 mM H_2O_2 in phosphate buffer. Extinction coefficient of 39.4 $mM^{-1}cm^{-1}$ was used to calculate activity. Enzyme activity was expressed in µM H_2O_2 min^{-1}.

Superoxide dismutase (SOD; EC 1.15.1.1)

Superoxide dismutase activity was measured by the photochemical method as described by Beauchamp and Fridovich (1971). One unit of SOD activity was defined as the amount of enzyme required to cause 50% inhibition of the rate of nitro blue tetrazolium (NBT) reduction at 560 nm in the presence of riboflavin and light. The reaction mixture contained 45 mM potassium phosphate buffer, pH 7.0, containing 0.1 mM EDTA and 13 mM methionine, 0.17 mM NBT in ethanol, 0.007 mM riboflavin and enzyme aliquot. Blanks were kept in the dark and the others were illuminated for 15 min. One unit of SOD is the amount of extract that gives 50% inhibition to the rate of NBT reduction.

Glutathione-S-transferase (GST; EC 2.5.1.18)

Glutathione-S-transferase activity was measured according to the method of Mannervik and Guthenberg (1981) by following the changes in the absorbance at 340 nm in a mixture containing 0.17 mM sodium phosphate buffer, pH 6.5, 1 mM GSH, 1 mM 1-chloro-2,4-dinitrobenzene (CDNB) in ethanol and enzyme extract. EU = the amount of enzyme that catalyses the formation of 1 µmol of S-2,4-dinitrophenylglutathione min^{-1}.

2.5. Determination of lipid peroxidation

The lipid peroxidation products were estimated by the formation of thiobarbaturic acid reactive substances (TBARS) and quantified in terms of malondialdhyde (MDA) as described by Haraguchi et al. (1995). 200 mg ground seeds were homogenized in 2 ml of 0.1% (w/v) trichloroacetic acid (TCA), followed by centrifugation at 12,000×g for 20 min. The supernatant (1 ml) obtained was mixed with an equal volume of TCA (10%) containing 0.5% (w/v) TBARS or no TBARS as blank, and heated at 95°C for 30 min and then cooled in ice. The reaction product was centrifuged at 12,000×g for 15 min and the supernatant absorbance was measured at 532 and 600 nm. After subtracting the non-specific absorbance (600 nm), the MDA concentration was determined by its molar extinction coefficient of 155 mM^{-1} cm^{-1} and the results are expressed as µmol/g d.w.

2.6. Assay of hydrogen peroxide content

Hydrogen peroxide was measured by the method described by Capaldi and Taylor (1983) with a slight modification. The ground seeds were extracted by 5% TCA (2.5 ml per 0.5 g powder) with 50 mg active charcoal at 0°C, and centrifuged for 10 min at 15,000xg. The supernatant was collected, neutralized with 4 N KOH to pH 3.6 and used for H_2O_2 assay. The reaction mixture contained 200 µl of seed extract, 100 µl of 3.4 mM 3-methylbenzothiazoline hydrazone (MBTH). The reaction was initiated by adding 500 µl of horseradish peroxidase solution (90 U per 100 ml) in 0.2 M sodium acetate (pH 3.6). Two minutes later 1400 µl of 1 N HCl was added and the absorbance was read at 630 nm after 15 min.

2.7. Determination of reduced glutathione (GSH)

Samples powder were extracted in 6% m-phosphoric acid (pH 2.8) containing 1 mM EDTA. The GSH content of sample extracts was measured by reaction with 5, 5`dithiobis -2-nitrobenzoic (DTNB) reagent to give a compound that absorbed at 412 nm. The GSH concentration was determined from a standard curve according to Silber et al. (1992).

2.8. Determination of soluble proteins

Soluble proteins were measured by the Bio-Rad micro assay modification of the Bradford (1976) procedure using crystalline bovine serum albumin as a reference.

2.9. Statistical analysis

All analysis were performed in triplicate (n=3). Statistical analysis was done using SPSS (version 10) program. Mean and standard error were descriptive measures of quantitative data using the analysis of variance test (ANOVA) for independent samples. P-values <0.05 were considered significant.

3. RESULTS AND DISCUSSION

Plants are well known to possess effective enzymatic and non-enzymatic detoxifying systems continually involved in the cellular protection against reactive oxygen species (ROS) coming from both the environment and cell metabolism. Exposure of

Vicia faba L. seeds to different sources of ionizing irradiation had different effects on antioxidant enzymes, peroxidase, ascorbate peroxidase, catalase, superoxide dismutase and glutathione-S-transferase as well as MDA, H_2O_2 and GSH contents as shown in (Tables 1-2).

3.1. Enzymes activities

The activities of POD, APOX, CAT, SOD and GST enzymes were significantly (P<0.05) stimulated and this stimulation reached its maximum at a dose level of 5.0 kGy for enzymes POD, APOX, CAT and SOD, but for GST enzyme at a dose level 10.0 kGy. For He-Ne laser without or with different doses (2.5, 5.0, 10.0 and 20.0 kGy) of γ-irradiation, enzyme induction was significantly (P<0.05) stimulated and positively correlated with the dose levels of γ-irradiation in combination with He-Ne treatment. Significant (P<0.05) stimulation in POD, APOX, CAT, SOD and GST activities were observed and they were more pronounced than in the case of using irradiation alone. The same trend was obtained with the diode laser. The antioxidant enzymes POD, CAT and SOD function as effective quenchers for ROS and their level may also determine the sensitivity of plants to lipid peroxidation (Smirnoff, 1993). Superoxide dismutase is an essential component (coenzyme) in plants and represents an antioxidative defense system since it transforms hydrogen peroxide to water and oxygen (Cakmak and Horst, 1991). Moreover Cho *et al*. (2000) found that the expression patterns of GST, SOD, POD and CAT genes exhibited increased transcripts with γ-irradiation of *Nicotiana tabacum*. Slooten *et al*. (1995) reported that the stimulation in SOD activity in response to stresses is possibly attributed to the de-novo synthesis of the enzymatic protein. Laser pretreatment can accelerate the physiological and biochemical metabolism and accordingly enhance the growth of seedlings. Antioxidative enzymes, non-enzymatic antioxidant and lipid peroxidation showed that proper laser pretreatment of wheat seeds may provide protection for wheat from drought stress damage as mentioned by Qi *et al*. (2008). Furthermore Cai *et al*. (1994) and Qi *et al*. (2000) reported that laser irradiation was able to build up the activities of SOD, APOX and CAT. The results obtained in the present study also demonstrate clearly that catalase and SOD protect plant cells from the destructive effects of reactive oxygen species and constitute key components of the cellular antioxidant defense systems. In this concern, Xiong *et al*. (2009) found that SOD first converts superoxide anions into hydrogen peroxide, which is then removed by catalase**.** The changes in the enzymatic activity of some winter wheat varieties were also observed by Galova (1996) after the irradiation of kernels by He-Ne laser light. Thus the results of this study suggested

Table 1
Effect of gamma and laser irradiation on POD, APOX, SOD, CAT and GST activities of *Vicia faba* L.

Treatment	POD Unit/mg protein	APOX Unit/mg protein	CAT (μ mol/mg protein/min)	SOD Unit/mg protein	GST Unit/mg protein
Control	3.5 ± 0.13^{j}	2.5 ± 0.17^{k}	2.1 ± 0.16^{j}	8.3 ± 0.20^{g}	8.2 ± 0.23^{i}
2.5 kGy	6.8 ± 0.16^{gh}	4.3 ± 0.18^{i}	5.2 ± 0.24^{h}	13.1 ± 0.33^{f}	13.4 ± 0.21^{g}
5.0 kGy	12.7 ± 0.2^{c}	7.2 ± 0.26^{f}	8.84 ± 0.15^{d}	16.9 ± 0.35^{de}	18.34 ± 0.21^{e}
10.0 kGy	10.9 ± 0.3^{d}	6.4 ± 0.23^{g}	7.63 ± 0.19^{e}	15.8 ± 0.27^{e}	19.6 ± 0.39^{de}
20.0 kGy	9.3 ± 0.18^{e}	5.5 ± 0.17^{h}	6.82 ± 0.17^{f}	13.6 ± 0.36^{f}	16.64 ± 0.36^{f}
He-Ne 5 min	5.84 ± 0.29^{hi}	3.42 ± 0.14^{j}	3.9 ± 0.23^{i}	12.8 ± 0.35^{f}	11.7 ± 0.38^{h}
He-Ne+ 2.5 kGy	7.9 ± 0.28^{f}	6.1 ± 0.24^{g}	6.4 ± 0.20^{fg}	16.4 ± 0.23^{e}	14.8 ± 0.45^{g}
He-Ne+ 5.0 kGy	13.6 ± 0.27^{c}	9.3 ± 0.16^{d}	10.2 ± 0.23^{c}	20.32 ± 1.09^{c}	20.3 ± 1.13^{d}
He-Ne+ 10 kGy	16.9 ± 0.45^{b}	12.2 ± 0.26^{c}	14.2 ± 0.33^{b}	24.18 ± 1.8^{b}	26.7 ± 1.9^{c}
He-Ne+ 20 kGy	19.7 ± 1.28^{a}	17.4 ± 0.33^{a}	18.6 ± 0.39^{a}	30.2 ± 1.9^{a}	32.83 ± 1.86^{a}
Diode 5 min	5.28 ± 0.37^{i}	3.25 ± 0.13^{j}	3.6 ± 0.22^{i}	11.9 ± 0.82^{f}	10.8 ± 0.33^{h}
Diode +2.5 kGy	7.4 ± 0.28^{fg}	5.1 ± 0.25^{h}	6.08 ± 0.31^{g}	16.1 ± 0.32^{e}	14.2 ± 0.34^{g}
Diode +5.0 kGy	13.1 ± 0.38^{c}	8.4 ± 0.14^{e}	9.67 ± 0.31^{c}	18.6 ± 1.61^{cd}	19.9 ± 0.48^{de}
Diode +10 kGy	16.3 ± 0.27^{b}	11.8 ± 0.42^{c}	13.69 ± 0.38^{b}	22.32 ± 1.8^{b}	25.2 ± 1.44^{c}
Diode +20 kGy	19.2 ± 1.74^{a}	16.9 ± 0.41^{b}	18.19 ± 0.92^{a}	29.5 ± 2.08^{a}	31.43 ± 1.8^{b}
LSD P < 0.05	1.027	0.414	0.574	1.913	1.57

[a,b,c,.....]Means within same column followed by different letters are significantly different at P<0.05. Values are means of three replicates (±SE)

Table 2
Effect of gamma and laser irradiation on the MDA, H_2O_2 and GSH contents of *Vicia Faba* L.

Treatment	MDA µmol/g d.w	H_2O_2 µmol/g d.w	GSH mg/g d.w
Control	3.9 ± 0.15^{j}	2.3 ± 0.14^{h}	3.1 ± 0.17^{h}
2.5 kGy	12.5 ± 0.28^{f}	8.4 ± 0.18^{f}	7.63 ± 0.09^{f}
5.0 kGy	20.3 ± 0.21^{c}	16.3 ± 0.9^{d}	11.4 ± 0.17^{d}
10.0 kGy	28.7 ± 0.81^{b}	24.9 ± 0.76^{b}	9.6 ± 0.34^{e}
20.0 kGy	38.2 ± 1.4^{a}	36.3 ± 2.5	8.5 ± 0.26^{ef}
He-Ne 5 min	4.4 ± 0.12^{ij}	2.8 ± 0.14^{h}	4.6 ± 0.23^{g}
He-Ne+ 2.5 kGy	6.21 ± 0.14^{h}	4.9 ± 0.16^{g}	8.02 ± 0.14^{f}
He-Ne+ 5.0 kGy	10.7 ± 0.17^{g}	8.2 ± 0.25^{f}	13.3 ± 0.18^{c}
He-Ne+ 10 kGy	12.3 ± 0.14^{f}	12.7 ± 0.18^{e}	17.2 ± 1.0^{b}
He-Ne+ 20 kGy	18.02 ± 1.4^{e}	18.3 ± 0.19^{c}	25.4 ± 0.95^{a}
Diode 5 min	5.08 ± 0.19^{i}	3.02 ± 0.1^{h}	4.4 ± 0.14^{gh}
Diode +2.5 kGy	6.4 ± 0.19^{h}	5.2 ± 0.21^{g}	7.2 ± 0.25^{f}
Diode +5.0 kGy	11.13 ± 0.26^{g}	9.04 ± 0.16^{f}	12.6 ± 0.24^{c}
Diode +10 kGy	12.9 ± 0.24^{f}	13.5 ± 0.27^{e}	16.3 ± 0.12^{b}
Diode +20 kGy	19.3 ± 0.67^{d}	19.4 ± 0.22^{c}	24.18 ± 1.31^{a}
LSD P < 0.05	1.003	1.223	1.36

$^{a,b,c,.....}$Means within same column followed by different letters are significantly different at P < 0.05. Values are means of three replicates (±SE)

that APOX, GST and POD activities along with SOD activity play an important protective role in the $O_2^{\cdot-}$ and H_2O_2 scavenging process.

3.2. Lipid peroxidation content

There was a significant (P<0.05) increase in the concentration of MDA (Table 2), and this increase was more pronounced at dose level 20.0 kGy (38.2 µmol/g d.w) compared to control (3.9 µmol/g d.w). Meanwhile, laser treatment by He–Ne laser or diode laser alone caused a slight increase (P<0.05) in MDA content (4.4 and 5.08 µmol/g d.w respectively) compared to control (3.9 µmol/g d.w). However, γ-irradiation followed by laser (He–Ne laser or diode laser) showed that the increase in MDA concentration was notably lower (P<0.05) than that of γ-irradiation treatments alone (Table 2). Gamma irradiation could lead to an accumulation of free radicals such as $O_2^{\cdot-}$ and H_2O_2 which unbalance the system of free radical elimination and thus induce lipid peroxidation. As a result, the cells of plants are injured and the MDA concentration increased (Murphy, 1990 and Joshi *et al*., 1991). In this concern, Štajner *et al*. (2007) stated that the use of γ-irradiation at a dose up to 10 kGy did not provoke significant changes in lipid peroxidation and caused a slight increase which could be the consequence of the appropriate antioxidant response. Also Horvathova *et al*. (2007) reported that irradiation cased a considerable increase in the TBAS values of oregano extract prepared from the sample irradiated by gamma irradiation 30.0 kGy. Laser irradiation of *Vicia faba* L. seeds could raise SOD, POD and CAT activities and could eliminate the accumulation of poisonous free radicals and prevent lipid peroxidation. Qi *et al*. (2000) reported that the use of the laser pretreatment could be used to protect the cells of the broad bean from UV-B-induced damage. The reduction of MDA concentration, which proceeds through different ways, such as increasing SOD, POD and CAT activity, is very important for plants.

3.3. Hydrogen peroxide content

As with lipid peroxidation, H_2O_2 content significantly (P<0.05) increased in all the treatments (Table 2) and this increase reached its maximum at dose level 20.0 kGy (36.3 µmol/g d.w) compared to control (2.3 µmol/g d.w.). On the other hand, He-Ne or diode laser treatments combined with γ-irradiation significantly decreased (P<0.05) in comparison with γ-irradiation treatments alone. There is a correlation between MDA and H_2O_2 contents and the scavenging efficiency of the antioxidant enzymes. The concentration of H_2O_2 increased in various pumpkin tissues such as leaves, petioles and hypocotyls after γ-irradiation (Wi *et al*., 2007). Moreover Qi *et al*. (2002) and Han *et al*. (2002) suggested that He–Ne laser prevent plants from an increase in MDA and H_2O_2 concentration.

In the present study, the decreasing in H_2O_2 content in the seed pretreatment with gamma irradiation before laser might be due to the great

increase in the activity of POD, which uses H_2O_2 as substrate.

3.4. Reduced glutathione (GSH) content

In the case of glutathione content, there were significant ($P<0.05$) increases (Table 2) by γ-irradiation at dose levels 2.5, 5.0, 10.0 and 20.0 kGy. Furthermore, with a combination of γ-irradiation and He-Ne and diode laser resulted in a marked increase ($p<0.05$) in glutathione content which was more pronounced than that of gamma irradiation alone. GSH is involved in the maintenance of the redox status in plant cells and organs (Horemans *et al.*, 2000). Moreover, Cotter and Sawyer (1961) found that γ-irradiation significantly increased the glutathione content of potato tubers and glutathione was dose dependent. The glutathione pool was slightly affected by solar UV-B exposure. Increased thiol content by UV-B irradiation was reported in many experiments hence, only GSSG content was decreased by UV-B irradiation (Galatro *et al.*, 2001 and Costa *et al.*, 2002). The formation of the reactive oxygen species (ROS) is prevented by an antioxidant system involving the activity of low molecular mass antioxidants (ascorbic acid, glutathione, tocopherols).Enzymes regenerate the reduced forms of antioxidants and antioxidant enzymes such as SOD, POD and CAT (Štajner *et al.*, 2009). Research on the effects of γ-irradiation has been done with little information available on antioxidant systems in plants. This preliminary study may pave way for further investigation on the basis of such variability with biochemical correlation thereof to cast more light on the possible use of such irradiation in biotechnological advancement in the agricultural field.

4. CONCLUSIONS

In conclusion, this study showed that increases in GST, CAT, SOD and POD activities in *Vicia faba* L. seeds could be attributed to ionizing irradiation stress. The results may also suggest that the combination of γ-irradiation and laser irradiation is more effective for increasing the ability of the antioxidant enzymes to protect against stress and plays a central protective role in the $O_2^{\cdot-}$ and H_2O_2 scavenging process. Indeed, the mechanisms of laser pretreatment effects on seedling physiology are not clear at present and will be investigated in the future.

REFERENCES

Aebi H. 1983. Catalase, in H Bergmeyer (Ed.) *Methods of Enzymatic Analysis*, vol **3.** Verlag Chemie, Weinheim, Adamse, pp. 273-277.

Al-Kaisey MT, Mohammed MA, Alwan AKH, Mohammed, MH. 2002. The effect of gamma irirradiation on the viscosity of two barley cultivars for broiler chicks. *Irradiation Physics and Chemistry* **63,** 295-297

Asada K. 1992. Ascorbate peroxidase-a hydrogen peroxide scavenging enzyme in plants. *Physiologia Plantarum* **85,** 235– 241.

Bari ML, Nazuka E, Sabina Y, Todoriki S, Isshiki K. 2003. Chemical and irirradiation treatments for killing Escherichia coli O157: H7 on alfalfa, radis, and mung bean seeds. *Journal of Food Protection* **66,** 767-774.

Beauchamp C, Fridovich I. 1971. Superoxide dismutase improved assays and an assay applicable to acrylamide gels. *Analytical Biochemistry* **44,** 276-287.

Bradford MM. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry* **72,** 248-254

Cai SW, Qi Z, Ma XL. 2000. The effect of He–Ne laser irirradiation on soluble protein synthesis of corn seedling. *Chinese Journal Lasers* **27,** 284-288.

Cai SW, Zhao XS, Lu FT, Ma XL. 1994. The influence of He–Ne laser irirradiation on the active oxygen metabolism of corn seedlings. *Chinese Journal Lasers* **21,** 767-769.

Cakmak I, Horst WJ. 1991. Effect of aluminum on lipid peroxidation, superoxide dismutase, catalase and peroxidase activities in root tips of soybean (Glycine max). *Journal of Plant Physiology* **83,** 463-468.

Capaldi DJ, Taylor KE. 1983. A new peroxidase color reaction: oxidative coupling of 3-methyl-2-benzothiazolinone hydrazone (MBTH) with its formaldehyde azine application to glucose and choline oxidases. *Analytical Biochemistry* **129,** 329–336.

Chaomei Z, Yanlin M. 1993. Irradiation induced changes in enzymes of wheat during seed germination and seedling growth. *Acta Agriculturae Nucleatae Sinica* **7,** 93-97.

Chen Y, Yuea M, Wanga X. 2005. Influence of He–Ne laser irirradiation on seeds thermodynamic parameters and seedlings growth of *Isatis indogotica*. *Plant Science* **168,** 601-606.

Cho YS, Lee HS, Pai HS. 2000. Expression patterns of diverse genes in response to gamma irirradiation in *Nicotiana tabacum*. *Journal of Plant Biology* **43,** 82-87.

Costa H, Gallego SM, Tomaro ML. 2002. Effect of UV-B irradiation on antioxidant defense system in sunflower cotyledons. *Plant Science* **162,** 939-945.

Cotter DJ, Sawyer RL. 1961. The effect of -irirradiation on the incidence of black spot and ascorbic acid, glutathione and tyrosinase content of potato tubers. *American Potato Journal* **38,** 58-65.

Foyer CH, Descourvieres P, Kunert KJ. 1994a. Protection against oxygen radicals: an important defense mechanism study in transgenic plants. *Plant, Cell & Environment* **17,** 507–523.

Foyer CH, Lelandais M, Kunert KJ. 1994b. Photooxidative stress in plants. *Physiologia Plantarum* **92,** 696-717.

Galatro A, Simontacchi M, Puntarulo S. 2001. Free radical generation and antioxidant content in chloroplasts from soybean leaves exposed to ultraviolet-B. *Physiologia Plantarum* **113,** 564-570.

Galova Z. 1996. The effect of laser beams on the process of germinating power of winter wheat grains. Roczniki AR, *Poznañ, CCCLXXXVI*, ser. Rol. **49,** 39-43.

Gressel J, Galun E. 1994. Genetic Controls of Photooxidant Tolerance, in Foyer CH, Mullineaux PM (Ed.) *Causes of photooxidative stress and amelioration of defense systems in plant*. Boca Raton, CRC Press, pp. 237–274.

Han R, Wang XL, Yue M. 2002. Influence of He–Ne laser irirradiation on the excision repair of cyclobutyl pyrimidine dimers in the wheat DNA. *Chinese Science Bulletin* **47,** 435-438.

Haraguchi H, Saito T, Okamura N, Yagi A. 1995. Inhibition of lipid peroxidation and superoxide generation by diterpenoids from *rosmarinus offcinalis*. *Planta Medica* **61,** 333-336.

Horemans N, Foyer CH, Asard H. 2000. Transport and action of ascorbate at the plant plasma membrane. *Trends in Plant Science* **5,** 263-267.

Horvathova J, Suhaj M, Polovka M, Iasta Brezov V, Šimko P. 2007. The influence of gamma-irradiation on the formation of free radicals and antioxidant status of oregano (*Origanum vulgare* L.). *Czech Journal of Food Sciences* **25,** 131–143.

Joshi PN, Biswal B, Biswal NC. 1991. Effect of UV-A on aging of wheat leaves and role of phytochrome. *Environmental and Experimental Botany* **3,** 267-276.

Kunert KJ, Foyer CH. 1994. Thiol/disulphide exchange in plants, in De Kok LJ, Stulen I, Rennenberg H, Brunhold C, Rausen W (Eds.) *Sulfur Nutrition and Assimilation in Higher Plants*: Regulatory, Agricultural and Environmental Aspects, SPB Academic Publishers, The Hague, pp. 132-164.

Mannervik B, Guthenberg C. 1981. Glutathione transferase (Humanplacenta). *Methods in Enzymology* **77,** 231-235.

Markolf HN. 1996. Laser and matter, in Markolf HN (Ed.) *Laser Tissues Interactions*, Springer-Verlag, Berlin, Germany, pp. 8–15.

Murphy TM. 1990. Effect of broad-band ultraviolet and visible irradiation on hydrogen peroxide formation by cultured rose cells. *Physiologia Plantarum* **80,** 63-78.

Nakano Y, Asada K. 1981. Hydrogen peroxide is scavenged by ascorbate specific peroxidase in spinach chloroplasts. *Plant Cell Physiology* **22,** 867-880.

Noctor G, Foyer CH. 1998. Ascorbate and glutathione: keeping active oxygen under control. *Annual Review of Plant Physiology and Plant Molecular Biology* **49,** 249-279.

Podleœny J. 2002. Effect of laser irradiation on the biochemical changes in seeds and the accumulation of dry matter in the faba bean. *International Agrophysics* **16,** 209-213.

Qi Z, Liu X, Tian X, Yue M. 2008. Effects of CO_2 laser pretreatment on drought stress resistance in wheat. *Journal of Photochemistry and Photobiology* **90,** 17-25.

Qi Z, Yue M, Han R. 2002. The damage repair role of He–Ne laser on plants exposed to different intensities of UV-B irradiation. *Journal of Photochemistry and Photobiology* **75,** 680-686.

Qi Z, Yue M, Wang XL. 2000. Laser pretreatment protects cells of broad bean from UV-B irradiation damage. *Journal of Photochemistry and Photobiology* **59,** 33–37.

Silber R, Farber M, Papopoulos E, Nervla D, Liebes L, Bruch M, Bron R. 1992. Glutathione depletion in chronic lymphocytic leukemia β-lymphocytes. *Blood* **80,** 2038-2040.

Singh RK, Chandra P, Singh J, Singh DN. 1993. Effect of gamma-ray on Physio-biochemical parameters of sugar cane. *Journal of Nuclear Agriculture & Biology* **22,** 65-69.

Slooten L, Capiau K, Van Camp W, Van Montagu M, Sybesma C, Inze D. 1995. Factors affecting the enhancement of oxidative stress tolerance in transgenic tobacco over expressing manganese superoxide dismutase in the chloroplasts. *Plant Physiology* **107,** 373-380.

Smirnoff N. 1993. The role of active oxygen in the response of plants to water deficit and desiccation. *New Phytologist* **125,** 27–58.

Štajner D, Milošević M, Popović BM. 2007. Irradiation effects on phenolic Content, Lipid and protein oxidation and scavenger ability of soybean seeds. *International Journal of Molecular Sciences* **8,** 618-627.

Štajner D, Popović BM, Taški K. 2009. Effects of γ-irradiation on antioxidant activity in soybean seeds. *Central Journal of Biology* **4,** 381–386.

Vitória AP, Lea PJ, Azevedo RA. 2001. Antioxidant enzymes responses to cadmium in radish tissues. *Phytochimstry* **57,** 701-710.

Wada H, Koshiba T, Matsui T, Sato M. 1998. Involvement of peroxidase in differential sensitivity to irradiation in seedlings of two *Nicotiana* species. *Plant Science* **132,** 109-119.

Wi SG, Chung BY, Kim J, Kim J, Baek M, Lee J, Kim YS. 2007. Effects of gamma irradiation on morphological changes and biological responses in plants. *Micron* **38,** 553-564.

Willekens H, Chamnongpol S, Schraudner M, Langebartels C, Van Montagu M, Inze D, Van Camp W. 1997. Catalase is a sink for H_2O_2 and is indispensable for stress defense in C3 plants. *EMBO Journal* **16,** 4806-4816.

Xiong Q, Xing Z, Feng Z, Tan Q, Bian Y. 2009. Effect of ^{60}Co γ-irradiation on postharvest quality and selected enzyme activities of *Pleurotus nebrodensis*. *LWT - Food Science and Technology* **42,** 157-161.

Recibido: 11/11/09
Aceptado: 22/1/10