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Involvement of peroxidase activity in various sensitivity to gamma irradiation in scions of Cabernet Sauvignon and Merlot cvs (*Vitis vinifera* L.)

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Abstract

Peroxidase (POD) is an enzyme detected in large quantities throughout plant cells. Under the various abiotic stress conditions such as treatments of cadmium (Cd), copper (Cu), manganese (Mn), gaseous fluoride (F), sulfur dioxide (SO_2) and also gamma (γ) irradiation to different plants lead to enhancement in POD activity as a stress marker. This research showed that POD activity in leaves from scions of Cabernet Sauvignon and Merlot cvs increased and there was a positive correlation between POD activity and rising doses of gamma irradiation. Findings of current study revealed that POD activities in leaves of scions irradiated with higher gamma doses were greater than those irradiated with lower gamma doses and these results enable researchers to use POD activity as stress marker for gamma irradiation applications as well as other stress factors. In general, root and shoot characteristics of Cabernet Sauvignon and Merlot cvs were adversely affected from gradually ascending gamma irradiation doses from 10 to 40 Gy.

Key words: POD, grapevine, gamma irradiation, characteristics of root and shoot, chlorophyll a, chlorophyll b, total chlorophyll, carotenoids, stoma density.

Introduction

Grape has been an integral part of human society for thousands of years and important fruit crop grown in the world today in terms of both total production area and dollar value [1]. This industry requires cultivars with high productivity and some superior attributes such as high grape quality, resistance to different pests and diseases, better grafting affinity with different rootstocks and better adaptation to various climatic conditions. Natural mutations and traditional breeding methods have been used to obtain new grape cultivars which have superior attributes [2]. Since natural mutations occur for a long time, different researches are conducted in various plant cultivars to take form artificial mutations, that are easier, cheaper and more variable [3-5]. For this purpose, physical and chemical mutagens which can generally cause some problems like pigment loss and delay of shooting and rooting in plants are used. One of the physical agents that create artificial mutations is low doses of gamma irradiation which may induce physiological and biochemical changes [6]. Radiation is one of the well known physical mutagens utilized for generation of genetic variation in plant breeding studies [7]. However, high radiation doses which increase the free radical level in the living cells on M_1 generation led to reductions in plant height, root length and plant numbers alive [8].

Gamma rays belong to ionizing radiation and interact with atoms or molecules to create free radicals which may be damaged or modify crucial components of plant cell in cells. They have been variously reported to affect the morphology, anatomy, biochemistry and physiology of plants depending on the irradiation level. Among these effects can be variations in the plant cellular structure and metabolism such as accumulation of phenolic compounds, modulation of the antioxidative system and alteration in photosynthesis [9-11].

It has generally been accepted that reactive oxygen species (ROS), such as hydrogen peroxide (H_2O_2), superoxide anion ($O_2^{\cdot -}$), hydroxyl radicals (OH) and singlet oxygen, are produced by water radiolysis [12, 13]. H_2O_2, which is one of these ROS, is a normal metabolite in cells under the optimal plant growth conditions and not particularly cytotoxic, but when its concentrations are raised by different environmental stresses and ionizing radiation, it can lead to cell lethality [14]. Peroxidase (POD), superoxide dismutase (SOD) and catalase (CAT) represent endogenous enzymatic defense system of the plant cell that become active in the course of cell injury.

PODs have different physiological roles in plant cell and take part in many reactions including lignification, cross linking of cell elongation and phenol oxidation which are linked to growth reductions [15]. POD enzyme has been known as biochemical indicator due to activity of POD enzyme and changes of its composition with physiological status of organism [16] and its activity has been also accepted for stress marker for plants in researches with excessive iron, copper and zinc [17], sodium chloride[18] and several other stress factors [19].

Several studies have shown that there is a close relation with POD activity and physiological processes like respiration, photosynthesis and transpiration. Besides, POD enzyme has also potential to serve as an indicator of stress in plants [20, 21] and POD is one of the considerable systems for enzymatic removal of hydrogen peroxide (H_2O_2) stress-induced toxic oxygen species in plants [22].

Reduction in the concentrations of chlorophyll a and b and carotenoids on exposure to biotic and abiotic stressors have been examined in many plant species [23-25]. The major concern of present research was to ascertain whether there are any variations induced by different gamma irradiation doses among the POD activities in leaves, root and shoot characteristics between Cabernet Sauvignon and Merlot cvs.

Materials and Methods

This research was carried out in research field of Horticultural Department of Agricultural Faculty of Namik Kemal University in Turkey (40º59'N, 27º34'E) during the vegetation period of 2008 year. Scions were obtained from Merlot and Cabernet Sauvignon grape cvs, which are eight years old grapevines grafted on 1103 P rootstock and trained to bilateral cordon. For this aim, winter pruning was performed in early February after winter frost had passed. One year old canes with 5-6 nodes from winter pruning were kept dormant by storing them in a cool place at +4ºC with 85 - 90% RH. Later, these one year old canes were exposed to different doses of gamma irradiation (control, 10, 20, 30 and 40 Gy) on March 25th, 2008. Radiation applications were performed at Trakya University, Medical Faculty and Department of Radiation Oncology in Edirne, Turkey. In order to apply gamma radiation, it was utilized from a ^{60}Co source at an average dose rate of 2.21 Gy/min (Cirus model). After gamma radiation applications, canes with six nodes were changed into one-eyed and planted into rooting canals (5 m x 0.5 m x 0.5 m) filled up with perlite on March 26th, 2008. Mean values of pH and EC of perlite used for rooting of cuttings were respectively 6.31 and 188 µS/cm. In the course of rooting and shooting of one-eyed scion, only irrigation practice was performed until scions were planted out from their places on June 28, 2008. At the end of study, peroxidase enzyme activity in leaves (POD activity, U/l), bud bursting percentage (BBP, %), mean shoot length (MSL, cm), mean fresh shoot weight (MFSW, g), mean total leaf area per scion (MTLA, cm^2), stoma density on leaf (SD, stoma no./mm^2), chlorophyll a content (Chl-a, µg/g FW), chlorophyll b content (Chl-b, µg/g FW), total chlorophyll content (Total Chl, µg/g FW), caretenoid content (Car, µg/g FW), rooting percentage (RP, %), mean root length (MRL, cm) and mean fresh root weight (MFRW, g) were assessed for shoots and roots from gamma irradiated scions of Cabernet Sauvignon and Merlot cvs.

Determination of stoma density (SD, stoma no./mm^2): At the end of research, stoma imprints were taken from the places near the central main vain that are located underside of leaves. In order to take proper imprints from leaves, it was utilized from nail polish and was applied to underside surfaces of leaf being studied and waited until nail polish completely dried. Later, nail polish patch was gently peeled from the leaf by pulling to obtain leaf impression and stoma density on leaf impression was examined under a light microscope to 40X.

Determination of mean total leaf area per scions (MTLA, cm^2): All leaves of scions planted out from perlite medium were scanned with a pc scanner and assessed with a pc packing program [26]. Afterwards, obtained mean values were converted from dm^2 to cm^2.

Determination of Chl-a, b, Total Chl and Car content (µg/g fresh leaf mass) in leaves: Prior to pigment analysis, 0.5 g fresh leaf samples from leaves rinsed with distilled tap water were taken. The chlorophyll and carotenoid pigments in leaves were extracted with 80% acetone and $CaCO_3$ by macerating the leaves with a mortar and pestle and centrifuged at 3000 rpm for 5 min. After dilution was performed at certain rates, the absorption of the extracts at wavelengths of 470 nm, 645 nm and 662 nm were measured with a spectrophotometer. After related dilution factors, the contents (µg/g fresh leaf mass) of Chl-a, Chl-b, Total Chl and Car were calculated using the equations of Lichtentaler and Wellburn [27]:

$$\text{Chl-a} = 11.75.A_{662} - 2.35.A_{645}$$
$$\text{Chl-b} = 18.61.A_{645} - 3.96.A_{662}$$
$$\text{Total Chl} = \text{Chl-a} + \text{Chl-b}$$
$$\text{Car} = 1000.A_{470} - 2.27.\text{Chl-a} - 81.4.\text{ Chl-b}/227$$

Determination of peroxidase activity (POD activity, U/l) in leaves: One g of fresh leaf sample was homogenized with 5 ml of 0.1 M sodium phosphate buffer (pH 7.0). After centrifugation of 13,000 rpm for 40 min, the supernatant was used as enzyme extract for POD activity determination [28].

POD activity in leaves of Cabernet Sauvignon and Merlot cvs was assayed according to method of Pütter [29]. POD activity was measured by following the change of absorption at 436 nm due to guaiacol oxidation. The activity was assayed for 2 min in a reaction mixture comprised of phosphate buffer (3 ml), guaiacol (0.05 ml), H_2O_2 (0.03 ml) and suitable amount (0.1 ml) of enzyme extract from leaves. Afterwards, POD activity was determined according to a formula indicated by Pütter [29].

After gamma irradiation, leaf samples from bud burst scions were collected at stage 11 (4 leaves separated) and 17 (12 leaves separated) of phenological growth stages of grapevine according to scale of Eichhorn and Lorenz[30] to determine POD activity in leaves and POD activities were measured for these two phenological growth stages of Cabernet Sauvignon and Merlot cvs (Table 1).

Statistical analysis: The experiment was arranged in factorial study with completely randomized block design with 4 replicates, including 15 cuttings per replicate [31]. After different gamma radiation doses, variations in characteristics of root and shoots belonging to two grape cvs were determined using TARIST pocket program [32]. Least significant difference (LSD) test was used for mean separation and significant differences were determined at $P \leq 0.01$.

Table 1. The dates of phenological growth stages in scions of Merlot and Cabernet Sauvignon cvs.

	Merlot					Cabernet Sauvignon				
Stage	Control	10 Gy	20 Gy	30 Gy	40 Gy	Control	10 Gy	20 Gy	30 Gy	40 Gy
11	April 25	April 28	April 30	May 5	May 14	April 27	April 30	May 3	May 7	May 17
17	May 30	June 3	June 6	June 12	June17	June 3	June 5	June 8	June 14	June 19

Results and Discussion

POD, CAT and SOD are generally increased in various plant species by the application of ionizing radiation [34-38]. As known, POD is one of the significant antioxidant enzymes in terms of cell protection under the stress conditions as well as ionizing radiation application. Wada *et al.* [37] report that especially the potential activity of POD to remove toxic H_2O_2 contributes to the difference in response to radiation between two Nicotiana species.

In our study, POD activity in leaves of Cabernet Sauvignon and Merlot cvs were determined at stage 11 and 17 according to scale of Eichhorn and Lorenz [30] after various doses of gamma irradiation were applied.

The responses of Cabernet Sauvignon and Merlot cvs to stress induced by gamma radiation were compared with regard to POD activity in leaves (Fig. 1). It was observed that the POD activity in leaves of both cvs had a positive association with the rising doses of gamma irradiation application. Results of present study, which were consistent with Wi *et al.* [39] showed that increasing doses of gamma irradiation increased the POD activities. While POD activities in leaves were the highest at early stage of shoot (stage 11), later, they were gradually reduced along with shoot development (stage 17). Responses of POD activity in leaves from scions of Cabernet Sauvignon and Merlot cvs exposed to different doses of gamma irradiation were found to be higher in Merlot cv. than in Cabernet Sauvignon cv. and POD activity increased with elevating stress conditions caused by rising doses of the applied gamma irradiation ranging from control to 40 Gy (Fig. 1).

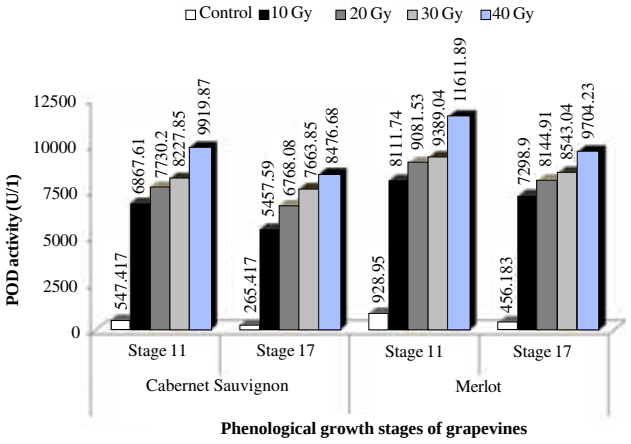

Figure 1. POD activities in leaves of Cabernet Sauvignon and Merlot cvs, exposed to various doses of gamma irradiation at two successive growth stages of grapevines.

Table 2 shows the variations in bud bursting percentage (BBP, %), mean shoot length (MSL, cm), mean fresh shoot weight (MFSW, g), mean total leaf area per scion (MTLA, cm^2/scion), stoma density on leaf (SD, stoma no./mm^2), chlorophyll a content (Chl-a, µg/g FW), chlorophyll b content (Chl-b, µg/g FW), total chlorophyll (Total Chl, µg/g FW), carotenoid content (Car, µg/g FW), rooting percentage (RP, %), mean root length (MRL, cm) and mean fresh root weight (MFRW, g) of Cabernet Sauvignon and Merlot grape cvs in responses to different gamma irradiation doses.

There were no statistically significant differences in BBPs in both cvs within the gamma irradiation ranges of control and 40 Gy. BBP ranged from 43.75 (40 Gy) to 100% (control, 10, 20, 30 Gy) for Cabernet Sauvignon and 12.51 (40 Gy) to 100% (control,10, 20 and 30 Gy) for Merlot cv. (Table 2).

Statistically significant differences were observed between MSLs of cvs ($p \leq 0.05$). Compared to control, MSL was reduced considerably with the increasing doses of gamma irradiation in both cvs. The highest decreases in MSL were at dose of 40 Gy for Cabernet Sauvignon and Merlot cvs, followed by doses of 30, 20 and 10 Gy for both cvs (Table 2).

MFSW of cvs are presented in Table 2 and were reduced by all gamma irradiation doses except for control in Cabernet Sauvignon and Merlot cvs. The lowest values in MFSW were obtained from doses of 40 Gy for both cvs and these were respectively followed by doses of 30, 20 and 10 Gy for both cvs.

As indicated by Wi *et al.* [11] while high dose irradiation such as 50 Gy inhibited seedling growth considerably, no significant morphological aberrations were observed in phenotype of the plants irradiated with relatively low doses ranging from 1 to 5 Gy of gamma rays**.**

As seen in Table 2, compared to control, gamma irradiation applications caused reductions in MTLA per scion from bud burst scions of Cabernet Sauvignon and Merlot cvs. The lowest values of MTLA per scion were respectively measured for doses of 40, 30, 20 and 10 Gy in both cvs.

The effects of gamma irradiation doses on SD on leaves of

Table 2. Effects of various gamma irradiation doses on root and shoot characteristics of scions of Cabernet Sauvignon and Merlot cvs.

	Cabernet Sauvignon					Merlot				
	Gamma irradiation doses (Gy)									
	Control	10	20	30	40	Control	10	20	30	40
BBP (%)	100	100	100	100	43.75	100	100	100	100	12.51
MSL (cm)	16.31a	14.93a	11.64b	6.12cd	1.51e	10.59b	8.69bc	3.42de	3.30de	0.63e
MFSW (g)	5.53	4.65	3.69	2.79	1.10	3.94	2.70	1.16	1.02	0.34
MTLA (cm^2)	283.33	269.19	239.55	228.38	156.76	276.68	255.71	226.24	222.61	44.70
SD (No./mm^2)	203.02a	172.83b	166.46bc	152.29bc	144.45c	220.66a	214.29a	172.62b	156.89bc	151.47bc
Chl-a (µg/g FW)	22.120	18.810	16.807	16.430	12.650	15.710	14.237	13.580	10.023	9.823
Chl-b (µg/g FW)	9.470	9.327	7.423	7.400	6.310	6.727	6.073	5.243	4.013	3.760
Total Chl (µg/g FW)	31.593	28.137	24.233	23.827	18.957	22.437	20.303	18.827	14.033	13.583
Car (µg/g FW)	5.340	3.927	3.737	3.717	3.017	4.057	3.380	2.807	2.553	2.500
RP (%)	100a	100a	96.88a	87.50ab	56.25c	100a	100a	90.63ab	78.13b	9.38d
MRL (cm)	14.55	13.85	13.77	10.00	8.76	11.05	9.97	6.96	4.80	0.45
MFRW (g)	9.21a	5.37b	4.42bc	3.76cd	1.77ef	3.43cd	2.35de	0.74f	0.47f	0.27f

Values at the same rows with the same letter are equal according to the least significant difference test with a P≤0.01.
MSL_{LSD} 3.221, SD_{LSD} 24.337, RP_{LSD} 16.120, $MFRW_{LSD}$ 1.586, BBP Bud bursting percentage, MSL Mean shoot length, MFSW Mean fresh shoot weight, MTLA Mean total leaf area, SD Stoma density, RP Rooting percentage, MFRW Mean fresh root weight.

Cabernet Sauvignon and Merlot cvs are shown in Table 2. Results revealed that compared to control gamma irradiation at rising doses led to statistically significant reductions in SDs of both cvs ($p \leq 0.01$).

Chlorophyll a, b and carotenoids are chief photosynthetic pigments for plants and they have great importance in photosynthesis. Chl-a, Chl-b, total Chl and Car contents in leaves of Cabernet Sauvignon and Merlot cvs under the stress conditions derived from gamma irradiation are presented in Table 2 and no statistically significant differences were observed. Compared to control, Chl-a contents were reduced by all of increasing doses of gamma irradiation in both cvs. Maximum decreases in Chl-a content were shown at dose of 40 Gy, followed by 30, 20 and 10 Gy for both cvs.

As displayed in Table 2, increasing doses of gamma irradiation reduced Chl-b contents of Cabernet Sauvignon and Merlot cvs compared to control. Among the gamma irradiation doses, least effective dose on Chl-b content was 10 Gy, followed by 20, 30 and 40 Gy.

Applications of gamma irradiation also led to reductions in Total Chl contents in both cvs and their contents were in lower levels compared to control (Table 2). The lowest levels of total Chl were respectively found at doses of 40, 30, 20 and 10 Gy in both cvs.

With gradually increasing gamma irradiation doses, Car contents in leaves of Cabernet Sauvignon and Merlot cvs were decreased compared with control (Table 2). Minimum mean values in Car content were respectively obtained from 40, 30, 20, 10 Gy for both cvs.

With regard to RP of both cvs, statistically significant differences were observed among the gamma irradiation doses ($p \leq 0.01$). The best RPs were obtained from control and 10 Gy application (100%) in Cabernet Sauvignon and Merlot cvs and RPs were gradually decreased by increasing gamma irradiation doses (Table 2).

As presented in Table 2, although no statistically significant differences were observed in different doses of gamma irradiation in MRL, values of control were higher than in other irradiation doses and MRLs were decreased with the rising gamma irradiation doses in both cvs.

Values of MFRW were significantly different ($p \leq 0.01$) (Table 2). MFRWs were significantly decreased by elevating doses of gamma irradiation compared to control in Cabernet Sauvignon and Merlot cvs.

Conclusions

Although ascending gamma irradiation doses did not considerably affect BBPs and RPs in scions of Cabernet Sauvignon and Merlot cvs.; other examined criteria were contrarily influenced by rising gamma irradiation doses from 10 to 40 Gy. According to results of examined parameters, it seems that Cabernet Sauvignon cv. is more tolerant to radiation than Merlot cv. and gamma irradiation to scions caused more severe damages to Merlot cv. than to Cabernet Sauvignon cv. The results showed that increasing doses of gamma irradiation lead to negative effects on the root and shoot characteristics of scions in Cabernet Sauvignon and Merlot cvs. Radiation applications, especially high doses, can bring about direct and indirect damage in living system by way of the various radicals in irradiated cell. In our research, the gamma irradiation applied in lower doses did not origin remarkable differences as compared to the control in POD activity in leaves, shoot and root characteristics of Cabernet Sauvignon and Merlot cvs. As a result, the findings of present study clearly showed that POD activity in leaves of Cabernet Sauvignon and Merlot cvs. was correlated with abiotic stress condition caused by gamma irradiation and it can be said that POD activity in leaf seems to be proper tool to determine stress from high doses of gamma irradiation.

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