Journal of Medicinal Plants Research Vol. 4(23), pp. 2573-2582, 4 December, 2010
Available online at http://www.academicjournals.org/JMPR
DOI: 10.5897/JMPR10.633
ISSN 1996-0875 ©2010 Academic Journals

Full Length Research Paper

Seasonal changes in leaf and twig antioxidant systems in Damask rose (*Rosa damascena* Mill.)

Bahareh Kashefi[1]*, Seyed Reza Tabaei-Aghdaei[2], Mohammad Matinizadeh[3], Amir Mousavi[4], Aliashraf Jafari[5] and Leila Mirjani[2]

[1]Department of Horticulture, Islamic Azad University- Science and Research Branch, P. O. Box 14515- 775, Tehran, Iran.
[2]Biotechnology Research Department, Research Institute of Forests and Rangelands, P. O. Box 13185-116, Tehran, Iran.
[3]Department of Forest, Research Institute of Forests and Rangelands, P. O. Box 13185-116, Tehran, Iran.
[4]Department of Plant Biotechnology, National Institute of Genetic Engineering and Biotechnology, P.O. Box 14155-6343, Tehran, Iran.
[5]Department of Gene Bank, Research Institute of Forests and Rangelands, P. O. Box 13185-116, Tehran, Iran.

Accepted 11 October, 2010

The present study aimed to investigate the antioxidant activity of the various tissue types in *Rosa damascena* and determine the seasonal variations. The existence of statistically significant differences in the specific activities of enzymes among *R. damascena* accessions and tissue type was observed annually. The content of enhanced peroxidase and catalase in leaves has been shown to undergo seasonal changes with relatively low levels on young leaves in spring and strong activities on mature leaves in autumn. Meanwhile, the expansion of season from spring to winter showed a decrease in twig peroxidase activity, but catalase activities enhanced before raining in the autumn and then it decreased. Based on the results, *R. damascena* tissues, such as leaves and twigs indicated different responses including activation of antioxidative enzymes to seasonal change and during acclimatization to environment. There were significant differences in the specific activities of enzymes among *R. damascena* accessions.

Key words: Antioxidant activity, catalase, enzyme, harvesting time, peroxidase seasonal changes.

INTRODUCTION

Rosa L. *as a* major genus in Rosaceae consists of almost 200 species with more than 18000 cultivars (Gudin, 2000). Damask rose (*Rosa damascena* Mill.) is an important *Rosa* species, which are known economically for their strong fragrance, oil production and their medicinal properties (Mahmood et al., 1996; Ardogan et al., 2002; Achuthan et al., 2003; Basim and Basim, 2003; Ozkan et al., 2004). In recent years, antioxidant, antibacterial and antimicrobial activities of essential oil *R. damascena* have been demonstrated (Achuthan et al., 2003; Ozkan et al., 2004). Antioxidants are compounds that can delay/inhibit the oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidative chain reactions (Velioglu et al., 1998) or alternatively scavenging the free radicals generated during oxidative destruction. Antioxidant defense system in plants contains enzymatic and non–enzymatic antioxidants. The enzymatic system consists of enzymes such as POD, CAT, SOD, ARx, MDAR, DHAR and GR (Keles and Oncel, 2002). Antioxidant activity is mainly related to production of chemical agents that are used for the protection of the plant. Observation of the free radical scavenging action of the plant may serve as a monitor for stressor or conditions (e.g. seasonal and climatic changes) that lead to generation of these antioxidant compounds. In fact, changes of antioxidant compounds and enzyme activities

*Corresponding author. E-mail: Bahareh.Kashefi@yahoo.com. Tel: 0098 9125319540.

Abbreviations: APX, Ascorbate peroxidase; **CAT**, catalase; **DHAR**, dehydroascorbate reductase; GR, glutathione reductase; H_2O_2, hydrogen peroxide; **MDAR**, monohydroascorbate reductase; **POD**, peroxidase; **ROS**, reactive oxygen species; **SOD**, superoxide dismutase.

reflect the impact of environmental stresses on plant metabolism and affected the antioxidant defense capacity of cells. As indicated above, antioxidant activity exhibits variations depending upon the physiological and biochemical effects (Herbinger et al., 2002; Syvacy and Sokmen, 2004; Beltagi, 2008). Meanwhile, enzyme activities aimed as important index of plant response and behavior to seasonal alterations. Variable in both time and location makes the seasonal environment difficult to predict responses of plants to the changing conditions of the environment (Sen and Mukherji, 2009).

POD is an important multifunctional enzyme and constitutes about 15% of all cellular proteins (Chibbar et al., 1984). It is involved in a number of important physiological processes and ubiquitous in plants (Sergeeva et al., 1987). PODs regulate H_2O_2 levels and ROS production within cells (Passardi et al., 2005) and catalyse the reduction of H_2O_2 by employing various substrates (De Gara, 2004; Hiraga et al., 2001; Passardi et al., 2005). CATs are consisting of monofunctional proteins that are specifically localized in peroxisomes (Engel et al., 2006; Feierabend, 2005; Willekens et al., 1997). Activities of CAT and non-specific POD is directly countered to production of H_2O_2 (Foyer and Halliwell, 1976) and decompose H_2O_2 to H_2O at different cellular locations (Asada, 1999; Mittler, 2002). The balance between ROS production and activities of antioxidative enzyme determines whether oxidative signaling and/or damage will occur (Moller et al., 2007).

Consequently, the present study aimed to investigate the antioxidant activity of the leaves and twigs (various tissue types) in *R. damascena* and to determine the seasonal variations. Damask rose accessions were collected from 10 provinces of Iran and cultivated in Damavand region. Previous studies showed that they may include multiple genotypes (Tabaei-Aghdaei et al., 2006; 2007). This research was directed towards the study of seasonal and annual changes in 12 accessions of Damask rose in relation to enzyme activities. Since enzymatic activities and the responses of the accessions were cleared difference and emphasis on the interaction among them. Also, it detected that one of them are sensitive and need more changes in enzymatic activities for protecting themselves in new condition. On the other hand, different tissues have various behaviors to the environmental changes. Therefore, this effort was performed for the reaction of each tissue to these alterations among the accessions.

MATERIALS AND METHODS

Plant materials

Twelve Damask Rose accessions originating from ten provinces of Iran were cultivated under randomized complete block design with three replications at Rangelands Research Station of Hamand Absard, Damavand., Iran. The state is located at 65 km from Tehran (co-ordinates, 35°44´N, 52°05´E and elevation 1960 m).

The minimum and maximum temperature was -24°C in January-February and 37°C in July-August. Average rainfall of a year was 333 mm and of first 3 months of year (development and flowering stage of plants) were 13 and 130 mm in 2008 and 2009, respectively.

In this research, samples were harvested at critical phenological stages of plant growth. Enzymatic activities in leaf and twig were evaluated during 2008 and 2009. The 3 phenological stages for leaf sampling included 50% flowering in spring, warmest time in summer and before rains in autumn. Twigs were taken at 4 stages, including above stages and in winter. Fresh leaves and twigs were sampled and the measurements were carried out.

Enzyme assays

For enzyme extracts and assays, about 1 g of leaves was rubbed and plugged in extraction buffer (pH 7.5) for 24 to 72 h (Ebermann and Stich, 1982). The homogenate was centrifuged at 3000 rpm for 15 min, and supernatant was collected and used for enzyme assays. POD activity assay was based on the method of Chance and Maehly (1955) with phosphate buffer (pH 6.0) which was determined by measuring the oxidation of guaiacol as an electron donor to tetraguaiacol in the presence of H_2O_2 at 420 nm. CAT activity was determined according to the method described by Eising and Gerhardt (1989) containing phosphate buffer (pH 7.0) with modifications which measure the decline of a decrease in absorbance at 240 nm for 1 min following the decomposition of H_2O_2.

Statistical analyses

Data presented are mean values ± S.E.M. for three replicates. The means of the treatments were separated using Duncan's new multiple ranges test (DMRT) at a 0.05 significant level. GLM procedure analysis was used in Statistical Analysis System (SAS) (SAS Institute, Inc., Cary, NC, USA). Cluster analysis was performed based on all characteristics, using Ward's method (Ward, 1963).

RESULTS

Significant differences were observed for specific activities of enzymes among *R. damascena* accessions, various harvesting time, and two years (Table 1). There were significant interactions among years, accessions and sampling stages. POD activities of leaves varied annually in the same condition and were higher in 2008 compared to 2009, except for Tehran1 that showed the highest activity (4.65 activity/g FW) in the second year. Strongest POD activities were showed in Fars1 and Tehran1 (3.54 and 3.39 activity/g FW, respectively) in the first year and the lowest ones was in Kermanshah1 (1.81 activity/g FW) in the second year (Figure 1). Results were expressed various effects of CAT activity among acessions in two years. So that Ardebil1 and Fars1 had maximum and Tehran1 had minimum CAT activities in two years (Figure 2). These results elucidated an inverse trend of POD and CAT activities among accessions, especially in Tehran1. The content of enhanced POD and CAT in leaves has been shown to undergo seasonal changes, with relatively low levels in young leaves in spring and strong activities in mature leaves in autumn

Table 1. Variance analysis of leaf enzymes activities in 12 damask rose accessions at Damavand (2008 to 2009).

Source	DF	Mean square	
		Peroxidase (activity/g FW)	Catalase (activity per min)
Year	1	8.93**	0.27*
Accession	11	4.04**	1.02**
Year * Accession	11	1.94**	0.069
Stage	2	36.47**	2.02**
Accession * Stage	22	1.81**	0.31**
Year * Stage	2	7.70**	2.78**
Year * Accession * Stage	22	0.59	0.1
rep	2	1.14	0.10
Error	141	0.75	0.07
CV%		31.93	24.08

* and ** significant differences at 5% and 1%, significantly.

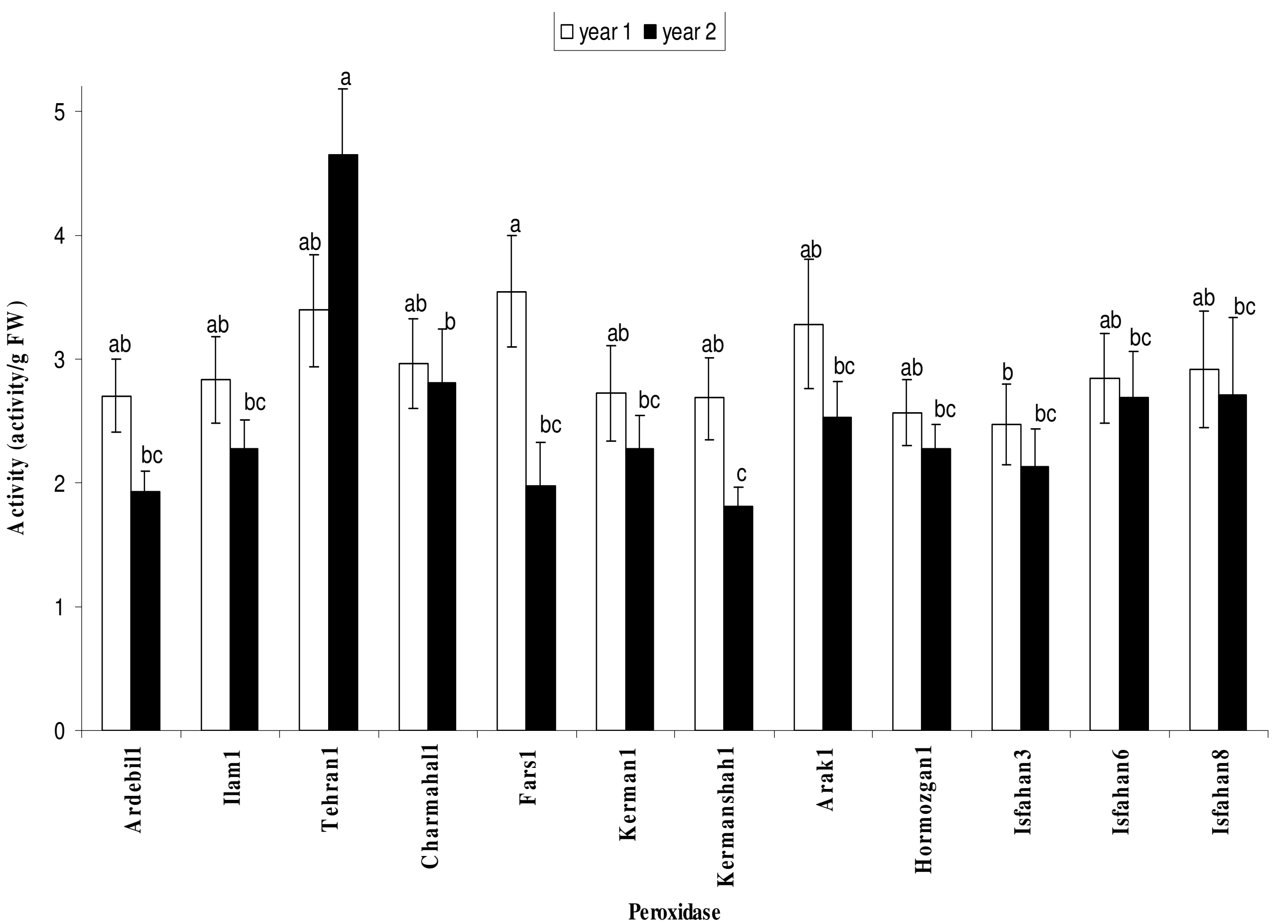

Figure 1. Changes of leaf peroxidase activity (activity/g FW) in 12 Damask Rose accessions in 2 years. Vertical bars represent the ±SEM. Different letters in each bar differ significantly according to DMRT test (0.05 level).

(Figure 3). Comparing the accessions, Tehran1 showed the highest POD activities in 3 stages (3.26, 3.49 and 5.31 activity/g FW) and Fars1 and Ardebil1 (1.26 and 1.75 activity/g FW, respectively) had the lowest activities in spring. Ardebil1 showed exceptional activities, whereas it had strong activities (3.05 activity/g FW) in warmest time in summer (Figure 4). Meanwhile, Ardebil1 and Fars1 (2.17 and 1.81 activity per min) had highest CAT activity and Tehran1 indicated lowest activities (0.67 activity per min) in autumn, respectively (Figure 5).

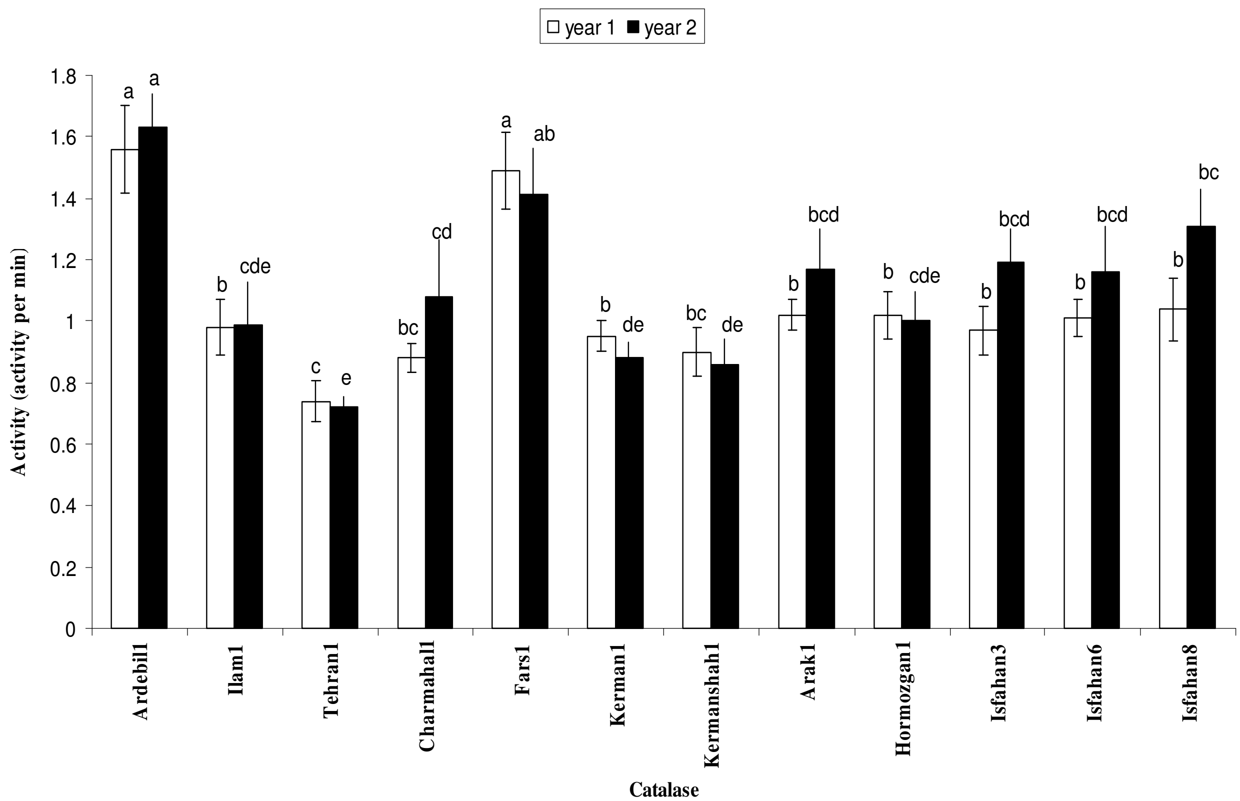

Figure 2. Changes of leaf catalase activity (activity per min) in 12 Damask Rose accessions in 2 years. Vertical bars represent the ±SEM. Different letters in each bar differ significantly according to DMRT test (0.05 level).

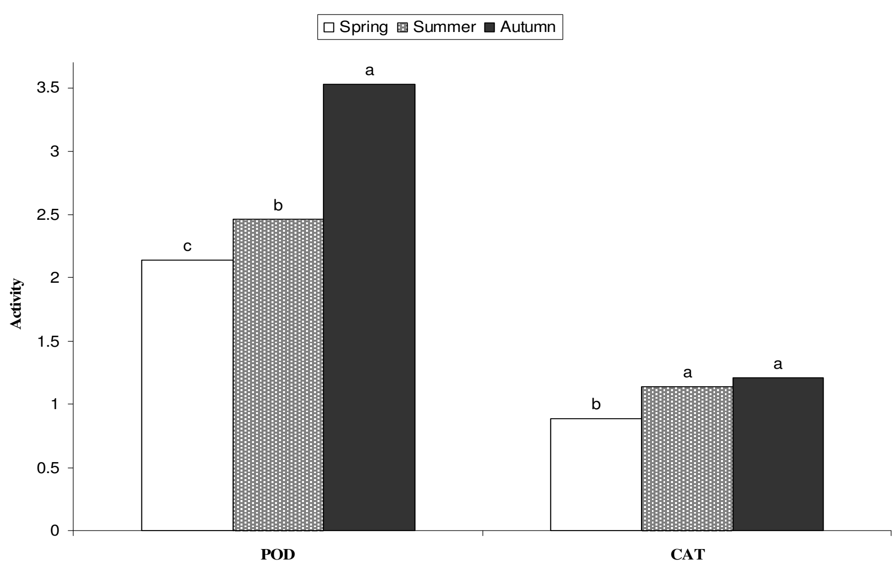

Figure 3. Seasonal changes of leaf peroxidase (activity/g FW) and catalase (activity per min) activities in 12 Damask rose accessions. Vertical bars represent the ±SEM. Different letters in each bar differ significantly according to DMRT test (0.05 level).

Significant differences were observed between POD and CAT leaf activities in Tehran1 and Kermanshah1 accessions, so that it indicated the converse activities among 3 phenological stages. Meanwhile, changes of twig enzyme activities indicated variation among acessions and different harvesting time with high interactions

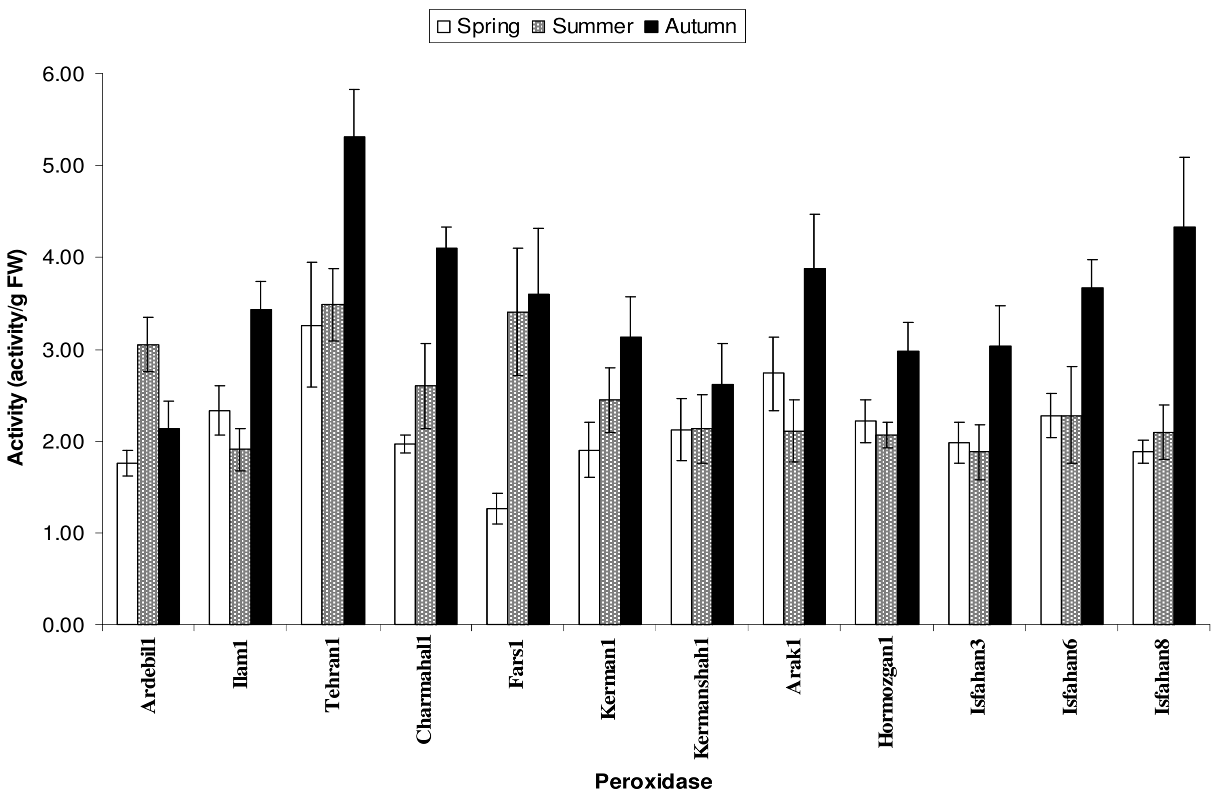

Figure 4. Seasonal changes of leaf peroxidase activity (activity/g FW) at 3 stages in 12 Damask rose accessions in 2 years. Vertical bars represent the ±SEM. Different letters in each bar differ significantly according to DMRT test (0.05 level).

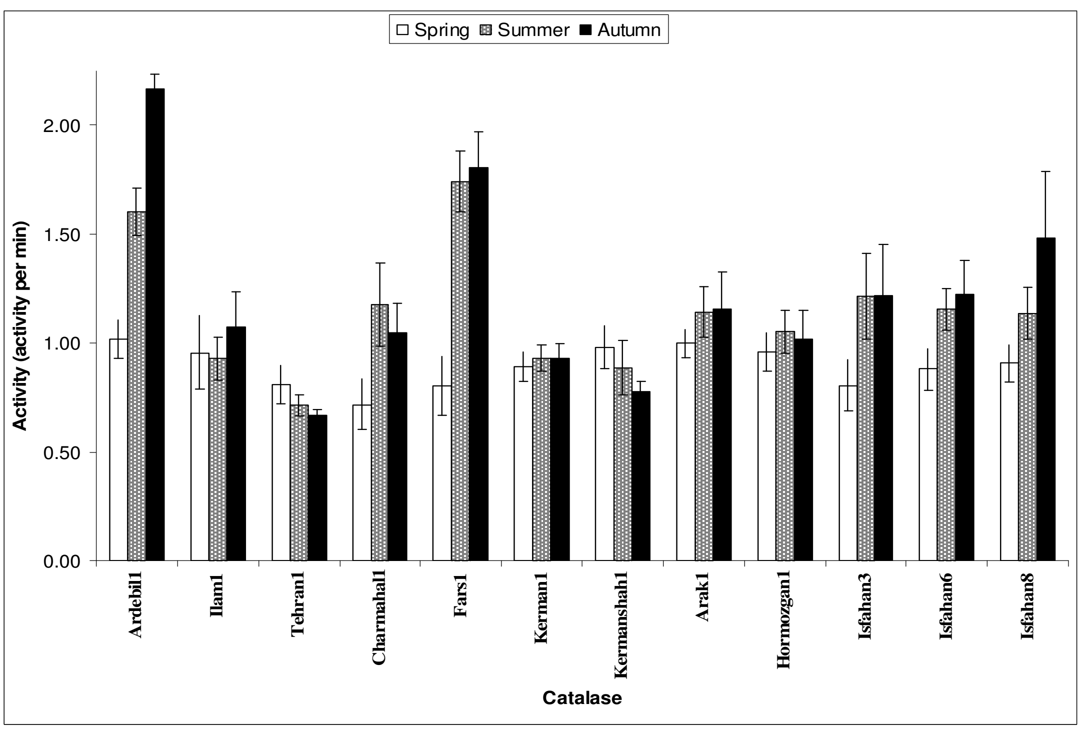

Figure 5. Seasonal changes of leaf catalase activity (activity per min) at 3 stages in 12 Damask rose accessions in 2 years. Vertical bars represent the ±SEM. Different letters in each bar differ significantly according to DMRT test (0.05 level).

Table 2. Variance analysis of twig enzymes activities in 12 damask rose accessions at Damavand (2009).

Source	DF	Mean square	
		Peroxidase (activity/g FW)	Catalase (activity per min)
Accession	11	26.75**	0.06**
Stage	3	143.42**	0.62**
Accession * Stage	33	3.86*	0.01
rep	2	2.32	0.03
Error	90	2.45	0.012
CV%		26.18	24.54

* and ** significant differences at 5 and 1%, significantly.

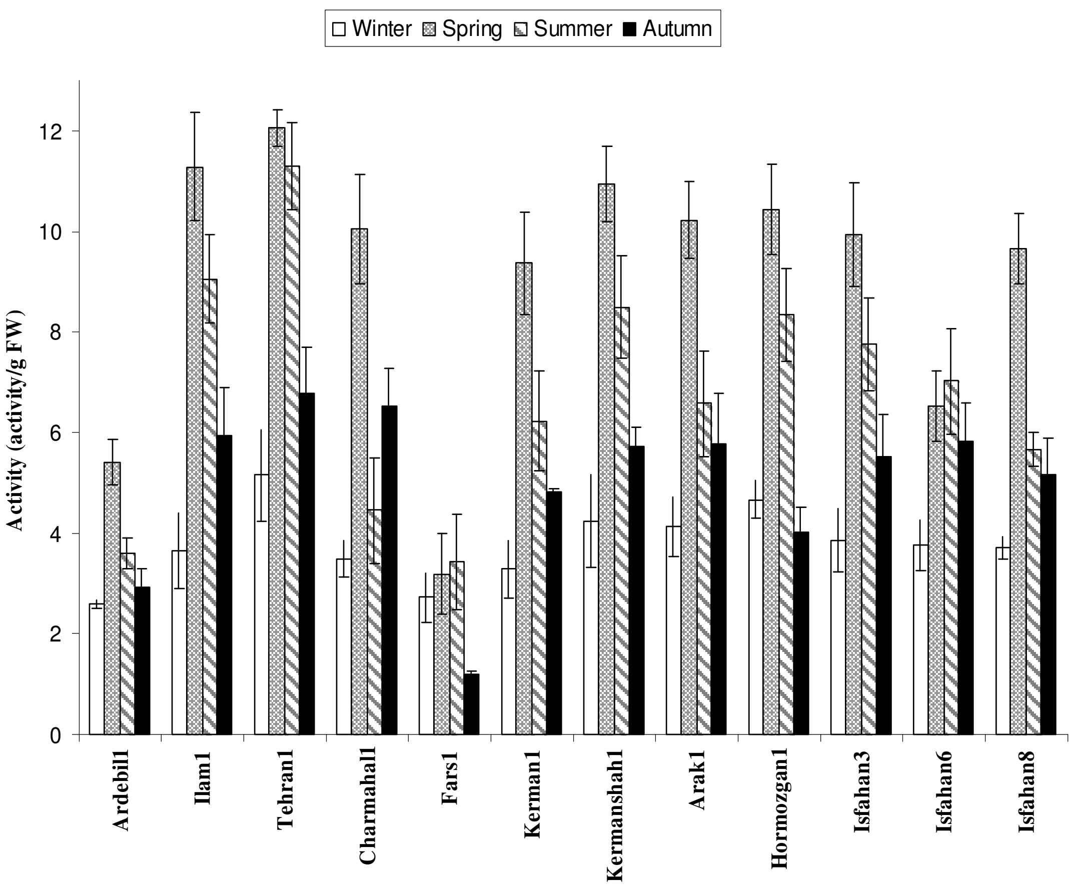

Figure 6. Seasonal changes of twig peroxidase activity (activity/g FW) in 12 Damask rose accessions in 2009. Vertical bars represent the ±SEM. Different letters in each bar differ significantly according to DMRT test (0.05 level).

(Table 2). POD activities in twigs were highest for Tehran1 at 50% flowering in spring (12.05 activity/g FW) and lowest ones were obtained for Ardebil1 when leaves fallen in winter (2.59 activity/g FW), except for Fars1 that showed the lowest activity (1.21 activity/g FW) before rains in autumn. Altogether, Tehran1 had maximum (5.15, 12.05, 11.3 and 6.78 activity/g FW) and Fars1 showed the minimum (2.72, 3.19, 3.42 and 1.21 activity/g FW) twig POD activities among harvesting time in winter, spring, summer and autumn, respectively (Figure 6). The difference was indicated that the season was expansion from spring to winter, POD activities were decreased, but

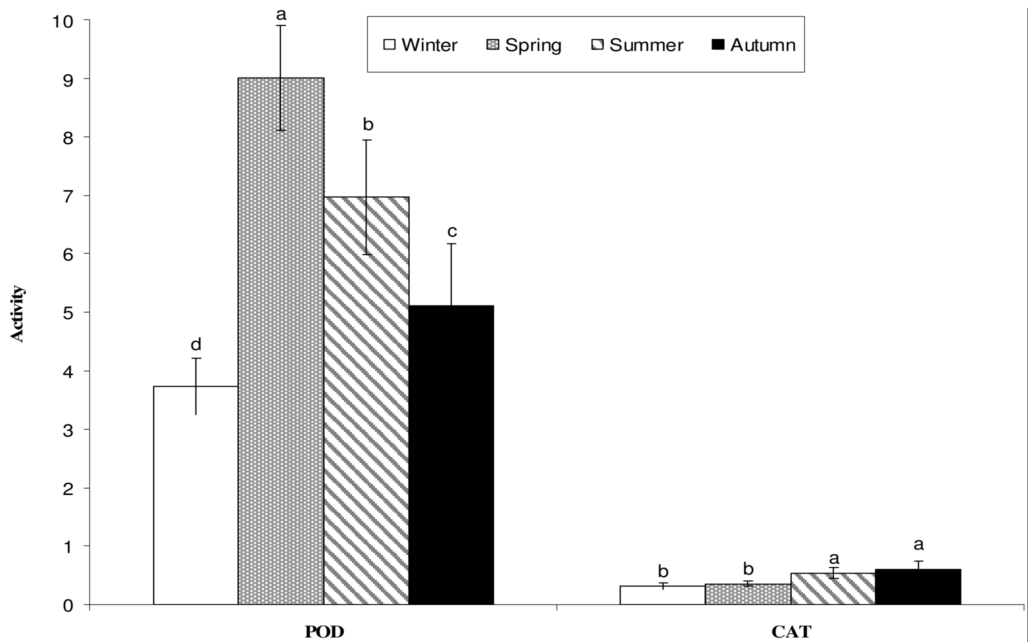

Figure 7. Seasonal changes of twig peroxidase (activity/g FW) and catalase activities (activity per min) in 12 Damask rose accessions. Vertical bars represent the ±SEM. Different letters in each bar differ significantly according to DMRT test (0.05 level).

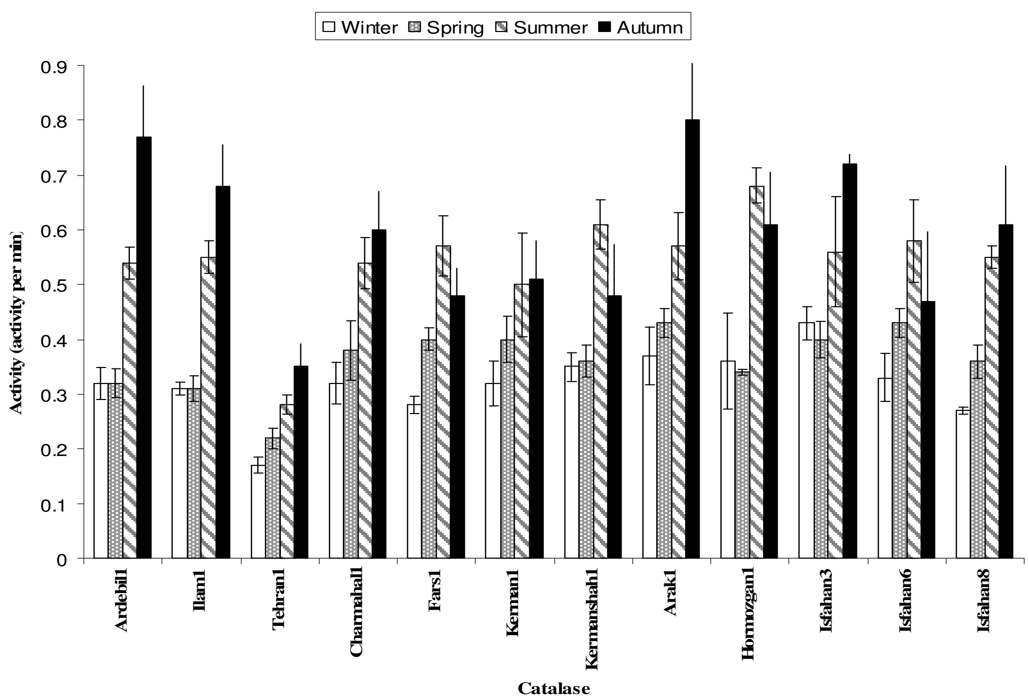

Figure 8. Seasonal changes of twig catalase activity (activity per min) in 12 Damask rose accessions in 2009. Vertical bars represent the ±SEM. Different letters in each bar differ significantly according to DMRT test (0.05 level).

CAT activities were enhanced to raining in autumn then it decreased (Figure 7). On the other hand, highest twig CAT activity was observed in Arak1, before rains in autumn and the lowest activity occurred in Tehran1 when leaves were fallen in winter, in contrast of POD activity (Figure 8). It is interesting that, POD activity exhibited

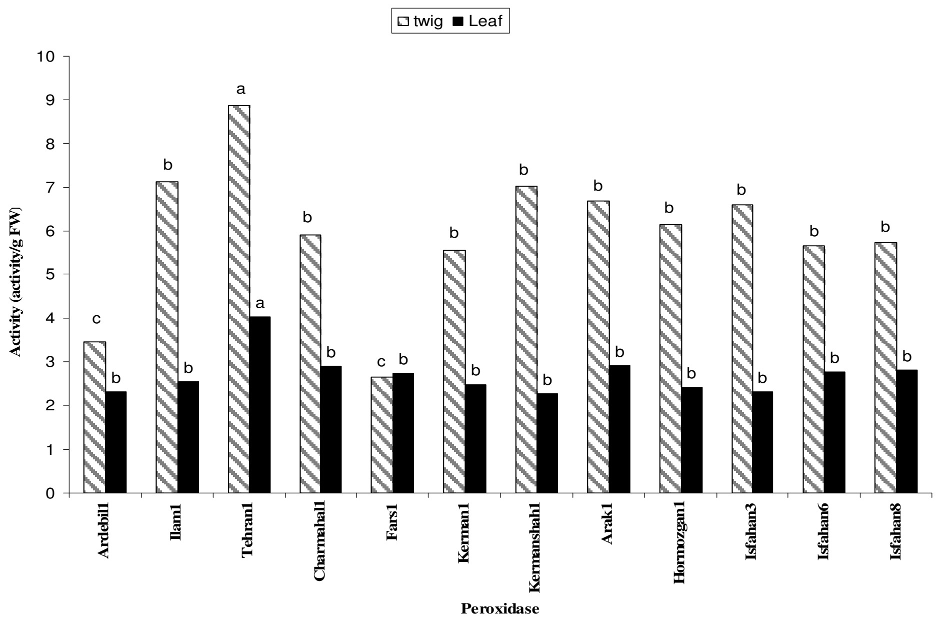

Figure 9. Changes of leaf and twig peroxidase activity (activity/g FW) in 12 Damask Rose accessions. Vertical bars represent the ± SEM. Different letters in each bar differ significantly according to DMRT test (0.05 level).

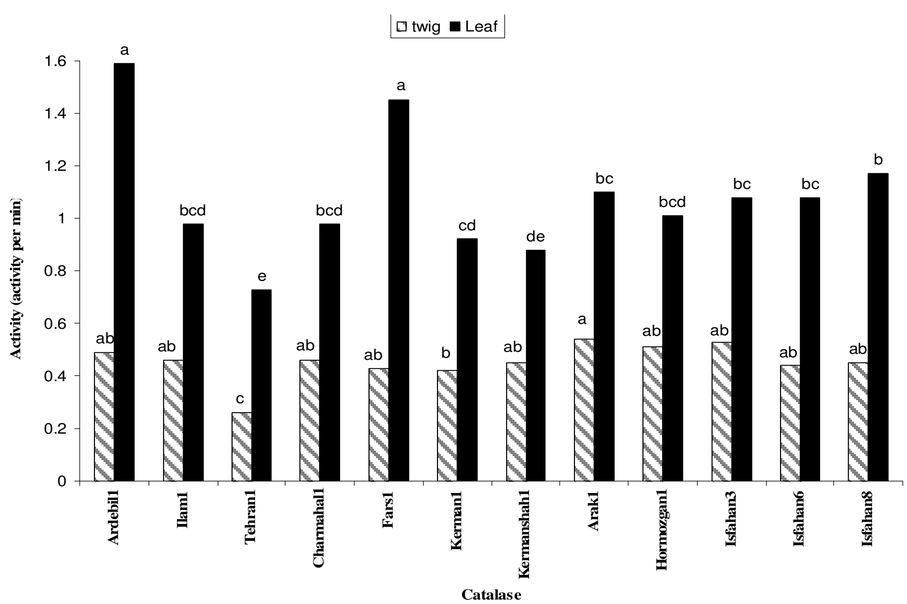

Figure 10. Changes of leaf and twig catalase activity (activity per min) in 12 Damask Rose accessions. Vertical bars represent the ±SEM. Different letters in each bar differ significantly according to DMRT test (0.05 level).

higher and CAT activity detected lower by the *R. damascena* twig extract than the leaf extract (Figures 9 and 10).

DISCUSSION

Seasonal alteration can be illustrated with changes in plant metabolism and affect on biosynthetic pathways (Hatano et al., 1986; Salminen et al., 2001). The results presented in this research were obtained from plants undergoing 2-year study of seasonal changes effects on the enzymatic activities in 12 Damask rose accessions. Antioxidants protect cells from biochemical damage caused by ROS, whose production increases during imbalances within and between bioenergetics' pathways

(Wise, 1995). Swanberg and Verhoeven (2002) reported the seasonal changes at antioxidant activity in the leaves of *Taxus cuspidate*. The existence of statistically significant differences in the specific activities of enzymes among *R. damascena* accessions could be attributed to the alteration in the amount of these enzymes in leaves on various harvesting time in two years. Also, main effect of seasonal acclimatization on the difference in POD and CAT enzymatic activities, suggesting a general capability of *R. damascena* plants of flexible adjustment in environmental conditions. The result was reinforced increase of leaf POD and CAT activities from spring to autumn, so that plants can be acclimated for high temperature in summer and prepared for winter conditions. So, decrease of raining in the first year was the reason of enhanced POD activity compare to the second year. On the other hand, these parallel changes of enzymes activities, indicating the synergistic function in protecting leaf cells against oxidative injury. Increases in the activity of leaf antioxidant systems have been suggested to be important in the acclimation of plants to winter conditions (Anderson et al., 1992; Logan et al., 1998). Vuleta et al. (2010) showed greater POD activity in leaves harvested in summer relative to their spring or autumn counterparts. Many studies have indicated the importance of guaiacol PODs in plant protection against various abiotic stresses. Mehlhorn et al. (1996) would render the guaiacol PODs in other less acidic-cellular sites effective as ascorbate-dependent $H_2O_2^-$ detoxifying enzymes. Esfandiari et al. (2009) detected increased activities of CAT, APX and GR bring ROS production and scavenging systems activity into a balance, hence preventing oxidative stress in the cells. Finally, damage to membranes is decreased and the cell is rendered into more desirable conditions.

A comparison of various responses in leaf and twig during the season indicated the expected differences in antioxidant activity. Perhaps different behaviors of tissue type could affect and caused these changes. However, in contrast to the CAT enzyme that removes the bulk of H_2O_2 in peroxisomes, PODs, like other peroxidases, scavenge the H_2O_2 that is produced in the cell wall, vacuoles and the apoplast (De Gara, 2004; Willekens et al., 1997). Results showing POD and CAT activities in twigs were counterpart of the leaves. It may be due to the relative presence or distribution of active components in the extracts. Another hypothesis might be explained by the fact that during development of twig cells (high rate of assimilation) a higher level of peroxidase will be required. Therefore, it showed higher POD activity in twig compare to leaf and CAT activity was inversed. Different responses of accessions to seasonal changes were related to their originated and adaptation region. Antioxidants have to play a direct role in protecting plants in an unsuitable environment (Lohrmann et al., 2004). Plants are less sensitive to climate changes, possibly because of their natural property and very high adaptability to external conditions (Sen and Mukherji, 2009; Khabarova et al., 2010). However, sensitive accessions increased their enzyme activities to protecting the vital cycle and continue their life. POD is an important enzyme for defensive purposes (Passardi et al., 2005; Herbinger et al., 2002). The observations emphasized that Tehran1 had the greatest activity of POD and where found in the leaf among all accessions examined, as well as in the twig during annual and seasonal patterns. It is able to explain this result, since it may be that this location has few distances to the Damavand region. Therefore, they are more similar condition than the other regions. So, we can introduce Tehran1 as the most compatible Damask rose accessions to this condition.

Conclusion

Subsuming these results suggested that in face of seasonal changes and acclimatization to environment, different responses of tissue type, leaf and twig, in *R. damascena* accessions are activated as consequences of antioxidative enzymes activities. Also, this research allows documenting responses of Damask Rose acessions to genotypic differences by enzymatic activities. However, further separation and identification should be carried out for a better understanding. Other test for antioxidant activity will be carried out in the future to discover the full potential of these accessions. Further important traits are recommended to be evaluated and needs to be continued to understand complex characteristic responses of the plant and indicate genes involved in plant adaptation and tolerance to environmental conditions.

ACKNOWLEDGMENT

This study was supported by the grant No. 89/571 of the Research Institute of Forest and Rangeland of Iran (RIFR).

REFRENCES

Achuthan CR, Babu BH, Padikkala J (2003). Antioxidant and hepatoprotective effects of *Rosa damascena*. Pharmaceut. Bio. 41:357-361.
Anderson JV, Chevone BI, Hess JL (1992). Seasonal variation in the antioxidant system of eastern white pine needles. Evidence for thermal dependence. Plant Physiol. 98: 501–508.
Ardogan BC, Baydar H, Kaya S, Demirci M, Ozbasar D, Mumcu E (2002). Antimicrobial activity and chemical composition of some essential oils. Arch. Pharm. Res. 25:860-864.
Asada K (1999). The water–water cycle in chloroplasts: scavenging of active oxygen and dissipation of excess photons. Annu. Rev. Plant Physiol. Plant Mol. Biol. 50:601–639.
Basim E, Basim H (2003). Antibacterial activity of *Rosa damascena* essential oil. Fitoterapia. 74:394-396.
Beltagi MS (2008). Exogenous ascorbic acid (vitamin C) induced anabolic changes for salt tolerance in chick pea (*Cicer arietinum* L.) plants. Afri. J. Plant Sci. 2(10):118-123.
Chance B, Maehly AC (1955). Assay of catalases and peroxidases, Methods enzymol. 2:764-775.

Chibbar RN, Van Huystee RB (1984). Characterization of Peroxidase in Plant Cells. Plant Physiol., 75:956–958.

De Gara L (2004) Class III peroxidases and ascorbate metabolism in plants. Phytochem. Rev., 3:195–205.

Ebermann R, Stich K (1982). Peroxidase and amylase isoenzymes in the sapwood and heartwood of trees. Phytochemistry. 21:2401-2402.

Eising R, Gerhardt B (1989). Catalase synthesis and turnover during peroxisome transition in the cotyledons of *Helianthus annuus* L. Plant Physiol., 89:1000-1005.

Engel N, Schmidt M, Lütz C, Feierabend J (2006). Molecular identification, heterologous expression and properties of light-insensitive plant catalases. Plant Cell Environ., 29:593–607.

Esfandiari E, Alavi-Kia SS, Bahmani A, Aazami MA (2009). The effect of light on ROS scavenging systems and lipid peroxidation under cold conditions in saffron (*Crocus sativus* L.). Afr. J. Agric. Res., 4(4):378-382.

Feierabend J (2005). Catalases in plants: molecular and functional properties and role in stress defence. In: Smirnoff N (ed) Antioxidants and Reactive Oxygen Species in Plants. Oxford: Blackwell Publishing Ltd, pp. 101–140.

Foyer CH, Halliwell B (1976). Presence glutathione and glutathione reductase in chloroplasts: a proposed role in ascorbic acid metabolism. Planta. 133:21-25.

Gudin S (2000). Plant Breeding Reviews. In: Janick J (ed) John Wiley and Sons. Inc. 17:159-189.

Hatano T, Kira R, Yoshizaki M, Okuda T (1986). Seasonal changes in the tannins of *Liquidambar formosana* reflectings their biogenesis. Phytochemistry. 25:2787-2789.

Herbinger K, Tausz M, Wonisch A, Soja G, Sorger A, Grill D (2002). Complex interactive effects of drought and ozone stress on the antioxidant defence systems of two wheat cultivars. Plant Physiol. Biochem., 40:691-696.

Hiraga S, Sasaki K, Ito H, Ohashi Y, Matsui H (2001). A large family of class III plant peroxidases. Plant Cell Physiol., 42:462–468.

Keles Y, Öncel I (2002). Response of antioxidative defense system to temperature and water stress combinations in wheat seedlings. Plant Sci., 163:783–790.

Khabarova O, Savin I, Medvedeva M (2010). Successful modeling of the environmental changes' influence on forests' vegetation over North Eurasia. Poster at the Euro. Planetary Sci. Con. EPSC Abstracts. Rome. Italy. 5:524.

Logan BA, Grace SC, Adams WW III, Demmig-Adams B (1998). Seasonal differences in xanthophyll cycle characteristics and antioxidants in *Mahonia repens* growing in different light environments. Oecologia. 116:9–17.

Lohrmann NL, Logan BA, Johnson AS (2004). Seasonal acclimatization of antioxidants and photosynthesis in Chondrus crispus and Mastocarpus stellatus, two co-occurring red algae with differing stress tolerances. Biol. Bull., 207:225–232.

Mahmood N, Piacente S, Pizza C, Burke A, Khan AI, Hay AJ (1996). The Anti-HIV Activity and Mechanisms of Action of Pure Compounds Isolated from *Rosa damascena*. Bioch. Biophys. Res. Comm., 220:73-79.

Mehlhorn H, Lelandais M, Korth HG, Foyer CH (1996). Ascorbate is the natural substrate for plant peroxidases. FEBS Lett. 378:203–206.

Mittler R (2002). Oxidative stress, antioxidants and stress tolerance. Trends Plant Sci., 7:405–410.

Møller IM, Jensen PE, Hansson A (2007). Oxidative modifications to cellular components in plants. Annu. Rev. Plant Biol., 58:459–481.

Ozkan G, Sagdic O, Baydar NG, Baydar H (2004). Antioxidant and antibacterial activities of *Rosa damascena* flower extracts. Food Sci. Technol. Int., 10:277-281.

Passardi F, Cosio C, Penel C, Dunand C (2005). Peroxidases have more functions than a Swiss army knife. Plant Cell Rep., 24:255–265.

Salminen J, Ossipov V, Haukioja E, Pihlaja K (2001). Seasonal variation in the content of hydroyisable tannins in leaves of *Betula pubescens*. Phytochemistry. 57:15-22.

Sen S, Mukherji S (2009). Season-controlled changes in biochemical constituents and oxidase enzyme activities in tomato (*Lycopersicon esculentum* Mill.). J. Envir. Biol., 30(4):479-483.

Sergeeva LI, Podol'nyi VV, Pole P (1987). Changes in the Oxidase Activity in Tobacco Trapezond Associated with Transition to Flowering. Fiziol. Rast., 34(2):329–334.

Swanberg A, Verhoeven A (2002). Seasonal acclimation of leaf antioxidant systems in the evergreen *Taxus cuspidate* growing in sun and shade. J. Minnesota Acad. Sci., 66:30.

Sývacý A, Sökmen M (2004). Seasonal changes in antioxidant activity, total phenolic and anthocyanin constituent of the stems of two Morus species (*Morus alba* L. and *Morus nigra* L.). Plant Growth Regul., 44:251–256.

Tabaei-Aghdaei SR, Babaei AR, Khosh-Khui M, Jaimand K, Rezaee MB, Assareh MH, Naghavi MR (2007). Morphological and oil content variations amongst Damask rose (*Rosa damascena* Mill.) landraces from different regions of Iran. Sci. Hortic., 113:44-48.

Tabaei-Aghdaei SR, Hosseini Monfared H, Fahimi H, Ebrahimzadeh H, Jebelly M, Naghavi MR, Babaei AR (2006). Genetic variation analysis of different populations of *Rosa damascena* Mill. In NW. Iran using RAPD markers. Iranian J. Bot., 12(2):121-127.

Velioglu YS, Mazza G, Gao L, Oomah BD (1998). Antioxidant activity and total phenolics in selected fruits, vegetables, and green products. J. Agr. Food Chem., 46:4113–4117.

Vuleta A, Manitašević Jovanović S, Šešlija D, Tucić B (2010). Seasonal dynamics of foliar antioxidative enzymes and total anthocyanins in natural populations of *Iris pumila* L. J. Plant Ecol., 3(1):59–69.

Ward JHJ (1963). Hierarchical grouping to optimize an objective function. J. Am. Statist. Assoc., 58:236–244.

Willekens H, Chamnongpol S, Davey M, Schraudner M, Langebartels C (1997). Catalase is a sink for H_2O_2 and is indispensable for stress defence in C3 plants. EMBO J., 16:4806–4816.

Wise RR (1995). Chilling-enhanced photooxidation: the production, action, and study of reactive oxygen species produced during chilling in the light. Photosynth. Res., 45:79–97.