

Full Length Research Paper

Superoxide dismutase and α -amylase changes of Damask rose (*Rosa damascena* Mill.) tissues seasonally

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This effort aims to set new record and study the activities of two metabolically important distinct antioxidant enzymes, superoxide dismutase (SOD) and amylase in leaves and twigs of Damask rose (*Rosa damascena* Mill.). This research is done in various seasons to trace the seasonal shifts in plant metabolism. The results of our experimental work would be indicative of the seasonal variation patterns of *R. damascena* accessions in the concentration/activity of the estimated enzymes, which would in turn determine their functional importance in two types of tissues. SOD and amylase activities of leaves changed annually in the same condition and the first year had higher activities than the second year, except Kerman and Kermanshah. The content of SOD in leaf showed the strongest levels at 50% flowering in spring and the lowest ones were cleared in summer. The highest amylase levels were obtained at the warmest time in summer, while the lowest levels were obtained in autumn. These results elucidated that SOD activities were stronger than amylase activities among accessions in three phenological stages. There were evident differences between SOD and amylase activities in tissues. So, results elucidated that SOD activities in twigs were higher than leaf except in Kerman¹ and Kermanshah¹. Meanwhile, this research showed the strongest amylase activities in leaf. Enzyme activities in twig expressed variation among accessions and four phenological stages with high interactions. Overall, results determined that SOD activities were highest in comparison to amylase in twig.

Key words: Enzymatic activity, oxidative stress, superoxide dismutase, amylase, seasonal changes.

INTRODUCTION

Many species and varieties of which are cultivated all over the world as attractive plants. Roses are known as the king of flowers and it is the most desirable ornamental plant that has been cultivated methodically. *Rosa damascena* Mill is the most important species related to the Rosaceae family and is well known as a medicinal herb. Damask rose is producing high-value aromatic oil in

conventional medicine which is used in the pharmaceutical, flavour and fragrance industries (Basim and Basim, 2003; Ercisli, 2004). Iran has been referred to as one of its origins and cultivation and consumption of *R. damascena* has a long history in this country (Kashefi et al., 2012). Plants under environmental stress may present increased levels of certain compounds. Antioxidation activity represents the ability of scavenging free radical and offering hydrogen atom. Oxidative metabolism and special H₂O₂ is involved in a wide variety of reactions and signaling overflows essential for all aspects of plant growth and the integration of activity ranging from the

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development of individual root hairs, to xylem differentiation and lignification to wall loosening and wall cross-linking, to root/shoot coordination and stomata function (Halliwell and Gutteridge, 1990; Cheeseman, 2007). Reactive oxygen species are involved in the development of various growth stages.

ROS scavengers such as superoxide dismutase and antioxidants are formed in different cell compartments such as chloroplasts, mitochondria and peroxisomes in small amounts and they can effectively decline damage from oxidation (Richards and Sharma, 1991). Balance between ROS production and scavenging is monitored by antioxidant defense system in plants (Bowler et al., 1992).

Metalloenzyme SOD is the most effective intracellular enzymatic antioxidant which is ubiquitous in all aerobic organisms and in all subcellular compartments prone to ROS mediated oxidative stress. It is well established that various environmental stresses often lead to the increased generation of ROS where SOD has been proposed to be important in plant stress tolerance and provide the first line of defense against the toxic effects of elevated levels of ROS (Gill and Tuteja, 2010). Some enzymes show different types of environmental stress, such as amylase and ascorbate peroxidase.

They have protected the plants against such danger and thus, promote their survival (Taiz and Ziger, 2002). Over the past few decades, considerable research has been made about extracellular α -amylase being produced by a wide variety of micro-organisms (Pandey et al., 2000). In plants, α -amylases split starch and produce smaller carbohydrates in case of more energy need (bud burst, exposure of stress, wintertime) or when photosynthesis machinery does not exist or decreases its activity.

Consequently, the present study permits to determine the seasonal changes by investigation of the antioxidant activity in the leaves and twigs (various tissue types) on *R. damascena*. Damask rose accessions were collected from 10 provinces of Iran and cultivated in Damavand region. Previous studies cleared that they may include multiple genotypes (Tabaei-Aghdaei et al., 2006) and had various antioxidant activities (Kashefi et al., 2010, 2012).

This research was directed towards the study of seasonal and annual changes in 12 accessions of Damask rose in association to enzyme activities. Since enzymatic activities and the responses of the accessions were showed difference and emphasis on the interaction among them.

Also, it indicated that one of them are sensitive and require 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 aims to record and study the activities of two metabolically important distinct antioxidant enzymes SOD and amylase in leaves and twigs

of Damask rose (*Rosa damascena* Mill.), an important plant with high medicinal attributes and aromatic oil in various seasons to trace the seasonal shifts in plant metabolism.

MATERIALS AND METHODS

Twelve Damask Rose accessions originating from ten provinces of Iran (Ardebil, Ilam, Tehran, Charmahal, Fars, Kerman, Kermanshah, Arak, Hormozgan and Isfahan) were cultivated 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 to August. Average rainfall of a year was 333 mm and of the first 3 months of the year (development and flowering stage of plants) were 13 and 130 mm in 2008 and 2009, respectively. The experiments were designed as a randomized complete block design with three replications.

In this study, enzymatic activities in leaf and twig were estimated during 2008 and 2009. Samples were harvested at critical phenological stages of plant growth. These three 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 the aforementioned stages in winter. Fresh leaves and twigs were sampled and the measurements were carried out.

Enzyme assays

For prepared enzyme extracts, about 1 g of leaves was rubbed and plugged in extraction buffer for 24 to 72 h (Ebermann and Stich, 1982). The homogenate was centrifuged at 3000 rpm for 15 min, and supernatant was selected and utilized for enzyme evaluation. Superoxide dismutase was assayed following the modified method of Beauchamp and Fridovich (1971) by measuring its ability to inhibit photochemical reduction of nitro blue tetrazolium chloride (NBT). SOD activity was determined in reaction mixture (3 ml) with phosphate buffer. Test-tubes were kept under fluorescent lamps and then transferred to the dark. The absorbance was indicated at 560 nm and activity was expressed as enzyme units/mg. One unit of superoxide dismutase activity was defined as that amount of enzyme which caused 50% inhibition of the initial rate of the reaction in the absence of enzyme. α -amylase activity was assayed by measuring the reducing sugar release during the reaction, using starch as the substrate according to the Somogyi-Nelson method (Nelson, 1944). The reaction mixture contained 50 μ l of 1.1% soluble starch in 2 mM imidazole- HCl buffer and 250 μ l of enzyme solution. The reaction was stopped by adding dinitrosalicylic acid solution after incubation at various temperatures. The reaction mixture was then heated in boiling water and absorbance at 540 nm was measured after cooling in ice and diluting with distilled water.

Statistical analyses

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

RESULTS AND DISCUSSION

The results of our experimental work showed the

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

Source	DF	Mean square	
		Superoxide dismutase (Unit/mg)	Amylase (Unit/ml)
Year	1	6.01**	0.008
Accession	11	0.16**	0.007**
Year * Accession	11	0.19**	0.005*
Stage	2	2.99**	0.12**
Accession * Stage	22	0.15**	0.006**
Year * Stage	2	1.5**	0.02**
Year * Accession * Stage	22	0.11**	0.002
rep	2	0.1	0.001
Error	130	0.04	0.003
CV%		19.52	25.34

*, **, Significant differences at 5 and 1% respectively.

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

Source	DF	Mean square	
		Superoxide dismutase (Unit/mg)	Amylase (Unit/ml)
Accession	11	0.03*	0.002**
Stage	3	0.06**	0.02**
Accession * stage	33	0.04**	0.001*
rep	2	0.01	0.0004
Error	70	0.016	0.0008
CV%		10.77	23.77

*, **, Significant differences at 5 and 1% respectively.

seasonal variation patterns of *R. damascena* accessions in the concentration/ activity of the estimated enzymes, which would in turn determine their functional importance in two types of tissues during different times of the year. There were significant interactions among years, accessions and phenological stages (Tables 1 and 2). SOD activities of leaves changed annually in the same condition and the first year had higher activities than the second year, except Kerman1 and Kermanshah1. The lowest SOD activities were indicated in Isfahan8 and Isfahan6 (0.68 and 1.05 Unit/mg) in the second year (Figure 1). Also, the average between two years were resolved that Kermanshah1 and Kerman1 (1.21 and 1.19 Unit/mg respectively) had maximum activities. The concentration of SOD in leaf showed the strongest levels at 50% flowering in spring (1.385 Unit/mg) and the lowest ones were cleared in Isfahan6 (0.694 Unit/mg) and Isfahan8 (0.714 Unit/mg) in summer (Table 3).

Results expressed various effects of amylase activity among accessions in two years, but almost their activities were higher in the first year. Ardebil1 had greatest and Kermanshah1 had the least (0.16 Unit/mg) amylase activities in the first and second year respectively (Figure

2). The highest levels were obtained in Ardebil1 (0.294 Unit/ml) and Kerman1 (0.292 Unit/ml) at warmest time in summer and Tehran1 appeared the lowest (0.130 Unit/ml) in autumn (Table 3). These results elucidated that SOD activities were stronger than amylase activities among accessions in the three phenological stages, since spring and summer (1.31 and 0.92 Unit/mg respectively) had maximum and minimum levels of SOD activities. The highest and lowest activities of amylase activities were obtained in summer (0.25 Unit/ml) and autumn (0.17 Unit/ml) respectively (Figure 3).

There were evident differences between SOD and amylase activities in tissues and as such, the results elucidated that SOD activities in twigs were higher than leaf except in Kerman1 and Kermanshah1. Tehran1 and Kermanshah1 (1.26 and 1.28 Unit/mg respectively) and Arak1 and Hormozgan1 (1.1 and 0.961 Unit/mg respectively) proved highest and the least SOD activities in twig and leave respectively (Figure 4). Meanwhile, this research explained the strongest amylase activities in leaf. Thus, Ardebil1, Fars1 and Isfahan8 (0.244, 0.242, 0.240 Unit/ml respectively) had the highest and Kermanshah1 (0.178 Unit/ml) had lowest amylase

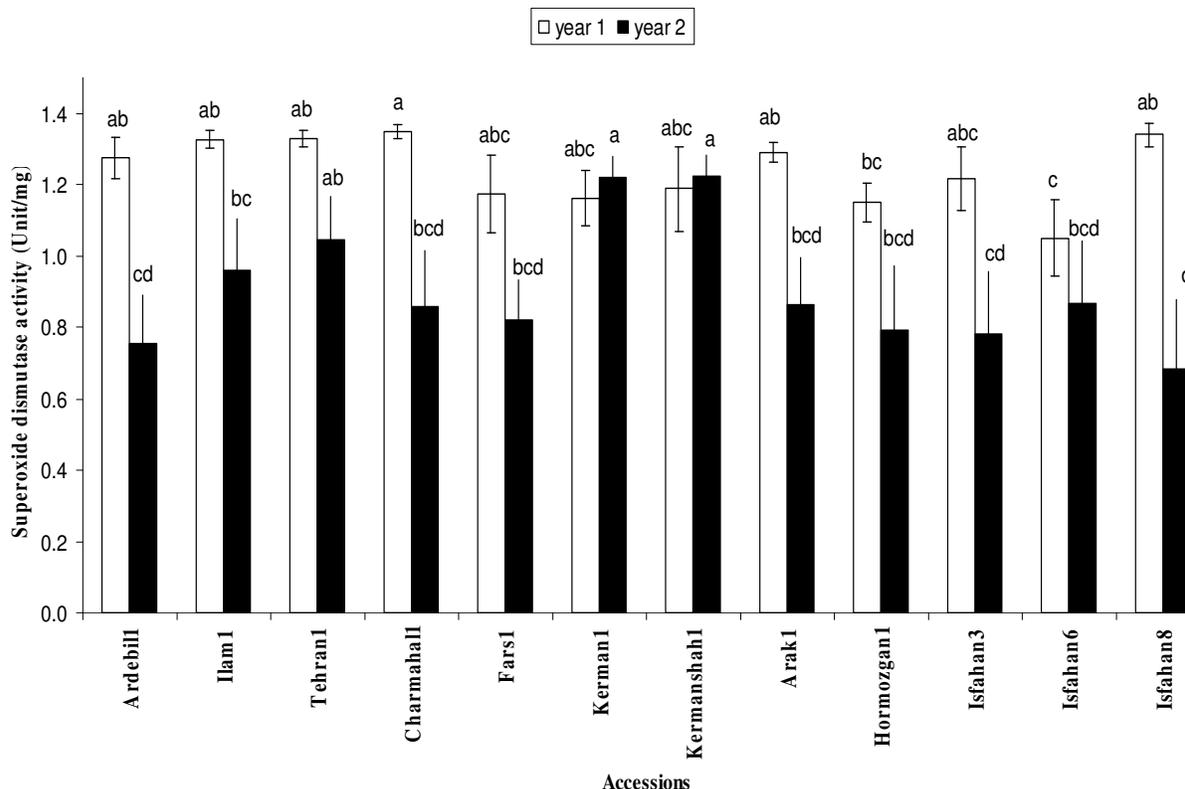


Figure 1. Changes of leaf superoxide dismutase activity (Unit/mg) in 12 Damask Rose accessions in 2 years. Vertical bars represent the \pm SEM. Different letters in each bar differ significantly according to DMRT test ($p=0.05$ level).

Table 3. Seasonal changes of leaf superoxide dismutase (Unit/mg) and amylase (Unit/ml) activity at 3 stages in 12 Damask rose accessions in 2 years.

Accessions	SOD (Unit/mg)			Amylase (Unit/ml)		
	Spring	Summer	Autumn	Spring	Summer	Autumn
Ardebil1	1.33 ^{ab}	0.807 ^a	0.907 ^{ab}	0.167 ^c	0.294 ^a	0.276 ^a
Ilam1	1.342 ^a	0.88 ^a	1.212 ^{ab}	0.228 ^{ab}	0.243 ^{ab}	0.172 ^b
Tehran1	1.333 ^{ab}	0.957 ^a	1.272 ^a	0.274 ^a	0.222 ^{ab}	0.129 ^b
Charmahal1	1.315 ^{ab}	0.785 ^a	1.212 ^{ab}	0.236 ^{ab}	0.249 ^{ab}	0.187 ^b
Fars1	1.147 ^b	0.872 ^a	0.975 ^{ab}	0.248 ^{ab}	0.285 ^a	0.193 ^b
Kerman1	1.312 ^{ab}	1.232 ^a	1.053 ^{ab}	0.261 ^{ab}	0.292 ^a	0.139 ^b
Kermanshah1	1.2 ^{ab}	1.295 ^a	1.127 ^{ab}	0.238 ^{ab}	0.166 ^b	0.151 ^b
Arak1	1.278 ^{ab}	1.115 ^a	0.833 ^{ab}	0.222 ^{ab}	0.204 ^{ab}	0.154 ^b
Hormozgan1	1.356 ^a	0.79 ^a	0.803 ^{ab}	0.208 ^{bc}	0.223 ^{ab}	0.158 ^b
Isfahan3	1.377 ^a	0.815 ^a	0.805 ^{ab}	0.245 ^{ab}	0.259 ^{ab}	0.172 ^b
Isfahan6	1.385 ^a	0.694 ^a	0.768 ^b	0.229 ^{ab}	0.270 ^a	0.147 ^b
Isfahan8	1.375 ^a	0.714 ^a	0.838 ^{ab}	0.251 ^{ab}	0.284 ^a	0.19 ^{3b}

Different letters in each column differ significantly according to DMRT test ($p=0.05$ level).

activities in leaves, but Isfahan6 (0.146 Unit/ml) and Ilam1 (0.076 Unit/ml) had most and a few levels of amylase in twig (Figure 5). Enzyme activities in twig expressed variation among accessions and four phenological stages with high interactions (Table 2). Overall, results concluded that SOD activities were highest in

comparison to amylase in twig. The magnitude of changes in SOD activity between winter and summer was substantially raised but it declined in autumn. Amylase activity was the strongest in summer and reduced in the fourth stage (Figure 6). SOD activities in twigs were the highest in Fars1, Tehran1 and Kermanshah1 (1.32, 1.3

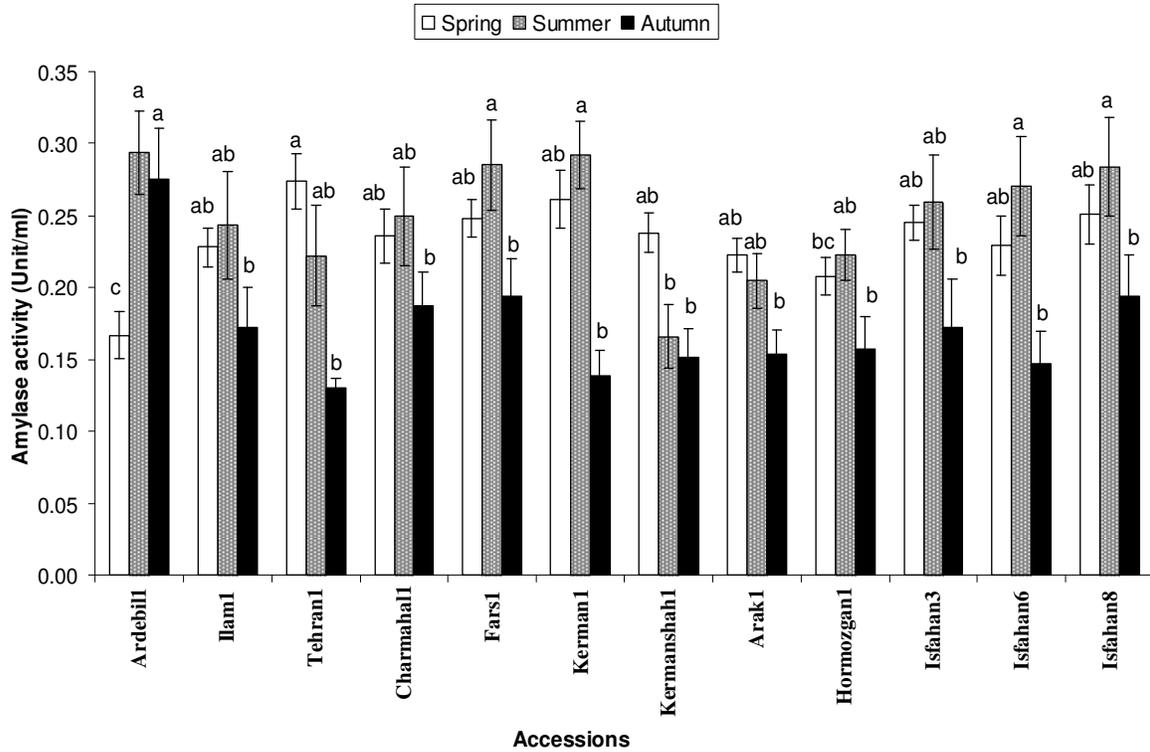


Figure 2. Changes of leaf amylase activity (Unit/ml) in 12 Damask Rose accessions in 2 years. Vertical bars represent the \pm SEM. Different letters in each bar differ significantly according to DMRT test ($p = 0.05$ level).

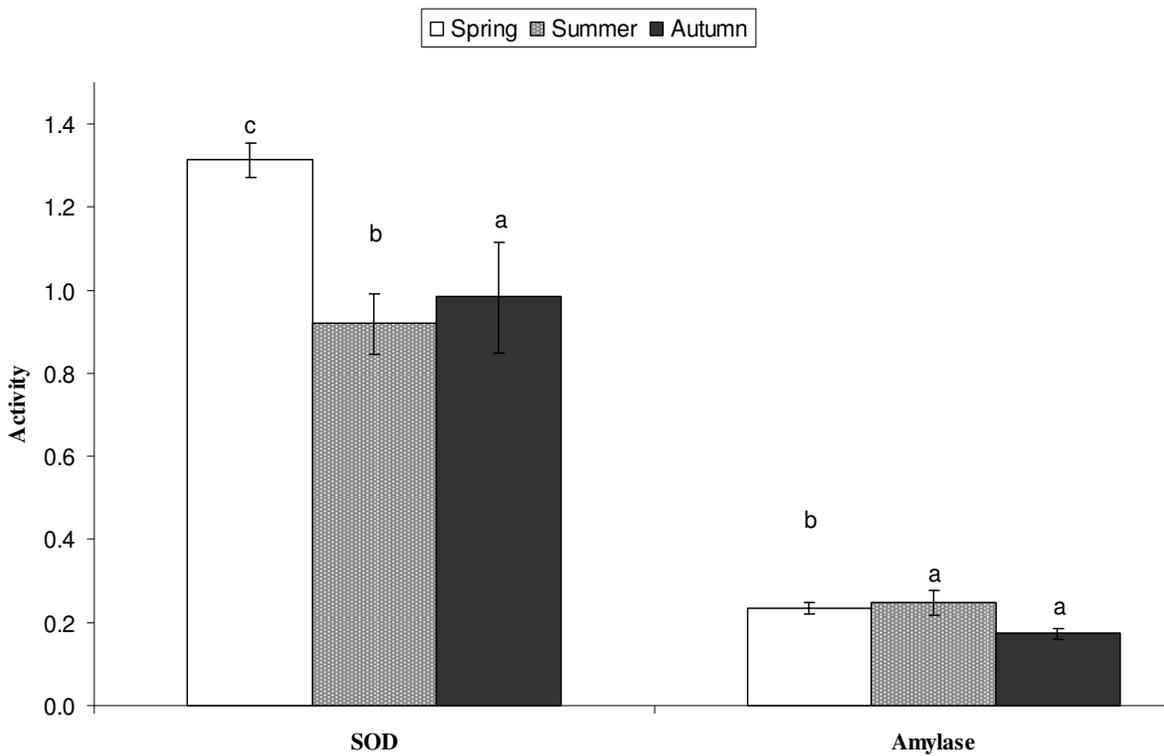


Figure 3. Seasonal changes of leaf superoxide dismutase (Unit/mg) and amylase (Unit/ml) activities in 12 Damask rose accessions. Vertical bars represent the \pm SEM. Different letters in each bar differ significantly according to DMRT test ($p = 0.05$ level).

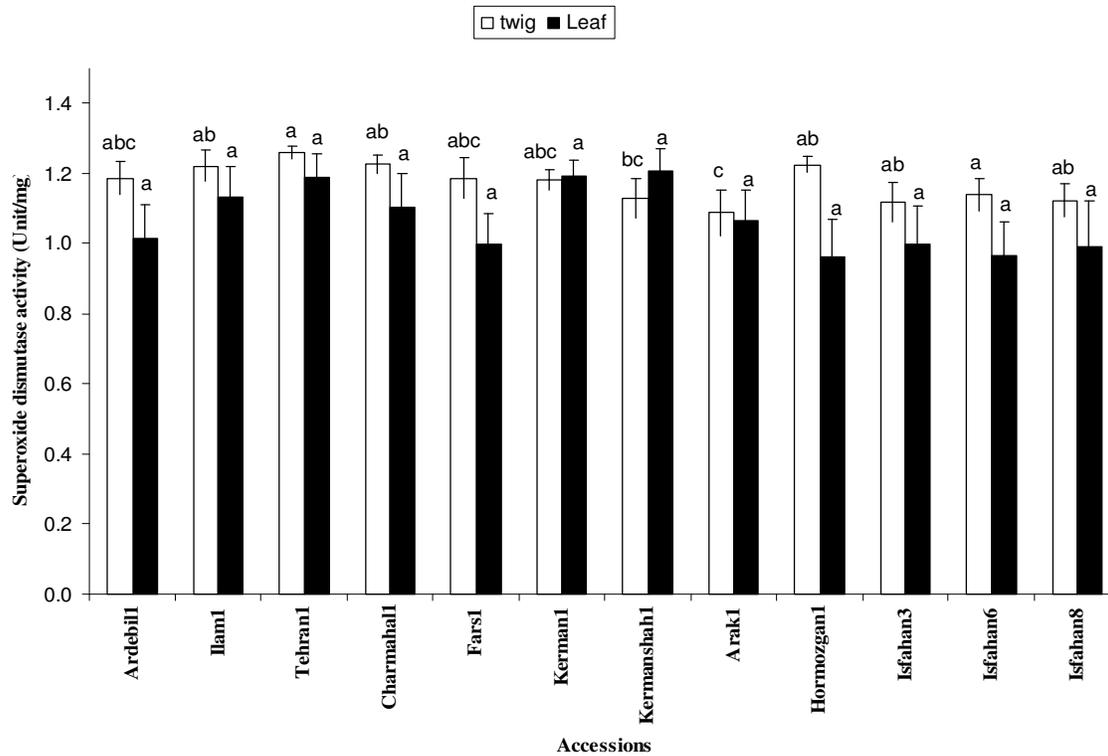


Figure 4. Changes of leaf and twig superoxide dismutase activity (Unit/mg) in 12 Damask Rose accessions. Vertical bars represent the \pm SEM. Different letters in each bar differ significantly according to DMRT test ($p=0.05$ level).

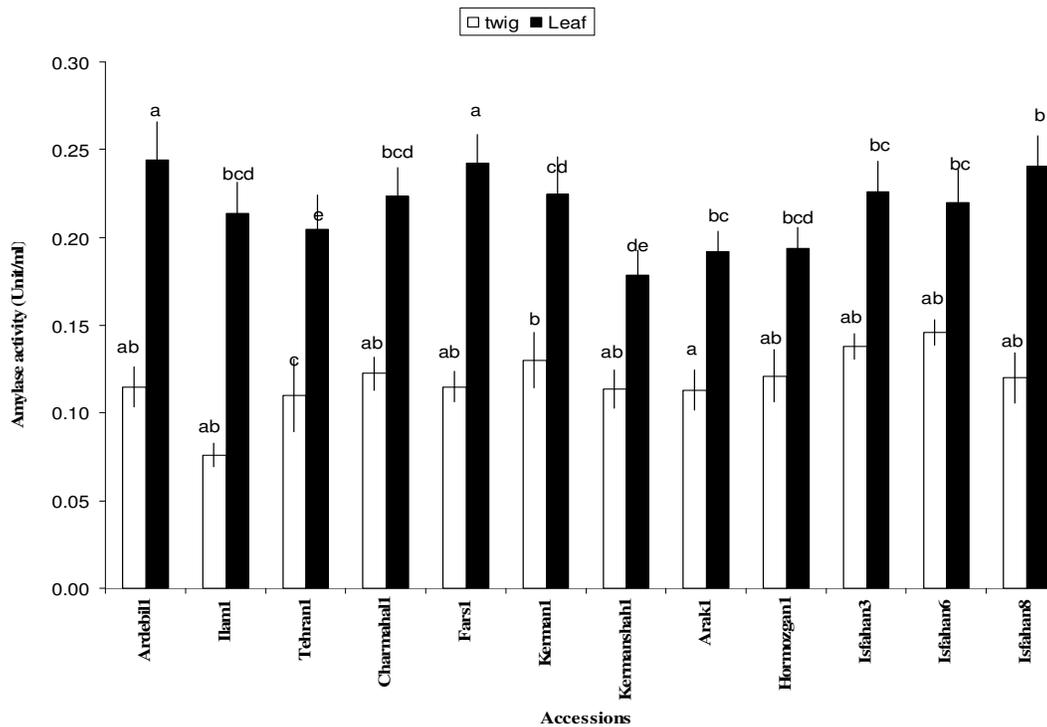


Figure 5. Changes of leaf and twig amylase activity (Unit/ml) in 12 Damask Rose accessions. Vertical bars represent the \pm SEM. Different letters in each bar differ significantly according to DMRT test ($p=0.05$ level).

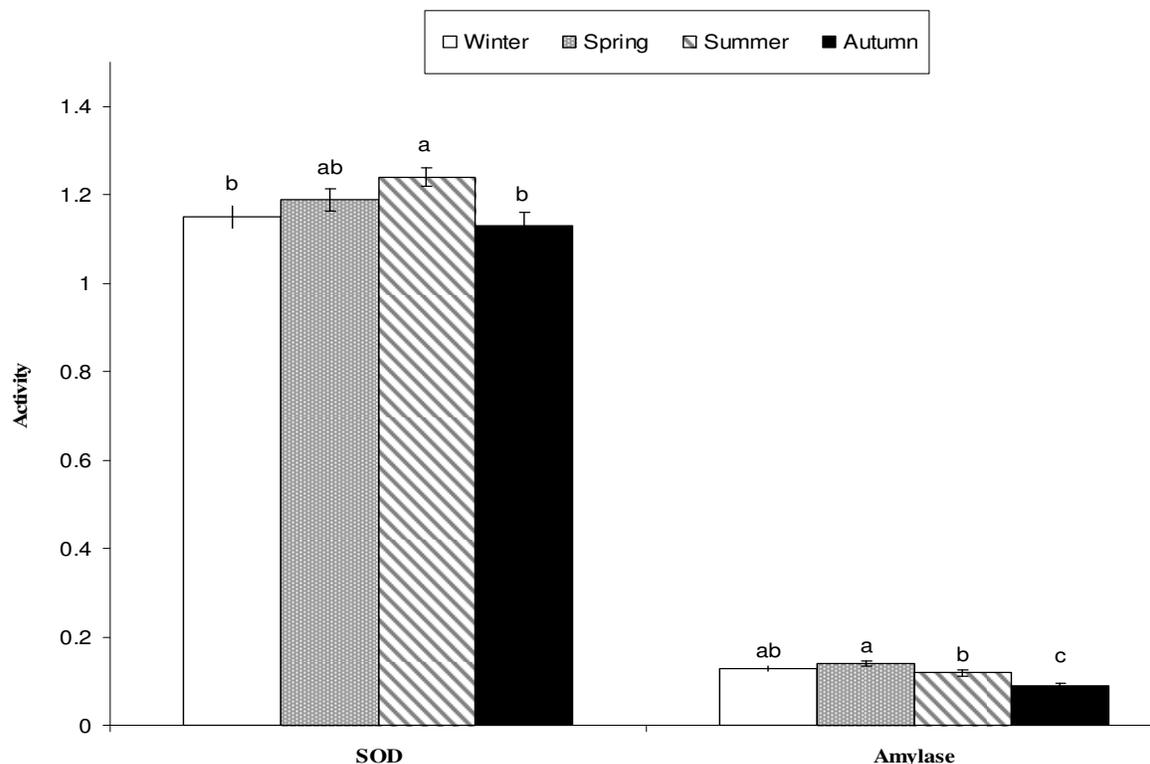


Figure 6. Seasonal changes of twig superoxide dismutase activity (Unit/mg) and amylase activities (Unit/ml) in 12 Damask rose accessions. Vertical bars represent the \pm SEM. Different letters in each bar differ significantly according to DMRT test ($p=0.05$ level).

Table 4. Seasonal changes of twig superoxide dismutase (Unit/mg) and amylase (Unit/ml) activity at 4 stages in 12 Damask rose accessions in 2009.

Accessions	SOD (Unit/mg)				Amylase (Unit/ml)			
	Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn
Ardebil1	1.28 ^{abc}	1.27 ^a	1.28 ^a	0.95 ^{bc}	0.137 ^{ab}	0.137 ^b	0.12 ^a	0.07 ^{cde}
Ilam1	1.15 ^{abcde}	1.26 ^{ab}	1.28 ^a	1.22 ^{ab}	0.087 ^b	0.73 ^c	0.08 ^a	0.067 ^{cde}
Tehran1	1.29 ^{ab}	1.30 ^a	1.27 ^a	1.20 ^{ab}	0.125 ^{ab}	0.18 ^{ab}	0.13 ^a	0.04 ^e
Charmahal1	1.21 ^{abcd}	1.23 ^{ab}	1.26 ^a	1.22 ^{ab}	0.13 ^{ab}	0.133 ^{bc}	-	0.095 ^{bcd}
Fars1	1.32 ^a	1.10 ^{abc}	1.18 ^a	1.14 ^{ab}	0.13 ^{ab}	0.12 ^{bc}	0.13 ^a	0.08 ^{cde}
Kerman1	1.17 ^{abcde}	1.14 ^{abc}	1.28 ^a	1.21 ^{ab}	0.18 ^a	0.143 ^b	0.1 ^a	0.077 ^{cde}
Kermanshah1	1.06 ^{cde}	1.28 ^a	1.30 ^a	0.87 ^c	0.133 ^{ab}	0.147 ^b	0.11 ^a	0.063 ^{de}
Arak1	1.11 ^{bcde}	1.26 ^{ab}	1.21 ^a	0.82 ^c	0.14 ^{ab}	0.13 ^{bc}	0.105 ^a	0.08 ^{cde}
Hormozgan1	1.20 ^{abcde}	1.17 ^{ab}	1.23 ^a	1.26 ^a	0.13 ^{ab}	0.210 ^a	0.12 ^a	0.083 ^{cde}
Isfahan3	1.00 ^e	1.02 ^{bc}	1.18 ^a	1.26 ^a	0.133 ^{ab}	0.135 ^b	0.16 ^a	0.13 ^{ab}
Isfahan6	1.03 ^{de}	1.18 ^{ab}	1.13 ^a	1.23 ^a	0.15 ^{ab}	0.13 ^{bc}	-	0.155 ^a
Isfahan8	1.07 ^{cde}	0.91 ^c	1.26 ^a	1.18 ^{ab}	0.113 ^b	0.16 ^{ab}	0.1 ^a	0.113 ^{abc}

Different letters in each column differ significantly according to DMRT test ($p=0.05$ level).

and 1.3 Unit/mg respectively) and the lowest was indicated in Arak1 (0.82 Unit/mg). Tehran1 (1.26 Unit/mg) and Arak1 (1.1 Unit/mg) had maximum and minimum activity in the average of four phenological stages (Table 4).

Hormozgan1 (0.21 Unit/ml) and Tehran1 (0.18 Unit/ml) had greatest amylase activity at 50% flowering in spring and Tehran1 (0.04 Unit/ml) had the least in autumn. Isfahan6 (0.15 Unit/ml) and Ilam1 (0.08 Unit/ml) had highest and lowest activity in the average of four seasons

(Table 4).

Plants are sometimes exposed to unsuitable environmental conditions. Physiological plasticity enables plants to withstand such seasonal fluctuations within the limits of tolerance (Sen and Mukherji, 2007). Environmental effects causes oxidative stress and induce the excess of free radicals. Part of free radicals promotes changes in macromolecules, such as DNA and proteins and others operate as a strong evolutionary force signal releasing the protective antioxidant systems of plants (Ballaré, 2003). Biochemical constituents and enzyme activities serve as important indices of plant response and behavior to seasonal variations (Sen and Mukherji, 2007). If there is a serious instability in any cell compartment between the production of ROS and antioxidant defense, oxidative stress and damage occurs (Mittler, 2002). SODs constitute the first line of defense against ROS within a cell since they catalyze the dismutation of O_2^- radicals to molecular O_2 and H_2O_2 (Meloni et al., 2003).

The main effect of seasonal patterns on the difference in SOD and amylase enzymatic activities, were recommended a general capability of *R. damascena* plants of flexible adjustment in environmental conditions. SOD activity in twigs was contrary of the leaves. Researchers explained that seasonal variation in the field conditions significantly influenced the specific activity of antioxidative enzymes SOD, CAT and POD in the leaf tissue of naturally growing *I. pumila* plants (Vuleta et al., 2010). Swanberg and Verhoeven (2002) reported the seasonal changes at antioxidant activity in the leaves of *Taxus cuspidate*. Kashefi et al. (2010) rendered that POD and CAT activities varied in seasonal variation of damask rose. Also, it was reported that total SOD activities have not shown any seasonal changes (Wingsle and Hällgren, 1993). Meanwhile, SOD enzymes activities d under low and high temperature stresses (Schoner and Krause, 1990). Our results revealed a greater increase in SOD activity of the twigs in comparison to the leaves at the warmest time in summer. It is suggesting that the strongest activity of twigs could be due to more susceptible temperature changes than the leaves. Another hypothesis may be associated to the relative presence or distribution of active components in the extracts. Langjun et al. (2006) investigated about high temperature effects on antioxidant activity and indicated that activities of APX and SOD increased in the two *Festuca arundinacea* cultivars after 10 days treatment and later declined after 20 days treatment. They suggested that the two stressed plants had an effective system for detoxifying active oxygen species at 10 days treatment and this system progressively deteriorated after 20 days. It could be that SOD activity in leaf caused injury during heightened temperature during summer, but it reacted to decrease of rainfall and the activity improved in the first year. It seems Kerman and Kermanshah did not obey the patterns between tissues and years.

Perhaps this act is associated to warm and cold climatic conditions respectively and relative acclimatization to unfavorable environment in these two regions.

In many higher plants, sucrose and starch are the primary photosynthetic end products of photosynthesis. Sucrose is the main carbohydrate translocated from leaves to sink tissues, and starch is a temporary storage carbohydrate that accumulates in the chloroplast. In Rosaceae species, sorbitol in addition to sucrose and starch is a primary photosynthetic end product and the most important translocated form of photoassimilate (Zhou et al., 2001). In this study, it was found out that photosynthetic end products are synthesized by several related enzymes for carbohydrate metabolism in leaves such as amylases (Li and Li, 2005). Thus, we could opine that amylase content can be enhanced in leaves as compared to twigs. The occurrence of amylases, particularly α -amylase, using the storage carbohydrate starch as substrate, catalyses the production of precursors for energy supply and the building procedure of cell components like oligosaccharides and glucose (Matinizadeh, 2005). So, amylase causes starch degradation in order to balance the supply of energy which is no longer supplied due to the reduced photosynthetic process in summer. Therefore, the results show that the greatest amylase activity of leaves occurs in summer. Since most of the twig growth occurs in spring, amylase activity is necessary for energy supply, and in this situation, a high level of amylase activity was found, but its levels in leaves and twigs reduced as a result of the reducing temperature in autumn. This study also indicated that the amount of rain which reduced in the first year had effect on amylase activity, and this effect was observed to be higher than that of the second year in all accessions except Tehran, Charmahal, Kerman and Arak. Different behaviors in these accessions may probably be as a result of their climatic conditions and hardening to unsuitable environments.

Overall, this study introduced Kerman and Kermanshah as various accessions for SOD and amylase activity between tissues. It seems that these accessions correlate to their climatic conditions.

Conclusion

This study recommended that different responses of tissues, seasonal patterns and acclimatization to climatic condition in *R. damascena* accessions are activated as consequences of antioxidant enzymes activities. Also, this research allows documenting responses of Damask Rose accessions to genotypic differences. However, further important characters are suggested to be evaluated and require to be followed to understand the complex trait responses of the plant and determine genes involved in plant adaptation and tolerance to environmental conditions. Other findings for enzymatic activity

need to be compared with the results of this study in order to determine the final potential of these accessions.

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Abbreviations: **APX**, Ascorbate peroxidase; **CAT**, Catalase; **DNA**, deoxyribonucleic acid; **H₂O₂**, hydrogen peroxide; **POD**, peroxidase; **ROS**, reactive oxygen species; **SOD**, superoxide dismutase.

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