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Some physiological and enzymatic characterizations of Damask Rose accessions (*Rosa damascena* Mill.)

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Abstract

Climate can be a potent selective agent on life history and physiology of plants. *Rosa damascena* Mill. is an important aromatic, ornamental and medicinal species with a high genetic variation in Iran. In order to investigate the phenological stages, 12 damask rose accessions were collected from Damavand region. Some physiological (proline, chlorophyll, leaf area, stomata density and dry weight percentage) and enzymatic characteristics were analyzed in 3 phenological stages during two years (2008-2009). Results revealed significant differences and interactions among accessions, years and seasons. Thus, this study showed the highest proline content and peroxidase activity in the second year but other traits indicated maximum level in the first year. Furthermore, third phenological stage was the strongest for some traits except proline and leaf area content. The results of this research allowed us to document the responses of Damask rose accessions to environmental condition and genotypic differences and to introduce Tehran and Fars as the hardest and the most sensitive accessions, respectively.

Keywords: Catalase; Chlorophyll; Compatibility; Leaf area; Peroxidase; Phenological stage; Proline; Stomata.
Abbreviations: CAT- Catalase; Chl- Chlorophyll; H_2O_2- hydrogen peroxide; LA- Leaf area; POD- Peroxidase; PRO- Proline.

Introduction

Rose has an economic value in ornamental, pharmaceutical and cosmetic trades. *Rosa* L., as a major genus in Rosaceae, includes over 200 species that are widely distributed in Europe, Asia, Middle East, and North America (Gudin, 2000). Damask rose (*Rosa damascena* Mill.) is the most important *Rosa* species. It was originally introduced from the Middle East especially, Damascus into Western Europe. It is thought that the origin and centre of diversity of Damask roses could be found in this region (Rusanov et al., 2005). Iran is also known as one of the origins of the cultivation of this plant (Chevallier, 1996). This crop is used to produce rose oil, rose water, rose concrete and rose absolute (Guterman et al., 2002) and it is also known for cooling, soothing, astringent and anti-inflammatory effects (Mnimh, 1995). Climate can be a potent selective agent on life history and the physiology of plants. Although clinal differentiation in morphological and physiological traits is well documented for native plants, less is known about exotics (Linhart and Grant, 1996). Due to their negative impact on plant development and the increasing of the lands affected, it became particularly important to identify and select the best adapted cultivated plants for improving yield in the more sensible areas (Djibril et al., 2005). Wide distribution, adaptability, cultivability, and stability are important factors in evaluating the potential of plants for commercial uses. Also, biochemical, physiological, anatomical, morphological and molecular markers parameters in higher plants have been developed as effective indices for tolerant screening in plant breeding programs and detected genetic diversity (Ashraf and Foolad, 2007). There are many defense mechanisms in plants helping plants to survive and grow under severe environmental conditions (Chaum and Kirdmanee, 2009). Limiting factors for photosynthesis, including stomatal and non-stomatal factors, affect the biochemical processes through decreasing stomatal conductance and causing water deficiency, respectively (Tissue et al., 2005). Plants respond to differences in resource availability through several physiological mechanisms, including alteration in leaf area, changes in carbon and other element allocation patterns, and changes in photosynthetic efficiency, nutrient and water use efficiency (Chapin, 1991). Consequently, the main objective of this study was to investigate the physiological and enzymatic responses of *R. damascena* accessions to determine and recommend the adaptative accessions in Damavand region. In this way, Damask rose accessions were collected from 10 provinces of Iran and cultivated in

Damavand region. Previous studies show that they may include multiple genotypes (Tabaei-Aghdaei et al., 2007). This research was directed towards the study of responses of 12 Damask rose accessions to location and the relation among physiological and enzymatic activities. Since these traits and variation among accessions were emphasized to different behaviors among them to translocation from native areas. Moreover, this effort was detected the accessions which are the best for breeding programs with ability to adapt in new areas.

Results

Variance analysis showed significant differences among *R. damascena* accessions of some physiological and enzymatic characteristics (Table 1).

Physiological characteristics

The year was found to have a significant effect on all traits. The entire characteristics showed significant differences between accessions except proline content and furthermore, we showed large effect of phenological stage in traits, except chlorophyll and proline content. There were also interactions among years, accessions and phenological stages.

Proline and leaf area content

PRO content and LA increased in most accessions in the second year (Fig. 1 and Fig. 2). Kerman1 (141.7 µmol/mg FW) and Kermanshah1 (127.9 µmol/mg FW) accessions in the first and second stages had the highest PRO content in the second year and Fars1 and Isfahan3 had the lowest ones in the first year. Generally, first and third phenological stages showed maximum and minimum proline concentrations, respectively. Also, Ardebil1 (35.88 cm^2) and Fars1 (35.86 cm^2) had the highest LA and Ardebil1 and Kerman1 had the lowest in the second and first years, respectively. Maximum LA was in the first stage and minimum one was in the second stage. So, Fars1 indicated the strongest LA in the third and first stages.

Chlorophyll content and stomata density

Chl content and stomata density significantly enhanced in the first year compared to the second year (Fig. 3 and Fig. 4). Arak1 (46.37 µg/g FW) and Charmahal1 (46.07 µg/g FW) had maximum in the first year and Ilam1 (39.55 µg/g FW) and Isfahan8 (40.36 µg/g FW) had minimum Chl content in the second year. Generally, third and second stages had highest Chl content. The stomata density was the strongest in Kermanshah1 (2119 per mm^2) and Isfahan8 (2004 per mm^2) and the lowest ones were Fars1 (792.6 per mm^2) and Arak1 (1041 per mm^2) in the first and second years, respectively. Although, third stage had generally maximum stomata density, Kermanshah1 and Kerman1 had highest density in second and third phenological stages.

Enzymatic characteristics

Proxidase activity

POD activities were higher in the first year compared to the second year, except for Tehran1. It showed the strongest (4.65 activity/g DW) in the second year. On the other hand, Fars1 and Tehran1 (3.54 and 3.39 activity/g DW, respectively) had maximum POD activity in the first year and Kermanshah1 (1.81 activity/g DW) had minimum in the second year. Furthermore, the highest and lowest activity was indicated in third and first stages, respectively. Thus, Tehran1 showed main increase in the third stage.

Catalase activity

CAT activities showed different results in the two years. So, the highest activities were observed in Ardebil1 (47.67 and 49.91 Unit/mg FW) and Fars1 (45.51 and 43.20 Unit/mg FW) in the two years, respectively (Fig. 6). Meanwhile, they revealed that both of them had maximum activity in the third and second stages. Overall, we showed the highest activity in the third and the lowest one in the first stage. In addition, Cluster analysis classified the accessions, based on means of origin sites for studied traits, into two main groups (Fig. 7). Algorithm was showed ranked distances of accessions; therefore, there were great similarity between Tehran1 and Fars1. Other accessions indicated dissimilarity compared to these ones.

Discussion

The environment surrounding a plant may vary daily and seasonally. The level of variability is determined by many factors including climate, geographical location, geomorphological features, the nature of site disturbances, and the number and type of plant species. Therefore, the influence of the environment on the plant depends on the level of environmental variability, the predictability of the variation, changes in plant size and physiology through the time (Bazzaz, 1979). The results presented in this research were obtained from plants undergoing 2-year study effects on the physiological and enzymatic activities in 12 Damask rose accessions. Researchers reported characteristics change as plant responses to environmental factors (Slafer and Araus, 2007; Aliu et al., 2010). Significant differences were observed between the two years for all traits and accessions showed significant changes in physiological and enzymatic characters. In fact, accessions from origins with experiencing various ecological conditions may have different growth levels and show different responses to environmental stress (Raj et al., 2011). Thus, the main events in the climate decreased in rainfall (13 mm) and relatively high temperature in the first year, especially during growth and flowering period of Damask Rose. Consequently, it was reinforced the significant differences between the two years. PRO is a free amino acid that acts as an osmo–regulator of plant cells and may protect protein structure and membranes from damage in higher plants (Ozturk and Demir, 2002). This accumulation happens in tolerant genotypes and therefore it can be used for selection of resistant genotypes in unfavorable conditions (Ashraf and Haris, 2004). Intensity, amount, exposure duration, plant growth stage and stress affected PRO accumulation (Lichtenthaler et al., 1981). Results showed PRO concentration in leaves of most accessions was higher in the second year. Kerman1 detected the highest PRO content and greater response behavior. It produced that Kerman1 had different climate in comparison to Damavand region; therefore, PRO content was enhanced. Thus, increase in PRO as a compatible component could be a sign of adaptability or tolerance to unfavorable conditions which have been reported frequently (Zaharah and Razi, 2009). Meanwhile, PRO and other similar substances accumulate more readily, since accumulation was found to vary among plant organs (Karamanos, 1995). Unfavorable environment can limit leaf expansion and decrease leaf development (Ünyayar et al., 2004; Cha-um and Kirdmanee, 2009). In most accessions, LA in the first year was lower than the

Table1. Variance analysis of different traits in 12 Damask Rose accession s at Damavand (2008-2009).

Source of variation	DF	Mean Squares					
		PRO content	Stomata density	LA	Chl content	POD activity	CAT activity
Year	1	5347.35**	5370354**	894.38**	233.65**	8.93**	0.27*
Accession	11	734.31	1634494**	67.73**	37.75*	4.04**	1.02**
Year × Accession	11	953.88*	163939	102.05**	10.94**	1.94**	0.07
Stage	2	1320.02	7105794**	338.26**	853.57	36.47**	2.02**
Accession × Stage	22	207.82	158861	25.37	11.82	1.81**	0.31**
Year × Stage	2	2950.5**	2911951**	28.55	64.39*	7.70**	2.78**
Year × Accession × Stage	22	352.92	112018	26.08	11.16	0.59	0.10
Replication	2	1788.55	203512	16.37	59.65	1.14	0.10
Error	142	491.89	107786	23.15	18.24	0.75	0.07
CV (%)		20.44	20.64	18.51	9.94	31.93	24.08

* & ** : Significant differences at 5% and 1%, respectively.
PRO content (μmol/ mg FW), Stomata density (number/ mm^2), LA (cm^2), Chl content (μg/g FW), POD activity (activity/g DW), CAT activity (Unit/mg FW).

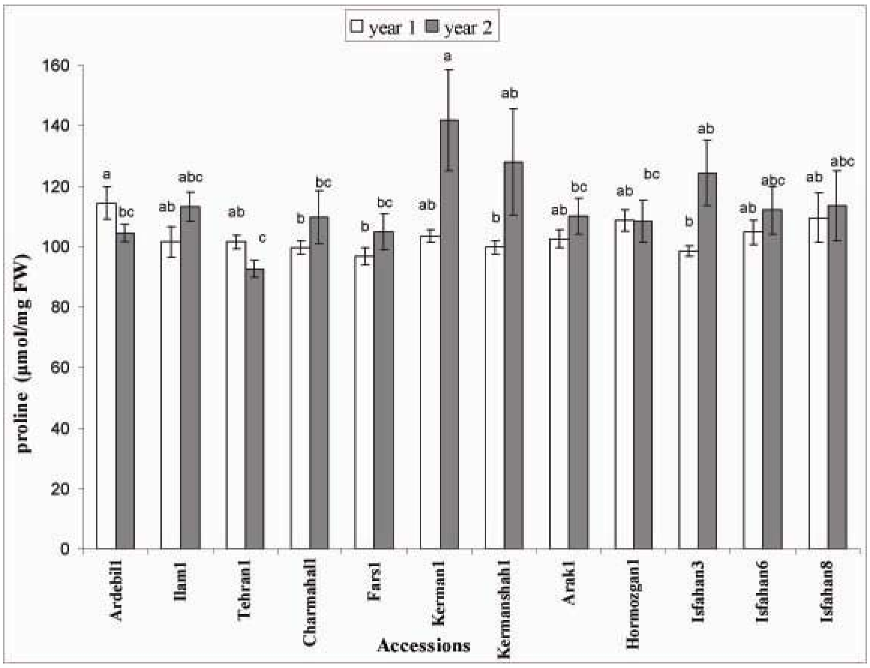

Fig 1. Changes of PRO concentration (μmol/mg FW) in 12 Damask Rose accessions in the 2 years. Vertical bars represent the ±SE. Different letters in each bar differ significantly according to DMRT test (p=0.05 level).

second year. This could be due to higher temperature and less rainfall. Ardebil1 was indicated higher sensitivity to climatic changes and had the lowest LA in the first year and highest LA in the second year. Generally, Fars1 and Kerman1 showed maximum and minimum LA, respectively. André (2003) reported stomata appear on the abaxial surface of rose leaves. So, this surface was used to determine stomata number. The number of stomata in the first year was higher than the second year. Furthermore, André has shown that different humidity affected frequency of stomata in rose leaves. The results showed it decreased when relative air humidity was enhanced. Chl is a basic catalyst in photosynthesis and changes in environmental conditions, especially the level of stress, affect the Chl concentration. Glutamate enzyme plays an important role in Chl production and stimulates PRO biosynthesis (Handa et al., 1986). These results elucidated an inverse trend of Chl and PRO concentration so, increase in Chl content and decline PRO rate were obtained in the first year. Other important indicators are antioxidants which protect cells from biochemical damage caused by ROS. They increase during imbalances between bioenergetics' pathways (Wise, 1995). Significant differences among enzymatic activities of *R. damascena* accessions could be reasons for the flexible adjustment in environmental conditions. POD is an important multifunctional enzyme that reflects environmental and physiological stresses. So, sensitive plants were showed the increase of POD activities under stress conditions (Fazeli et al., 2007). CATs are consisting of momofunctional proteins that affect plant growth so, CAT activity changes dramatically under unfavorable conditions (Ti-da et al., 2006; Xin and Xiu, 2007; Yang et al., 2008). Activities of CAT and non- specific POD are directly countered to production of H_2O_2 (Foyer and Halliwell, 1976). The result was reinforced the increase of antioxidant enzyme activities with the expansion of seasons. It could be acclimated plants to high

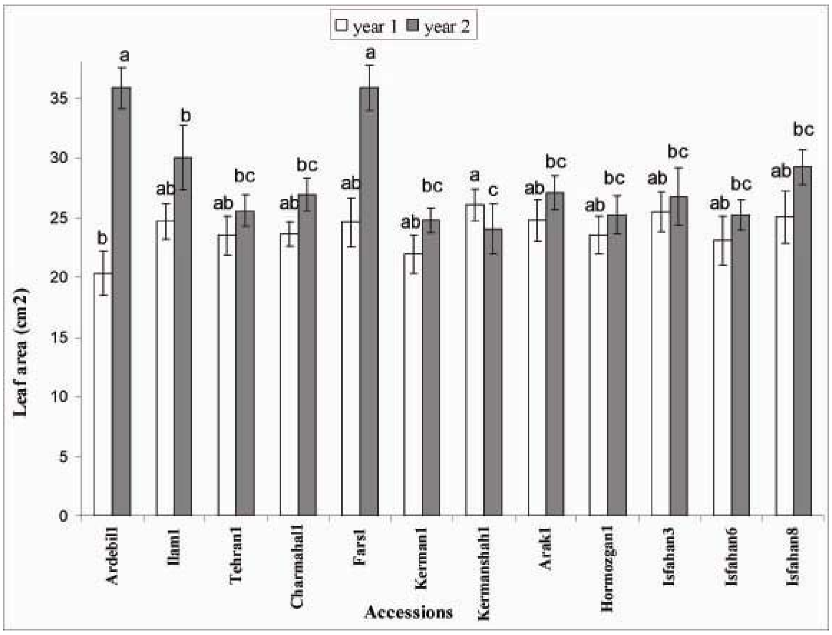

Fig 2. Changes of LA (cm^2) in 12 Damask Rose accessions in the 2 years. Vertical bars represent the ±SE. Different letters in each bar differ significantly according to DMRT test (p=0.05 level).

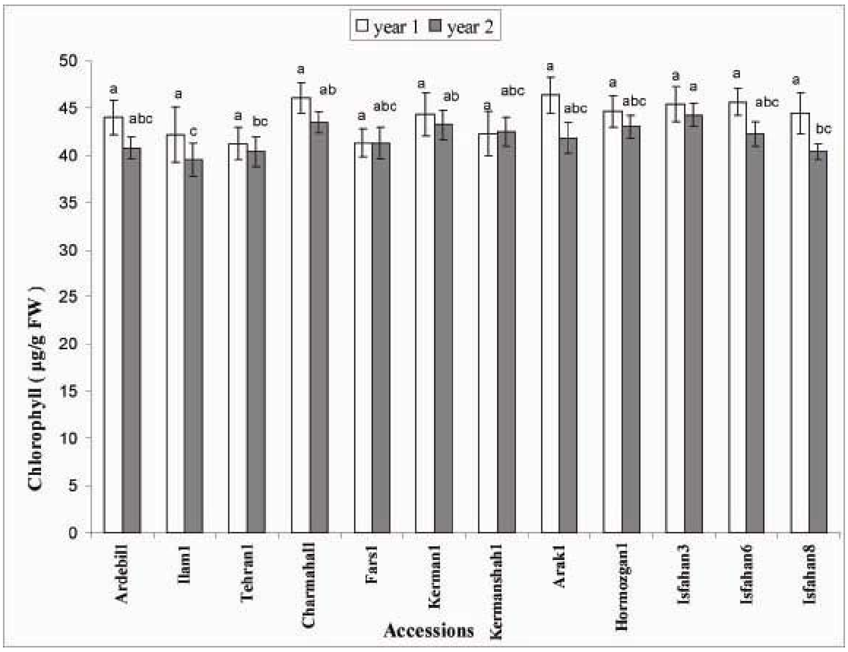

Fig 3. Changes of total Chl (µg/g FW) in 12 Damask Rose accessions in the 2 years. Vertical bars represent the ±SE. Different letters in each bar differ significantly according to DMRT test (p=0.05 level).

temperature in summer and prepared for winter conditions. Furthermore, the decrease of raining in the first year was the reason of enhanced POD activity compared to the second year. These hypotheses were confirmed by other researches (Bian and Jiang, 2009; Liu et al., 2009). In contrast to the CAT that removes the bulk of H_2O_2 in peroxisomes, PODs, scavenge the H_2O_2 that is produced in the cell wall, vacuoles and the apoplast (De Gara, 2004; Ozden et al., 2009). So, results elucidated an inverse trend of POD and CAT activities among accessions, especially in Tehran1. Different physiological and enzymatic responses of accessions could be related to their originated. 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). Antioxidants have to play a direct role in protection plants to unsuitable environment especially POD an important enzyme for defensive purposes (Passardi et al., 2005). The observations were emphasized that Tehran1 had the greatest activity of POD where found in the leaf among all accessions examined. Thus, this location has few distance to Damavand region. Therefore, they are more similar conditions than the other regions and we can introduce Tehran1 as the most compatible

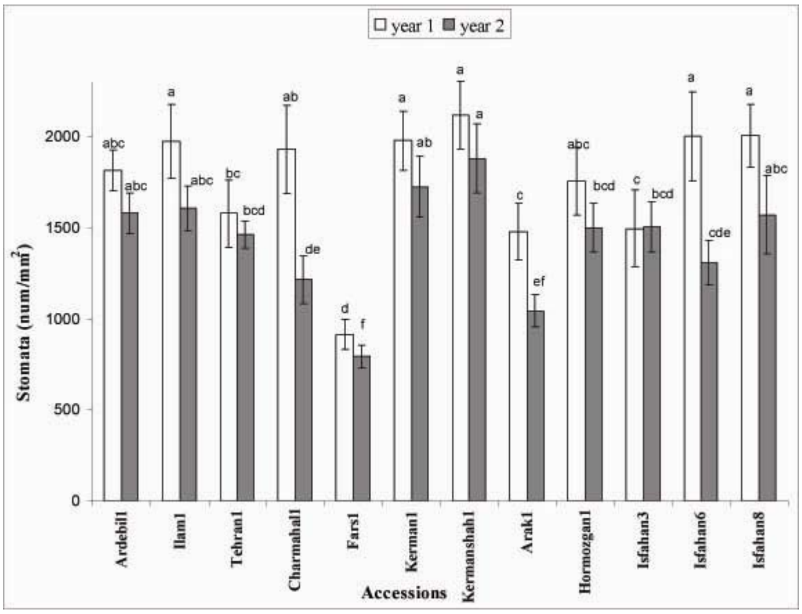

Fig 4. Changes of stomata density (number/ mm^2) in 12 Damask Rose accessions in the 2 years. Vertical bars represent the ±SE. Different letters in each bar differ significantly according to DMRT test (p=0.05 level).

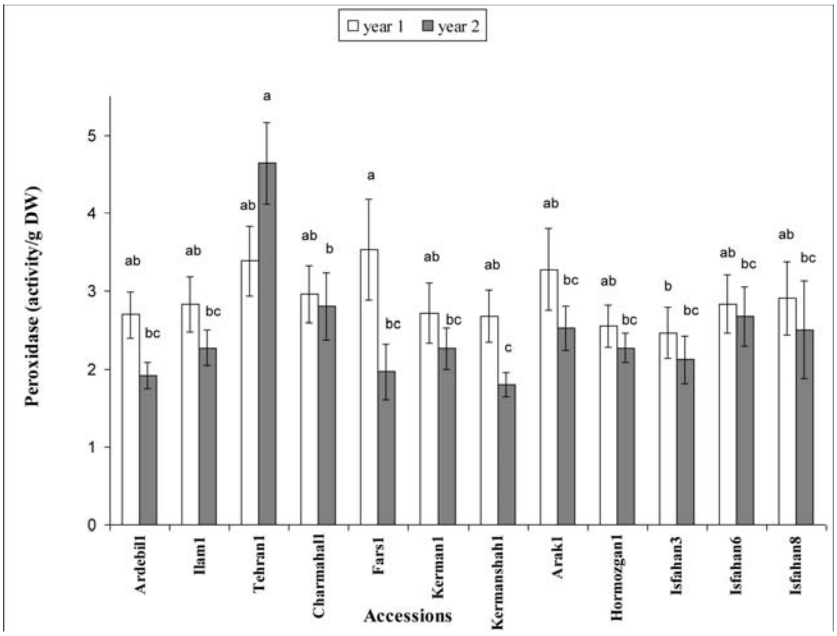

Fig 5. Changes of POD activity (activity/g DW) in 12 Damask Rose accessions in the 2 years. Vertical bars represent the ±SE. Different letters in each bar differ significantly according to DMRT test (p=0.05 level).

Damask rose accession to this condition. Also, variance and cluster analyses were indicated that Fars1 was classified separate of the other accessions.It may be the reason for inadaptability of this accession to Damavand region.

Materials and Methods

Plant materials

Twelve Damask Rose accessions from different parts of Iran (Ardebil, Ilam, Tehran, Charmahal, Fars, Kerman, Kermanshah, Arak, Hormozgan and Isfahan) were cultivated using randomized complete block design with three replications at Rangelands Research Station of Hamand Absard in Damavand, Iran. The research site is at a distance of 65 km from Tehran (co-ordinates, 354420N, 520535E and elevation 1960 m). The minimum and maximum temperature is about -24 °C in January and February and 37 °C in July and August. The Average rainfall in the first three months of year (development and flowering period of plants) was recorded 13 mm and 130 mm in 2008 and 2009, and the average rainfall of 333 mm in the year. In this research, some

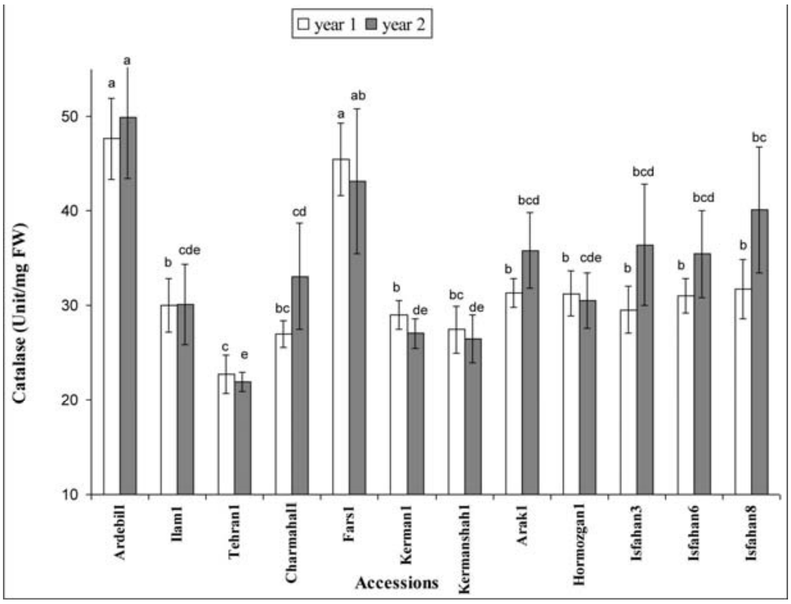

Fig 6. Changes of CAT activity (Unit/mg FW) in 12 Damask Rose accessions in the 2 years. Vertical bars represent the ±SE. Different letters in each bar differ significantly according to DMRT test (p=0.05 level).

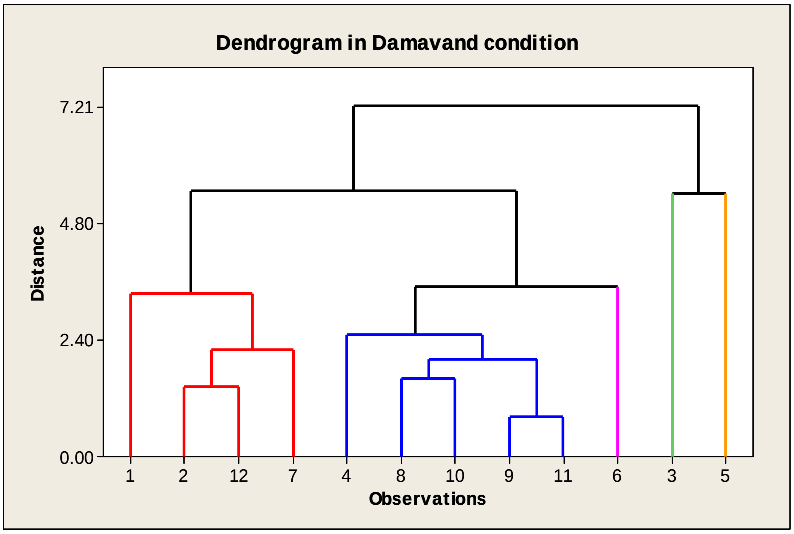

Fig 7. Classification of 12 Damask Rose accessions based on physiological characters and enzymatic activities using Ward clustering procedure. (observations data means 1: Ardebil1, 2: Ilam1 3: Tehran1 4: Charmahal1 5: Fars1 6:Kerman1 7: Kermanshah1 8: Arak1 9: Hormozgan1 10: Isfahan3 11: Isfahan6 12: Isfahan8).

physiological traits and enzymatic activities were evaluated based on different phenological stages during 2008 and 2009. Three phenological stages included 50% flowering in spring, warmest time in summer and before rains in autumn. Fresh leaves were sampled and the measurements were carried out.

Proline estimation

The Proline content of the leaves was assessed according to the method of Bates et al. (1973). Leaf samples (0.5 g) were extracted with 3% sulphosalicylic acid (10 ml) and filtered through filter paper. Then, 2 ml of the extracted solution was combined with 2 ml glacial acetic acid and ninhydrin reagent (125 mg ninhydrin in 3 ml of glacial acetic acid and 2 ml 6M H_3PO_4) and incubated at 100 °C for 1 h. The reaction was terminated by placing the container in an ice bath. The reaction mixture was vigorously mixed with 4 ml toluene. After warming at 25 °C, the chromophore was measured by spectrophotometer Bausch and Lomb at 520 nm, using toluene as a blank. PRO content was calculated from standard curve, using purified PRO as a standard. Results were expressed in µmol/ mg fresh weight (FW).

Total chlorophyll

Total Chlorophyll was measured by chlorophyll meter (Minolta SPAD-502). Chl content was shown in µg/g FW.

Leaf area

Leaf area was measured with an electronic area meter (240V.AC, GATEHOUSE., UK). Upon sampling, five leaf discs were taken from fully expanded young leaves for each accession from each replication. The values were used to determine LA in cm^2.

Stomata density

Epidermis imprints on abaxial leave were used to count stomata, and the imprints were later removed using transparent adhesive tape and were placed on a microscope slide. The counting was replicated on three plants for each accession. The stomata were counted using a light microscope with a 40 x 10 magnification lenses. The obtained values were expressed in number of stomata per mm^2.

Dry weight percentage

Dry weight percentage of leaves was determined in hot-air oven at 105 °C for 3 h and then the leaves were incubated in desiccators before the measurement of dry weight.

Enzymatic assays

For enzymatic extracts and assays about 1 g of leaves were rubbed and plugged in an extraction buffer for (pH 7.5) 24-72 h (Ebermann and Stich, 1982). The homogenate was centrifuged at 3000 rpm for 15 min, and supernatant was collected and used for enzymatic assays.

Proxidase activity

Proxidase activity assay was based on the method of Chance and Maehly, (1955) with phosphate buffer (pH 6.0) which uses measuring the oxidation of guaiacol as an electron donor to tetraguaiacol in the presence of H_2O_2 at 420 nm.

Catalase activity

Catalase 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 in absorbance at 240 nm for 1 min following the decomposition of H_2O_2.

Statistical analyses

Data were analyzed using randomized complete block design and data presented are mean values ± S.E. for three replicates. The mean values were compared 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 carried out based on all characteristics, using Ward's method (Ward, 1963).

Conclusion

The results of this research allowed us to document the responses of Damask rose accessions to environmental condition and genotypic differences. Other important traits are recommended to be evaluated. Also, this research needs to be continued to understand complex physiological and biochemical responses of the plant to stresses and evaluate stress-responsive genes involved in plant adaptation and tolerance to environmental conditions.

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