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Comparison of 18 Iranian caprifig cultivars based on some morphological and biochemical parameters

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Abstract: Caprifig is a valuable candidate for fig breeding programs as it typically grows naturally under non-optimal conditions. The present study was carried out to evaluate the biochemical/morphological characteristics of 18 caprifig cultivars indigenous to the Darab region/southern Iran with 4 replications in a completely randomized block design. From each cultivar, healthy uniform leaf samples and spring fruits were taken and analyzed. Our results showed that 'Naneghasem' had the highest leaf α-tocopherol and polyphenol concentration. The highest leaf ascorbic acid concentration was obtained from the Gol Khengi cultivar (17.03 µg g^{-1} fresh weight). Also, the contents of chlorophyll, carotenoids, and anthocyanins were significantly different among the studied cultivars. Various cultivars had different absorption potentials for essential elements as macro and micronutrients concentration in the leaves were statistically different in various caprifigs; 'Naneghasem' had the highest Ca (4.46 mg g^{-1} dry weight) and Fe (67.71 mg kg^{-1} dry weight) concentration and the highest leaf K concentration (22.46 mg g^{-1} dry weight) was observed in 'Mahali Layzengan'. In conclusion, 'Naneghasem' was evaluated as a cultivar which seems to be more morphologically- and biochemically-distant from other studied caprifig varieties and probably more adaptable/tolerant to environmental conditions.

1. Introduction

The fig, a deciduous tree of the Moraceae family and native to the southern Arabian Peninsula in the subtropical region, is an important horticultural crop that is grown for dry and fresh consumption. Dried figs are an excellent source of amino acids, vitamins, minerals, polyphenols, and crude fiber (5.8%, w/w) (Pourghayoumi *et al*., 2016). Satisfactory dry fig production is highly dependent on sufficient pollination. Caprifig is the only fig species that has both pistillate and staminate flowers. Growers collect the fruits of caprifigs, place them in cans, and hang the cans on Smyrna-type figs; *Blastophaga psenes* wasps, which live in the syconium of caprifigs, leave the profichi and enter the female flowers of the edible

figs and pollinate their pistillate flowers (Rahemi and Jafari, 2008).

In recent years, fig cultivation has developed rapidly due to the wide adaptability, early fruit and high yield, fewer pests and diseases, and simple management of this crop in various parts of the world (Zhang *et al*., 2020). This increases the importance of protecting and developing fig germplasm. Maintaining possible local varieties for commercial cultivation requires characterization of the available germplasm.

This type of thermophilic tree could be useful to adapt to the consequences of global warming, as fig trees can grow in dry regions where many other species may not survive (Sugiura *et al*., 2007). Fig trees have not been subjected to extensive breeding programs; consequently, many fig populations have rich diversity that cannot be fully exploited until correctly identified (Perez-Jiménez *et al*., 2012).

One of the first steps in breeding is the selection of promising species/cultivars. Previous studies show differences between various common fig cultivars (Aradhya *et al*., 2010; Perez-Jiménez *et al*., 2012; Zhang *et al*., 2020). These differences suggest varied levels of acclimation and/or tolerance potential to abiotic stress conditions. The plant's enzymatic and non-enzymatic antioxidant potential plays a vital role in this regard (Bonyanpour and Jamali, 2020). Maintenance of high antioxidant capacity to scavenge toxic reactive oxygen species (ROS) has been associated with better adaptation of plants to environmental conditions (Sharma *et al*., 2012). In addition, different cultivars have different abilities to uptake nutrients. The concentration of macro- and micronutrients in plant organs and tissues correlates with the plant's potential to tolerate prevailing environmental conditions (Jamali *et al*., 2016; Jamali and Bonyanpour, 2017; Tian *et al*., 2021).

Iran has one of the richest fig germplasm resources in the world; however, the majority of previous studies have focused on edible commercial cultivars, while studies on caprifig varieties are rare. Caprifig can be a valuable resource for breeding programs because it grows naturally under less than optimal conditions and is not domesticated. Strengthening research on caprifig germplasm's genetic/chemical diversity is of scientific importance for germplasm conservation, efficient breeding, and satisfactory production.

Typically, cultivar characterization studies involve determining a vast range of morphological, biochemical, molecular, etc., parameters to find promising varieties for future breeding projects. As part of a series of similar investigations on caprifig cultivars, the present study aimed to compare 18 cultivars indigenous to the Darab/Southern Iran region, focusing on phonological parameters and biochemical characteristics associated with non-optimal growing conditions.

2. Materials and Methods

Plant material

Uniform and healthy plants of eighteen mature autochthonous caprifig cultivars were selected. They were planted and grown in a completely randomized block design with 4 replications; each replication had 4 plants with a row spacing of 2.5 m and an inter-row spacing of 3.5 m in a rain-fed collection orchard in the Darab region, Fars province, southern Iran. Average annual climate parameters in the experimental region were: precipitation: 200 mm, relative humidity Max: 55%, Min: 18%, temperature Max: 42°C, Min: 4°C. The soil of the orchard was sampled and analyzed for soil texture, mineral content, organic matter, pH, and EC (Table 1). Cultivars included: 1-Mashgholamrezai (MGR), 2-Darabi (DRB), 3-Abbasali (ASA), 4-Khazraie (KZR), 5-Ieji (IJ), 6-Gol Khengi (GK), 7-Rastaghi (RS), 8-Naneghasem (NGM), 9-Mahali Layzengan (MLZ), 10-Cheshmbolboli (CMB), 11-Pouzehdonbali (PZD), 12-Danesibi (DS), 13-Pasras (PR), 14-Johari (JR), 15-Maseeh (MS), 16-Cho (CO), 17-Pasbehdari (PSD), and 18-Suzu (SZ). Leaf samples were taken from different orientations (north, south, west, and east) of the trees; 25 fully expanded mature leaves from each side of all trees (100 leaves per tree as bulk samples) were transported to the

Table 1 - Analysis of soil samples in the experimental region

Soil depth (cm)	Soil texture	Soil mineral content (mg kg^{-1})							Organic carbon (%)	EC (dS m^{-1})
		Nitrate	Ca	Mg	K	Fe	Zn	Mn		
0-50	Loamy clay	37	1110	170	220	7.00	1.7	5.50	0.70	0.72

laboratory. Leaves were of spring bloom, the middle third of the branch, at the height of 1-1.5 m, including the petiole. Leaves with abnormal symptoms, including mechanical lesions caused by diseases or pests as well as chlorosis, were avoided. Routine cultural practices were carried out during the experimental period.

Measurements

The following parameters were determined in caprifig cultivars for two consecutive years, with an average reported.

Leaf dry matter content

Three uniform leaves were washed with tap and distilled water. After drying with a clean towel, they were weighed with a digital scale to get fresh weight (FW) and then dried in an oven at 70°C for 72 hours and re-weighed to get dry weight (DW). The percentage of leaf dry matter was calculated according to the following formula:

$$\text{Leaf dry matters (\%)} = [\text{Leaf DW (g)}/\text{leaf FW (g)}] \times 100$$

Leaf relative water content (LRWC)

Ten leaf discs from each treatment were weighed (FW), then hydrated to saturation (constant weight) for 48 hours at 5°C in darkness (turgid weight, TW). Leaf discs were dried in an oven (DW). The relative water content was calculated according to the following formula (Jamali and Eshghi, 2015):

$$\text{LRWC (\%)} = (\text{FW-DW})/(\text{TW-DW}) \times 100$$

Leaf electrolyte leakage

Leaf electrolyte leakage (EL) was determined by recording leaf leachate's electrical conductivity (EC) in double-distilled water at 40 and 100°C. Leaf samples were cut into uniform-sized disks and placed in test tubes containing 10 ml of double-distilled water. The test tubes were kept at 40°C for 30 minutes and at 100°C for 15 minutes, and their respective electrical conductivities (EC1 and EC2) were measured using a conductivity-meter (METROHM Conductometer 644, Switzerland):

$$\text{Electrolyte leakage (\%)} = (\text{EC1}/\text{EC2}) \times 100$$

Leaf soluble carbohydrates

Leaf samples of 0.5 g (DW) were homogenized in 5 ml ethanol (95%) and centrifuged at 4500 × g for 15 min. The supernatant was removed from the sample, and the residue was resuspended in 5 ml ethanol (70%). The supernatant was centrifuged for final extraction. Both supernatants were combined. The anthrone sulfuric acid assay was used for determination (Irigoyen *et al*., 1992). An aliquot of 100 µl was added to 3 ml of the anthrone-sulfuric acid solution; the mixture was shaken, heated in a boiling water bath for 10 min, then cooled at 4°C. The absorbance at 625 nm was determined spectrophotometrically. Glucose (0-100 mg/l, Merk) was used as a standard (Jamali and Bonyanpour, 2017).

Leaf chlorophyll and carotenoids concentration

Leaf discs of 0.25 g were extracted in 2.5 ml of acetone (80%) and then centrifuged at 6,000 × *g* for 10 min. The supernatant was used to prepare a final volume of 50 ml of leaf extract. Extraction of the leaf tissue with the buffer was continued until decoloration. The absorbance of the extract was measured at 470, 645, and 663 nm spectrophotometrically. Acetone (80%) was used as a blank. Finally, the content of chlorophyll and carotenoids was calculated using the following equations (Lichtenthaler, 1987):

$$\text{Leaf total chlorophyll concentration (mg. g}^{-1}\text{ FW)} = [(7.15A663 + 18.71A645) \times v / 1000 \times W]$$

$$\text{Carotenoids (mg. g}^{-1}\text{ FW)}: 1000A470 - 1.82Chla - 85.02Chlb / 198$$

A = absorbance at ƛ (nm), W = sample weight.

Leaf anthocyanins concentration

Total anthocyanins in the leaves were determined by the pH differential method using a spectrophotometer and two buffer systems: potassium chloride buffer, pH 1.0 (0.025 M), and sodium acetate buffer, pH 4.5 (0.4 M). 0.25 g leaf samples were extracted with 1 ml methanol: water: concentrated HCl solution (80:20:1 v/v/v). 0.4 ml of leaf extract was mixed with 3.6 ml of corresponding buffers and read against water as blank at 510 and 700 nm. Absorbance (A) was calculated as

$$A = (A515 - A700)\ pH\ 1.0 - (A510 - A700)\ pH\ 4.5$$

Then total leaf anthocyanins concentration was calculated using the following equation:

$$\text{Anthocyanin (µg. g}^{-1}\text{ FW)} = (A \times Mw \times DF \times 1000) / e$$

Where A is the absorbance of the diluted sample, DF is the dilution factor (10), Mw is the molecular weight of cyanidin-3-glucoside (449.2), and e = 26,900 L/mol.cm, the molar extinction coefficient of cyanidin-3- glucoside.

Leaf α-tocopherol concentration

The α-tocopherol was extracted using Chong *et al*. (2004) method. 200 mg lyophilized sample was homogenized in 1 ml acetone at 4°C with a pre-chilled mortar and pestle. 0.5 ml hexane was added, and the homogenate was first vortexed for 30 s, centrifuged at 1000 × *g* for 10 min. The upper hexane

layer was removed while the acetone layer containing α-tocopherol remained in the vial. A second aliquot of 0.5 ml hexane was added, and the extraction process was repeated three times. α-tocopherol was determined by the method of Kanno and Yamauchi (1997).

To 0.2 ml of pooled extract a 0.4-ml aliquot of 0.1% (w/v) 3-(2-pyridyl)-5,6- diphenyl-1,2,4-triazine was added. The volume was made up to 3 ml with absolute ethanol, 0.4 ml 0.1% (w/v) ferric chloride ($FeCl_3.6H_2O$) was added, and the content was gently mixed under dim light in a dark room to avoid photochemical reduction. After a 4-minute reaction at room temperature, 0.2 ml 0.2 M orthophosphoric acid was added, and the mixture was left for another 30 min. Absorbance was determined spectrophotometrically at 554 nm and reported as µg g^{-1} FW. The blank was prepared the same way; absolute ethanol was used instead of the sample. α-tocopherol (Sigma Chemical) was used as a standard.

Glutathione concentration

Two hundred mg of tissue was homogenized in 2 ml of ice-cold 5% TCA. The homogenate was centrifuged at 17,000 × g for 30 min at 4°C. A volume of 75 µl of the supernatant was added to a cuvette containing 300 µl of phosphate buffer (0.2 M, pH 8.0) and 750 µl of 0.6 mM DTNB (5,5´-dithiobis- 2-nitrobenzoic acid) in phosphate buffer. The absorbance at 412 nm was read, and glutathione concentration was derived against a standard curve prepared with known amounts of GSH in 5% TCA (Moron *et al*., 1979).

Leaf total polyphenols

This parameter was determined using the Folin-Ciocalteu reagent. One gram of freeze-dried leaf samples was placed in an Eppendorf tube, mixed with 1 ml of methanol (80%), ground at 4°C, then centrifuged at 10000 × *g* for 15 minutes. The extract was mixed with 0.5 ml of Folin-Ciocalteu reagent; diluted 1:1 with water, and then 1 ml of a 5% sodium carbonate solution was added. Absorbance was measured at 725 nm and expressed as mg per g FW after 30 minutes. Gallic acid was used as the standard phenolic compound (Bonyanpour and Jamali, 2020).

Lipid peroxidation

A sample of 0.2 g was homogenized in 2 ml 0.1% trichloroacetic acid (TCAA) solution and then centrifuged at 15,000 × *g* for 10 min at 4°C, the 0.5 ml of supernatant was added to 1.5 mL 0.5% TBA in 20% TCAA, followed by incubation of the mixture at 95 °C in a shaking water bath for 30 min. The tubes were placed in an ice-water bath to stop the reaction. The samples were re-centrifuged at 10,000 × *g* for 5 min, and the absorbance of the supernatant was determined spectrophotometrically at 532 nm and corrected for non-specific turbidity by subtracting the absorbance at 600 nm. The malondialdehyde (MDA) concentration was calculated using the extinction coefficient of 155mM^{-1} cm^{-1} (Cakmak and Horst, 1991).

Leaves proline concentration

Leaf samples were homogenized with 3% sulfosalicylic acid. The homogenate was centrifuged at 2500 × *g* for 20 min. The supernatant was treated with acid ninhydrin and acetic acid, boiled for 60 minutes, and then the absorbance of the solution was recorded at 520 nm. Leaves proline contents were expressed as µmol·g^{-1} FW. Proline amino acid was used as standard (Bates *et al*., 1973).

Leaf ascorbic acid concentration

This parameter was measured by the method of Omaye *et al*. (1979). To 1 g of freeze-dried leaf sample, 10% ice-cold TCA was added and centrifuged at 3500 × g for 20 min at room temperature. The supernatant (1 ml) was mixed with 0.2 ml of DTC reagent and incubated at 37°C for 3 h. Then 1.5 ml of ice-cold 65% sulfuric acid was added and mixed, and the solutions were allowed to stand at room temperature for another 30 min. The color developed was read at 520 nm spectrophotometrically and reported as µg g^{-1} FW.

Macro and micronutrients' concentration

Oven-dried leaf samples (0.5 g) were ground and ashed at 550°C in a porcelain crucible for seven h. The white ash was mixed in 2 M hot HCl, filtered, and finally made up to 50 mL with distilled water. Nitrogen (N) concentration was measured using the Kjeldahl digestion method. Potassium (K) concentration was determined using the flame emission method using a Sherwood Scientific Ltd model 360 flame photometer. An atomic absorption spectrophotometer (AA 6200, double beam atomic absorption spectrophotometer Shimadzu, Kyoto, Japan) was used to determine Ca, Mg, and micronutrient element, including Fe, Zn, and Mn concentrations. Phosphorus (P) concentration was determined colorimetrically (Kalra, 1998).

Leaf biometrics

Leaf area was determined using a leaf area meter

(Kaiser RS1) and expressed as mm^2; leaf and petiole length by using a digital caliper and expressed as mm.

Fruit fresh and dry weight

Spring fruits were harvested and weighed using a digital scale and reported as gram. Then they were oven-dried and weighed again.

Experimental design and statistical analysis

The experiment was carried out in a completely randomized block design with four replications. Data were analyzed by ANOVA test using SPSS (Ver. 9.1); means were compared using Duncan's multiple range test at 5% probability level. Cluster analysis was also performed according to Ward's minimum-variance method using SPSS (Ver. 9.1) to classify the cultivars.

3. Results

The leaf glutathione concentration in NGM (67.01 μg g^{-1} FW) was nearly 50% higher than MS (32.32 μg g^{-1} FW). CO (64 μg g^{-1} FW) was not statistically different when compared with NGM. The leaf MDA concentration in KZR (12.12 μg g^{-1} FW) was lower than MS and CO cultivars. Other caprifig trees were not statistically different (Table 2).

The leaf ascorbic acid concentration in the GK cultivar (17.03 μg g^{-1} FW), was 59% higher than DRB (7.14 μg g^{-1} FW). RS, CMB, PZD, JR, MS, and CO cultivars were not statistically different in comparison with GK. The leaf proline concentration in MS (6.77 μg g^{-1} FW) was not statistically different in comparison with GK, NGM, CMB, PR, JR, and MS cultivars. The leaf total polyphenols concentration in NGM (26.71 mg g^{-1} FW), was 56% higher when compared with the MGR cultivar. PZD, JR, and PSD were not statistically different compared to the NGM cultivar (Table 2).

The leaf dry weight in NGM was 26% higher when compared with MGR. CMB, PZD, MS, CO, PSD, and SZ cultivars were not statistically different in comparison with NGM. Leaf total sugars were not statistically different in all cultivars. LRWC in the NGM cultivar was 8.4% higher when compared with MS . IJ, GK, MLZ, DS, CO, and SZ were not statistically different in comparison with NGM. Leaf EL in MGR (22.63%) was significantly higher than DS (17.62%). This parameter was not statically different in other cultivars (Table 3).

Leaf nitrogen concentration was not statistically different in all cultivars. The leaf P concentration in DS (3.32 mg g^{-1} DW) was higher in comparison with PR, JR, and CO; other cultivars were not different.

Table 2 - The concentration of some non-enzymatic antioxidants and MDA in leaves of studied cultivars

Cultivar	Glutathione (μg g^{-1} fresh weight)	MDA (μg g^{-1} fresh weight)	α-Tocopherol (μg g^{-1} fresh weight)	Ascorbic acid (μg g^{-1} fresh weight)	Proline (μg g^{-1} fresh weight)	Polyphenol (mg g^{-1} fresh weight)
MGR	45.02 fg [z]	15.00 abc	92.92 d	7.40 c	2.83 d	17.06 e
DRB	44.30 fg	16.00 abc	110.41 ab	7.14 c	3.65 cd	17.17 e
ASA	53.3 de	14.07 bc	102.13 bc	8.88 c	3.33 d	18.19 e
KZR	57.20 cd	12.12 c	69.82 e	10.12 bc	4.41 bcd	18.88 e
IJ	50.10 ef	17.07 abc	97.04 cd	10.21 bc	3.45 cd	22.09 bcd
GK	61.11 bc	15.19 abc	72.20 e	17.03 a	5.55 abc	18.29 e
RS	53.44 d	15.41 abc	105.45 bc	12.00 abc	4.14 bcd	17.21 e
NGM	67.01 a	12.14 c	122.18 a	8.22 c	6.71 a	26.71 a
MLZ	48.15 ef	13.25 c	114.12 a	9.06 c	2.92 d	19.82 de
CMB	40.22 gh	17.15 abc	115.45 a	16.33 a	6.20 ab	18.85 e
PZD	52.36 de	18.02 abc	105.31 bc	12.14 abc	4.38 bcd	25.42 ab
DS	61.10 bc	14.14 bc	100.05 bc	7.63 c	2.78 d	23.41 bc
PR	45.16 fg	13.14 c	77.14 e	10.47 bc	5.61 abc	22.22 bcd
JR	37.35 hi	14.20 bc	98.32 cd	12.42 abc	5.99 ab	24.56 ab
MS	32.32 i	20.37 ab	77.11 e	15.15 ab	6.77 a	20.37 cde
CO	64.00 ab	21.07 a	100.07 cd	15.71 ab	4.70 abcd	20.17 cde
PSD	40.05 gh	15.3 abc	105.50 bc	9.16 c	3.77 cd	20.22 abc
SZ	41.07 gh	13.33 c	115.00 a	8.05 c	3.42 cd	22.15 bcd

[z] Means followed by the same letters within columns are not different at 5% probability using Duncan's test.

Table 3 - Leaf relative water content, electrolyte leakage, dry matter, and total sugars in studied caprifig cultivars

Cultivar	Leaf dry matter (%)	Leaf total sugars (mg g^{-1} dry weight)	Leaf relative water content (%)	Electrolyte leakage (%)
MGR	26.22 b [z]	27.33 a	77.92 d	22.63 a
DRB	28.30 b	26.05 a	81.21 abc	22.04 ab
ASA	27.33 b	27.17 a	78.89 bcd	21.87 ab
KZR	26.55 b	29.55 a	77.92 cd	19.12 ab
IJ	27.44 b	26.77 a	83.04 a	20.00 ab
GK	28.15 b	27.37 a	80.08 abcd	22.03 ab
RS	27.61 b	28.47 a	79.52 bcd	21.43 ab
NGM	33.05 a	29.14 a	83.60 a	17.96 ab
MLZ	28.15 b	30.13 a	81.11 abc	19.06 ab
CMB	30.31 ab	28.45 a	79.63 bcd	22.33 ab
PZD	29.55 ab	29.02 a	77.32 d	22.34 ab
DS	27.22 b	27.37 a	80.29 abcd	17.62 b
PR	29.71 ab	28.02 a	77.14 d	20.77 ab
JR	28.44 b	30.00 a	78.32 cd	19.72 ab
MS	29.81 ab	28.88 a	77.11 d	19.75 ab
CO	28.88 ab	30.11 a	81.00 abc	21.72 ab
PSD	29.02 ab	27.14 a	79.67 bcd	19.55 ab
SZ	28.87 ab	28.64 a	82.22 ab	19.15 ab

[z] Means followed by the same letters within columns are not different at 5% probability using Duncan's test.

The leaf K concentration in MLZ (22.46 mg g^{-1} DW) which was not statistically different compared with ASA, KZR, GK, RS, NGM, PZD, DS, PR, JR, MS, CO, PSD, and SZ. The leaf Ca concentration (4.46 mg g^{-1} DW) in NGM was 25% higher than PR. CMB had the highest leaf Mg concentration (2.48 mg g^{-1} DW); KZR had 42% lower leaf Mg concentration. Other cultivars were not statistically different (Table 4).

The leaf Fe concentration in NGM (67.71 mg kg^{-1} DW) was significantly higher than KZR, MLZ, PR, JR, MS, PSD, and SZ. The leaf Zn concentration in NGM (27.31 mg kg^{-1} DW) was not statistically different in comparison with DRB, MLZ, PSD, and SZ. Leaf Mn concentration was not statistically different in all cultivars (Table 5).

The leaf total chlorophyll concentration in NGM (1.43 mg g^{-1} FW) was not statistically different compared with DRB, ASA, KZR, MLZ, DS, MS, PSD, and SZ (Fig. 1). The leaf concentration of carotenoids in PSD was significantly higher than ASA, KZR, GK, CMB, PZD, RS, JR, and MS. Other cultivars were not statistically different in comparison with PSD (Fig. 2). The concentration of leaf anthocyanins in MGR was significantly higher than PSD. This parameter was not significantly different in other cultivars (Fig. 3).

Table 4 - Leaf macronutrients in studied caprifig cultivars

Cultivar	N (mg g^{-1} dry weight)	P (mg g^{-1} dry weight)	K (mg g^{-1} dry weight)	Ca (mg g^{-1} dry weight)	Mg (mg g^{-1} dry weight)
MGR	22.12 a [z]	2.60 ab	16.33 c	3.76 bc	2.00 ab
DRB	20.13 a	3.10 ab	17.16 bc	3.77 bc	2.22 ab
ASA	20.15 a	3.05 ab	19.65 abc	3.97 abc	2.02 ab
KZR	21.41 a	2.52 ab	18.27 abc	4.44 a	1.55 b
IJ	21.05 a	2.97 ab	17.05 bc	3.65 bc	1.95 ab
GK	21.77 a	2.55 ab	21.20 abc	3.80 bc	1.77 ab
RS	22.30 a	2.92 ab	18.85 abc	4.20 ab	1.67 ab
NGM	22.12 a	3.00 ab	20.38 abc	4.46 a	2.35 ab
MLZ	19.64 a	3.14 ab	22.46 a	3.95 abc	2.20 ab
CMB	20.51 a	2.46 ab	16.44 c	3.67 bc	2.48 a
PZD	22.35 a	2.81 ab	18.71 abc	3.72 bc	2.36 ab
DS	19.19 a	3.32 a	18.22 abc	3.90 abc	1.83 ab
PR	22.66 a	2.45 b	19.70 abc	3.55 c	2.11 ab
JR	20.44 a	2.37 b	19.00 abc	3.63 bc	2.07 ab
MS	20.11 a	2.88 ab	20.77 abc	4.18 ab	1.61 ab
CO	22.00 a	2.44 b	18.00 abc	3.76 bc	2.18 ab
PSD	21.02 a	3.11 ab	21.56 ab	4.02 ab	2.14 ab
SZ	21.48 a	3.23 ab	22.07 ab	3.89 abc	2.25 ab

[z] Means followed by the same letters within columns are not different at 5% probability using Duncan's test.

Table 5 - Leaf micronutrients in studied caprifig cultivars

Cultivar	Fe (mg kg^{-1} dry weight)	Zn (mg kg^{-1} dry weight)	Mn (mg kg^{-1} dry weight)
MGR	60.66 ab [z]	23.33 bcd	47.22 a
DRB	60.47 ab	26.50 ab	50.16 a
ASA	62.23 ab	22.74 cd	49.44 a
KZR	58.36 b	22.46 cd	50.33 a
IJ	63.33 ab	23.00 bcd	49.31 a
GK	64.44 ab	21.20 d	48.56 a
RS	60.96 ab	22.85 cd	47.91 a
NGM	67.71 a	27.31 a	48.05 a
MLZ	57.22 b	25.44 abc	50.20 a
CMB	61.22 ab	22.07 cd	50.27 a
PZD	63.42 ab	23.08 bcd	49.22 a
DS	60.75 ab	22.77 cd	48.71 a
PR	59.29 b	23.12 bcd	48.05 a
JR	57.33 b	21.90 cd	49.66 a
MS	59.40 b	22.35 cd	50.23 a
CO	60.05 ab	22.68 cd	51.44 a
PSD	58.63 b	24.88 abcd	50.63 a
SZ	59.34 b	25.14 abc	47.33 a

[z] Means followed by the same letters within columns are not different at 5% probability using Duncan's test.

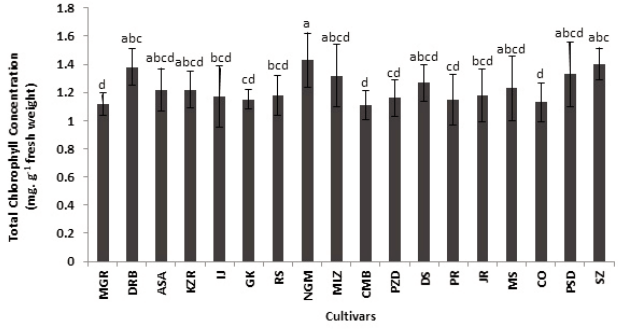

Fig. 1 - Total chlorophyll concentration in studied caprifig cultivars. Columns with the same letters are not statistically different at 5% probability using Duncan's test. Vertical bars indicate standard errors (n=4).

The highest fruit FW and DW were obtained from DS. NGM had the highest leaf length (154.66 mm). PR, MS, PZD, CMB, RS, and KZR were not statistically different. The leaf area (11581.2 mm^2) and petiole length (66.69 mm) in NGM were significantly higher than all other cultivars (Table 6).

The cluster analysis based on Ward's method divided the cultivars into three major clusters, including 8 cultivars in cluster 1, 9 cultivars in cluster 2, and 1 cultivar in cluster 3. The first cluster consisted of

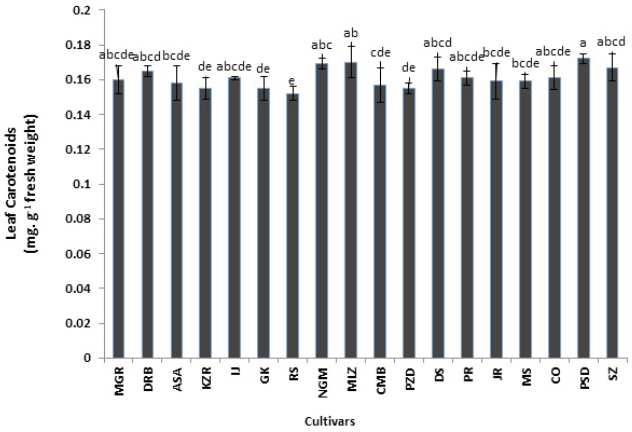

Fig. 2 - Concentration of leaf carotenoids in studied caprifig cultivars. Columns with the same letters are not statistically different at 5% probability using Duncan's test. Vertical bars indicate standard errors (n=4).

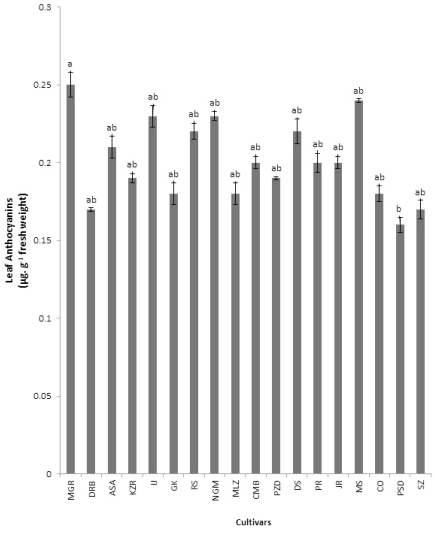

Fig. 3 - Concentration of leaf anthocyanins in studied caprifig cultivars. Columns with the same letters are not statistically different at 5% probability using Duncan's test. Vertical bars indicate standard errors (n=4).

two subclusters: subcluster 1 (MGR, IJ, ASA, and DS) and subcluster 2 (DRB, PSD, SZ and MLZ). Cluster 2 also had 2 subclusters: subcluster 1 (KZR, MS, and RS) and subcluster 2 (GK, CO, CMB, JR, PZD, and PR). Cluster 3 had only one cultivar: NGM (Fig. 4).

4. Discussion and Conclusions

Non-enzymatic antioxidants were statistically different among investigated cultivars in our study, which was consistent with previous studies. Jamali

Table 6 - Fruit fresh and dry weight and leaf and petiole length, and leaf area in studied caprifig cultivars

Cultivar	Fruit fresh weight (g)	Fruit dry weight (g)	Leaf length (mm)	Leaf area (mm^2)	Petiole length (mm
MGR	4.77 hi [z]	0.90 fg	78.97 g	1879.5 j	23.45 h
DRB	5.77 gh	1.09 ef	89.70 fg	3217.3 efg	29.36 fgh
ASA	6.48 efg	1.20 de	83.54 g	2232.6 hij	23.08 h
KZR	7.60 cde	1.21 de	121.63 bc	6352.9 b	37.46 cd
IJ	4.94 hi	0.95 fg	98.97 ef	2005.9 ij	28.60 fgh
GK	8.06 cd	1.48 bc	107.57 de	4433.1 d	def 10/33
RS	8.64 c	1.76 a	118.04 bcd	7090.9 b	50.72 b
NGM	6.63 efg	0.94 fg	154.66 a	11581.2 a	66.69 a
MLZ	4.01 i	0.72 g	79.28 g	2814.7 fgh	26.57 gh
CMB	7.12 def	1.09 ef	116.12 bcd	3698.2 def	38.30 cd
PZD	5.92 fgh	0.96 f	117.25 bcd	4373.1 d	35.95 de
DS	12.40 a	1.71 a	98.90 ef	2522.8 ghij	30.79 efg
PR	6.39 efg	1.03 ef	113.67 bcd	4554.1 d	37.77 cd
JR	6.37 efg	1.07 ef	99.53 ef	3980.3 de	32.84 defg
MS	10.88 b	1.58 ab	125.09 b	6518.2 b	42.68 c
CO	6.76 efg	1.02 ef	112.09 cd	5459.2 c	43.36 c
PSD	6.67 efg	1.36 cd	81.50 g	3334.1 efg	28.24 fgh
SZ	4.94 hi	0.9 fg	87.78 fg	2965.6 fgh	29.02 fgh

[z] Means followed by the same letters within columns are not different at 5% probability using Duncan's test.

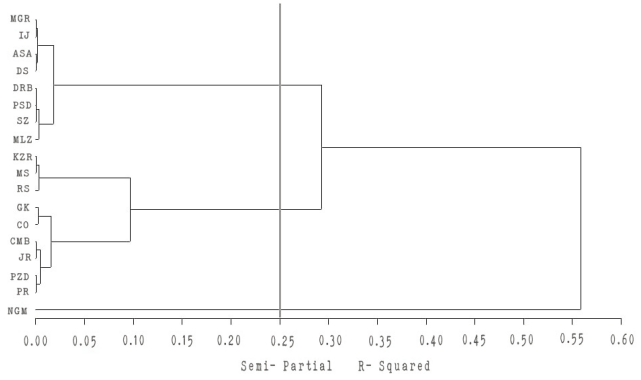

Fig. 4 - Cluster analysis dendrogram of 18 Iranian caprifig cultivars based on Ward's method.

and Bonyanpour (2017) determined leaf mineral composition and also some biochemical parameters, i.e., concentration of non-enzymatic antioxidants in leaves, in seven Iranian pomegranate cultivars for selecting probable more tolerant cultivars. They found that cultivars with a higher concentration of non-enzymatic antioxidants (e.g., glutathione, α-Tocopherol, proline, etc.) had better adaptability to the prevailing environmental conditions. As mentioned earlier, various species and cultivars have inherently different potential to tolerate non-optimal growth conditions. One of the possible reasons for this difference is their enzymatic/non-enzymatic antioxidant responses and/or their ability to absorb macro/micro nutrients. Therefore, determination of these characteristics can be used as biochemical markers for cultivar comparison/screening.

Gholami *et al*. (2012) compared four fig cultivars (Deyme Ahvaz, Sabz Estahban, Siah, and Shahanjir) under normal, drought, and recovery conditions. According to their results, some of the non-enzymatic and enzymatic antioxidants in leaves differed significantly under optimal conditions. Their findings show that the fig cultivars demonstrated a clear difference in their response to water stress and recovery. They evaluated Deyme Ahvaz as a more tolerant cultivar than Sabz Estahban. Deyme Ahvaz had higher leaf concentration of glutathione and anthocyanin in comparison to Sabz Estahban under non-stress conditions.

Samec *et al*. (2021) reviewed the role of phenolic compounds in inducing tolerance to non-optimal conditions in plants. They reported that the potential tolerance of a plant species to stress conditions can be associated with this diverse family of chemicals which is universally present in plants and includes

more than 8000 molecules. Phenolic compounds play an essential role in plant responses, especially in defense mechanisms. They have strong antioxidant properties and neutralize the detrimental effects of reactive oxygen species (Balasundram *et al*., 2006). The absolute concentrations of these chemicals in plant organs vary significantly depending on the determination method, cultivar, or tissue, but it can be concluded that higher phenol concentrations correlate with increased stress tolerance.

Arteaga *et al*. (2020) have proposed the use of proline as a biochemical marker for rapid and simple large-scale screening of different genotypes for drought and salt tolerance. Transgenic plants, especially those overexpressing genes for proline accumulation, show higher adaptation to abiotic stresses (El Moukhtari *et al*., 2020; Ghosh *et al*., 2022. Similarly, endogenous proline concentration has been linked to a relatively higher stress tolerance when comparing different cultivars by various authors in different species (Kapuya *et al*., 1985; Misra and Gupta, 2005; Goharrizi *et al*., 2020).

The lower MDA content in NGM, RS, or MLZ may be due to the higher content of antioxidants such as α-tocopherol in these two caprifigs. This is consistent with previous studies (Assaha *et al*., 2015; Amoah *et al*., 2019; Nawaz and Wang, 2020).

Except for leaf N and Mn concentration, other macro and micronutrients were significantly different in various caprifig cultivars. This was in agreement with previous studies on different fig cultivars (Anac *et al*., 1982; Aksoy *et al*., 1987; Askin *et al*., 1998; Hakerlerler *et al*., 1998; Bougiouklis *et al*., 2020).

The higher leaf DW or LRWC in NGM could be attributed to the higher leaf concentration of K, Ca, Fe, or Zn in this caprifig in comparison with other studied cultivars. This was in agreement with previous studies as various cultivars absorb macro/micronutrients differently. Hegwood (1972) reported a significant varietal effect on leaf mineral composition during full bloom and harvest in eleven snapbean cultivars. Jordão *et al.* (1999) studied 15 olive cultivars and found that the mean effect of cultivar on leaf concentrations of essential elements, including N, P, K, Ca, Mg, S, Mn, Zn, and B, was significant. There are also many other examples of the effects of cultivar or root system of different species on leaf mineral composition (Tsipouridis and Thomidis, 2005; North and Cook, 2006; Tomala *et al*., 2008; Kviklys *et al*., 2012).

This difference in endogenous macro/micronutrient composition can affect numerous aspects of plant growth and development. In other words, the presence of a particular macro- or micronutrient (e.g., Ca) above a critical concentration can alter the plant's response to non- optimal growing conditions (Pilbeam and Morley, 2007; Taiz and Zeiger, 2010). For instance, Fageria (2013) reported an increased root DW in 12 lowland rice genotypes after adding K fertilizer. They found that increase in root DW at high K levels was 246% compared to the low level of K.

Leaf chlorophyll and carotenoids concentration were significantly different in studied cultivars. Chlorophyll concentration is a sensitive biochemical marker indicating cellular metabolic state (Chutipaijit *et al*., 2011). Previous studies have shown that chlorophyll concentration and stability correlate with plants' high tolerance potential against abiotic stresses such as salinity or drought (Hasanuzzaman *et al*., 2013). Carotenoids protect the photosynthesis apparatus by scavenging free radicals keeping its integrity against photo-oxidative damages (Dall'Osto *et al*., 2007; Andrade-Souza *et al*., 2011). As an ABA precursor, higher carotenoid concentration means lower photo-oxidative damage and elevated potential for regulating plant growth under stress conditions (Götz *et al*., 2002; Han *et al*., 2008).

Caprifig cultivars in our study were clustered into three main groups indicating lower level of variability within clusters and higher variability levels between clusters. Morphological and biochemical markers have long been used for screening and characterizing different genotypes, as they are the first steps in describing/classifying any germplasm (Cantini *et al*., 1999). Previous studies on figs show the usefulness of these markers in documenting variability among genotypes (Salhi-Hannachi *et al*., 2006; Saddoud *et al*., 2008; Podgornik *et al*., 2010).

NGM had the highest leaf glutathione, α-Tocopherol, proline, polyphenols, Ca, and Zn concentrations. Leaf length, leaf area, and petiole length were the highest in this cultivar. Cluster analysis indicated that NGM was the only cultivar in cluster 3. In conclusion, NGM was evaluated as a genotype which seems to be more distant (morphologically and biochemically) from other studied caprifigs and probably more adaptable/tolerant. Our findings can be used as an overture for complementary studies for selecting promising genotypes for breeding and introducing new tolerant fig cultivars.

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