Morphological and biochemical changes in two parsley varieties upon water stress

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Abstract The study was carried out to determine the responses of two parsley (*Petroselinum crispum* L.) hybrids; plain and curly- leafed to water stress. Besides the control, three irrigation treatments (10, 30 and 50 % of control water amount) were applied. Plant yield (leaf area, stem length and diameter, number of leaves) and quality (fresh and dry weights and concentrations of chlorophyll, carotenes, anthocyanins and vitamin C) were measured. The responses of plant yield and quality varied according to the hybrid and water treatment. The high quality was observed in curly-leafed parsley if compared to plain- leafed parsley, which had higher yield (stem length and diameter, leaf area). Moreover, water stress improved quality but decreased yield parameters in both hybrids.

Keywords Parsley · Yield · Quality · Stress · Chlorophyll · Carotene · Anthocyanins

Introduction

Parsley is one of the most important vegetables produced in the world. Its varieties differ according to the commercial organ. There are leafed parsley such as plain and curly-leafed varieties and fleshy roots parsley such as Hamburg varieties (Petropoulos et al. 2006, 2008).

In spite of its high daily consumption in Syrian Arab Republic, there is not yet accurate data concerning parsley cultivated area and production. The parsley importance is

attributed to its high vitamins concentration (mainly vitamin C), antioxidants (Pennington and Church 1985), and some mineral elements such as iron (Kmiciek and Lisiewska 1999) as well as volatile oils that play an important role in the pharmaceutical and food industries (Sellar 2001; Wander and Bouwmesster 1998).

Previous studies indicated that the effect of water stress varies according to the parsley variety (Petropoulos et al. 2006) and growth stage. Plants exposure to water stress during the vegetative growth (before flowering stage) exhibit a decrease in plant height and leaf area (Abbaszadeh et al. 2008; Sharifi et al. 2005; Taheri et al. 2008), dry matter production (Aliabadi et al. 2009) and number of leaves (Misra and Srivastava 2000; Rhizopoulou and Diamantoglou 1991). Such effect was attributed to the diminution of light interception because of reduced expansion rate and number of leaves (Fatima et al. 1999), or the temporary leaf wilting or wrapping during the stress period. The exposure of plants to water stress during the reproduction stage reduce the period between a seed formation and fullness (Barnabas et al. 2007; Earl and Davis 2003; Mohamed et al. 2002; Scalabrelli et al. 2007). In general, water stress reduces the plant fresh and dry weight and such impact is attributed to the disorder in water absorption and transfer, as well as to the disruption of leaf gas exchange and other metabolic processes (Gamalei 2002; Sarker et al. 2005).

Providing a permanent source of water is a priority to increase the production and improve the quality of the cultivated vegetables such as parsley, which is classified as a sensitive plant to water stress (especially if it is planted in the summer and last spring). Beside a very low rate of rainfall, the Mediterranean regions are suffering from water sources. Such conditions make it difficult to provide the water for agriculture. Therefore, application of a specific irrigation program is one of the important practices to

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control the balance between production and quality. Up to now, there are few studies dealing with the impact of water stress in parsley growth and quality. Hence the objectives of this research were (i) to compare some productivity and quality parameters of two parsley hybrids, (ii) to study the impact of water stress in productivity and quality of parsley hybrids, throughout some morphological and biochemical criteria.

Materials and methods

Plant material and growth conditions

Two parsley hybrids were planted; the curly-leaved parsley (*Petroselinum crispum* cv. Moss curled) referred by (M) and the plain-leaved parsley (*Petroselinum crispum* cv. Neoplitanum) referred by (A).

The experiment was conducted from mid September 2010 until the end of April 2011 in Faculty of Agriculture in Damascus University. At the beginning of September, the seeds were soaked into warm water (30 °C) for 24 hours for planting the next day in boxes (30×60 cm) containing a mixture of soil and compost (1:1 v/v).

The plants were irrigated once a week. Water stress was imposed at the beginning of November 2010. In order to determine the requisite water amount, a primary test was carried out, where boxes containing a mixture of soil and compost were weighted (dry weight). Then it was saturated and allowed to drain freely until there was no change in weight. The difference between this weight and box dry weight was used to calculate 100 % of water holding capacity. Thus, the amount of water added to the control (C) was 1 liter (i.e., 100 %). The water stress treatments were 10, 30 and 50 % of the control. Eight treatments were carried out according to the hybrid (A and M) and water stress treatment (10-30-50 and C): A-10, A-30, A-50, A-C, M-10, M-30, M-50 and M-C.

Morphological and chemical measurements

The harvest was carried out once every three weeks from 10 November 2010. Plants were harvested by cutting the stems of each plant separately at 1 cm overground. Harvested plants were placed in numbered nylon bags in order to achieve the measurements in laboratory. For the biochemical analysis, leaves were placed in liquid nitrogen and stored at a temperature of - 20 °C.

After cutting, plant fresh weight (g) of seven plants per replicate, was measured immediately using a sensitive balance (Sartorius, 0.1±0.001 g, India). Leaf area (cm²) was recorded for the whole plant (10 plants / replicate) using a Li-Cor 3100 area meter (Li-Cor, Lincoln, NE, USA). The

plant dry weight was determined after drying in oven at a temperature of 110 °C for three days (until weight stability). The plant water concentration (PWC %) was estimated according to the equation of Gonzalez and Gonzalez-Vilar (2003) :

$$PWC\% = \left(\frac{\text{wet weight} - \text{dry weight}}{\text{wet weight}} \right) * 100$$

Stems length were measured using a tape measure, while the diameter was measured using a digital caliper (500-181U Mitutoyo, precision 1/100th, UK). The average number of leaves per stem was recorded.

To determine the leaf chlorophyll concentration (a+b), 0.2 g of leaves powder was homogenized by adding 7 ml of acetone (80 %). The mixture was centrifuged at 3400 g for 20 minutes at 4 °C (Tabletop model, IEC 215, USA). The resulting supernatant was used to determine the absorbance at 664 and 647 nm using the spectrophotometer (Shimadzu Mini-1240 UV–Vis, USA). If the absorbance is greater than 1, the resulting supernatant is diluted by 10 % using acetone (80 %). The leaves chlorophyll concentration was determined according to Adelusi et al. (2006):

$$\text{chlorophyll}_a = 13.19 A_{664} - 2.57 A_{647}$$

$$\text{chlorophyll}_b = 22.1 A_{647} - 5.26 A_{664}$$

Where: A_{664} and A_{647} are the absorbance at 664 and 647 nm, respectively.

To determine β -carotene concentration, Teow et al., 2007 method was used. The carotene concentration was analyzed by high performance liquid chromatography (HPLC, Hamilton, UK). The samples were placed in the sample tray cooled to 6 °C and covered by aluminum foil to minimize light. Samples (20 μ l) were injected onto a reverse phase column (Chandler and Schwartz, 1998). Separation was performed at 35 °C with a mobile phase of methanol, acetonitrile and chloroform (42.5/42.5/15 v/v). The flow rate was maintained at 1.2 ml/min. Peaks were monitored at 450 nm. Standard solutions of β -carotene with concentration from 0.5 to 10 μ g/ml were used to obtain a standard curve.

Total anthocyanin concentration was measured by the pH-differential method described by Rodriguez-Saona et al. (1998). The absorbance was measured at 530 and 700 nm with distilled water as a blank. The absorbance difference between the pH 1.0 and pH 4.5 samples was:

$$A = (A_{530} - A_{700})_{pH1.0} - (A_{530} - A_{700})_{pH4.5}$$

The monomeric anthocyanin concentration (mg/100 g fresh product, expressed as cyaniding-3-glucoside equivalents) was calculated with the following formula:

Anthocyanin content

$$= (A \times MW \times DF \times 100 \times V_{\text{flask}}) / (\epsilon \times L \times m_{\text{sample}})$$

Where: $MW=449.2$ (g/mol) and $\epsilon=26.900$ are respectively, the molecular weight and molar absorptivity of cyanidin-3-glucoside which was used as a standard. DF and L are dilution factor (final volume/initial volume) and path length (1 cm), respectively. V_{flask} is volume (ml) of volumetric flask, which was brought extract/sample solution. m_{sample} is the weight of powder fresh weight (5 g).

Vitamin C concentration was measured according to Murshed et al. (2008) where the absorbance was measured at 525 nm in a micro-plate reader (Power Wave HT Microplate Spectrophotometer, BioTek).

Experimental design and statistical analysis

The experiment was designed as randomized complete sectors (two hybrids * four water stress treatments). Each treatment included three replicates. Each replicate was consisted of 10 plants. In order to calculate the mean values and standard errors, the R statistical version 2.5.1 (The R Project for Statistical Computing, Lyon, France) was used. Since this experiment represents a factorial design with variety (two levels) and water regime (four levels), the pairwise Wilcoxon test was used to determine the significant differences between the studied variables on 95 % confidence level.

Results

Yield parameters

The variations of fresh and dry weight of plant (g), PWC%, average of leaves number per stem, stems length and diameter (mm) were presented using the radar plot. Two polygons with six corners were established, one for each cultivar (Fig. 1a and b for plain- and curly leafed hybrid, respectively). Each line from the center to a corner of the polygon represents the relative scale for one parameter as value of a treated sample / value of the untreated sample.

Concerning the plant fresh weight, it was higher significantly by 54 % in A-C as compared to M-C. The plant fresh weight was decreased from 66.06 to 42.04 g in A-C and A-10, respectively, and from 45.20 to 18.13 g in M-C and M-10, respectively. Regarding the plant dry weight, it was higher by two folds in A-C (4.58 ± 0.92 g) as compared to M-C. The water stress decreased significantly the dry weight in both hybrids. No significant change in the average of leaves number per stem was observed whatever the hybrid and water stress treatment (Fig. 1a and b). The plant water concentration (PWC %) was varied significantly ($P < 0.05$) according to the hybrid and stress treatment (Fig. 2a

Fig. 1 Average of plant fresh and dry weight (g), PWC %, number of leaves per stem, stems length and diameter (mm) for 15 plants per treatment. Polygons (a) and (b) are plain- and curly leafed hybrid, respectively. Each corner is one variable for seven harvests

and b). The PWC of M-C was higher (88.33 ± 1.26 %) as compared to A-C (86.05 ± 1.65 %). The impact of water stress on PWC was more pronounced in curly- leafed hybrid, particularly in M-10 and M-30, which decreased significantly to reach to 80.03 ± 3.08 and 85.71 ± 1.75 %, respectively (Fig. 2b).

The stem length was longer twice in A-C than M-C (66.22 mm). On the other hand, the stem length was decreased gradually (Fig. 1a and b) with stress intensity from 132.1 in A-C to 95.78 mm in A-10, and from 66.22 to 36.56 mm in M-C and M-10, respectively. The stem diameter of M-C (2.973 mm) was significantly ($P < 0.05$) higher by 47 % than A-C. The water stress increased significantly the stem diameter to 1.920 mm in A-10 and to 3.379 mm in M-10 (Fig. 1a and b).

Leaf area (cm²)

The results showed changes of the leaf area according to the hybrid and stress treatment (Fig. 2a). The higher leaf area is appeared in the plain- leafed hybrid (A-C) (28.04 ± 7.72 cm²) as compared to all other treatments. The leaf area has been affected significantly with water stress ($P < 0.05$). It decreased with increasing severity of stress to 15.35 ± 5.58 and 5.73 ± 4.10 cm² in A-10 and M-10, respectively.

Fig. 2 Average of leaf area (cm²) (a), the leaves carotene concentration (mg / g fresh weight) (b), the leaves anthocyanins concentration (mg / g fresh weight) (c) and the leaves vitamin concentration (mg / g fresh weight) (d) according to the treatment. Points and vertical lines represent the mean of 15 plants per treatment $\pm$ the standard error. Each point represents seven harvests. The different characters indicate the significant differences between the treatments at 95 % confidence level

Leaf carotenes concentration

Figure 2b shows the leaf carotenes concentration as affected by the hybrid and water treatments. The analysis of variance showed that leaf carotenes concentration of M-10 was higher significantly ($P < 0.05$) as compared to all treatments. The leaf carotenes concentration of M-C (0.0069 mg / g fw) was higher by 72 % ($P < 0.05$) than that of A-C (0.0050 mg / g fw). This variable was increased significantly ($P < 0.05$) with water stress to reach to 0.0059, 0.0069 and 0.0078 mg / g fw in A-50, A-30 and A-10, respectively, and to 0.0081, 0.0122 and 0.0156 mg / g fw in M-50, M-30 and M-10, respectively.

Leaf anthocyanins concentration

Changes of leaf anthocyanins concentration were observed according to the hybrid and water stress (Fig. 2c). While A-C and M-C had an approximate leaf anthocyanins concentration (0.015 mg / g fw) ($P > 0.05$), water stress affected relatively this criterion. In the plain- leaved hybrid, water stress led to a significant increase ($P < 0.05$) of about 2 and 0.7 folds of leaf anthocyanins concentration in A-10 and A-30, respectively, as compared to A-C. While in curly- leaved hybrid, water stress led to an increase of 4 and 5 folds in leaf anthocyanins concentration of M-30 and M-10, respectively, as compared to M-C.

Leaf vitamin C concentration

Leaf vitamin C concentration (Fig. 2d) was higher significantly ($P > 0.05$) in M-10 as compared to all treatments

except M-30. The variance analysis between the hybrids showed significant differences between M-C (2.28 ± 0.09 mg / g fw) as compared to A-C (1.9 ± 0.11 mg / g fw). Water stress increased leaf vitamin C concentration in both hybrids. Significant increases ($P < 0.05$) of about 1.5 folds were observed in A-50 and M-50 as compared to the controls.

Chlorophyll concentration

Changes of leaf chlorophyll concentration were observed according to the hybrid and water stress (table 1). Significant differences ($P < 0.05$) were appeared between M-C treatment, which had higher leaf chlorophyll concentration (0.0263 ± 0.0019 mg / g fw) as compared to all treatments except M-30. Water stress affected relatively this criterion. In the plain- leaved hybrid, water stress led to a significant

Table 1 The leaf chlorophyll concentration (mg/g FW). The values are the means of 20 plants per treatment for seven harvests $\pm$ the standard error. The different characters indicate the significant differences between the treatments at 95 % confidence level

Treatment	Leaf chlorophyll concentration
A-10	0.0133 ± 0.0018 ^{de}
A-30	0.0120 ± 0.0039 ^{de}
A-50	0.0131 ± 0.0012 ^e
A-C	0.0173 ± 0.0025 ^{cd}
M-10	0.0186 ± 0.0036 ^{bcd}
M-30	0.0229 ± 0.0022 ^{ab}
M-50	0.0213 ± 0.0020 ^{bc}
M-C	0.0263 ± 0.0019 ^a

decrease ($P<0.05$) of 75 % in leaf chlorophyll concentration, regardless the water stress intensity, as compared to the control. While in curly-leaved hybrid, water stress led to a decrease of 40 % in the leaf chlorophyll concentration in M-30 and M-50 and of 45 % in M-10 as compared to the control.

Correlation matrix of the variables studied

The relationships between the parameters studied in this research were estimated. At first, the models were assessed for each treatment separately, and then the variance analysis between the models was achieved according to Wallach (2006). Since no significant differences were observed, it was possible to combine all treatments in one model. Of the most important relationships, there are three linear models; (i) between leaf area and fresh weight (Fig. 3a) ($R^2=0.91$), (ii) between fresh and dry weight of leaves (Fig. 3b) ($R^2=0.81$) and (iii) between leaf area and dry weight (Fig. 3c) ($R^2=0.81$).

Stem diameter (2r) versus stem length (L) was plotted as a double logarithmic scatter (Fig. 4), because the stem volume (V) is proportional to the square of the stem diameter and to the stem length ($\pi r^2 L$) (Fig. 4 inserted). In spite of its components variations, no significant differences were observed in the stem volume, not only for hybrid but although for water stress.

Discussion

Comparison of the hybrids

The results of this research showed the difference between growth, productivity and quality of parsley plants. Although

Fig. 3 Correlation between leaf area and fresh weight (a), dry and fresh weight (b) and dry weight and leaf area (c) for all treatments

Fig. 4 Relation logarithmic between stem length and diameter for all treatments. The inserted figure represents the evolution of the stem volume according to the hybrid and water treatment

Kmiecik and Lisiewska (1999) have indicated the significant differences between the productivity of plain and curly-leaved hybrids of parsley planted in northern Europe, Petropoulos et al. (2008) found that the cultivation of such hybrids in Mediterranean basin did not lead to any differences in productivity. In our study, significant differences in the productivity and quality parameter have been shown between the two types of parsley hybrids.

Water stress effect on growth and quality

The parsley is considered a sensitive plant to low soil moisture (Mordy and Atta 1999). Although it is one of yield parameter, leaves number (plastochron) did not vary under stress. The effect of stress in the plastochron differs according to the plant.

While it was not affected in wheat (Grieve et al. 1994; Jerônimo et al. 2005) and lettuce (Scholberg et al. 2000), it was decreased in purple basil (Gamalei 2002; Moeini Alishah et al. 2006) and tomato (Najla et al. 2009). The other plant yield parameters (leaves area, fresh and dry weight, as well as stem length and diameter reduction) were reduced under water stress. Stomatal closure is often invoked as the main cause for photosynthesis decline under water stress (Alarcon et al. 1994; Romero-Aranda et al. 2001; Xu et al. 1999; Yeo et al. 1991). However, CO₂ uptake may be limited also by non-stomatal limitations (reduced mesophyll conductance, photochemical and enzymatic limitations), especially when water stress persists for long periods (Flexas et al. 2006; Peguero-Pina et al. 2008).

Calculating the stem volume showed how the plant regulates this parameter by increasing one of its components (stem length) and decreasing the other (stem diameter) as a way of acclimation. Moreover, the small size of the leaves means that their ability to intercept light and thus their photosynthetic efficiency is reduced, that is reflected on the plants growth (Gamalei 2002).

Some studies attributed parsley popularity due to its high concentration of β -carotene and vitamin C (Rubatzky et al. 1999). Parsley quality (fresh and dry weight, chlorophyll, anthocyanin, carotene and vitamin C concentrations) varied with hybrid and water stress. Previous studies on the leafy vegetables showed a significant decrease in leaves chlorophyll and carotene concentrations (Misra and Srivastava 2000) under water stress. Water stress hurts the chloroplasts, which directly leads to a decrease for chlorophyll (Aspinall and Paleg 1981). It must be pointed that β -carotene is found in chloroplasts (Wahid et al. 2007) which suggests a correlation between the pigment of chlorophyll and carotene. The chlorophyll concentration, in this study, was lower 4 folds as compared to *Pelargonium odoratissimum* (Khalid et al. 2010) which ranged between 0.15 to 0.09 mg / g fw according to water treatment. The value of β -carotene concentration in our study (0.003 and 0.02 mg / g fw) was less that cited by the USDA. Such difference could be attributed to the method used for analysis.

In the other hand, there is growing evidence that anthocyanins may serve a dual role as antioxidants in leaves (Neill et al. 2002; Steyn et al. 2002), therefore many studies considered often the anthocyanin accumulation as a visible sign of stress (Steyn et al. 2002). The anthocyanin concentration has been found to range from 2 to 40 mg/100 g FW according to species, cultivars, environmental conditions and plant age (Rodriguez-Saona et al. 1998; Steyn et al. 2002). This value agrees with that observed in our study.

Vitamin C is one of the antioxidants that suppress the ROS (reactive oxygen species) induced by stress (Murshed et al. 2008) and plays a key role in cell division and expansion (Kato and Esaka 1999; Smirnov 1993). It is increased with stress (Devarshi and Khanna-Chopra 2006; Nayyar and Gupta

2006). In our study, the vitamin concentration value agreed with that reported in previous study for parsley (123–165 mg/100 g FW) (Mordy and Atta 1999; Rubatzky et al. 1999).

Conclusions

It should be pointed out that the curly-leafed parsley demonstrated higher quality (concentration of fresh and dry matter, chlorophyll, anthocyanin, β -carotene and vitamin C) than plain leafed which had higher yield (leaf area, fresh and dry weight and stem length and diameter). Moreover, water stress improved quality characters and decreased yield.

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