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PHYSIOLOGICAL STUDY OF VARIOUS CULTIVARS OF *PUNICA GRANATUM* (L.) WITH AN EYE TO ACCLIMATIZATION TO ROMANIA'S ENVIRONMENTAL CONDITIONS

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Abstract

The phytotherapeutic and economic importance given to Punica granatum (L.) *in the last decades indicates the rebirth of this species. In Romania, the species is barely known by farmers and hobby growers, while the pomegranate products are widely consumed. This research is supposed to ensure the physiological data that will be the basis for the development of pomegranate crop technology for eight P. granatum cultivars that were introduced for acclimatization in the experimental fields of University of Agronomic Sciences and Veterinary Medicine of Bucharest. In this study, physiological processes are described, insisting on photosynthesis, transpiration and respiration in interrelation with the chlorophyll content, water and dry matter content existing in leaves. The measurements were performed during the main growth and flowering phenophases, starting late March 2016. The results showed a connection between the photosynthesis process, leaf dry matter and leaf chlorophyll content, transpiration process and leaf water content, respiration process and the flowering phenophase (in some cultivars, not in all).*

Key words*: phenophase, photosynthesis, respiration, transpiration, chlorophyll.*

INTRODUCTION

Punica granatum (L) is a fruit-bearing shrub, belonging to the Punicaceae family, having its origins in Middle East. From its origin, considered now Iran and Afghanistan, the pomegranate spread east to India and China and west to Mediterranean countries such as Turkey, Egypt, Tunisia, Morocco, Greece, Italy and Spain. It is assumed that Spanish missionaries brought the pomegranate to the American continent in the 1500's (Hodgson, 1917; LaRue, 1980).

In Romania, the shrub can be cropped especially in the areas with a warm climate such as Dobrogea and Banat.

Pomegranate is the symbol and heraldic device of the ancient city of Granada in Spain, from which the city gets its name. The genus name, *Punica*, was the Roman name for Carthage, where the best pomegranates were known to grow. Pomegranate is known by the French as grenade, the Spanish as granada, and literally translates to seeded ("granatus") apple ("pomum") (Jurenka, 2008).

In the past decade, numerous studies on the antioxidant, anticarcinogenic, and anti-inflammatory properties of pomegranate constituents have been published, focusing on treatment and prevention of cancer, cardiovascular disease, diabetes, bacterial infections or antibiotic resistance (Jurenka, 2008).

Considering lack of water resources and intensification of abiotic stresses such as drought and salinity, importance of pomegranate has increased in recent years, since this species is a tolerant fruit crop and thrives well under arid and semi-arid climatic conditions (LaRue, 1980; Jamali and Bonyanpour, 2017).

The *P. granatum* shrubs have an effective control of the plant water status by reducing transpiration due to stomatal closure and maintaining a consistent maximum stem with changes in evaporative demand (Intrigliolo et al., 2011; Parvizi et al., 2016).

This species can not be cropped only in regions with water deficit, but in seasonally wetlands as well.

The photosynthetic rate, as well as transpiration and respiration processes depend on various parameters like chlorophyll content, leaf water and dry matter content, abiotic factors (temperature, light intensity, humidity), plant age or plant phenophases (Burzo et al., 2005).

The present research, whose results are recorded in the writing, aims to thoroughly fathom the physiological and biochemical particularities of certain *P. Granatum* cultivars of different origins, more precisely to compare certain physiological parmeteres during the species' vegetation period, informations that will allow the crop technology acquaintance for this species' acclimatization to Romania's pedo-climatic conditions.

MATERIALS AND METHODS

Plant material was represented by eight *P. granatum* cultivars*: Hicaz, Kandahar, Nikitski ranni, Echen, Mollar, Shahvar, Dolce* and *Local selection* cultivar.

The botanical characterization of the selected *P. granatum* cultivars was based on macroscopic (visual) observations made directly in the study field.

Thus, the studied plants are characterized as deciduous shrubs, with a gray-brown bark, that grow between 0.7 and 1 meter tall and have 3 to 5 branches with twigs of a length between 3 and 60 cm.

The leaves are simple, petiolated, entire, lanceolate, oposite, coriaceous and have a length of 3 to 7 cm and a width of 1 to 2 cm.

The plant assortment lies on a 60 m^2 surface and comprise a total of 13 plants of kindred age (aprox. 6 years old), originated in Europe and Middle East, planted at a 2.5 m distance between plants and 2.7 m distance between the rows (also, there is no plant support system) in the experimental field belonging to Fruit Growing Department of the Faculty of Horticulture.

The measurements were performed during the main growth and flowering phenophases, on similar leaves belonging to the middle level of the analyzed plants, starting late March 2016 (up until late June 2016), 3 times a week - for the phisyological processes - between 9:00 am and 10:00 a.m, at a light intensity with values between 1083 and 1586 µmol/CO_2/m^2/s and an air temperature of 17.5 up to 19.7°C.

Also, the analysis of photosynthetic pigments and total water and dry matter content were completed twice a month, the determination of photosynthetic pigments was made using the leaves belonging to the upper level (young leaves) of the *P. granatum* plants taken in study.

Photosynthesis as well as transpiration and respiration process intensities were determined with the LCA-4 electronic analyser and expressed in µmol/CO_2/m^2/s (photosynthesis and respiration) and mmol/H_2O/m^2/s (transpiration).

The photosynthetic pigments content was determined using 80% acetone extraction and by colorimetry at wavelengths of 470 nm, 663 nm and 646 nm. The results were calculated using the Lichtenthaler & Wellburn's formula (1983) and the results obtained expressed in mg/100g fresh weight (FW).

The total water and dry matter content were determined through gravimetric analysis. And expressed in percentages.

RESULTS AND DISCUSSIONS

The intensity of the Photosynthesis process in *P. granatum* leaves during the Growth Phenophase

The maximum photosynthetic rate is recorded, for most species, before growth cessation of the leaves, when they reached 37 - 90% of their foliar surface (Burzo et al., 2004).

From Table 1 data analysis, it is observed that the photosynthesis process recorded similar values at *Echen* and *Mollar* cultivars (8.26 µmol/CO_2/m^2/s). They carried out the photosynthesis process with the highest intensities, 1.30 times higher than *Hicaz* and *Dolce*, 1.23 times higher than *Kandahar*, 1.94 times higher than *Shahvar* and 1.51 times more intense than *Local selection* cultivar.

Table 1. The intensity of the Photosynthesis process in *P. granatum* leaves during the Growth Phenophase

Cultivar	Light intensity (µmol/m^2/s)	Temperature (°C)	Photosynthesis intensity (µmol/CO_2/m^2/s)
Hicaz	1245	18.0	6.32
Kandahar	1189	18.1	6.68
Nnikitski ranni	1204	17.7	8.01
Echen	1153	17.5	8.26
Mollar	1083	18.6	8.26
Shahvar	1073	19.0	4.25
Dolce	1126	17.7	6.32
Local selection	1119	17.8	5.45

Nikitski ranni also recorded a high photosynthetic rate of 8.01 µmol/CO_2/m^2/s.

The *Shahvar* cultivar, during the growth period, carried out the photosynthesis process with the lowest intensity, compared to the other studied cultivars, namely 4.25 µmol/CO_2/m^2/s.
Of note were *Hicaz*, *Dolce* and *Kandahar* cultivars, whose photosynthesis processes were carried out with related intensities, the values being between 6.32 and 6.68 µmol/CO_2/m^2/s.
Of the obtained data, it was found that the photosynthesis process was influenced by the type of cultivar taken in study, representing an internal factor that the specialty literature signals in modifying dynamics of the photosynthesis process (Burzo et al. 2005; Dobrescu, 2007; Bădulescu, 2016).

The intensity of the Photosynthesis process in *P. granatum* leaves during the Flowering Phenophase

During the appearance of the first flowers at *Hicaz*, *Kandahar*, *Nikitski ranni* and *Local Selection* cultivar, the photosynthesis process was carried out with a high intensity in all studied cultivars.
The highest photosynthesis process intensity was determined at *Hicaz*, its value being 11.58 µmol/CO_2/m^2/s, followed by the *Echen*, 10.33 µmol/CO_2/m^2/s and *Nikitski ranni*, 9.71 µmol/CO_2/m^2/s (Table 2).

Table 2. The intensity of the Photosynthesis process in *P. granatum* leaves during the Flowering Phenophase

Cultivar	Light intensity (µmol/m^2/s)	Temperature (°C)	Photosynthesis intensity (µmol/CO_2/m^2/s)
Hicaz	1402	18.8	11.58
Kandahar	1514	19.7	8.37
Nnikitski ranni	1415	18.2	9.71
Echen	1425	19.5	10.33
Mollar	1505	18.5	9.03
Shahvar	1498	18.2	5.19
Dolce	1567	19.1	7.86
Local selection	1586	18.5	9.16

The lowest intensity was recorded at *Shahvar* cultivar (5.19 µmol/CO_2/m^2/s).
There was recorded an increase of the photosynthesis process during flowering phenophase, compared to growth phenophase, thus: at the flowering phenophase moment, the *Hicaz* cultivar intensified its photosynthesis process 1.83 times, *Kandahar* 1.25 times, *Nikitski ranni* 1.21 times, *Echen* 1.25 times, *Mollar* 1.09 times, *Shahvar* 1.22 times, *Dolce* 1.24 times and *Local selection* cultivar 1.44 times.
The *Hicaz* cultivar (with the highest photosynthetic rate) carried out the photosynthesis process with an intensity of 1.38 times higher than *Kandahar*, 1.19 times higher than *Nikitski ranni*, 1.12 times higher than *Echen*, 1.28 times higher than *Mollar*, 2.23 times higher than *Shahvar*, 1.46 times higher than *Dolce* and 1.29 times higher than *Local Selection*.

Total leaf Chlorophyll content of *P. granatum* during the Growth Phenophase

Following the chlorophyll pigment analysis, the highest content in total leaf chlorophyll was found at *Nikitski ranni* cultivar, 38.78 mg/100g FW, followed by *Echen* cultivar, 28.14 mg/100g FW (Table 3).

Table 3. Total leaf Chlorophyll content of *P. granatum* during the Growth Phenophase

Cultivar	Total Chlorophyll (mg/100g FW)
Hicaz	23.29
Kandahar	26.41
Nnikitski ranni	38.78
Echen	28.14
Mollar	22.80
Shahvar	24.44
Dolce	22.09
Local selection	26.38

Regarding these 2 cultivars, there was a relevancy between the total leaf chlorophyll content and the intensity of the photosynthesis process. Therefore, the photosynthesis processes of these cultivars showed the highest intensity, given the highest content of chlorophyll (Tables 3, 1).
As for *Hicaz*, *Kandahar*, *Mollar*, *Shahvar*, *Dolce* and the *Local Selection* cultivars, there was no interdependence between the total leaf chlorophyll content and the intensity of the photosynthesis processes.

Total leaf Chlorophyll content of *P. granatum* during the Flowering Phenophase

During the flowering phenophase, it was noted a different accumulation of chlorophyll pigments of the analyzed cultivars. The highest values were shown at *Kandahar* (which flourished), *Nikitski ranni* (also flourished) and *Shahvar*, and the lowest were recorded at *Echen* (Table 4).

Table 4. Total leaf Chlorophyll content of *P. granatum* during the Flowering Phenophase

Cultivar	Total Chlorophyll (mg/100g)
Hicaz	27.33
Kandahar	30.89
Nnikitski ranni	32.27
Echen	22.90
Mollar	28.89
Shahvar	32.63
Dolce	28.15
Local selection	26.39

The *Hicaz*, *Dolce* and *Mollar* cultivars recorded a higher chlorophyll content during flowering phenophase, compared to growth phenophase, with the remark that *Dolce* and *Mollar* reported a similar increase rate of chlorophyll content. These cultivars showed a connection between the chlorophyll content and the intensity of the photosynthesis process. At the *Dolce* cultivar, the photosynthesis process intensified with the same rate that the chlorophyll content increased (1.25 times) (Tables 4, 2).

It is worth mentioning that during the flowering phenophase, the chlorophyll content of *Local selection* cultivar did not change compared to the growth phenophase and the photosynthesis process was not influenced by this biochemical indicator. Therefore, these two physiological parameters were not influenced by the phenophase at this cultivar.

The intensity of the Transpiration process in *P. granatum* leaves during the Growth Phenophase

Data analysis revealed an intense transpiration at *Echen* cultivar (4.82 mmol/H_2O/m^2/s), followed by *Shahvar* (4.58 mmol/H_2O/m^2/s), and *Mollar* (4.13 mmol/H_2O/m^2/s) (Table 5).

Referring to *Echen* cultivar, the intensity of transpiration process was: 1.41 times lower at *Hicaz*, 1.96 times lower at *Kandahar*, 2.49 times lower at *Nikitski ranni*, 1.16 times lower at *Mollar*, 1.05 times lower at *Shahvar*, 2.12 times lower at *Dolce*, and 1.32 times lower at *Local selection* cultivar.

The lowest transpiration intensity was recorded at *Nikitski ranni*, of 1.93 mmol/H_2O/m^2/s .

Analyzing the amount of water present in leaves and the intensity of transpiration process, it was observed that the *Echen* cultivar correlates the considerable quantity of water present in leaves with the high intensity of transpiration process (Table 5, 7).

The studied cultivars carried out the transpiration process with different values, albeit they showed a similar water content in their leaves. This occurrence is explained by the absorption of a distinct amount of light energy caused by the difference in leaf size of each analyzed cultivar (the light intensity directly influences the transpiration process through its caloric effect) (Burzo and Dobrescu, 2011; Dobrescu, 2007).

Table 5. The intensity of Transpiration process in *P. granatum* leaves during the Growth Phenophase

Cultivar	Light intensity (µmol/m^2/s)	Temperature (°C)	Transpiration intensity (mmol/H_2O/m^2/s)
Hicaz	1245	18.0	3.30
Kandahar	1189	18.1	2.45
Nnikitski ranni	1204	17.7	1.93
Echen	1153	17.5	4.82
Mollar	1083	18.6	4.13
Shahvar	1073	19.0	4.58
Dolce	1126	17.7	2.27
Local selection	1119	17.8	3.64

The intensity of the Transpiration process in *P. granatum* leaves during the Flowering Phenophase

During the flowering phenophase, according to specialty literature data, plant water requirements are higher and stimulate water absorption at the root level, which enhances the transpiration process (Burzo, 2016; Burzo et al., 2005; Dobrescu, 2007).

The transpiration process was carried out with a distinct intensity from one cultivar to another: the highest transpiration intensities, with values between 5.15 and 5.56 mmol/H_2O/m^2/s were recorded at *Shahvar* and *Mollar* cultivars, followed by *Echen* and *Local Selection* cultivars of 4.34 and 4.90 mmol/H_2O/m^2/s.

The lowest values were registered at *Nikitski ranni* and *Hicaz* of 3.50 and 3.60 mmol/H_2O/m^2/s (Table 6).

Table 6. The intensity of Transpiration process in *P. granatum* leaves during the Flowering Phenophase

Cultivar	Light intensity (µmol/m²/s)	Temperature (°C)	Transpiration intensity (mmol/H_2O/m²/s)
Hicaz	1402	18.8	3.60
Kandahar	1514	19.7	4.12
Nnikitski ranni	1415	18.2	3.50
Echen	1425	19.5	4.34
Mollar	1505	18.5	5.56
Shahvar	1498	18.2	5.15
Dolce	1567	19.1	3.92
Local selection	1586	18.5	4.90

The transpiration process was strongly influenced in its development by the phenophase, stating that in all cultivars the transpiration intensity increased during the flowering phenophase, compared to growth phenophase, for example 1.12 times, respectively 1.34 times at *Shahvar* and *Mollar* cultivars.
High transpiration values during the flowering phenophase can be explained due to both high light intensity and high temperature, external factors that directly influence this process.

Total leaf water and dry matter content of *P. granatum* during the Growth Phenophase

During growth phenophase, the highest water content was determined at *Echen* cultivar, of 68.18%, which was 1.05 times higher than the other studied cultivars (Table 7).

Table 7. Total leaf water and dry matter content of *P. granatum* during the Growth Phenophase

Cultivar	Water content (%)	Dry matter content (%)
Hicaz	64.88	35.12
Kandahar	65.62	34.38
Nnikitski ranni	64.40	35.60
Echen	68.18	31.82
Mollar	65.89	34.11
Shahvar	65.71	34.29
Dolce	62.87	37.13
Local selection	64.55	35.45

At *Echen*, the leaf water content had positively influenced the process of photosynthesis which recorded the highest intensity (it is known that the water represents a key matter for the photosynthesis process) (Tables 7, 1).

Also, at *Echen* cultivar, it was recorded a high transpiration intensity, this process being conditional on the leaf water content (Tables 7, 5).
The *Dolce* cultivar showed the lowest leaf water content, of 62.87%, compared to the other analyzed cultivars. It was noted that, for this cultivar, the low leaf watter content diminished the intensity of photosynthesis and transpiration processes (Tables 7, 1, 5).
The rest of the studied *P. granatum* cultivars showed an approximately equal leaf water content, with values between 64.8% and 65.8%.
The highest leaf dry matter content was registered at *Dolce* cultivar, of 37.13%, followed by *Nikitski ranni* and "*Local Selection*".
It was found that the *Echen* cultivar, whose leaves had the lowest dry matter content, of 31.82%, can not be explained because the intensity of the photosynthesis process was higher (it is known that photosynthesis process leads to the accumulation of leaf dry matter) (Tables 7, 1).
Data obtained showed that the *Kandahar*, *Mollar* and *Shahvar* cultivars had a similar leaf dry matter content, therefore this indicator does not allow the correlation with the photosynthesis process, process that presented fluctuations of values for each individual studied cultivar (Tables 7, 1). It is considered that part of leaf dry matter resulted from photosynthesis was translocated to growing vegetative organs.
The accumulation of dry matter in plant organs is mostly the result of the photosynthesis efficiency. Also, the amount of dry matter in *P. granatum* leaves varies, depending on the cultivar type, cultivar age, or the leaves position on the branches.

Total leaf water and dry matter content of *P. granatum* during the Flowering Phenophase

During the flowering phenophase, the leaf water content of *P. granatum* cultivars showed a decreasement of approximately 1.04 times at all studied cultivars compared to the growth phenophase (Table 8).
Also, it was found that the leaf water content did not influence the transpiration process which registered different intensities from one cultivar to another (Tables 8, 6).

Table 8. Total leaf water and dry matter content of *P. granatum* during the Flowering Phenophase

Cultivar	Water content (%)	Dry matter content (%)
Hicaz	62.59	37.41
Kandahar	64.26	35.74
Nnikitski ranni	58.11	41.89
Echen	61.15	38.85
Mollar	63.27	36.73
Shahvar	62.53	37.47
Dolce	61.60	38.40
Local selection	63.18	36.82

At *Hicaz*, *Kandahar* and *Local Selection*, the leaf water content had values between 62.59% and 64.26%, lower than the ones registered during the growth phenophase. These values are in interrelation with the high intensities of the transpiration process (some of the water quantity present in leaves was eliminated) (Table 8, 6).

The lowest leaf water content was determined at *Nikitski ranni*, of 58.11%.

During the flowering phenophase, the highest leaf dry matter content was recorded at *Echen* cultivar, which was 1.22 times higher compared to growth phenophase. Also its photosynthesis process intensified 1.25 times (probably, the biosynthesized organic substance following the photosynthesis process was present in the dry matter content) (Tables 8, 2).

Withal, the leaf dry matter content of *Hicaz, Kandahar, Nikitski ranni, Mollar, Shahvar, Dolce, Local selection* registered an increasement, compared to the growth phenophase, explained by a high photosynthesis process intensity (Tables 8, 2). Yet these cultivars' leaf dry matter content was 1.15 times lower that *Echen*'s.

Therefore, this biochemical indicator can characterize and differentiate the analyzed cultivars, influencing their metabolism and, the degree of acclimatization.

The intensity of the Respiration process in *P. granatum* leaves during the Growth Phenophase

During the growth phenophase, the respiration process was carried out with different intensity values at the analyzed cultivars: between -2.46 (*Mollar*) and -3.36 μmol/CO_2/m^2/s (*Shahvar*) (Table 9).

Table 9. The intensity of Respiration process in *P. granatum* leaves during the Growth Phenophase

Cultivar	Light intensity (μmol/m^2/s)	Temperature (°C)	Respiration intensity (μmol/CO_2/m^2/s)
Hicaz	1245	18.0	- 2.77
Kandahar	1189	18.1	- 2.94
Nnikitski ranni	1204	17.7	- 3.18
Echen	1153	17.5	- 2.59
Mollar	1083	18.6	- 2.46
Shahvar	1073	19.0	- 3.36
Dolce	1126	17.7	- 3.13
Local selection	1119	17.8	- 3.06

The lowest respiration intensity was registered in *Mollar* cultivar leaves, that also had the slightest biometry (based on macroscopic observations).

The *Shahvar* cultivar had the highest respiration process intensity.

The biochemical energy requirement for biosynthesis of organic substances involved in the growing process made the *Shahvar* cultivar have the largest biometry (based on microscopic observations).

The intensity of the Respiration process in *P. granatum* leaves during the Flowering Phenophase

During the flowering phenophase there was a difference in the floral evocation of the analyzed cultivars, as follows: *Hicaz*, *Kandahar*, *Nikitski ranni* and *Local selection* cultivar were characterized by the appearance of the first flowers while the other cultivars were not.

It was revealed that the respiration process had higher intensities in flowery cultivars compared to the ones whose floral evocation did not happen (Table 10).

Analyzing the cultivars in terms of metabolism, namely the photosynthesis and respiration values, representing the anabolic side, respectively the catabolic side, it was found that the early flowery cultivars had a more intense metabolism compared to those of which the flowering process did not manifest during the determination.

Table 10. The intensity of Respiration process in *P. granatum* leaves during the Flowering Phenophase

Cultivar	Light intensity ($\mu mol/m^2/s$)	Temperature (°C)	Respiration intensity ($\mu mol/CO_2/m^2/s$)
Hicaz	1402	18.8	- 3.75
Kandahar	1514	19.7	- 4.85
Nnikitski ranni	1415	18.2	- 4.50
Echen	1425	19.5	- 3.55
Mollar	1505	18.5	- 2.88
Shahvar	1498	18.2	- 3.56
Dolce	1567	19.1	- 3.61
Local selection	1586	18.5	- 4.04

CONCLUSIONS

The *Hicaz*, *Nikitski ranni*, *Echen*, *Mollar* cultivars are the first ones that entered the vegetative period, justifying a more intense metabolic reactions during the research.

Only *Hicaz*, *Nikitski ranni*, *Kandahar* and *Local Selection* cultivars registered the floral evocation during the research, marking the beginning of acclimatization process

Regarding *P. granatum* species, the physiological processes and biochemical parameters are directly influenced by the type of cultivar and by the phenophase.

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