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Ecophysiological aspects of some sweet cherry cultivars from North East Romania

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Abstract: The physiological performance, growth and competitive ability of fruit trees are increasingly affected by the effects of global climate change, very different at a regional level, which mainly causes considerable changes in water availability. The purpose of this study was the evaluation of three sweet cherry cultivars from the Research Station for Fruit Growing (RSFG) Iași, Romania under the climatic conditions of 2022–2023 by performing physiological investigations into the water regime and the transpiration process through the stomatal conductance, the water content of the leaves as well as their dehydration rate after 24 hours. The obtained data were statistically interpreted taking three experimental factors into account: factor A consisted of three distinct phenological stages (full flowering, fruit about 80% of the final size and fruit ripening); factor B constituted by the three cultivars ('Van', 'Andreiaș' and 'Margonia'); and factor C was constituted by the canopy area from the samples (internal and external). The experimental variants statistically interpreted by Duncan's test ($P \leq 0.05$) registered significant differences. The Pearson correlation coefficient (R^2) between the measured variables obtained positive distinctly significant values of $R^2 = 0.686$ (with the stomatal conductance) and negative distinctly significant values of $R^2 = -0.874$ (with the water content). The obtained results will support the development of predictive models for different irrigation and breeding strategies to improve the sweet cherry production in temperate continental climates.

Keywords: chlorophyll; gas exchange; *Prunus avium* L.; transpiration; water regime

The sweet cherry (*Prunus avium* L.) is a highly valuable fruit tree crop, highly appreciated by consumers. Its cultivation requires smaller amounts of water than other fruit trees (Montiel et al. 2010), which is an important factor in areas where water is often a limiting factor for production. The sweet cherry tree is a rustic fruit crop which finds optimal conditions in Romania to manifest its agrobiological potential (Iurea et al. 2019). The agroclimatic projection for the coming years is expected to show that there will be an increase in the average temperature with drought events occurring in the phenological stages of high evapotranspiration demand, irregular precipitation and a decrease in the groundwater availability of fruit crops (Blaya Ros et al. 2020; Bhattacharjee et al. 2022). In Romania, there are areas where there has been a linear increase in the average annual temperature from 10.5 °C in 2005 to 12.4 °C (2007 and 2012). Compared to the average temperature of the period 1960–1990

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(10.6 °C), the average annual temperature has increased by + 1.8 °C during the March-April period (Cosmulescu, Gruia 2016; Paltineanu, Chitu 2020). The increased variability of annual and seasonal extreme temperatures and uneven precipitation during the growth season in the global context of climate change in the regions of Romania was also noted by Chitu et al. (2015).

The sweet cherry precipitation requirement can fluctuate depending on other factors (other climatic factors, rootstocks), between 550 mm and 1 000 mm of precipitation is required per year (Neilsen et al. 2014); an adequate water supply is imperative during the flowering and fruit development phenology stages, as well as in the post-harvest period (Predieri et al. 2003). For the sweet cherry, low water availability has a visible effect of reducing the tree vigour, slowing the shoot growth, premature tissue ageing, shortening the tree growth and development stages, having weaker fruit bud differentiation, causing irregularities of production and fruit size and causing physiological imbalances with a pathogenic aspect (Quero-Garcia et al. 2007). Physiological changes occurring at the cellular level cause a reduction in the intensity of the transpiration and photosynthesis process, as well as in the accumulation of soluble dry matter.

Monitoring the plant water stress indicators is considered to better integrate the environmental conditions, plant water content, and the evaporative demand to which it responds. Although its measurement cannot be automated, the leaf stomatal conductance represents the flow of water through the plant and the plant's response to water use (Blanco et al. 2018). The withering of the leaves reduces the photosynthetic activity of the plants and the negative effects are highlighted by the reduction in the transpiration, photosynthesis, the opening of the stomata, and thus in the reduction in the CO_2 intake (Jităreanu et al. 2009). The stomata have major influences on plants, the ecosystem productivity and drought tolerance. Depending on the climatic factors, but also on the species, cultivar, phenological stage and canopy type, the leaves of trees with a greater open stomatal pore area have higher stomatal conductance and higher rates of photosynthetic CO_2 assimilation and water removal through transpiration (Henry et al. 2019). In the sweet cherry, being a non-climacteric stone fruit tree species, as well as in other early cultivars of the genus *Prunus*, whose fruits develop rapidly, phenological stage II is indistinguishable and or even overlaps stages I and III. For this reason, it is not recommended to have a water deficit at any stage of fruit growth in the early and extra-early cultivars, where the physiological indicators need to be frequently monitored (Marsal et al. 2010).

Sweet cherry trees are characterised from the rainfall point of view by their significant sensitivity to water stress during pre-harvest, when fruit growth can be slowed (Blaya Ros et al. 2020). The leaf, being the main organ of plant photosynthesis, provides almost the entire metabolite requirement for the plants. Water stress varies according to the cultivar, tree age, phenophase and organ (Burzo, Dobrescu 2011). Considering that, in the soil-plant-atmosphere relationship, the plant represents an intermediate system located in a degree of water potential between the soil and the atmosphere, any measurement of a plant's water status indicators will inevitably depend on the water status of the soil and air and it is worth evaluating over the long term (Blanco et al. 2018).

The main objective of this study was to evaluate the water regime of some sweet cherry cultivars without an irrigation system. For this, the seasonal variation of some physiological water indicators such as, stomatal conductance, leaf water content and dehydration rate, were followed in three distinct phenological stages as well as in different areas of the canopy. To help achieve these objectives, the physiological response of the studied sweet cherry trees in the climatic conditions of North-Eastern Romania was characterised over a period of two years in order to recommend the best technological and crop productivity strategy in areas in which irrigation is not possible.

MATERIAL AND METHODS

Description of the study site. The study was carried out in the North-East of Romania, in Iași county (47°20'N; 27°60'E), in the experimental field of the Research Station for Fruit Growing (RSFG) Iași, where the natural relief was a sloping plateau with a predominantly cambic chernozem soil with good natural drainage. The climatic conditions during the period of the experiment were obtained by means of the meteorological system AgroExpert. In the period 1990–2020, the average annual temperature was 10.6 °C and the precipitation quantified an average amount of 543.2 mm. In the year 2022, the average annual temperature recorded was 11.4 °C, +1.7 °C higher than the multiannual average (1961–2011), and the total precipitation had a value of 440 mm with a deviation of –77.5 mm from the multiannual value.

Biological material. The research was conducted during the years 2022–2023 using, as the biological material, three cultivars of sweet cherry: 'Van' (native from Canada, with parentage: 'Empress Eugenie'× unknown), 'Andreiaș' (obtained through the controlled hybridisation of 'HC 24/4' × 'Boambe de Cotnari' at Iasi, North-East of Romania) and 'Margonia' (obtained through open pollination of 'Van' at Iasi, North-East of Romania) existing in the germplasm fund from RSFG Iași-Romania. The trees in this experimental lot were grafted onto high-vigour *Prunus mahaleb* L. seedlings as the rootstock and were planted at a distance of 5 × 4 m without support or an irrigation system. The trees were planted in 2014 and were formed as a free palmette flattened in the direction of the row, with a canopy maintained at an average diameter of 2.3 m and a maximum height of 2.5 m. Each cultivar had nine trees, was structured and studied in three repetitions with three trees per repetition.

Experimental procedures. Physiological determinations were carried out in three different phenological stages, according to the Biologische Bundesanstalt, Bundessortenamt and Chemical Industry (BBCH) scale (Meier, 2001) at 65 (full flowering); 78 (fruit about 80% of final size) and 89 (fruit ripening) and in different areas of the canopy (internal and external). The leaf samples were from both the inside and outside of the canopy, from randomly selected branches from a full height. For each repetition, ten readings and samples were taken.

In order to estimate the transpiration process and the rate of gas exchange through the stomata of the leaves, measurements of the stomatal conductance (gs) were carried out with a portable foliar porometer (Model SC-1, Decagon Devices Inc., Pullman, WA, USA) using the method of determining the flow of water vapour from the surface of the leaf in the atmosphere in the local conditions described by Pietragalla and Pask (2012). Ten readings were taken for each replicate, on leaves from separate shoots at hourly intervals between 09:00 and 19:00, in the three phenological stages. The porometer was calibrated following the instructions given in the manual at the beginning of each measurement session and repeated if there was an appreciable change in temperature and/or humidity, being recorded at each measurement.

The chlorophyll content was estimated using a non-destructive method with a portable chlorophyll meter (Konica Minolta SPAD-502Plus, Osaka, Japan). The SPAD index was determined by averaging twenty readings per sample. This instrument determines the relative quantity of chlorophyll present in the leaf tissue in the red and infrared regions (at a wavelength of 650 nm and 940 nm) (Yamamoto et al. 2002).

The conditions of the average temperature and humidity at the leaf level in the canopy where the stomatal conductance and chlorophyll content were performed were: in 65 BBCH (May 01), t °C = 20–22 °C and U% = 70%; in 75 BBCH (May 20), t °C = 25 °C and U% = 71.3% and in 89 BBCH (June 20), t °C = 26.5 °C and U% = 75.6%.

The relative water content (RWC) analysis of the sweet cherry leaves was performed by the laboratory oven drying method described by Zhang et al. (2012). Fresh leaves of each sample were weighed using an analytical balance and recorded as the FM (fresh matter), then dried at 80 °C for 2–72 hours. The weighed dry matter was recorded as DM (dry matter). The leaf water content was calculated with the equation: RWC (%) = (FM – DM)/ FM × 100.

The rate of foliar dehydration was performed at intervals of 1, 2, 3, 4 and 24 hours, determining the percentage of water lost in the first hour and at 24 hours at a constant controlled temperature set at 21 °C (Jităreanu et al. 2009). The water content of the plant leaves was calculated by the formula (1):

$$FD\ (\%) = \frac{x_{1-n}}{x_0} \times 100 \qquad (1)$$

Where: FD – foliar dehydration, %; x_0 – the first weighing of the leaf (g); x_{1-n} – leaf weight (g) after certain periods of time (one to 24 hours).

The statistical analysis was organised according to three experimental factors: factor A consisted of three distinct phenological stages according to BBCH: a1 – 65 (full flowering); a2 – 78 (fruit about 80% of final size); a3 –89 (fruit ripening), the B factor constituted the three cultivars (b1 –'Van'; b2 – 'Andreiaș' and b3 – 'Margonia') and the C factor constituted the canopy area, respectively c1 – internal zone and c2 – external zone. The data were interpreted statistically by the Duncan's multiple range test at $P \leq 0.05$, using SPSS software version 28.

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To estimate the relationship between the physiological determinations of the water regime and the amount of chlorophyll, Pearson's correlation coefficient (R^2) was also calculated.

RESULTS AND DISCUSSION

In the conditions of the environment in North-East Romania during the years 2022–2023, the stomatal conductance (gs) recorded in the sweet cherry during the vegetation period was 8.44–9.46 mol m/s on average (Table 1). Based on the results of the stomatal conductance (gs), an indicator of the gas exchange during the physiological process of transpiration, it was highlighted that the maximum values were recorded in both years of the study in the experimental variant a3b3c1, meaning in the fruit ripening phenophase (89 BBCH) by the 'Margonia' cultivar in the interior of the canopy. The minimum values were recorded in the phenophase of the fruit growth (75BBCH). The stomatal conductance response characteristics to the meteorological or physiological changes showed seasonal variations. The variability in the stomatal conductance increased in accordance with the humidity recorded at the leaf level.

The differences between the experimental variants are statistically significant, having values between a minimum of 6.60 mol m/s (in 2022 for the 'Andreiaș' cultivar, in the phenophase of fruit growth outside the canopy) and 17.84 mol m/s (also in 2022 year, but for 'Margonia' inside the canopy at fruit ripening). According to Yoon and Richter (1990), seasonal changes in stomatal conductance in fruit trees and stomatal responses to dehydration in cherry cultivars appear to be influenced by the fruit development and ripening. A fact highlighted in this case as well, in Figure 1A, where all the cultivars registered a visible increase in the sap flow during the ripening period (89BBCH).

The various values of the water status indicators in the sweet cherry trees are high depending on the environmental conditions, canopy architecture, soil and the variability of the cultivars (Blanco et al. 2019). Also, a small decrease in the stomatal conductance may reveal a protective mechanism against water and heat stress, allowing plant water conservation and improving the plant water use efficiency.

Table 1. Stomatal conductance and the chlorophyll content of the studied experimental variants (2022–2023)

The experimental variant	Stomatal conductance (mol m/s)		Chlorophyll content (SPAD units)	
	2022	2023	2022	2023
a1b1c1	7.75^{f}	8.65^{de}	24.44^{g}	21.39^{fg}
a1b1c2	7.45^{fg}	7.81^{efgh}	25.28^{fg}	23.07^{f}
a1b2c1	7.12^{fg}	8.35^{def}	25.28^{fg}	20.76^{fg}
a1b2c2	6.81^{fg}	7.28^{ghi}	27.16^{e}	23.31^{f}
a1b3c1	7.71^{f}	7.88^{efg}	23.81^{g}	17.21^{h}
a1b3c2	6.78^{fg}	6.70^{i}	24.35^{g}	19.48^{gh}
a2b1c1	7.09^{fg}	7.27^{ghi}	30.92^{d}	29.79^{de}
a2b1c2	6.85^{fg}	6.93^{i}	31.15^{d}	29.76^{de}
a2b2c1	7.17^{fg}	7.50^{fghi}	31.15^{d}	32.32^{cd}
a2b2c2	6.60^{g}	7.24^{ghi}	25.65^{efg}	31.42^{de}
a2b3c1	7.67^{f}	7.00^{hi}	30.29^{d}	30.09^{de}
a2b3c2	6.83^{fg}	6.98^{hi}	27.66^{e}	28.90^{e}
a3b1c1	14.83^{c}	10.88^{bc}	37.83^{a}	38.83^{a}
a3b1c2	10.58^{e}	8.20^{ef}	37.28^{a}	38.51^{a}
a3b2c1	16.79^{b}	11.43^{b}	33.60^{c}	35.09^{bc}
a3b2c2	12.43^{d}	9.14^{d}	34.94^{bc}	36.91^{ab}
a3b3c1	17.84^{a}	12.52^{a}	36.67^{ab}	37.11^{ab}
a3b3c2	11.91^{d}	10.25^{c}	37.03^{a}	37.39^{ab}

a1 – 65BBCH; a2 – 75BBCH; a3 – 89BBCH; b1 – 'Van'; b2 – 'Andreiaș'; b3 – 'Margonia'; c1 – canopy interior; c2 – canopy exterior; $^{a-i}$significant differences (Duncan's test, $P < 0.05$), SPAD – soil plant analysis development

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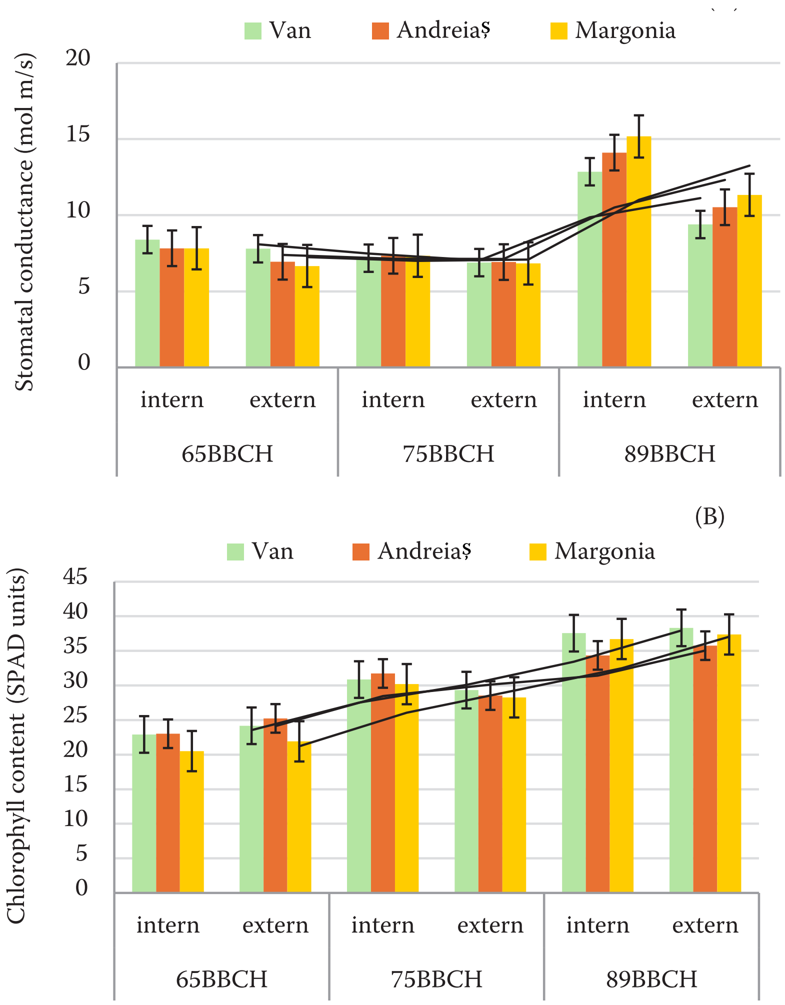

Figure 1. Stomatal conductance (A) and chlorophyll content (B) in the studied sweet cherry cultivars (2022–2023)

The variation between cultivars shows that the local 'Margonia' cultivar shows, on average, the highest conductance value during the season from bloom to ripening and, as for the area of the canopy, outside it, the flow of vapour is lower compared to the internal area (Figure 1A).

To determine the differences in the leaf chlorophyll concentration between the studied experimental variants, leaf Soil Plant Analysis Development (SPAD) readings were obtained (Table 1). The leaf SPAD readings showed that the chlorophyll content increased progressively with the unfolding of the monitored phenophases, from minimum values at flowering (23.81 SPAD units in 2022 and 17.21 SPAD units in 2023) to maximum values during the fruit ripening in the interior of the canopy (37.83–38.83 SPAD units). The highest chlorophyll content was recorded in the 2023 conditions with the 'Van' cultivar in the fruit ripening phenophase inside the canopy (Table 1).

Similar results with sweet cherry trees were obtained in other studies (but mostly in irrigated trees), where the highest SPAD values in the leaf during the fruit ripening phenophase were measured in the inner part of the trees (Moreno et al. 2001; Vosnjak et al. 2022). As in the study by Roper and Kennedy (1986), the chlorophyll content in the 65BBCH phenological stage indicates that sweet cherry leaves become photosynthetically competent early in their development and can thus supplement reserve carbohydrates for growth early in the season.

The seasonal variation in the chlorophyll content continuously increased starting with the flowering (Figure 1B), highlighting a decrease in the average values in the external part of the canopy. The 'Van' cultivar recorded the maximum values for this indicator.

The relative water content (RWC) of the sweet cherry leaves was measured to quantify the water status of the investigated genotypes (Table 2). According to the results, the RWC had values that varied from 61.56% (at 'Andreiaș' cultivar in the 89 BBCH phenophase at the exterior of the canopy in the 2023 condi-

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Table 2. Relative water content and dehydration rate (DR) of the leaves of the studied experimental variants (2022–2023)

The experimental variant	RWC (%)		DR (%)	
	2022	2023	2022	2023
a1b1c1	66.50^{abcde}	69.86^{abcd}	50.17^{abcde}	38.84^{de}
a1b1c2	65.97^{bcde}	69.36^{abcd}	47.42^{bcde}	42.55^{cde}
a1b2c1	67.51^{abcde}	70.16^{ab}	53.06^{abcd}	54.38^{a}
a1b2c2	66.45^{abcde}	70.62^{a}	54.58^{abc}	51.24^{ab}
a1b3c1	71.15^{a}	70.07^{abc}	57.20^{ab}	45.75^{bcd}
a1b3c2	70.70^{ab}	69.35^{abcd}	59.99^{a}	48.10^{abc}
a2b1c1	65.16^{cde}	68.51^{cd}	47.71^{bcde}	40.78^{de}
a2b1c2	65.54^{cde}	68.39^{d}	42.40^{def}	43.96^{cde}
a2b2c1	68.37^{abcd}	68.83^{bcd}	41.72^{defg}	39.97^{de}
a2b2c2	66.50a^{bcde}	69.25^{abcd}	42.84^{cdef}	41.07^{cde}
a2b3c1	69.24^{abcd}	69.36^{abcd}	42.85^{cdef}	37.52^{e}
a2b3c2	68.70^{abcd}	68.71^{bcd}	43.33^{cdef}	42.81^{cde}
a3b1c1	64.67^{cde}	62.30^{ef}	28.70^{gh}	42.85^{cde}
a3b1c2	63.94^{de}	61.83^{ef}	31.41^{fgh}	38.38^{e}
a3b2c1	62.36^{e}	62.27^{ef}	27.10^{h}	38.54^{e}
a3b2c2	62.64^{e}	61.56^{f}	27.12^{h}	29.13^{f}
a3b3c1	63.96^{cde}	63.46^{e}	44.36^{cde}	28.69^{f}
a3b3c2	64.71^{cde}	62.36^{ef}	38.42^{efgh}	27.72^{f}

RWC – relative water content; FD – foliar dehydration; a1 – 65BBCH; a2 – 75BBCH; a3 – 89BBCH; b1 – 'Van'; b2 – 'Andreiaș'; b3 – 'Margonia'; c1 – crown interior; c2 – crown exterior; $^{a–h}$significant differences (Duncan's test, $P < 0.05$)

tions) to 71.15% (at 'Margonia' cultivar in the flowering stage in the 2022 conditions). The statistical analysis revealed significant differences in the water content of the experimental variant leaves. Since the leaves' water content cannot fully characterise the water regime of the plants, the determination of the rate of dehydration during 24 hours on sweet cherry leaves was performed (Table 2). Therefore, the lowest water content was lost in the phenophase of fruit ripening over 24 hours (27.10% in 2022 and 27.72% in 2023).

This situation of the sweet cherry trees' water status in the fruit ripening phenophase (the leaves have a higher total dry matter content and the dehydration rate is much slower) was described by Yoon and Richter (1990) in that the last days of pre-harvest coincide with the beginning of the differentiation in the fruit buds and the maturation of the wood so that there is competition for the assimilates between the different physiological processes making the trees very susceptible to water deficit during this time. The ability to retain water during dehydration is an important strategy for plant tolerance to drought stress (Viljevac et al. 2013).

The relationship between the stomatal conductance (gs) and the chlorophyll content expressed in SPAD units for both years (average of 2022 and 2023) in the analysed experimental variants was significant, having a positive correlation coefficient of 0.6862 (Figure 2). The variability in the stomatal conductance depended directly on the content and function of the chlorophyll, the degree of dependence of the two indicators being significant.

The dependence of the stomatal conductance variability on the chlorophyll concentration increased proportionally with the seasonal change of the phenological stages, from flowering (where the leaf temperature was 20–22 °C and the humidity was 70%) to the time of fruit ripening (where the temperature was 26.6 °C and the humidity was 75.6%). These results indicate that, accounting for the seasonal weather changes in the plant's physiological properties, it is important in assessing the water, energy, and CO_2 fluxes between plants and the atmosphere.

Other studies indicate that during early leaf ontogeny, the chlorophyll content is also limited by associated light responses; whereas, as the leaves become mature, photosynthesis may be enhanced by the stomatal opening response, as evidenced by Roper and Kennedy (1986). The increase in CO_2 assimilation during leaf development could possibly be due

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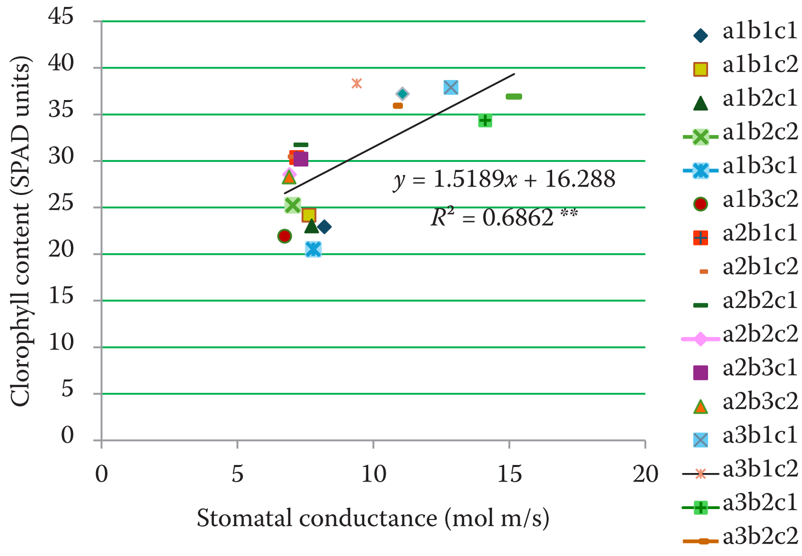

Figure 2. Dependence of the chlorophyll content to the stomatal conductance in the leaves of the studied experimental variants
a1 – 65BBCH; a2 – 75BBCH; a3 – 89BBCH; b1 – 'Van'; b2 – 'Andreiaș'; b3 – 'Margonia'; c1 – canopy interior; c2 – canopy exterior; **positive correlation – distinctly significant n = 18, P5% = 0.47

to the stomatal opening and may also lead to an increase in the rate of photosynthesis. The effects of drought and water stress on the photosynthesis are direct (such as limitations of the transpiration and gas exchange through the stomata and mesophyll and changes in the photosynthetic metabolism). The phenological stage's specific trend of leaf transpiration followed changes in the stomatal conductance with significantly higher water losses, but without imprinting a reduction in the photosynthesis (Flexas et al. 2007).

In order to establish the relationship between the photosynthetic activity represented by the chlorophyll content and the water content of the leaves with the rate of dehydration (Figure 3), a high significant correlation coefficient (R^2) was obtained. Thus, in the relation RWC : SPAD units, R^2 = 0.8738 and in the relation FD : SPAD units, R^2 = 0.8968. This type of correlation shows that if one variable increases in its values, the other variable decreases in its values by an exact linear rule (Ratner 2009).

The chlorophyll concentration index measured using SPAD units indicated that there were significant differences in the variation in the chlorophyll content, induced by water restrictions at the foliar level in the three phenological stages of the evaluated cultivars.

The chlorophyll content of our experimental variants shows a stronger association with the water status parameters which indicate that water stress restricts the water regime from the leaf surface (Figure 4). In addition, it reduces the efficiency of the photosynthesis and limits the productivity of crops.

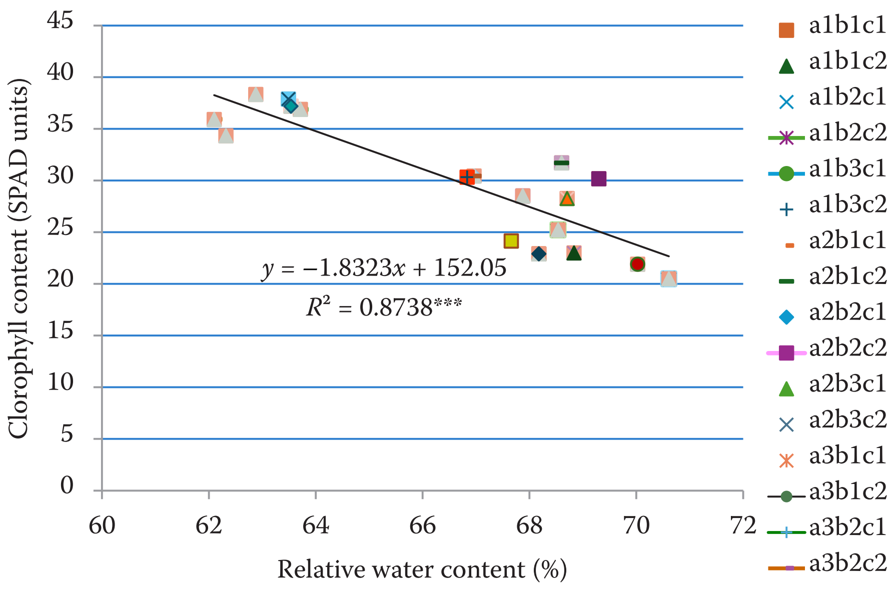

Figure 3. Dependence of the chlorophyll content to the relative water content
a1 – 65BBCH; a2 – 75BBCH; a3 – 89BBCH; b1 – 'Van'; b2 – 'Andreiaș'; b3 – 'Margonia'; c1 – canopy interior; c2 – canopy exterior; **positive correlation – distinctly significant n = 18, P5% = 0.47

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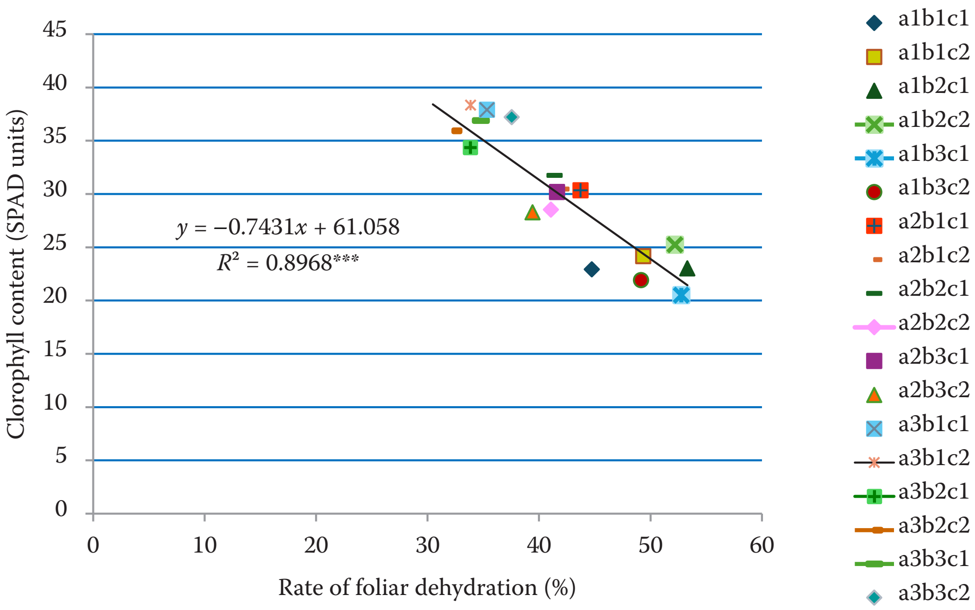

Figure 4. Dependence of the chlorophyll content to rate of foliar dehydration

a1 – 65BBCH; a2 – 75BBCH; a3 – 89BBCH; b1 – 'Van'; b2 – 'Andreiaș'; b3 – 'Margonia'; c1 – canopy interior; c2 – canopy exterior; ***positive correlation – high significant (n = 18, P5% = 0.47)

When cherry trees are stressed by water deficit, the turgor of the leaves decreases. The response of the stomata to this fact is to close the ostioles reducing the rate of transpiration, which increases the efficiency of water use in plants (Houghton et al. 2022) and reduces their photosynthetic efficiency.

CONCLUSIONS

Following the study of the dynamics of the water regime, general models of the water exchange in sweet cherry leaves were established in the climatic conditions of North-East Romania without an irrigation system and the following conclusions were formulated:

- The studied cultivars can be characterised with a high capacity to maintain the state of turgor in conditions of reduced water availability, although there were conditions of water and thermal stress.
- Although, in the phenophase of fruit ripening, there was a more dramatic decrease in the total water in the leaves than in the other phases of the sweet cherry development, the defence mechanism of the trees was to slow down the dehydration process (water loss) at the foliar level.

In the studied parameters, among the evaluated experimental variants, the cultivar 'Andreiaș' (factor B, b2) stood out for its resistance to water stress following the water indicators, and regarding the surface of the canopy, inside the canopy (factor C, c1), the physiological processes are much intensified.

- The results show a high efficiency of leaf water use, especially at times and in the environments with high evapotranspiration requirements. In the environmental context, where water is a limited resource, the real possibility of carrying out additional studies on resistance to water and thermal stress in the context of climate change must be evaluated.

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