

ANALYSIS OF SOME MORPHO-PHYSIOLOGICAL FOLIAR INDICATORS OF BLACK CURRANT (*RIBES NIGRUM L.*) IN PEDOCIMATICS CONDITIONS FROM NORTHEASTERN ROMANIA

**ANALIZA UNOR INDICATORI MORFO-FIZIOLOGI FOLIARI AI
COACĂZULUI NEGRU (*RIBES NIGRUM L.*) ÎN CONDIȚIILE
PEDOCIMATICE DIN NORD ESTUL ROMÂNIEI**

***MICULSCHI Cristina, GRĂDINARIU G.,
ISTRATE M., CIOBOTARI Gh.***

University of Agricultural Sciences and Veterinary Medicine Iassy, Romania

Abstract: Precipitation and high summer temperatures are limiting factors of the currant crop in northeastern Romania. Knowledge of these stress factors influence is important in identifying resistant or tolerant varieties. Objective of the present research was to study the physiological and morphological response of four varieties of the species *Ribes nigrum L.* in ecological conditions in this area, in three culture technologies: with drip irrigation, soil amendment with hydrophilic polymers and natural conditions. Were determined: leaf area (cm^2), stomata number, relative water content (RWC, %).

Key words: blackcurrant, leaf area, relative water content, stomata density.

Rezumat: Nivelului precipitațiilor și temperaturile ridicate din timpul verii sunt factori limitativi ai culturii coacăzului în NE-ul României. Cunoașterea influenței acestor factori de stress este importantă în identificarea soiurilor rezistente sau tolerante. Obiectivul cercetărilor de față a fost studierea răspunsul fiziolologic și morfologic a patru soiuri ale speciei *Ribes nigrum L.* la condițiile ecologice din acest areal, în trei tehnologii de cultură: cu irrigare prin picurare, cu amendarea solului cu polimeri hidrofilici și în condiții naturale. S-au determinat: suprafața foliară (cm^2), numărul de stomate/ mm^2 , conținutul relativ de apă (RWC, %).

Cuvinte cheie: coacăz negru, suprafața foliară, conținutul relativ de apă (RCW %), densitatea stomatică.

INTRODUCTION

Major abiotic stress factors for the culture of black currant in the north-east of Romania is the level of rainfall and the high summer temperatures. The water content of plants is important in plants protection against drought and frost damage, and to regulate physiological processes (Westwood, 1993).

Water stress may result in plant organs (roots, stems, leaves and fruits) a series of reactions aimed at adapting to an unfavorable environment. So the leaves may change as the number of stoma and leaf area. Stomata are epidermis formations with important rol in the physiological processes (photosynthesis and transpiration), in

plant adaptation and productivity of plants (Brownlee, 2001). It was estimated that 99% of root absorbed water is lost through in leaves transpiration (Muruvvet Ilgin and Semih Caglar, 2009). Stomatal density variation in response to changes of precipitation and drought was observed by several researchers (Lecoeur *et al.* 1995, Zhao *et al.*, 2001, Galme's *et al.*, 2007, Yang *et al.*, 2007). Moreover, several studies have shown that water scarcity has led to increased density stomatal (Yang and Wang, 2001) and to decrease their size (Quarrie and Jones, 1977, Spence *et al.*, 1986). In contrast to these findings, Greenwood *et al.* (2003) noted that the stomatal indices of *Neolitsea dealbata* leaf (Lauraceae) in tropical forests is not sensitive to changes in climatic factors (precipitation or temperature).

In drought conditions there is a limit CO₂ și O₂ exchange in leaves, a change the relationship between chlorophyll and carotenoid pigments (Anjum *et al.*, 2003, Faroq *et al.*, 2009). It is known that photosynthetic rate decreases with decreasing hydric potential of leaves (Lawlor and Cornic, 2002), but not yet shown, if this effect is due to close main stomata or metabolic deficiency. However it is accepted that water stress is decisive factor for the reduction of photosynthesis (Faroq *et al.*, 2009).

Differences between genotypes about morpho-physiological behavior in drought conditions were observed in a number of species (Bota and Medrano, 2001), but not black currant varieties. Therefore, the objective of this study was to examine the morpho-physiological response (stomatal density, leaf area and relative water content) of four varieties of black currant (Deea, Ronix, Abanos, Tsema,) to identify the best genotype response at existing culture conditions.

MATERIAL AND METHOD

Biological material: four black currant varieties, Deea, Ronix, Abanos, Tsema, from orchard of fruit bushes of the Horticultural Department of University of Agricultural Sciences and Veterinary Medicine Iassy in August 2009; in each decade were harvested ten leaves from the top, middle and bottom of the bushes. August was chosen because the leaves have the maximum area and the level of precipitation recorded in the vegetation period.

In this study we used three types of culture technology, two of which were intended to reduce water stress in the soil for bushes. The first technology was in natural ecological conditions (A), the second option - with drip irrigation (B), and the third involved the soil with synthetic polymers (Aquasorb). The last method has sought better use of rain water.

This study aims to determine the variation of leaf parameters (leaf water status, stomata number, average leaf area) for to set the differences that arise between four blackcurrant varieties of adaptability of plants in stress climatic condition.

Leaf area: was used gravimetric method, the results are expressed in square centimeters.

Stomata density: For stomata counting was used the method of Schechter *et al.*, 1992. Number of stomata was observed for 5 microscope slides with 3 repetitions for each leaf sample by using a light microscope with a 40x and 10 magnification.

The obtained values were calculated as stomata numbers per mm².

Leaves water status: Relative water content (RWC) was calculated with following equation: (FW-DW)/(SW-DW)×100, where FW is the leaf fresh weight, DW the dry weight of leaves after drying at 105 C for 8 hours, and SW is the turgid weight of leaves after being soaked in water for 4h at room temperature, approximately 20 C (Gonzalez and

Gonzales, 2001). For water content and dry weight in leaves there was used gravimetric method, by drying the probes in the drying stove at 105°C for 24 hours.

Statistical analysis: In this study using the averages and standard errors for all data. For to establish the correlation between morpho - physiological parameters we used to analyze linear regression.

RESULTS AND DISCUSSIONS

Enviroment condition

Black currant is a „moisture-loving” species that gives good results in areas with higher annual average rainfall of 700 mm (Mihalca *et al.*, 1981). Climatic conditions of North Eastern Romania is characterized by insufficient rainfall to the requirements of black currant, as in Iassy area is approx. 300-400 mm precipitation during vegetation period, compared to 500-600 mm as necessary (figure 1). Uneven distribution of the annual amount of precipitation, loss of water from precipitation through surface runoff or low capacity for holding its ground, requires finding ways to solve water shortage in the soil.

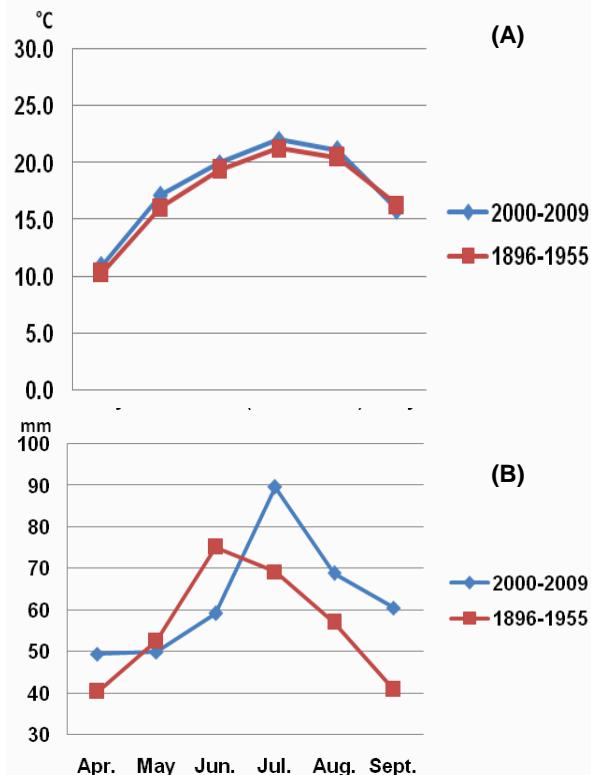


Fig. 1 Monthly average temperature (°C)(A) and the monthly total rainfall (mm/month) (B) in Iassy-Romania

Leaf morpho-physiological response

Stoma number per unit area can be considered a form of adaptation of genotypes to environmental conditions. It is demonstrated that the stomatal density (per/mm²) at walnut varieties increases with altitude (Caglar *et al.*, 2004), but at the apricot in dry regions this structural parameter decreased (Olmez *et al.*, 2006).

In our researches we found a variation in stomatal density between varieties (table 1). The highest number of stoma (359.1 / mm², respectively 358.3 / mm²) was found in Abanos var. and Ronix var.. Lowest stomatal density per unit leaf area was recorded in non-irrigated crops, approximately 230/mm².

Table 1
Leaf area, stomatal density and relativ water content of black currant genotypes

Blackcurrant genotype/ culture technology		Leaf area averages and standart errors (cm^2) $\bar{X} \pm S_{\bar{X}}$	Stomata averages and standart errors of leaf (per mm^2) $\bar{X} \pm S_{\bar{X}}$	Relative water content (RWC %) $\bar{X} \pm S_{\bar{X}}$
Deea	irrigated	21.78 ± 0.2	272.6 ± 6.8	81.7 ± 0.86
	unirrigated	7.40 ± 0.1	230.4 ± 6.7	87.4 ± 0.81
	with Aquasorb	16.56 ± 0.2	251.2 ± 6.8	84 ± 0.76
Abanos	Irrigated	10.91 ± 0.3	306.3 ± 20.8	72.4 ± 0.57
	unirrigated	10.17 ± 0.15	245.5 ± 12.15	81.9 ± 0.5
	with Aquasorb	10.58 ± 0.2	359.1 ± 20.0	74.7 ± 0.49
Ronix	Irrigated	20.19 ± 0.4	305.0 ± 15.3	79.3 ± 0.63
	Unirrigated	17.25 ± 0.7	235.1 ± 18.9	87.9 ± 0.21
	with Aquasorb	18.88 ± 1.1	358.3 ± 20.5	81.8 ± 0.56
Tsema	Irrigated	8.53 ± 0.8	328.6 ± 12.2	71.2 ± 0.32
	unirrigated	6.32 ± 0.55	234.4 ± 17.6	76.2 ± 0.6
	with Aquasorb	6.87 ± 0.2	313.3 ± 20.0	74.2 ± 0.44

The analysis of data on average leaf area and stomatal density with simple linear regression shows that there is less of a 0.0118% chance that the cases be due to chance (Figure 2). It is well known important role of stomata in the process of photosynthesis (for gas and water exchange, and hence the assimilate production), but our results demonstrates that a surface large leaf does not involve a hight number of stoma per unit of leaf area of black currant varieties studied ($r^2=0.0102$).

Xul and Zhou, 2008, showed that moderate drought conditions inhibit the leaves growth and development, and the stomatal density increases, while severe drought leads to significant reductions in both leaf area and number of stoma. This fact, confirmed by other researchers (Ganzachian *et al.*, 2007) lead us to establish favorable technological variant for each variety. Irrigation culture of black currant ensure a superior leaf area compared with the other two types of culture technology. The results from the use of polymer Aquasorb allows us to say that it may be a

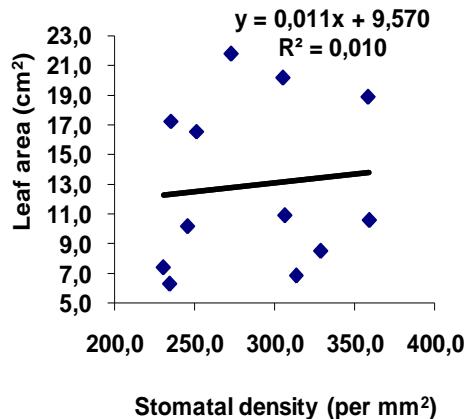


Fig. 2. Correlation of leaf area with stomatal density

solution to reduce water shortage for Abanos var. and Ronix var., but not for Deea var., because the leaf area decrease and the number of stoma increase ($251.2/\text{mm}^2$ to 272.2 mm^2).

These findings may indicate the beginning of a moderate water stress. Tsema var. recorded a very low leaf area compared to other varieties studied in all technology variants analysed.

Relative water content (RCW) is a physiological indicator of plant water balance (Gonzalez and Gonzalez, 2001). Our results show no correlation between relative water content with stomatal density or with leaf area (fig. 3 and 4). These findings can be explained by the existence of different dimensions of stomata, or by the different thickness of leaves. But these issues will be a theme for future study.

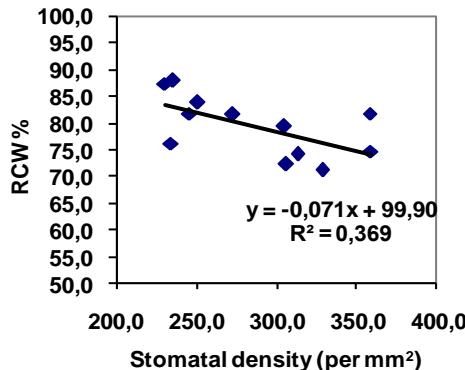


Fig. 3 Response of relativ water content content with the leaf area

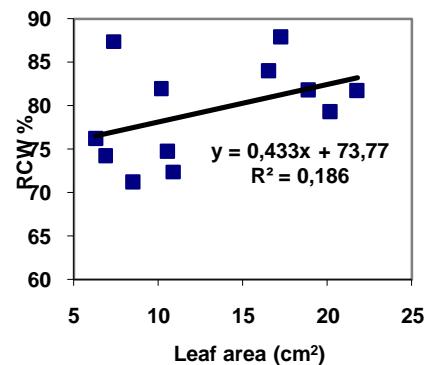


Fig. 4 Corelation of relativ water (RCW %) (RCW %) at stomatal density

CONCLUSIONS

Our study demonstrated the effects of insufficient hydric factor on some morpho-physiological foliar parameters (leaf area, stomatal density and relativ water content) for four black currant varieties (Deea, Abanos, Ronix, Tsema). Thus it is noted as a severe decrease in the number of stoma, relative water content and leaf area for plants unirrigated. The irrigation ensures at all four varieties, higher values of the parameters analyzed. The soil improved with Aquasorb can be a solution to improve the soil water reserve available to plants through a better use of water from precipitation.

Between the four black currant varieties studied, Tsema var. was recorded lower leaf area (over 50% less) in all three variants of cultivation analysis, which can reveal the poor adaptation to pedoclimatics conditions.

REFERENCES

1. Anjum F., Yaseen M., Rasul E., Wahid A. and Anjum S., 2003 - Water stress in barley (*Hordeum vulgare L.*). II. Effect on chemical composition and chlorophyll contents. *Pakistan J. Agric. Sci.*, 40: 45–49.

2. Bota J., Flexas J., Medrano H., 2001 - *Genetic variability of photosynthesis and water use in Balearic grapevine cultivars.* ANN. APPL. BIOL. 138: 353-361.
3. Brownlee C., 2001 - *The long and short of stomatal density signal.* Trend in Plant Sci., 6: 441-442.
4. Caglar S., Suttyemez M., Bayazit S., 2004 - *Stomatal density in some selected walnut (*Juglans regia*) types.* J. Akdeniz Univ. Agric. Fac. 17: 169-174.
5. Farooq, M., A. Wahid, N. Kobayashi, D. Fujita and S.M.A. Basra, 2009 - *Plant drought stress: effects, mechanisms and management,* Agron. Sustain. Dev., 29: 185–212.
6. Gazanchian A, Hajheidari M, Sima NK, Salekdeh GH., 2007 - *Proteome response of *Elymus elongatum* to severe water stress and recovery.* Journal of Experimental Botany 58: 291–300.
7. Gonzalez L., Gonzalez-Vilar M., 2001 - *Determination of relative water content,* Handbook of plant ecophysiology techniques. Dordrecht: Kluwer Academic,
8. Galme's J., Flexas J., Save' R., Medrano H., 2007 - *Water relations and stomatal characteristics of Mediterranean plants with different growth forms and leaf habits: responses to water stress and recovery.* Plant and Soil 290: 139–155.
9. Greenwood D. R., Scarr M. J., Christophe D. C., 2003 - *Leaf stomatal frequency in the Australian tropical rainforest tree Neolitsea dealbata (Lauraceae) as a proxy measure of atmospheric pCO₂,* Mammology, Palaeoclimatology, Palaeoecology 196: 375-393.
10. Lawlor D.W. and G. Cornic, 2002 - *Photosynthetic carbon assimilation and associated metabolism in relation to water deficits in higher plants.* Plant Cell Environ., 25: 275–294
11. Lecoeur J., Wery J., Turc O., Tardieu F., 1995 - *Expansion of pea leaves subjected to short water-deficit: cell number and cell-size are sensitive to stress at different periods of leaf development.* Journal of Experimental Botany 46: 1093–1101.
12. Mihalca Gh., Botez M., Vieru R., Florescu I., 1981 - *Cultura și valorificarea coacăzului.* EdituraCeres, București
13. Muruvvet İlgin and Semih Caglar, 2009 - *Comparison of leaf stomatal features in some local and foreign apricot (*Prunus armeniaca* L.) genotypes.* African Journal of Biotechnology Vol. 8 (6): 1074-1077.
14. Olmez HA, Ak BE, Gulcan R, 2006 - *The relationship between stomata density and fruit quality of some apricot varieties growing in different altitudes in Malatya province.* Acta Hort 701: 163-166.
15. Quarrie SA, Jones HG., 1977 - *Effects of abscisic acid and water stress on development and morphology of wheat.* Journal of Experimental Botany 28: 192–203.
16. Spence RD, Wu H, Sharpe PJH, Clark KG., 1986 - *Water stress effects on guard cell anatomy and the mechanical advantage of the epidermal cells.* Plant, Cell and Environment 9: 197–202.
17. Schechter I, Proctor JTA, Elfving DC, 1992 - *Morphological difference among apple leaf types.* Hortscience, 27: 101-103.
18. Xu Zhenzhu and Zhou Guangsheng, 2008 - *Responses of leaf stomatal density to water status and its relationship with photosynthesis in a grass.* Journal of Experimental Botany, Vol. 59, No. 12: 3317–3325.
19. Zhao RX, Zhang QB, Wu XY, Wang Y., 2001 - *The effects of drought on epidermal cells and stomatal density of wheat leaves.* Inner Mongolia Agricultural Science and Technology 6: 6–7.
20. Yang L, Han M, Zhou G, Li J., 2007 - *The changes of water-use efficiency and stoma density of *Leymus chinensis* along Northeast China Transect.* Acta Ecologica Sinica 27: 16–24.
21. Yang HM, Wang GX., 2001 - *Leaf stomatal densities and distribution in *Triticum aestivum* under drought and CO₂ enrichment.* Acta Phytoecologica Sinica 25: 312–316.
22. Westwood M.N., 1993 - *Temperate Zone Pomology. Physiology and Culture.* Timber Press, Third Edition. Portland, Oregon.