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EFFECT OF THE ALTITUDE AND SUMMER PRUNING ON THE ANATOMICAL STRUCTURE OF GRAPE BERRY EXOCARP OF VRANEC CULTIVAR (*VITIS VINIFERA* L.)

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Abstract

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A study was carried out on the effect of the altitude and summer pruning on the anatomical structure of grape berry exocarp of *Vitis vinifera* L., cv. Vranec. It was established that the different altitudes – Gevgelija (50 m asl), Veles (280 m asl) and Skopje (595 m asl) did not have a significant effect on the changes in the epidermal layer thickness. Applied summer pruning had a stronger effect on the histological structure in the region with the lowest altitude. Hypodermal layer thickness increased from the region with the lowest altitude – Gevgelija (43.8 μm) towards the region with the highest altitude – Skopje (55.9 μm). The differences between the studied characteristics in the three regions were statistically significant.

Key words: vine cultivar Vranec; altitude; anatomical structure; grape berry; exocarp

Introduction

Grapevine (*Vitis vinifera* L.) is extremely sensitive to the conditions of the external environment and their impact affects the quality of the grape yield. The climate of a certain region and the microclimate in vine plantations change significantly depending on the altitude. It strongly affects the ampelographic characteristics of the grape cultivars grown. The altitude has an effect on the morphological and anatomical characteristics of the different vegetative and generative organs (berries, shoots, roots) (Semerdjieva et al., 2015). Creasy and Creasy (2009) mentioned that the environmental factors, more important of them being light, temperature, altitude, slope, etc., influence fruit yield and berry quality of grapevine. The same authors mentioned that some agricultural practices, such as summer pruning, are applied to improve the light regime, the air flow and the disease protection. The fruits of *V. vinifera* are juicy berries (Artyushenko et al., 1986). The berry is differentiated into skinny exocarp (the dermal system), fleshy mesocarp and endocarp. The fleshy part of the berry derives from the growing mesocarp cells. In the evolutionary development of the grape berry pericarp it is thought that the histological structure and characteristics are the result of purposeful breeding and selection (Van der Pijl, 1982; Hardie and O'Brien, 1988; Hardie et al., 1996). Detailed information on the reproductive capacity of grapevine cultivars is presented in the study of Pratt (1971). The author established that the adventitious buds of the species are latent and they enter winter dormancy, when they have two to three inflorescence primordia, depending on the cultivar. The development of microscopic techniques led to expanding the data about the histological structure of the grape berry. Considine and Knox (1979, 1979) carried out a detailed study on the exocarp and on pistil develop-

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ment. Stomatal characteristics and the structure of the cuticle layer of the grape berry were studied by Bessis (1972, 1972), Chambers and Possingham (1963). Holloway (1982) also studied the formation and characteristics of the cuticle layer in the grape berry. The author found that the cuticle is formed from a secretion of monomer lipids penetrating through the outer epidermal walls. He suggested that they oxidize on the epidermal surface and interact with the released enzymes.

The shape, colour and texture of the grape berry are an important morphological, ampelographic and inherent varietal characteristic (Timush et al., 1986). Polymorphism in grape berry is the most economically significant phenomenon. The yield and the suitability for various uses are related to it. According to Coombe (1992), Coombe and Carthy (2000) and Davies and Robinson (2000) grape berries exhibit a double sigmoid growth. It includes three development stages: first – the stage of rapid development, second – delayed stage of poor or no growth and third – the stage of continuing growth and berry ripening. Changes in biochemical composition and morphology of the grape berry occur at each of the three stages. In world literature there are no data about studies on the effect of the altitude on the anatomical structure of the grape berry. Information on the impact of summer pruning on the histological features of the berry was also not found. The assessment of the genotype-environment interaction and the pericarp structure are of key importance to disease and pest resistance of grapes and the yield quality. The purpose of the present study was to assess the effect of the altitude and summer pruning on the anatomical parameters of the grape berry exocarp of *Vitis vinifera* L., cv. Vranec.

Material and Methods

Material

The grape berry of *Vitis vinifera* L., autochthonous Montenegrin cv. Vranec, was used in the study. According to its botanical description, the cultivar belongs to the Black Sea ecological and geographical group (*Convarietas pontica* Negr.), Balkan subgroup (*Subconvarietas balcanica* Negr.), (Nastev, 1985) (Figure 1). In Macedonia it is the main cultivar for producing red wines, but it is also planted in Croatia and Serbia, although on a limited scale (Bozhinovic, 1996, 2010; Milosavljevic, 2012).

Characteristics of the Region

The experiments were carried out in vine plantations grown in three regions of the Republic of Macedonia at different altitudes.

1) Gevgelija (50 m asl). The area is located in the Mediterranean climatic region. It is characterized by long hot summers and winters. The average air temperature is 14.0°C and during the vegetation season – 18.2°C. The annual temperature sum in this area is about 5200°C. The absolute maximum temperature is often higher than 44°C and the absolute minimum rarely falls below -10°C. According to many-year data on average monthly air temperature, it is classified as an arid area, the annual rainfall being 682.4 mm. In July and August there is a dry period. Air humidity is 66.4%. This area is characterized by high monthly average insolation.

Fig. 1. Cluster of Vranec cultivar

2) Veles (280 m asl). The area belongs to the Continental-Mediterranean climatic region and it is characterized by slightly warmer climate. The average annual air temperature is 13.3°C and during the vegetation season – 17.9°C. During summer the monthly temperature is high reaching up to 43°C. Winters are milder and frosty days are few. The area is characterized by the lowest annual rainfall in the Republic of Macedonia – 284.1 mm. According to the aridity index the region is arid, the strongest intensity being established in the months of June, July and August. The average annual air humidity is 70.2%.

3) Skopje (595 m asl). Skopje valley also falls within the Continental-Mediterranean climatic region. It combines the influences of Sub-Mediterranean and Eastern Continental climate; however the influence of the continental climate is stronger. The average annual temperature is 12.5°C and during the vegetation season – 17.1°C. Skopje valley is characterized by a large number of frosty days. The annual amount of precipitation is 460,9 mm. According to the aridity index it is also an arid climatic region with a dry period in July and August. The average air humidity is 67.9%. Average monthly insolation varies from 10.3 hours in July to 2.0 hours in December or 7.6 hours in average during the vegetation season.

Planting Schemes

The planting distance of grapevines in the region of Skopje and Veles is 3.20×1.20 m and in Gevgelija – 3.20×1.00 m. Vineyards were trained to double Guyot system, loaded at pruning with two spurs with two winter buds each and two canes with eight buds each.

Histological Studies

Berries were collected for the analysis in the autumn of 2014 and 2015 at technological maturity of the grapes. The experiment included the following variants: 1) control – no summer pruning applied; 2) by applying defoliation from the base of the fruiting canes to the place of bunch formation, carried out in mid-August at 80% berry veraison; 3) by thinning carried out at the end of July, leaving 6 grape clusters per vine; 4) thinning at the end of July, leaving 10 clusters per vine.

Samples (berries) of the separate variants were fixed in FAA for 24 hours (formaldehyde, glacial acetic acid, ethyl alcohol and distilled water in a ratio of 6:1:20:40). After 24 hours the samples were washed with 50% ethyl alcohol into three series, and then placed for storage in FA (70% ethyl alcohol and 40% formaldehyde in a ratio of 19:1).

Transverse section of the exocarp (berry skin) was made from the side of the berry in the sector between the pedicel and the tip. Semi-durable microscopic samples were prepared. Microscope observations were carried out with light microscope 'Amplival'. Measurements were performed with eyepiece micrometer and pictures were taken with digital light microscope 'Motic BA 210'. Thicknesses of the cuticle, epidermis and hypodermal layer were measured in μm, at 16x40 increased (eyepiece X lenses).

Experimental Design and Statistics

The modules of two-way analysis of variance and Duncan's test, SPSS 17 software, were used for the analysis of the experimental data. Assessment of environmental variability of the studied histological parameters was carried out by an algorithm following the method of Wricke (1962, 1966), which enabled to determine the genotype-environment interaction (Mokreva et al., 2001).

Ecological coefficients were calculated by variants in the three regions, the low values indicating greater ecological plasticity and vice versa.

Results and Discussion

The characteristics of the cuticle, epidermis and hypodermal layer were established in the different experimental variants as a result of the histological study of the berry exocarp, cv. Vranec. The cuticle is the outermost layer of exocarp. It is an important diagnostic feature that determines fruit resistance to diseases and helps protect the berry against water loss. Beneath the cuticle is a single-layer epidermis with thickened tangential and periclinal walls (Figures 2, 3, 4). The cells of the epidermal layer control the gas-exchange processes and store certain organic compounds. The hypodermal layer is beneath the epidermis. It is composed of 6-7 layers of densely distributed collenchyma cells with tangentially thickened walls. The shape of those cells varies from oval to isodiametric. Stained areas of anthocyanin pigments and colorless areas are observed in the hypodermal layers. According to Timush et al. (1986) those anthocyanin pigments conglomerate in anthocyanin bodies. The same authors proposed berry classification based on the coloration and anatomical characteristics of the pericarp. From the observation made it was found that according to the anthocyanin bodies and coloration of the exocarp, cv. Vranec can be attributed to the third type of the proposed classification – cell juice is colored in three-six layers of blue, blue-violet and separate cells of the epidermis are colored in red. Beneath the hypodermal layer is the berry mesocarp, which consists of large oval parenchymal cells.

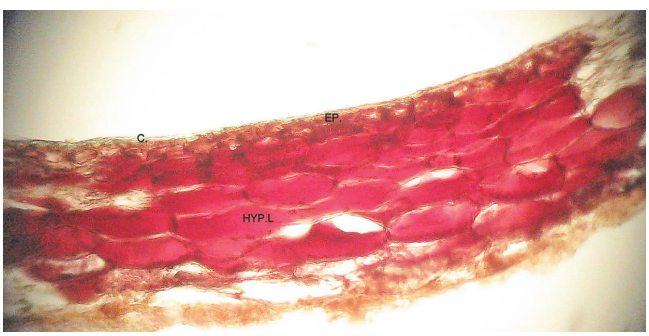

Fig. 2. Transverse section on grape berry exocarp of Vranec cultivar, Gevgelija (50 m asl), LM, magnification 16x10 (layer epidermis EP, cuticle C, hypodermal layer HYP.L)

After mathematical processing of the data, it was found that the cuticle thickness of the samples collected from areas of different altitudes varied from 6.6 μm (Veles) to 7.4 μm (Gevgelija), (Table 1). The increase of the altitude resulted in a slight reduction of the cuticle layer thickness. The highest values were established in the cuticle of the samples collected from the control variants of all three altitudes. In the separate variants with summer pruning operations, data show that there is not a clear tendency towards an increase or a drastic reduction of the cuticle thickness. The highest

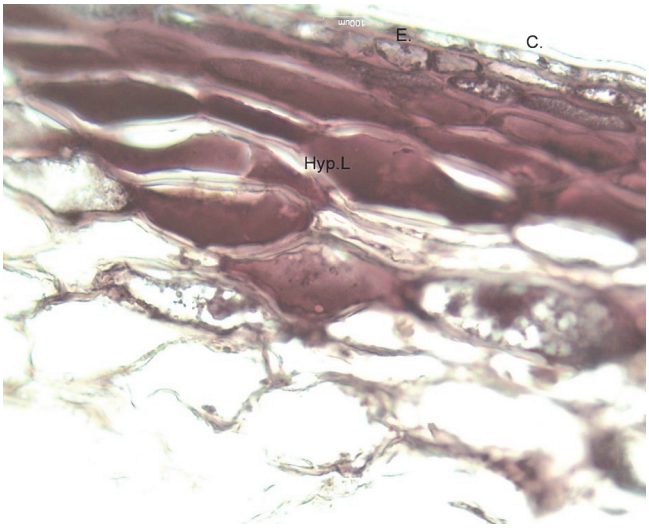

Fig. 3. Transverse section on grape berry exocarp of Vranec cultivar, Veles (280 m asl), LM, magnification 16x10 (layer epidermis EP, cuticle C, hypodermal layer HYP.L)

values (8.6 µm) were established in the samples collected from the vineyard in the region of Gevgelija (50 m), Variant 2 (defoliation) (Table 2). This is probably due to the low altitude and the better sun exposure of the berries thanks to defoliation. The lowest cuticle thickness was reported in the samples of Variant 3 (6 clusters), (Table 3). Data obtained were statistically significant. The variation coefficient (V%) of cuticle was within 18.5-39.1 and it can be considered that the statistical sampling is relatively homogeneous.

The characteristics of the epidermal complex of plants are genetically determined and they are directly related to the taxonomic classification of the species (Barthlott, 1981). The same author noted that the different ecological conditions in the habitats have an insignificant effect on the characteristics of the epidermal cells. The results of the present study also confirmed that statement. The epidermal thickness in the studied variants of different altitudes varied within a limited range, from 8,8 µm (Gevgelija) to 9,6 µm (Veles), (Table 3). The lowest values were reported in the samples collected from the grapevine plantation in Gevgelija, located at the lowest altitude (50 m). In the variants with summer pruning the epidermal layer thickness again varied slightly, i.e. within 8.0 µm-10.0 µm. The lowest values of the epidermal thickness were reported in the variants with defoliation, located in the region of Skopje (8.0 µm) and in the variant with 6 grape clusters per vine in the habitat near Gevgelija (8.0 µm). Only the differences between the variant with 6 clusters and the

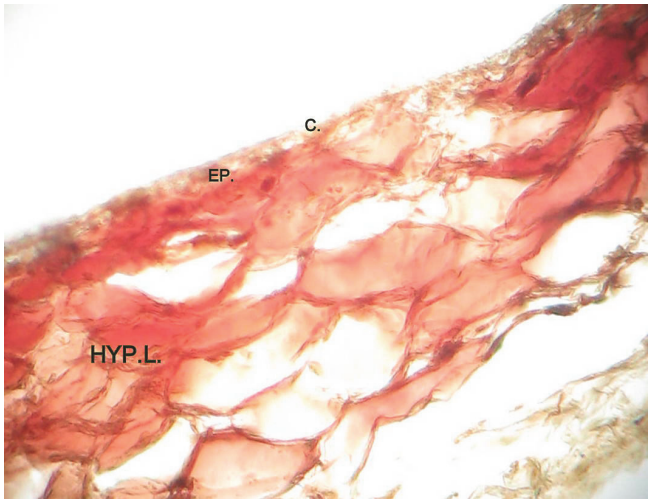

Fig. 4 Transverse section on grape berry exocarp of Vranec cultivar, Skopje (595 m asl), LM, magnification 16x10 (layer epidermis EP, cuticle C, hypodermal layer HYP.L)

Table 1
Multidirectional comparison of variance – 2014-2015

Variance	Average value		
	Cuticle in µm	Epidermis in µm	Hypodermal layer in µm
Gevgelija (50 m asl).	7.4 ^{b}	8.8 ^{b}	43.8 ^{c}
Veles (280 m asl)	6.6 ^{b}	9.6 ^{a}	52.9 ^{b}
Skopje (595 m asl)	6.7 ^{a}	9.2 ^{b}	55.9 ^{a}

a, b, c.. level of proof of variance and Duncan's test, Error α = 0.05

Table 2
Two-way analysis of variance and Duncan's test – 2014-2015

Parameters/ Variance	Regions/ altitude	Thickness of µm		
		Cuticle	Epidermis	Hypodermal layer
Control	Gevgelija (50 m asl).	6.9 ^{bcd}	9.0 ^{c}	48.9 ^{d}
	Veles (280 m asl)	7.9 ^{ab}	10.3 ^{a}	50.9 ^{d}
	Skopje (595 m asl)	7.8 ^{abc}	10.0 ^{ab}	64.9 ^{a}
Defoliation	Gevgelija (50 m asl).	8.6 ^{a}	8.8 ^{cd}	44.6 ^{e}
	Veles (280 m asl)	5.8 ^{d}	9.0 ^{cd}	44.5 ^{e}
	Skopje (595 m asl)	5.8 ^{d}	8.0 ^{d}	43.1 ^{e}
6 grape clusters per vine	Gevgelija (50 m asl).	5.7 ^{d}	8.0 ^{d}	43.1 ^{d}
	Veles (280 m asl)	6.4 ^{d}	9.0 ^{c}	55.7 ^{c}
	Skopje (595 m asl)	6.3 ^{d}	10.0 ^{ab}	57.9 ^{bc}
10 grape clusters per vine	Gevgelija (50 m asl).	8.5 ^{a}	9.3 ^{bc}	38.7 ^{f}
	Veles (280 m asl)	6.4 ^{d}	10.1 ^{ab}	60.8 ^{b}
	Skopje (595 m asl)	6.8 ^{cd}	8.7 ^{c}	57.6 ^{bc}

a, b, c.. level of proof of variance and Duncan's test, Error α = 0.05

Table 3
Multidirectional comparison of variance

Variance	Average value		
	Cuticle in µm	Epidermis in µm	Hypodermal layer in µm
Control	7.5 [a]	9.8 [a]	54.9 [a]
Defoliation	6.7 [bc]	8.6 [c]	44.0 [c]
6 grape clusters per vine	6.1 [c]	9.0 [bc]	52.2 [b]
10 grape clusters per vine	7.2 [ab]	9.3 [ab]	52.4 [b]

a, b, c.. level of proof of variance and Duncan's test, Error α = 0.05

control variant were statistically significant. The coefficient of variation of the epidermal thickness is within 0.0 to 22.7, indicating that the deviation of the values of that characteristic is low and the samples are homogeneous (Snedecor, 1961). The results of the present study are directly related to the ontogenetic fruit setting. The samples in the present study were collected at the time of technological maturity of the grape clusters. In this period of fruit development the histological processes of formation of the different tissues have almost finished. That is why the values obtained for the epidermal layer thickness are very close. In the initial stages of fruit setting, the conditions of plant development were almost the same.

Measurements of the hypodermal layer thickness show that the highest values were reported in the samples of the control variant in the region of Skopje (64.9 µm). The lowest values were established in the samples collected from the vineyards located at the lowest altitude Gevgelija (48.9 µm). It should be mentioned that the hypodermal layer thickness increased with altitude. A similar tendency was established in the variant with 6 grape clusters. In the different variants with summer pruning the results show that similar values of the hypodermis were reported in the variant with defoliation (43.1-44.5 µm). The most controversial data were obtained in the variant with 10 clusters. The thickest hypodermal layer was established in the samples from the region of Veles (60.8 µm) and the lowest thickness was measured in the samples from Gevgelija (38.7 µm).

The many-sided comparison of the studied characteristics about the effect of green pruning on the anatomical structure of exocarp showed that in the variants with defoliation, the plants responded by reduced thickness of the epidermal layer, cuticle and hypodermal layer.

The lowest values of the ecological coefficient in all three characteristics were reported in the region of the highest altitude, i.e. Skopje (595 m asl), followed by the habitat of the species in Veles (280 m asl) and Gevgelija (50 m asl), (Figure 5).

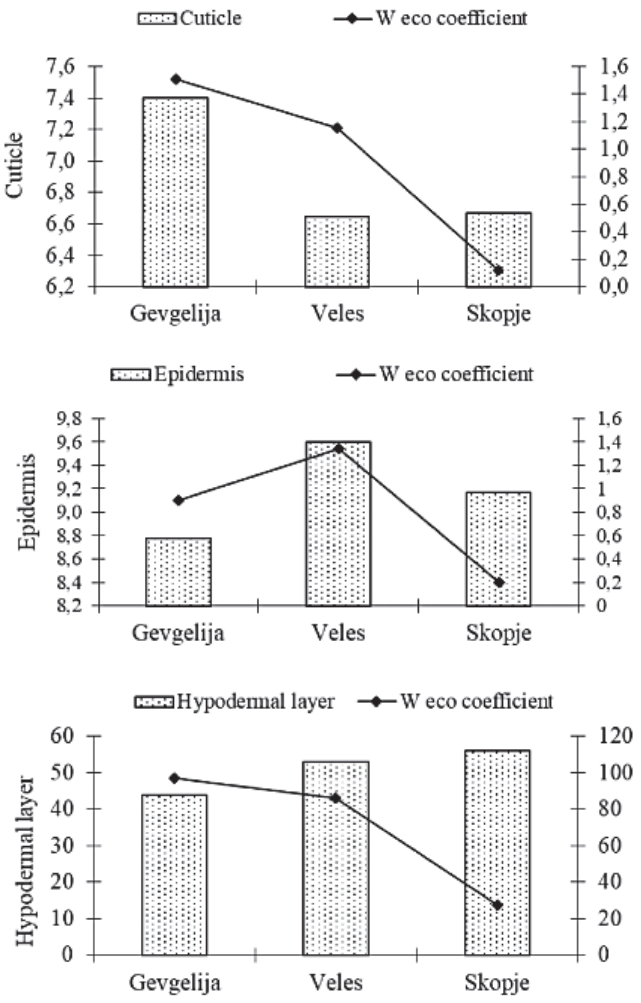

Fig. 5. Ecological plasticity of the studied histological indices in all growing regions of cv. Vranec

Conclusion

The different altitudes did not have a significant effect on the epidermal layer thickness. The green pruning carried out in the region with the lowest altitude Gevgelija (50 m asl) had an effect on the histological characteristics of the grape berry exocarp in the grapevine cultivar Vranec. The cuticle layer thickness decreased and the hypodermal layer increased with altitude.

The applied summer pruning induced an increase of the hypodermal layer of the grape berry from the region with the lowest altitude (Gevgelija) to the region with the highest altitude (Skopje). The differences between the values of the studied characteristics in the three regions are statistically significant.

References

Artyushenko, Z., A. Feodorov, O. Nikiforov, M. **Zhurmanov,** 1986. Atlas of descriptive morphology of higher plants, *Fruit. Ed. Nauka*, Leningrad, 390 pp.

Barthlott, W., 1981. Epidermal and seed surface characters of plants: systematic applicability and some evolutionary aspects. *Nord. J. Bot.,* **1** (3): 345-354.

Bessis, R., 1972a. Etude de l'évolution des caractères morphologiques des cires cuticulaires au cours de la vie du fruit de la Vigne. *Comptes Rendus de l'Académie des Sciences*, Paris Série D, **274**: 1911-1914.

Bessis, R., 1972b. Etude de l'évolution des stomates et des tissus péristomatiques du fruit de la Vigne. *Comptes Rendus de l'Académie des Sciences*, Paris Série D, **274**: 2158-2161.

Božinovic, Z., 1996. Ampelography. *Ed. Academic Skopje*, 266 pp (Mk).

Božinovic, Z., 2010. Ampelography. 2nd revised edition, Skopje, 318 (106) pp (Mk).

Chambers, T.C. and **J.V. Possingham,** 1963. Studies of the fine structure of the wax layer of Sultana grapes. *Australian Journal of Biological Sciences*, **16**: 818-825.

Considine, J.A. and R.B. Knox, 1979a. Development and histochemistry of the cells, cell-walls and cuticle of the dermal system of the fruit of the grape *Vitis vinifera* L. *Protoplasma,* **99**: 347-365.

Considine, J.A. and R.B. **Knox,** 1979b. Development and histochemistry of the pistil of the grape, *Vitis vinifera*. *Annals of Botany*, **43**: 11-22.

Coombe, B.G., 1992. Research on development and ripening of the grape berry. *American Journal of Enology and Viticulture*, **43**: 101-110.

Coombe, B.G. and M.G. McCarthy, 2000. The dynamics of grape berry growth and physiology of ripening. *Aust. J. Grape Wine Res.*, **6**: 131-135.

Creasy, G.L. and L.L. Creasy, 2009. Grapes. *CABI North American Office*.

Davies, C. and P.S. Robinson, 2000. Differential screening indicates a framatic change in mRNA profiles during grape berry ripening. Cloning and characterization of cDNAs encoding putative cell wall and stress response proteins. *Plant Physiology,* **122:** 803-812.

Hardie, W.J. and T.P. O'Brien, 1988. Considerations of the biological significance of some volatile constituents of grape (*Vitis* spp.). *Australian Journal of Botany,* **36**: 107-117.

Hardie, W.J., T.P. O'Brien and V.G. Jaudzems, 1996. Morphology, anatomy and development of the pericarp after anthesis in grape, *Vitis vinifera* L. *Australian Journal of Grape and Wine Research*, **2**: 97-142.

Holloway, P.J., 1982. The chemical constitution of plant cutins. In: D.F. Cutler, K.L. Alvin, and C.E. (eds.) The Plant Cuticle. Price. Linnean Society London, Symposium, *Academic Press*: London, Series No. 10.

Milosavljevic, M., 2012. Bioetnika vinove loze. Samostalna izdavacka agencija *NIK-PRESS,* Beograd. (Sr).

Mokreva, T., **V. Roychev and D. Dimova,** 2001. Possibilities of analyzing genotype-environment interaction in agricultural crops using MS Excel, Agricultural University – Plovdiv. *Scientific Works*, XLVI (1): 79-84, ISSN 1312-6318.

Nastev, D., 1985. Ampelography. Ed: *Institute of Economy and Social Activities of SRM 'Managing practice'*, Skopje, 276 pp. (Mk).

Pratt, C., 1971. Reproductive anatomy in cultivated grapes – a review. *American Journal of Enology and Viticulture*, **22**: 93-109.

Semerdjieva, I., V. Roichev, T. Mokreva and T. Trifonova, 2015. Comparative histological examination of the leaves of parental cultivar and F_1 generation of hybrid Alicant Bushe x Rusalka 1 (V*itis vinifera* L.). Agricultural university – Plovdiv, *Scientific Works*, **LIX** (4): 233-242.

Snedecor, D.U., 1961. Statistical Methods Applied in Research in Agriculture and Biology. *Selhozizdat*, Moscow, 503 pp.

Timush, A.I. (ed.), V.A. Andrunakievich, D.G. Batыr, I.D. Blazh, I.T. Bogdesko, P.P. Bozu, D.V. Gizu, B.V. Glushko, A.A. Gudыm, A.B. Drumia, G.I. Eremei and V.G. Efremov, 1986. Encyclopedia of the Vine**,** Kishinev, pp. 317-320 (Ru).

Van der Pijl, L., 1982. Principles of Dispersal in Higher Plants, *Springer-Verlag*: Berlin.

Wricke, G., 1962. Über eine Methode zur Erfassung der ekologischen steubreite in Feldversuchen. *Z. Pflanzenzuchtung*, **47:** 92-96.

Wricke G., 1966. Über eine Biometris ehe methode zur Erfassung der ekologischen. *Anpass ung-Acia Agric. Scand. Suppl.*, **16:** 98-101.

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