## THE IMPACT OF MICRONUTRIENTS IN THE PROTECTIVE COMPOUNDS ACCUMULATION IN THE VINE ORGANS

# ACUMULAREA COMPUȘILOR PROTECTORI ÎN ORGANELE VIȚEI DE VIE ÎN FUNCȚIE DE FERTILIZAREA EXTRARADICULARĂ CU MICROELEMENTE

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Abstract. The unfavorable conditions of growth cause significant deviation in intensity of absorption and inclusion of nutrients in the metabolism. Realization of the potential of vine frost resistance and wintering can be enhanced by micronutrients applying, which may serve as a trigger for the accumulation of protective compounds in plant tissues. It has been shown in conditions of production that foliar fertilization of plants in vegetation period contributes to essential changes in the content of free amino acids and carbohydrates in leaves and shoots, the composition of the sap after the winter. The accumulation of stress protective substances (prolin, glutamic acid, glutamine, monosaccharides) after fertilization of plants with complex of micronutrients leads to the formation and fuller manifestation of potential of vine resistance to wintering.

**Key words:** resistance, vine, trace elements, xylem exudates, free amino acids, carbohydrates.

Rezumat. Realizarea potențialului de rezistență la ger și la iernare a plantelor de viță de vie poate fi sporită prin aplicarea micronutrienților, care pot servi ca un trigger pentru acumularea compușilor protectori în țesuturile plantelor. În condiții de producere a fost evidențiat că fertilizarea foliară a plantelor în perioadă de vegetație contribuie la schimbări esențiale în conținutul acizilor aminici liberi și carbohidraților în frunze și lăstari, precum și în componența sevei. Acumularea substanțelor stres-protectoare (prolină, acid glutamic, glutamină, monozaharide), după fertilizarea plantelor cu complexul de microelemente, a contribuit la formarea și manifestarea mai amplă a rezistenței viței de vie la iernare.

Cuvinte cheie: rezistență, viță de vie, microelemente, sevă, aminoacizi liberi, carbohidrați.

#### INTRODUCTION

The winters in Moldova are extremely severe for grape plant dormancy, the temperatures dropping down to  $-25^{\circ}\text{C} - -27^{\circ}\text{C}$  and sometimes even to  $33^{\circ}\text{C}$  - $36^{\circ}$  C. The winter of 2009-2010 is an eloquent prove of that. The meteorological and agrometeorological conditions of that winter in the Republic of Moldova were special, with the temperatures that were lower than usual and plenty of snow. The absolute minimal air temperature during the winter constituted minus  $31^{\circ}$  C (January 26, Bălți), the phenomenon registered on the average once per 25-30 years. But the critical minimum for the recognized noble cultivars, especially table ones, is within the range of

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-18°C – -22°C. This fact is a vivid demonstration of the importance and necessity to fortify researches on the physiology and biochemistry of the grape resistance to frost and hibernation. The literature data and those obtained at the Institute of Genetics and Plant Physiology of the Moldovan Academy of Sciences prove that the plant resistance to the action of unfavorable factors may be enhanced through the improvement of nutrient regime and regulation of the photosynthetic process. The accumulation of vegetative mass, yield amount, and production quality are conditioned by the degree of macro- and microelement involvement in metabolism. The mechanisms ensuring trace element actions in the formation and realization of plant resistance are multiple and need further studies. One of them can be accomplished through participation of the compounds with a stress protective action, i.e. glucides, free amino acids etc. in the accumulation in plant.

The aim of this study is to reveal the contribution of the micronutrients that are applied individually and in combination for grape foliar fertilization to the accumulation of protective compounds in grape organs in order to realize the wintering resistance potential.

#### **MATERIAL AND METHOD**

The research was carried out on grape (cv. Codrinschi) in the years 2008 to 2010. The foliar microfertilizer treatment was conducted in three terms (1 – before flowering, 2 and 3 – at the stage of intensive growth with an interval of 12 to 14 days). Unfertilized plants served as control. Leaves for analyses were sampled three and six days after the foliar treatment, annual and perennial shoots - in March, sap- at the budding stage. The following analytical methods were used: the content of free amino acids using an AAA-300 analyzer, the carbohydrate content according to Bertan; the shoot growth and maturation was determined according to the M.A.Lazarevskii (1963) and Ion Alexandrescu (1998) method. The evaluation of the plant resistance to wintering was performed in field conditions after the dormancy according to the method developed by M.V.Cernomoret, particularly for the grape crop (1995).

#### RESULTS AND DISCUSSIONS

1. The content of free amino acids (FAA) and glucides in grape leaves. The total content of FAA in plant issues is quite a mobile indicator and depends on many factors including the plant condition, vegetation stage, nutrition conditions etc. The FAA content was measured during the vegetation in plant blade (alcohol extract) and in apoplast (water extract). The findings demonstrate that the total content of FAA, especially that of non-essential ones, decreases in leaves three days after foliar microelement fertilization; the same trend is observed in apoplasts, where 3% to 8% of the total content is present in leaf blade, with the exception of the treatment where the Microcom-V complex is applied (figure 1). A tendency of increase in the tryptophan, proline, hystidine, glycine, cystic acid content is observed. The FAA level grows in the leaves of the fertilized treatments during the vegetation, in comparison with the witness.

The total glucide content in grape leaves changes quite significantly during the vegetation. The estimation of mono- and disaccharides in dynamics demonstrated a positive impact of the microfertilizers containing Fe, Ni or the six-microelement complex, Microcom-V, in the process of glucides synthesis (figure 2). The

application of microelements in combination (Microcom-V) or in a chelate form (Dissolvin) is more effective according to the findings.

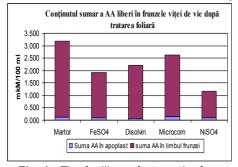


Fig. 1 - The fertilizer efect on the free content amino acid content in grape leaves three days following plan t treatment

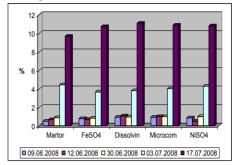


Fig. 2 - The fertilizer effect on the glucide in grape leaves

Table 1

The analyses performed three and six days after foliar fertilization of plants demonstrate that treatment with Fe-containing solutions contributes to a decrease of glucides, especially disaccharides in leaves immediately after treatment and a subsequent increase in leaves and grape berries. The ratio of di- and monosaccharides in the Fe treatment is lower in leaves and higher in grape berries in comparison with the control. The Ni influence on the carbohydrate compound content and the ratio is less significant in grape organs.

2. FAA content in wintering organs. The estimation of free amino acids in grape organs at the end of the rest period demonstrates that the total content is significantly higher in annual shoots than in perennial ones. Grape foliar fertilization at the critical developmental stages during plant vegetation has been shown to influence the content of FAA in wintering organs (table 1).

The total free amino acid content in annual and perennial grape shoots, 17.02.2010. mu/100 mg

17:02:25 10, mp/100 mg									
Treatments/Indices	∑ Free AA	∑ non- essential AA	∑ essential AA	∑S- containingAA					
Annual shoots									
1. Witness	2,2151	1,2408	0,5132	0,0238					
2. FeSO <sub>4</sub>	2,2096	1,1861	0,7214	0,0183					
<ol><li>Dissolvin</li></ol>	1,6514	1,0451	0,3189	0,0186					
4. Microcom	3,2194	2,0479	0,7245	0,0268					
5. NiSO <sub>4</sub>	3,4189	2,1749	0,7297	0,0244					
Perennial shoots									
1. Witness	1,5347	0,8861	0,3509	0,0103					
2. FeSO <sub>4</sub>	1,4004	0,8516	0,2967	0,0089					
3. Dissolvin	1,4126	0,8141	0,3373	0,0153					
4. Microcom	1,9271	1,1529	0,4722	0,0193					
5. NiSO <sub>4</sub>	1,4315	0,8054	0,3537	0,0141					

The Microcom-V fertilization enhanced the FAA amount in wintering organs, especially in annual shoots. The growth of FAA content in wintering shoots might be a

protective reaction of plants to a strong previous drop in the air temperature. Our earlier findings demonstrated that foliar microelement fertilization during preceding vegetation accelerated the hydrolysis of sugars in grape wintering shoots (Veliksar S., Toma S., 2003; Veliksar et al., 2007), the resistance to freezing increasing.

The qualitative FAA analysis has demonstrated that glutamic acid makes 40.89% of the total content in annual shoots, which confirms the main role of this AA in grape metabolism (table 2). A considerable growth of this FAA in plant shoots is observed in the treatments fertilized with the microelement complex and NiSO<sub>4</sub>. The content of  $\gamma$ -aminobutyric acid in plant tissues is usually reduced, but grows at the action of stressogenic abiotic factors. An increased amount of this acid, making 0.2866 mµ/100 mg (20.08% of the total FAA content) was found in annual shoots, which might be associated with the action of lower temperatures that occurred in January. It is important that the relative content of  $\gamma$ -aminobutyric acid decreased in the fertilized treatments. The problem of decarboxylation of glutamate and the role of  $\gamma$ -aminobutyric acid as an osmoprotector in the plant resistance to lower temperatures is discussed in the paper of E. Mazzucotelli et al. (2006). The increase of the so called stress acids (Haldemann et al., 1988) – proline, alanine – in shoots is in favor of our supposition about the positive role of Microcom in plant adaptation to lower temperatures. The quantitative FAA analysis in perennial shoots demonstrated the same tendency as in annual shoots (table 3).

3. The content of FAA and glucides in grape sap. Table 4 summarisez the findings of the FAA content estimation in grape sap in spring (30.03.2010) in an ascendent flow. The reduction of the total FFA content in plant sap from the treatments fertilized with Microcom-V observed during three years (2008-2010) allows us to assume that the microelements, especially the microelement complex, Microcom-V applied during vegetation intensify the processes of protein synthesis in wintering organs, that takes place during the period of exit from vegetative rest (Burzo et al., 1999). The results of the qualitative FAA content analysis in sap demonstrates an increased content of S-containing amino acids – glutamic acid, proline, valine, alanine. Noteworthy, the absolute and relative content of glutamic acid, which is a general type of N transport with the ascendent flow at the beginning of vegetation, increases significantly in the treatments fertilized with Fe, Microcom-V and N.

Total free amino acid content in grape sap, mu/100mg

Table 4

Treatments/indices		∑ non-essential	∑ essential	∑ S-containing AA	
Treatments/indices	∑ free AA	_ AA	_ AA		
1 Witness	1,3979	0,2455	0,2835	0,9404	
2 FeSO4	4,7394	2,1674	1,2538	1,5585	
3 Dissolvin	0,4047	0,1202	0,0990	0,2283	
4 Microcom	0,9891	0,4341	0,2419	0.3494	
5 NiSO4	0,5839	0,2378	0,1541	0,2033	

Table 2

Table 3

Content of FAA in annal grape shoots, cv. Codrinskii, 17.02.2010

Treatment Amino acids	Witness mµ/100mg	% of ∑	FeSO₄ mµ/100mg	% of ∑	Dissolvin mµ/100mg	% of Σ	Microcom- V mµ/100 mg	% din Σ	NiSO₄ mkM/100mg	% din ∑
Cystic acid	0.0082	0.37	0.0045	0.20	0.0028	0.17	0.0063	0.20	0.0053	0.16
Aspartic acid	0.0841	3.80	0.1006	4.55	0.0811	4.91	0.1379	4.28	0.1112	3.25
Glutamic acid	0.9058	40.89	0.8883	40.20	0.6964	42.17	1.5207	47.24	1.6258	47.55
Proline	0.0329	1.49	0.0328	1.48	0.0457	2.77	0.0747	2.32	0.0830	2.43
Valine	0.0285	1.29	0.019	0.86	0.0214	1.30	0.0285	0.89	0.0379	1.11
Phenylalanine	0.0112	0.51	0.0094	0.43	0.0075	0.45	0.0099	0.31	0.0137	0.40
γ-aminobutyric acid	0.4448	20.08	0.2891	13.08	0.2777	16.82	0.4293	13.33	0.4995	14.61
Arginine	0.3606	16.28	0.6017	27.23	0.1929	11.68	0.5505	17.10	0.5403	15.80
ΣFAA	2.2151	100.00	2.2096	100.00	1.6514	100.00	3.2194	100.00	3.4189	100.00

The qualitative amino acid content in grape perennial shoots, cv. Codrinskii, 17.02.2010

Treatments Amino acids	Witness mµ/100m g	% of ∑	FeSO₄ mµ/100m g	% of ∑	Dissolvin mµ/100m g	% of ∑	Microcom mµ/100mg	% din Σ	NiSO <sub>4</sub> mkM/100m g	% din ∑
Cystic acid	0.0034	0.02	0.0016	0.11	0.0040	0.28	0.0047	0.24	0.0032	0.22
Aspartic acid	0.0989	0.64	0.0841	6.01	0.1256	8.89	0.1890	9.81	0.0854	5.97
Glutamic acid	0.5935	38,7	0.5596	39.96	0.4513	31.95	0.7369	38.24	0.5231	36.54
Proline	0.0290	0.19	0.0301	2.15	0.0256	1.81	0.0400	2.08	0.0428	2.99
Valine	0.0096	0.06	0.0079	0.56	0.0161	1.14	0.0177	0.92	0.0109	0.76
Phenylalanine	0.0149	0.10	0.0085	0.61	0.0155	1.10	0.0129	0.67	0.0106	0.74
γ -aminobutyric acid	0.2866	18.73	0.2445	17.46	0.2460	17.41	0.2843	14.75	0.2614	18.26
Arginine	0.2720	1.77	0.2390	17.07	0.2354	16.66	0.3766	19.54	0.2843	19.86
ΣΑΑ	1.5347	100.0 0	1.4004	100.00	1.4126	100.00	1.9271	100.00	1.4315	100.00

The content of glucides in grape sap at the budding stage in relation to plant fertilization during the precedent vegetation period grows in comparison with the control, specially in the treatment with chelate form Fe (Dissolvin) and with the microelement complex (Microcom-V).

It has been found that the number of viable buds constituted only 38.39% in the cotrol (the plants that were not treated with fertilizer). In the plants treated with Microcom-V, this index reached a value of 44.56%, a significant increase of 16.07% being recorded. It should be also mentioned that a high number of injured buds making 44.56% was detected in the treated plants, by 11,88% higher in comparison with the control. The action of the negative critical temperature of -27° registered on January 26, 2010 has been found to provoke a loss of 39,13% of buds in the control, while in the treated plants it made only 30.29%, which is by 22.59% lower in comparison with the control. The resistance to wintering was studied in the grape plants under study in field conditions in the middle of the third decade of May. It has been revealed that the number of the buds developed on annual shoots in the witness treatment constituted 56.86%, while in that with the plants treated with Microcom-V it was 68.15%, the increase making 19.9%.

#### **CONCLUSIONS**

- 1. Foliar treatment of grape with the complex microelement product Microcom-V induces significant modifications in the processes of synthesis, accumulation, translocation, and storage of protective substances (glucides, free amino acids) in the organs and tissues of the plants exposed to the action of low negative temperatures during winter.
- 2. The microelement complex Microcom-V as a post-action is suggested to serve a trigger in starting the transition proces from vegetative rest to the vegetative period.

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