EFFECT OF TRACE ELEMENTS AND PGPB *AZOTOBACTER CHROOCOCCUM* AND *PSEUDOMONAS FLUORESCENS* APPLICATION ON GRAPE RESISTANCE TO THE WINTERING

EFECTUL APLICĂRII MICROELEMENTELOR ŞI PGPB *AZOTOBACTER CHROOCOCCUM* ŞI *PSEUDOMONAS FLUORESCENS* ASUPRA REZISTENŢĂ VIŢEI DE VIE LA IERNARE

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Abstract. *Vine resistance to the wintering is one of the main conditions of high productivity and longevity of vineyards. Realization of the genetically based potential of resistance may be enhanced by improving plant nutrient regime. Obtained results have shown that foliar fertilization of plants in the growing season with micronutrients adding metabolites and suspensions of bacteria Azotobacter chroococcum and Pseudomonas fluorescens is causing significant changes in metabolic processes during the growing season and period of dormancy, which contribute to increase of vine resistance to wintering.*
Key words: vine resistance, micro fertilizer Microcom-VA, bacteria, photosynthetic pigments, carbohydrates, bud viability.

Rezumat. *Rezistenţă viţei de vie la iernare este una din condiţiile principale a productivităţii şi longevităţii podgoriilor. Realizarea potenţialului genetic de rezistenţă poate fi sporită prin îmbunătăţirea regimului nutritiv a plantelor. Rezultatele cercetărilor obţinute au demonstrat, că fertilizarea foliară a plantelor în perioadă de vegetaţie cu microelemente cu adăugarea metaboliţilor şi suspensiilor a bacteriilor Azotobacter chroococcum şi Pseudomonas fluorescens induce schimbări esenţiale în procesele metabolice pe parcursul perioadei de vegetaţie şi în timpul iernării, ce contribuie la sporirea rezistenţei a viţei de vie la iernare.*
Cuvinte cheie: viţă de vie, rezistenţă, microîngrăşăminte Microcom-VA, bacterii, pigmenţi fotosintetici, carbohidraţi, viabilitatea mugurilor.

INTRODUCTION

Resistance is a feature of plants wintering, influenced by a number of factors during the winter, first by frost and so-called winter drought (Cernomoreț *et al.*, 2000; Кондо, 1970; Погосян, 1975; Туманов, 1979). According to multiple data from the literature and field practices vine resistance to wintering depends largely on the plant mineral nutrition (Bratco, 1999; Veliksar and Toma, 2003; Waraich *et al.*,

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2012). Studies conducted over 10 years, showed that foliar fertilization of grape with a new set of trace elements Microcom-VA, created special for grape, increases the shoot growth and maturation, induces significant modifications in the processes of synthesis, accumulation, translocation, and storage of protective substances (sugars, free amino acids, phosphorus compounds) in the organs and tissues of the plants exposed to the action of low negative temperatures during winter. It contributes to the formation and a fuller manifestation of genetically based potential of frost and winter resistance, increase of plant productivity and longevity (Veliksar *et al.*, 2013).

In recent years appeared quite a lot publications about the influence of biological products, obtained on the basis of bacteria PGPB (plant growth promoting bacteria) on the metabolism of cultivated plants (Bhardwaj *et al.*, 2014; Шабаев, 2012). Analysis of data on mechanisms of PGPB action on plants (Bordiec *et al.,* 2011; Bhardwaj *et al.*, 2014) confirms the necessity of deepen research in this area aimed at revealing of certain physiological aspects and genotypic peculiarities for achieving more complete realization of the plant adaptive potential and prevention of the environment contamination.

Our investigations showed opportunity to improve the nutritive regime of the vine by the simultaneous application of the halved dose of Microcom-VA and metabolites of bacteria strains PGPB. Application of suspension of Pseudomonas *fluorescens* and *Azotobacter chroococcum* together with micronutrients have increased the availability of nutrients to plants, the metabolic activity of some processes, improved the quality of planting material and decreased the quantity of applied nutrients (Veliksar *et al.*, 2014). The last gives possibility to reduce the negative impact of fertilizers excess on the environment and to increase viability of vineyards.

In this paper the results of the investigation of the trace elements and metabolites of the bacteria *Pseudomonas fluorescens* and *Azotobacter chroococcum* on the vine resistance to wintering are presented.

MATERIAL AND METHOD

The researches have been performed in field conditions on the experimental plot of the Institute of Genetics, Physiology and Plant Protection of the ASM, cv. Codrinschi. The foliar fertilization of plants by the micro fertilizer (Microcom-VA-0,15%, that is 1/2 of the recommended doze) and metabolites or suspension of *Azotobacter chroococcum* and *Pseudomonas fluorescens* (titre - 10^7 CFU / ml) was conducted three terms: before flowering and twice - at the stage of intensive growth with an interval of 12 to 14 days. Water treated plants were used as control. The leaves for analyses were sampled during the vegetation period six days after the foliar treatment, thoroughly rinsed with water, allowed drying and used for analysis. The shoots and buds were sampled at the end of vegetation and on the end of deep dormancy periods. The following analytical methods were used: photosynthetic pigments content was measured using Chlorophyll Content Meter CCM-200 plus, the carbohydrate content - according to Bertan, proline content - according to Bates (1973). Evaluation of the winter resistance was carried out under field conditions in

accordance with the method developed by Cernomoreț (1985, 2000). The results were analyzed statistically according to Statistica-7.

RESULTS AND DISCUSSIONS

Resistance of vine to wintering depends largely on the intensity of some metabolic processes of plants in vegetation period, growing and maturation of shoots. The high content of photosynthetic pigments in leaves and their active absorption capacity of PAR influences positive on the intensity of sugars accumulation - the primary products of photosynthesis, on the growth, development and productivity of plants in summer, as well as during the process of growth and hardening of vine, its preparation for winter (Жакотэ, 1974; Черноморец *et al.*, 1984).

Foliar fertilization of plants has contributed to increasing of summary content of photosynthetic pigments in the leaves of the vine in the second half of the growing season. On July 21 content of chlorophyll in the leaves of variants with application of halved dose of Microcom-VA plus suspension of microorganisms or metabolites was 13.27 and 14.22 mg/g fresh weight (tab. 1) - 117.7 and 119.7 % compared to control.

Table 1

Total content of photosynthetic pigments in grape leaves under the influence of foliar fertilization by trace elements and bacteria, v. Codrinschi, mg/g f.w.

Variants	09.06.2013	11.07.2013	21.07.2013
Control	8.59±0.19	13.45 ±0.16	11.88±0.48
Foliar fertilization by Microcom-VA, 0.5 doza	8.69±0.52	13.24 ±0.19	12.70±0.37
Foliar fertilization by metabolites of *Ps. fluorescens* + *Az.chroococcum*	9.02 ±0.26	12.62 ±0.33	12.51±0.54
Metabolites of *Ps. fluorescens* + *Az.chroococcum*+ Microcom VA, 0.5	8.46 ±0.53	13.92 ±0.08	13.27±0.35
Suspensia of *Ps. fluorescens* + *Az.chroococcum*+ Microcom VA, 0.5	9.17 ±0.29	14.47 ±0.52	14.22±0.043

It was revealed considerable increase in the degree of growth and maturation of shoots under the foliar fertilization. It has been confirmed the beneficial effect of micronutrient complex Microcom-VA on the growth of shoots - +4.92 % to the control (tab. 2). Effect of trace elements on the growth and maturation of the shoots increased in case of application of trace elements complex in combination with products of microorganisms, especially - in the form of suspension. The degree of the shoots maturation was increased from 3.09% to 9.11% compared to the control (tab. 2). Timely completion of the annual growth and maturation of shoots is one of the basic conditions for the formation of frost resistance of vine.

Table 2

Effect of foliar fertilization on the growth and maturation of vine shoots, v. Codrinschii, 2013

Variants	length of shoots, cm	length of maturated shoots, cm	± to the control	grade of maturation %	± to the control
Control	134.13± 3.12	93.90±2.04	-	70.01	-
Foliar fertilization by Microcom-VA, 0.5 doza	165.40 ±2.74	123.93± 1.53	+30.03	74.93	+4.92
Metabolites of *Ps. fluorescens* + *Az. chroococcum*	158.93± 1.78	117.77± 1.67	+23.87	74.10	+3.09
Metabolites of *Ps. fluoresc.*+ *Az. chrooc.*+Microcom VA, 0.5	169.07± 3.06	133.77± 2.79	+39.87	79.12	+9.11
Suspensia of *Ps. fluorescens* + *Az.chrooc.*+ Microcom+VA, 0.5	171.37± 2.65	132.37 ±2.13	+39.47	77.24	+7.23

One of the essential moments for grape preparation at the wintering period is October. Suspending flow of carbohydrates and their translocation into the perennial shoots and roots in late October is stipulated by plants entrance to the dormancy period, occlusion of sieve tubes of callose. In October 2014 after the first autumn night frost (-5^{o} C) content of carbohydrates in the leaves and shoots of vine was determined. The increased content of sucrose in the leaves and annual shoots was manifested in variants with plant fertilization by metabolites or suspension of bacteria and Microcom-VA – 112.65 and 117.74%.

Next selection of shoots for the analysis was performed at the end of the deep dormancy - January 27th, at the -7° C. The content of monosaccharide and disaccharide, which are known as a compatible osmolytes, increased compared to the control, especially after the simultaneous treatment of plants by suspension of bacteria and compound of trace elements Microcom+VA. Sum of sugars was in that variant 5.82 % (tab. 3) or 123.83% compared to the control.

Table 3

Effect of foliar fertilization on the sugars content in shoots, 27 January 2014, t. -7 ^{o} C, v. Codrinschii, %

Variants	monosac-charide	disacchari-de	sum of sugars	% to control
Control	3.10±0.11	1.60±0.56	4.70	100
Foliar fertilization by Microcom-VA, 0.5 doza	3.62±0.19	2.09±0	5.71	121.49
Metabolites of *Ps. fluorescens* + *Az. chroococcum*	2.65±0.32	1.72±0.02	4.37	92.98
Metabolites of *Ps. fluoresc.*+ *Az. chrooc.*+Microcom VA, 0.5	3.42±0.56	1.87±0.02	5.29	112.55
Suspensia of *Ps. fluorescens* + *Az.chrooc.*+ Microcom+VA, 0.5	3.90±0.34	1.92±0	5.82	123.83

There has been a significant increase of starch and less pronounced – hemicelluloses in variants fertilized with micronutrients and bacteria metabolites. Starch cleavage does not occur at the moment. After the literature date in the period of dormancy starch cleavage and formation of glucose, fructose and sucrose in vine strings

is more intense after the temperature of -10° C. Starch reserve stored in xylem not are hydrolyzed because of strong cell walls lignification and blocking of enzymes, that's why it do not participate in the protection of vine from frost. The content of hemicelluloses in plants treated with micronutrients and product of bacteria increased compared to control. It is considered as a form of carbohydrate reserves, hydrolysis of hemicelluloses occurs at lower temperatures.

The obtained data allow us to assume that the treated plants accumulated more reserves carbohydrate and are able to resist lower negative temperatures. This assumption was confirmed at the influence of lower temperature on 31 January 2014 (-24^o C) and subsequent determination of the number of dead buds in spring.

Simultaneously with the carbohydrates it was determined content of compatible osmolytes – proline - into the shoots and buds. It was established that the foliar fertilization of plants during the vegetation period has contributed to increasing of the content of proline into the shoots and buds during the dormancy, particularly after applying metabolites or suspension of bacteria with Microcom.

Determination of one of the main indicators of vine resistance to wintering - bud viability - on spring, after the period of dormancy, showed, that the number of viable buds grew evident under the foliar fertilization, from 3.48% to 9.92% compared to control (tab. 4). Least of all died buds were on the plants fertilized during vegetation period by suspension or metabolites of bacteria with complex of trace elements - 57.36 and 55.84% viable buds (control - 47.44%). Generally amount of died buds was higher than previous years due to very low temperature on the end of winter.

Table 4

Effect of foliar fertilization and frost (-24^o C on 31 January 2014) on the state of grape buds on the spring, v. Codrinschii, %

Variants	buds died, %		buds alive, %	
	M± m	± to control	M± m	± to control
Control	52.56± 1.23		47.44±1.47	
Foliar fertilization by Microcom-VA, 0,5 doza	47.31±1.13	-5.25	52.69±1.25	+5.25
Metabolites of *Ps. fluorescens* + *Az. chroococcum*	49.08±1.16	-3.48	50.92±1.34	+3.48
Metabolites of *Ps. fluoresc.*+ *Az. chrooc.*+Microcom VA, 0.5	42.64± 0.92	-9.92	57.36± 1.13	+9.92
Suspensia of *Ps. fluorescens* + *Az.chrooc.*+ Microcom+VA, 0.5	44.16±0.78	-8.40	55.84±0.81	+8.40

Highlighting the positive effect of trace elements and products of bacteria *Az. chroococcum* and *Ps. fluorescens* on the accumulation of carbohydrates, compatible osmolytes content (sucrose and proline) in the organs of plants under low temperature indicates about the stimulator role of the mentioned products in achieving greater degrees of grape potential to resistance realization. We suppose that plants fertilized with trace elements and suspension and metabolites of bacteria facilitate the activities of one of the specialized mechanisms of plant resistance to low temperatures - compatible osmolytes accumulation.

CONCLUSION

It was revealed that the complex of trace elements Microcom-VA in halved dose and the products of bacteria *Azotobacter chroococcum* and *Pseudomonas fluorescens* (suspension and metabolites), which contain large amounts of biologically active substances, applied simultaneously for foliar fertilization of the vine thrice on the vegetation, induce changes in the metabolic processes during vegetation and dormancy periods, increase the growth and maturation of shoots, contributing to increasing of vine resistance to the wintering.

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