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Genetics and Selection

Study of the Outcomes of the Permanent Effect of Copper Ions on Natural Populations of Wine Yeast

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ABSTRACT. Resistance of natural populations of Anaga (Kakheti) and Surebi (Guria) wine yeast to copper ions has been studied. 250 strains, components of each population, have been investigated. Both populations have been found polymorphic to the investigated trait. The Anaga population, in contrast to the Surebi population, has been subjected to the effect of copper ions for decades. As a result of natural selection, a great number of resistant strains have been accumulated in the Anaga population. A genetic analysis has established that out of 10 strains of the Anaga population 6 contain two resistance determining SMF1 and SMF2 dominant genes. The studied strains of the Surebi population have only one active locus. © *2009 Bull. Georg. Natl. Acad. Sci.*

Key words: *wine yeast, natural population, strains, inheritance, copper ions.*

Living organisms have long been known to be subjected to the effect of heavy metals, including that of copper ions. The genetic system controlling the resistance of yeast fungi to copper ions has been well studied [1, 2]. The application of copper-containing agents in Georgian vineyards has a centuries-old history [3, 4].

The purpose of the study was to investigate the wine yeast populations that have been subjected to the effect of copper ions and to correlate them to the populations that have almost not been subjected to such a factor.

Wine yeast populations of Anaga (Kakheti) *Rkatsiteli* grape variety and Surebi (Guria) *Isabella* grape variety were chosen for the purpose. In contrast to *Rkatsiteli* grape varieties, *Isabella* grape variety is a semi-cultural one, resistant to fungus diseases and is not, therefore, subjected to treatment by fungicides. The isolation of strains from the wine lees was carried out by means of the method described in literature [3-5]. The species status in the isolated strains was established as per [6, 7]. 10 micro-populations in each population were analyzed. 25-25 strains, belonging to the wine yeast (*Saccharomices cerevisiae* var.vini), were derived from the specific population and analyzed. The relationship of the strains to copper ions was determined in a solid standard nutrient medium [8]. The nutrient medium was supplemented with copper sulphate at different rates. The concentration of copper sulphate in the medium varied between 1 mM to 7 mM. The yeast fungus cells could not grow in a medium with higher molarity. Three-day cultures were introduced into the test (copper sulfate-free) and copper sulfate media by means of a replicator. The cultures were incubated at 25^{o} C temperature. The obtained results were analyzed on the 5th day.

The relationship of the Anaga wine yeast population strains to copper ions is given in Table 1 below. The population-contained micro-populations differ in abundance of resistant and sensitive strains. The sensitivity range is between 1 mM to 7 mM. Stable strains are found in the micro-populations I, III and VIII. It is noteworthy that only sensitive strains are concentrated in

Table 1

Relation of wine yeast of Anaga population to copper ions

Micropopulation	mM	1			2			3			4			5			6			7		
	growth	+	±	−	+	±	−	+	±	−	+	±	−	+	±	−	+	±	−	+	±	−
I	quant	25	0	0	25	0	0	21	3	1	19	1	5	10	9	6	8	9	8	1	2	22
	%	100	0	0	100	0	0	84	12	0,4	76	4	20	40	36	24	32	36	32	4	8	88
II	quant	18	2	5	12	7	6	5	3	17	1	4	20	0	9	11	0	6	19	0	0	25
	%	72	8	20	48	28	24	20	12	68	4	16	80	0	36	44	0	24	76	0	0	100
III	quant	23	2	0	17	3	5	11	10	4	8	12	5	4	14	7	1	8	15	1	3	21
	%	92	8	0	68	12	20	44	40	16	32	48	20	16	56	28	4	32	60	4	12	84
IV	quant	21	4	0	15	1	9	12	8	5	5	12	8	3	7	15	1	1	23	0	1	24
	%	84	16	0	60	4	36	48	32	20	20	48	32	12	28	60	4	4	92	0	4	96
V	quant	20	5	0	18	6	1	13	6	6	7	13	5	1	9	15	1	4	20	0	0	25
	%	40	20	0	72	24	4	52	24	24	28	52	20	4	36	60	4	16	80	0	0	100
VI	quant	19	5	1	14	9	2	9	3	13	6	6	13	1	2	22	0	5	20	0	0	25
	%	76	20	4	56	36	8	36	12	52	24	24	52	4	8	88	0	20	80	0	0	100
VII	quant	21	1	3	17	3	5	5	12	8	6	13	6	2	7	16	0	7	18	0	0	25
	%	84	4	12	68	12	20	20	48	32	24	52	24	8	28	64	0	28	72	0	0	100
VIII	quant	21	4	0	11	13	1	9	12	4	7	7	11	2	9	12	1	4	20	1	2	22
	%	84	16	0	44	52	4	36	48	16	28	28	44	8	36	48	4	16	80	4	8	88
IX	quant	25	0	0	24	1	0	18	2	5	15	3	7	10	7	8	5	6	14	0	4	21
	%	100	0	0	96	4	0	72	8	20	60	12	28	40	28	16	20	24	56	0	16	84
X	quant	20	4	1	18	5	2	7	3	15	1	4	21	2	1	22	1	6	18	0	0	25
	%	80	16	4	72	20	8	28	12	60	4	16	84	8	4	88	4	24	72	0	0	100

Note: + good growth on the medium; ± weak growth on the medium; - absence of growth on the medium

Table 2

Relation of wine yeast of Surebi population to copper ions

Micropopulation	mM	1			2			3			4			5		
	growth	+	±	−	+	±	−	+	±	−	+	±	−	+	±	−
I	quant	24	1	0	18	2	5	11	8	6	3	2	20	0	5	20
	%	96	4	0	72	8	20	44	32	24	12	8	80	0	20	80
II	quant	25	0	0	20	5	0	17	5	3	11	5	9	3	7	15
	%	100	0	0	80	20	0	68	20	12	44	20	36	12	28	60
III	quant	18	4	3	11	4	10	3	12	9	1	9	15	0	0	25
	%	72	16	12	44	16	40	12	48	36	4	36	60	0	0	100
IV	quant	25	0	0	19	1	5	15	3	7	13	2	10	1	2	22
	%	100	0	0	76	4	20	60	12	28	52	8	40	4	8	88
V	quant	20	3	2	13	7	5	9	11	5	6	6	13	0	6	19
	%	80	12	8	52	28	20	36	44	20	24	24	52	0	24	76
VI	quant	18	2	5	16	2	7	4	9	12	1	10	14	0	0	25
	%	72	8	20	64	8	28	16	36	20	4	40	56	0	0	100
VII	quant	23	2	0	17	3	5	10	7	8	4	6	15	1	3	21
	%	92	8	0	68	12	20	40	28	32	16	24	60	4	12	84
VIII	quant	25	0	0	21	3	1	6	18	1	0	13	12	0	1	24
	%	100	0	0	84	12	4	24	72	4	0	52	48	0	4	96
IX	quant	17	3	5	10	7	8	5	3	17	2	2	21	0	0	25
	%	68	12	20	40	28	32	20	12	68	8	8	84	0	0	100
X	quant	21	2	2	14	6	5	5	13	7	1	15	9	0	4	21
	%	84	8	8	56	24	20	20	52	28	4	60	36	0	12	84

Note: + good growth on the medium; ± weak growth on the medium; - absence of growth on the medium

the micro-populations II, VI and VII. No strain could grow well in a medium with the 6 mM concentration of ions, although they contain such strains that manifested poor growth rate in the same medium.

The sensitivity range of the strains – components of the Surebi *Isabella* population - was found to be much limited against that of the Anaga population and was between 1 mM to 5 mM (see Table 2). Comparatively resistant strains were found in the micro-populations II, IV and VII; in particular, 5 strains used to grow in the 5 mM medium. The most sensitive were found to be members of the micro-population VIII. No strain could grow in the nutrient medium with the 5 mM concentration of copper ions.

In 10-10 resistant strains of the Anaga and Surebi populations, inheritance of the resistance-determining trait has been studied (see Tables 3 and 4). Resistance to heavy metals is being controlled by two dominant genes SMF1 and SMF2 of the SMF gene family [1, 9, 10]. The genetic line 31-1-7B (genotype MATa trp1arg4ade8ura3 SMF1) developed by Professor S. Fogel [11] was used to produce hybrids. A genetic analysis was carried out in auxotroph segregants. Segregation between the isolates sensitive and resistant to copper ions in the ten strains of the Surebi population was found to be in the 1:1 proportion. It is indicative of the monogenic inheritance of the trait. In 6 out of 10 investigated strains of the Anaga population, segregation was in the 3:1 proportion. Such segregation is indicative of the digenic inheritance of the trait. Thus, these strains contain concurrently two dominant genes that determine the trait. The studied populations reveal polymorphism in terms of resistance to copper ions. The Anaga population wine yeast is characterized by higher polymorphism against the Surebi one.

In local wine yeast populations, the resistance to heavy metals (Cu, Mn, Pb) has been studied and the intergenic polymorphism has been identified [3,4]. In studying resistance to copper ions in 8 different Kakheti populations, three morphs were identified, the trait being determined by one or seldom two dominant genes [5]. In studying two Kakheti populations (Shilda, Kvemo Kedi), their polymorphism in terms of resistance to copper ions was established. This phenomenon in the population was determined by two dominant genes [12]. The Anaga population studied by us was found to be similar in its structure to other Kakheti populations, whereas the Surebi population differed from other populations met in Western Georgia. Natural selection has led to the formation in the Anaga population of strains resistant to copper ions and to the growth of resistance in them.

Table 3

Inheritance of resistance to copper ions in cultures of Anaga population of wine yeast

Hybrid	Number of analyzed segregants	Relation to copper ions		Segregation	X^2
		Cup^r	Sup^s		
SF -1	100	71	29	3:1	0.85
SF -2	100	53	47	1:1	0.36
SF -3	100	22	78	3:1	0.36
SF -4	100	77	23	3:1	0.21
SF -5	100	57	43	1:1	1.96
SF -6	100	42	58	1:1	2.56
SF -7	100	47	53	1:1	0.36
SF -8	100	24	76	3:1	0.05
SF -9	100	20	80	3:1	1.33
SF -10	100	21	79	3:1	0.85

Table 4

Inheritance of resistance to copper ions in cultures of Surebi population of wine yeast

Hybrid	Number of analyzed segregants	Relation to copper ions		Segregation	X^2
		Cup^r	Sup^s		
Su -1	100	43	57	1:1	1.96
Su -2	100	56	44	1:1	1.44
Su -3	100	48	52	1:1	0.16
Su -4	100	47	53	1:1	0.36
Su -5	100	45	55	1:1	1.00
Su -6	100	49	51	1:1	0.08
Su -7	100	56	44	1:1	1.44
Su -8	100	42	58	1:1	2.56
Su -9	100	53	47	1:1	0.36
Su -10	100	52	48	1:1	0.16

გენეტიკა

ღვინის საფუვრის ბუნებრივ პოპულაციებზე სპილენძის იონების პერმანენტული ზემოქმედების შედეგების შესწავლა

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ნაშრომში შესწავლილია ანაგისა (კახეთი) და სურების (გურია) ღვინის საფუვრის ბუნებრივი პოპულაციების მდგრადობა სპილენძის იონებისადმი. გამოკვლეულია თითოეული პოპულაციის შემადგენელი 250 შტამი. შესწავლილი ნიშნისადმი ორივე პოპულაცია პოლიმორფული აღმოჩნდა. ანაგის პოპულაცია სურების პოპულაციისგან განსხვავებით მრავალი ათეული წლის განმავლობაში ექვემდებარებოდა სპილენძის იონების ზემოქმედებას. ბუნებრივი გადარჩევის მოქმედებამ გამოიწვია რეზისტენტული შტამების მაღალი სიხშირით დაგროვება ანაგის პოპულაციაში. გენეტიკური ანალიზით დადგენილია, რომ ანაგის პოპულაციის 10 შტამიდან 6 რეზისტენტობის განმსაზღვრელ ორ SMF1 და SMF2 დომინანტ გენს შეიცავს. სურების პოპულაციის შესწავლილ შტამებს მხოლოდ ერთი აქტიური ლოკუსი გააჩნიათ.

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