ON CHARACTERISATION OF A PIGMENT APPARATUS IN INTRODUCED CULTIVARS OF GRAPE IN BELARUS

KABASHNIKOVA L.F.[1], L.M. ABRAMCHIK[1], V.N. MAKAROV[1], G.E. SAVCHENKO[1]
e-mail: kabashnikova@ibp.org.by

Abstract. *The photosynthetic apparatus of twenty grape cultivars introduced in Belarus during vegetation period was investigated. An essential intervarietal diversity has been revealed regarding chlorophylls and carotenoids content. For almost all un hardy cultivars, the pigment content gained its maximal value already in June, though for the hardy ones it has been still increasing in August. For most of investigated cultivars, the content of photosynthetic pigments falls short of characteristic values for grape plants vegetating in warmer climate zones. The light harvesting system of photosynthetic machinery during the whole vegetation period resembled that of shade-tolerant plants. Most anthocyanins have been detected exclusively in the leaves of red-fruit cultivars. High content of malonic dialdehyde as indicator of oxidative stress, especially at the early stages of vegetation, is typical for most of grape cultivars introduced in Belarus.*
Key words: grape, introduced cultivars, photosynthetic pigments, anthocyanins, lipid peroxidation

INTRODUCTION

Analysis of empirical data for introduction of grapevines (*Vitis vinifera* L.) in various geographical regions clearly evidences high dependency of a cultivar's appearance on the current ecological conditions. On the other hand, it has been shown that content of the photosynthetic pigments in the grapevine leaves may serve as an indicator of physiological state of plants and thus indicate if the plant fell under the stress (Abdallach F.B. et al., 2006; Blanchfield A.L. et al., 2006; Gornik K. et al., 2008).

Here, we report our efforts to characterize pigment apparatus of grapevine cultivars introduced in Belarus where climate conditions are marginal to those which such a light- and heat-demanding species would ideally require.

MATERIAL AND METHOD

Twenty different cultivars of grapevines introduced in RB were investigated. The screened cultivars were outdoor-grown and varied by their resistance to low-temperature stress, grape's color and application particulars (Ustinov V.N., Rusovskaja A.V., 2009). Leaves of the middle layers of the plants were analyzed throughout the different vegetation phases of the season 2009. Content of photosynthetic pigments, anthocyanins, and degree of peroxidation of lipids were determined according to (Kabashnikova L.F et al., 2011).

[1] The Institute of Biophysics and Cell Engineering of the National Academy of Sciences of Belarus

RESULTS AND DISCUSSIONS

1. Photosynthetic pigments

Quantitative analysis of the photosynthetic pigments in leaves of the screened grapevine cultivars (table 1) shows significant intervarietal dispersion regarding content of chlorophyll (*a*+*b*) (9,5–33,0 µg/cm^2) and carotenoids (2,3–6,5 µg/ cm^2, data not shown).

Table 1

Chlorophylls content (*a + b*, g/cm^2) in the middle layer leaves of twenty grape cultivars introduced in Belarus during vegetation period 2009

Resistens to cold by Ustinov V.N., Rusovskaja A.V., 2009	Cultivar	Vegetation period			
		June	July	August	September
Highly-resistant, technical type	*Alfa*	11,71±0,99	11,29±1,57	14,67±1,55	12,35±2,98
	Golubok	16,25±0,24	21,72±1,71	18,61±1,38	19,93±2,70
	MN 1094	18,10±0,34	-	16,23±1,01	14,15±0,02
Resistant, technical type	*Bianka*	13,73±0,56	12,68±0,35	16,43±0,66	-
	Dushisty	10,37±1,03	14,52±2,12	9,51±0,72	16,19±2,85
	Kristall	16,12±1,40	10,99±0,72	16,18±1,15	9,573±2,28
	Platovsky	19,20±0,68	15,55±0,88	10,94±0,41	9,99±0,82
Resistant, table type	*Supaga*	18,74±1,29	19,26±1,65	27,23±0,25	23,17±0,32
Moderately resistant, technical type	*Muskat desertny*	20,02±1,47	19,51±2,79	16,61±0,59	17,15±0,00
	Muskat Niny	10,21±0,59	18,93±0,45	10,59±0,02	-
	Suvenir Vaskovskogo	13,82±0,38	13,60±1,56	13,75±0,60	12,07±1,80
Moderately resistant, table type	*Viktoria*	19,13±0,14	25,59±5,07	33,70±0,64	27,23±0,95
	Iyulsky	20,96±0,95	18,85±0,94	16,20±0,18	12,96±1,83
	Krasa Severa	17,75±1,56	19,49±3,07	14,92±0,79	12,27±0,04
	Neptun	22,20±0,64	19,92±2,11	13,22±0,04	10,83±0
Low-resistant technical type	*Portugiser*	18,00±1,18	15,69±0,85	12,67±0,49	15,00±1,85
Low-resistant, table type	*Aleshenkin*	15,96±1,27	20,12±0,42	12,73±0,21	13,07±0,00
	Dekabrsky	17,70±0,28	22,81±3,19	16,12±1,28	13,93±0,46
	Novoukrainsky ranny	13,53±0,91	18,82±1,02	11,59±0,54	12,23±0
Non-resistant, table type	*Dunav*	13,46±0,75	-	12,00±1,34	15,06±1,50

Nevertheless, for vast majority of the investigated cultivars even the top values were significantly surpassed by those of cultivars usually vegetated in more warm climate zones (Abdallach F.B. et al., 2006; Blanchfield A.L. et al., 2006; Gornik

K. et al., 2008), and appeared to be twice lower then those found in grown up cereal leaves (Abramchik L.M. et al., 2008).

In spite of difference in content of chlorophylls within the group of highly-resistant cultivars, the type of kinetics for uptake of chlorophylls was identical. Its main feature is significantly increased uptake of the chlorophylls in the second half of the vegetating period. For instance, according to this criterion, "*Victoria*" could be classified as hardy cultivar. Within the group of hardy and moderately hardy cultivars of technical type, essential differences in the pigments uptake dynamic were observed. Thus, for all most cold susceptible cultivars, the pigment content reached its peak already in June and then steadily decreased until the end of the season. However, photosynthetic pigments of the vast majority of the examined cultivars didn't degrade drastically even by the end of September. Such a trend is not in line with that which would be expected for the most of mono- and dicots in which chlorophyll decompose even in the leaves of the upper layer quite quickly in course of a harvest maturing. Noteworthy, a fairly low ratio of chlorophyll *a* to chlorophyll *b* featured all investigated cultivars (table 2).

Table 2

Variation in composition of photosynthetic pigments and correlation indexes (*r*) between total content of chlorophylls and carotenoids (A) and ratio of chlorophyll *a* to chlorophyll *b*, and carotenoids content (B) measured in the middle layer leaves of grape cultivars during vegetation period 2009

Сорт	Chlorophylls a/b		Chlorophylls (a+b)/ Carotenoids		*r*	
	June	August	June	August	A	B
Alfa	2,51±0,35	2,03±0,65	2,68±0,39	3,36±0,35	0,49	-0,31
Golubok	2,89±0,02	2,46±0,04	3,54±0,09	4,31±0,41	-0,54	-0,68
MN 1094	2,68±0,01	2,58±0,01	3,6±0,16	3,60±0,16	1,00	0,99
Bianka	2,81±0,12	2,51±0,06	3,49±0,32	4,11±0,02	0,99	0,81
Dushisty	2,45±0,28	2,75±0,12	3,07±0,13	2,75±0,16	0,83	-0,47
Kristall	2,81±0,04	2,37±0,01	3,19±0,11	4,20±0,11	0,86	0,77
Platovsky	2,62±0,22	2,43±0,03	3,94±0,39	3,91±0,19	0,99	0,88
Supaga	2,68±0,04	2,52±0,03	3,89±0,28	4,78±0,05	0,86	-0,75
Muskat desertny	2,83±0,05	2,65±0,02	3,95±0,26	4,20±0,17	0,83	-0,12
Muskat Niny	2,02±0,02	2,76±0,06	3,36±0,14	3,07±0,13	0,98	0,77
Suvenir Vaskovskog	2,65±0,02	2,47±0,01	3,59±0,17	3,90±0,01	0,71	0,07
Viktoria	3,02±0,05	2,38±0,04	2,97±0,02	5,44±0,19	-0,46	0,36
Iyulsky	2,8±0,07	2,51±0,01	3,66±0,06	3,87±0,07	0,99	0,59
Krasa Severa	2,84±0,01	2,59±0,05	4,06±0,10	4,10±0,20	0,95	0,57
Neptun	2,96±0,02	2,36±0,06	3,63±0,04	3,96±0,02	0,97	0,97
Portugiser	2,85±0,06	2,25±0,01	3,55±0	4,67±0,17	0,97	0,52
Aleshenkin	3,03±0,02	2,61±0,04	3,48±0,06	3,74±0,05	0,40	0,21
Dekabrsky	2,79±0,08	2,48±0,00	3,18±0,12	4,22±0,07	0,66	0,43
Novoukrain-sky ranny	2,66±0,01	2,58±0,05	3,43±0,28	4,08±0,32	0,57	-0,46
Dunav	2,73±0,02	2,56±0,05	3,2±0,13	3,79±0,12	0,99	0,11

For the most of cultivars it was below 2,6 and notably varied in course of vegetation. The highest variability (from 2.0 to 3.0) was observed for green-fruit cultivars *Muskat Niny* and *Aleshenkin*. In general, light harvesting system of the cultivars grown in open soil resembled this of shade-resistant plants.

The ratio between chlorophylls and carotenoids content at the early stage of vegetation (table 2) varied within the range of 3,5; in the middle of vegetation period it increased to the value ≥4,5 without significant cultivar differences, and at the end of vegetation period it lowered for most of cultivars, mainly due to the predominant decrease of chlorophyll content. A tendency was observed towards decreased value of the ratio between net chlorophylls and carotenoids content in grapevine leaves under biotic stress in the field (Blanchfield A.L. et al., 2006).

Under normal growth conditions, correlation index (***r***) between chlorophylls and carotenoids content is usually around 1. But not all the grapevine cultivars revealed such correlative interrelation (table 2, column A). For the cultivars with high chlorophyll content (considering the excess of energy absorbed by chlorophyll), the lack of positive correlation between accumulation of chlorophylls and carotenoids is probably associated with an underbalance between the components of a plant antioxidant system, in which carotenoids play a dominating role in the neutralization of active oxygen forms.

Interplay between light-harvesting and chlorophyll-protecting systems is the important feature of plant cultivars. It is characterized by a correlation index between changes in proportion of chlorophylls *a* and *b*, and carotenoids content. The data obtained, reveal a significant variability of ***r*** for these parameters (table 2, column B). Thus, high positive values of ***r*** were determined for four hardy cultivars of technical type. Seven cultivars of different cold-resistance groups were characterized by negative values, and another four cultivars did not reveal any correlations. Likely, interplay between two pigment systems can be disharmonized under stress if additionally carotenoids are synthesized, not chlorophylls. If that is the case, detected diversity for the values of ***r*** for different cultivars introduced in the same climate zone can specifically reflect their individual response on temperature stress during the vegetation course.

2. Anthocyanins

The major amount of anthocyanins playing an important role in stress response was found mainly in the leaves of red-fruit cultivars *Neptun* and *Dunav* (15-17 μg per gram of fresh mass, table 3). For the other cultivars in the first half of a vegetation period, this value did not exceed 8,5 μg per gram of fresh mass being next lower order to that for the leaves of rye, triticale, and tomatoes (Kabashnikova L.F. et al., 2011). In the majority of grapevine cultivars, anthocyanins were revealed only at the end of vegetation season. Though being not always evident by direct viewing, the bulk of anthocyanins is localized in petioles.

3. Degree of lipid peroxidation

Degree of lipid peroxidation was evaluated relying on the content of malonic dialdehyde (MDA). In general, degree of lipid peroxidation in severed grapevine leaves (table 3) significantly exceeded the values determined for cereal plants (Abramchik L.M. et al., 2008), especially at the final stage of plant ontogenesis. As no correlation with cold resistance was observed for all examined cultivars, it is entirely possible that different cultivars of heat-demanding grapevine are similarly affected by rather low night temperatures during outdoor growing.

Table 3

Content of anthocyanins and products of lipides peroxidation (malonic dialdehyde, MDA) in the middle layer leaves of grape cultivars

Resistance to cold by Ustinov V.N., Rusovskaja A.V., 2009	Cultivar	Anthocyanins, µg per gram of fresh mass, June	Anthocyanins, µg per gram of fresh mass, September	MDA, µmol per gram of fresh mass, June
Highly-resistant, technical type	*Alfa*	4,8±0,60	≤ 0,1	15,28±0,50
	Golubok	6,7±0,70	9,58 ±0,48	6,80±0,14
Resistant, technical type	*Bianka*	6,1±0,80	≤ 0,1	3,92±0,13
	Kristall	2,6±0,40	≤ 0,1	17,37±0,95
Resistant, table type	*Supaga*	≤ 0,1	≤ 0,1	2,72±0,74
Moderately resistant, technical type	*Muskat desertny*	≤ 0,1	17,36±1,40	6,67±0,12
	Muskat Niny	4,1±0,30	≤ 0,1	12,64±0,04
	Suvenir Vaskovskogo	8,5±1,10	36,12±1,43	17,96±0,95
Moderately resistant, table type	*Viktoria*	1,8±0,20	20,89±0,65	14,57±0,21
	Iyulsky	1,7±0,10	10,56±0,80	6,92±0,15
	Krasa Severa	3,1±0,50	12,91±0,92	8,71±0,35
	Neptun	17,4±0,20	21,11±0,64	15,73±0,42
Low-resistant, technical type	*Portugiser*	4,3±0,90	19,76 ±1,63	3,92±0,15
Low-resistant, table type	*Aleshenkin*	0,9±0,10	15,61±0,34	12,57±0,22
	Dekabrsky	0,8±0,10	13,02±1,45	15,86±0,12
Non-resistant, table type	*Dunav*	15,6±0,40	15,60±1,41	7,76±0,28

CONCLUSIONS

1. An essential intervarietal diversity has been revealed regarding chlorophylls and carotenoids content. For most of investigated cultivars, the content of photosynthetic pigments falls short of characteristic values for grape plants vegetating in warmer climate zones. Low content of photosynthetic pigments compared to other cultures, e.g. cereals, is indicative for a pigment system of light-demanding grapes grown in Belarusian climate.

2. For almost all unhardy cultivars, the pigment content gained its maximal value already in June, though for the hardy ones it has been still increasing in

August. No pronounced degeneration of photosynthetic pigments has been detected by the end of September.

3. Correlation indexes (***r***) between chlorophylls and carotenoids content have been found to deviate from high positive values for a number of cultivars.

4. The light harvesting system of photosynthetic machinery during the whole vegetation period resembled that of shade-tolerant plants with rather low ratio of chlorophyll *a* to chlorophyll *b* (less then 2, 6).

5. Most of examined grape cultivars demonstrated extremely small content (next lower order compared to the leaves of rye, triticale, and tomatoes) of anthocyanins known to play a major role in stress response.

6. High content of malonic dialdehyde, especially at the early stages of vegetation, is typical for most of grape cultivars introduced in Belarus and indicates intensive oxidative processes in lipid membranes giving evidence that all examined cultivars of heat-loving grapevine have undergone stress. This resulted in increased degree of lipid peroxidation and affected the process of accumulation of photosynthetic pigments.

Acknowledgement. *This work was conducted with financial support of Belarusian Republican Foundation for Fundamental Research (Grant B09SCST-009).*

REFERENCES

1. **Abdallach F.B., Elloumi N., Mezghani I., Garrec J.P, Boukhris M., 2006** – *Industrial fluoride pollution of Jerbi grape leaves and the distribution of F, Ca, Mg, and P in them*. Research Report Fluoride. Vol. 39, pag. 43–48.
2. **Abramchik L.M., Serdjuchenko E.V., Zenevich L.A., Kabashnikova L.F., 2008** – *Influence of high intensity light and heat stress on structural and functional state of barley photosynthetic membranes (Hordeum vulgare L.)*. Proceeding of NAS of Belarus, Biology science (in Rus.). № 3, pag. 59-62.
3. **Blanchfield A.L., Robinson S.A., Renzullo L.J., Powell K.S., 2006** – *Can leaf pigment composition help us identify grapevines infested with Phylloxera?* Funct. Plant Biol. Vol. 33, pag. 507–513.
4. **Gornik K., Grzesik M., Romanowska–Duda B., 2008 –** *The effect of chitosan on rooting of grapevine cuttings and on subsequent plant growth under drought and temperature stress*. J of Fuit and Ornamental Plant Research. Vol. 16, pag. 333–343.
5. **Kabashnikova L.F., Abramchik L.M., Makarov V.N., et al., 2011** – *Characteristics of a pigment apparatus of introduced cultivars of grape*. Vestnik of the foundation for fundamental research (in Rus.). № 1, pag. 31–43.
6. **Ustinov V.N., Rusovskaja A.V., 2009** – *Vinogradorstvo. Prakticheskoye posobiye* (in Rus.) Minsk: Lasurak. 208 pag.