Phenotyping under extreme weather conditions and microsatellite based genotyping of some Hungarian grape cultivars

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Summary

Phenology is an indicator of plant response to the environment. In viticulture growth stages timing is important for site and cultivar selection, vineyard planning and cultural practices management. In the last years, autochthonous cultivars obtained an increased interest and, therefore, in this work we studied the phenological performances of Hungarian old varieties. The data recorded in 2012 were compared with the ones published 60 years ago. The examined genotypes were identified by SSR markers. Extremely high temperature and low precipitation occurred during the 2012 vintage. The uncommon weather conditions affected the length of the vegetation period less than expected. However, the time period between flowering and harvest decreased significantly. In general, minor varieties seem to be more sensitive to extreme conditions than international cultivars, according to their eno-carpological characteristics.

K e y   w o r d s : grapevine; growth cycle; climate; viticulture; phenological stages.

Introduction

Climate effect on grapevine growth timing can be described by phenological records. In general, it is accepted that increasing temperature during the vegetation period will shorten the time between phenophases. The time between the phenological stages of vine annual growth cycle varies with grape cultivar, meteorological conditions and location (Jones and Davis 2003). Knowledge of plant phenology is crucial in viticulture for site and cultivar selection, with the purpose of a rational vineyard establishment design. Phenotype is genetically determined but the environmental conditions could significantly modify it (Keller 2010). As a major example, phenology is strongly influenced by environmental conditions. Our aim was to define indications for the future Hungarian viticulture by comparing plant development under extreme weather conditions (such as the ones observed in 2012 in Hungary), with the available data from the past decades. Furthermore, we wanted to characterize the old Hungarian varieties by microsatellite markers as suggested in the COST FA1003 project.

Material and Methods

The investigation was set up on the experimental vineyard of Georgikon Faculty, Keszthely, Hungary (longitude: 17.24 degree; latitude: 46.75 degree; elevation: 110 m above Sea level). Meteorological data were collected with iMETOS® (Pessl Instruments, Austria) automatic weather station, what was installed in the experimental vineyard. Phenological stages were recorded according to the BBCH scale (Lorenz *et al.* 1994) as suggested in the framework of the COST Action FA1003-GRAPENET project (Rustioni *et al.* 2014a). Twenty-one accessions were included in the trial. Seventeen varieties were old Hungarian cultivars: 'Fehér járdovány', 'Fehér lisztes', 'Hárslevelű', 'Juhfark', 'Kék bakator', 'Királyleányka', 'Kocsis Irma', 'Kossuth', 'Kövér szőlő' , 'Leányka', 'Mathiász Jánosné muskotály', 'Mátyás király', 'Mirkovácsa', 'Pintes', 'Piros gohér', 'Piros szlanka', 'Sárfehér'. Four reference varieties were also included: 'Cabernet franc', 'Chasselas blanc', 'Pinot noir' and 'Sultanina'. Phenological data recorded in 2012 were compared to the ones already available in Németh Ampelography (1970) where phenological data from 1950 to 1957 concerning most of the varieties described are reported. Each dataset included the day of the year of budburst, beginning of flowering, full blooming, start of veraison, harvest date and leaf fall. The berry sugar content was noted at the date of harvest.

Eno-carpological data were also recorded in 2012 following Rustioni *et al.* (2014b).

The morphologically characterized grape varieties were genotyped in 9 microsatellite loci. DNA isolation and microsatellite analyses were carried out according to Halász *et al.* (2005). Nine nuclear SSR primer pairs VVS2, VVMD5, VVMD7, VVMD25, VVMD27, VVMD28, VVMD32, VrZag62, VrZag79 were chosen based on Gen-Res081, GrapeGen06 EU projects. PCR was performed in a Bio-Rad iCycler or a T100 thermolcycler (Bio-Rad, Ltd. Hungary)) in a 25 μL volume. The reaction mixture contained 20 ng DNA template, 1 μM of each primer, 75 μM of each dNTP, 2 mM $MgCl_2$, 1x reaction puffer and 1 unit Taq polymerase (WestTeam Biotech, Hungary). The following PCR profile was applied: precycle: 2 min at 95 °C; 40 cycles of denaturation 30 s at 95 °C, 30 s annealing at 56 °C and 1 min extension at 72 °C; postcycle: 5 min at 72 °C. The PCR products were separated on sequencing gel (8 % polyacrylamide gel) and analyzed with ALF Express II DNA Analyzer (Amersham Biosciences). Allele

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sizes were estimated by Fragment Analyzer Software (Amersham Biosciences) using Cy5-labeled internal size standards.The raw SSR allele data were N-coded according to the recommendation of GrapeGen06 program and the uploading criteria of the European Vitis Database (Maul *et al.* 2012).

Results and Discussions

The examined genotypes are different according to the DNA analysis. Some examples of the obtained information are reported in Tab. 1. All the examined cultivars were genetically different, thus, each of them represents a unique genotype. Our data demonstrated they are not related each other: the differences among them were 3 to 8 alleles (Tab. 2). The largest number of sharing alleles occurred in

the VVS2 SSR primer pairs: one allele of this primer was replicated in all the cultivars. The highest polymorphism was given by the VVMD28 SSR marker: this locus showed differences in all the coded allele 1 and 2.

The 2012 weather conditions were unfavorable. Late spring frost, drought and heat waves occurred during the whole season in our research site. The precipitations reached 100 mm during all the vegetation period, while the 35 years (1961-1995) average was 400 mm. The Growing Degree Day value reached 1550 and we had 12 days above 35 °C during the summer (Figure). Based on our results bud break had a 10-12 d delay when compared with previous data in most of the varieties (Tab. 3). In spite of that, flowering was almost in the same period as for a 7 years average recorded 60 years earlier. Veraison started 5 to 7 d earlier and grape harvesting occurred 2 weeks earlier. We have to note that harvest date is affected by viticultural and

Table 1

Nine nuclear SSR allele results of five cultivars

Cultivar/Primer		Kocsis Irma	Kossuth	Mathiász Jánosné muskotály	Mátyás király	Mirkovacsa
VVMD5	coded allel 1	N+4	N+12	N+4	N+8	N+4
	coded allel 2	N+16	N+16	N+8	N+12	N+18
VVMD7	coded allel 1	N+22	N+18	N+8	N+22	N+8
	coded allel 2	N+2	N+18	N+18	N+18	N+14
VVMD25	coded allel 1	N+4	N+6	N+6	N+6	N+4
	coded allel 2	N+20	N+20	N+14	N+6	N+20
VVMD27	coded allel 1	N+10	N+6	N+4	N+6	N+4
	coded allel 2	N+20	N+20	N+14	N+14	N+4
VVMD28	coded allel 1	N+32	N+2	N+28	N+18	N+16
	coded allel 2	N+50	N+36	N+52	N+26	N+42
VVMD32	coded allel 1	N+7	N+7	N+5	N+7	N+17
	coded allel 2	N+37	N+37	N+29	N+17	N21
VVS2	coded allel 1	N+10	N+10	N+10	N+10	N+10
	coded allel 2	N+10	N+30	N+10	N+10	N+10
VrZag62	coded allel 1	N+10	N+18	N+20	N+12	N+20
	coded allel 2	N+28	N+28	N+28	N+2	N+28
VrZag79	coded allel 1	N+8	N+8	N+18	N+8	N+12
	coded allel 2	N+16	N+20	N+22	N+20	N+12

Table 2

Number of matching alleles among the different pairwise of samples.

| Sample 1	Sample 2	Score	No. alleles used in comparison	No. alleles matching between individuals	No. MD Sample 1	No. MD Sample 2
Kocsis Irma	Kossuth	44.44 %	18	8	0	0
Kossuth	Mátyás király	44.44 %	18	8	0	0
Mathiász Jánosné muskotály	Mirkovacsa	38.89 %	18	7	0	0
Kocsis Irma	Mirkovacsa	33.33 %	18	6	0	0
Mathiász Jánosné muskotály	Mátyás király	33.33 %	18	6	0	0
Kocsis Irma	Mátyás király	27.78 %	18	5	0	0
Kocsis Irma	Mathiász Jánosné muskotály	22.22 %	18	4	0	0
Kossuth	Mathiász Jánosné muskotály	22.22 %	18	4	0	0
Kossuth	Mirkovacsa	16.67 %	18	3	0	0
Mátyás király	Mirkovacsa	16.67 %	18	3	0	0
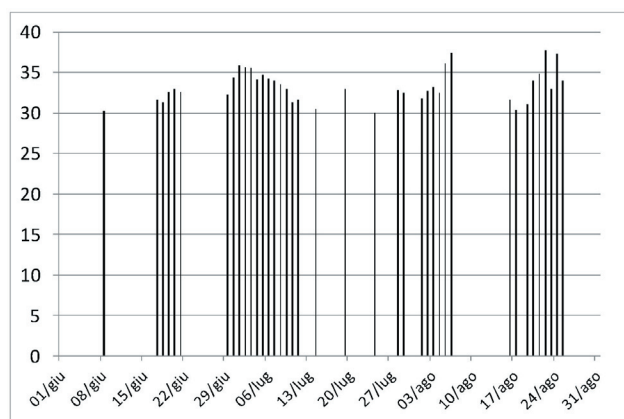
Figure: Maximum daily temperatures of extremely hot days, which were 30 °C or above in 2012.

enological goals also, which could be changed during the years. In spite of that, it seems our results strengthen the findings that one of the consequences of climate change (related to temperature increase) is a shift of some phenological stages to earlier times during the season (Wolfe *et al.* 2005). However, the beginning of leaf discoloration has started later and leaf fall ended in the last days of November, as usual. This could have positive consequences on the plant reserve accumulation. Our data suggests that the speed of plant development during the vegetation period has significantly been modified in 2012 when compared to 60 years ago data. However the total length of the vegetation period did not change significantly. This behavior could be related to the daylight hours during the vegetative period which should have been kept the same among the years, confirming that light is one of the most powerful determinants of plant phenology (Mc Clung 2001). Also the eno-carpological data were recorded, such as berry weight, cluster weight, seed number, sugar content, total polyphenols content. Some results are reported in Tab. 4. Most of the studied traits showed a high variability among cultivars. However, the results indicate that environment sensitive cultivars respond with less sugar accumulation (e.g. 'Kossuth') or smaller cluster weight (e.g.: 'Chasselas blanc').

Conclusions

Independently of the cultivar, the traditional Hungarian grape varieties showed the same phenological changes during the 2012 vegetation period when compared to the 60 years ago data. The plant development was modified between specific phenological stages, but the whole vegetation period length did not change significantly. Because the berry ripening was anticipated, we could suggest, for example, that varieties which requires more heat, radiation etc. could be grown under our conditions, or that, nowadays, yield safety will be much higher. These results will bring the attention of grape growers to autochthonous Hungarian cultivars which were abandoned in the last century.

Acknowledgement

Table 3

Comparison of two cultivars phenophases
(expressed in day of the year) in 2012 and 60 years ago

BBCH code	Description	Cultivars			
		Hárslevelű		Kövér szőlő	
		1957	2012	1957	2012
08	Bud burst	105	114	102	109
67	Full blooming	163	161	157	158
81	Beginning of ripening	232	225	229	215
89	Harvest (Brix)	293 (17.2)	297 (15.8)	248 (22.0)	261 (17.3)
97	End of leaf fall	326	327	322	327

Table 4

Eno-carpological average data of some examined cultivars in 2012

Characteristics	Cultivars/varieties		
	Kövér szőlő	Kossuth	Chasselas blanc
Date of harvest (day/month)	18/09	03/09	06/09
Berry colour	blanc	blanc	blanc
Sugar content (°Brix)	17.3	12.9	17.2
Titratable acidity (g·L^{-1} tartaric acid)	7.46	6.59	4.35
Cluster weight (g)	133.14	145.85	95.19
Berry weight (mg)	2889	2954	2585
Number of seeds/berry	2.0	2.8	1.9
Total phenolic content (mg·kg^{-1} of grape)	432.87	862.94	568.92

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