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PHYLLI – an international database for grape phylloxera (*Daktulosphaira vitifoliae* Fitch)

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Abstract: PHYLLI is a database of standardized microsatellite reference alleles to manage phylloxera genotypes. Protocols for the aspects of analysis (DNA extraction, PCR methods, visualization and allele description) are presented with appropriate flexibility. The open database is a major step towards developing strategies and devising measures to control aggressive phylloxera strains. This is the first report to introduce the PHYLLI database and discuss its advantages for both vineyard management and academic research.

Key words: genotype, phylloxera, grapevine, SSR, biotypes

Introduction

Grape phylloxera, *Daktulosphaira vitifoliae* (Fitch), is a highly destructive insect pest of susceptible or partially resistant grapevine varieties where it may feed on roots and leaves (Forneck and Huber, 2009). Galls (nodosities) are induced in the meristematic zone of the root tip and deformed swollen root tissues (tuberosities) are formed on lignified root parts in response to phylloxera feeding. In association with soil borne saprophytes (Edwards *et al*., 2007) and other abiotic stresses, nodosities and tuberosities lead to severe yield losses and eventual death of the plant (Powell *et al*., 2013).

Over the past 30 years, evidence for the existence of different phylloxera strains has become more apparent because of differences in their performance on a range of *Vitis* genotypes (Kocsis *et al*., 1999; Forneck *et al*., 2001; Herbert *et al*., 2010; Korosi *et al*., 2011). These strains have caused tremendous economic losses recently (Powell *et al*., 2013) emphasizing the need to identify and monitor strain diversity and seasonal population changes in vineyards. A wide-range of strains has been reported in Europe, Australia, Canada, South Africa, China and the USA. Because standardized protocols for DNA based genotyping don't yet exist, it is not possible to accurately characterize and compare the distribution of all strains in different countries.

The link between phenotype (in terms of performance and effects on the host) and genotype has often not been fully determined. Furthermore a standardized system to monitor phylloxera population development and genetics is lacking. Such monitoring is essential to

devise pest risk assessments that lead to efficient pest management strategies; they are also important in quarantine strategies and the assessment of vineyard market values.

Microsatellite (simple sequence repeat SSR) markers for grape phylloxera have been used since 2002 and continue to be optimized (e.g. Corrie *et al*., 2002; Vorwerk & Forneck, 2006; Lin *et al*., 2006; Riaz *et al*., 2014). SSR markers are versatile genetic tools as they produce reproducible data across different laboratories. However, drawbacks include large allele dropouts, difficulty in scoring due to stutter (slip strand mispairing during PCRs), null alleles and homoplasy of alleles. In 2014, the ISHS Phylloxera Working Group proposed the use of ten SSR markers and well-known phylloxera genotypes as references for data comparison across different labs.

The aims of the PHYLLI project are: (1) to define and name phylloxera genotypes suitable for comparative studies; (2) to provide access to genotype information in an open database (PHYLLI); and (3) to develop a robust genotyping protocol based on the choice of SSR primers available.

Genotyping: basic experimental design

SSR-primer set

Because we considered SSR markers to be the neural point of a successful and reproducible method, great care and time was taken to select a subset of SSR markers from those available. The primers were selected from published (Riaz *et al*., 2014; Corrie *et al*., 2002; Vorwerk & Forneck, 2006) and unpublished databases (Papura, 2014). In 2014, the group chose a subset of 10 from the repository of 200 SSR markers that had passed initial screening. The selection criteria were: (1) allele size, (2) allele frequency, (3) technical robustness for scoring and amplification, and (4) suitability for multiplexing (Table 1).

DNA extraction procedure

In order to optimize the process of SSR genotyping, the method of DNA extraction can be done according to the individual lab's practice (if robust SSR primers are chosen). Based on a literature research DNA extraction techniques for single-individual extractions employed are either CTAB-based according to Lin & Walker (1996), commercial animal or fungal kits, or a Chelex-based protocol (Forneck *et al*., submitted). All of these extraction methods are effective if they are employed with the set of 10 SSR primer pairs.

PCR protocols & machines

Both protocol and technical equipment are flexible and may be used according to a lab's best practices. PHYLLI does not require defined cycling protocols or specific polymerases. An annealing temperature of 60 °C for all primers is suggested. PCRs may be run on a single or multiplex basis. Optimized protocols for multiplex PCRs are currently under development.

Visualization of alleles

Visualization and detection of alleles is critical for accurate and reproducible results. Within the group, flat gel and capillary systems (e.g. Li-COR, ABI Prism, Advanced Sequencing) and vertical gel electrophoresis are being used. There is mutual agreement favoring plate-based mass screening and most of the group's members employ ABI-machines. Regardless of the choice of machine, a set of standard phylloxera samples are required to calibrate allele calling among runs on the same machines and runs among different machines in different labs. The group suggests 6 reference samples.

Table 1. SSR Primers employed for the standardized protocol in PHYLLI. (∑ Alleles = Number of alleles; H_{obs} = observed heterozygosity)

Name	Repeat	References	∑ Alleles	$H_{obs.}$
Dvit 3	(AT)9(GT)11	Corrie *et al*., 2002	3	0.0.03175
Dvit 6	(AAT)9	Vorwerk and Forneck, 2006	5	0.63492
DV 4	(GTT)9	Papura, unpubl.	6	0.65079
DV 8	(TG)8	Papura, unpubl.	3	0.47619
DV 11	(CT)7	Papura, unpubl.	2	0.03175
PhyIII 30	(TCT)12	Riaz *et al*., 2014	6	0.63492
PhyIII 36	(TAA)11	Riaz *et al*., 2014	4	0.20635
PhyIII 55	(ATT)11	Riaz *et al*., 2014	6	0.53968
PhyIV 4	(AATA)5	Riaz *et al*., 2014	5	0.16129
DVSSR4	(CT)12	Lin *et al*., 2006	6	0.46774

Reference samples for calibration of alleles

Six samples are suggested. Each sample is derived from a single founder clonal line to provide DNA for all participating labs. The selected lines are heterozygous and provide a wide range of alleles for the selected 10 SSR markers for comparative studies. The DNA samples will be mailed to each participating lab.

Genotyping: Experimental efficacy

Studies performed at BOKU (Mammerler, 2016) with 10 SSR primers and 63 samples found that the selected set of 5-6 primers will identify more than 95% of multi locus genotypes (MLGs) (Figure 1). These results also indicate that a smaller set of primers is sufficient to genotype phylloxera populations within a given sample range. Further studies of other groups are underway to test the efficacy of the primer combinations in a wider range of samples. The goal of this platform is to obtain feedback from all research groups so that the most efficient primer combinations can be selected in the near future.

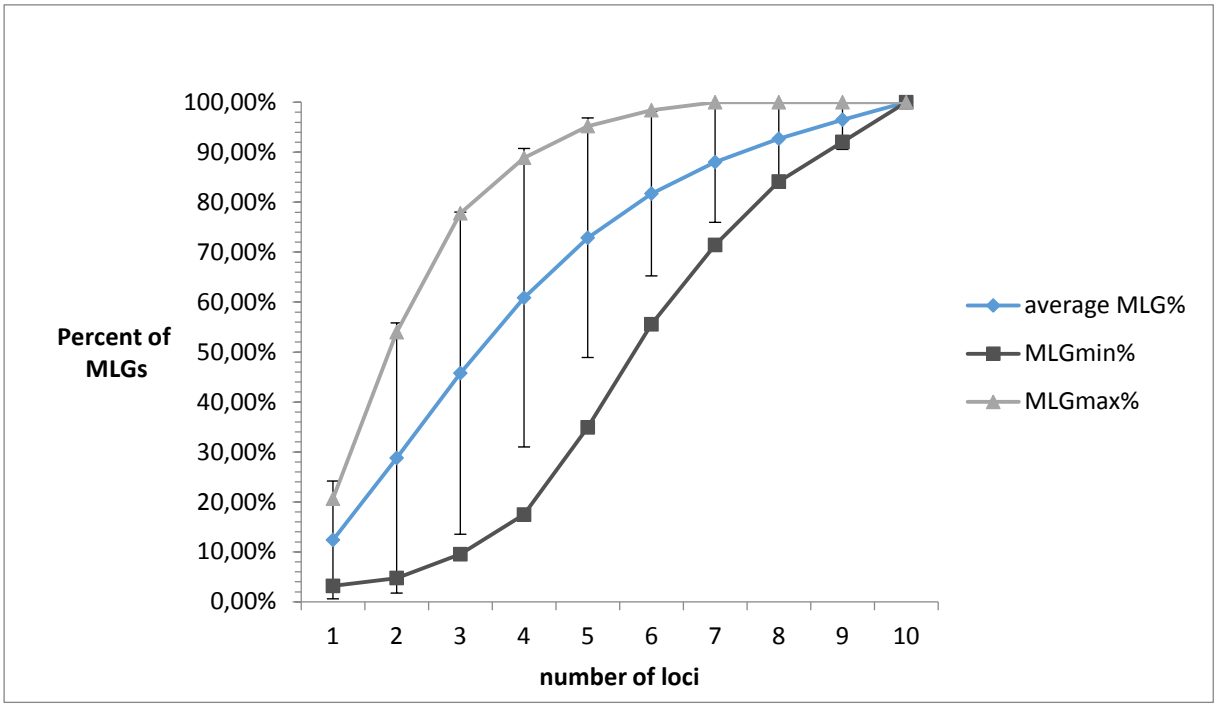


Figure 1. Percent increase in distinguishable genotypes. The primer accumulation curve was calculated by selecting all combinations Cln from l = 10 available primers with $1 \leq n \leq 10$ in GenClone software. The percent of distinguishable genotypes describes the number of grape phylloxera genotypes in percent that are discriminated by ABI-Prism technology. Starting with one primer in each step another primer was added up to a total of ten primers. The number of analyzed genotypes was N = 103. Maximum number of distinguishable genotypes: 63 = 100%. MLG, Multi Locus Genotype.

Database PHYLLI

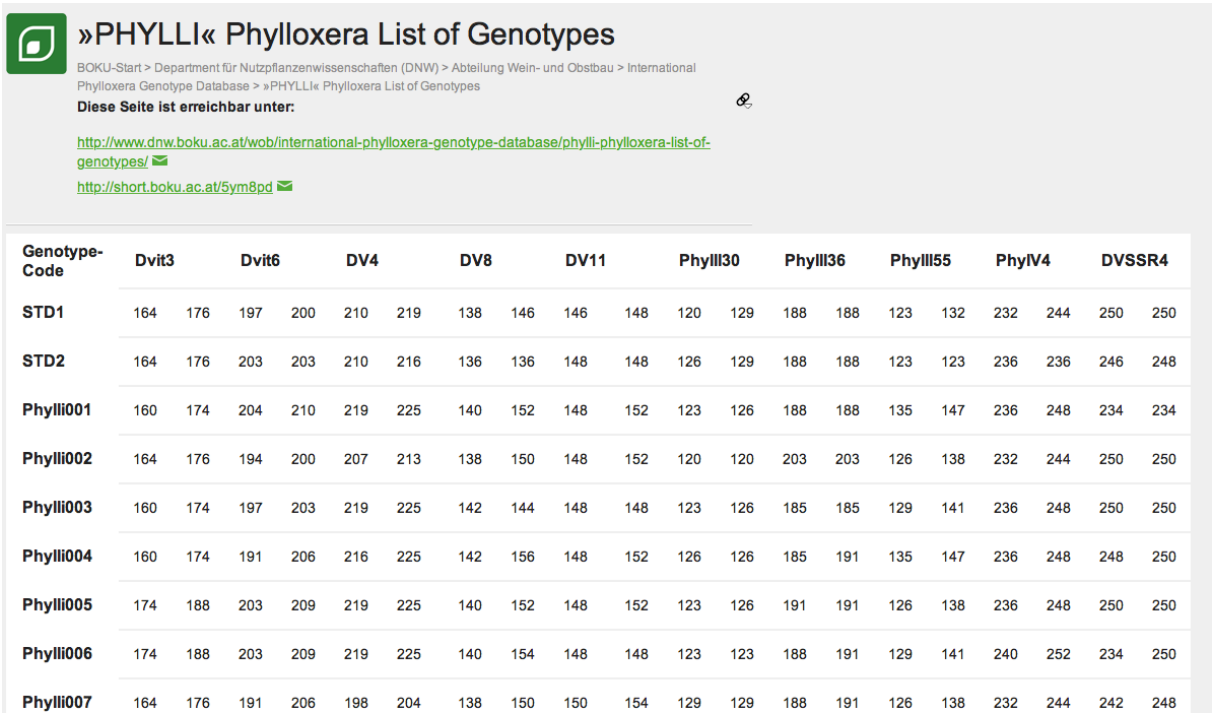
Data upload

PHYLLI will use standard excel file formatting (previously designed) to facilitate automated data upload and data management. Each genotype that will become part of the database will be assigned a unique genotype code number. All records with identical genotype numbers will be noted, but not given a new code (Figure 2). The database is open to all participating labs and will be hosted by the University of Natural Resources and Life Sciences, Vienna.

Database benefits

The development of a database that provides "true" unambiguously named and comparable genotype information for phylloxera strains will allow researchers to compare and tracking of phylloxera MLGs for the first time.

The assessment of the frequency of MGLs will allow the analysis of phylloxera migration (by human mediated means). Moreover, population genetic studies encompassing both space and time at the global level will be possible. PHYLLI will also allow researchers to identify changes in the host adaptation of existing strains and the introductions of new phylloxera strains as well as to track abundant MLGs "super clones". Such efforts will allow quicker development, adoption and application of risk management strategies, detection and surveillance approaches and quarantine policies.



»PHYLLI« Phylloxera List of Genotypes

BOKU-Start > Department für Nutzpflanzenwissenschaften (DNW) > Abteilung Wein- und Obstbau > International Phylloxera Genotype Database > »PHYLLI« Phylloxera List of Genotypes

Diese Seite ist erreichbar unter:

http://www.dnw.boku.ac.at/wob/international-phylloxera-genotype-database/phylli-phylloxera-list-of-genotypes/

http://short.boku.ac.at/5ym8pd

Genotype-Code	Dvit3		Dvit6		DV4		DV8		DV11		PhylII30		PhylII36		PhylII55		PhylV4		DVSSR4	
STD1	164	176	197	200	210	219	138	146	146	148	120	129	188	188	123	132	232	244	250	250
STD2	164	176	203	203	210	216	136	136	148	148	126	129	188	188	123	123	236	236	246	248
PhylII001	160	174	204	210	219	225	140	152	148	152	123	126	188	188	135	147	236	248	234	234
PhylII002	164	176	194	200	207	213	138	150	148	152	120	120	203	203	126	138	232	244	250	250
PhylII003	160	174	197	203	219	225	142	144	148	148	123	126	185	185	129	141	236	248	250	250
PhylII004	160	174	191	206	216	225	142	156	148	152	126	126	185	191	135	147	236	248	248	250
PhylII005	174	188	203	209	219	225	140	152	148	152	123	126	191	191	126	138	236	248	250	250
PhylII006	174	188	203	209	219	225	140	154	148	148	123	123	188	191	129	141	240	252	234	250
PhylII007	164	176	191	206	198	204	138	150	150	154	129	129	188	191	126	138	232	244	242	248

Figure 2. PHYLLI database and unique genotype coding.

Future expansion of PHYLLI

Phylloxera biotypes

In addition to the genotype descriptions, PHYLLI should also contain a phenotype description, detailing the host range and aggressiveness of the genotypes (biotypes) listed. At this point, the genetic background of biotype variants is not clear but it may be based on allelic genotypes.

Currently no standard protocol to biotype phylloxera exists but discussion in the ISHS phylloxera working group on adjustments to the bioassay systems and development of a homogenous nomenclature for phylloxera biotypes has commenced. Once this has been achieved PHYLLI may provide additional information on host ranges as a key step for the quantification and control of aggressive strains.

Phylloxera biological data

Information on the life stages (larval instar stages, sexual vs. asexual morphs) of the specimen sampled may be of additional value for further fundamental research as will the information on the date of sampling (spring vs. fall populations) and relative abundance on hosts.

Phylloxera host plant

As biotypes are defined according their performance on host plants, information on the host plants would be of great importance. These data may help identify molecular markers associated with a particular host feeding performance. This includes the location (leaves and/or roots) of the feeding site and host plant parentage.

Phylloxera environmental factors
Environmental factors (e.g. temperature, aridity) that directly affect phylloxera biology are of importance for the interpretation of data in a wider context. Additional environmental factors (e.g. soil, chemical and physical properties) may contribute to the distribution of biotypes and/or genotypes (Powell *et al*., 2003). An empirical overview may help to develop experimental designs capable of studying possible environmental interactions worldwide.

Conclusion

The innovative aspect of PHYLLI is that researchers can employ flexible genotyping procedures to generate precise and comparable data sets of phylloxera MLGs. The process is resource efficient and provides information for fundamental and applied research questions. It has direct benefit for biosecurity pest risk management and serves quarantine policies. These benefits may be greatly enhanced by including additional information on the biology, host and environment of the genotypes studied.

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