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Proceedings of the 7th International Workshop on Grapevine Downy and Powdery Mildew

June 30th-July 4th, 2014. Vitoria/Gasteiz.





Arabako Foru Aldundia
Diputación Foral de Álava

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Highthroughput phenotyping for downy mildew resistance applied to marker assisted pre-breeding in grapevine

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Introduction: In this work we present the recent implementation carried out at the Fondazione Edmund Mach (FEM) aiming to optimize the screening of large segregating populations or germplasm collections for downy mildew resistance. This phenotyping method was inspired from previous protocols present in literature, subsequently optimized and adapted to FEM facilities and objectives. This project has started about one year ago and we intend to extend the analysis to other populations in the following years.

Main Text: It is widely known that viticulture is an ancient practice that nevertheless still represent a pivotal role as both economic income and cultural tradition. Unfortunately, due to the high susceptibility to many fungal pathogens of cultivated varieties, disease outbreaks can cause a serious economic loss and strategies for controlling them still rely on a massive use of chemicals. Since decades, breeding programs have exploited natural resistance of wild Vitis species and selection of hybrds, which are more resistant to abiotic and biotic stresses, is an ongoing activity to limit the use of chemicals. Since traditional breeding is time consuming and requires intensive greenhouse and/or extensive field space, in particular in perennial plants such as grapevine, the research projects that support breeding programs are more and more focused on the discovery of new molecular tools, such as new markers. Many papers [1, 2] have been published on the use of molecular biology and new genetic/genomic techniques in plant breeding. Thanks to the support of this molecular tools it is possible to analyse a huge quantity of data in a rather small time, however phenotyping, requires much longer times and precise seasons, because of the need to follow the pathogen biological cycle, this creates an evident bottleneck in the advance of the research. In the last years, however, the importance of phenotyping is gaining relevance in the scientific community, stressed also by the institution of a European Plant Phenotyping Network (EPPN).

FEM has targeted on-going programs focusing on breeding strategies in grapevine to develop new molecular markers, thus our group focused on the implementation of a high-throughput phenotyping method. At the institute, this method has been applied during the summer 2013 the first time and its usefulness will be confirmed in the following years. Phenotyping of all genotypes was conducted according to documented leaf disc bioassay protocol [3, 4], adapted to the available infrastructures and the project main goals. To decide a suitable and definitive protocol, pilot tests have been carried out to assess the influence of the dark period span on sporulation and necrosis development. We have observed that an exposition to medium length dark period favours a more abundant sporulation, while limits necrosis development. Following these observations, we have decided to favour sporulation on necrosis appearance and keep samples in dark condition for the first days post infection.

We have phenotyped 9 segregating populations (*Vitis vinifera* L. X *Vitis* hybrid) for a total of 419 F1 individuals, 13 parental and 4 control genotypes. The size of the population is rather variable, depending on germination rate and success in controlled pollination. Controls were chosen based on literature where their resistance type has been thoroughly described and we selected two susceptible, one resistant and one average resistant genotype (Pinot noir, Muscat Ottonel, *Vitis riparia* Gloire de Montpellier, *Vitis rupestris* du Lot, respectively). Parental genotypes, as wood cuttings, and segregating individuals, as seedlings, were harvested in the same greenhouse at 25°C and natural light conditions.

All populations were infected with a downy mildew spores inoculum of the same origin in a concentration of 100,000 sp/ml. The sporangia were collected in the summer 2012 and derived from natural infections of *P. viticola* on different varieties of *V. vinifera*, in an untreated experimental field at FEM. The inoculum, preserved at -20°C, was propagated on *vinifera* X *vinifera* seedlings one week before each planned bioassay. For each genotype leaf 4 and 5 (from the apex) were sampled for laboratory analysis and rinsed in distilled water. Two discs of 2 cm diameter were excised and put in Petri dishes with the abaxial surface up until inoculation. The bottom of all dishes was covered in advance with filter paper damped with 5ml sterile distilled water kept at 4°C. Discs were then sprayed with a spore suspension of 100,000 sp/ml. Soon after infection, samples were incubated at 21°C in a growth chamber and kept in total dark conditions for the first 3 days post infection (dpi). Afterward conditions switched in a 16/8 hrs (light/dark) photoperiod. The whole experimental planning was repeated once later in the season, to obtain a final scoring of 8 data points per genotype per observation.

Visual observation of downy mildew symptoms was carried out at 4 and 6 dpi and pictures of each sample were taken from 4 dpi to 7 dpi. The degree of infection was evaluated according to two parameters: sporulation density (SD) and disease progress (DIS), this last one represents an adaptation of the OIV 452 for leaf disc bioassay. For both parameters, genotypes have been divided in different classes 1, 3, 5 ,7 and 9 according to the degree of resistance and in general the highest the class, the strongest the resistance [5]. Evaluation of classes of resistance for each genotype plant

was conducted on the basis of the survey of each single leaf disc and then averaged for data analysis. The two parameters were integrated in a single one (INT) to have a measure of both the density and the disease progression in one single data, which thus represent a global assessment of the phenotype scored. This latter was then considered a third parameter and used for further data analysis.

First results showed that the studied segregating populations are differently allocated in the resistance classes. In some populations, progeny amount is too low to establish a confident distribution tendency, as the weak number of individuals introduces a strong bias. However if we consider the integrated data, for example, even in the more numerous progenies (pop A and pop I) it is clear that there is no random distribution of the population, but a trend to follow a binomial or normal distribution. This might depend on the different resistance sources inherited in the population and suggests that genes with different level of influence and interaction may lay underneath the studied resistance mechanisms (Fig. 1). Pictures taken throughout the entire experiment will be analysed with the Image J software [6] in order to provide a quantitative assessment of downy mildew sporulation on leaves. The data obtained with image analysis will be also compared to visual scoring to compare the two methods. The possibility to evaluate downy mildew progress with a continuous parameter will likely give a more precise assessment of resistance level distribution in the populations studied. Moreover, this method will generate a additional parameter represented by the disease progress in time.

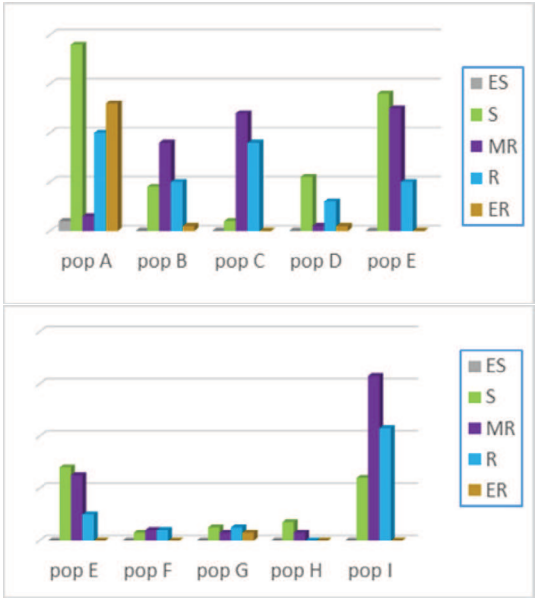

Fig. 1. Histogram showing population distribution according to the INT parameter at 6 dpi. All genotypes were included in the following classes, ES: extremely susceptible, S: susceptible, MR: medium resistant, R: resistant, ER: extremely resistant.

We aimed at developing a hight-hroughput method both to uniformly phenotype various segregating populations and to obtain few targeted parameters representing important steps of the disease resistance evaluation. The main goal of this project is to use all the phenotypic parameters to test a novel approach to detect genomic regions responsible for the expression of resistance traits. This will finally lead both to the definition of new molecular markers and to the check of the consistency of already existing markers in different populations. Results will boost implementation of marker-assisted pre-breeding applied to downy mildew.

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