The Journal of Animal & Plant Sciences, 20(4): 2010, Page: 243-247
ISSN: 1018-7081

USE OF MORPHOLOGICAL MARKERS TO IDENTIFY FOLIAR DISEASE RESISTANCE IN GRAPEVINE

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ABSTRACT

Morphological characteristics of 324 vines, developed from a cross between 'Italia' and 'Mercan' grapevine varieties with various levels resistance to powdery and downy mildew were examined for a possible association with disease resistance using a tree-based discriminant analysis. Petiole coloration was the main discriminant for downy mildew. When the pigmentation was low, anthocyanin coloration of the tip was needed to screen susceptible plants from the resistant ones. With the anthocyanin pigmentation medium to strong, 63.5% of the plants were selected as resistant. Plants with darker petiole color required mature leaf profile and width of the petiolar sinus to be factored in to successfully discriminate the susceptible ones from the resistant plants. Anthocyanin coloration of the tip was the main discriminant variable for powdery mildew. Alongside of a strongly colored shoot tip, the petiole color and the shape of the mature leaf blade had to be incorporated into selection process. It seems that anthocyanin in petioles or shoot tip might play a role selecting resistant or susceptible genotypes from the population.

Key words: grapevine, fungal disease, classification tree analysis, vegetative traits, anthocyanin.

INTRODUCTION

Throughout the history of selection for better plant species or varieties, people have been using their senses and better judgment, biochemical compounds, and molecular markers. Depending on the time, money and effort to be put in, work could be cumbersome and take long period of time. The most encountered problem in selecting best individuals in processes in which fruit is involved is that it might take too long to see the actual fruit. Vegetative characters at this point might be a way to circumvent this problem.

Downy mildew, caused by *Plasmopara viticola* (Berk. et Curtis ex. de Bary Berl. et de Toni), is one of the most destructive grapevine diseases, that occurs worldwide, particularly in warm and humid climates. Grapevine powdery mildew is caused by the fungus *Uncinula necator* and it affects crop resulting in loss of eating and wine quality. Our primary objective is to search for the possibility of using morphological markers on a grapevine to identify resistant or susceptible individuals to grape fungal diseases in a F_1 population. The method we chose for that is classification tree analysis. Classification and regression tree analyses have found application in medical diagnosis (Goldman *et al.,* 1998), meteorology (Burrows 1991), plant pathology (Baker 1993), soil regionalization (Mertens *et al.,* 2002) and species distribution (Vayssieres *et al.,* 2000).

MATERIALS AND METHODS

Plant Material: A total of 324 F_1 progenies obtained from a cross between 'Italia' and 'Mercan' parents, developed in 1992 under the project 'Producing New Grape Types with Standard Specifications and Resistant against Downy Mildew and Powdery Mildew, by Means of Cross Breeding Method' were used. 'Italia' is a table grape (*Vitis vinifera* L.) cultivar grown in many regions. 'Mercan' is a white, small berried juice variety (*Vitis labrusca*) grown in Black Sea region, where the climate is very humid all year round. The parents and the derived progenies were grown on their roots at the Viticulture Research Institute, Tekirdağ, Turkey.

Trait observations: A total of 17 vegetative traits, such as anthocyanin intensity on shoot tip, cold resistance of buds, bud break time, young leaf upper surface color, young leaf upper surface hairiness, young leaf lower surface color, mature leaf lower surface hairiness, mature leaf upper surface appearance, mature leaf form, mature leaf shape, mature leaf contour, shape of petiolar sinus, sinus width on mature leaf, dentation of mature leaf, petiole color, petiole hairiness and cane color was recorded. All trait characterizations were done for the parents and the progeny for two growing years. The description of morphological traits was done according to the methods of IPGRI, UPOV, OIV (1997).

Foliar fungal disease resistance traits (powdery and downy mildew) were measured on the vines under natural infection. No pest management strategy against the diseases was employed and natural epidemics of mildew diseases were allowed to progress. Resistance of the F_1 plants to powdery mildew and downy mildew was determined by the method of Eibach (1994) and Anonymous (1983), respectively. Twenty mature leaves were taken from the middle section of the shoots in July for a period of two consecutive years. Every hybrid plant from the selection block was rated regarding the level of fungus attack on leaves.

Statistical Analysis: For all traits, the scores were calculated as mean value over a two-year period, per individual. Morphological traits which showed variation among the progenies were determined as the anthocyanin intensity on shoot tip, young leaf upper surface color, mature leaf form, mature leaf blade shape, shape of petiolar sinus, and petiolar sinus width on mature leaf, petiole color and cane color.

The leaves which scored 5, 7 and 9 were classified as susceptible (1), or 1 and 3 as resistant (2) for powdery mildew. Susceptible plants (1) were those scored as 3>4>5, and resistant ones (2) were those rated 1>2 for downy mildew before statistical analysis.

Classification tree analysis was performed on the data to develop an effective method for explanatory analysis of data sets consisting of vegetative characteristics of a F_1 population together with fungal disease occurrence.

RESULTS AND DISCUSSION

Anthocyanin pigmentation on petiole was the main discriminate for downy mildew (Fig. 1). When the anthocyanin accumulation is low on petiole, anthocyanin coloration of the tip was needed to screen susceptible plants from the resistant ones. With the anthocyanin pigmentation medium to strong, 63.5% of the plants were selected as the resistant. Plants with darker petiole color required mature leaf profile and width of the petiolar sinus to be indexed in to successfully discriminate the susceptible ones from the resistant plants. These additional variables enabled selection of 77.8% susceptible ones when leaves were flat or rolled upward with wide open petiolar sinus. Only 66.7% plants were screened as resistant individuals when leaf margins were rolled downward with closed petiolar sinuses.

Anthocyanin coloration of the tip was the main discriminator variable for powdery mildew (Fig. 2). However, when it was weak, the power was not great enough to select both susceptible and resistant plants. Alongside a strongly colored shoot tip, the petiole color and the shape of the mature leaf blade also had to be incorporated. Green petiole was helpful to distinguish the resistant ones at 71.4%. However, when it was brown to red, the shape of the mature leaf blade had to be more circular to select 62.5% of the resistant individuals, or cordate to wedge-shaped to select 62.3% of them.

The overall percentage for correct prediction for downy mildew was 63.6 and 65.4% for susceptible and resistant plants, respectively. The overall power of this analysis to discriminate powdery mildew resistant plants from susceptible ones was 86.3% (Table 1).

This study analyzes the utilization of grapevine vegetative characteristics to positively select foliar fungal disease (powdery and downy mildew) resistant of genotypes using tree-based discriminant analysis. The relationship was the strongest between petiole color and downy mildew. Anthocyanin coloration of the shoot tip, mature leaf profile and width of the petiolar sinus were the other variable needed to select downy mildew resistant phenotypes. At least 6 out of 10 plants could be successfully selected against the disease resistance. Anthocyanin coloration appeared to be a major component of powdery mildew resistance in the grapevine. Other strong indicators were petiole color and the shape of the mature leaf blade. The selective success for powdery mildew resistance was higher and at least 8 plants out of 10 could be selected.

Phenolic compounds are needed for pigmentation, growth, reproduction, resistance to pathogens and for many other functions. Some authors have pointed out that phenolics are often stored at strategically important sites where they play a signaling role, and often a direct role, in defense (Lattanzio *et al,.* 2006). Anthocyanins are water soluble pigments derived from flavonoids (Chalker-Scott 1999). Various intrinsic and environmental factors have been linked to anthocyanins (Mol *et al.,* 1996), such that deficiencies in nitrogen (Do and Cormier 1991), and phosphorus (Dedaldechamp *et al.,* 1995), low pH (Suzuki 1995) were shown to increase anthocyanin in grapevine. Wounding, pathogen infection and fungal elicitors have been also found in relation with increased anthocyanin in different plants (Ferreres *et al.,* 1997; Dixon *et al.,* 1994). In our present study, as the anthocyanin pigmentation in petiole or shoot tip increased, the ability of selecting resistant genotypes from the population increased. Nitrogen or phosphorus deficiency is not considered a factor here, because all genotypes were grown in one plot under same cultural management.

One of the disadvantages encountered here is that the number of characteristics that showed variations among the individuals was limited. If it had been higher, the relationships found would have been different. The number of the individuals on which the study was conducted was, on the other hand, deemed sufficient enough to draw these conclusions.

Various data sets have been used by researchers to analyze relationships between various type of characteristics and more important features of a plant, such as yield and disease resistance using analyses of correlation, tree clustering-classification and regression tree (CART), correspondence and multidimensional scaling (MDS). For instance, Jeandet *et al.* (1992) discussed the use of phytoalexin induction and *in vitro* methods as a tool for selection of *Vitis* resistant genotypes for grey mould in early screening tests.

Table 1. Predicted and observed values of disease resistance of 324 grapevine hybrids (Italia x Mercan) after classification tree analysis

Observed	Powdery mildew				Downy mildew		
	Predicted				Predicted		
	1,00	2,00	Percent Correct	Observed	1,00	2,00	Percent Correct
1,00	105	60	63,6%	1,00	43	76	36,1%
2,00	55	104	65,4%	2,00	28	177	86,3%
Overall Percentage	49,4%	50,6%	64,5%	Overall Percentage	21,9%	78,1%	67,9%

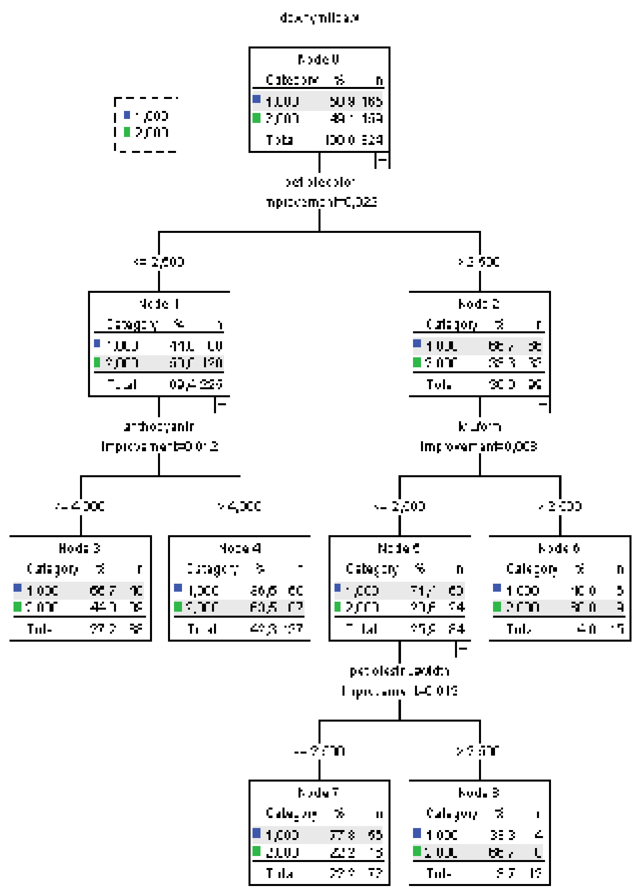

Fig. 1. Classification tree of 324 F_1 plants developed by crossing 'Italia' and 'Mercan' grapevine varieties against downy mildew resistance (1: susceptible, 2: resistant).

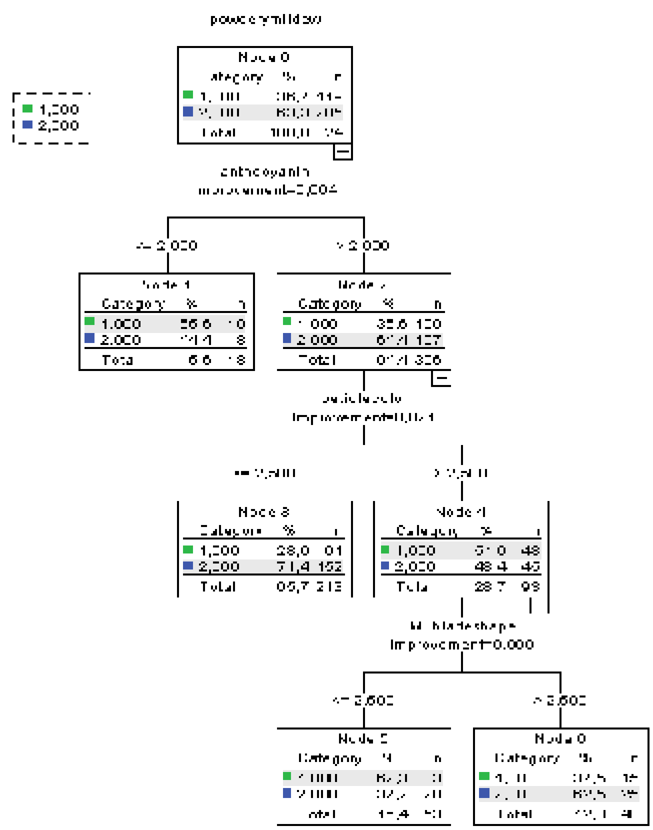

Fig. 2. Classification tree of 324 F_1 plants developed by crossing 'Italia' and 'Mercan' grapevine varieties against powdery mildew resistance (1: susceptible, 2: resistant).

Mlikota-Gabler *et al.* (2003) studied the association between *B. cinerea* disease resistance and morphological, anatomical and chemical characteristics of berries and found that the number of pores on berry surface was negatively correlated, while the number and the thickness of epidermal and hypodermal cell layers and cuticle and wax contents were positively correlated. Kortekamp and Zyprian (2003) established a strong correlation between peroxidase activity in leaves of *in vitro* plants and resistance to *Plasmapora viticola*. Mundy and Beresford (2007) studied the relation of *Botrytis cinerea* to berry nitrogen and sugar concentration using regression analysis. Batovska *et al.* (2009) examined metabolic profiles of acetone and butanol extracts obtained from the leaves of a wine grape cultivar 'Storgozia' and found that the more polar compounds were related to sensitivity, and only hexadecanoic and the monohydroxy carboxylicacids were related to resistance to downy mildew in grapevine.

More recent studies involved tree clustering or scaling analyses to find a relationship between various parameters. Calonnec *et al.* (2004) quantified effects of powdery mildew (*Uncinula necator*) on grape yield, juice and wine quality for Cabernet Sauvignon. MDS successfully grouped batches of Cabernet Sauvignon according to disease severity. Welter *et al.* (2007) studied quantitative trait loci affecting leaf morphology and fungal disease resistance in a F1 population of 'Regent' and 'Lemberger'. However, they did not associate leaf morphology QTLs to the disease resistance.

Conclusion: Using vegetative characteristics with respect to disease resistance provides a point of view for breeders to improve plant populations. Using robust statistical methods certainly make it easy for researchers to establish a relationship between the predictor variables and the desired characters, mainly improved yield and disease resistance. These methods also enable using as many and different type of variables as possible to identify factors underlying the characteristics under investigation. However, in order to determine the strength, direction and significance of their association with more certainty, the investigation should extend to different segregation populations.

Acknowledgment: We wish to thank Mehmet Mendeş (Canakkale Onsekiz Mart University) for statistical analysis.

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