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The use of molecular markers for pyramiding resistance genes in grapevine breeding

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Summary

The practical application of pyramiding resistance genes by the use of molecular markers was investigated in a F1 progeny derived from the cross of *VHR 3082-1-42* x 'Regent'. *VHR 3082-1-42* is a cross between *Muscadinia rotundifolia* x *Vitis vinifera*, backcrossed another four times with V. vinifera (Pauquet *et al.* 2001). It carries the *Run1*-gene which causes resistance to powdery mildew and the *Rpv1*-gene which is related to resistance against downy mildew. Both genes were introduced from *Muscadinia rotundifolia* (Bouquet *et al.* 2000; Wiedemann-Merdinoglu *et al.* 2006). 'Regent' is a new cultivar with quantitative resistance against downy and powdery mildew (Eibach and Töpfer 2003) released in Germany in 1996 for commercial use. 119 individuals of the F1 progeny were screened with a molecular marker for the *Run1*-gene (Donald *et al.* 2002), with two SSR-markers for the *Rpv1*-gene (Wiedemann-Merdinoglu *et al.* 2006) and with several markers from 'Regent' that showed good correlation to powdery and downy mildew resistance (Zyprian *et al.* 2002, Salakhutdinov *et al.* 2003, Akkurt 2004, Fischer *et al.* 2004, Akkurt *et al.* 2007). Phenotypic evaluation for downy mildew resistance was done by artificial inoculation of leaf discs, and for powdery mildew by natural infection in a greenhouse. Comparison of the phenotypic data with the results of the molecular marker analyses showed a clear correlation between the degree of resistance and the presence of the resistance related alleles. According to the phenotypic data, 20 genotypes of the offspring were free of powdery and downy mildew infections. Based on a marker-assisted evaluation, out of these 20 genotypes a subset of four carried all the resistance related alleles for powdery and downy mildew indicating that resistance genes from both parents were effectively combined.

K e y w o r d s : downy mildew, powdery mildew, *Vitis*, resistance, marker assisted selection, breeding.

Introduction

The use of molecular markers is becoming increasingly important for breeding purposes in a lot of agricultural crops like wheat (Adhikari *et al.* 2004, Gupta *et al.* 2005, Yang *et al.* 2005, Sardesai *et al.* 2005), rice (Sharma *et al.* 2004, Ashikari and Matsuoka 2006) maize (Widstrom *et al.* 2003) but also grapes (Striem *et al.* 1996, This *et al.* 2000, Doligez *et al.* 2002, Mejia and Hinrichsen 2003). Of particular relevance is this method for polygenic traits such as resistance against the mildews in grapevine. From the breeding point of view, it is highly desirable to combine as many resistance genes as possible in a new cultivar in order to make resistance as sustainable as possible. Based only on phenotypic evaluation data, it is hardly feasible to track the accumulation of resistance genes in a new breeding line. The use of molecular markers provides a new tool for breeders and may help to overcome this problem (Dalbó 1998, Dalbó *et al.* 2001, Luo *et al.* 2001, Pauquet *et al.* 2001, Fischer *et al.* 2004).

Material and Methods

Investigations were carried out on a F1 progeny of 119 individuals derived from a cross between *VRH3082-1-42 x* 'Regent'. Population was grown with one plant per genotype in a greenhouse with natural soil and at the time of investigation the plants were four years old. The female parent *VRH3082-1-42* can be traced back to an initial cross between *Muscadinia rotundifolia G52* x Malaga seedling No. 1 and four further backcrosses with *Vitis vinifera* cultivars (Pauquet *et al.* 2001). It carries the *Run1*-gene which is responsible for resistance against powdery mildew and is linked to the CAPS-marker GLP1-12 (Donald *et al.* 2002). It also carries the *Rpv1*-gene which confers good resistance against downy mildew (Wiedemann-Merdinoglu *et al.* 2006). 'Regent' is a new cultivar with complex resistance against powdery and downy mildew, released in Germany for commercial use in 1996 (Eibach and Töpfer 2003).

Phenotypic screening for downy mildew was performed with artificial inoculation tests. One leaf per plant was collected from a well developed shoot in the middle of the cane. For harmonizing leaf age the 7th leaf from shoot top was harvested respectively. Four leaf discs per leaf were generated. They were placed in a plastic box on wet filter paper. 20 µl of a spore suspension with a concentration of 50.000 spores/ml was used for artificial inoculation. Screening of downy mildew infection was done one week after infection. Ratings followed the OIV descriptor list for grapevine cultivars and *Vitis* species (Anonymous 1983) while not the degree of resistance but the degree of infection was rated (1 = no infection, 9 = heavy infection). Experiments were done twice. Natural appearance of powdery mildew infection on leaves was rated in a greenhouse in 2005 and 2006 (1 = no infection, 9 = heavy infection).

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For both mildews the highest degree of infection for the individual genotypes was used for further calculation.

DNA was isolated from young, healthy leaves following the protocol of THOMAS *et al.* (1993) or using the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany). According to the protocol of DONALD *et al.* (2002), the CAPS marker GLP1-12 was used to evaluate the progeny for the presence or absence of the *Run1*-gene. Screening for the *Rpv1*-gene was done using two linked SSR-markers, VMC_8g9 and VMC_1g3.2, according to WIEDEMANN-MERDINOGLU *et al.* (2006). FISCHER *et al.* (2004) developed a genetic map using the progeny of a cross between 'Regent' x 'Lemberger'. A major QTL for downy mildew was located on linkage group 18 and another one for powdery mildew on linkage group 15. Three SSR-markers located within the QTL for downy mildew and three SSR-markers located within the QTL for powdery mildew were used for genetic analysis. In addition the SCAR-marker ScOR A7-760 linked to the powdery mildew QTL (AKKURT *et al.* 2007) was included in the investigations.

PCR for SSR loci were performed in standard reactions of 10 µl. Primer pairs were labelled with ABI fluorescent dyes and analyzed by capillary electrophoresis on an ABI 3100 Genetic Analyser. PCR for the SCAR marker was carried out in standard assays in a total volume of 25 µl. Products were separated on a 1.5 % agarose gel.

Results and Discussion

No powdery mildew infection was observed on 48 % of the genotypes of the F1 progeny (Fig. 1 A). By dividing them into two subgroups with and without the *Run1*-gene, all the genotypes carrying the *Run1*-gene proved to be free of powdery mildew infection (Fig. 1 B). However those genotypes not carrying the *Run1*-gene were distributed over all classes of infection, with 9 % showing either no infection or severe infection (Fig. 1 B). The frequency distribution of the classes of infection for those genotypes carrying neither the *Run1*-gene nor the individual resistance related alleles of 'Regent' is demonstrated in Fig. 2 A. Considering the absence of all resistance related alleles of 'Regent', no individual was rated as free of infection. Compared to this group, the presence of the individual resistance related alleles of 'Regent' led to a significant shift of the frequency distribution towards the lower infection classes (Fig. 2 B). 19 % of the individuals with all four resistance related markers of 'Regent' proved to be free of infection. All individuals with the combination of the presence of the *Run1*-gene and the absence of individual resistance related alleles of Regent were free of powdery mildew infection (Fig. 2 C). Phenotypically this group didn't differ from the group of individuals which carried the *Run1*-gene as well as the individual resistance related markers of 'Regent' (Fig 2 D). Genotypically it can be stated that, within the latter group, powdery mildew resistance genes derived from both parents are accumulated.

Fig. 3 demonstrates the frequency distribution of the progeny for downy mildew. Thirty-six percent of the population showed no downy mildew infection while 6 % showed severe infection (Fig. 3 A). The genotypes carrying the *Rpv1*-markers exhibit a frequency distribution shifted towards the lower infection classes compared to the individuals without the *Rpv1*-markers (Fig. 3 B).

The frequency distribution for the degree of infection for those genotypes carrying neither the *Rpv1*-markers nor the individual downy mildew resistant related markers of 'Regent' is shown in Fig. 4 A. Seventy-four percent of the individuals were rated 7 or worse when all the resistance related markers from 'Regent' were absent. The comparison with the group of genotypes having the individual 'Regent'-markers shows a significant shift of the frequency distribution towards the lower infection classes (Fig. 4 B). When all 'Regent'-markers were present, 96 % of the individuals were rated within the infection classes 1 and 3. The effect of the presence of the *Rpv1*-gene is demonstrated in Fig. 4 C. Compared to Fig. 4 A, there is a considerable shift towards the lower infection classes. 87 % of the vines were rated within the infection classes 3 and 5 but none was within infection class 1. The comparison of the groups shown in Fig. 4 B and Fig. 4 C demonstrates that the effect of the 'Regent'-related resistance markers led to a higher degree of resistance in the offspring than the *Rpv1*-gene related markers. The combination of the presence of the *Rpv1*-markers and the individual 'Regent'-markers is shown in Fig. 4 D. When all the 'Regent'-markers were present, these individuals showed no infection of downy mildew. This demonstrates that the combination of both

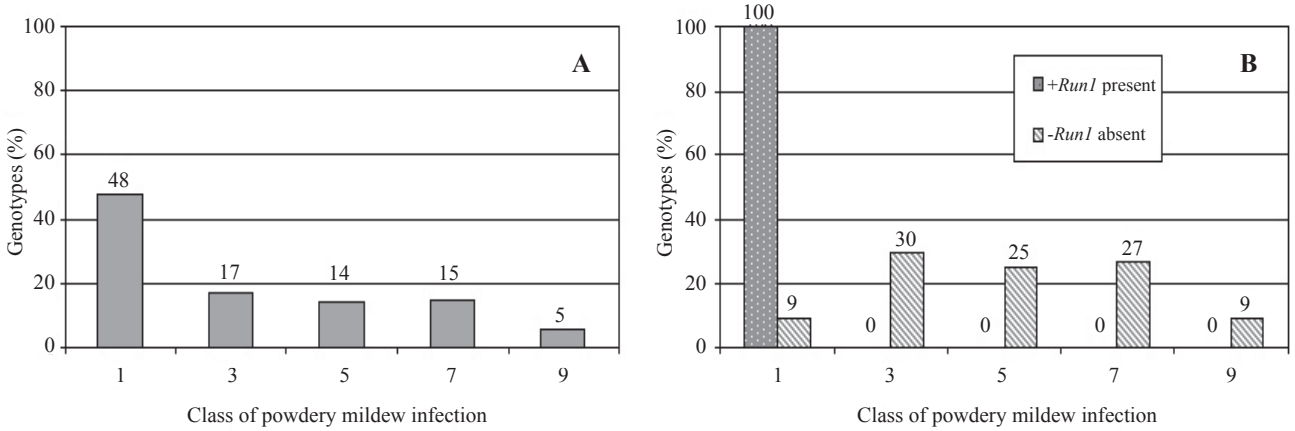

Fig. 1: Frequency distribution for powdery mildew infection (1 = no infection, 9 = heavy infection); **A**: entire progeny, **B**: progeny divided into genotypes with and without the *Run1*-gene.

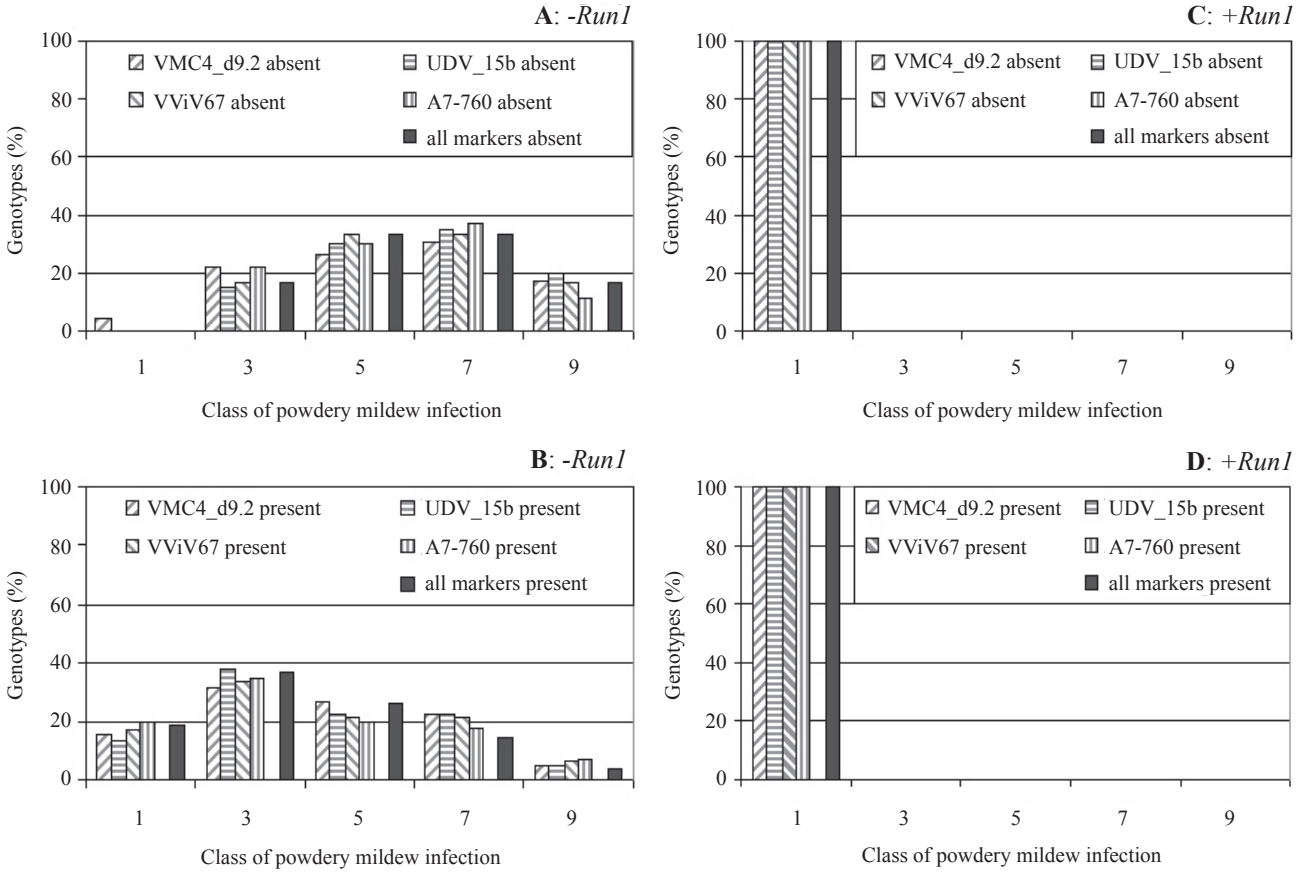

Fig. 2: Frequency distribution of powdery mildew infection (1 = no infection, 9 = heavy infection) for different groups of seedling genotypes: **A**: genotypes not carrying the *Run1*-gene and sorted by the absence of individual powdery mildew resistance related alleles of 'Regent'; **B**: genotypes not carrying the *Run1*-gene and sorted by the presence of individual powdery mildew resistance related alleles of 'Regent'; **C**: genotypes carrying the *Run1*-gene and sorted by the absence of powdery mildew resistance related alleles of 'Regent'; **D**: genotypes carrying the *Run1*-gene and sorted by the presence of individual powdery mildew resistance related alleles of 'Regent'.

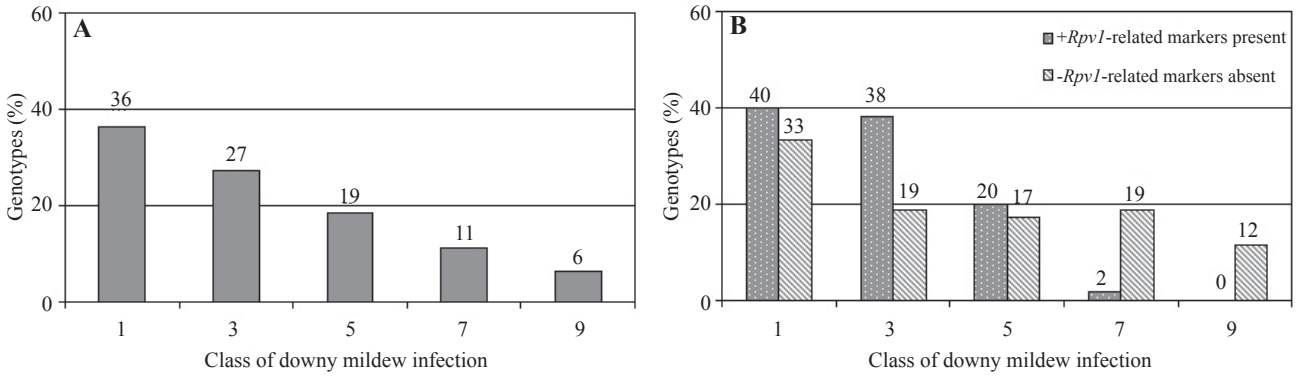

Fig. 3: Frequency distribution for downy mildew infection; **A**: total progeny, **B**: progeny divided into genotypes with and without the *Rpv1*-related markers VMC_8g9 and VMC_1g3.2.

resistance sources had an additive effect on the degree of resistance. Comparing the effect of the different resistance sources for both mildew diseases it can be stated that the effect of the resistance genes originated from 'Regent' is higher for downy mildew while for powdery mildew the effect of the *Run1*-gene is much higher, it even covers phenotypically the effect of the resistance genes originating from 'Regent'. Taking into account both mildew diseases, phenotypic screening led to 20 individuals which were totally free of any infections. By using marker assisted selection, a subset of four genotypes could be identified which carry each the *Run1*-gene and the *Rpv1*-related markers along with the entire set of powdery and downy mildew resistance related alleles originating from 'Regent'. Thus all markers associated with mildew resistance genes of both parents are present in the four individuals. By using only these vines for further breeding work it can be expected that the proportion of resistant individuals in the progeny will be higher and hence the efficiency of resistance breeding will increase.

Since marker assisted selection is still labour- and cost-intensive, a combined scheme of phenotypic and genetic

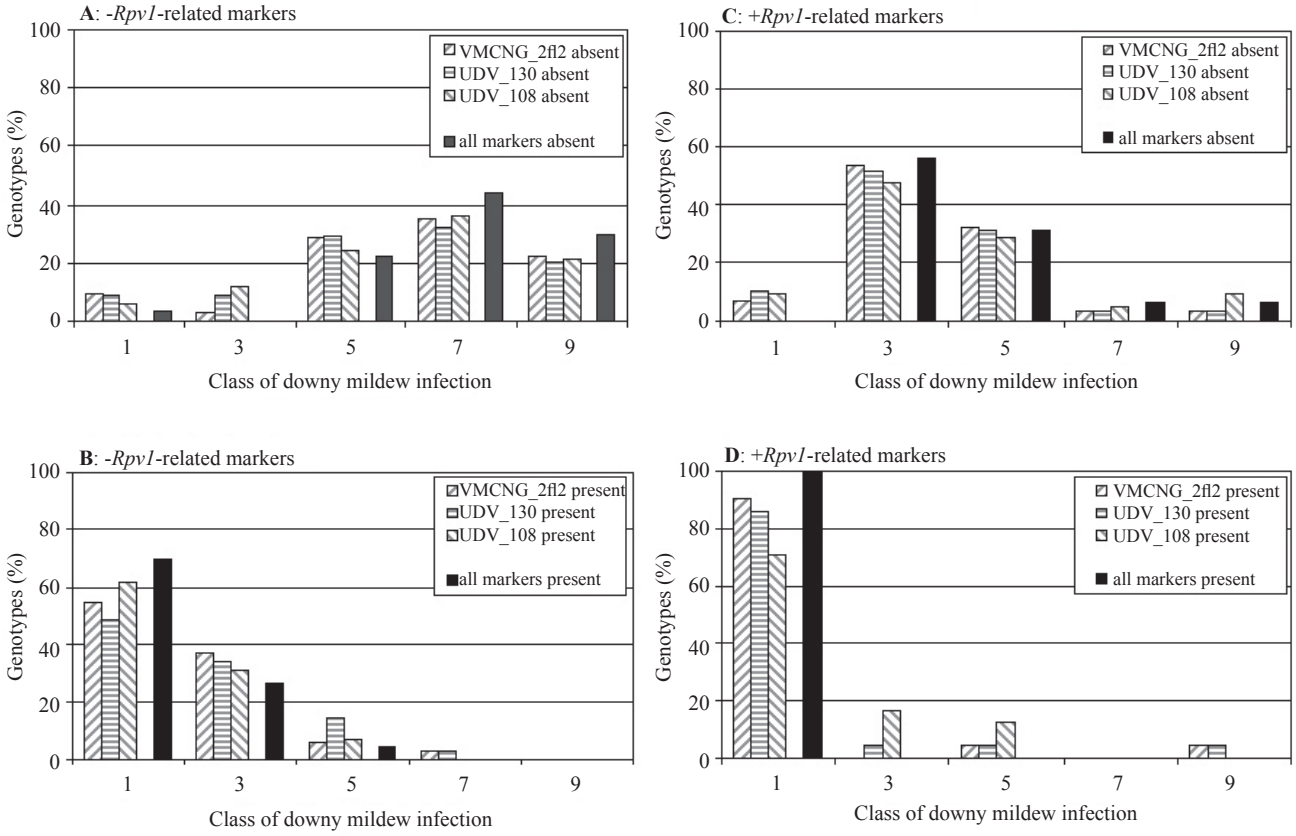

Fig. 4: Frequency distribution of downy mildew infection (1 = no infection, 9 = heavy infection) for different groups of seedling genotypes; **A**: genotypes not carrying the *Rpv1*-related markers and sorted by the absence of individual downy mildew resistance related alleles of 'Regent'; **B**: genotypes not carrying the *Rpv1*-related markers and sorted by the presence of individual downy mildew resistance related alleles of 'Regent'; **C**: genotypes carrying the *Rpv1*-related markers and sorted by the absence of downy mildew resistance related alleles of 'Regent'; **D**: genotypes carrying the *Rpv1*-related markers and sorted by the presence of individual downy mildew resistance related alleles of 'Regent'.

selection is proposed. Fig. 5 clarifies the procedure of such a combined selection procedure applied to the investigated population as an example. In the first step the selection of downy mildew resistance can be executed rather easily by artificial inoculation of the seedlings with spores of downy mildew. In a second screening step those seedlings showing no infections are subjected to powdery mildew inoculation. Only the remaining seedlings without infections of downy and powdery mildew are processed for marker assisted selection. In the example demonstrated in Fig. 5, there are 20 seedlings, which is around 15 % of the initial seedling population. After the following marker assisted selection steps, four seedlings were identified posessing, as already mentioned, the entire set of powdery and downy mildew related alleles of both parents.

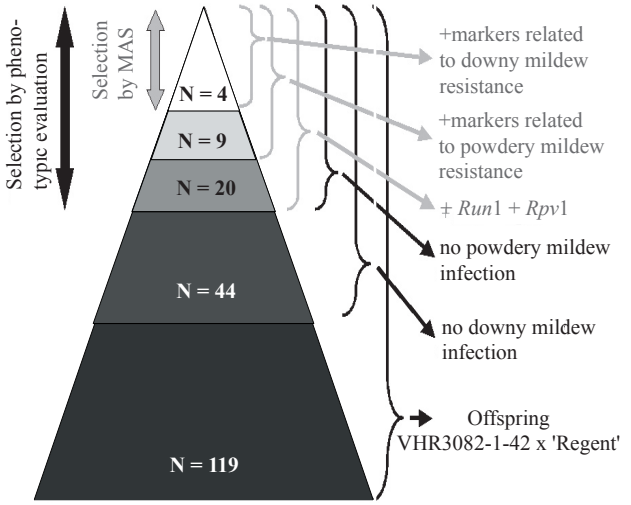

Fig. 5: Scheme for pyramiding mildew resistance genes by a combination of phenotypic evaluation and marker assisted selection (MAS). MAS reduced the number of elite individuals from 20 to 4.

Conclusions

The investigations carried out in an offspring of the cross *VRH3082-1-42* x 'Regent' show that the *Run1*-marker GLP1-12 is very tightly linked to powdery mildew resistance while the *Rpv1*-gene related markers are linked to downy mildew resistance. The evaluation of genotypes carrying neither the *Run1*-marker nor the *Rpv1*-gene related markers shows that the markers VMC_4d9.2a, UDV_015b,VVIv67 and ScOR A7-760 are related to powdery mildew resistance (19 % no infection class 1, 37 % slight infection class 3). The markers UDV_130, VMCNG2_f12 and UDV_108 are related to downy mildew resistance (71 % no infection, 25 % slight infection class 3). This relation is stronger compared to the markers for powdery

mildew. The application of marker assisted selection for the mildew resistance related markers leads to a pyramiding of resistance genes. For breeding purposes a combination of phenotypic evaluation and marker assisted selection is suggested.

References

Anonymous; 1983: Descriptor List for Grapevine Varieties and *Vitis* Species. Off. Int. Vigne Vin, Paris.

Adhikari, T. B.; Yang, X.; Cavaletto, J. R.; Hu, X.; Buechley, G.; Ohm, H. W.; Shaner, G.; Goodwin, S. B.; 2004: Molecular mapping of Stb1, a potentially durable gene for resistance to septoria tritici blotch in wheat. Theor. Appl. Genet. **109**, 944-953.

Akkurt, M.; 2004: Entwicklung molekularer Marker für Oidium (*Uncinula necator*)-Resistenz bei der Weinrebe. Thesis Univ. Karlsruhe.

Akkurt, M.; Welter, L.; Maul, E.; Töpfer, R.; Zyprian, E.; 2006: Development of SCAR markers linked to powdery mildew (*Uncinula necator*) resistance in grapevine (*Vitis vinifera* L.). Mol. Breed. **19**, 103-111.

Ashikari, M.; Matsuoka, M.; 2006: Identification, isolation and pyramiding of quantitative trait loci for rice breeding. Trends Plant Sci. **11**, 344-350.

Bouquet**,** A.; Pauquet, J.; Adam-Blondon, A. F.; Torregrosa, L.; Merdinoglu, D.; Wiedemann-Merdinoglu, S.; 2000: Vers l'obtention de variétés de vigne résistantes à l'oidium et au mildiou par les méthodes conventionnelles et biotechnologiques. Prog. Agric. Vitic. **117**, 383-389.

Dalbó, M. A.; 1998: Genetic Mapping, QTL Analysis and Marker-assisted Selection for Disease Resistance Loci in Grapes. Thesis, Cornell Univ., Geneva, USA.

Dalbó**,** M. A.; Ye, G. N.; Weeden, N. F.; Wilcox, W. F.; Reisch, B. I.; 2001: Marker-assisted selection for powdery mildew resistance in grapes. J. Am. Soc. Hortic. Sci. **126**, 83-89.

Doligez, A.; Bouquet, A.; Danglot, Y.; Lahogue, F.; Riaz, S.; Meredith, C. P.; Edwards, K. J.; This, P.; 2002: Genetic mapping of grapevine (*Vitis vinifera*) applied to the detection of QTLs for seedlessness and berry weight. Theor. Appl. Genet. **105**, 780-795.

Donald, T. M.; Pellerone, F.; Adam-Blondon, A. F.; Bouquet**,** A.; Thomas, M. R.; Dry, I. B.; 2002: Identification of resistance gene analogs linked to a powdery mildew resistance locus in grapevine. Theor. Appl. Genet. **104**, 610-618.

Eibach, R.; Töpfer, R.; 2003: Success in Resistance Breeding: „Regent" and its Steps into the Market, 687-692. Proceedings of the VIIIth Int. Conf. Grape Genet. Breed. Kecskemét, Hungary.

Fischer, M. B.; Salakhutinov, I.; Akkurt, M.; Eibach, R.; Edwards, K. J.; Töpfer, R.; Zyprian, E.; 2004: Quantitative trait locus analysis of fungal disease resistance factors on a molecular map of grapevine. Theor. Appl. Genet. **108**, 501-515.

Gupta, S. K.; Charpe, A.; Koul, S.; Prabhu, K. V.; Haq, Q. M.; 2005: Development and validation of molecular markers linked to an Aegilops umbellulata-derived leaf-rust-resistance gene Lr9, for marker-assisted selection in bread wheat. Genome **48**, 823-830.

Luo, S. L.; He, P. C.; Zhou, P.; Zheng, X. Q.; 2001: Identification of molecular genetic markers tightly linked to downy mildew resistant genes in grape. Acta Genet. Sinica China **28**, 76-82.

Meija, N.; Hinrichsen, P.; 2003: A new, highly assertive SCAR marker potentially useful to assist selection for seedlessness in table grape breeding. Acta Hortic. **603**, 559-564.

Pauquet, J.; Bouquet, A.; This, P.; Adam-Blondon, A. F.; 2001: Establishment of a local map of AFLP markers around the powdery mildew resistance gene ***Run1*** in grapevine and assessment of their usefulness for marker assisted selection. Theor. Appl. Genet. **103**, 1201-1210.

Salakhutdinov, I.; Fischer, B.; Akkurt**,** M.; Eibach, R.; Töpfer, R.; Zyprian, E.; 2003: Genetische Kartierung der Weinrebe. Perspektiven für Forschung und moderne Rebenzüchtung. Deutsches Weinbau-Jahrbuch **54**, 53-64.

Sardesai, N.; Nemacheck, J. A.; Subramanyam, S.; Williams, C. E.; 2005: Identification and mapping of *H32*, a new wheat gene conferring resistance to Hessian fly. Theor. Appl. Genet. **111**, 1167-1173.

Sharma, P. N.; Torii, A.; Takumi, S.; Mori, N.; Nakamura, C.; 2004: Marker-assisted pyramiding of brown planthopper (Nilaparvata lugens Stal) resistance genes *Bph1* and *Bph2* on rice chromosome 12. Hereditas **140**, 61-69.

Striem**,** M. J.; Ben-Hayyim, G.; Spiegel-Roy, P.; 1996: Identifying molecular genetic markers associated with seedlessness in grape. J. Am. Soc. Hortic. Sci. **121**, 758-763.

This, P.; Lahogue, F.; Adam-Blondon, A.F.; Doligez, A.; Bouquet, A.; 2000: Towards marker-assisted selection of seedlessness in grapevine. Acta Hortic. **528**, 221-229.

Thomas, M. R.; Scott, N.; 1993: Microsatellite repeats in grapevine reveal DNA polymorphisms when analyzed as sequence-tagged sites (STSs). Theor. Appl. Genet. **86**, 985-990.

Widstrom, N. W.; Butron, A.; Guo, B. Z.; Wilson, D. M.; Snook, M. E. Cleveland, T. E.; Lynch, R. E.: 2003: Control of preharvest aflatoxin contamination in maize by pyramiding QTL involved in resistance to ear-feeding insects and invasion by *Aspergillus* spp. Eur. J. Agron. **19**, 563-572.

Wiedemann-Merdinoglu, S.; Prado, E.; Coste,P.; Dumas, V.; Butterlin, G.; Bouquet, A.; Merdinoglu, D.; 2006: Genetic Analysis of Resistance to Downy Mildew from *Muscadinia rotundifolia*. 9th Int.Conf. Grape Genet. Breed., Udine, Italy.

Yang, Z.; Gilbert, J.; Fedak, G.; Somers, D. J.; 2005: Genetic characterization of QTL associated with resistance to *Fusarium* head blight in a doubled-haploid spring wheat population. Genome **48**, 187-196.

Zyprian, E.; Eibach, R.; Töpfer, R.; 2002: Comparative Molecular Mapping of Fungal Disease Resistance Factors in Segregating Populations of Grapevine, 73-78. Proc. VIIIth Int. Conf. Grape Genet. Breed., Kecskemét, Hungary.

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