

Received 15 March 2002

Accepted 26 April 2002

THE USE OF MOLECULAR MARKERS IN APPLE BREEDING FOR DISEASE RESISTANCE

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Abstract: Molecular markers have become a useful tool simplifying and speeding up breeding work. They are also helpful in the genetic analysis of complex agronomic traits. The investigations concern the use of already available SCAR markers for molecular analysis of breeding materials. The markers are the apple powdery mildew resistance gene *Pl2*, originating from *Malus zumi*, and the apple scab resistance gene *Vf*, from *M. floribunda* 821. The clone U 211 was found to be highly resistant to mildew under field conditions and transmitted a high level of resistance to the majority of its progeny. The presence of the *Vf* gene conferring resistance to scab was confirmed by molecular analysis. As the molecular markers for the *Pl2* gene are not present in the DNA of U 211, it is probable that the clone U 211 is a new source of mildew resistance.

Key Words: Molecular Markers, Disease Resistance, Apple Breeding

INTRODUCTION

Breeding apple plants with multiple, durable resistances is an alternative way to control fungal pathogens and reduce any environmental impact due to pesticide use. The fungi *Venturia inaequalis* (Cke.)Wint and *Podosphaera leucotricha* (Ell. Et Ev.) Salm. are the most important apple tree pathogens in many areas of the world. Selection for mildew resistance is complicated because the results depend on many factors – the age of the plant, environmental conditions, and the race of the pathogen. Marker assisted selection (MAS) is faster and more reliable. The clone U 211, selected in the Department of Pomology and Basic Natural Sciences in Horticulture (DPBNSH) SGGW, was found to be highly resistant to mildew under field conditions and transmitted a high level of

resistance to the majority of its progeny. The clone U 211 originates from the open pollination of 'Primula', which carries the *Vf* gene from *M. floribunda* 821. The phenotypic assessment showed that it is also resistant to scab. Resistance screening of the progeny 'Idared' x U 211 was conducted over a three year period in the nursery [1] and since then it is performed in the orchard. Analyses are conducted on this progeny with the aim of indentifying the molecular markers for QTLs conferring resistance to powdery mildew in the clone U 211. This paper describes the molecular analysis of disease resistance in the breeding materials of DPBNSH using available SCAR markers for apple scab and powdery mildew.

MATERIALS AND METHODS

The analyses were carried out on plants of the progeny 'Idared' (susceptible) x U 211 (resistant) and on the parental plants. *M. zumi* from the collection of DPBNSH was also analysed because this tree was found to be infected with powdery mildew. The DNA was extracted from the leaves of analysed individuals using the CTAB protocol [2] with minor modifications. PCR amplification conditions for N18SCAR and U02SCAR (the markers for the *PI2* gene) were as described in [3]. The DNA of 'Idared' was a negative control and the DNA from the plant (PI) with the *PI2* gene was a positive control. Analysis with AL07SCAR (*Vf* gene marker) was conducted following the procedure in [4]. The DNA of 'Idared' constituted the negative control and the DNA of 'Primula' was used as the positive control. The PCR products were separated on 1.5% agarose gel, stained with ethidium bromide and visualized under UV light.

RESULTS AND DISCUSSION

The molecular marker AL07SCAR was found in the clone U 211 and in the positive control ('Primula'), and was absent in the negative control. The marker was also present in all 22 of the arbitrarily chosen individuals of the progeny 'Idared' x U 211 (Fig. 1A.). All the progeny were selected on the basis of their phenotype for scab resistance. Susceptible individuals were discarded before planting in the nursery. Molecular analysis conducted with AL07SCAR confirms the presence of the *Vf* gene in U 211 and constitutes a validation of the phenotypic selection of the progeny for scab resistance. Because of the co-dominant character of AL07SCAR, we are able to distinguish heterozygotes (PCR products as two bands on the gel) from dominant homozygotes for this gene (one band on the gel) [4]. The clone U 211 was found to be a heterozygote (Fig. 1A.). As 'Idared' is a recessive homozygote (susceptible to scab) for *Vf*, the resistant individuals from the progeny 'Idared' x U 211 are expected to be heterozygotes like one of parents. These expectations were confirmed by AL07SCAR analysis (Fig. 1A).

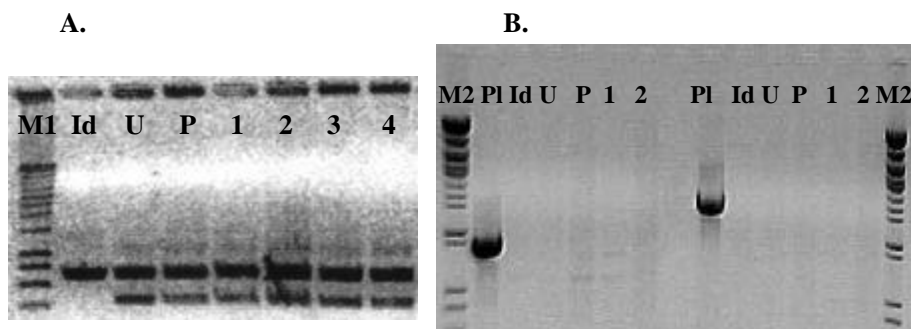


Fig. 1. The results of molecular analysis with AL07SCAR (A), N18SCAR and U02SCAR (B) conducted on 'Idared' (Id), the clone U 211 (U), 'Primula' (P), individuals from the progeny 'Idared' x U 211 (represented by 1-4 on A and 1, 2 on B) and the plant with the *PI2* gene (PI). M1 - size marker SmartLadder (Eurogentec), M2 - size marker Lambda DNA/Eco 471 (MBI Fermentas).

The molecular markers N18SCAR and U02SCAR were not found in U 211. However, these markers are present in the positive control (PI) and absent in the negative control (Id) (Fig. 1B). The RAPD marker OPAT20 for the gene *PI1* from *M. robusta* [5] was not found in U 211 either [6]. This suggests that the resistance of the U 211 is not due to the presence of *PI2* from *M. zumi* nor *PI1* from *M. robusta*. It confirms the assumption that the clone U 211 is a new source of mildew resistance.

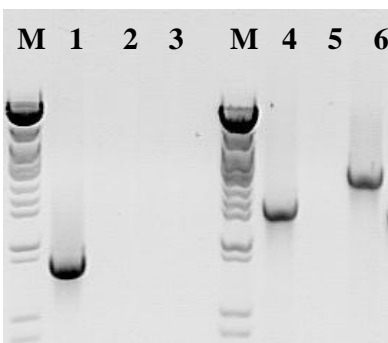


Fig. 2. The results of molecular analysis with N18SCAR (1-3) and U02SCAR (4-6) conducted on the plant with *PI2* gene (1, 4), 'Idared' (2, 5) and *M. zumi* from DPBNSH (3, 6). M - size marker Lambda Eco 471 (MBI Fermentas).

The analysis with N18SCAR and U02SCAR was also carried out with the DNA of *M. zumi* from the collection of DPBNSH SGGW. The *PI2* gene is flanked by U02SCAR and N18SCAR, respectively located 8 and 7 cM from the gene [3]. N18SCAR was not found in the analysed individual. In the case of the PCR reaction with primers generating the marker U02SCAR, a product of another

length was obtained (Fig. 2). This result suggests that the powdery mildew susceptibility of this tree is due to the absence of the *P12*, gene or to a mutation in this area, rather than to the pathogen breaking down its resistance.

We found the SCAR markers N18 and U02 easier to score and more reproducible between laboratories than the RAPD marker OPAT20. These results concur with the observations for molecular markers of the *Vf* gene [7]. This study shows the usefulness of SCAR markers in the analysis of breeding materials. However, because of the high degree of genetic diversity within *Malus* sp., each marker analysis requires verification individually.

Acknowledgements. M. Stankiewicz is supported by the Foundation for Polish Science. This study was supported in part by grant 5 P06C 032 19 from the State Committee for Scientific Research (KBN).

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