Development of an efficient MAS pipeline for multiple disease resistant genes in apple

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Introduction

The majority of the cultivated apple varieties are susceptible to the most common diseases, such as apple scab (*Venturia inaequalis*), powdery mildew (*Podosphaera leucotricha*), fire blight (*Erwinia amylovora*). As a result, modern dessert apple production in high-density, low-stem orchards requires a high number of applications of plant protection products throughout the season. Since the early 1990s, the apple breeding program at Agroscope in Switzerland has a strong focus on disease resistance breeding. Over the years, marker-assisted selection (MAS) has been successfully used to stack and combine resistance (*R*-) genes and QTLs for apple scab, powdery mildew, rosy apple aphid and fire blight.

Method

Seedlings form 23 different crosses were raised according to Bühlmann-Schütz et al. (2023). 1526 seedlings were tested with a total of 41 (three SCAR, six SSR and 32 SNP) molecular markers associated to resistance loci (Table 1) based on the presence or absence of the specific resistance in their pedigree. Genotyping with simple-sequence repeat (SSR) and sequence characterized amplified region (SCAR) markers was done at Ecogenics GmbH (www.ecogenics.ch) using multiplex PCR assays with fluorescently labelled primers. Genotyping with single nucleotide polymorphism (SNP) markers was performed at LGC Genomics Ltd. (www.lgcgroup.com) using KASP™ PCR assays. Seedlings were selected according to the desired marker combination in favour of stacking different *R*-genes / QTLs for the same and different pest and diseases.

Table 1 Molecular markers (SNP, SSR, SCAR) used in spring 2024 for 14 different *R*-genes/QTLs. Unless explicitly stated, SNP markers were used.

Pathogen/pest	Locus (*R*-Source)	Markers used (type)	Publication
Venturia inaequalis	*Rvi2* (TSR34T15)	FbsnRvi2-1_M417, FBsnRvi2-2_M341, FBsnRvi2-3_M58, FBsnRvi2-4_R489, FBsnRvi2-5_M366, FBsnRvi2-6_1_M95, FBsnRvi2-6_2_M133, FBsnRvi2-7_Y292, FBsnRvi2-8_R243, Vh2(OPL19-438) (SCAR), Vh2(CH05e03 -173) (SSR)	Jänsch et al. 2015; Bus et al. 2005
	Rvi4 (TSR33T239)	FBsnRvi4-1_K146, TNL1_Rvi4_R131	Jänsch et al. 2015
	Rvi6 (*Malus floribunda* 821)	M8S_Rvi6_Y124, M18_Rvi6_Y32, M8S_Rvi6_R156, Vf(CHVf1-164) (SSR)	Jänsch et al. 2015; Bus et al. 2005
	Rvi10 (A 723-6)	Vf(CHVf1-143) (SSR)	Hemmat et al. (2003)
	Rvi11 (*Malus baccata* jackii)	FBsnRvi11-1_Y111, FBsnRvi11-2_R357	Jänsch et al. 2015
	Rvi12 (Hansen's Baccata #2)	Rvi12_23_523_170, Rvi12_24_482_318	Padmarasu et al. 2014
Podosphaera leucotricha	*Pl1* (*Malus robusta*)	three markers	unpublished data
	Pl2 (*Malus zumi*)	FBsnPl2-1_Y245, FBsnPl2-1_R531	Jänsch et al. 2015
	Plm (Mildew Immune Selection)	CH02d12 (SSR)	Bus et al. 2010
Dysaphis plantaginea	*Dpfl* (Florina)	Dpfl_SNP_205, Dpfl_SNP_398, Dpfl_SNP_585	Pagliarani et al. 2016
Erwinia amylovora	*FB_MR5* (*Malus x robusta* 5)	FB-MR5_SNP_M106, FB-MR5_SNP_R209	Jänsch et al. 2015
	Fb_E (Evereste)	FBsnFBE-1_Y230, FBsnFBE-2_Y495	Jänsch et al. 2015
	FB_Mfu10 (*Malus fusca*)	Fb_Mfua(FRM4-156) (SSR), Fb_Mfub(CH03d11-109) (SSR)	Emeriewen et al. 2014
	FB_F7 (Fiesta)	SNP_FB_0716011, SNP_FB_0716013, FBF7(AE10-380) (SCAR), FBF7(GE-8019-403) (SCAR)	Khan et al. 2007; van de Weg et al. 2018

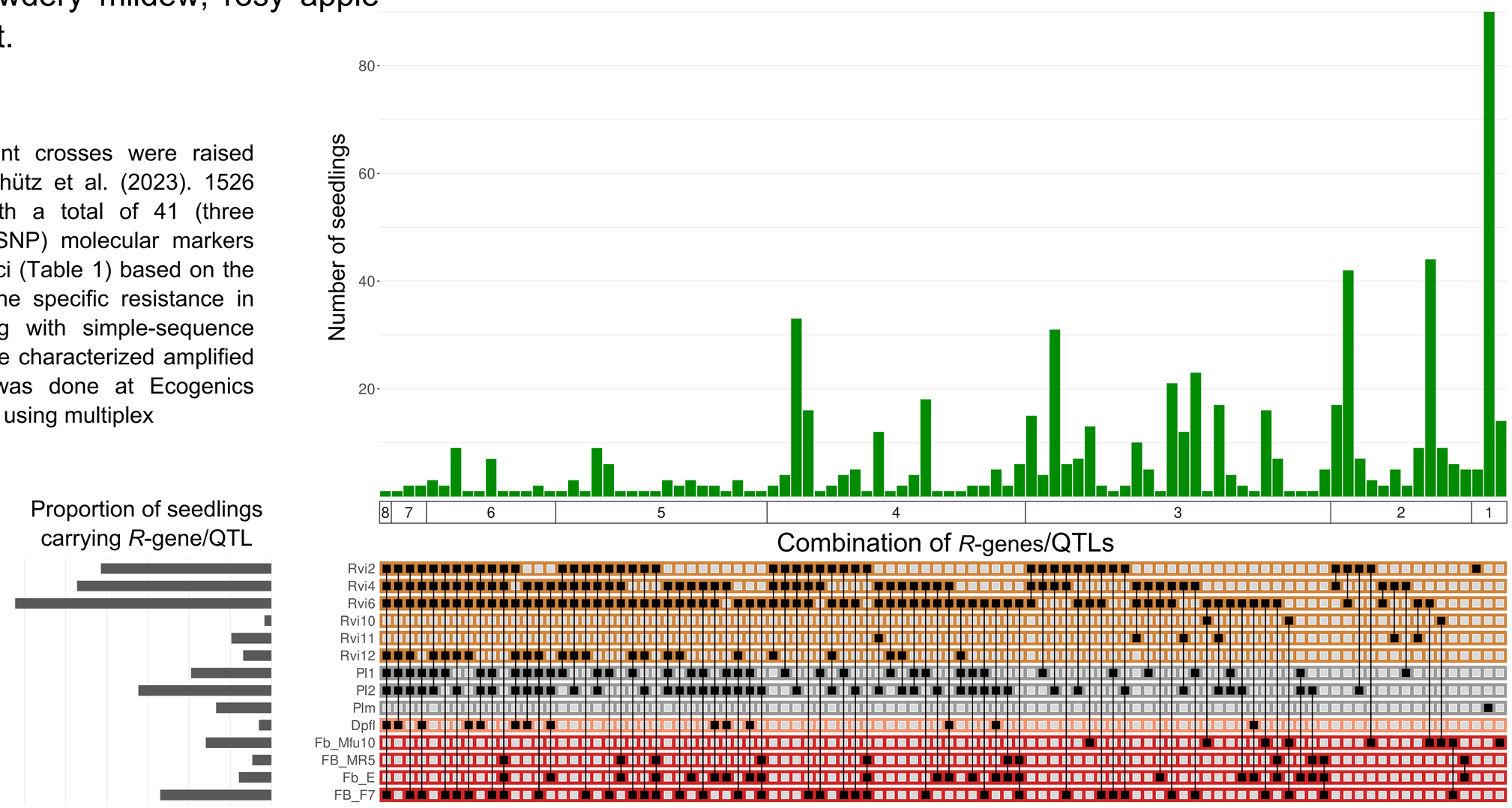

Figure 1 Overview of 671 selected (out of 1526 tested) seedlings in Spring 2024. The upper half of the figure (green bars) shows the number of seedlings selected with the combinations of *R*-genes/QTLs indicated in the lower part of the figure (black cells). The colour of the frame of the cells indicates the resistance to apple scab (orange), powdery mildew (grey), rosy apple aphid (pink) or fire blight (red). The bar plot in the lower left corner shows the proportion of selected seedlings carrying the respective *R*-gene/QTL.

Results

A maximum of eight *R*-genes/ QTLs were found in one seedling (Figure 1). In most of the progenies, three to four *R*-genes/ QTLs were combined.

Conclusion

Marker-assisted selection (MAS) is used successfully to stack and combine *R*-genes and QTLs for apple scab, powdery mildew, rosy apple aphid and fire blight. The state-of-the-art MAS pipeline currently in use enables time- and cost-efficient selection of young seedlings in the greenhouse and container field before grafting and field evaluation.

References

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