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Thomas Wöhner, Mohammed Eldin Ali, Andreas Peil

Wildspecies
Apple: *Malus ×robusta*

Julius Kühn-Institut, Federal Research Centre for Cultivated Plants

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submitted
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Abstract

This datasheet aims to describe the *Malus* hybrid species *Malus ×robusta* (Carrière) Rehder. This secondary species possesses beneficial resistances to diseases. International breeding programs like the breeding program at the Julius Kühn-Institut (JKI), Institute for Breeding Research on Horticultural and Fruit Crops in Dresden-Pillnitz (ZGO) focus on the genotype *Malus ×robusta* No. 5.

As the name "*robusta*" implies, *Malus ×robusta* 5 represents an important genetic resource for breeding of disease resistance. As donor for fire blight resistance it mainly became importance in the Geneva® USDA/Cornell Apple Rootstock Breeding program of the Cornell University, New York. Currently, the molecular background of the resistance mechanism is investigated at ZGO.

The datasheet contains the classification and a description of a few accessions of *Malus ×robusta* (Carrière) Rehder. The *Malus ×robusta* accessions maintained at the German National Fruit Collection which belongs to ZGO are characterized and evaluated for resistance to some important diseases of apple. The results of a genetic finger print enable the differentiation between the accessions of *Malus ×robusta* (Carrière) Rehder.

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Malus ×*robusta* (Carrière) Rehder – Synonyms

The synonyms are the same as published by Potter et al. (2007) and the web site of the United States Department of Agriculture (USDA).

Malus microcarpa var. *robusta* Carrière

Pyrus baccata var. *cerasifera* Regel

Malus cerasifera Schneider

Sibirischer Holz-Apfel, Kirsch-Holz-Apfel (germ.)

Sibirian crap apple (engl.)

Robusta apel (swed.)

Taxonomy

Malus ×*robusta* (Carrière) Rehder belongs to the order Rosales, to the Subfamily Amygdaloideae (previously Spiraeoideae), to Tribe Maleae, and in between the Subtribe Malinaea to the Genus *Malus*. *Malus* ×*robusta* (Carrière) Rehder is a natural *Malus* hybrid species (secondary species) of the primary species *Malus baccata* (L.) Borkh. and *Malus prunifolia* (Willd.) Borkh., whose origin is located in Siberia and Central Asia. (Forsline et al. 2003).

Order: Rosales
→ Family: Rosaceae
→ Subfamily: Amygdaloideae
→ Tribe: Maleae
→ Subtribe: Malinae
→ Genus: Malus
→ Species : *Malus* ×*robusta*

General Morphology

The general morphology is described referred to Fitschen (1990), Krüssmann (1977) and Roloff (1996).

Tree habit
Malus ×robusta is a small to medium-sized, deciduous, twiggy tree with a weak to strong vigor. Its upright to spreading crown is formed by the characteristic fruit bearing long- and short shoots.

Shoots
The branches can droop slightly. Pubescence is medium on the upper side of the shoot surface to absent. The bark shines medium to strong. The size of the elliptic lenticels as well as the shape of the buds (acute and rounded) can vary.

Leaves
The green elliptic leaves could reach a length of 8 to 11 cm. The leaf shape is sharp pointed and the margin is serrated. The leaf blade is narrower in *Malus ×robusta* than in *M. baccata* and pubescence is weak to absent on lower side.

Flower
The flower width can range from 3 to 5 cm in diameter, colored from white to pink. Flowering time starts in middle of April and ends in beginning of May under the conditions in Dresden. The arrangement of the crenate petals varies from free to overlapping. The number of styles is 4 to 5. The type of inflorescence is corymbiform with a number of 3 to 8 single flowers on long and short shoots. The pedicel is pubescent. The position of stigmas is at the same level or above relative to anthers.

Fruit
The length of the fruit stalk is between 2 to 3.5 cm (long). The fruits are globose or ellipsoid with a diameter of 1 to 3 cm. The color can vary between red and yellow, sometimes also bluish. The color of the flesh can differ from greenish, yellowish to crème. The calyx is sometimes discarded and can be closed to completely open.

The variation of these characteristics is shown in **Figure 1-4,** where tree habit, shoots, leaves, flowers and fruits of different accessions of *Malus ×robusta* are presented.

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Malus ×*robusta* – accessions

The origin of single accessions is rarely described in literature. The latest and most detailed compendium for crab apples was published by Fiala (1994).

Below the general origin of the *Malus* genotypes *M.* ×*robusta* No. 5, *M.* ×*robusta* 'Persicifolia' and *M.* ×*robusta* 'Erecta' is described referred to Fiala (1994) in a slightly restated form.

Malus ×*robusta* No. 5

M. ×*robusta* No. 5, also known as 'Arnold-Canada' or Ottawa No.524 or *M.* ×*robusta* 5, originates from a seed collected near Beijing. In 1927 the accession was introduced to the US. The Arnold Arboretum (Boston, Massachusetts, USA) obtained this seedling from Russia. In 1947 the accession was introduced to Canada through the Department of Agriculture (Ottawa, Ontario).

M. ×*robusta* No. 5 differs from the general description of *M.* ×*robusta* by its expansive crown and upright to spreading habit (**Fig. 1e**). The fruits are slightly larger and are colored yellow with a more light intensity of red over color (**Fig. 4e**). The leaves are larger, wider and more oval (Fiala, 1994) turning to a bright yellow in autumn. In the apple wild species collection of ZGO, *M.* ×*robusta* 5 is designated with the accession number MAL0991.

Malus ×*robusta* 'Persicifolia'

M. ×*robusta* 'Persicifolia' was collected as a seed by William Purdon on the road to Wutai Shan near Beijing (China) and introduced to USA in 1910. The Arnold Arboretum obtained this genotype in 1913 by J. Veitch & Sons (Veitch Nurseries, UK).

This accession displays a more shrub-like habit (**Fig. 1a**). The branches are slender and upright. The leaves are slim and dark green, lightly glossy. The serrated leaves have a length of 7 cm and resemble peach leaves (**Fig. 3a**). Flower buds are pink. Single flowers are colored white with of 4 cm in diameter. The elliptic shaped fruits are light red. The color of the flesh varies from yellow to green or brownish to green. The diameter of the fruits is about 2 cm (**Fig. 4a**). Fruit setting in general is very high and they often remain on the tree until February or March.

In the apple wild species collection of ZGO, *M.* ×*robusta* 'Persicifolia' is designated with the accession number MAL0205.

M. ×*robusta* 'Erecta'

The Arnold Arboretum obtained this accession as seed by C.S. Sargent in 1904 from Beijing (China). *M.* ×*robusta* 'Erecta' has an upright tree habit. Flower buds are white and with little pink inclusions. The flowers are white with a diameter of 4 cm and can occur as single and double flowers. Fruit color can vary from yellow over red up to deep purple. Their diameter is about 2.3 cm. *M.* ×*robusta* 'Erecta' alternates very strong in bloom.

Morphological characterization of the *Malus ×robusta* accessions of the Julius Kühn-Institute

The *Malus ×robusta* accessions present in the wild species collection of the Julius Kühn-Institute (MAL0207, MAL0595, MAL0711, MAL0205, MAL0991) and there morphological characteristics are described under the climatic and growth conditions in Dresden-Pillnitz (**Table 1, 2**). The soil type is brown soil composed of sandy loams to loamy sands. The annual average precipitation is 680 mm and the average temperature is 9°C. All accessions are grafted on 'Bittenfelder Sämling' planted with 3.5 m space within the rows and 5 m distance between the rows.

Traits where evaluated using the Guidelines of the International Union for the Protection of new Varieties of Plants (UPOV) and were described in the thesis of Mohamed Eldin Ali (2011), too.

Figure 1 displays tree habit, shoot tips and shoots are shown in **Figure 2,** leaves are presented in **Figure 3** and **Figure 4** shows flowers and fruits of the *Malus ×robusta* accessions held in the collection of ZGO.

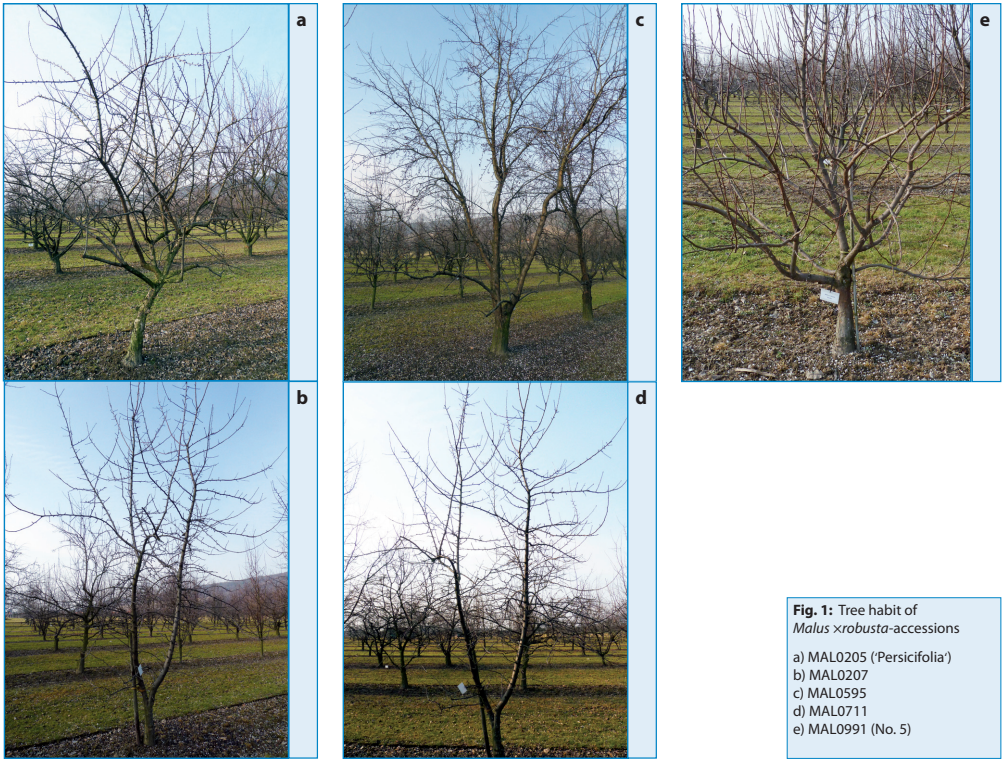

Fig. 1: Tree habit of *Malus ×robusta*-accessions

a) MAL0205 ('Persicifolia')
b) MAL0207
c) MAL0595
d) MAL0711
e) MAL0991 (No. 5)

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a	b	c	d	e

Fig.2: Shoot tips (above); one year old shoots (below)
a) MAL0205 ('Persicifolia')
b) MAL0207
c) MAL0595
d) MAL0711
e) MAL0991 (No. 5)

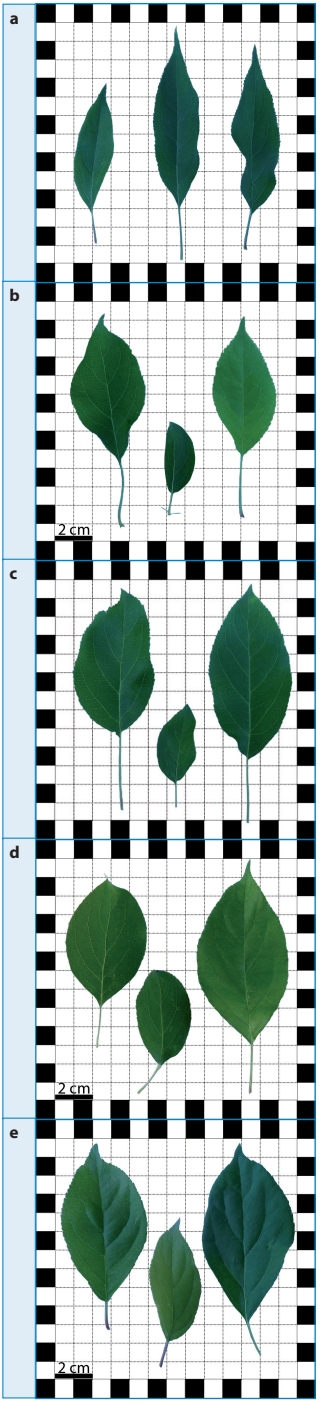

Fig.3: Leaves
a) MAL0205 ('Persicifolia')
b) MAL0207
c) MAL0595
d) MAL0711
e) MAL0991 (No. 5)

Table 1: Morphological evaluation of leaf-, tree- and shoot characteristics of *Malus* ×*robusta*- accessions

Morphological charateristic		Accession Designation MAL0205 'Persicifolia'	MAL0207	MAL0595	MAL0711	MAL0991 No. 5
Leaf (Fig. 3)	Length (cm)	7,2	8,6	8,5	9,9	5,7
	Width (cm)	3,2	4,9	5,0	5,3	2,6
	Incisions of margin	serrate	serrate	serrate	serrate	serrate
	Green color of upper side	medium	light	medium	light	dark
	Glossiness of upper side	strong	strong	strong	strong	strong
	Pubescence on lower side	weak	medium	medium	medium	weak
Tree (Fig. 1)	Habit	upright	spreading	spreading	spreading	upright
	Vigor	weak	weak	strong	strong	weak
One-year-old shoot (Fig. 2)	Thickness (mm)	3,4	3,2	2,9	3,8	3,5
	Color on sunny side	grey green	grey green	brown green	grey green	grey green
	Pubescence	absent	weak	absent	weak	medium
	Shape of lenticels	broad elliptic	broad elliptic	elliptic	broad elliptic	broad elliptic

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Fig.4: : Flowers (left); fruits (right)
a) MAL0205 ('Persicifolia')
b) MAL0207
c) MAL0595
d) MAL0711
e) MAL0991 (No. 5)

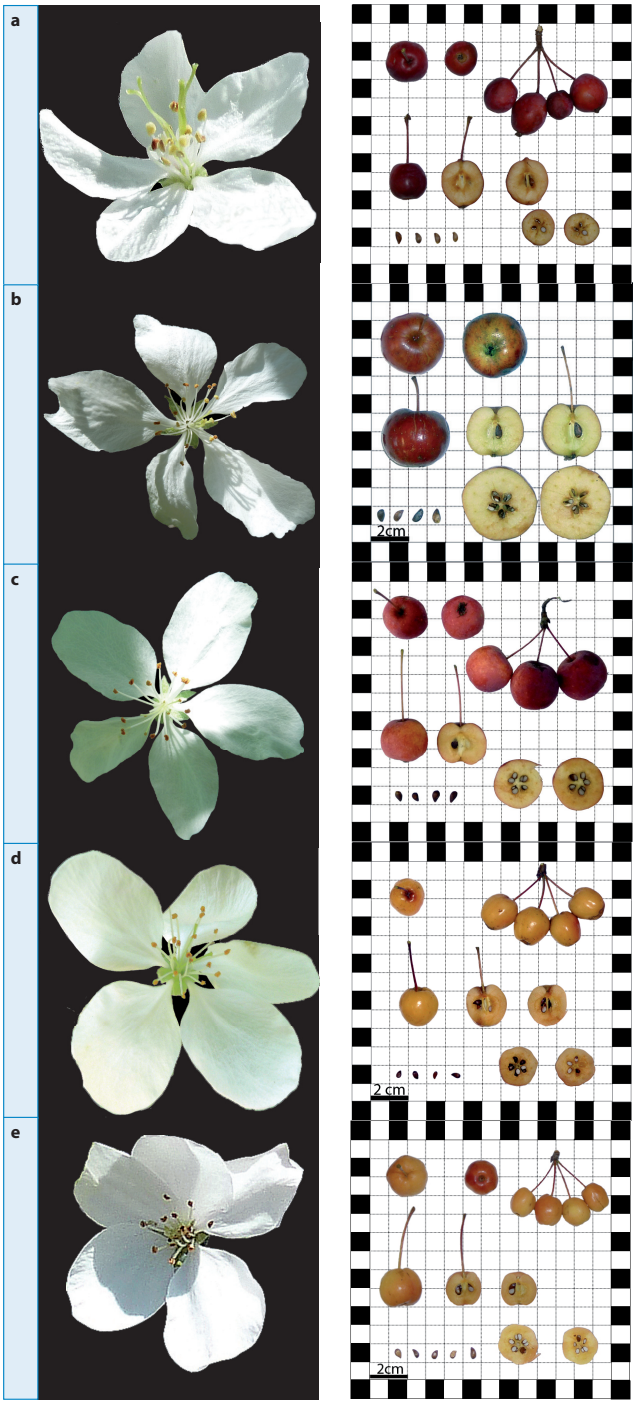

Table 2: Morphological evaluation of flower- and fruit characteristics of *Malus* ×*robusta*- accessions

Morphological characteristics		Accession Designation MAL0205 'Persicifolia'	MAL0207	MAL0595	MAL0711	MAL0991 No. 5
Flower (Fig. 4)	Unopened flower: color	white	light pink	white	dark pink	dark pink
	Number of flowers per cluster	7	7	7	7	6
	Petals: relative position of margins	touching	touching	touching	touching	touching
	Number of petals	single flower	single flower	single flower	single flower	single flower
	Diameter (cm) (horizontal position of petals)	3,3	5,4	4,0	3,8	4,6
	Petal: shape	broad elliptic	broad elliptic	elliptic	broad elliptic	broad elliptic
	Petal: color	white	white	white	white	white
	Position of stigmas relative to the anthers	above	same level	same level	same level	same level
Fruit (Fig. 4)	General shape	ellipsoid conic	globose	globose	oblong conic	globose conic
	Height (cm)	1,7	3,1	2,0	2,2	1,7
	Diameter (cm)	1,6	3,7	2,2	2,0	1,8
	Predominant color	dark red	medium red	purple	orange	yellow
	Color of flesh	yellowish	yellowish white	yellowish	yellowish	yellowish
	Bloom of skin	strong	weak	strong	weak	weak
	Length of stalk (cm)	2,7	1,9	1,9	2,3	1,8

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Molecular genetic analysis of the *Malus* ×*robusta* accessions

A genetic fingerprint was performed for the *Malus* ×*robusta* (Carrière) Rehder accessions of the Julius Kühn-Institute in Dresden-Pillnitz and additional accessions as well as *Malus* ×*robusta* types of the National Germplasm Repository (Geneva, New York) and accessions of the Institute of Pomology and Floriculture (Skierniewice, Polen).

A total of 12 microsatellites belonging to a set of reference markers of the European Cooperative Program for Plant Genetic Resources (ECPGR) were used (van Teuren et al. 2011). Amplification of fragments using multiplex PCR, fragment analysis with a capillary sequencer and analysis of the different alleles using the Software Fragment Analyzer are described in Peil et al. (2012). A comparison of genetic fingerprints enables the identification of identical *Malus* ×*robusta* genotypes in different national collections (trueness-to-type). The investigated genotypes and their origin are listed in **Table 3**. The results of the molecular investigation are presented in **Table 4**. The results of the marker analysis revealed some *Malus* ×*robusta* accessions with identical fingerprints. The fragment lengths of the different fingerprints of each accession are listed in **Table 4**.

Table 3: *Malus* ×*robusta*- accessions

Accessionnumber	Type/cultivar
MAL0205[1]	'Persicifolia'
MAL207[1]	
MAL0595[1]	
MAL0711[1]	
MAL0991[1]	No. 5
PI 588825[2]	No. 5
PI 215542[2]	'Yellow Siberian'
PI 589278[2]	'Leucocarpa'
PI 588904[2]	'Cherry Crab'
PI 588936[2]	'Erecta'
PI 589286[2]	'Fastigiata'
PI 589003[2]	'Korea'
PI 588993[2]	
PI 589383[2]	'Persicifolia'
PI 589424[2]	'Persicifolia'
PI 590068[2]	'Persicifolia'
M. ×*robusta* US[3]	
M. ×*robusta* SZW[3]	

[1] Accessions of the germplasm collection of the Julius Kühn-Institut, Pillnitz, Dresden
[2] provided by the National Germplasm Repository, Geneva, New York
[3] provided by the Institute of Pomology and Floriculture, Skierniewice, Polen
Genotypes with identical Fingerprint are marked (without white) with the same color

The accessions of the Julius-Kühn Institute

The fingerprint of the *Malus* ×*robusta* accession MAL0991 (No. 5) as well as the fingerprint of MAL0205 ('Persicifolia') matched completely with their analogs of the American collection.

Accession MAL0711 shows a high accordance to *Malus* ×*robusta* No. 5. Only one allele of marker GD12 was amplified in *Malus* ×*robusta* No. 5 and the fragment amplified by Ch04e05 is one base shorter compared to MAL0991.

Accession MAL0207 matched with three markers and MAL0595 matched with four markers to other accessions of *Malus* ×*robusta*. A complete identity was not found. This implies that accessions MAL0207, MAL0595 and MAL0711 are unique genotypes within the collection of the JKI.

Table 4: Genetic fingerprints of different *Malus ×robusta*- accessions, performed with 12 microsatellite markers in four multiplex-PCRs

Multiplex		1				2			3			4	
Linkage group		17	8	4	9	13	1	7	11	10	3	1	12
SSR-Marker		CH01h01[1]	CH01h10[1]	CH04c07[1]	CH01f03b[1]	GD147[3]	Hi02c07[2]	CH04e05[1]	CH02d08[1]	CH02c11[1]	GD12[3]	CH02c09[1]	CH01f02[1]
Accession	Type												
MAL0207		111 134	91 118	108 110	160 184	143 152	104 112	185 203	225 254	197 208 230	150 168	234	170 180
MAL0711		91 101	89 112	108 110	172	153	114 116	183	213 215	197 208 222	152 154	249	176 180
MAL0595		91 120	98 112	110 112	151 160	141	112	191 211	213 219	197 208 218	150 154	256	188 220
MAL0205 PI 589383 PI 589424 PI 590068	'Persicifolia' 'Persicifolia' 'Persicifolia' 'Persicifolia'	120	110 112	98 110	151 172	141	114 116	183 205	215	197 208 234	160	252	176
MAL0991 PI 588825 *M. ×robusta* US	No. 5 No. 5	91 101	89 112	108 110	172	153	114 116	184	213 215	197 208 222	152	249	176 180
PI 215542	'Yellow Siberian'	118 120	98 112	108 110	172 180	135 141	114	199 224	213 215	197 218 220	150	234	188 205
PI 588904	'Cherry Crab'	117 120	90 98	108 110	151 160	135 143	104	183 191	213 215	197 207 236	141 150	246 256	170 220
PI 589278	'Leucocarpa'	124 134	98 103	102 108	151 172	139	128 140	197 213	217 256	197 209 234	150	228 246	186 207
PI 589286 PI 588936	'Fastigiata' 'Erecta'	90	110 112	98 110	151 172	141	116 120	183 199	213 215	197 234	160	252	170
PI 589003 PI 588993	'Korea'	117 134	91 97	108 120	151 180	141	132 148	183 203	221 256	197 220 244	150	256	180 237
M. ×robusta SZW		91 120	88 90	106 110	172	135	114 118	183 211	213	197 207 237	184	258	180 205

Accessions with the same color (without white) possess identical fingerprints
1 Liebhard et al. (2003)
2 Silfverberg-Dilworth et al. (2006)
3 Hokanson et al. (1998)

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Phenotypic and genotypic evaluation of the *Malus* ×*robusta* accessions of the JKI

The *M.* ×*robusta* accessions of the Gene Bank of the Julius Kühn-Institute were phenotypically evaluated for natural occurrence of apple pathogens (powdery mildew, apple scab, sooty blotch, brown rot and flyspeck disease) in a plot not sprayed with fungicides in the years 2009 and 2010 as described in Eldin (2011). Evaluation was performed using the scoring scales presented in **Table 5**. Screening of accessions for resistance to fire blight was executed using artificial inoculation of grafted scions in the greenhouse (Peil et al. 2007).

Additionally, the *Malus* ×*robusta* (Carrière) Rehder accessions of the apple wild species collection of the Julius Kühn-Institute in Dresden-Pillnitz were investigated with molecular markers for rosy leaf curling aphid, apple scab, powdery mildew and fire blight resistance genes (**Table 6**). Results of the phenotypic evaluation of apple pathogens in the orchard of the ZGO are shown in **Table 5**. The molecular evaluation with molecular markers for resistance genes is shown in **Table 6**.

For all *Malus* ×*robusta* accessions no symptoms of powdery mildew where observed in the years 2009 and 2010. Marker analysis resulted in the positive evidence for the marker of the powdery mildew resistance gene *Pl1* for all analyzed accessions. MAL0207 and MAL0595 were medium to highly susceptible to apple scab. Accessions MAL0711, 0205 and 0991 showed no symptoms in both years. Accession MAL0205 was positively tested for the gene specific SSR marker Ch-Vf1 (*Rvi 6*, 159bp).

Accession MAL0207 is most susceptible to *Erwinia amylovora*, the casual agent of fire blight, with a total shoot necrosis of 58%. Accession MAL0991 (No. 5) is resistant to fire blight. The presence of the fire blight QTL detected in 'Fiesta' was proven with the markers AE10-375 and GE8019 enclosing the resistance QTL. Accessions MAL0711 and MAL0595 showed only AE 10-375 whereas only marker GE80-19 was detected in MAL0207. The resistance QTL of *Malus* ×*robusta* No. 5 (Fb_Mr5) was detected in the accessions MAL0711 and 0991 using the molecular markers Ch03e03 amd Fem18, enclosing the resistance locus on chromosome 3 in *M.* ×*robusta* No.5. The marker for the presence of the resistant gene to rosy leaf curling aphid (*Sd-1,*181bp) was detected for the accession MAL0207.

Acknowledgement

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Table 5: Phenotypic evaluation of different *Malus* ×*robusta*- accessions on several apple pathogens

Disease	Scoring-scale	Reference	Accession Designation MAL0205 'Persicifolia'	MAL0207	MAL0595	MAL 0711	MAL0991 No. 5
Powdery mildew	0 - 5[1]	Dunemann et al. (2007)	0	0	0	0	0
Scab	1 - 7[2]	Eldin Ali (2011)	1	7	6	1	1
Brown rot	1 - 9[3]	Eldin Ali (2011)	1	1	7	3	1
Flyspeck	0 - 1[4]	Andrews et al. (2001)	0	0	0	0	0
Sooty blotch	0 - 3[5]	Andrews et al. (2001)	1	1	1	3	1
Fire blight	0 - 100 %[6]	Peil et al. (2007)	14	58	10	19	0

1 Infestation of leaves (0: without, 1: very weak, 2: weak, 3: medium, 4: strong, 5: very strong)
2 Infestation of leaves (1: without, 2: very weak, 3: weak, 4: weak to medium, 5: medium, 6: medium to strong, 7: strong)
3 Infestation of the fruit surface (1: 0 %, 3: 1 - 25 %, 5: 26 - 50 %, 7: 51 - 75 %, 9: 76 - 100 %)
4 Infestation of the fruit surface (0: without disease, 1: susceptible)
5 Infestation of the fruit surface (0: without disease, 1: < 1 % of the fruit surface affected, 2: 1 - 5 % of the fruit surface affected, 3: > 5 % of the fruit surface affected)
6 Total shoot necrosis (0 - 100 %, Percentage of shoot necrosis relative to the total shoot length)

Table 6: Genotypic evaluation of different *Malus* ×*robusta*- accessions with molecular markers for resistance loci

Disease	Resistance locus	Marker	Reference	Accession Designation MAL0205 'Persicifolia'	MAL0207	MAL0595	MAL0711	MAL0991 No. 5
Powdery mildew	PL1	AT20 Scar	Markussen et al. (1995)	+	+	+	+	+
	PL2	N18 Scar	Seglias et al. (1997)	-	-	-	-	-
Fire blight	FbF7	Ae10-375	Kahn et al. (2006)	-	-	+	+	-
		GE 80-19	Chalenge et al. (2005)	-	+	-	-	-
	FbR5	Ch03e03	Peil et al. (2007)	-	-	-	+	+
		Fem 18	Fahrentrapp et al. (2012)	-	-	-	+	+
Scab	Rvi2	Ch02b10	Hemmat et al. (2002)	-	-	-	-	-
	Rvi4	Ch02c02	Bus et al. (2005)	-	-	-	-	-
	Rvi6	Ch-Vf1	Vinatzer et al. (2004)	+	-	-	-	-
	Rvi17, Rvi10	Ch-Vf1	Bus et al. (2012)	+	-	-	-	-
Rosy leaf curling aphid	Sd	Sd-1	Bus et. al. (2008)	-	+	-	-	-

(+: present, -: not present)

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