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Short title: Loss of susceptibility

Genetic loss of susceptibility: a costly route to disease resistance?

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The susceptibility of plants to microbial pathogens involves molecular interactions between microbial effectors and host targets. In most cases, pathogen effectors prevent recognition or suppress host defence. However, successful suppression of host defence is not always sufficient for pathogenesis, which requires further host components that meet the demands of pathogen development and nutrition. Additionally, the plants possess negative regulators of immune response to avoid autoimmunity and unnecessary investment into defence in environments with little disease pressure. Consequently, disease susceptibility can be lost by mutation of negative regulators of defence but also of other host factors, that otherwise support the successful pathogen. Here, we review genetic loss of susceptibility to adapted microbial pathogens and focus on examples of lost susceptibility to powdery mildew. We

discuss costs of resistance and potential consequences for application in breeding and biotechnology.

Introduction

Plant resistance is common in nature to diseases caused by microbial agents. This is explained by the fact that microbes have to evolve pathogenicity and virulence factors to recognize a plant as a suitable host and to overcome preformed and pathogen-induced plant defence. Co-evolution of the pathogen with its hosts can cause a high degree of adaptation to a limited number of plants (Jones & Dangl, 2006). Host specificity is particularly wide-spread amongst biotrophic fungal pathogens, which develop long-lasting interactions of specialized feeding cells, haustoria, with living host cells.

Once a pathogen has overcome preformed defence barriers, it faces pathogen-induced plant defence. This is activated by recognition of either the pathogen itself or alterations of host cell structures or functions by the pathogen. Plants monitor their cell surface for molecular patterns that indicate the presence of non-self. This happens via the detection of either conserved non-self molecules, designated as microbe- or pathogen-associated molecular patterns (MAMPs or PAMPs), or by detection of non-self activities that release endogenous so-called damage-associated molecular patterns (DAMPs) from plant structures. Pattern recognition receptors and receptor-like proteins are the most prominent proteins that function in these processes. Their ligands are PAMPs such as bacterial flagellin or fungal chitin, or DAMPs such as plant-derived peptides or oligosaccharides (Fig. 1a). General non-self recognition appears partially redundant and is fundamental to diverse kinds of pathogen race-nonspecific resistance (Boller & Felix, 2009). For suppression of induced defence, pathogens secrete effector molecules, which can result in effector-triggered susceptibility (ETS, Figs. 1b, 1d). Effectors normally function in the pathogen but change the host´s response to pathogen infection in a way that supports compatibility. Consequently, plant immunity also involves direct or indirect recognition of pathogen effectors by host resistance proteins. Most plant resistance (R) proteins function as receptor of effectors or of host proteins damaged by

71 the effector (de Wit *et al.* 2009). Such direct or indirect effector recognition then results in
72 effector-triggered immunity (ETI). In contrast to PAMP-triggered immunity (PTI), ETI is
73 pathogen race-specific, because effectors are more diverse and evolve rapidly. Additionally,
74 effectors, which trigger immunity, can be eliminated, or other effectors can again supress ETI
75 such that an evolutionary arms race takes place. Pathogens hence evolve virulence by
76 avoidance and suppression of PTI and ETI (Jones & Dangl, 2006).
77 Suppression of immunity appears to be a prerequisite for pathogenesis. However, it may not
78 always be sufficient. This may be the case particularly if a biotrophic pathogen establishes
79 feeding structures, such as the haustorium of a powdery mildew (PM) fungus, in intact host
80 cells and has a long-lasting interaction with its host. In such a situation, pathogen effectors
81 may not only overcome immunity but may additionally change plant cell architecture for
82 accommodation of the pathogen. The haustorial complex consists of the fungal haustorium
83 and the partially or fully host-derived neck band, an extrahaustorial matrix and an
84 extrahaustorial membrane (Green *et al.*, 2002). The host thus must contribute to the formation
85 of this complex, albeit possibly under hostile control by pathogen effectors. Additionally it is
86 suggested that infected leaf areas constitute nutrient sinks from which pathogens feed or are
87 fed via host nutrient transporters (Fotopoulos *et al.*, 2003; Chen *et al.*, 2010). Hence,
88 structural and metabolic reprogramming of the host (Fig.1) accompanies suppression of
89 immunity in a compatible interaction with fungal biotrophs.

90 Loss of susceptibility

91 In most cases, immunity is dominantly inherited. This is most obvious for monogenic race-
92 specific resistances based on ETI. Quantitative (partial) resistance is of complex genetic
93 nature and may involve allele-dosage effects. However, there is also recessively inherited
94 resistance to fungal biotrophs. Recessively inherited resistance can be considered as loss of
95 susceptibility (LOS). In this respect, one should distinguish between LOS-mutants, which

96 show constitutive or primed defence to the pathogen, from those, which cannot support a
97 compatible interaction. The first may suffer from compromised control over defence
98 mechanisms (compare Figure 1 c and d), whereas the latter may be susceptibility mutants in a
99 more narrow sense (compare Figure 1 e and f). They may reflect a high demand of the
100 pathogen for contribution from an intact host to pathogenesis. Obligate biotrophs such as PM
101 apparently lost many genes during co-evolution with their hosts because the plant can
102 compensate for the lack of certain metabolic pathways (Spanu *et al.*, 2010). Loss or
103 dysfunction of host factors involved in such pathways (Figs. 1e, 1f) then may limit
104 susceptibility. Such LOS is difficult to circumvent for the pathogen because at this point
105 evolution is likely irreversible – the pathogen has entered a dead end. Consequently, LOS
106 should confer durable resistance.
107 From a mechanistic point of view, susceptibility can be lost when a negative regulator of
108 disease resistance, such as POWDERY MILDEW RESISTANT4 (PMR4) loses function, and
109 the corresponding mutant shows constitutive or primed defence. Hence, although resistance is
110 recessively inherited due to loss of PMR4 function, immunity requires activated defence
111 responses by the host. Accordingly, genetic experiments show that LOS in pmr4 mutants
112 requires genetic elements of salicylic acid signalling (Nishimura *et al.*, 2003). Similarly, PM
113 resistance in *mlo* mutants requires functional ROR2, a component of the secretory machinery
114 in barley (Collins *et al.*, 2003), and further components of host defence in Arabidopsis
115 (Consonni *et al.*, 2006; Consonni *et al.*, 2010). Other types of LOS are characterized by
116 reduced pathogenesis because of insufficient host support for fungal development or
117 nourishment. Barley monomeric G-protein RACB and barley alcohol dehydrogenase 1 are
118 host proteins that potentially support fungal accommodation and biotrophy rather than control
119 defence (Schultheiss *et al.*, 2002; Hoefle *et al.*, 2011; Pathuri *et al.*, 2011). One might expect
120 that LOS in a more narrow sense should be accompanied by pleiotropy in terms of plant

development or metabolism, whereas mutants with enhanced PM defence may additionally show susceptibility to necrotrophs. The trade-off of basal resistance to biotrophs and susceptibility to necrotrophs is best explained because salicylic acid is involved in both.

There are only few examples for accepted LOS mutants in a narrow sense, possibly because it is difficult phenotypically to uncouple less fungal success from more effective defence. At the cellular level, for instance, formation of callosic cell wall appositions and a certain frequency of subsequent single cell death typically accompany failure of fungal penetration on susceptible barley. Hence, if compatibility is limited due to LOS, enhanced plant defence might result from the failure of the fungus to proceed to a status that allows for effective delivery of suppressors.

The *powdery mildew resistance* mutants *pmr5* and *pmr6* of Arabidopsis show PM resistance without obvious activation of defence pathways (Vogel *et al.*, 2002, 2004). These mutants show cell wall alterations, and one may speculate that an altered host cell wall lacks proper cues for fungal development or releases an altered spectrum of DAMPs during fungal penetration such that defence is locally activated without primed defence signalling. This type of LOS causes growth retardation of the resistant mutants. Recently, it was suggested that Arabidopsis accession Te-0 could be a naturally occurring LOS genotype because it limits fungal sporulation without showing obvious enhanced defence reactions (Fabro & Alvarez, 2012).

Future studies may show whether susceptibility proteins are involved in ETS or are part of a plant developmental or physiological program PM hitchhikes on without the requirement for direct molecular interference (compare Figs. 1e and 1f). It should also be generally analysed whether putative LOS mutants show normal responses to PAMPs and whether LOS is specific to a certain pathogen species or class rather than showing pleiotropic effects in interaction with other pathogens.

Recent examples of LOS

Table 1 contains selected recent descriptions of host genes required for full susceptibility to PM. Some of them are described below. Previous review articles contain further examples of potential susceptibility factors (Hückelhoven, 2005; O´Connell & Panstruga, 2006; Pavan *et al.*, 2010).

The barley monomeric G-protein RACB is a susceptibility factor for penetration by *Blumeria graminis* f.sp. *hordei* (*Bgh*, Schultheiss *et al.*, 2002). RACB is required for fungal invasion and subsequent expansion of haustoria in epidermal leaf cells (Hoefle *et al.*, 2011). At the mechanistic level, RACB and directly interacting proteins such as MICROTUBULE ASSOCIATED GAP1 and ROP BINDING KINASE1 organize arrays of cortical microtubules (Hoefle *et al.*; 2011, Huesmann *et al.*, 2012). Microtubules have a function in basal penetration resistance (Kobayashi *et al.*, 1997). Active RACB is suggested to loosen local arrays of microtubules for better penetration by *Bgh*. Because knock down of *RACB* supports basal penetration resistance, it is difficult to uncouple LOS from enhanced defence. However, recently it was shown that stable transgenic knock down of *RACB* also prevents normal outgrowth of hairs from the root epidermis (Hoefle *et al.*, 2011). This supports the view that *Bgh* might co-opt RACB´s functions in local cell expansion during ingrowth into epidermal cells of barley. Consequently, loss of RACB is accompanied by developmental failure but not by spontaneous expression of classical pathogenesis-related genes (Björn Scheler and R.H. TU München, unpublished).

Recently, it was shown that the barley endoplasmic reticulum-resident cysteine-rich receptor-like kinase HvCRK1 is involved in negative control of basal resistance to *Bgh*. Interestingly, expression of HvCRK1 is induced by hydrogen peroxide and depends on a functional *MLO* susceptibility gene. Transient knock down of *HvCRK1* via RNAi reduces the susceptibility

index to penetration by *Bgh* by more than 50% in a susceptible *MLO* background (Rayapuram *et al.*, 2012). HvCRK1 might thus be a part of an MLO-dependent regulon, which negatively controls basal resistance to *Bgh*. It is not yet known whether constitutive loss of HvCRK1 function would come along with pleiotropic effects.

Barley *BAX INHIBITOR-1* (BI-1) is another gene, whose expression is modulated by MLO, although it does not strictly depend on MLO. Transient or stable silencing of *BI-1* limits fungal penetration success (Eichmann *et al.*, 2010), whereas over-expression supports penetration even into fully resistant *mlo* mutants (Hückelhoven *et al.*, 2003). BI-1-like plant LIFEGUARD proteins are further host factors, which, when silenced, limit susceptibility to penetration by *Bgh* and when over-expressed support susceptibility (C.W., R.E., and R.H., TU München, unpublished results). Arabidopsis BI-1 further interacts *in planta* with the monooxygenase CYP83A1, and *cyp83a1* mutants show LOS to *Erysiphe cruciferarum* (C.W., R.E., and R.H., TU München, unpublished results). BI-1 further attenuates ETI of barley and Arabidopsis (Eichmann *et al.* 2006, Kawai-Yamada *et al.* 2009), and mammalian BI-1 is a direct effector target of pathogenic *Escherichia coli* for blocking apoptosis (Hemrajani *et al.*, 2010). BI-1 proteins might thus be conserved proteins involved in disease susceptibility or control of innate immunity. Stable silencing of *BI-1* in barley was not obviously costly for the plant when unchallenged. However, in Arabidopsis, loss of BI-1 leads to enhanced sensitivity to fungal toxins and abiotic stress (Ishikawa *et al.*, 2011). Vice versa, over-expression of green fluorescent protein-tagged barley BI-1 limits susceptibility to *Fusarium graminearum* (Babaeizad *et al.*, 2009).

Another example of a susceptibility gene is Arabidopsis *MYB3R4*. MYB3R4 is involved in DNA endoreduplication, which is locally activated in parts of the leaf successfully colonized

196 by PM (Chandran *et al.*, 2009). PM can infect *MYB3R4* loss of function mutants, but disease
197 development is attenuated. It was suggested that functional feeding sites of biotrophs require
198 DNA endoreduplication and metabolic reprogramming for establishment of hypertrophy and
199 nutrient sinks (Wildermuth, 2010). This may also involve alcoholic fermentation, which is
200 transcriptionally activated at such feeding sites and involved in susceptibility to PM
201 (Wildermuth, 2010; Pathuri *et al.*, 2011). Loss of MYB3R4 results in mild developmental
202 defects, whereas simultaneous loss of related MYB3R4 and MYB3R1 results in severe
203 developmental failure (Haga *et al.*, 2007; Haga *et al.*, 2011). Hence, trade-off of LOS might
204 be less severe when functional redundancy buffers pleiotropic effects.
205 Recently, it was described that the Arabidopsis phytochrome-associated protein phosphatase
206 type C (PAPP2C) negatively regulates basal resistance to PM (Wang *et al.*, 2012). PAPP2C
207 was identified in a yeast two hybrid screening using the atypical PM R protein RPW8.2 as
208 bait. PAPP2C and RPW8.2 also interact *in planta* and both control salicylic acid-dependent
209 defence with opposing outcomes. Silencing PAPP2C leads to spontaneous cell death and
210 strongly limits PM. Data suggest that PAPP2C is a susceptibility factor and that RPW8.2 and
211 PAPP2C act antagonistically.
212

213 ## Evolution of susceptibility genes: disposition for disease?

214 In human immunology, it is quite well accepted that there is a genetic disposition for
215 infectious diseases, which only partially derives from immunodeficiency (e.g. Azad *et al.*,
216 2012). In plants however, there is little evidence for natural polymorphisms of susceptibility
217 (*S*) genes, whereas diversity of race-specific *R* genes is increasingly well understood (Ellis *et*
218 *al.*, 2000). In this context, it is important to mention that dominant *S*-genes have been shown
219 to operate in plant responses to host-specific toxins secreted by cell death-inducing fungi
220 (Wolpert *et al.*, 2002; Lorang *et al.*, 2004; Stukenbrock *et al.*, 2009). For *S*-genes to biotrophs

this is not yet established, but one might expect future classification of race-specific *S*-genes and non-race specific *S*-genes. The first might code for effector targets, and LOS thus may not affect all fungal genotypes. The latter would code for more general regulators of immunity and factors supporting the pathogen (compare Fig. 1).

The race-nonspecifically acting *mlo11* allele is an example of a naturally occurring LOS phenomenon that has been domesticated by farmers in Africa (Jørgensen, 1992; Piffanelli *et al.*, 2004). Another example of naturally occurring variation of susceptibility derives from polymorphisms at the Arabidopsis *ACD6* locus, which greatly determines susceptibility to downy and powdery mildew (Todesco *et al.*, 2010). Otherwise, little is known about natural diversity of putative *S*-genes to PM.

A host susceptibility factor may put the plant under strong pathogen pressure. Hence, selection should eliminate susceptibility alleles. Since this is apparently not the case, *S*-genes should have important functions apart from being involved in pathogenesis. In turn, pleiotropy often accompanies loss of *S*-gene function. From the pathogen´s point of view, it might be advantageous to target host susceptibility factors, which are fundamental to host function and therefore evolve slowly. It would be interesting to learn more about allelic variation at susceptibility loci because our knowledge on susceptibility is extremely limited and largely builds on Arabidopsis null mutants and gene silencing in barley. One may further speculate that conserved susceptibility factors, which are effector targets, might be perfect guardees for R-proteins (Fig. 1g) that indirectly recognize effector functions via host protein guarding (van der Biezen & Jones, 1998). The interaction of PAPP2C with RPW8.2 possibly reflects such a mechanism. Understanding host susceptibility might thus also pave the way for better understanding of ETI. It is also possible that conserved *S*-genes become subject of gene duplications to build an evolutionary playground and to allow for the development of molecular decoys that mimic effector targets (van der Hoorn & Kamoun, 2008).

246 The question arises whether targets of pathogen effectors generally are products of *S*-genes.
247 This is clearly not the case. On the contrary, ETS involves suppression of host immunity
248 often via direct inhibition of components of PTI or ETI. Consequently, loss of immunity-
249 related targets of effectors usually results in a gain of susceptibility rather than in LOS.
250 However, negative regulators of host immune responses often are susceptibility factors and
251 represent potential effector targets. Conservation of immune-modulators must be important
252 for the plant in environments where it faces challenge by more than one stress, e.g. biotic and
253 abiotic stress or biotrophs and necrotrophs, which the plant cannot defend at the same time in
254 the same tissue. MLO for example is considered a modulator of host defence responses
255 (Wolter *et al.*, 1993; Büschges *et al.*, 1997), and PM-resistant *mlo* mutants are super-
256 susceptible to cell death inducing pathogens and toxins (Jarosch *et al.*, 1999; Kumar *et al.*,
257 2001; Consonni *et al.*, 2006). Therefore, polymorphism of host *S*-genes might be under
258 influence from geographic factors and local disease pressure.
259

260 ## Costs of resistance and potential of application

261 LOS is often accompanied by cost of resistance in form of pleiotropy. This is a major hurdle
262 for application of LOS in terms of classical mutation breeding. However, nowadays
263 TILLING may allow for finding alleles of *S*-genes that show partial loss of function and
264 cause mild pleiotropy, when compared to full knock out alleles. Similarly, natural diversity of
265 *S*-genes can be addressed by candidate sequencing associated with phenotyping disease
266 resistance and pleiotropy, such that genomic resources become accessible for targeted
267 inbreeding and stacking of weak *S*-alleles. One can speculate that some quantitative trait loci
268 for PM resistance built on weak *S*-alleles.
269 Another strategy for application of LOS might come from better understanding of
270 susceptibility at the mechanistic level. This might be done via genetic suppressor screens (e.g.

Freialdenhoven *et al.* 1996; Collins *et al.* 2003) or analysis of the protein interaction environment, in which susceptibility factors operate (e.g. Kim *et al.*, 2002; Hoefle *et al.*, 2011; Huesmann *et al.*, 2012). Such approaches might identify further susceptibility factors but also proteins that antagonise susceptibility factors and open new potentials for support of basal resistance.

Transgenic suppression of *S*-genes by targeted knock down is successfully applied in research (e.g. Eichmann *et al.*, 2010; Hoefle *et al.*, 2011; Wang *et al.*, 2012). However, strong silencing of *S*-genes may be accompanied by similar pleiotropy as full knock out. Here, promising approaches for application of LOS rely on partial silencing or on silencing on demand driven by PAMP-activated or tissue-specific promoters. Similar applications are plausible with expression of dominant negative alleles of *S*-genes that might derive from artificial evolution approaches.

Conclusion

Susceptibility to biotrophs seems to be a double-edged sword. A susceptible plant will suffer from disease but it likely survives because the biotroph may not eradicate its host. If a plant genotype lost susceptibility, it may suffer from pleiotropic effects and may be out-competed by susceptible neighbours at least in environments without extreme disease pressure. However, between extremely susceptible genotypes and fully resistant LOS mutants, there is a lot of space for future studies on how susceptibility is actually established and how much the host contributes to it. Our current difficulties to apply LOS in plant protection are due to our incomplete knowledge on the mechanistic principles of susceptibility and on the natural diversity of *S*-genes. Hence, future research on host susceptibility may further open our eyes for intimate interconnection of host and pathogen functions and pave the way for trapping obligate biotrophs on their evolutionary one-way track.

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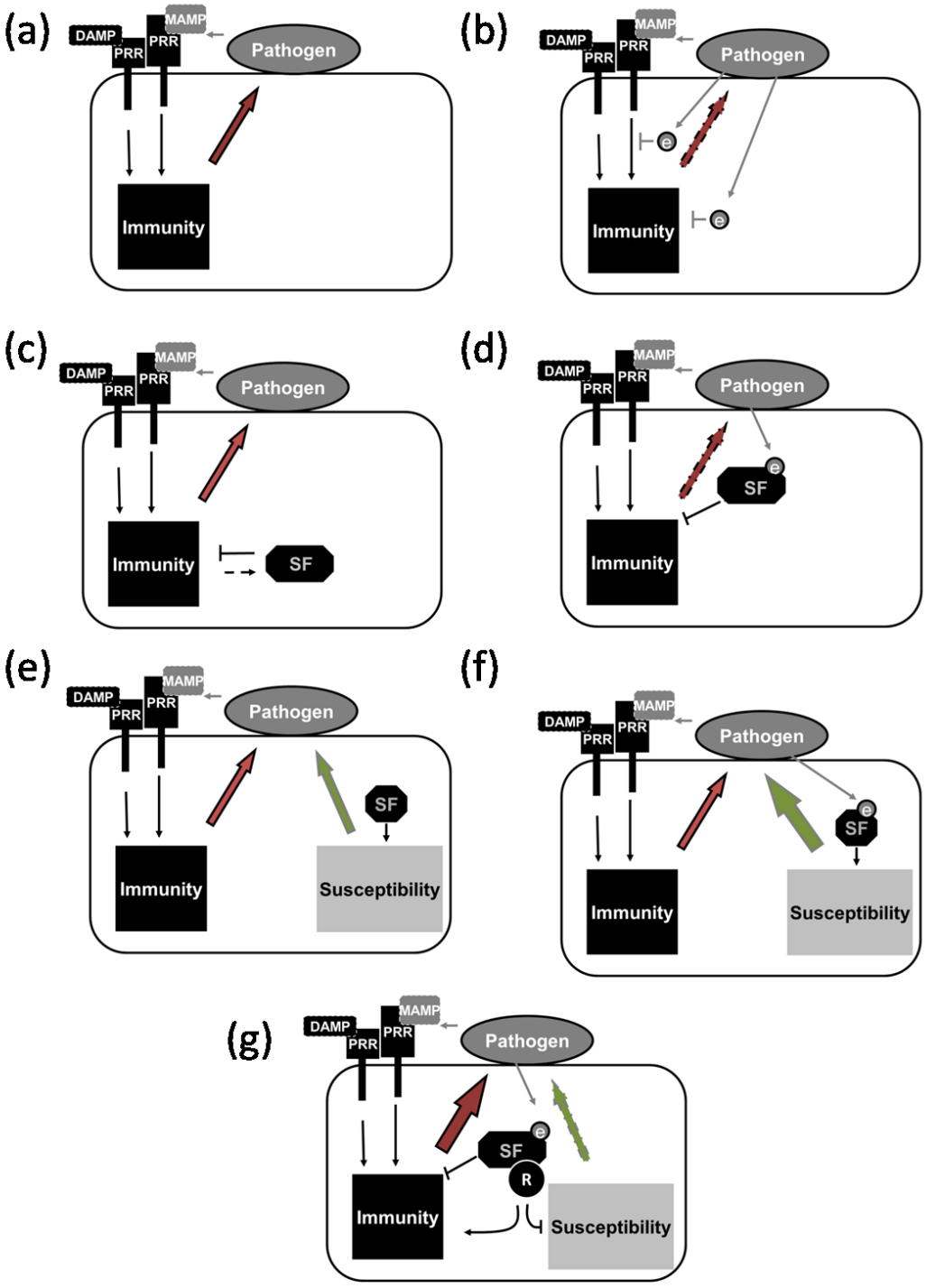

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484 **Figure 1** Hypothetical functions of susceptibility factors to biotrophs in the context of plant
485 immunity. (a) Basal resistance to biotrophs involves PAMP/MAMP and DAMP-triggered
486 immunity, which is initiated on ligand binding to pattern recognition receptors (PRRs).
487 Transcriptional and metabolic re-programming of the host then leads to defence responses
488 (red arrow) against the extracellular pathogen. (b) The pathogen secretes effectors, e, to

suppress basal defence responses by interfering i.a. with signal transduction or defence responses. (c) Host immunity is under constitutive or induced negative control. The endogenous host factors operating in negative control of defence contribute to disease susceptibility and are therefore considered susceptibility factors (SF). (d) A host SF that acts in negative control of immunity is manipulated by a pathogen effector, which therefore can suppress immunity. (e) The host provides immunity-unrelated SFs, which serve demands of the biotrophic pathogen. (f) An immunity-unrelated SF is addressed by a pathogen effector to foster susceptibility. (g) Any type of SF may be guarded by resistance proteins (R) for triggering immunity (ETI) in response to effector action on the SF.

500 **Table 1. Recent examples of susceptibility factors to PM.**

Susceptibility factor	Protein features	Potential function in susceptibility	Pleiotropy , trade-off	Reference
Barley				
HvBI-1 (BAX inhibitor-1)	ER-resident membrane protein	Suppression of penetration resistance and cell death	Potentially enhanced susceptibility to necrotrophs	Babaeizad *et al.* 2009; Eichmann *et al.* 2010
HvRACB	ROP GTPase	Support of haustorium accommodation and regulation of polarity	Developmental defects	Schultheiss *et al.* 2002; Hoefle *et al.* 2011
HvADH1	Alcohol dehydrogenase	Carbohydrate metabolism/fermentation	Potentially enhanced susceptibility to abiotic stress	Pathuri *et al.* 2011
HvBLN1 (blufensin1)	Secreted small peptide	Negative regulation of penetration resistance	Not analysed	Meng *et al.* 2009
HvSLN (Slender)	DELLA-type transcriptional repressor of gibberellic acid responses	Cell death regulation	Developmental defects	Saville *et al.* 2012
HvCRK1	DUF26 domain cysteine-rich receptor-like kinase	Defence regulation downstream of MLO	Not analysed	Rayapuram *et al.* 2012
Arabidopsis				
AtATG2 (autophagy-related 2)	Autophagosome biogenesis	Regulation of autophagy and SA-dependent defence	Early senescence	Wang *et al.* 2011
AtMYB3R4	Transcription factor	Regulation of DNA endoreduplication/ hypertrophy	Mild developmental defects	Chandra *et al.* 2009
AtFERONIA	Malectin-receptor-like kinase	Control of host cell entry	Developmental defects	Kessler *et al.* 2010
AtPAPP2C (phytochrome-activated protein phosphatse 2C)	Protein phosphatase	Negative regulation of SA-dependent defence and RPW8.2	Developmental defects	Wang *et al.* 2012

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