1 # Costs and Benefits of Transgenerational Acquired

2 # Resistance in Arabidopsis.

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4 Ana López Sánchez[1,2]*, David Pascual Pardo[1], Leonardo Furci[1], Michael R. Roberts[3], and

5 Jurriaan Ton[1]*

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7 [1]Plant Production and Protection Centre, Department of Animal and Plant Sciences, The

8 University of Sheffield, UK. [2]Present Address: Genética Molecular de Plantas, Centro Nacional

9 de Biotecnología, Spain. [3]Lancaster Environment Centre, Lancaster University, UK.

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11 *Corresponding authors: Jurriaan Ton: Department of Animal and Plant Sciences, Alfred

12 Denny Building, The University of Sheffield, Western Bank, Sheffield, S10 2TN. Ana López

13 Sánchez: Genética Molecular de Plantas, Centro Nacional de Biotecnología, C/ Darwin 3,

14 Campus de Cantoblanco, 28049 Madrid, Spain.

15

16 Email addresses: ALS: ana.lopez@cnb.csic.es; DPP: d.p.pardo@sheffield.ac.uk; LF:

17 leonardo.furci@oist.jp; MRR: m.r.roberts@lancaster.ac.uk; JT: j.ton@sheffield.ac.uk.

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25 **Abstract**

26 Recent evidence suggests that stressed plants employ epigenetic mechanisms to transmit
27 acquired resistance to their progeny. However, little is known about the evolutionary and
28 ecological significance of this transgenerational acquired resistance (TAR). In this study, we
29 have used a full factorial design to study the specificity, costs and stability of TAR following
30 exposure of *Arabidopsis thaliana* to increasing stress intensities by a biotrophic pathogen, a
31 necrotrophic pathogen, and soil salinity. All stresses incrementally reduced parental growth,
32 while salt stress additionally impacted reproductive success. Biotrophic and necrotrophic
33 pathogens, but not salt, increased resistance of progeny against the stress experienced by their
34 parents (*i.e*., in matched environments). In mis-matched environments, however, pathogen-
35 elicited TAR was associated with costs from increased susceptibility to other stresses.
36 Furthermore, the stability of pathogen-elicited TAR over one stress-free generation and its
37 associated costs were proportional to parental disease severity, suggesting that plants use stress
38 intensity as an environmental proxy to adjust TAR investment. We conclude that pathogen-
39 elicited TAR is an adaptive and deterministic parental effect that is associated with ecological
40 costs. Accordingly, our study provides evolutionary and ecological context to the epigenetic
41 TAR response.

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43 **Key words**: Adaptive parental effects; Arabidopsis; Phenotypic plasticity; Plant stress;
44 Transgenerational acquired resistance.

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46 **Introduction**

47 Phenotypic plasticity allows organisms to modify their biochemical, physiological or
48 morphological traits to survive in changing environments (Schlichting, 1986). While
49 phenotypic plasticity has mostly been studied within the lifespan of organisms, there is
50 increasing evidence that life history experiences of individuals can influence traits in their
51 progeny. These include simple direct maternal effects, such as nutrient provisioning to progeny.
52 However, some transgenerational effects can persist over multiple generations and involve

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53   heritable epigenetic changes (Lämke & Bäurle, 2017; Bošković & Rando, 2018). These

54   epigenetic responses have the potential to provide adaptive benefits to progeny, thereby

55   enhancing evolutionary fitness of the parents. When facing environmental changes, organisms

56   can adopt various transgenerational strategies to optimise their fitness. When environments

57   change frequently and are unpredictable, parents may adopt a bet-hedging strategy to increase

58   the variability within their progeny (Crean & Marshall, 2009). By contrast, when environments

59   undergo directional and stable changes, which present a more predictable cue about future

60   environmental conditions, parents could enhance reproductive fitness by transmitting specific

61   adaptive traits to their progeny (Marshall & Uller, 2007; Proulx & Teotonio, 2017).
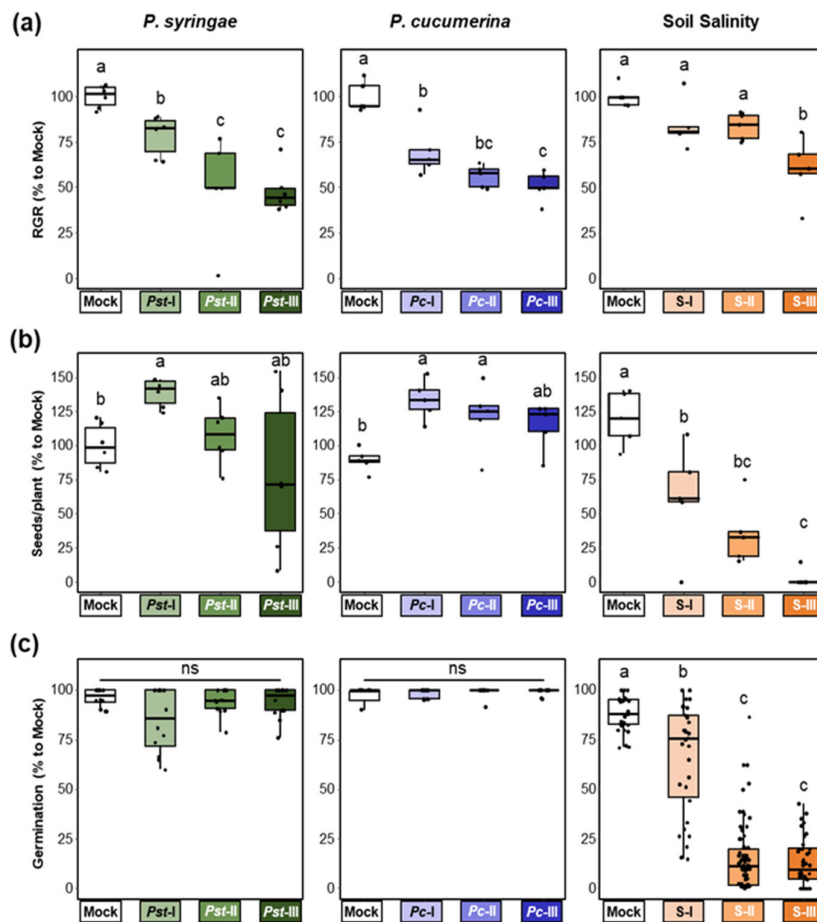
62   Transgenerational responses to stress have been reported in both plants and animals;

63   ranging from maladaptive pathological effects of environmental pollutants, to adaptive

64   immunological traits that increase resistance against pests and diseases (Holeski *et al.*, 2012;

65   Luna *et al.*, 2012; Rasmann *et al.*, 2012; Perez & Lehner, 2019; Tetreau *et al.*, 2019). In plants,

66   transgenerational induced resistance to disease is often referred to as 'transgenerational

67   acquired resistance' (TAR), which is typically based on a sensitisation, or 'priming', of the

68   immune system, mediating a faster and/or stronger immune response (Wilkinson *et al.*, 2019).

69   We have previously demonstrated that bacterial speck disease, caused by the hemi-biotrophic

70   pathogen *Pseudomonas syringae* pv. *tomato* (*Pst*), elicits TAR that can be maintained over two

71   stress-free generations in the self-fertilising annual plant Arabidopsis (*Arabidopsis thaliana*)

72   (Luna *et al.*, 2012; Stassen *et al.*, 2018). Although the exact epigenetic mechanisms

73   underpinning TAR are still under investigation, the induction and/or transmission of the

74   resistance requires DNA demethylation at transposable elements, and is associated with

75   genome-wide changes in DNA methylation (Luna & Ton, 2012; Lopez Sanchez *et al.*, 2016;

76   Stassen *et al.*, 2018; Furci *et al.*, 2019). These results are supported by numerous other studies

77   reporting transgenerational changes in DNA methylation in response to environmental stress

78   (Lämke & Bäurle, 2017; Wilkinson *et al.*, 2019).

79   Despite the fact that epigenetic modifications are attracting much attention as a

80   mechanism for transgenerational phenotypic plasticity, there is still controversy over whether

81   such responses are adaptive (Herman & Sultan, 2011; Uller *et al.*, 2013; Burggren, 2015; Crisp

82   *et al.*, 2016). Transgenerational phenotypic plasticity to abiotic conditions, such as light and

83   water availability, have been shown to provide improved fitness when the environments of
84   parents and progeny are matched (Galloway & Etterson, 2007; Herman *et al.*, 2012). However,
85   when parent and progeny environments are mismatched, transgenerational effects can be
86   maladaptive, which may explain why many epigenetic modifications are erased during sexual
87   reproduction (Iwasaki & Paszkowski, 2014; Crisp *et al.*, 2016; Gehring, 2019). For TAR to be
88   genuinely adaptive, theory provides three key predictions. First, TAR should be elicited by
89   specific stresses that the parents can recover from and that generate corresponding specific
90   phenotypic changes in their progeny. Secondly, since TAR is an inducible response, there
91   should be associated costs that may only become apparent in mismatched environments.
92   Finally, parents should have the ability to distinguish strong, reliable cues with high predictive
93   value about future environments. There is limited evidence to support some of these
94   predictions. For instance, while progeny from *P. syringae*-infected Arabidopsis exhibit TAR
95   against another biotrophic pathogen, *Hyaloperonospora arabidopsidis* (*Hpa*), this same
96   progeny showed enhanced susceptibility to the necrotrophic fungus *Alternaria brassicicola*
97   (Luna *et al.*, 2012). Similarly, progeny from spider mite-infested Arabidopsis were primed for
98   defence against both spider mites and aphids but suffered increased susceptibility to *P. syringae*
99   (Singh *et al.*, 2017). However, none of these studies employed reciprocal designs to
100   systematically test predictions relating to specificity and costs within a single experimental
101   framework. Moreover, it remains unknown whether plants can perceive stress intensity as an
102   environmental proxy to estimate the likelihood that the same stress is still present in the
103   progeny environment and adjust the strength and/or durability of TAR accordingly.

104   Here, we have addressed the above hypotheses by characterising TAR responses of
105   Arabidopsis to different types and intensities of biotic and abiotic stress. Using a full factorial
106   reciprocal experimental design, we have examined the specificity of TAR by quantifying the
107   impacts of three parental stresses in both matched and mismatched progeny environments. We
108   show that TAR elicited by biotrophic and necrotrophic pathogens is not associated with
109   reductions in reproductive fitness and provides resistance benefits to progeny in matched
110   environments. However, these specific TAR responses were associated with ecological costs
111   that become apparent in mismatched environments from enhanced susceptibility to other
112   stresses. By contrast, abiotic stress by soil salinity failed to elicit TAR against salt but elicited

113   non-specific resistance in mismatched environments against biotrophic and necrotrophic

114   pathogens, which was offset by major reproductive costs from dramatically reduced seed

115   production and viability. Finally, we demonstrate that the transgenerational stability and costs

116   of pathogen-elicited TAR are proportional to the disease intensity experienced by the parents

117   and discuss these results in context of current evolutionary theory about transgenerational

118   phenotypic plasticity.

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Results

122   **Dose-dependent effects of biotic and abiotic stresses on plant fitness parameters.**

123   To test our central hypotheses, we produced populations of Arabidopsis progeny that in the

124   parental generation had been exposed to three different stresses: the (hemi)biotrohic pathogen

125   *Pseudomonas syringae* pv. *tomato* DC3000 (*Pst*), the necrotrophic pathogen *Plectosphaerella*

126   *cucumerina* (*Pc*), and salt stress (Fig. 1). All parents came from a single common ancestor to

127   minimise (epi)genetic variation. For each stress type, we applied four severity levels (mock

128   plus three increasing levels of the stress) and estimated their effects on different fitness

129   parameters (Fig. 2).

Fig. 1. Full factorial experimental design of the study. *Arabidopsis thaliana* plants (accession Col-0) from a common ancestor were exposed to increasing stress intensities (Mock, Low, Medium and High) by the (hemi)biotrophic bacterial pathogen *Pseudomonas syringae* pv. *tomato* (green), the necrotrophic fungal pathogen *Plectosphaerella cucumerina* (blue), or soil salinity (NaCl; orange). Plants in this parental generation (P) were evaluated for impacts on fitness parameters. Four plants per stress level were selected to generate F1 populations, which were analysed for transgenerational changes in resistance against all 3 stresses, in order to determine the specificity of transgenerational acquired resistance (TAR), potential costs arising from increased susceptibility, and dose-dependency of TAR intensity on parental stress. Four individual plants from 3 independent F1 populations were randomly selected to set seed in the absence of stress. The resulting F2 populations were analysed for resistance against the parental stress to examine dose-dependent effects on TAR stability. Circles indicate individual plants; small (thin-lined) boxes indicate F1/F2 populations derived from a common ancestor in the previous generation; big (bold-lined) boxes indicate pooled F1/F2 populations from a common ancestor 2 generations earlier.

130   All stresses induced a dose-dependent decline in relative growth rate (RGR), confirming that

131   the plants perceived and responded to the stresses in a dose-dependent manner (Fig. 2a). By

132   contrast, seed production showed a different response to the stresses. The lowest levels of

133   disease by *Pst* and *Pc* stimulated seed production, whereas the highest stress levels by these

134   diseases had no statistically significant effect on seed production (Fig. 2b). This suggests that

135   Arabidopsis can adapt to both diseases by compensating the reduced growth during pathogen

136   exposure with increased seed production at the end of its life cycle. Conversely, increasing

137   levels of soil salinity caused a dose-dependent reduction in seed production (Fig. 2b), indicating

138   that Arabidopsis does not recover as efficiently from this stress as it does from disease by *Pst*

139   or *Pc*. Similar patterns were observed for seed viability, where *Pst* and *Pc* failed to have an

140   effect (Fig. 2c and Fig. S1a,b), whereas soil salinity caused a dramatic dose-dependent decline

141   in seed viability (Fig. 2c and Fig. S1c), which was absent in F2 seeds after one stress-free F1

142   generation (Fig. S1d).

Fig. 2. Differential impacts of three (a)biotic stresses on parental fitness parameters. Plants in the parental generation (4.5-weeks-old) were exposed to varying stress intensities by *P. syringae* pv *tomato* (Mock, Pst-I, Pst-II, Pst-III), *P. cucumerina* (Mock, Pc-I, Pc-II, Pc-III) or soil salinity (Mock, S-I, S-II, S-III) over a 3-week period before transferring to long-day conditions to trigger flowering and set seed. Boxplots show the interquartile range (IQR; box) ± 1.5xIQR (whiskers), including median (horizonal line) and replication units (single dots). **(a)** Impacts on relative growth rate (RGR) during the period of stress exposure. Data represent RGR values of single plants (n= 5-6) normalised to the average RGR of Mock-treated plants (100%). Different letters indicate statistically significant differences (ANOVA + Tukey post-hoc test, α=0.05). **(b)** Impacts on seed production. Data represent seed numbers per plant (n= 5-6) normalised to average value of Mock-treated plants (100%). Different letters indicate statistically significant differences (*Pst*: Welch ANOVA + Games-Howell posthoc test, α=0.05; *Pc* and salt: ANOVA + Tukey post-hoc test, α=0.05). **(c)** Impacts on seed viability. Seed viability was determined 5 days after planting of surface-sterilised and stratified seeds onto 0.2x Murashige and Skoog (MS) agar plates. Data represent mean germination percentages per plate (25 seeds/plate) of seed batches from 4 similarly treated parents (n=15-60). Different letters indicate statistically significant differences (Welch ANOVA + Games-Howell post-hoc test; α=0.05). Viability data for seed batches from individual plants are presented in Fig. S1a-c.

143 **Parental stress leads to beneficial or neutral impacts on resistance of progeny in matched**

144 **environments.**

145 Next, we investigated TAR in F1 progeny against the same stress to which the parents had been

146 exposed (matched environments). Parents exposed to disease by biotrophic *Pst* produced F1

147 progeny that were more resistant to both *Pst* (Fig. **3a** and Fig. **S2a**), and the biotrophic

148 Oomycete *Hyaloperonospora arabidopsidis* (*Hpa*; Fig. **3b** and Fig. **S2b**). These findings

149 support our previous results (Luna *et al.*, 2012) and demonstrate that *Pst*-elicited TAR is not

150 specific at the level of pathogen species, but that it protects against taxonomically unrelated

151 pathogens with similar biotrophic lifestyles.

Fig. 3. Intensity and transgenerational stability of *Pseudomonas syringae* pv. *tomato*-elicited TAR in matched environments. Parental plants had been exposed to different disease severities by the biotrophic bacterium *Pseudomonas syringae* pv. *tomato* (*Pst*; Mock, *Pst*-I, *Pst*-II, *Pst*-III). F1 and F2 plants were analysed for resistance against the same pathogen (*Pst*) and/or the biotrophic Oomycete *Hyaloperonospora arabidopsidis* (*Hpa*). (a) TAR against *Pst* in F1 progeny at 3 days post inoculation (dpi). Boxplots show the interquartile range (IQR; box) ± 1.5xIQR (whiskers), including median (horizonal line) and replication units (dots). Data represent ^{10}Log-transformed bacterial titres (Log cfu cm^{-2}) in leaves of single plants within F1 populations from similarly treated parents (n=42). Different letters indicate statistically significant differences (Welch ANOVA + Games-Howell test, α=0.05). Data for individual F1 populations are shown in Fig. **S2a**. (b) TAR against *Hpa* in F1 progeny. *Hpa* colonisation was quantified at 6 dpi by assigning trypan-blue stained leaves to 4 *Hpa* resistance classes (I: healthy; II: hyphal colonisation only; III hyphal colonization with conidiospores; IV hyphal colonisation with conidiospores and oospores). Stacked bars show leaf frequency distributions within F1 populations from similarly treated parental plants (n=600-1000). Different letters indicate statistically significant differences (Pairwise Fisher's exact tests + Bonferroni FDR, α=0.05). Data for individual F1 populations are shown in Fig. **S2b**. (c) TAR against *Hpa* in F2 progeny at 6 dpi after one stress-free F1 generation. Stacked bars show leaf frequency distributions across *Hpa* resistance classes within F2 populations that share a common parental ancestor (n=300-350). Different letters indicate statistically significant differences (Pairwise Fisher's exact tests + Bonferroni FDR; α=0.05). Data for individual F2 populations are shown in Fig. **S2c**.

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153 To determine whether Arabidopsis develops TAR in response to necrotrophic pathogens, we

154 tested F1 progeny from parents exposed to increasing disease by the necrotrophic fungus *Pc*

155 for resistance against the same pathogen. Compared to progeny from mock-inoculated parents,

156 all but the lowest severity of parental disease resulted in a statistically significant suppression

157 of *Pc* lesion development in F1 progeny (Fig. **4a** and Fig. **S3a**).

Fig. 4. Intensity and transgenerational stability of *Plectosphaerella cucumerina*-elicited TAR in matched environments.
Parental plants had been exposed to different disease severities by necrotrophic *Plectosphaerella cucumerina* (*Pc*; Mock, *Pc*-I, *Pc*-II, *Pc*-III). F1 and F2 plants were analysed for resistance against the same pathogen. Lesion diameters were determined in 4 leaves/plant at 15 days post inoculation (dpi) and the average lesion diameter per plant was used as statistical unit of replication. Boxplots show the interquartile range (IQR; box) ± 1.5xIQR (whiskers), including median (horizonal line) and replication units (dots). **(a)** TAR against *Pc* in F1 progeny. Data represent lesion diameters (mm) of plants within F1 populations from similarly treated parents (n=40). Different letters indicate statistically significant differences (Welch ANOVA + Games-Howell test, α=0.05). Data for individual F1 populations are shown in Fig. **S3a**. **(b)** TAR against *Pc* in F2 progeny after a stress-free F1 generation. Data represent lesion diameters of plants within F2 populations that share a common parental ancestor (n=20). Different letters indicate statistically significant differences (ANOVA + Tukey post-hoc test; α=0.05). Data for individual F2 populations are shown in Fig. **S3b**.

158

159　　Finally, we investigated the transgenerational effects of soil salinity in matched environments.

160　　To this end, F1 progeny from parents exposed to increasing NaCl concentrations in the soil

161　　were analysed for root growth inhibition on agar medium supplemented with 50 mM and 100

162　　mM NaCl, which is a common method to quantify salt tolerance in Arabidopsis (Verslues *et*

163　　*al.*, 2006; Claeys *et al.*, 2014). F1 populations from differently treated parents showed small

164　　but statistically significant differences in root growth on agar medium containing 0 mM and

165　　50 mM NaCl (Fig. **S4a**), which were absent in the F2 generation (Fig. **S4b**). However, these

166　　differences in root growth appeared non-adaptive, since the degree of NaCl-induced root

167　　growth inhibition compared to roots on control plates (0 mM NaCl) was similar between

168　　populations from all parental treatments (Fig. **5a**,**b** and Fig. **S4c,d**). Thus, under our conditions,

169　　progeny from salt-stressed plants did not express TAR in matched environments.

Fig. 5. Lack of salt-elicited TAR in matched environments. Parental plants had been exposed to different stress intensities by soil salinity (NaCl; Mock, S-I, S-II, S-III). Salt tolerance of F1 and F2 plants was quantified by root growth reduction (%) over a 5-day period on NaCl-containing agar medium relative to the average root growth on agar medium without NaCl. Boxplots show the interquartile range (IQR; box) ± 1.5xIQR (whiskers), including median (horizonal line) and replication units (dots). (a) Unaltered tolerance of F1 plants to 50 and 100 mM NaCl. Data represent growth reduction percentages of single plants within F1 populations from similarly treated parents (n=60). ns: no statistically significant differences (ANOVA; α=0.05). Root growth data for individual F1 populations are shown in Fig. **S4a**; root tolerance data for individual F1 populations are shown in Fig. **S4c**. (b) Unaltered tolerance of F2 plants to 50 and 100 mM NaCl after one stress-free F1 generation. Data represent growth reduction percentages of single plants within F2 populations that share a common parental ancestor (n=18-20). ns: no statistically significant differences (ANOVA; α=0.05). Root growth data for individual F2 populations are shown in Fig. **S4b**; root tolerance data for individual F2 populations are shown in Fig. **S4d**.

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TAR is associated with costs that become apparent in mismatched environments.

To investigate the resistance phenotypes of our F1 populations against stresses other than the parental stress (mismatched environments), we used a reciprocal experimental design based on the three parental stress treatments (Fig. **1**). We have previously reported that *Pst*-elicited TAR is associated with increased susceptibility to the necrotrophic fungus *A. brassicicola* (Luna *et al.*, 2012). In agreement with this finding, F1 progeny from *Pst*-infected plants developed larger lesions after inoculation with the necrotrophic *Pc* (Fig. **6a** and Fig. **S5a**). Furthermore, F1 progeny from *Pst*-exposed parents showed a statistically significant increase in root growth inhibition by 50 mM NaCl (Fig. **6b** and Fig. **S5b,c**), indicating increased sensitivity to salt stress. Next, we investigated F1 progeny from *Pc*-infected parents for resistance against biotrophic *Hpa* and salt stress. F1 populations from parents exposed to the two highest severities of *Pc* disease showed increased susceptibility to *Hpa* (Fig. **6c** and Fig. **S6a**) but were

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183   unaffected in salt tolerance (Fig. **6d** and Fig. **S6b,c**). Together, these results indicate that the
184   potential benefits of pathogen-induced TAR are traded off against costs of increased
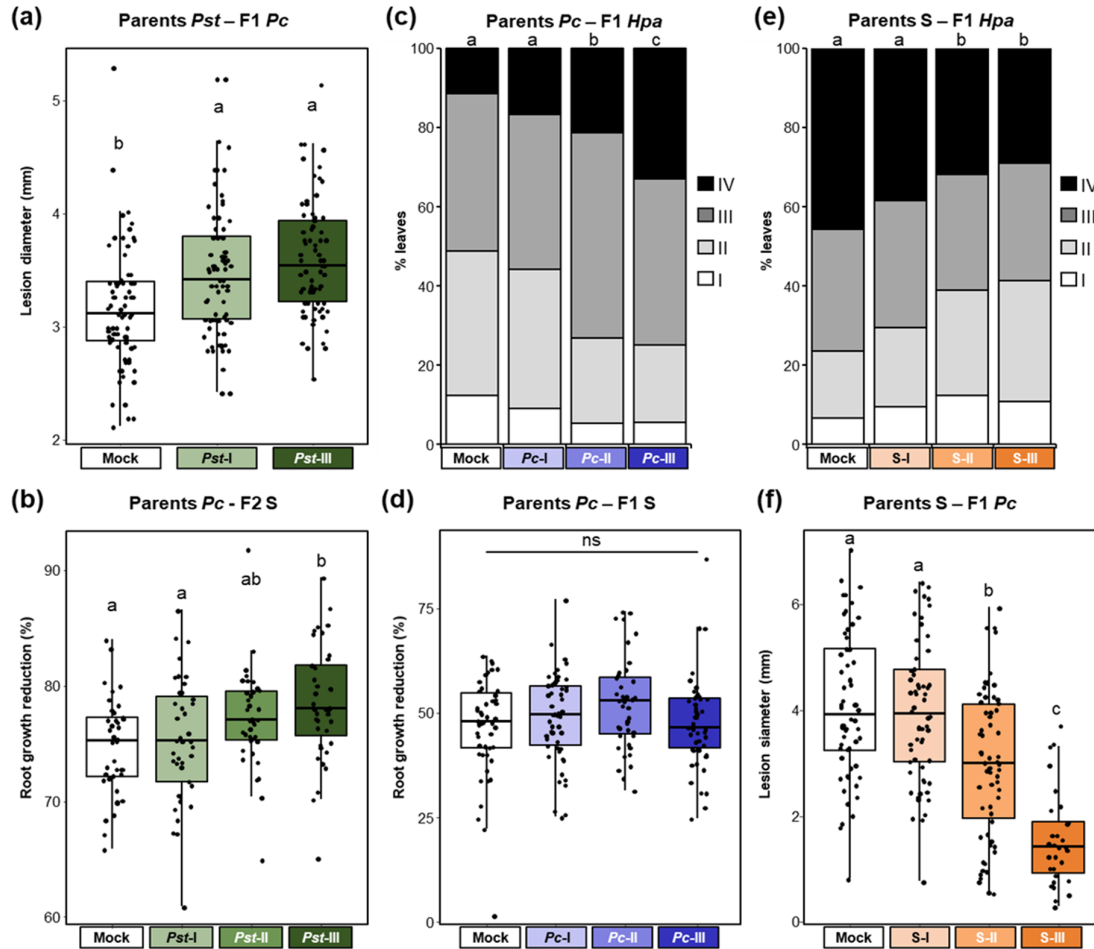185   susceptibility to other stresses that become apparent in mismatched environments.

Fig. 6. Costs and benefits of TAR in mismatched environments. Parental plants had been exposed to different stress severities by *Pst* (green), *Pc* (blue) or soil salinity (orange). F1 plants were tested for resistance against different stresses than the parental stress. **(a)** Increased *Pc* susceptibility in F1 progeny from *Pst*-exposed parents. Box plots show lesion diameters (mm) of plants within F1 populations from similarly treated parental plants (n=76-80). See legend of Fig. **4** for details. Different letters indicate statistically significant differences between parental treatments (ANOVA + Tukey post-hoc test; α=0.05). Data for individual F1 populations are shown in Fig. **S5a**. **(b)** Reduced salt tolerance in F1 progeny from *Pst*-exposed parents. Box plots show root growth reduction percentages by 50 mM NaCl of plants within F1 populations from similarly treated parental plants (n=40). See legend of Fig. **5** for details. Different letters indicate statistically significant differences (ANOVA + Tukey post-hoc test; α=0.05). Root growth data for individual F1 populations at 0, 50 and 100 mM NaCl are shown in Fig. **S5b**; tolerance data for individual F1 populations to 50 and 100 mM NaCl are shown in Fig. **S5c**. **(c)** Increased *Hpa* susceptibility in F1 progeny from *Pc*-exposed parents. Stacked bars show leaf frequency distributions across *Hpa* resistance classes within F1 populations from similarly treated parents (n=400-500). See legend of Fig. **3b** for details. Different letters indicate statistically significant differences (pairwise Fisher's exact tests + Bonferroni FDR, α=0.05). Data for individual F1 populations are shown in Fig. **S6a**. **(d)** Unaltered salt tolerance in F1 progeny from *Pc*-exposed parents. Box plots show root growth reduction percentages by 50 mM NaCl of plants within F1 populations from similarly treated parental plants (n=47). See legend of Fig. **5** for details. ns: no statistically significant differences (ANOVA; α=0.05). Root growth data for individual F1 populations at 0, 50 and 100 mM NaCl are shown in Fig. **S6b**; tolerance data for individual F1 populations to 50 and 100 mM NaCl are shown in Fig. **S6c**. **(e)** Non-specific TAR against *Hpa* in F1 progeny from NaCl-exposed parents. Stacked bars show leaf frequency distributions across *Hpa* resistance classes within F1 populations from similarly treated parents (n=350-800). See legend of Fig. **3b** for details. Different letters indicate statistically significant differences (Pairwise Fisher's exact tests + Bonferroni FDR, α=0.05). Data for individual F1 populations are shown in Fig. **S7a**. **(f)** Non-specific TAR against *Pc* in F1 progeny from NaCl-treated parents. Box plots show lesion diameters (mm) of plants within F1 populations from similarly treated parental plants (n=30-60). See legend of Fig. **4** for details. Different letters indicate statistically significant differences between parental treatments (ANOVA + Tukey post-hoc test; α=0.05). Data for individual F1 populations are shown in Fig. **S7b**.

186   Surprisingly, F1 progeny from parents exposed to the highest degrees of soil salinity showed
187   increased resistance to both biotrophic *Hpa* and necrotrophic *Pc* (Fig. **6e,f** and Fig. **S7a,b**).
188   This finding argues against the concept of TAR specificity, since the benefits, rather than the
189   costs, were apparent in mismatched environments. However, the evolutionary significance of
190   this non-specific TAR by salt stress must be considered against the lack of adaptive effects in
191   the matched environment (Fig. **5**) and the severe fitness costs arising from reduced plant
192   growth, seed production and seed viability (Fig. **2**).

193

194   **Stress intensity acts as a weighted indicator for TAR investment.**
195   Since TAR is associated with costs in mismatched environments, we considered the possibility
196   that plants can adjust TAR investment in accordance to the reliability of the environmental
197   stress signal. We hypothesised that severe stress is perceived as a more reliable predictor of the
198   progeny environment, resulting in stronger TAR investment. In matched environments, *Pst*-
199   and *Pc*-elicited TAR was strongest in F1 progeny from parents exposed to the highest stress
200   levels, although the difference in TAR intensity between the lowest and highest parental stress
201   intensities was not statistically significant (Fig. **3a,b**, Fig. **S2a,b**, Fig. **4a** and Fig. **S3a**).
202   Similarly, in mismatched environments, F1 progeny from parents exposed to the highest level
203   of *Pst* disease showed increased *Pc* susceptibility compared to progeny from parents exposed
204   to the lowest level of *Pst* disease; however, this difference was not statistically significant (Fig.
205   **6a** and Fig. **S5a**). By contrast, the increased sensitivity of F1 progeny from *Pst*-exposed parents
206   to salt showed statistically significant differences that were proportional to the levels of
207   parental disease severity (Fig. **6b** and Fig. **S5c**). Similarly, *Hpa* susceptibility in F1 progeny
208   from *Pc*-exposed parents (Fig. **6c** and Fig. **S6a**), as well as non-specific TAR in F1 progeny
209   from salt-exposed parents, showed statistically significant differences that were proportional
210   to the level of parental stress (Fig. **6e,f**, Fig. **S7**). Thus, although the intensity of pathogen-
211   elicited TAR in matched environments of F1 progeny does not show a statistically significant
212   dose effect, the response is associated with dose-dependent costs that become evident in
213   mismatched environments. Finally, we investigated whether the transgenerational stability of
214   TAR into the F2 generation is proportional to the level of parental stress in matched
215   environments. To this end, we determined pathogen-elicited TAR in F2 progeny after one

216 stress-free F1 generation. In contrast to F1 progeny (Fig. **3b** and Fig. **S2b**), F2 progeny from

217 parents exposed to the lowest levels of *Pst* disease no longer showed TAR against *Hpa* (Fig.

218 **3c** and Fig. **S2c**). Furthermore, only one F2 population from parents exposed to intermediate

219 levels of *Pst* disease had maintained a statistically significant TAR response, whereas all F2

220 populations from parents exposed to the highest levels of *Pst* disease had maintained a

221 statistically significant TAR response (Fig. **3c** and Fig. **S2c**). F2 populations from parents

222 exposed to low and intermediate levels of *Pc* disease all failed to show TAR (Fig. **4b** and Fig.

223 **S3b**). However, ANOVA of pooled F2 populations from similarly treated parental plants, as

224 well as nested ANOVA with F2 population as a random variable, revealed a statistically

225 significant effect of parental stress treatment (Fig. **S3b**), indicating a residual amount of TAR

226 in F2 populations from *Pc*-exposed parents. This is further supported by the observation that

227 F2 populations from parents exposed to the highest degree of *Pc* disease also showed the

228 highest level of *Pc* resistance (Fig. **4b** and Fig. **S3b**). Hence, TAR in response to relatively high

229 levels of disease by *Pst* or *Pc* can persist into the F2 generation, whereas TAR elicited by low

230 or intermediate disease levels is reverted or weakened after one stress-free F1 generation.

231 Together, these results demonstrate that the intensity, costs and/or transgenerational stability

232 of TAR have a dose-dependent relationship with parental stress intensity, which supports our

233 hypothesis that plants use stress intensity as a weighted indicator of TAR investment.

234

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Discussion

237 Evolutionary models predict that parental effects on specific traits act as an adaptive

238 mechanism to increase fitness in changeable environments (Leimar & McNamara, 2015;

239 Pigeault *et al.*, 2016; Proulx & Teotonio, 2017). However, despite numerous reports of

240 transgenerational effects of stress in plants, it has remained uncertain whether these responses

241 are adaptive (Uller *et al.*, 2013; Burggren, 2015; Crisp *et al.*, 2016). Although the importance

242 of full factorial designs to address this question has been stressed (Marshall & Uller, 2007;

243 Bonduriansky *et al.*, 2012; Uller *et al.*, 2013; Burgess & Marshall, 2014; Tetreau *et al.*, 2019),

244 most TAR studies in plants have focused on the underpinning mechanisms and overlooked the

245   evolutionary and ecological significance of the response. Our study employed a full factorial

246   reciprocal design with different (a)biotic stresses to address three predictions about the

247   evolutionary and ecological relevance of TAR: *i*) if TAR is adaptive, it will be specific to the

248   parental environment and in response to a stress that the parents can recover from, *ii*) TAR will

249   impose costs in mismatched environments, *iii*) parents will use stress severity to predict

250   progeny environments and adjust adaptive TAR investment accordingly.

251   Most previous reports about TAR are based on experiments in which resistance was

252   tested in matched environments and therefore do not address the specificity of the response.

253   The reciprocal design of our study (Fig. **1**) allowed us to examine progeny resistance

254   phenotypes in both matched and mismatched environments. In the case of disease stress, we

255   found strong evidence that TAR is specific. Disease by biotrophic *Pst* bacteria elicits TAR

256   against taxonomically unrelated *Hpa* with a similar biotrophic lifestyle (Fig. **3b,c**) but fails to

257   protect against necrotrophic *Pc* or abiotic salt stress (Fig. **6a,b,c**). Similarly, parental disease

258   by *Pc* elicited TAR against the same necrotrophic fungus (Fig. **4**) but not against biotrophic

259   *Hpa* and abiotic salt stress (Fig. **6c,d**). This specificity supports the notion that pathogen-

260   elicited TAR is an adaptive response. By contrast, we found no evidence for increased salt

261   tolerance in F1 or F2 progeny from salt-exposed parents (Fig. **5**), even though F1 progeny from

262   salt-exposed parents showed non-specific resistance against biotrophic and necrotrophic

263   pathogens (Fig. **6e**,**f**). Previous studies have shown that abiotic stress causes non-specific

264   transgenerational tolerance to other abiotic stresses (Boyko *et al.*, 2010; Rahavi *et al.*, 2011),

265   but not biotic stresses. The lack of specific TAR against salt stress (Fig. **4**), along with the

266   associated reduction in reproductive fitness (Fig. **2b,c**), suggests that salt-elicited TAR is non-

267   adaptive and unlikely provides a selective advantage in natural environment. The discrepancy

268   between salt- and pathogen-elicited TAR can be explained by differences in parental response

269   to these stresses (Fig. **2a,b,c**). While all three stresses caused an immediate reduction in plant

270   growth, *Pst* and *Pc* had no, or even stimulatory, effects on reproductive fitness (Fig. **2a,b,c**),

271   indicating that Arabidopsis uses induced resistance to mitigate *Pst* and *Pc* stress, compensating

272   potential fitness loss from reduced growth with increased seed production at the end of its life

273   cycle. This ability to recover from stress constitutes a reliable cue that TAR will improve fitness

274   of progeny in the same environment, thereby justifying TAR investment. By contrast, the

275   progressive loss of seed production and viability upon increasing levels of stress from soil

276   salinity (Fig. **2b**,**c**) indicates that the parental plants do not recover well from this stress.
277   Investment in a transgenerational response is therefore not beneficial, even in a matched
278   environment. This hypothesis is supported by modelling which shows that TAR in
279   invertebrates occurs at intermediate levels of disease stress, but not when there are more severe
280   impacts on mortality (Pigeault *et al.*, 2016).

281   Costs are central to the evolution of adaptive transgenerational responses. The fact that
282   pathogen-elicited TAR is inducible and reversible in the absence of stress implies that the
283   response is associated with costs (Fig. **3,4**) (Stassen *et al.*, 2018). Previous work has identified
284   transgenerational impacts of parental stress on vegetative and reproductive development (e.g.
285   Rahavi *et al.*, 2011; Suter & Widmer, 2013; Groot *et al.*, 2016), but it remains unclear how far
286   these changes influence fitness. While we did not observe consistent effects on plant growth or
287   seed set in F1 and F2 progeny from disease-exposed plants (data not shown), the reciprocal
288   design of our experiments strongly indicates ecological costs arising from increased
289   susceptibility to other stresses (Fig. **6a,b,c,d**). Antagonism between plant defence pathways
290   against biotrophic pathogens, necrotrophic pathogens and abiotic stress is well-documented
291   (Koornneef & Pieterse, 2008; Pieterse *et al.*, 2012), and transgenerational persistence of these
292   effects have been reported previously (Luna *et al.*, 2012; Singh *et al.*, 2017). Accordingly, we
293   propose that negative cross-talk between defence pathways imposes a major cost on adaptive
294   TAR responses to pathogens.

295   Although examples of transgenerational phenotypic plasticity are now widespread,
296   there are instances where researchers have failed to identify such effects in Arabidopsis
297   (Pecinka *et al.*, 2009; Suter & Widmer, 2013). Indeed, evolutionary theory predicts that
298   transgenerational plasticity is not a universal trait and that its occurrence depends highly on
299   genotype, mode of reproduction, ecological niche and life history traits, as well as the nature
300   and consistency of the eliciting stress (Crisp *et al.*, 2016; Groot *et al.*, 2016). Compared to fixed
301   genetic adaptation, it can be expected that transgenerational phenotypic plasticity offers a
302   suitable adaptation strategy under variable environments. However, it is unlikely that either
303   adaptation strategy will be under positive selection in highly variable, unpredictable
304   environments, since the frequency of incurred costs would outweigh the specific benefits.
305   Adaptive parental effects would therefore more likely emerge when the same type of

306   environmental stress occurs regularly (Tricker, 2015). Under such conditions, stress-exposed
307   plants can optimise fitness either by maximising their own immediate performance to the
308   detriment of their progeny ('selfish parental effects'), or by modifying progeny traits to provide
309   enhanced performance in the altered environment (Marshall & Uller, 2007). The latter strategy
310   can take form in either a diversified bet-hedging strategy, or a more deterministic provision of
311   specific adaptive traits, such as pathogen-elicited TAR, which is tailored to the parental
312   environment (Marshall & Uller, 2007; Crean & Marshall, 2009; Proulx & Teotonio, 2017). Not
313   only do evolutionary models predict that transgenerational phenotypic plasticity is likely to
314   evolve in fluctuating environments (Leimar & McNamara, 2015; Pigeault *et al.*, 2016; Proulx
315   & Teotonio, 2017), the model developed by Proulx and Teotonio (2017) suggests that
316   deterministic (adaptive) parental effects like pathogen-elicited TAR provide increased fitness
317   over a wider range of environmental parameters than a randomising bet-hedging strategy.

318   Central to the provision of adaptive transgenerational traits is the ability to make
319   accurate and reliable predictions about future progeny environments. While this aspect has
320   been emphasised in both evolutionary theory and modelling of parental effects (Burgess &
321   Marshall, 2014; Leimar & McNamara, 2015), it has rarely been addressed experimentally. The
322   few studies to have included this concept applied the same stress repeatedly over multiple
323   generations rather than applying different stress intensities within the same generation. In one
324   of the most comprehensive studies of this type, Groot et al. (2016) found complex interactions
325   between parental (P), grandparental (GP) and great-grandparental (GGP) salt stress in
326   Arabidopsis. When the stress was applied to only one generation, P effects were typically
327   stronger than GP and GGP effects. For treatments over multiple generations, the impacts of GP
328   and GGP stress were additive to P treatments for some traits, but antagonistic for others.
329   Furthermore, the transgenerational effects in the study by Groot et al. (2016) varied between
330   controlled environments and field conditions, making it difficult to conclude whether the
331   effects were adaptive. In our study, varying levels of three different stresses were applied
332   within one generation, providing a straightforward design to assess whether parents can
333   distinguish stress severities and adjust the transgenerational response accordingly. Our
334   pathogen treatments resulted in dose-dependent impacts on relative growth rate during the
335   treatment period (Fig. **2a**), indicating that Arabidopsis perceives these stresses in a dose-

336   dependent manner. Furthermore, analysis of the transgenerational stability of TAR provided

337   evidence for a dose-dependent relationship with parental disease severity. Although F1

338   populations from both *Pst-* and *Pc*-exposed parents expressed TAR to statistically similar

339   levels across stress levels (Fig. **3a,b** and Fig. **4a**), TAR only persisted over a stress-free

340   generation when elicited by the highest stress levels (Fig. **3c** and Fig. **4b**). Furthermore, in

341   mismatched environments, there was a dose-dependent effect on the costs of pathogen-elicited

342   TAR: both salt sensitivity of F1 progeny from *Pst*-infected parents and *Hpa* susceptibility of

343   F1 progeny from *Pc*-infected parents correlated with the severity of parental disease stress

344   treatment (Fig. **6b,c**). Overall, these results support our hypothesis that plants perceive disease

345   severity as a predictive cue to adjust TAR investment.

346   Collectively, our study demonstrates that parental investment in pathogen-elicited TAR

347   provides fitness benefits in matched environments and costs in mismatched environments. This

348   stress-specific TAR is dependent on the intensity of the stress experienced by the parents,

349   which holds predictive value for future progeny environments. Accordingly, our findings are

350   consistent with the evolutionary prediction that pathogen-elicited TAR is a genuine adaptive

351   trait in Arabidopsis. In one of the most convincing cases of adaptive parental effects in plants,

352   Galloway and Etterson (2007) used field-based studies to demonstrate adaptive

353   transgenerational plasticity in response to the light environment. It will now be of interest to

354   undertake ecological field studies and verify our laboratory experiments in support of TAR as

355   an adaptive transgenerational effect in nature.

356

357

Materials and Methods

358

Plant material and growth conditions.

359

360   All *Arabidopsis thaliana* lines described in this study are in the genetic background of

361   accession Col-0 (NCBI, Tax ID 3702). To exclude confounding effects of TAR from stress in

362   previous generations, all lines originated from a common ancestor of a population that had

363   maintained under stress-free conditions (mock-inoculated) in two previous generations (Luna

364   *et al.*, 2012). Except for the stress treatments, all plants were grown under similar conditions

16

365   (see Supplementary Methods in Supporting Information for details). To generate F1

366   populations, 6-8 parental plants of 4.5-weeks-old were subjected to mock/stress treatments over

367   a duration of 3 weeks, after which 4 parental plants with representative symptoms were moved

368   to long-day conditions (16 h light/8 h darkness) to set seed and generate F1 populations (Fig.

369   **1**). Three individual plants from each F1 population were kept apart under stress-free

370   conditions to set seed, resulting in 3 F2 populations from each F1 population and a total of 12

371   F2 populations per parental treatment (Fig. **1**). Details of all F1 and F2 populations are

372   presented in Table S1 in the Supporting Information.

373

374   **Stress treatments of parental plants.**

375   Inoculation with biotrophic *Pseudomonas syringae* pv. *tomato* (*Pst*) was performed at 3-4 day

376   intervals over a total period of 3 weeks, as detailed in the Supplementary Methods. Plants were

377   subjected to different *Pst* disease pressures: no disease (Mock; 6 subsequent inoculations with

378   the mock suspension), low disease (*Pst*-I; 2 inoculations with *Pst* followed by 4 mock

379   inoculations), medium disease (*Pst*-II; 4 inoculations with *Pst* followed by 2 mock inoculations

380   solution), and high disease (*Pst*-III; 6 subsequent inoculations with *Pst*). To ensure

381   necrotrophic infection by *Plectophaerella cucumerina* (*Pc*), inoculation was performed by

382   placing 6 µl-droplets (10^6 spores ml^{-1}) onto fully expanded leaves of approximate similar age

383   (Petriacq *et al.*, 2016), as detailed in the Supplementary Methods. Plants were subjected to

384   different *Pc* disease pressures: no disease (Mock; 6 leaves were mock-inoculated), low disease

385   (*Pc*-I; 2 leaves *Pc*-inoculated and 4 leaves mock-inoculated), medium disease (*Pc*-II; 4 leaves

386   *Pc*-inoculated and 2 leaves mock-inoculated), and high disease (*Pc*-III; 6 leaves *Pc*-inoculated).

387   After inoculation, plants were kept at 100% RH for 2 weeks until visible disease symptoms

388   appeared in >80% of the leaf surface (necrosis and chlorosis). To prevent sporulation and

389   ongoing disease progression, plants were returned to 60% RH before moving to long-day

390   conditions 1 week later. Salt stress was applied by soil-drenching with 100 mM NaCl solution.

391   Plants were subjected to different stress levels over the 3-week period: mock treatment (S-I;

392   drenched 6x with water), low stress (S-II; drenched 2x with NaCl and 4x with water), medium

393   stress (S-III; drenched 4x with NaCl and 2x with water), high stress (S-III; plants drenched 6x

394   with NaCl). Plants returned to a normal watering regime when moved to long-day conditions.

395

Quantification of fitness parameters.

397 Relative growth rate (RGR) was determined non-destructively by quantification of green leaf
398 area (GLA) before and after stress treatments, as detailed in the Supplementary Methods.
399 Reproductive fitness was estimated by seed production and seed viability, as described in the
400 Supplementary Methods.

401

Quantification of transgenerational resistance phenotypes.

403 To quantify resistance against biotrophic *Pst*, the inoculum was prepared and adjusted to $2x10^5$
404 CFU mL^{-1} (see Supplementary Methods). Inoculation was performed by syringe infiltration of
405 4 leaves/plant of approximate similar age. Bacterial growth was quantified at 3 days post
406 inoculation (dpi) by dilution plating on selective agar plates (see Supplementary Methods).
407 Inoculation with biotrophic *Hpa* and quantification of *Hpa* resistance was performed as
408 described previously (Lopez Sanchez *et al.*, 2016; see also Supplementary Methods).
409 Quantification of salt tolerance was based on root growth analysis on agar plates containing
410 0mM, 50 mM and 100 mM NaCl. Assays were conducted as described previously (Verslues *et*
411 *al.*, 2006; Claeys *et al.*, 2014) with minor modifications (see Supplementary Methods).

412

Statistical analysis.

414 Analytical statistics was performed using R studio (v 1.1.456, https://rstudio.com/), supporting
415 R software (v 3.5.1, https://www.r-project.org/). Statistical significance of treatment effects on
416 continuous variables was analysed by linear models; statistical significance of treatment effects
417 on categorical variables (class frequencies) was analysed by Fisher's exact tests. Details about
418 data transformations, statistical models, and R software packages are described in the
419 Supplementary Methods.

420

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427

428

Competing interests statement

430   There are no competing interests of a financial or non-financial nature.

431

432

Author Contributions

434   JT and MR conceived the project; JT and ALS designed and supervised the experiments; ALS,

435   DP and LF performed bioassays; JT performed statistical analyses; MR, ALS and JT wrote the

436   manuscript. All authors reviewed and approved the final manuscript. All authors declare no

437   competing interests.

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439

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543

544

Figure legends

545

546 **Fig. 1. Full factorial experimental design of the study.** *Arabidopsis thaliana* plants

547 (accession Col-0) from a common ancestor were exposed to increasing stress intensities (Mock,

548 Low, Medium and High) by the (hemi)biotrophic bacterial pathogen *Pseudomonas syringae*

549 pv. *tomato* (green), the necrotrophic fungal pathogen *Plectosphaerella cucumerina* (blue), or

550 soil salinity (NaCl; orange). Plants in this parental generation (P) were evaluated for impacts

551 on fitness parameters. Four plants per stress level were selected to generate F1 populations,

552 which were analysed for transgenerational changes in resistance against all 3 stresses, in order

553 to determine the specificity of transgenerational acquired resistance (TAR), potential costs

554 arising from increased susceptibility, and dose-dependency of TAR intensity on parental stress.

555 Four individual plants from 3 independent F1 populations were randomly selected to set seed

556 in the absence of stress. The resulting F2 populations were analysed for resistance against the

557 parental stress to examine dose-dependent effects on TAR stability. Circles indicate individual

558 plants; small (thin-lined) boxes indicate F1/F2 populations derived from a common ancestor in

559 the previous generation; big (bold-lined) boxes indicate pooled F1/F2 populations from a

560 common ancestor 2 generations earlier.

561

562 **Fig. 2. Differential impacts of three (a)biotic stresses on parental fitness parameters.**

563 Plants in the parental generation (4.5-weeks-old) were exposed to varying stress intensities by

564 *P. syringae* pv *tomato* (Mock, *Pst*-I, *Pst*-II, *Pst*-III), *P. cucumerina* (Mock, *Pc*-I, *Pc*-II, *Pc*-III)

565 or soil salinity (Mock, S-I, S-II, S-III) over a 3-week period before transferring to long-day

566 conditions to trigger flowering and set seed. Boxplots show the interquartile range (IQR; box)

567 ± 1.5xIQR (whiskers), including median (horizonal line) and replication units (single dots). **(a)**

568 Impacts on relative growth rate (RGR) during the period of stress exposure. Data represent

569 RGR values of single plants (n= 5-6) normalised to the average RGR of Mock-treated plants

570 (100%). Different letters indicate statistically significant differences (ANOVA + Tukey post-

571 hoc test, α=0.05). **(b)** Impacts on seed production. Data represent seed numbers per plant (n=

572 5-6) normalised to average value of Mock-treated plants (100%). Different letters indicate

573 statistically significant differences (*Pst*: Welch ANOVA + Games-Howell posthoc test,

574 α=0.05; *Pc* and salt: ANOVA + Tukey post-hoc test, α=0.05). **(c)** Impacts on seed viability.

575 Seed viability was determined 5 days after planting of surface-sterilised and stratified seeds

576 onto 0.2x Murashige and Skoog (MS) agar plates. Data represent mean germination

577 percentages per plate (25 seeds/plate) of seed batches from 4 similarly treated parents (n=15-

578 60). Different letters indicate statistically significant differences (Welch ANOVA + Games-

579 Howell post-hoc test; α=0.05). Viability data for seed batches from individual plants are

580 presented in Fig. **S1a-c.**

581

582 **Fig. 3. Intensity and transgenerational stability of *Pseudomonas syringae* pv. *tomato*-**

583 **elicited TAR in matched environments.** Parental plants had been exposed to different disease

584 severities by the biotrophic bacterium *Pseudomonas syringae* pv. *tomato* (*Pst*; Mock, *Pst*-I,

585 *Pst*-II, *Pst*-III). F1 and F2 plants were analysed for resistance against the same pathogen (*Pst*)

586 and/or the biotrophic Oomycete *Hyaloperonospora arabidopsidis* (*Hpa*). **(a)** TAR against *Pst*

587 in F1 progeny at 3 days post inoculation (dpi). Boxplots show the interquartile range (IQR;

588 box) ± 1.5xIQR (whiskers), including median (horizonal line) and replication units (dots). Data

589 represent [10]Log-transformed bacterial titres (Log cfu cm^{-2}) in leaves of single plants within F1

590 populations from similarly treated parents (n=42). Different letters indicate statistically

591 significant differences (Welch ANOVA + Games-Howell test, α=0.05). Data for individual F1

592 populations are shown in Fig. **S2a**. **(b)** TAR against *Hpa* in F1 progeny. *Hpa* colonisation was

593   quantified at 6 dpi by assigning trypan-blue stained leaves to 4 *Hpa* resistance classes (I:

594   healthy; II: hyphal colonisation only; III hyphal colonization with conidiospores; IV hyphal

595   colonisation with conidiospores and oospores). Stacked bars show leaf frequency distributions

596   within F1 populations from similarly treated parental plants (n=600-1000). Different letters

597   indicate statistically significant differences (Pairwise Fisher's exact tests + Bonferroni FDR,

598   α=0.05). Data for individual F1 populations are shown in Fig. **S2b**. **(c)** TAR against *Hpa* in F2

599   progeny at 6 dpi after one stress-free F1 generation. Stacked bars show leaf frequency

600   distributions across *Hpa* resistance classes within F2 populations that share a common parental

601   ancestor (n=300-350). Different letters indicate statistically significant differences (Pairwise

602   Fisher's exact tests + Bonferroni FDR; α=0.05). Data for individual F2 populations are shown

603   in Fig. **S2c**.

604

605   **Fig. 4. Intensity and transgenerational stability of *Plectosphaerella cucumerina*-elicited**

606   **TAR in matched environments.** Parental plants had been exposed to different disease

607   severities by necrotrophic *Plectosphaerella cucumerina* (*Pc*; Mock, *Pc*-I, *Pc*-II, *Pc*-III). F1 and

608   F2 plants were analysed for resistance against the same pathogen. Lesion diameters were

609   determined in 4 leaves/plant at 15 days post inoculation (dpi) and the average lesion diameter

610   per plant was used as statistical unit of replication. Boxplots show the interquartile range (IQR;

611   box) ± 1.5xIQR (whiskers), including median (horizonal line) and replication units (dots). **(a)**

612   TAR against *Pc* in F1 progeny. Data represent lesion diameters (mm) of plants within F1

613   populations from similarly treated parents (n=40). Different letters indicate statistically

614   significant differences (Welch ANOVA + Games-Howell test, α=0.05). Data for individual F1

615   populations are shown in Fig. **S3a**. **(b)** TAR against *Pc* in F2 progeny after a stress-free F1

616   generation. Data represent lesion diameters of plants within F2 populations that share a

617   common parental ancestor (n=20). Different letters indicate statistically significant differences

618   (ANOVA + Tukey post-hoc test; α=0.05). Data for individual F2 populations are shown in Fig.

619   **S3b**.

620

621   **Fig. 5. Lack of salt-elicited TAR in matched environments.** Parental plants had been

622   exposed to different stress intensities by soil salinity (NaCl; Mock, S-I, S-II, S-III). Salt

623   tolerance of F1 and F2 plants was quantified by root growth reduction (%) over a 5-day period

624   on NaCl-containing agar medium relative to the average root growth on agar medium without

625   NaCl. Boxplots show the interquartile range (IQR; box) ± 1.5xIQR (whiskers), including

626   median (horizonal line) and replication units (dots). **(a)** Unaltered tolerance of F1 plants to 50

627   and 100 mM NaCl. Data represent growth reduction percentages of single plants within F1

628   populations from similarly treated parents (n=60). ns: no statistically significant differences

629   (ANOVA; α=0.05). Root growth data for individual F1 populations are shown in Fig. **S4a**; root

630   tolerance data for individual F1 populations are shown in Fig. S4c. **(b)** Unaltered tolerance of

631   F2 plants to 50 and 100 mM NaCl after one stress-free F1 generation. Data represent growth

632   reduction percentages of single plants within F2 populations that share a common parental

633   ancestor (n=18-20). ns: no statistically significant differences (ANOVA; α=0.05). Root growth

634   data for individual F2 populations are shown in Fig. S4b; root tolerance data for individual F2

635   populations are shown in Fig. **S4d**.

636

637   **Fig. 6. Costs and benefits of TAR in mismatched environments.** Parental plants had been

638   exposed to different stress severities by *Pst* (green), *Pc* (blue) or soil salinity (orange). F1 plants

639   were tested for resistance against different stresses than the parental stress. **(a)** Increased *Pc*

640   susceptibility in F1 progeny from *Pst*-exposed parents. Box plots show lesion diameters (mm)

641   of plants within F1 populations from similarly treated parental plants (n=76-80). See legend of

642   Fig. **4** for details. Different letters indicate statistically significant differences between parental

643   treatments (ANOVA + Tukey post-hoc test; α=0.05). Data for individual F1 populations are

644   shown in Fig. **S5a**. **(b)** Reduced salt tolerance in F1 progeny from *Pst*-exposed parents. Box

645   plots show root growth reduction percentages by 50 mM NaCl of plants within F1 populations

646   from similarly treated parental plants (n=40). See legend of Fig. **5** for details. Different letters

647   indicate statistically significant differences (ANOVA + Tukey post-hoc test; α=0.05). Root

648   growth data for individual F1 populations at 0, 50 and 100 mM NaCl are shown in Fig. **S5b**;

649   tolerance data for individual F1 populations to 50 and 100 mM NaCl are shown in Fig. **S5c**. **(c)**

650   Increased *Hpa* susceptibility in F1 progeny from *Pc*-exposed parents. Stacked bars show leaf

651   frequency distributions across *Hpa* resistance classes within F1 populations from similarly

652   treated parents (n=400-500). See legend of Fig. **3b** for details. Different letters indicate

653   statistically significant differences (pairwise Fisher's exact tests + Bonferroni FDR, α=0.05).

654   Data for individual F1 populations are shown in Fig. **S6a**. **(d)** Unaltered salt tolerance in F1

655   progeny from *Pc*-exposed parents. Box plots show root growth reduction percentages by 50

656   mM NaCl of plants within F1 populations from similarly treated parental plants (n=47). See

657   legend of Fig. **5** for details. ns: no statistically significant differences (ANOVA; α=0.05). Root

658   growth data for individual F1 populations at 0, 50 and 100 mM NaCl are shown in Fig. S6B;

659   tolerance data for individual F1 populations to 50 and 100 mM NaCl are shown in Fig. **S6c**. **(e)**

660   Non-specific TAR against *Hpa* in F1 progeny from NaCl-exposed parents. Stacked bars show

661   leaf frequency distributions across *Hpa* resistance classes within F1 populations from similarly

662   treated parents (n=350-800). See legend of Fig. **3b** for details. Different letters indicate

663   statistically significant differences (Pairwise Fisher's exact tests + Bonferroni FDR, α=0.05).

664   Data for individual F1 populations are shown in Fig. S7a. **(f)** Non-specific TAR against *Pc* in

665   F1 progeny from NaCl-treated parents. Box plots show lesion diameters (mm) of plants within

666   F1 populations from similarly treated parental plants (n=30-60). See legend of Fig. **4** for details.

667   Different letters indicate statistically significant differences between parental treatments

668   (ANOVA + Tukey post-hoc test; α=0.05). Data for individual F1 populations are shown in Fig.

669   **S7b**.

670

671

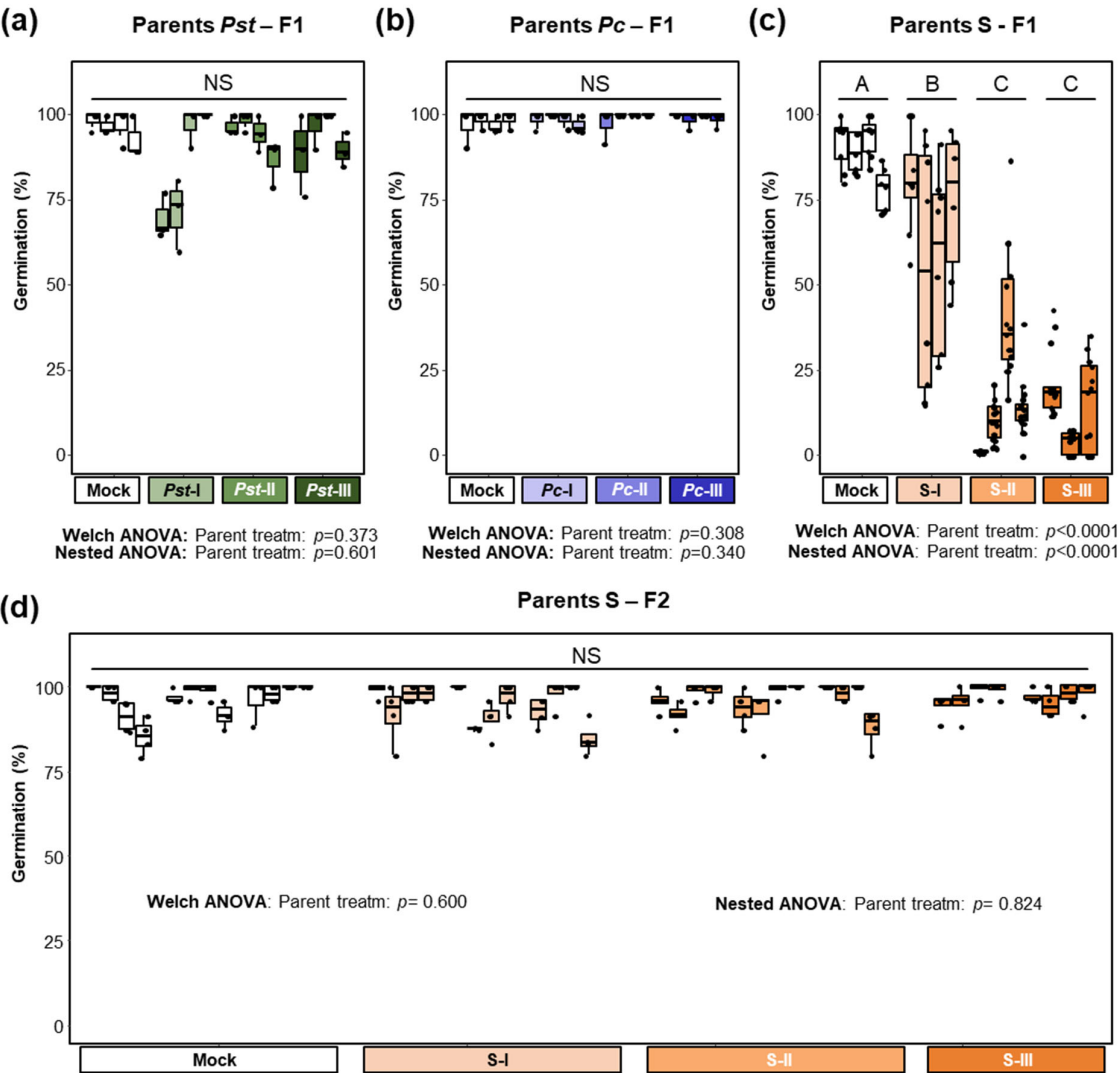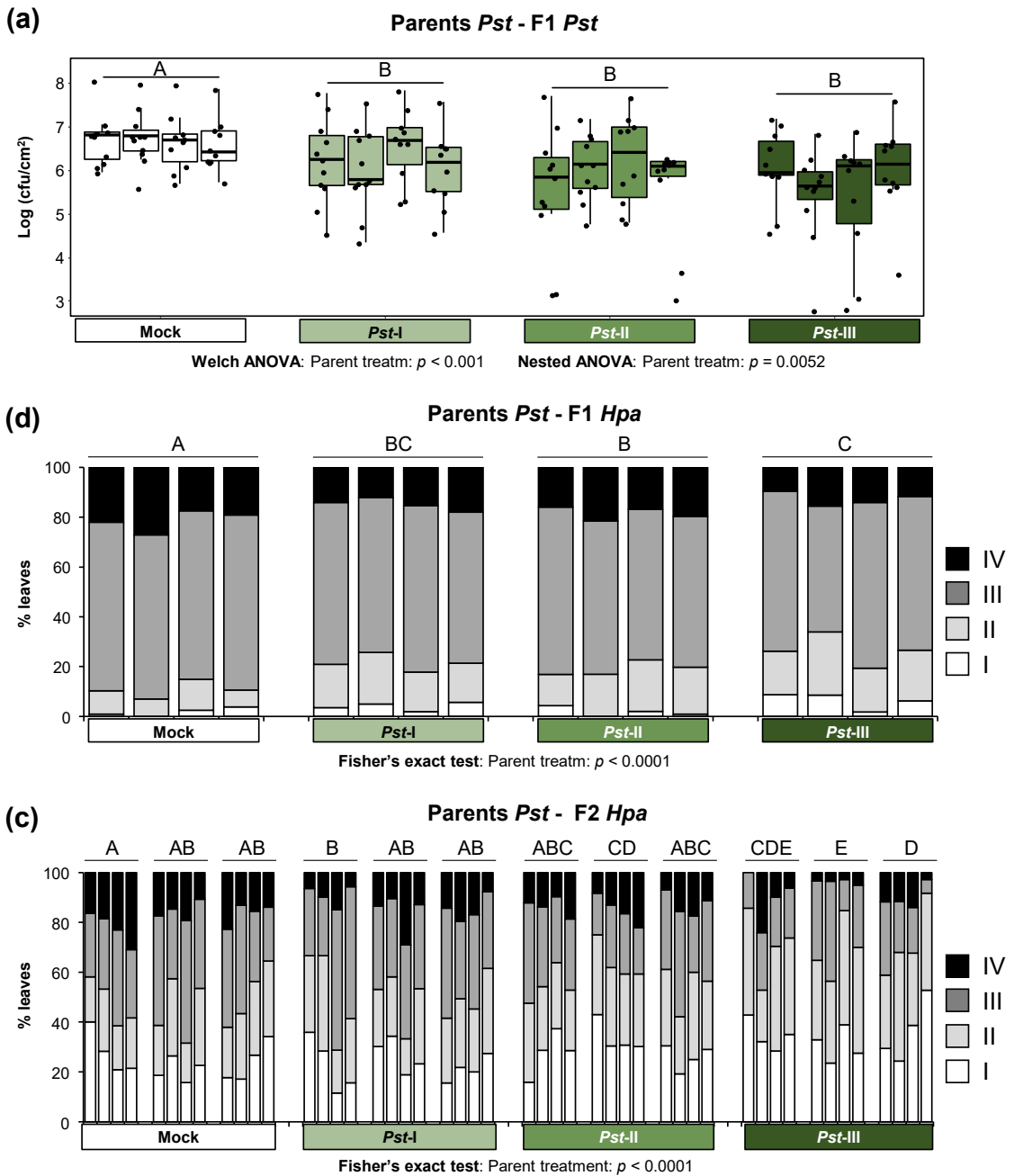672 **Supplementary Figures and Table**

673

674

675 **Fig. S1: Impacts of (a)biotic stresses on seed viability from individual plants.** See legend
676 of Fig. **2c** for details. Boxplots show the interquartile range (IQR; box) ± 1.5xIQR (whiskers),
677 including median (horizonal line) and replication units (dots). Data represent germination
678 percentages of F1 seed batches from *Pst*-exposed parents (green, **(a)**; n=3-4), F1 seeds from
679 *Pc*-exposed parents (blue, **(b)**; n=3-4), F1 seeds from salt-exposed parents (orange, **(c)**; n=8-
680 22) and F2 seeds from salt-exposed parents after one stress-free F1 generation (orange, **(d)**;
681 n=4-5). *P*-values indicate statistical significance of parent treatment by Welch ANOVA of

27

682   pooled populations from similarly treated parent plants (F1) or a common parental ancestor

683   (F2), as well as nested ANOVA with individual F1/F2 population as random factor. Different

684   letters indicate statistically significant differences between pooled populations (Welch

685   ANOVA + Games-Howell post-hoc test; α=0.05; NS: no statistically significant differences).

(a)

Parents *Pst* - F1 *Pst*

Welch ANOVA: Parent treatm: *p* < 0.001   Nested ANOVA: Parent treatm: *p* = 0.0052

(d)

Parents *Pst* - F1 *Hpa*

Fisher's exact test: Parent treatm: *p* < 0.0001

(c)

Parents *Pst* - F2 *Hpa*

Fisher's exact test: Parent treatment: *p* < 0.0001

686

Fig. S2. TAR in individual F1 and F2 populations from *Pst*-treated parents in matched environments. (a) *Pst*-elicited TAR against *Pst* in F1 plants at 3 dpi. See legend of Fig. **3a** for details. Boxplots show the interquartile range (IQR; box) ± 1.5xIQR (whiskers), including median (horizontal line) and replication units (dots). Data represent [10]Log-transformed bacterial

29

691   titres (Log cfu cm^{-2}; n=10-12) in leaves of single plants within individual F1 populations. *P*-
692   values indicate statistical significance of parent treatment by Welch ANOVA of pooled F1
693   populations from similarly treated parents, and nested ANOVA with individual F1 population
694   as random factor, respectively. Different letters indicate statistically significant differences
695   between pooled F1 populations from similarly treated parents (Welch ANOVA + Games-
696   Howell post-hoc test; α=0.05). **(b)** *Pst*-elicited TAR against *Hpa* in F1 plants at 6 dpi. See
697   legend of Fig. **3b** for details. Stacked bars show leaf frequency distributions across *Hpa*
698   resistance classes within individual F1 populations (n=70-250). *P*-value indicates statistical
699   significance of the parent treatment (Fisher's exact test). Different letters indicate statistically
700   significant differences between pooled F1 populations from similarly treated parents (pairwise
701   Fisher's exact tests + Bonferroni FDR, α=0.05). **(c)** *Pst*-elicited TAR against *Hpa* in F2 plants
702   at 6 dpi after one stress-free F1 generation. See legend of Fig. **3c** for details. Stacked bars show
703   leaf frequency distributions across *Hpa* resistance classes within individual F2 populations
704   (n=55-100). *P*-value indicates statistical significance of parent treatment (Fisher's exact tests).
705   Different letters indicate statistically significant differences between pooled F2 populations
706   from a common parental ancestor (pairwise Fisher's exact tests + Bonferroni FDR; α=0.05).
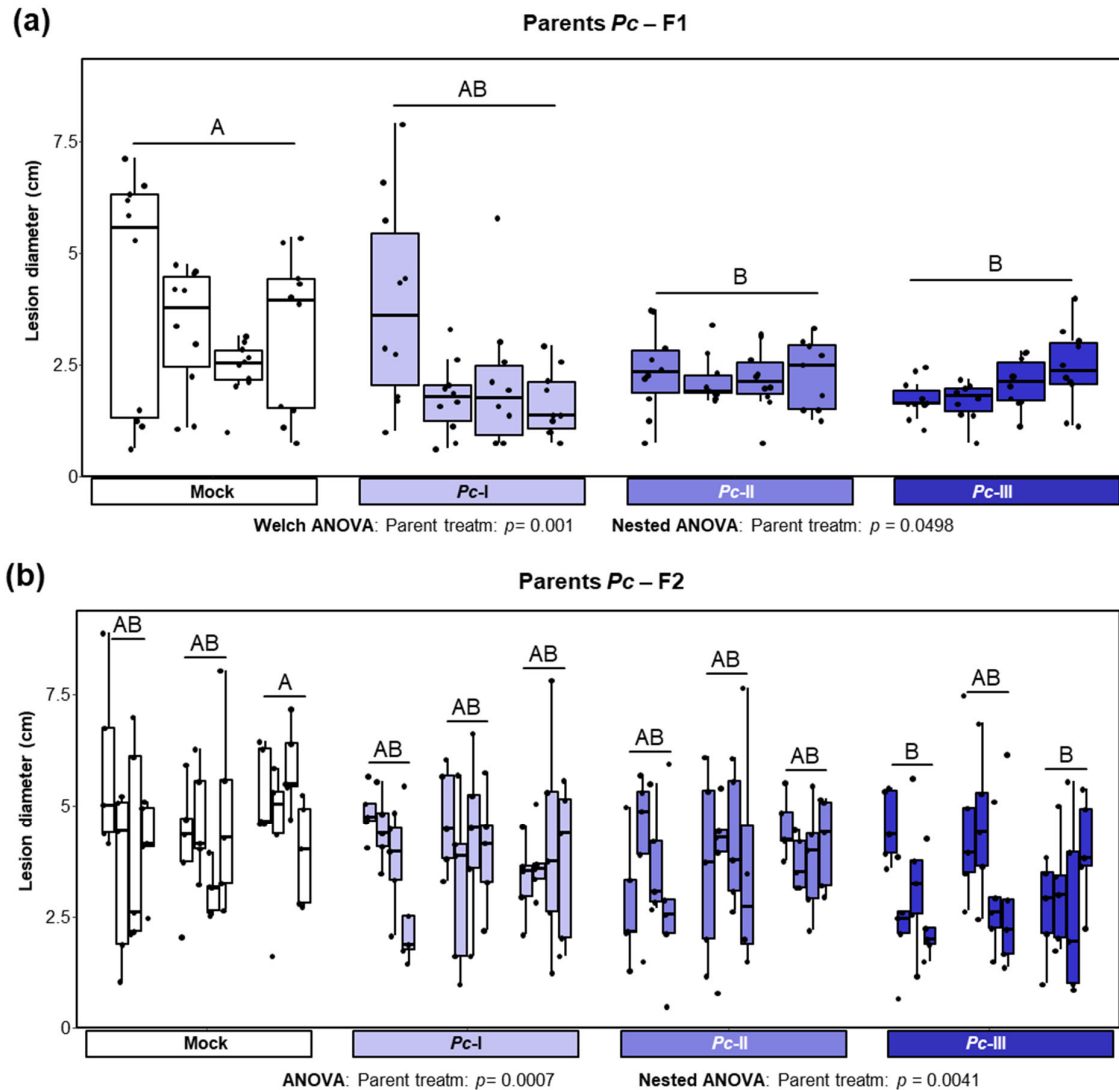
Fig. S3. TAR in individual F1 and F2 populations from *Pc*-exposed parents in matched environments. Data represent lesion diameters (mm) of plants within individual populations at 15 dpi. See legend of Fig. **4** for details. Boxplots show the interquartile range (IQR; box) ± 1.5xIQR (whiskers), including median (horizontal line) and replication units (dots). **(a)** *Pc*-elicited TAR against *Pc* in F1 plants (n=10). *P*-values indicate statistical significance of parent treatment by Welch ANOVA of pooled F1 populations from similarly treated parents, and nested ANOVA with F1 population as random factor, respectively. Different letters indicate statistically significant differences between pooled F1 populations from similarly treated

716   parents (Welch ANOVA + Games-Howell post-hoc test; α=0.05). **(b)** *Pc*-elicited TAR against

717   *Pc* in F2 plants (n=5). *P*-values indicate statistical significance of parent treatment by ANOVA

718   of pooled F2 populations from a common parental ancestor, and nested ANOVA with F2

719   population as random factor, respectively. Different letters indicate statistically significant

720   differences between pooled F2 populations from a common parental ancestor (ANOVA +

721   Tukey post-hoc test; α=0.05).
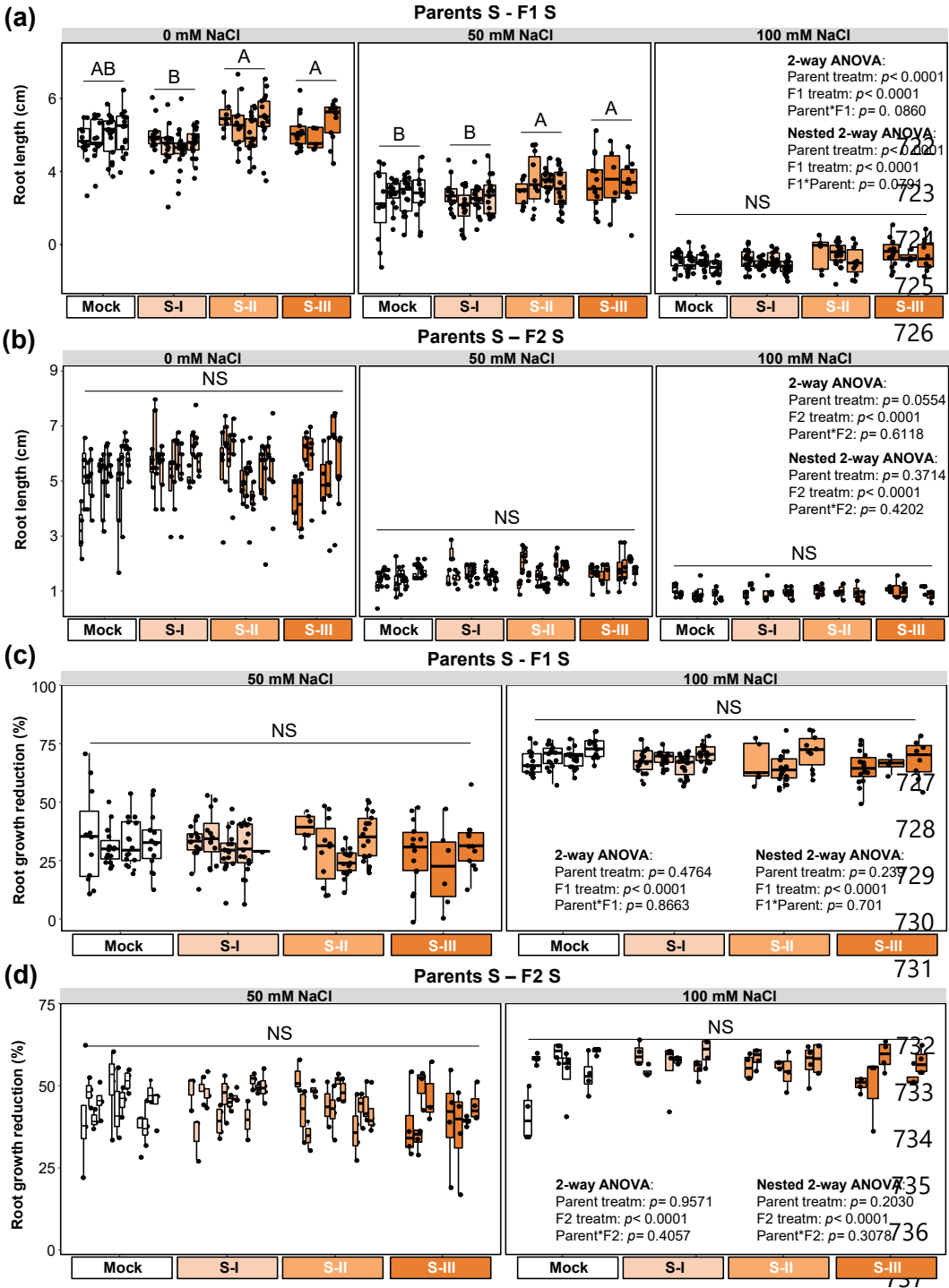
(a)

Parents S - F1 S

(b)

Parents S – F2 S

(c)

Parents S - F1 S

(d)

Parents S – F2 S

738

739 **Fig4. Transgenerational effects of soil salinity on root growth and salt tolerance in**

740 **individual F1 and F2 populations and matched environments.** All boxplots show the

741   interquartile range (IQR; box) ± 1.5xIQR (whiskers), including median (horizonal line) and

742   replication units (dots). **(a)** Root growth of F1 plants at 0, 50 and 100 mM NaCl. Data represent

743   root growth values (cm) of plants within individual F1 populations over a 5-day period (n=15).

744   *P*-values indicate statistical significance of parent treatment, F1 treatment and interaction by

745   2-way ANOVA of pooled F1 populations from similarly treated parents, and nested 2-way

746   ANOVA with F1 population as random factor, respectively. For each NaCl concentration,

747   different letters indicate statistically significant differences between pooled F1 populations

748   from similarly treated parents (ANOVA + Tukey post-hoc test; α=0.05; NS: no significant

749   differences). **(b)** Root growth of F2 plants at 0, 50 and 100 mM NaCl after one stress-free F1

750   generation. Data represent root growth values (cm) of plants within individual F2 populations

751   over a 5-day period (n=4-5). *P*-values indicate statistical significance of parent treatment, F2

752   treatment and interaction by 2-way ANOVA of pooled F2 populations from a common parental

753   ancestor, and nested 2-way ANOVA with F2 population as random factor, respectively. NS:

754   no statistically significant differences between pooled F2 populations from a common parental

755   ancestor (ANOVA + Tukey post-hoc test; α=0.05). **(c)** Tolerance of F1 plants to 50 and 100

756   mM NaCl. Tolerance was quantified by root growth reduction (%) relative to the mean root

757   growth at 0 mM NaCl of the corresponding F1 population (Fig. **S4a**). Data represent growth

758   reduction percentages of single plants within individual F1 populations (n=15). *P*-values

759   indicate statistical significance of parent treatment, F1 treatment and interaction by 2-way

760   ANOVA of pooled F1 populations from similarly treated parents, and nested 2-way ANOVA

761   with F1 population as random factor, respectively. NS: no statistically significant differences

762   between pooled F1 populations from similarly treated parents (ANOVA + Tukey post-hoc test;

763   α=0.05). **(d)** Tolerance of F2 plants to 50 and 100 mM NaCl after one stress-free F1 generation.

764   Tolerance was quantified by root growth reduction relative to the mean root growth at 0 mM

765   NaCl of the corresponding F2 population (Fig. **S4b**). Data represent growth reduction

766   percentages of single plants within individual F2 populations (n=4-5). *P*-values indicate

767   statistical significance of parent treatment, F2 treatment and interaction by 2-way ANOVA of

768   pooled F2 populations from a common parental ancestor, and nested 2-way ANOVA with F2

769   population as random factor, respectively. NS: no statistically significant differences between

770   pooled F2 populations from a common parental ancestor (ANOVA + Tukey post-hoc test;

771   α=0.05).

(a)

Parents *Pst* – F1 *Pc*

Nested ANOVA: Parent treatment: *p* = 0.0325
ANOVA: Parent treatments: *p*= 0.0009

(b)

Parents *Pst* – F1 S

2-way ANOVA:
F1 treatment: *p*< 0.0001
Parent treatment: *p*= 0.0008
Parent*F1: *p*= 0.0683

2-way nested ANOVA:
F1 treatment: *p*< 0.0001
Parent: *p*= 0.0369
Parent*F1: *p*= 0.0578

(c)

Parents *Pst* – F1 S

2-way ANOVA: F1 treatment: *p*< 0.0001; Parent treatment: *p*= 0.0002; Parent*F1: *p*= 0.1485
2-way nested ANOVA: F1 treatment: *p*< 0.0001; Parent treatment: *p*= 0.0286; Parent*F1: *p*= 0.1333
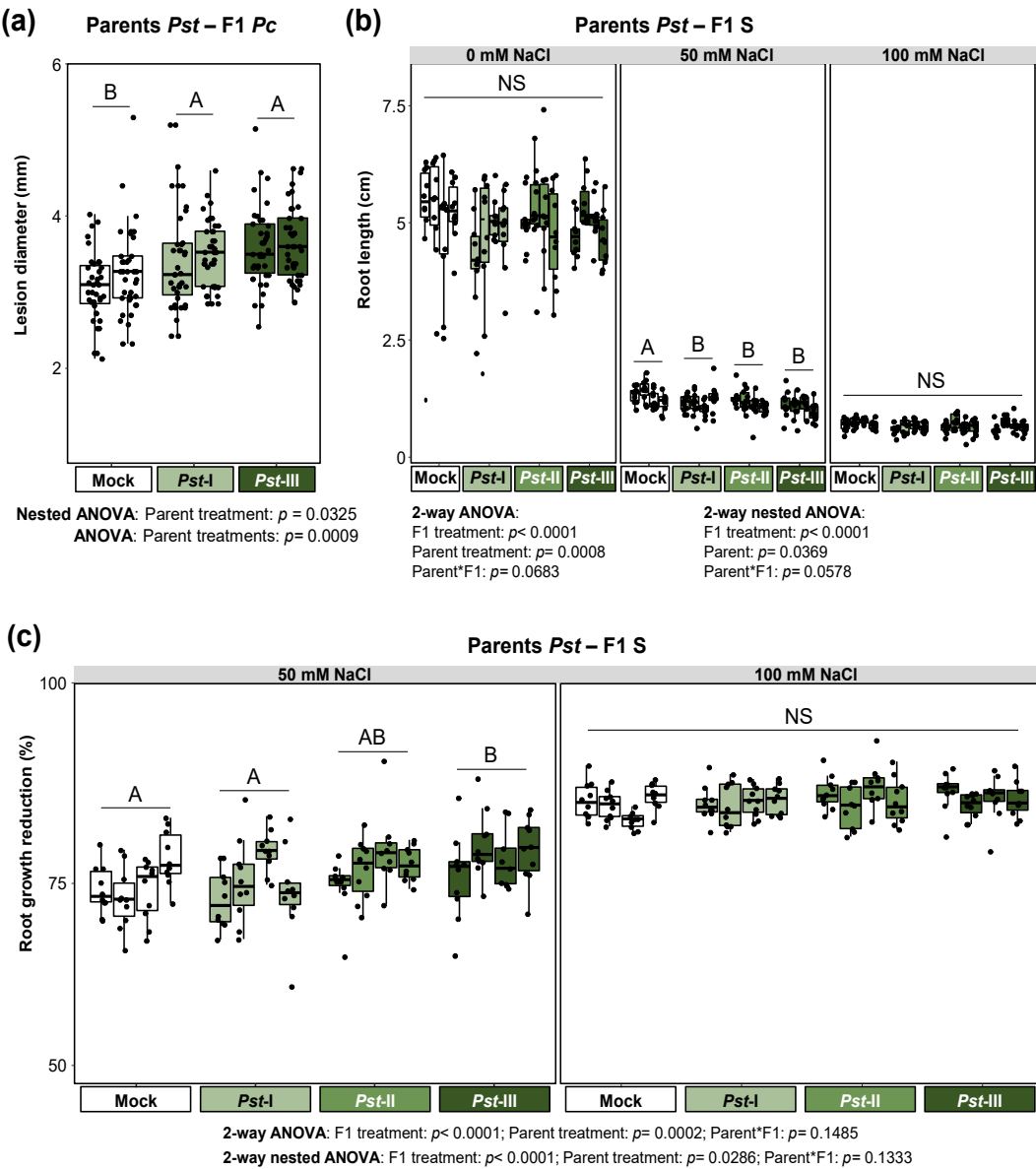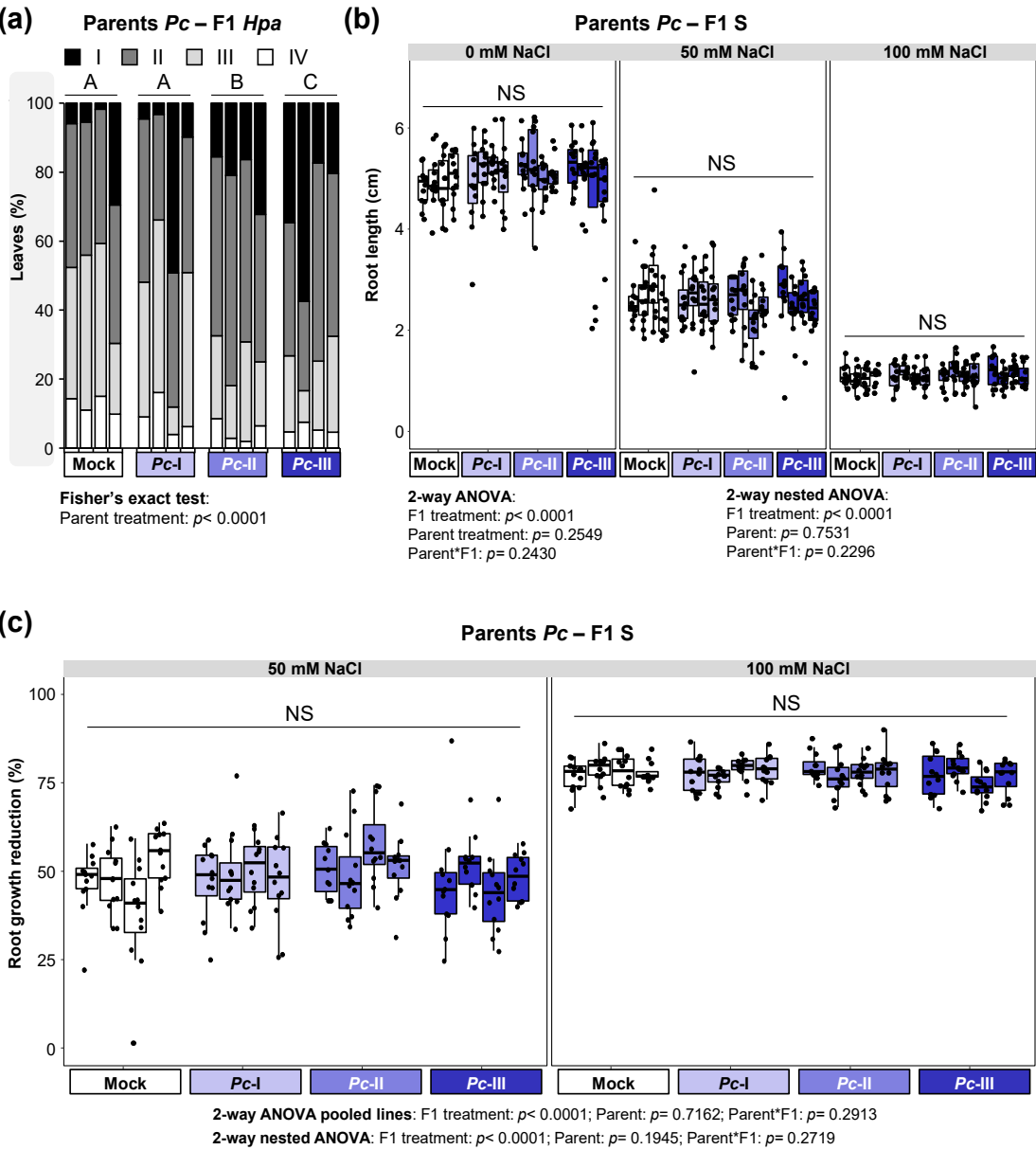
Fig. S5. Costs of *Pst*-elicited TAR in individual F1 populations and mismatched environments. All boxplots show the interquartile range (IQR; box) ± 1.5xIQR (whiskers), including median (horizonal line) and replication units (dots). See legends to Fig. **6a,b** for details. **(a)** *Pc* resistance of F1 plants from mock- and *Pst*-treated parents 6 dpi. Data represent lesion diameters (mm) of plants within individual F1 populations (n=38-40). *P*-values indicate statistical significance of parent treatment by ANOVA of pooled F1 populations from similarly

35

779   treated parents, and nested ANOVA with individual F1 population as random factor,

780   respectively. Different letters indicate statistically significant differences between pooled F1

781   populations from similarly treated parents (ANOVA + Tukey post-hoc test; α=0.05). **(b)** Root

782   growth of F1 plants from mock- and *Pst*-treated parents at 0, 50 and 100 mM NaCl. Data

783   represent root growth values (cm) of plants within individual F1 populations over a 5-day

784   period (n=10). *P*-values indicate statistical significance of parent treatment, F1 treatment and

785   interaction by 2-way ANOVA of pooled F1 populations from similarly treated parental plants,

786   and nested 2-way ANOVA with F1 population as random factor, respectively. For each NaCl

787   concentration, different letters indicate statistically significant differences between pooled F1

788   populations from similarly treated parents (ANOVA + Tukey post-hoc test; α=0.05; NS: no

789   statistically significant differences). **(c)** Tolerance of F1 plants from mock- and *Pst*-treated

790   parents to 50 and 100 mM NaCl. Tolerance was quantified by root growth reduction (%)

791   relative to the mean root growth value at 0 mM NaCl of the corresponding F1 population (Fig.

792   **S5b**). Data represent root growth reduction percentages of single plants within individual F1

793   populations (n=10). *P*-values indicate statistical significance of parent treatment, F1 treatment

794   and interaction by 2-way ANOVA of pooled F1 populations from similarly treated parents, and

795   by nested 2-way ANOVA with F1 population as random factor, respectively. For each NaCl

796   concentration, different letters indicate statistically significant differences between pooled F1

797   populations from similarly treated parents (ANOVA + Tukey post-hoc test; α=0.05; NS: no

798   significant differences).

(a) Parents *Pc* – F1 *Hpa*

(b) Parents *Pc* – F1 S

Fisher's exact test:
Parent treatment: *p*< 0.0001

2-way ANOVA:
F1 treatment: *p*< 0.0001
Parent treatment: *p*= 0.2549
Parent*F1: *p*= 0.2430

2-way nested ANOVA:
F1 treatment: *p*< 0.0001
Parent: *p*= 0.7531
Parent*F1: *p*= 0.2296

(c) Parents *Pc* – F1 S

2-way ANOVA pooled lines: F1 treatment: *p*< 0.0001; Parent: *p*= 0.7162; Parent*F1: *p*= 0.2913
2-way nested ANOVA: F1 treatment: *p*< 0.0001; Parent: *p*= 0.1945; Parent*F1: *p*= 0.2719

799

Fig. S6. Costs of *Pc*-elicited TAR in individual F1 populations and mismatched environments. See legends of Fig. **6c,d** for details. **(a)** *Hpa* resistance in F1 plants from *Pc*-exposed parents. Stacked bars show leaf frequency distributions across *Hpa* resistance classes within F1 populations from similarly treated parents (n=80-130). *P*-value indicates statistical significance of parental treatment (Fisher's exact test). Different letters indicate statistically

805   significant differences between pooled F1 populations from similarly treated parents (Pairwise

806   Fisher's exact tests + Bonferroni FDR; α=0.05). **(b)** Root growth of F1 plants from mock- and

807   *Pc*-treated parents at 0, 50 and 100 mM NaCl. Boxplots show the interquartile range (IQR;

808   box) ± 1.5xIQR (whiskers), including median (horizonal line) and replication units (dots). Data

809   represent root growth values (cm) of single plants within individual F1 populations over a 5-

810   day period (n=10). *P*-values indicate statistical significance of parent treatment, F1 treatment

811   and interaction by 2-way ANOVA of pooled F1 populations from similarly treated parents, and

812   nested 2-way ANOVA with F1 population as random factor, respectively. NS: no statistically

813   significant differences between pooled F1 populations from similarly treated parents (ANOVA

814   + Tukey post-hoc test; α=0.05). **(c)** Tolerance of F1 plants from mock- and *Pc*-treated parents

815   to 50 and 100 mM NaCl. Tolerance was quantified by root growth reduction (%) relative to the

816   mean root growth value at 0 mM NaCl of the corresponding F1 population (Fig. **S6b**). Boxplots

817   show the interquartile range (IQR; box) ± 1.5xIQR (whiskers), including median (horizonal

818   line) and replication units (dots). Data represent root growth reduction percentages of single

819   plants within individual F1 populations. *P*-values indicate statistical significance of parent

820   treatment, F1 treatment and interaction by 2-way ANOVA of pooled F1 populations from

821   similarly treated parents, and nested 2-way ANOVA with F1 population as random factor,

822   respectively. NS: no statistically significant differences between pooled F1 populations from

823   similarly treated parents (ANOVA + Tukey post-hoc test; α=0.05).
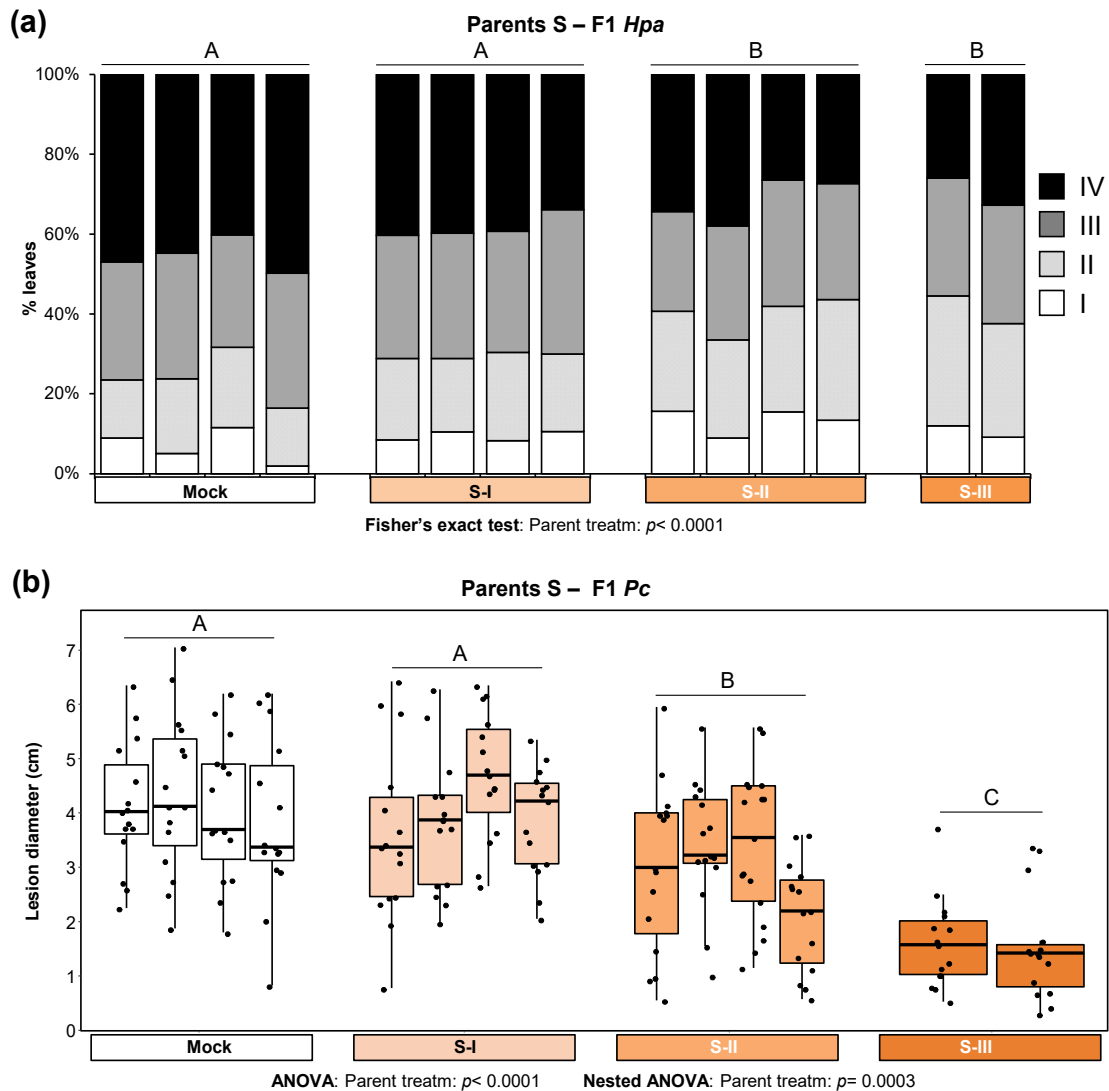
(a)

Parents S – F1 *Hpa*

(b)

Parents S – F1 *Pc*

824

Fig. S7. Non-specific TAR by soil salinity against *Pst* and *Pc* in individual F1 populations

825

and mismatched environments. See legend of Fig. **6e,f** for details. **(a)** Non specific TAR

826

against *Hpa* in F1 plants. Stacked bars show leaf frequency distributions across *Hpa* resistance

827

classes within individual F1 populations (n=100-225). *P*-value indicates statistical significance

828

of parental treatment (Fisher's exact test). Different letters indicate statistically significant

829

differences between pooled F1 populations from similarly treated parental plants (pairwise

830

Fisher's exact tests + Bonferroni FDR; α=0.05). **(b)** Non-specific TAR against *Pc* in F1 plants.

831

Boxplots show the interquartile range (IQR; box) ± 1.5xIQR (whiskers), including median

832

(horizontal line) and replication units (dots). Data represent lesion diameters (mm) of plants

833

834   within individual F1 populations (n=15). *P*-values on the right indicate statistical significance

835   of parent treatment by ANOVA of pooled F1 populations from similarly treated parental plants,

836   and nested ANOVA with individual F1 population as random factor, respectively. Different

837   letters indicate statistically significant differences between pooled F1 populations from

838   similarly treated parents (ANOVA + Tukey post-hoc test; α=0.05).

839

840   **Table S1.** Collection of Arabidopsis F1 and F2 populations (Col-0) that in the parental
841   generation had been subjected to varying stress intensities by *Pst*, *Pc* or soil salinity.

Parental treatment	Generation	No. of lines	Code
Pst-Mock	F1	4 lines	*Pst*-0, 1-4
Pst-Low	F1	4 lines	*Pst*-I, 1-4
Pst-Medium	F1	4 lines	*Pst*-II, 1-4
Pst-High	F1	4 lines	*Pst*-III, 1-4
Pst-Mock	F2	12 lines	*Pst*-0 M2, 1-12
Pst-Low	F2	12 lines	*Pst*-I M2, 1-12
Pst-Medium	F2	12 lines	*Pst*-II M2, 1-12
Pst-High	F2	12 lines	*Pst*-III M2, 1-12
F1 *Pc*-Mock	F1	4 lines	*Pc*-0, 1-4
F1 *Pc*-Low	F1	4 lines	*Pc*-I, 1-4
F1 *Pc*-Medium	F1	4 lines	*Pc*-II, 1-4
F1 *Pc*-High	F1	4 lines	*Pc*-III, 1-4
F2 *Pc*-Mock	F2	12 lines	*Pc*-0 M2, 1-12
F2 *Pc*-Low	F2	12 lines	*Pc*-I M2, 1-12
F2 *Pc*-Medium	F2	12 lines	*Pc*-II M2, 1-12
F2 *Pc*-High	F2	12 lines	*Pc*-III M2, 1-12
F1 Salt-Mock	F1	4 lines	S-0, 1-4
F1 Salt-Low	F1	4 lines	S-I, 1-4
F1 Salt-Medium [1]	F1	4 lines	S-II, 1-4
F1 Salt-High [1]	F1	4 lines	S-III, 1-4
F2 Salt-Mock	F2	12 lines	S-0 M2, 1-12
F2 Salt-Low	F2	12 lines	S-I M2, 1-12
F2 Salt-Medium	F2	12 lines	S-II M2, 1-12
F2 S-High	F2	8 lines	S-III M2, 1-12

842

843

844   **Supplementary Methods**

845

846   **General growth conditions.**

847   After stratification in water and darkness at 4ºC for 2-3 days, seeds were sown on *Jiffy-7* peat

848   pellets. All plants were initially grown under short-day conditions (8.5 hour -h- light/15.5 h

849   darkness) at 21 °C, 60% relative humidity (RH) and 100-125 µmol s^{-1} m^{-2} light intensity) and

850   watered to saturation by flooding the trays for 0.5 h and removing the excess of water

851   afterwards (3 times/week). After the stress exposure, parental plants (7.5-weeks-old) were

852   moved to long-day conditions (16 h light/8 h darkness) to trigger flowering and set seed. For

853   quantification of disease resistance in F1/F2 populations, plants were grown in individual pots

854   in a randomised block design, and regularly rotated within and between blocks within the

855   climate chamber (3 times/week) to prevent positional effects. For the quantification of NaCl

856   tolerance, agar plates containing multiple F1/F2 populations were rotated with similar

857   frequency.

858

859   **Microbes and inoculation protocols.**

860   *Pseudomonas syringae* pv. *tomato* strain DC3000 was cultivated from a frozen glycerol stock

861   for 48 h on King´s B (KB) agar plates, supplemented with 50 µg/ml rifampicin (Sigma-Aldrich,

862   R3501). Cells were collected from agar plates, resuspended and washed in 10mM MgSO$_4$

863   before adjusting the optical density spectrophotometrically (OD600) For stress induction, cells

864   were adjusted to to 5x10^7 colony-forming units (CFU)/mL and supplemented with 0.015%

865   Silwet L-77 (Lehle Seeds NC0138454) prior to spraying onto the rosettes. Mock inoculation

866   was performed by spraying equal amounts of 10mM MgSO$_4$ + 0.015% Silwet L-77). For

867   quantification of *Pst* resistance in F1/F2 progeny, the inoculum was adjusted to 2x10^5 CFU/mL

868   and syringe-infiltrated into 4 leaves/plant of approximate similar age. Plants were kept at 100%

869   RH for 1-2 h immediately after inoculation. *Plectosphaerella cucumerina* strain BMM (*Pc*)

870   was cultivated on half-strength Potato Dextrose Agar (BD Difco, BD-213400) for 3.5 weeks

871   in the dark. Spores were resuspended from agar plates in water and filtered through 2 layers of

872   Miracloth (Merck, 475855-1R) to remove mycelium debris. *Pc* inoculum was adjusted to 10^6

41

873   spores/ml in water, using a Neubauer haemocytometer. To ensure necrotrophic infection by the

874   fungus, inoculation was performed by placing 6 µl droplets (10^6 spores/ml) onto fully expanded

875   leaves of approximate similar age; mock inoculum was performed by of applying 6 µl water

876   droplets. The obligate biotrophic Oomycete *Hyaloperonosopora arabidopsidis* strain WACO9

877   was maintained and bulked on hypersusceptible NahG plants (Ws-0, Syngenta Agribusiness

878   Biotechnology Research, Line 3A). Sporulating plants were collected in 15-mL falcon tubes

879   containing demineralised water and gently shaken to extract conidiospores. The suspension

880   was then filtered through 2 layers of Miracloth and adjusted to 10^5 conidiospores/mL, using a

881   Neubauer haemocytometer. Three-week-old plants were inoculated by spraying the spore

882   suspension onto the shoots, after which plants were maintained at 100% RH.

883

884   **Analysis of relative growth rate.**

885   Relative growth rate (RGR) analysis was based quantification of green leaf area (GLA) before

886   and after stress treatments. Digital photos (Canon, 500D 15MP) were taken before and after

887   the stress treatment. Digital image analysis of GLA was performed using Adobe Photoshop

888   6.0. Green pixels corresponding to GLA were selected using a combination of "magic wand"

889   and "lasso" tools and converted into mm^2. For each plant, the following formula was used to

890   calculate RGR, where GLA_2 and GLA_1 are GLA values before and after stress exposure,

891   respectively, and (*t2 – t1*) represents the time-window (d):

892
$$RGR = \frac{(\ln GLA_2 - \ln GLA_1)}{(t_2 - t_1)}$$

893   RGR values were determined for 5- 6 plants per treatment and normalized to the average RGR

894   value of non-stressed plants (mock treatment; 100%). Reproductive fitness was estimated by

895   seed production and seed viability as described in the Supplementary Methods.

896

897   **Seed production and seed viability assays.**

898   To estimate reproductive fitness, seeds from 5- 6 plants per stress treatment were collected in

899   Aracons (Lehle seeds) and weighed. Seed weights for each plant were converted to numbers

900   of seeds, based on the mass of 100 counted seeds (~ 1.2 mg). Seed viability was determined

901   after sterilisation on agar plates (see for details). Seed viability was quantified on 0.2x

902   Murashige and Skoog (MS) agar (Duchefa, M0221), containing 1% sucrose and 6 g/L agar

903   (pH=5.7, adjusted with KOH). Vapour-phase sterilization was performed by incubating seeds

904   for 4 h in open Eppendorf tubes inside a glass vacuum desiccator (10.5 L), in which 100 ml of

905   bleach (Jantex, R-GG183) and 3 ml of HCl were mixed to produce chlorine gas. Plates were

906   stratified at 4°C in the dark for 3 d and transferred to short-day growth conditions. Seed

907   germination rates were determined in 3-17 replicate plates/population (~25 seeds/plate) at 5 d

908   after stratification. Seeds were considered germinated when green cotyledons were visible.

909

910   ***Pst* resistance assays.**

911   Bacterial growth in syringe-inoculated leaves (see above) was quantified at 3 days post

912   inoculation (dpi) by collecting 4 leaf discs/plant in 1.5-mL tubes with 600μL 10 mM $MgSO_4$,

913   using a cork borer (0.75 cm diameter). Leaf discs were homogenised in the tubes using plastic

914   pestles and transferred to 96-wells microtiter plates (Costar®) for serial dilutions in 10mM

915   $MgSO_4$. Twelve samples in each plate were serial-diluted 8 times (5-fold dilutions) and plated

916   onto selective KB agar plates, containing 50mg/mL Rifampicin (Sigma-Aldrich, R3501), using

917   96-wells Scienceware® replicator (Sigma-Aldrich). For each 96-wells plate, 2 technical

918   replicates were plated onto separate KB agar plates and incubated at 28°C for 2 days before

919   counting CFUs. For each biologically replicated sample (n=10-12), bacterial CFUs were

920   averaged between two technical replicates and 2-3 serial dilutions. For each plant, bacterial

921   CFUs were normalised to its leaf area (mm^2).

922

923   ***Hpa* resistance assays.**

924   Inoculated shoots were collected at 6 dpi in trypan blue solution (0.067% w/v trypan blue, 33%

925   w/v phenol, 33% v.v glycerol, 33% v.v DL-lactic acid, supplemented with 2 volumes 100%

926   ethanol), boiled for 60 sec, kept at room temperature (RT) for 15 min, boiled again for 30 sec,

927   and incubated at RT for 3 h. Shoots were de-stained in 60% Chloral hydrate (Sigma-Aldrich,

928   23100) for at least 12 h before scoring. Stained leaves were scored under a stereomicroscope

929   by assigning each leaf to one of the 4 different colonisation classes, which are based on distinct

930   stages of *Hpa* pathogenesis: class I: no hyphal colonisation visible; class II: hyphal colonization

931   without conidiophores; class III: hyphal colonization with conidiophore formation; class IV:

932   extensive hyphal colonization with conidiophores and sexual oospores. Resistance scoring was

933   based on ~60 seedlings/population (~240 leaves/ population) for F1 plants and ~20

934   seedlings/population (~80 leaves/population) for F2 plants, representing ~900 leaves/parental

935   stress treatment.

936

937   ***Pc* resistance assays.**

938   After droplet-inoculation of 4 leaves/plant of approximate similar age (see above), plants were

939   kept at 100% RH and monitored daily for disease progression, which appeared as necrotic

940   lesions surrounded by wider chlorotic halos at the sites of inoculation. Fungal colonisation was

941   quantified by average diameter of the tightly defined necrotic lesion area. The time-point of

942   scoring varied between experiments and was determined when average lesion diameters in a

943   subsample of 5-10 individuals from the control group (progeny of mock-inoculated plants) was

944   >3 mm. Four lesions per plant were averaged and used as unit of statistical replication.

945

946   **Salt tolerance assays.**

947   Salt tolerance assays were performed as described previously (Verslues *et al.*, 2006; Claeys *et*

948   *al.*, 2014) with modifications. Seeds were vapour-sterilized, stratified and germinated as

949   described for the seed viability assays. At 5 d after moving the 0.2x MS plates to short-day

950   light conditions, seedlings were transplanted onto new 0.2 x MS plates containing 0 mM, 50

951   mM or 100 mM NaCl. To avoid contact of leaves with the (NaCl-containing) MS agar,

952   seedlings were positioned along a straight line above which the agar was excised. The root tip

953   was marked on the plate as a reference point to determine root growth. At 5 d after

954   transplantation, the length of the newly formed root from the reference point was determined

955   for each individual plant. Salt stress was quantified as the percentage root growth reduction in

956   each individual plant relative to the average root length on 0 mM NaCl agar of the
957   corresponding line.

958

Statistical analysis.

960   Continuous variables were analysed by general linear models. Residuals were first analysed
961   for normal distributions, using Shakiro-Wilk tests and Q-Q plots. If residuals failed to show
962   normal distributions, data were either arcsine-transformed (percentage data), or Box-Cox
963   transformed, using the 'MASS' R package (MASS_7.3-51.5.tar.gz). Models were analysed for
964   heteroscedasticity, using Levene's tests ('car' R package - car_3.0-0.tar.gz-). When variances
965   were confirmed to be homogeneous, effects of parent treatment were tested by ANOVA models
966   ('nlme' R package - nlme_3.1-137.tar.gz). Statistical significance of parental treatment on F1
967   resistance against *P. syringae* (colony forming units; CFU) and *P. cucumerina* (lesion
968   diameters; mm) were determined by two separate models. In addition to ANOVA of pooled F1
969   lines from similarly treated parent plants, using 'aov' function in R base, we performed nested
970   ANOVA with F1 line as random variable, using the 'lme' (method = "REML") and
971   'anova.lme' function (type = "sequential"; adjustSigma = FALSE) from the 'nlme' R package.
972   Similarly, parental effects on F2 resistance were determined by ANOVA of pooled F2
973   populations from the same parent plant, as well as nested ANOVA with F2 population as
974   random variable. When both models indicated a statistically significant effect of parent
975   treatment, Tukey HSD post-hoc tests were performed to identify statistically significant
976   differences between pooled F1 populations from similarly treated parent plants or between
977   pooled F2 populations from the same parental ancestor, using the 'TukeyHSD' function in R
978   base. If data continued to show heteroscedasticity after transformation, parental effects were
979   studied by Welch ANOVA, followed by Games-Howell post-hoc tests, using the
980   'userfriendlyscience' R package (userfriendlyscience_0.7.2.tar.gz). Statistical effects of
981   parental salt treatment on root length (mm) and root length reduction (% relative to 0 mM NaCl
982   treatment) were analysed by two-way ANOVA, in order to separate the effects of parental salt
983   treatment (induction) from progeny salt treatment (challenge). In all cases, type II models were
984   used after having confirmed lack of statistically significant interactions between parent and
985   progeny treatments. The statistical significance of parental effects on root length and root

986   length reduction in F1 plants was determined by 2-way ANOVA of pooled F1 populations

987   from similarly treated parent plants ('aov' function followed by 'Anova' function; type = "II"),

988   as well as 2-way mixed-effect ANOVA with F1 population as random variable ('lmer' function

989   of 'lme4' R package followed by 'Anova' function; type = "II"). Similarly, parental effects on

990   root length and root length reduction in F2 plants were determined by 2-way ANOVA of pooled

991   F2 populations from the same parent plant, as well 2-way mixed-effect ANOVA with F2

992   population as random variable. When both 2-way ANOVA models indicated a statistically

993   significant effect by parental treatment, pooled F1 populations from similarly treated parent

994   plants were analysed for statistically significant differences at each progeny salt concentration

995   (0 mM, 50 mM and 100 mM), using ANOVA followed by Tukey HSD post-hoc tests.

996   Categorical variables (leaf frequencies within *Hpa* colonisation classes) were analysed for

997   statistical differences by Fisher's exact tests. Statistically significant effects by parental

998   treatment were assessed by pooling populations derived from similarly treated parents (F1) or

999   a common parental ancestor (F2) in the cross table. Statistical differences between population

1000   groups were determined by pairwise Fisher's exact tests after Bonferroni multiple testing

1001   correction, using the R package 'fifer' (fifer_1.1.tar.gz).

1002