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The genomic architecture of competitive response of *Arabidopsis thaliana* is highly flexible among plurispecific neighborhoods

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Author contribution statement

F.R., L.A. and D.R. supervised the project. E.B., L.A. and F.R. designed the experiments. E.B. and J.L. conducted the greenhouse experiment. J.L. and E.B. measured the phenotypic traits. C.L. analyzed the phenotypic traits. C.L. performed the GWA mapping. C.L. performed and analyzed the enrichment tests. C.L., D.R. and F.R. wrote the manuscript. All authors contributed to the revisions.

Keywords

plant-plant interactions, *Arabidopsis thaliana*, Genetic Variation, GWAS, Local population, plurispecific interactions

Abstract

Word count: 227

Plants are daily challenged by multiple abiotic and biotic stresses. A major biotic constraint corresponds to competition with other plant species. Although plants simultaneously interact with multiple neighboring species throughout their life cycle, there is still very limited information about the genetics of the competitive response in the context of plurispecific interactions. Using a local mapping population of *Arabidopsis thaliana*, we set up a Genome Wide Association study to estimate the extent of genetic variation of competitive response in 12 plant species assemblages, based on three competitor species (*Poa annua*, *Stellaria media* and *Veronica arvensis*). Based on five phenotypic traits, we detected strong crossing reaction norms not only between the three bispecific neighborhoods, but also among the plurispecific neighborhoods. The genetic architecture of competitive response was highly dependent on the identity and the relative abundance of the neighboring species. In addition, most enriched biological processes underlying competitive responses largely differ among neighborhoods. While RNA related processes might confer a broad range response toolkit for multiple traits in diverse neighborhoods, some processes such as signaling and transport might play a specific role in particular assemblages. Altogether, our results suggest that plants can integrate and respond to different species assemblages depending on the identity and number of each neighboring species, through a large range of candidate genes associated with diverse and unexpected processes leading to developmental and stress responses.

Contribution to the field

Despite the importance of plant-plant interactions on crop yield and plant community dynamics, our understanding of the genetic and molecular bases underlying natural variation of plant-plant interactions is largely limited in comparison to other types of biotic interactions. In addition, most studies reporting the genetic and molecular mechanisms of natural plant-plant interactions are based on bispecific heterospecific interactions. However, in natural communities and crop field, a focal plant rarely interacts with only one neighbor species, thereby strengthening the need to study plant-plant interactions in the context of plurispecific interactions. By setting up a Genome-Wide Association mapping study, the genetic architecture of competitive response of *Arabidopsis thaliana* was found to be highly flexible not only between bispecific neighborhoods, but also between bispecific and plurispecific neighborhoods. In addition, enriched biological processes underlying the competitive response largely differ between bispecific and plurispecific neighborhoods. Our results suggest that plants can integrate and respond to different species assemblages depending on the identity and number of each neighboring species, through a large range of genes associated mainly with RNA related processes, signaling and transport.

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In review

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In review

The genomic architecture of competitive response of *Arabidopsis thaliana* is highly flexible among plurispecific neighborhoods

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14

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19 **Abstract**

20 Plants are daily challenged by multiple abiotic and biotic stresses. A major biotic constraint
21 corresponds to competition with other plant species. Although plants simultaneously interact with
22 multiple neighboring species throughout their life cycle, there is still very limited information about
23 the genetics of the competitive response in the context of plurispecific interactions. Using a local
24 mapping population of *Arabidopsis thaliana*, we set up a Genome Wide Association study to estimate
25 the extent of genetic variation of competitive response in 12 plant species assemblages, based on three
26 competitor species (*Poa annua*, *Stellaria media* and *Veronica arvensis*). Based on five phenotypic
27 traits, we detected strong crossing reaction norms not only between the three bispecific neighborhoods,
28 but also among the plurispecific neighborhoods. The genetic architecture of competitive response was
29 highly dependent on the identity and the relative abundance of the neighboring species. In addition,
30 most enriched biological processes underlying competitive responses largely differ among
31 neighborhoods. While RNA related processes might confer a broad range response toolkit for multiple
32 traits in diverse neighborhoods, some processes such as signaling and transport might play a specific
33 role in particular assemblages. Altogether, our results suggest that plants can integrate and respond to
34 different species assemblages depending on the identity and number of each neighboring species,
35 through a large range of candidate genes associated with diverse and unexpected processes leading to
36 developmental and stress responses.

37 INTRODUCTION

38 ~~Because p~~Plant-plant interactions are recognized as a major factor mediating plant community
 39 structure, diversity and dynamics (Tilman 1985; Goldberg & Barton 1992; Chesson 2000; Martorell &
 40 Freckelton 2014). ~~Thus,~~ deciphering the genetic and molecular bases of plant-plant interactions appears
 41 fundamental to predict the evolutionary dynamics of plant communities in ecological time (Pierik et
 42 al., 2013; Frachon et al., 2017). This is especially relevant in the context of current anthropogenic
 43 modifications of plant assemblages, which may in part result from the intertwined effect of increased
 44 plant biomass and reduced plant diversity under climate warming (Baldwin et al., 2014) or from native
 45 species having different geographical range shifts under climate change (Bachelet et al., 2001; Gilman
 46 et al., 2010; Singer et al., 2013). ~~Furthermore, Because in absence of pesticides, the average potential~~
 47 ~~to reduction of e~~ crop yield ~~by weeds~~ is significantly higher ~~for weeds~~ than ~~by~~ any ~~other~~ crop pests ~~in~~
 48 ~~absence of pesticides~~ (Oerke et al., 2004; Neve et al., 2009). ~~, i~~ Identifying and characterizing the
 49 function of genes underlying ~~crop-weed plant plant~~ interactions appears ~~therefore also~~ fundamental to
 50 accelerate breeding programs aimed at ~~regulating weeds increasing crop competitiveness~~ (Worthington
 51 & Reberg-Horton 2013; Onishi et al., 2018). In addition, in the context of complementarity in using
 52 resources, optimizing species assemblages in crops may be facilitated by the understanding of the
 53 genetics underlying overyielding (Litrico & Violle 2015; Pakeman et al., 2015; Weiner et al., 2017).

54 ~~However, i~~In comparison to other types of biotic interactions such as plant response to virus,
 55 bacteria, fungi, oomycetes and to a lesser extent, herbivores ([Roux & Bergelson 2016](#); [Bartoli & Roux](#)
 56 [2017](#)), there is still very limited information about the genetics associated with natural variation of
 57 plant-plant interactions, i.e. when plants have been directly challenged by other plants ~~(and not in~~
 58 ~~artificial environments simulating plant plant interactions)~~ ([Bergelson & Roux 2010](#); [Bartoli & Roux](#)
 59 [2017](#)). For example, a recent review listed only 47 Quantitative Trait Loci (QTL) mapping studies
 60 (including three Genome Wide Association studies, GWAS) that have been designed to study the
 61 genetic architecture underlying natural variation of plant-plant interactions (Subrahmaniam et al.,
 62 2018). ~~In agreement with other types of biotic interactions (Roux & Bergelson 2016; Bartoli & Roux~~
 63 ~~2017), Natural genetic variation in plant-plant interactions are mainly driven by a complex genetic~~
 64 ~~architecture, ranging from the identification of few medium-effect QTLs to the identification of up to~~
 65 ~~tens of small-effect QTLs (Subrahmaniam et al., 2018). In a heterospecific context, A more than 80%~~
 66 ~~out two thirds~~ of these QTL mapping studies focused on asymmetric interactions (i.e. when one of
 67 the interacting partners benefits at the expense of the other), including [response to parasitic plants and](#)

68 weed suppressive ability mediated by allelopathy ~~and response to parasitic plants~~ (Subrahmaniam et
69 al., 2018). Surprisingly, despite the importance of competition in driving plant community
70 assemblages, only six QTL mapping studies (including two GWAS) focused on competitive
71 interactions in a heterospecific context, i.e. when both interacting species suffer significant cost by
72 investing in competing and therefore compromising on the benefit (Dudley 2015). ~~In agreement with~~
73 ~~other types of biotic interactions (Roux & Bergelson 2016; Bartoli & Roux 2017), plant-plant~~
74 ~~interactions are mainly driven by a complex genetic architecture, ranging from the identification of few~~
75 ~~medium-effect QTLs to the identification of up to tens of small-effect QTLs (Subrahmaniam et al.,~~
76 ~~2018). While informative, most of these QTL mapping studies were based on bispecific heterospecific~~
77 ~~interactions (i.e. one single pair of interacting species; Subrahmaniam et al., 2018). In the first GWAS~~
78 ~~conducted on bispecific heterospecific interactions (Baron et al., 2015), we used 48 natural accessions~~
79 ~~of *Arabidopsis thaliana* from the highly genetically polymorphic French TOU-A local population~~
80 ~~located in a highly competitive habitat (Frachon et al., 2017) and genotyped with a SNP-array of~~
81 ~~214,051 SNPs (Hancock et al., 2011; Horton et al., 2012). These accessions were grown in field~~
82 ~~conditions with four competitor species frequently associated with *A. thaliana* in natural plant~~
83 ~~communities (i.e. *Poa annua*, *Stellaria media*, *Trifolium arvense* and *Veronica arvensis*). Interestingly,~~
84 ~~the genetic architecture was found to be highly flexible between the four competitor species for each~~
85 ~~of the nine phenotypic traits measured on *A. thaliana* plants, including a proxy of total seed production~~
86 ~~(Baron et al., 2015).~~

87 While informative, most QTL mapping studies conducted in a heterospecific context were
88 based on bispecific interactions (i.e. one single pair of interacting species; Subrahmaniam et al., 2018).
89 However, throughout their life cycle, focal plants often interact simultaneously with several
90 neighboring species, either in crop fields or in natural communities (Wilson et al., 2012). This
91 highlights the need to study the genetic architecture underlying plant-plant interactions by considering
92 the response of a focal species to plurispecific interactions. In particular, whether the genetic
93 architecture underlying the response of a focal species in a plurispecific neighborhood corresponds to
94 the sum of QTLs that are specific to the presence of a neighbor species and/or to the emergence of new
95 QTLs, remains an open question (Subrahmaniam et al., 2018). In a first attempt to address this
96 question, a Genome-Environment Association (GEA) analysis was recently set up to finely map
97 genomic regions of the model plant *Arabidopsis thaliana* associated with various plant community
98 descriptors in 145 natural populations of *A. thaliana* located in south-west of France (alpha-diversity,
99 community composition, presence/absence of the 44 most prevalent plant species; Frachon et al.,

2019). The genetic architecture was highly dependent not only on the identity of a specific neighboring species, but also on the diversity and composition of the plant communities inhabited by *A. thaliana* (Frachon *et al.*, 2019). In agreement, the candidate genes underlying QTLs were highly dependent on the assemblage of plant species co-occurring with *A. thaliana* ([Methods in Supplementary Information](#), Supplementary Figure 1), suggesting a flexible genetic architecture between bispecific and plurispecific plant-plant interactions. ~~While informative, a~~ main drawback of GEA studies performed on biotic descriptors is [however](#) related to the confounding effects of abiotic factors such as climate variables and soil physico-chemical properties, thereby advocating for the need for a controlled common garden approach to confirm or not the main conclusions obtained from a GEA approach (Frachon *et al.*, 2019).

In this study, [we extended the study from Baron *et al.* \(2015\) by setting up](#) ~~we set up~~ a GWAS to compare the genetic architecture of competitive response of *A. thaliana* between bispecific and plurispecific neighborhoods. Based on a common garden experiment [performed in greenhouse conditions](#), we first estimated the extent of genetic variation of competitive response in a set of 91 [whole-genome sequenced](#) *A. thaliana* accessions from the ~~highly genetically polymorphic~~ [same](#) French TOU-A local population ~~located in a highly diverse competitive habitat~~ [previously used in Baron *et al.* \(2015\)](#) ~~(Frachon *et al.*, 2017)~~. These accessions were submitted to bispecific and plurispecific competition treatments based on all one-way, two-way and three-way combinations of ~~three species frequently associated with *A. thaliana* in natural plant communities in France, i.e. *Poa annua*, *Sclerotinia media* and *Veronica arvensis*~~. Based on the whole-genome sequences of the 91 accessions, we then run GWA mapping combined with a local score approach to compare the genetic architecture of competitive response of *A. thaliana* between bispecific and plurispecific neighborhoods. Finally, we compared the biological processes over-represented among the candidate genes identified in competitive response among bispecific and plurispecific neighborhoods and discussed the function of some candidate genes.

125

126 MATERIAL AND METHODS

127 Plant material

128 A set of 96 whole-genome sequenced accessions of *A. thaliana* collected in the TOU-A local
129 population (Toulon-sur-Arroux, Burgundy, France, 46°38'53.80"N - 4° 7'22.65"E) were used for the

130 purpose of this study. As previously described in Frachon et al. (2017), the TOU-A population is
131 highly polymorphic at both the phenotypic and genomic levels. Importantly, the very short Linkage
132 Disequilibrium (LD) observed in this population ($r_{0.5}^2 \sim 18\text{bp}$) allows the fine mapping of genomic
133 regions associated with natural variation of phenotypic traits down to the gene level (Brachi et al.,
134 2013; Huard-Chauveau et al., 2013; Frachon et al., 2017; Lonjon et al., 2020; Aoun et al., 2020). These
135 96 accessions were chosen amongst a set of 195 accessions in order to be representative of the genetic
136 diversity observed in the TOU-A population (Platt et al., 2010).

137 Maternal effects of the 96 accessions were reduced by growing one plant of each family for one
138 generation under controlled greenhouse conditions (16-h photoperiod, 20°C) in early 2011 at the
139 University of Lille. Given an estimated selfing rate of ~94% in this population (Platt et al., 2010), the
140 96 accessions were considered as mostly homozygous along the genome.

141 In this study, we used three neighboring species commonly associated with *A. thaliana* in
142 natural plant communities in France and detected in the TOU-A plant community (F. Roux, personal
143 observation). These species are the meadow grass *P. annua* (Poaceae) with a low spreading growth
144 form, the chickweed *S. media* (Caryophyllaceae) and the speedwell *V. arvensis* (Scrophulariaceae)
145 both with a crawling growth form. Seeds for these three species have been obtained from the
146 Herbiseeds company (<http://www.herbiseed.com/home.aspx>).

147

148 **Phenotypic characterization**

149 *Experimental design*

150 An experiment of 4,608 focal plants of *A. thaliana* and 12,672 neighbor plants was set up at the
151 University of Lille 1 (North, France), using a split-plot design arranged as a randomized complete
152 block design (RCBD) with 12 treatments nested within four blocks. These 12 competition treatments
153 correspond to (Figure 1):

154 - one control treatment where *A. thaliana* was grown alone (i.e. absence of interaction; hereafter named
155 treatment A).

156 - 10 interspecific interaction treatments corresponding to the full combination of the three neighboring
157 species *P. annua* (P), *S. media* (S) and *V. arvensis* (V): PPP, SSS, VVV, PPS, PPV, PSS, PVV, SSV,
158 SVV and PSV.

159 - one intraspecific interaction treatment (hereafter named treatment AAA). This treatment was
160 included in the experiment to test whether the differences observed between the treatment where *A.*
161 *thaliana* was grown alone (i.e. treatment A) and the treatments of interspecific interactions were not
162 only due to the presence of a neighbor plant, but were rather dependent on either the identity of the
163 neighboring species or the combination of neighboring species. With respect to the barochorous mode
164 of seed dispersal in *A. thaliana* (Weinig et al., 2006; Wender et al., 2005), the intraspecific interaction
165 treatment corresponds to intra-genotypic interaction.

166 Each 'block x competition treatment' combination was represented by 96 pots (7 cm x 7 cm x
167 7 cm, vol. ~250 cm³; TEKU MQC) filled with damp standard culture soil (Huminsubstrat N3, Neuhaus)
168 and disposed in staggered rows, each pot corresponding to one of the 96 TOU-A accessions. The 17th
169 of January 2013 (day 0), a minimum of five *A. thaliana* seeds were sown in the central position of each
170 pot. For all the treatments (with the exception of treatment A), seeds for neighboring plants were evenly
171 spaced, two cm away from the *A. thaliana* central position (Figure 1). Germination date of *A. thaliana*
172 focal seedlings was daily monitored until six days after sowing. At this time, five accessions had a poor
173 germination rate (between 0% and 6.25%) and were therefore discarded from further analyses. Plants
174 that germinated after 6 days (i.e. 1.16%) were also discarded from further analyses.

175 *A. thaliana* focal seedlings and neighboring seedlings were thinned to one per pot 18 to 20 days
176 after seed sowing. Plants were grown at 20 °C under natural light supplemented by artificial light to
177 provide a 16-hr photoperiod and were top watered without supplemental nutrients. The experience
178 lasted 87 days, from sowing to harvesting of the last plants.

179

180 *Measured phenotypic traits*

181 Three raw phenotypic traits were measured on each focal plant of *A. thaliana* at the time of their
182 flowering (FLO), which was measured as the number of days between germination and flowering dates.
183 The first trait corresponds to the height from the soil to the first flower on the main stem (H1F expressed
184 in mm). H1F is related to seed dispersal (Wender et al., 2005) and shade avoidance (Dorn et al., 2000)
185 in *A. thaliana*. The two other traits were used as proxies of resources accumulation. The maximum
186 diameter of the rosette was measured at the nearest millimeter (DIAM; Weinig et al., 2006). This trait
187 is a proxy of the growth of the rosette of the focal plant from germination to flowering. The above-
188 ground dry biomass (BIOMASS, expressed in grams, with a precision down to the tenth of a milligram)
189 was estimated by drying the aboveground portion for 48H at 60°C. We additionally quantified the

190 strategy adopted by *A. thaliana* in response to neighboring plants by calculating the ratio HD as the
 191 height of the first flower on the rosette diameter (i.e. H1F/DIAM). Values of HD below and above 1
 192 would correspond to an aggressive and escape strategy, respectively (Baron et al., 2015).

193 [Although all plants bolted at the end of the experiment \(i.e. 87 days after sowing\)](#). Plants that had
 194 not flowered [87 days after sowing at this time](#) (i.e. 1.15%) were assigned a flowering date value of 87.
 195 H1F and HD values were therefore not available for these plants. [All raw phenotypic data are available](#)
 196 [in Dataset 1](#).

197

198 **Statistical analysis**

199 *Exploring natural variation of plant-plant interactions at different levels of complexities*

200 Using the *lmer* function implemented in the *R* library [lmerTest \(Kuznetsova et al., 2017\)](#), the
 201 following mixed model was used to explore the genetic variation of response among the 91 TOU-A
 202 accessions [\(Methods in Supplementary Information\)](#):

$$203 \quad Y_{ijkl} = \mu_{trait} + \text{block}_i + \text{treatment}_j + \text{block}_i \times \text{treatment}_j + \text{accession}_k + \text{treatment}_j \\ 204 \quad \times \text{accession}_k + \text{germ}_l + \varepsilon_{ijkl}$$

205 where ‘Y’ is one of the phenotypic traits scored on focal *A. thaliana* plants, ‘μ’ is the overall phenotypic
 206 mean; ‘block’ accounts for differences in micro-environment among the four experimental blocks;
 207 ‘treatment’ corresponds to effect of the 12 treatments (A, AAA, PPP, PPS, PPV, PSS, PSV, PVV, SSS,
 208 SSV, SVV and VVV); ‘accession’ measures the effect of the 91 accessions; the interaction term
 209 ‘treatment x accession’ accounts for genetic variation in reaction norms across the 12 treatments; the
 210 term ‘germ’ is a covariate accounting for natural variation for the germination date between the 91
 211 accessions; and ‘ε’ is the residual term. All factors were treated as fixed effects [because levels of no](#)
 212 [factor were random samples from a population to which we intended to extrapolate. Given the split-](#)
 213 [plot design used in this study, the only exception was the ‘block x treatment’ term that was treated as](#)
 214 [random, thereby allowing to use its variance as the error term for testing the ‘block’ and ‘treatment’](#)
 215 [effects. The residual variance of the term ‘ε’ was used for the calculation of the *F*-values for the terms](#)
 216 [‘accession’ and ‘treatment x accession’.](#)

217 ~~For the calculation of F -values, terms were tested over their appropriate denominators. Given the~~
218 ~~split plot design used in this study, the variance associated with ‘block x treatment’ was used as the~~
219 ~~error term for testing the ‘block’ and ‘treatment’ effects.~~

220 For each treatment, estimated marginal means (EMM) were obtained for each accession using
221 the function *emmeans* implemented in the *R* library *emmeans* (Lenth et al., 2018) (Methods in
222 [Supplementary Information](#)). Because *A. thaliana* is a highly selfing species (Platt et al., 2010), EMM
223 correspond to genotypic values of accessions.

224 ~~To test whether the five phenotypic traits were not redundant, we estimated for each treatment~~
225 ~~the genetic correlation for each pair of traits, by calculating the correlation coefficient of Pearson based~~
226 ~~on genotypic values as described above.~~

227 Using the function *marker_h2_means* implemented in the *R* library *heritability* (Kruijer et al.,
228 [2016](#)), narrow-sense heritability of each phenotypic trait (h^2_{trait}) was estimated by fitting genotypic
229 values of accessions against the corresponding kinship matrix estimated on the whole set of 1,692,194
230 SNPs detected among the 91 accessions (Methods in Supplementary Information). [This set of SNPs](#)
231 [was obtained from the whole-genome sequencing of 195 accessions of the TOU-A population,](#)
232 [including the 91 accessions used in this study \(Frachon et al., 2017\).](#)

233

234 ~~[Detecting the presence of crossing reaction norms](#)~~ *Genetic correlations*

235 ~~For each phenotypic trait, we estimated the strength of ‘accession x treatment’ interactions by~~
236 ~~estimating across-environment genetic correlations for each pairwise treatment combination. Crossing~~
237 ~~reaction norms requires that (i) different accessions show different slopes across environments (with~~
238 ~~the extreme case of significant differences on the sign of the slope across environments), and (ii) the~~
239 ~~ranking of accessions largely differs between the two environments. This pattern corresponds to the~~
240 ~~cross-over effect (either symmetrical, or asymmetrical) (Lacaze et al., 2009). In order to estimate the~~
241 ~~strength of crossing reaction norms for each phenotypic trait, we estimated across-environment genetic~~
242 ~~correlations for each pairwise treatment combination~~ *Genetic correlations were estimated by*
243 *calculating the correlation coefficient of Pearson based on genotypic values (by using the *cor.test**
244 *function implemented in the *R* environment).* Significant crossing reaction norms were detected by
245 testing whether 95% confidence intervals of Pearson’s r were not overlapping with the value of 1.

246 ~~To test whether the five phenotypic traits were not redundant, we estimated for each treatment~~
 247 ~~the genetic correlation for each pair of traits, by calculating the correlation coefficient of Pearson-based~~
 248 ~~on genotypic values as described above.~~

249 **Genome-wide association mapping combined with a local score approach**

250 The effects of population structure on phenotype-genotype associations has been demonstrated
 251 to be limited in the TOU-A population (Brachi et al., 2013; Baron et al., 2015). Nevertheless, GWA
 252 mapping was run using a mixed-model approach implemented in the software Genome-wide Efficient
 253 Mixed Model Association (GEMMA, Zhou & Stephens 2012). This model includes a genetic kinship
 254 matrix as a covariate to control for the effect of the demographic history of the TOU-A population. In
 255 this study, we discarded SNPs with more than 17 missing values across the 91 accessions. In addition,
 256 because rare alleles may lead to an inflation of low p -values (Atwell et al., 2010; Brachi et al., 2010),
 257 we only considered SNPs with a minor allele relative frequency (MARF) $> 10\%$, leaving us with
 258 630,234 SNPs. ~~Because phenotypic traits were scored at the time of flowering time,~~ GEMMA was run
 259 independently on H1F, DIAM, HD and BIOMASS for each of the 12 treatments. H1F, DIAM, HD and
 260 BIOMASS were genetically correlated with FLO (mean value \pm standard errors across the 12
 261 treatments, BIOMASS: Pearson's $r = 0.768 \pm 0.043$, DIAM: Pearson's $r = 0.360 \pm 0.133$, H1F:
 262 Pearson's $r = -0.434 \pm 0.085$, HD: Pearson's $r = -0.625 \pm 0.015$). by including FLO genotypic values
 263 were thus included as a covariate in GEMMA analyses, thereby allowing controlling for putative
 264 effects of a confounding developmental stage (Uffelmann et al., 2021).

265 Thereafter, we implemented a local score approach on the set of p -values provided by GEMMA.
 266 The local score allows accumulating the statistical signals from contiguous genetic markers in order to
 267 detect significant genomic regions associated with phenotypic natural variation (Fariello et al., 2017).
 268 In a given QTL region, the association signal, through the p -values, will cumulate locally due to linkage
 269 disequilibrium between SNPs, which will then increase the local score (Bonhomme et al., 2019). The
 270 tuning parameter ξ was fixed at 2 (Bonhomme et al., 2019). Significant SNP-phenotype associations
 271 were identified by estimating a chromosome-wide significance threshold for each chromosome
 272 (Bonhomme et al., 2019).

273

274

275 **Identification of candidate genes associated with response to monospecific and plurispecific**
 276 **interactions**

277 To identify candidate genes associated with plant-plant interactions, we retrieved genes around
 278 significant zones identified by the local score approach. Using a custom script, we selected all genes
 279 inside the zones ~~and also as well as~~ the first gene upstream and the first gene downstream of these zones.
 280 The TAIR 10 database (<https://www.arabidopsis.org/>) was used as our reference, leaving us with 4,524
 281 unique candidate genes ($N_{\text{BIOMASS}} = 1,538$; $N_{\text{DIAM}} = 1,008$; $N_{\text{HIF}} = 1,559$; $N_{\text{HD}} = 1,572$; Supplementary
 282 Figure 2).

283 To identify the biological processes involved in response to the different neighborhoods for the
 284 different traits, we submitted the candidate gene list to the classification supervisor tool
 285 (http://bar.utoronto.ca/ntools/cgi-bin/ntools_classification_supervisor.cgi, Provart & Zhu 2003) to
 286 identify the biological processes and MapMan processes significantly over-represented ($P < 0.05$).

287

288 **RESULTS**

289 **Extent of natural genetic variation of response to bispecific and plurispecific interactions**

290 Highly significant genetic variation was found across the 12 treatments (Figure 1) for the five
 291 phenotypic traits scored on focal *A. thaliana* plants (Table 1, [Supplementary Table 1](#)). Similar results
 292 were observed without considering the treatments where *A. thaliana* was grown alone or with clones
 293 (Supplementary Tables [21](#) and [32](#)). Narrow-sense heritability SNP-based estimates were significant
 294 for 96.7% of the 60 ‘phenotypic trait * treatment’ combinations (Supplementary Table [43](#)) and ranged
 295 from 0.36 to 0.99 (mean = 0.72, median = 0.76), suggesting that a large fraction of the phenotypic
 296 variation observed within each treatment was driven by genetic differences among the local *A. thaliana*
 297 accessions (Figure 1). ~~Interestingly, heritability estimates were significantly negatively associated with~~
 298 ~~the number of species surrounding *A. thaliana* (linear regression, $t = -2.564$, $P = 0.01322$,~~
 299 ~~[Supplementary Figure 3](#)).~~

300 Importantly, as evidenced by highly significant ‘Treatment x Accession’ interactions, a strong
 301 genetic variation of reaction norms was found for the five phenotypic traits, with or without considering
 302 the treatments where *A. thaliana* was grown alone or with clones (Table 1, Supplementary Tables [1](#),

303 [24](#) and [32](#)). For the phenotypic traits DIAM, H1F, HD and BIOMASS, across-environment genetic
 304 correlations ranged from 0.06 to 0.97 (mean = 0.69, median = 0.73; Figure 2), indicating that the
 305 ranking of accessions for a given phenotypic trait largely differed among the 12 treatments. This pattern
 306 of crossing reaction norms is well illustrated for the height from the soil to the first flower for which
 307 the mean value was similar among the 12 treatments (Figure 1, Table 1, [Supplementary Table 1](#)). In
 308 contrast, across-environment genetic correlations for FLO were very close to the unity (min = 0.91,
 309 max = 0.97, mean = 0.95, median = 0.95; Figure 2), suggesting that the ranking of flowering time was
 310 similar among the 91 accessions across the 12 treatments. FLO was therefore discarded from further
 311 analyses.

312 For each treatment, genetic correlations among the four remaining phenotypic traits were
 313 significantly different from the unity (absolute values of Pearson's r : min = 0.02, max = 0.89, mean =
 314 0.53, median = 0.62), suggesting that the traits scored in this study partly behave independently
 315 (Supplementary Figure [34](#)).

316 Genetic architecture revealed by GWA mapping combined with a local score approach

317 To compare the genetic architecture underlying the competitive response of *A. thaliana*
 318 between bispecific and plurispecific neighborhoods, we used GWA mapping based on 630,234 SNPs
 319 (i.e. 1 SNP every 189bp) combined with a local score approach (Bonhomme et al., 2019). This
 320 combination of approaches was demonstrated to be efficient in the TOU-A population, allowing a fine
 321 description of the genetic architecture (in particular, the detection of QTLs with small effects)
 322 associated with quantitative disease resistance to the bacterial pathogen *Ralstonia solanacearum* and
 323 the cloning of [four](#) of the detected QTLs (Aoun et al., 2020; [Demirjian et al., 2021](#)).

324 The genetic architecture associated with natural variation of the traits DIAM, H1F, HD and
 325 BIOMASS was polygenic with a number of detected QTLs ranging from 13 for BIOMASS in the SVV
 326 treatment to 72 for H1F in the A treatment (mean = 32, median = 28) (Supplementary Table [54](#)). Across
 327 the 12 treatments, the mean number of detected QTLs was not significantly different between DIAM,
 328 H1F, HD and BIOMASS (Supplementary Table [54](#)).

329 For each phenotypic trait, the genetic architecture of competitive response of *A. thaliana* was
 330 highly flexible among the 12 treatments. Firstly, the genetic architecture of competitive response of *A.*
 331 *thaliana* to bispecific interactions was highly dependent on the identity of the neighboring species
 332 (Figure 3). For example, no significant QTL region was shared between the treatments PPP and SSS

333 for H1F or between the treatments PPP and SSS for HD (Figure 3). Secondly, the genetic architecture
 334 of competitive response of *A. thaliana* was also highly dependent on the number of species co-
 335 occurring with *A. thaliana*. For instance, two very neat peaks of association were identified for HD in
 336 the middle of chromosome 3 in the treatment PSV (Figure 4A). While the first peak was not identified
 337 in the treatments PPP, SSS and VVV, the second peak was identified in the treatment PPP but with a
 338 much lower association score (PSV: Lindley process value = 214, PPP: Lindley process value = 9.5).
 339 Thirdly, the genetic architecture of competitive response of *A. thaliana* to the presence of a specific
 340 pair of neighboring species largely differed not only from the genetic architecture identified in the
 341 corresponding bispecific interaction treatments, but also between the two related assemblages. As an
 342 illustration, we detected a neat association peak for H1F at the beginning of chromosome 1 in the
 343 treatment PSS (i.e. one *P. annua* individual + two *S. media* individuals), but neither in the treatment
 344 PPS (i.e. two *P. annua* individuals + one *S. media* individual), nor in the treatments PPP and SSS
 345 (Figure 4B).

346 From these QTLs, we retrieved 4,524 unique genes located within and in the vicinity of 1kb of
 347 the significant QTL regions detected for each ‘phenotypic trait x treatment’ (Dataset [24](#)). In agreement
 348 with the values of genetic correlations between these four traits (Supplementary Figure [43](#)), a large
 349 fraction of candidate genes was specific to a given treatment (BIOMASS: 77.4%, DIAM: 63.4%, H1F:
 350 77.2%, HD: 70.6%), suggesting that the genetic bases are largely distinct among the 12 treatments
 351 (Figure 5A, Supplementary Figure [45](#)). Therefore, in this study, the genetic architecture underlying
 352 intraspecific variability of the competitive response largely depends on both the composition and
 353 assemblage of the neighborhood of *A. thaliana*.

354

355 **Identification of enriched biological processes**

356 Across the 48 ‘phenotypic trait x treatment’ combinations, we identified 139 significantly over-
 357 represented gene ontology (GO, MapMan functional annotation) terms, which belong to 25 unique GO
 358 processes (Dataset [32](#)). Interestingly, RNA related processes correspond to the most abundant GO term
 359 over-represented found in 28 ‘phenotypic trait x treatment’ combinations, with eight treatments for
 360 BIOMASS, seven treatments for DIAM and HD and six treatments for H1F (Figure 5, Supplementary
 361 Figure [56](#), Dataset [32](#)). The second most represented GO terms are transport and minor CHO
 362 metabolism processes with 13 and 12 ‘phenotypic trait x treatment’ combinations, respectively (Figure

363 5, Supplementary Figure 56, Dataset 32), but with different distribution across traits. In one hand, the
 364 transport processes were represented in the four traits, while minor CHO metabolism processes was
 365 not found for BIOMASS in any of the 12 treatments. Conversely, C1-metabolism processes were
 366 mostly enriched for BIOMASS in six treatments out of the seven ‘phenotypic trait x treatment’
 367 combinations identified for these processes (Supplementary Figure 56). The remaining enriched
 368 MapMan processes are related to tetrapyrrole synthesis, miscellaneous, protein, redox, secondary
 369 metabolism, stress, lipid metabolism, hormone metabolism, cell, photosystem, signaling and not
 370 assigned genes (Figure 5, Supplementary Figure 56, Dataset 32).

371

372 DISCUSSION

373 What are the causes and consequences of variation in competitive ability is a long standing
 374 question in studies on the dynamics of structure and diversity of plant communities (Aarssen, 1992).
 375 Some experimental and theoretical studies suggest that intraspecific genetic variation in competitive
 376 response and effect in a heterospecific context can promote maintenance of within-species genetic
 377 variation and species coexistence at a fine spatial scale (Aarssen, 1992; Fridley et al., 2007). This may
 378 originate from the rank order of competitive ability among genotypes/species shifting from transitive
 379 to intransitive, depending in the identity of the genotypes of both species (Bossdorf et al. 2009; Baron
 380 et al., 2015). On the other hand, other theoretical studies predict that variation among genotypes may
 381 negatively affect species coexistence. For instance, an extension of intraspecific niche variation due to
 382 genetic variation in resources requirements would reduce niche differentiation among species (Hart et
 383 al., 2016). In addition, plant-plant interactions might constrain life history evolution of a focal species,
 384 thereby limiting its evolutionary potential to respond to other environmental challenges than plant-
 385 plant interactions (Wilson, 2014). While informative, most of these studies consider the interactions
 386 between two plants species. However, ~~Because~~ a focal ~~species~~plant rarely interacts with only one
 387 neighboring species either in crop fields or in more natural environments (up to 89 species, Wilson et
 388 al., 2012).~~†~~The genetics of plant-plant interactions and their consequences on the dynamics of plant
 389 coexistence need therefore to be studied in a community context. In this study, we compared the genetic
 390 architecture of the competitive response of *A. thaliana* between bispecific and plurispecific
 391 neighborhoods on a highly genetically polymorphic local population of *A. thaliana* known to interact
 392 *in situ* with the three neighboring species considered in this study.

393

394 ~~A flexible genetic architecture for competitive response among bispecific and plurispecific~~
 395 ~~neighborhoods~~ Extensive crossing reaction norms among diverse plant assemblages within the
 396 local population TOU-A

397 Several studies reported extensive genetic variation of the competitive ability of *A. thaliana* in
 398 the context of pairwise heterospecific interactions, both at the worldwide and local scales (Bossdorf et
 399 al., 2009; Brachi et al., 2012; Bartheleimer et al., 2015; Baron et al., 2015; Frachon et al., 2017; Frachon
 400 et al., 2019, Palacio-Lopez et al., 2020). Based on several phenotypic traits related to resource
 401 accumulation and life-history traits such as traits related to seed dispersal (Reboud et al., 2004; Wender
 402 et al., 2005), we also found extensive local genetic variation of the competitive responseability of *A.*
 403 *thaliana* in all the plurispecific neighborhoods tested in this study. More importantly, we detected
 404 strong crossing reaction norms not only among the three bispecific interaction treatments, but also
 405 among the different plurispecific neighborhoods ~~plant assemblages~~ surrounding the focal *A. thaliana*
 406 accessions. Altogether, these results suggest that the simultaneous interactions of *A. thaliana* with
 407 several plant partners can promote maintenance of the high genetic diversity observed in the TOU-A
 408 local population (i.e. only 5.6 times less than observed in a panel of 1,135 worldwide accessions)
 409 (Frachon et al., 2017). This diversity may in turn confer a high potential for *A. thaliana* to respond to
 410 future modifications of the assemblages in the TOU-A plant community.

411 Testing whether the presence of genetic variation in competitive response in plurispecific
 412 neighborhoods can promote species coexistence in the TOU-A plant community would require several
 413 additional phenotyping and analyses. For instance, it would be interesting to test for the presence of
 414 genetic variation in competitive effect of *A. thaliana* on the diverse competitor assemblages used in
 415 this study, thereby allowing testing whether a potential shift between transitivity and intransitivity
 416 depending on the identity of the accession of *A. thaliana* could vary between monospecific and
 417 plurispecific neighborhoods. In addition, while we analyzed separately the phenotypic traits measured
 418 in this study, it may be worthwhile to analyze them simultaneously in order to test whether the direct
 419 and indirect gradients of selection acting on the phenotypic traits depend on the number of competitor
 420 species and/or plant assemblage (Baron et al., 2015; Palacio-Lopez et al., 2020). In the context of major
 421 ecological strategies (i.e. Grime's Competitive-Stress-tolerant-Ruderal CSR theory; Grime, 1974;
 422 Grime, 1977), this may in turn help to test the importance of plasticity of trait syndromes in shifting
 423 ecological strategies among diverse plant assemblages (Takou et al., 2019). While *A. thaliana* is still

424 [considered as a pioneer species with low competitive abilities and a typical ruderal strategy, several](#)
 425 [recent studies revealed that *A. thaliana* can inhabit highly competitive environments over several](#)
 426 [generations \(Brachi et al., 2013; Bartoli et al., 2018; Frachon et al., 2019\).](#)

427 **A flexible genetic architecture for competitive response among bispecific and plurispecific**
 428 **neighborhoods**

429 In agreement with the strong crossing reaction norms observed among the three bispecific
 430 interaction treatments, a GWA mapping approach revealed that the genetic architecture of *A. thaliana*
 431 competitive response in bispecific neighborhoods was highly dependent on the identity of the neighbor
 432 species. Similar results were observed (i) in *A. thaliana* challenged with four neighboring species
 433 (including the three species used in this study) in field conditions (Baron et al., 2015), and (ii) in *Oryza*
 434 *sativa* challenged with the three weed species *Echinochloa oryzicola*, *Monochoria korsakowii* and
 435 *Schoenoplectus juncooides* in greenhouse conditions (Onishi et al., 2018). Altogether, these results
 436 suggest that the effect of the identity of the neighboring species on the genetic architecture of
 437 competitive response is conserved among focal plant species and across diverse phenotyping
 438 environments. The genetic architecture of *A. thaliana* competitive response was also highly flexible
 439 between bispecific and plurispecific neighborhoods, suggesting that the genetic response of a particular
 440 accession of *A. thaliana* to a plurispecific neighborhood can be hardly predicted from the observation
 441 of its genetic responses in the corresponding bispecific neighborhoods. These results confirm our
 442 previous conclusions obtained from a GEA approach performed on 145 whole-genome sequenced
 443 natural populations of *A. thaliana* located in south-west of France and described for various plant
 444 community descriptors (Frachon et al., 2019). Our results are also in line with previous [studies](#)
 445 reporting in *A. thaliana* (i) the limited overlap between QTLs underlying resistance to combined stress
 446 responses, such as drought plus the specialist herbivore *Pieris rapae*, and QTLs underlying resistance
 447 to the corresponding single stress response (Davila-Olivas et al., 2017⁶; Thoen et al., 2017⁶), and (ii)
 448 the distinct transcriptomic responses for single and combined stresses in diverse plant species, as
 449 illustrated for pathogen attack combined with heat stress (Suzuki et al., 2014; Dessaint et al., 2021).

450 In this study, three non-exclusive and interconnected hypotheses can be proposed to explain the
 451 emergence of new QTLs in plurispecific neighborhoods. They are based on (i) the putative generation
 452 of new signals or elimination/modification of pre-existing signals (e.g. light, aerial volatile organic
 453 compounds, root exudates and nutrient availability) in the context of plurispecific interactions, and (ii)
 454 their specific perception and subsequent signaling in *A. thaliana*. Firstly, the amount of some signals

455 produced by the neighboring species A is strongly reduced in a plurispecific neighborhood, thereby
456 leading to the strong reduction of the perception/signaling events activated in the context of bispecific
457 interactions. Secondly, the production of new or modified signals in a given neighboring species is
458 triggered by the presence of another neighboring species. Thirdly, the simultaneous presence of
459 different signals produced by the neighboring species A and B leads to the generation of new signaling
460 processes. The two latter cases correspond to the production of new signals that emerged from higher-
461 order interactions among the neighboring species (Levine et al., 2017).

462

463 **Biological pathways and candidate genes associated with competitive response depend on the** 464 **identity and assemblage of neighboring species**

465 While our experimental design identified a large set of genes (4,524 genes), we clearly observed
466 RNA process as a significant enriched biological process for most of the ‘phenotypic trait x treatment’
467 investigated, including RNA processing, RNA binding and a large variety of transcription factors, with
468 a more pronounced proportion of BHLH and homeobox genes. These transcription factors are known
469 to regulate multiple developmental processes, including stress and light response ([Bürglin Bürglin](#)
470 [Bürglin](#) & Affolter, [20162015](#); Guo et al., 2021) and might exert a crucial role for promoting plant
471 adaptation to diverse plant environments.

472 The second major over-represented biological process interaction category is related to
473 transport functions. Interestingly, 14 out of the 79 transport related proteins, identified in the seven
474 treatments for [ratio HD corresponding to the height of the first flower divided by rosette diameter](#)~~HD~~
475 ~~trait~~, correspond to ABC (ATP-binding cassette) transporter proteins (Dataset [2+](#)). Although none of
476 them have been functionally characterized, these transporters have been recently proposed as key
477 players of plant adaptation to their abiotic or biotic environment (Hwang et al., 2016). Interestingly,
478 different ABC transporter genes were identified in the case of bispecific interactions (ABC-B2 and
479 G13) as compared to plurispecific interactions (ABC-C3, C6, C7 and G26). Because of their diverse
480 substrate specificities, they might constitute essential components of perception/signaling pathways
481 activated during plurispecific plant-plant interactions. Among transport processes, nutrient foraging
482 might also be a major response strategy in the context of plurispecific plant-plant interactions (Pierik
483 et al., 2013).

484 Our observations, if considering all the enriched biological processes found in this study, are
485 partially in agreement with the previously reported seven categories of functions identified in artificial
486 environments simulating plant–plant interactions: photosynthesis, hormones, cell wall modification
487 and degradation, defense against pathogens, ABC transporters, histone modification and meristem
488 identity/life history traits (Subrahmaniam et al., 2018). However, transcriptional regulation appears
489 here as a major previously unidentified process, in line with a highly flexible genetic reprogramming
490 in response to different plant assemblage environments.

491 In conclusion, our results suggest that plants can integrate and respond to different species
492 assemblages depending on the identity and number of each neighboring species, through a large range
493 of genes associated mainly with transcription adjustment (RNA process) leading to developmental and
494 stress responses. Our experiment also highlights that intraspecific variation in response to plant induced
495 constraints, is mainly due to processes not proposed to be involved in plant-plant communications (i.e.
496 light, nutrients, volatile organic compounds and root exudates). Complementarily to our findings in
497 our GWA study, transcriptomic and proteomic analyses of *A. thaliana* plants exposed to monospecific
498 and plurispecific neighborhoods would help to identify genes and proteins that are differentially
499 regulated under these conditions. To our knowledge, no such studies comparing global changes in
500 protein and gene expression between bispecific and plurispecific neighborhoods have been reported so
501 far (Subrahmaniam et al., 2018). Another step to understand the mechanisms underlying natural
502 variation of plant-plant competitive responses would be (i) to functionally validate the identified
503 candidate genes. This would open the way to functional analyses to investigate (ii) the nature of the
504 signals perceived by the plant, and (iii) decipher the signaling pathways resulting from signal
505 perception, leading to the plant response.

506 **References**

- 507 [Aarssen, L.W. \(1992\). Causes and consequences of variation in competitive ability in plant communities.](#)
 508 [Journal of Vegetation Science, 3, 165-174.](#)
- 509 Aoun, N., Desaint, H., Boyrie, L., Bonhomme, M., Deslandes, L., Berthomé, R., & Roux, F. (2020). A complex
 510 network of additive and epistatic quantitative trait loci underlies natural variation of *Arabidopsis thaliana*
 511 quantitative disease resistance to *Ralstonia solanacearum* under heat stress. *Molecular Plant Pathology*,
 512 21, 1405-1420. <https://doi.org/10.1111/mpp.12964>
- 513 Atwell, S., Huang, Y. S., Vilhjálmsson, B. J., Willems, G., Horton, M., Li, Y., ... & Nordborg, M. (2010).
 514 Genome-wide association study of 107 phenotypes in *Arabidopsis thaliana* inbred lines. *Nature*, 465(7298),
 515 627-631.
- 516 Bachelet, D., Neilson, R. P., Lenihan, J. M., & Drapek, R. J. (2001). Climate change effects on vegetation
 517 distribution and carbon budget in the United States. *Ecosystems*, 4(3), 164-185.
- 518 Baldwin, A. H., Jensen, K., & Schönfeldt, M. (2014). Warming increases plant biomass and reduces diversity
 519 across continents, latitudes, and species migration scenarios in experimental wetland communities. *Global*
 520 *Change Biology*, 20(3), 835-850.
- 521 Bardou, P., Mariette, J., Escudié, F., Djemiel, C., & Klopp, C. (2014). jvenn: an interactive Venn diagram
 522 viewer. *BMC Bioinformatics*, 15(1), 1-7.
- 523 Baron, E., Richirt, J., Villoutreix, R., Amsellem, L., & Roux, F. (2015). The genetics of intra-and interspecific
 524 competitive response and effect in a local population of an annual plant species. *Functional Ecology*, 29(10),
 525 1361-1370.
- 526 Bartelheimer, M., Schmid, C., Storf, J., Hell, K., & Bauer, S. (2015). Interspecific competition in *Arabidopsis*
 527 *thaliana*: a knowledge gap is starting to close. *In Progress in botany* (pp. 303-319). Springer, Cham.
- 528 [Bartoli, C., Frachon, L., Barret, M., Rigal, M., Huard-Chauveau, C., Mayjonade, B., Zanchetta, C., Bouchez,](#)
 529 [O., Roby, D., Carrère, S. & Roux, F. \(2018\). In situ relationships between microbiota and potential](#)
 530 [pathobiota in *Arabidopsis thaliana*. *The ISME Journal*, 12, 2024-2038.](#)
- 531 Bartoli, C., & Roux, F. (2017). Genome-wide association studies in plant pathosystems: toward an ecological
 532 genomics approach. *Frontiers in Plant Science*, 8, 763.

533

- 534 ~~Bergelson, J., & Roux, F. (2010). Towards identifying genes underlying ecologically relevant traits in~~
535 ~~*Arabidopsis thaliana*. *Nature Reviews Genetics*, 11(12), 867-879.~~
- 536 Bonhomme, M., Fariello, M. I., Navier, H., Hajri, A., Badis, Y., Miteul, H., ... & Pilet-Nayel, M. L. (2019). A
537 local score approach improves GWAS resolution and detects minor QTL: application to *Medicago*
538 *truncatula* quantitative disease resistance to multiple *Aphanomyces euteiches* isolates. *Heredity*, 123(4),
539 517-531.
- 540 Bossdorf, O., Shuja, Z., & Banta, J. A. (2009). Genotype and maternal environment affect belowground
541 interactions between *Arabidopsis thaliana* and its competitors. *Oikos*, 118(10), 1541-1551.
- 542 Brachi, B., Aimé, C., Glorieux, C., Cuguen, J., & Roux, F. (2012). Adaptive value of phenological traits in
543 stressful environments: predictions based on seed production and laboratory natural selection. *PLoS*
544 *One*, 7(3), e32069.
- 545 Brachi, B., Villoutreix, R., Faure, N., Hautekèete, N., Piquot, Y., Pauwels, M., ... & Roux, F. (2013).
546 Investigation of the geographical scale of adaptive phenological variation and its underlying genetics in
547 *Arabidopsis thaliana*. *Molecular Ecology*, 22(16), 4222-4240.
- 548 Bürglin, T. R., & Affolter, M. (2016). Homeodomain proteins: an update. *Chromosoma*, 125(3), 497-521.
- 549 Chesson, P. (2000). Mechanisms of maintenance of species diversity. *Annual review of Ecology and*
550 *Systematics*, 31(1), 343-366.
- 551 Conway, J. R., Lex, A., & Gehlenborg, N. (2017). UpSetR: an R package for the visualization of intersecting
552 sets and their properties. *Bioinformatics*.
- 553 Davila-Olivas, N. H., Kruijer, W., Gort, G., Wijnen, C. L., van Loon, J. J., & Dicke, M. (2017). Genome-wide
554 association analysis reveals distinct genetic architectures for single and combined stress responses in
555 *Arabidopsis thaliana*. *New Phytologist*, 213(2), 838-851.
- 556 [Demirjian, C., Razavi, N., Desaint, H., Lonjon, F., Génin, F., Roux, F., Berthomé, R., & Vaillau F. \(2021\).
557 Study of natural diversity in response to a key pathogenicity regulator of *Ralstonia solanacearum* reveals
558 new susceptibility genes in *Arabidopsis thaliana*. DOI: 10.1111/mpp.13135](#)
- 559 Desaint, H., Aoun, N., Deslandes, L., Vaillau, F., Roux, F. and Berthomé, R. (2021), Fight hard or die trying:
560 when plants face pathogens under heat stress. *New Phytologist*, 229, 712-734.
561 <https://doi.org/10.1111/nph.16965>
- 562 Dorn, L. A., Pyle, E. H., & Schmitt, J. (2000). Plasticity to light cues and resources in *Arabidopsis thaliana*:
563 testing for adaptive value and costs. *Evolution*, 54(6), 1982-1994.

- 564 Dudley, S. A. (2015). Plant cooperation. *AoB Plants*, 7:[pv1113](#).
- 565 Fariello, M. I., Boitard, S., Mercier, S., Robelin, D., Faraut, T., Arnould, C.,... & SanCristobal, M. (2017).
566 Accounting for linkage disequilibrium in genome scans for selection without individual genotypes: the local
567 score approach. *Molecular Ecology*, 26(14), 3700-3714.
- 568 Frachon, L., Libourel, C., Villoutreix, R., Carrère, S., Glorieux, C., Huard-Chauveau, C., ... & Roux, F. (2017).
569 Intermediate degrees of synergistic pleiotropy drive adaptive evolution in ecological time. *Nature Ecology*
570 & *Evolution*, 1(10), 1551-1561.
- 571 Frachon, L., Mayjonade, B., Bartoli, C., Hautekèete, N. C., & Roux, F. (2019). Adaptation to plant communities
572 across the genome of *Arabidopsis thaliana*. *Molecular biology and evolution*, 36(7), 1442-1456.
- 573 ~~Gautier, M. (2015). Genome wide scan for adaptive divergence and association with population specific~~
574 ~~covariates. *Genetics*, 201(4), 1555-1579.~~
- 575 [Fridley, J.D., Grime, J.P. & Bilton, M. \(2007\). Genetic identity of interspecific neighbors mediates plant](#)
576 [responses to competition and environmental variation in a species-rich grassland. *Journal of Ecology*, 95,](#)
577 [908-9&\(.](#)
- 578 Gilman, S. E., Urban, M. C., Tewksbury, J., Gilchrist, G. W., & Holt, R. D. (2010). A framework for community
579 interactions under climate change. *Trends in Ecology & Evolution*, 25(6), 325-331.
- 580 Goldberg, D. E., & Barton, A. M. (1992). Patterns and consequences of interspecific competition in natural
581 communities: a review of field experiments with plants. *The American Naturalist*, 139(4), 771-801.
- 582 [Grime, J.P. \(1974\). Vegetation classification by reference to strategies. *Nature*, 250, 26-31.](#)
- 583 [Grime, J.P. \(1977\). Evidence for the existence of three primary strategies in plants and its relevance to ecological](#)
584 [and evolutionary theory. *The American Naturalist*, 111, 1169-1194.](#)
- 585 Guo, J., Sun, B., He, H., Zhang, Y., Tian, H., & Wang, B. (2021). Current Understanding of bHLH Transcription
586 Factors in Plant Abiotic Stress Tolerance. *International Journal of Molecular Sciences*, 22(9), 4921.
- 587 [Hancock, A.M., Brachi, B., Faure, N., Horton, M.W., Jarymowycz, L.B., Sperone, F.G., Toomajian, C., Roux,](#)
588 [F. & Bergelson, J. \(2011\). Adaptation to climate across the *Arabidopsis thaliana* genome. *Science*, 334, 83-](#)
589 [86](#)
- 590 [Horton, M.W., Hancock, A.M., Huang, Y.S., Toomajian, C., Atwell, S., Auton, A., Mulyati, N.W., Platt, A.](#)
591 [Sperone, F.G., Vilhjalmsón, B.J., Nordborg, M., Borevitz, J.O. & Bergelson, J. \(2012\). Genome-wide](#)

- 592 [patterns of genetic variation in worldwide *Arabidopsis thaliana* accessions from the RegMap panel. *Nature*](#)
 593 [Genetics, 44\(2\), 212-216.](#)
- 594 [Hart, S.P., Schreiber, S.J. & Levine, J.M. \(2016\). How variation between individual affects species coexistence.](#)
 595 [Ecology Letters, 19, 825-838.](#)
- 596 Huard-Chauveau, C., Perchepped, L., Debieu, M., Rivas, S., Kroj, T., Kars, I., ... & Roby, D. (2013). An atypical
 597 kinase under balancing selection confers broad-spectrum disease resistance in *Arabidopsis*. *PLoS*
 598 [Genetics, 9\(9\), e1003766.](#)
- 599 Hwang, J. U., Song, W. Y., Hong, D., Ko, D., Yamaoka, Y., Jang, S., ... & Lee, Y. (2016). Plant ABC
 600 transporters enable many unique aspects of a terrestrial plant's lifestyle. *Molecular Plant*, 9(3), 338-355.
- 601 [Kruijer, W., Flood, P., & Kooke, R. \(2016\). heritability: Marker-based estimation of heritability using individual](#)
 602 [plant or plot data.](#)
- 603 [Kuznetsova A, Brockhoff PB, Christensen RHB \(2017\). lmerTest Package: Tests in Linear Mixed Effects](#)
 604 [Models. Journal of Statistical Software, 82\(13\), 1–26. doi: 10.18637/jss.v082.i13.](#)
- 605 [Lacaze, X., Hayes, P.M. & Korol, A. \(2009\). Genetics of phenotypic plasticity: QTL analysis in barley, *Hordeum*](#)
 606 [vulgare. Heredity, 102, 163-173.](#)
- 607 [Lenth, R., Singmann, H., Love, J., Buerkner, P., & Herve, M. \(2018\). Emmeans: Estimated marginal means, aka](#)
 608 [least-squares means. R package version, 1\(1\), 3.](#)
- 609 Levine, J. M., Bascompte, J., Adler, P. B., & Allesina, S. (2017). Beyond pairwise mechanisms of species
 610 coexistence in complex communities. *Nature*, 546(7656), 56-64.
- 611 Litrico, I., & Violle, C. (2015). Diversity in plant breeding: a new conceptual framework. *Trends in Plant*
 612 [Science, 20\(10\), 604-613.](#)
- 613 Lonjon, F., Rengel, D., Roux, F., Henry, C., Turner, M., Le Ru, A., ... & Vailleau, F. (2020). HpaP ~~zs~~Sequesters
 614 HrpJ, an ~~e~~Essential ~~c~~Component of *Ralstonia solanacearum* ~~V~~virulence ~~t~~That ~~t~~Triggers ~~n~~Necrosis in
 615 *Arabidopsis*. *Molecular Plant-Microbe Interactions*, 33(2), 200-211.
- 616 Martorell, C., & Freckleton, R. P. (2014). Testing the roles of competition, facilitation and stochasticity on
 617 community structure in a species-rich assemblage. *Journal of Ecology*, 102(1), 74-85.
- 618 Neve, P., Vila-Aiub, M., & Roux, F. (2009). Evolutionary-thinking in agricultural weed management. *New*
 619 [Phytologist, 184\(4\), 783-793.](#)

- 620 Oerke, E. C., & Dehne, H. W. (2004). Safeguarding production—losses in major crops and the role of crop
621 protection. *Crop protection*, 23(4), 275-285.
- 622 Onishi, K., Ichikawa, N., Horiuchi, Y., Kohara, H., & Sano, Y. (2018). Genetic architecture underlying the
623 evolutionary change of competitive ability in Asian cultivated and wild rice. *Journal of Plant*
624 *Interactions*, 13(1), 442-449.
- 625 Pakeman, R. J., Karley, A. J., Newton, A. C., Morcillo, L., Brooker, R. W., & Schöb, C. (2015). A trait-based
626 approach to crop–weed interactions. *European Journal of Agronomy*, 70, 22-32.
- 627 [Palacio-Lopez, K., King, C.M., Bloomer, J. & Hovick, S.M. \(2020\). Natural selection on traits and trait](#)
628 [plasticity in *Arabidopsis thaliana* varies across competitive environments. *Scientific Reports*, 10, 21632.](#)
- 629 Pierik, R., Mommer, L., & Voesenek, L. A. (2013). Molecular mechanisms of plant competition: neighbour
630 detection and response strategies. *Functional Ecology*, 27(4), 841-853.
- 631 Platt, A., Horton, M., Huang, Y. S., Li, Y., Anastasio, A. E., Mulyati, N. W., ... & Borevitz, J. O. (2010). The
632 scale of population structure in *Arabidopsis thaliana*. *PLoS genetics*, 6(2), e1000843.
- 633 Provar, N., & Zhu, T. (2003). A browser-based functional classification SuperViewer for Arabidopsis
634 genomics. *Currents in Computational Molecular Biology*, 2003, 271-272.
- 635 Reboud, X., Le Corre, V., Scarcelli, N., Roux, F., David, J. L., Bataillon, T., ... & McKhann, H. (2004). Natural
636 variation among accessions of *Arabidopsis thaliana*: beyond the flowering date, what morphological traits
637 are relevant to study adaptation. *Plant Adaptation: Molecular Genetics and Ecology*, edited by QCB Cronk,
638 J. Whitton, RH Ree and IEP Taylor. NRC Research Press, Ottawa, Ontario, Canada, 135-142.
- 639 Roux, F., & Bergelson, J. (2016). The genetics underlying natural variation in the biotic interactions of
640 *Arabidopsis thaliana*: the challenges of linking evolutionary genetics and community ecology. *Current*
641 *Topics in Developmental Biology*, 119, 111-156.
- 642 Singer, A., Travis, J. M., & Johst, K. (2013). Interspecific interactions affect species and community responses
643 to climate shifts. *Oikos*, 122(3), 358-366.
- 644 Subrahmaniam, H. J., Libourel, C., Journet, E. P., Morel, J. B., Muñoz, S., Niebel, A., ... & Roux, F. (2018).
645 The genetics underlying natural variation of plant–plant interactions, a beloved but forgotten member of the
646 family of biotic interactions. *The Plant Journal*, 93(4), 747-770.
- 647 Suzuki, N., Rivero, R. M., Shulaev, V., Blumwald, E., & Mittler, R. (2014). Abiotic and biotic stress
648 combinations. *New Phytologist*, 203(1), 32-43.

- 649 [Takou, M., Wieters, B., Kopriva, S., Coupland, G., Lindstädtter, A. & de Meaux, J. \(2019\). Linking genes with](#)
650 [ecological strategies in *Arabidopsis thaliana*. *Journal of Experimental Botany*, 70\(4\), 1141-1151.](#)
- 651 Thoen, M. P., Davila-Olivas, N. H., Kloth, K. J., Coolen, S., Huang, P. P., Aarts, M. G., ... & Dicke, M. (2017).
652 Genetic architecture of plant stress resistance: multi-trait genome-wide association mapping. *New*
653 *Phytologist*, 213(3), 1346-1362.
- 654 Tilman, D. (1985). The resource-ratio hypothesis of plant succession. *The American Naturalist*, 125(6), 827-
655 852.
- 656 [Uffelmann, E., Huang, Q.Q., Munung, N.S., de Vries, J., Okada, Y., Martin, A.R., Martin, H.C., Lappalainen,](#)
657 [T., & Posthuma, D. \(2021\). Genome-Wide Associations studies. *Nature Reviews Methods Primers*, 1, 59.](#)
- 658 Weiner, J., Du, Y. L., Zhang, C., Qin, X. L., & Li, F. M. (2017). Evolutionary agroecology: individual fitness
659 and population yield in wheat (*Triticum aestivum*). *Ecology*, 98(9), 2261-2266.
- 660 Weinig, C., Johnston, J., German, Z. M., & Demink, L. M. (2006). Local and global costs of adaptive plasticity
661 to density in *Arabidopsis thaliana*. *The American Naturalist*, 167(6), 826-836.
- 662 Wender, N. J., Polisetty, C. R., & Donohue, K. (2005). Density-dependent processes influencing the
663 evolutionary dynamics of dispersal: a functional analysis of seed dispersal in *Arabidopsis thaliana*
664 (Brassicaceae). *American Journal of Botany*, 92(6), 960-971.
- 665 Wilson, J. B., Peet, R. K., Dengler, J., & Pärtel, M. (2012). Plant species richness: the world records. *Journal of*
666 *vegetation Science*, 23(4), 796-802.
- 667 [Wilson, A.J. \(2014\). Competition as a source of constraint on life history evolution in natural populations.](#)
668 [Heredity](#), 112, 70-78.
- 669 Worthington, M., & Reberg-Horton, C. (2013). Breeding cereal crops for enhanced weed suppression:
670 optimizing allelopathy and competitive ability. *Journal of Chemical Ecology*, 39(2), 213-231.
- 671 Zhou, X., & Stephens, M. (2012). Genome-wide efficient mixed-model analysis for association studies. *Nature*
672 *Genetics*, 44(7), 821-824.
- 673

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680

681 **Author contributions**

682 F.R., L.A. and D.R. supervised the project. E.B., L.A. and F.R. designed the experiments. E.B. and J.L.
683 conducted the greenhouse experiment. J.L. and E.B measured the phenotypic traits. C.L. analyzed the
684 phenotypic traits. C.L. performed the GWA mapping. C.L. performed and analyzed the enrichment
685 tests. C.L., D.R. and F.R. wrote the manuscript. All authors contributed to the revisions.

686

687 **Data Accessibility**

688 Phenotypic data ~~will be available in the Dryad database: doi:10.5061/dryad.XXX~~ are available In
689 [Dataset 1](#).

690

691 **Figure legends**

692 **Figure 1. Natural genetic variation of reactions norms of 89 TOU-A accessions across 12 plant-**
 693 **plant interaction treatments. (a)** Diagram illustrating the 12 treatments. **(b)** Height from the soil to
 694 the first flower on *A. thaliana* (H1F). Each line links the genotypic values of one of 89 TOU-A
 695 accessions. The two remaining accessions A1-69 and A1-117 are not represented due to missing
 696 genotypic values in the PPV and SSS treatments, respectively. For a given treatment, the mean H1F
 697 genotypic value among the accessions is represented by a red dot.

698

699 **Figure 2. Pairwise genetic correlation coefficients of Pearson among the 12 treatments for each**
 700 **phenotypic trait.** The dots correspond to the 66 pairwise treatment combinations. Black segments
 701 correspond to the mean value of Pearson's r .

702

703 **Figure 3. Identification of genomic regions associated with bispecific interactions in the TOU-A**
 704 **population. (a)** Manhattan plots of GWA mapping results for the PPP and SSS treatments for the
 705 'height of the first flower' (H1F, left-panel) and the ratio 'height of the first flower / the rosette
 706 diameter' (HD, right-panel). The x -axis indicates the physical position of the 630,234 SNPs along the
 707 five chromosomes. The y -axis indicates the Lindley process scores estimated from $-\log_{10} p$ -values from
 708 the mixed model implemented in the software GEMMA using SNPs with MARF > 10%.

709

710 **Figure 4. Comparison of the genetic architecture among bispecific and plurispecific interactions**
 711 **in the TOU-A population. (A)** Manhattan plots comparing GWA mapping results for the bispecific
 712 PPP, SSS and VVV treatments and the plurispecific interaction treatment PSV for the HD ratio. **(B)**
 713 Manhattan plots comparing GWA mapping results from the bispecific PPP to SSS treatments (PPP →
 714 PPS → PSS → SSS) for the H1F trait. The x -axis indicates the physical position of the 630,234 SNPs
 715 along the five chromosomes. The y -axis indicates the Lindley process scores estimated from $-\log_{10} p$ -
 716 values from the mixed model implemented in the software GEMMA using SNPs with MARF > 10%.

717

718 **Figure 5. Illustrations of the flexibility of genetic architecture and processes involved in HD**
719 **among the 12 treatments.** (A) UpSet plot illustrating the variability of genes underlying QTLs related
720 to HD among the 12 treatments. (B) Heatmap illustrating the different MapMan enriched processes
721 among the 12 treatments. (C) UpSet plot illustrating the variability of genes involved in RNA process
722 underlying the seven treatments enriched in RNA process. (D) UpSet plot illustrating the variability of
723 genes involved in transport process underlying the six treatments enriched in transport process. (E)
724 UpSet plot illustrating the variability of genes involved in signaling process underlying the three
725 treatments enriched in signaling process. Blue dots and barplots highlights genes that are specific to
726 only one treatment for the corresponding trait.

In review

727 **Table 1: Natural variation of four phenotypic traits scored on *A. thaliana* plants in 12 treatments.** Bold *P*-values indicate significant
 728 effects after FDR correction. FLO: flowering time, DIAM: maximum diameter of the rosette, H1F: height from the soil to the first flower on
 729 the main stem, HD = H1F / DIAM, BIOMASS: aboveground dry biomass.

730

Model terms	Traits									
	FLO		BIOMASS		DIAM		H1F		HD	
	<i>F</i>	<i>Pr(>F)</i>								
block	4.93	6.11E-03	1.81	1.42E-01	2.63	6.60E-02	1.81	1.64E-01	1.57	2.15E-01
germ	2.46	1.17E-01	0.38	5.37E-01	0.45	5.01E-01	0.33	5.67E-01	0.41	5.22E-01
treatment	12.56	7.21E-09	686.62	<1E-272	139.93	1.22E-24	1.84	8.55E-02	31.32	2.45E-14
accession	247.51	<1E-272	112.52	<1E-272	67.52	<1E-272	28.92	<1E-272	49.76	<1E-272
treatment x accession	1.98	2.12E-44	9.45	<1E-272	3.94	3.69E-183	1.67	8.74E-25	1.42	1.21E-12

731

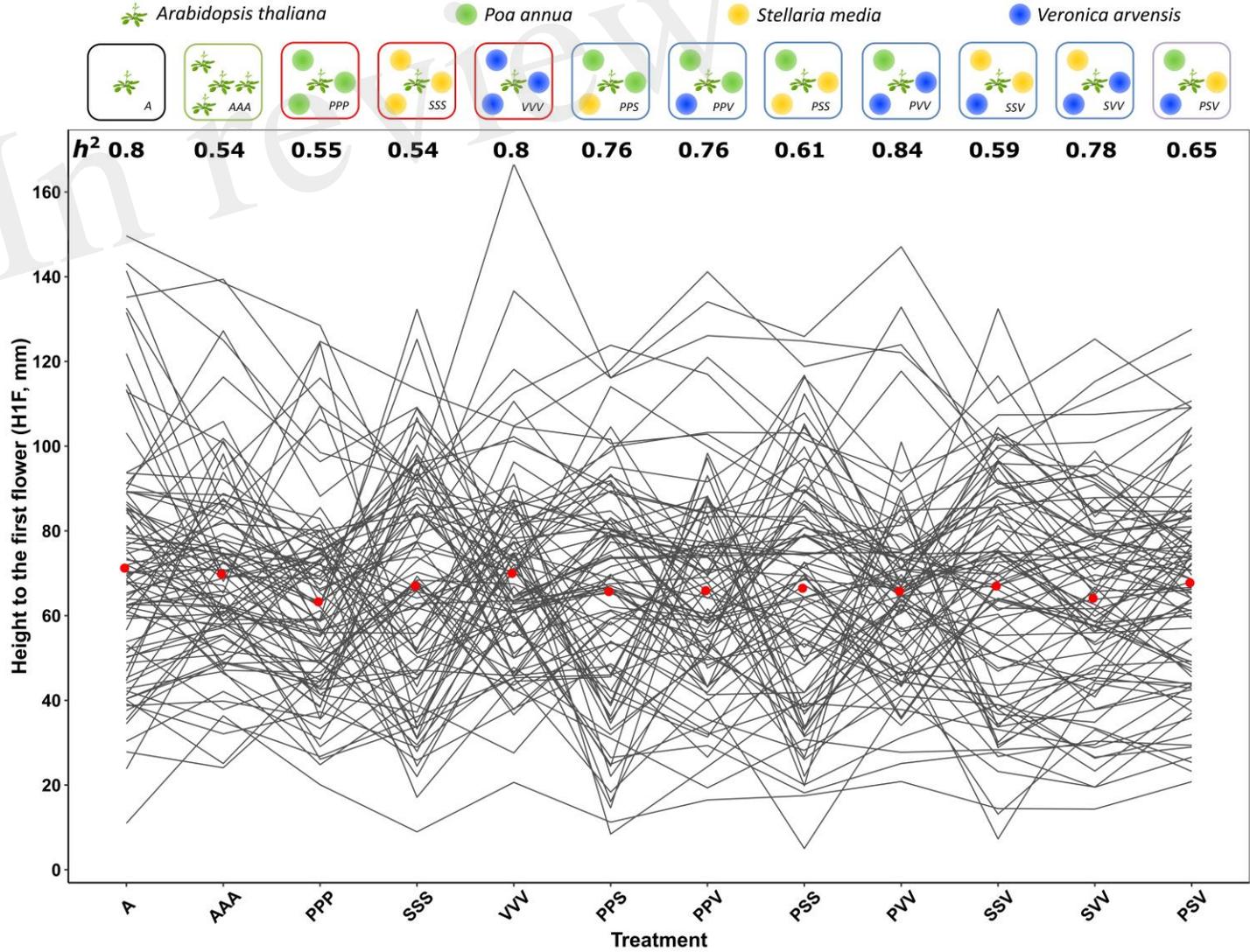
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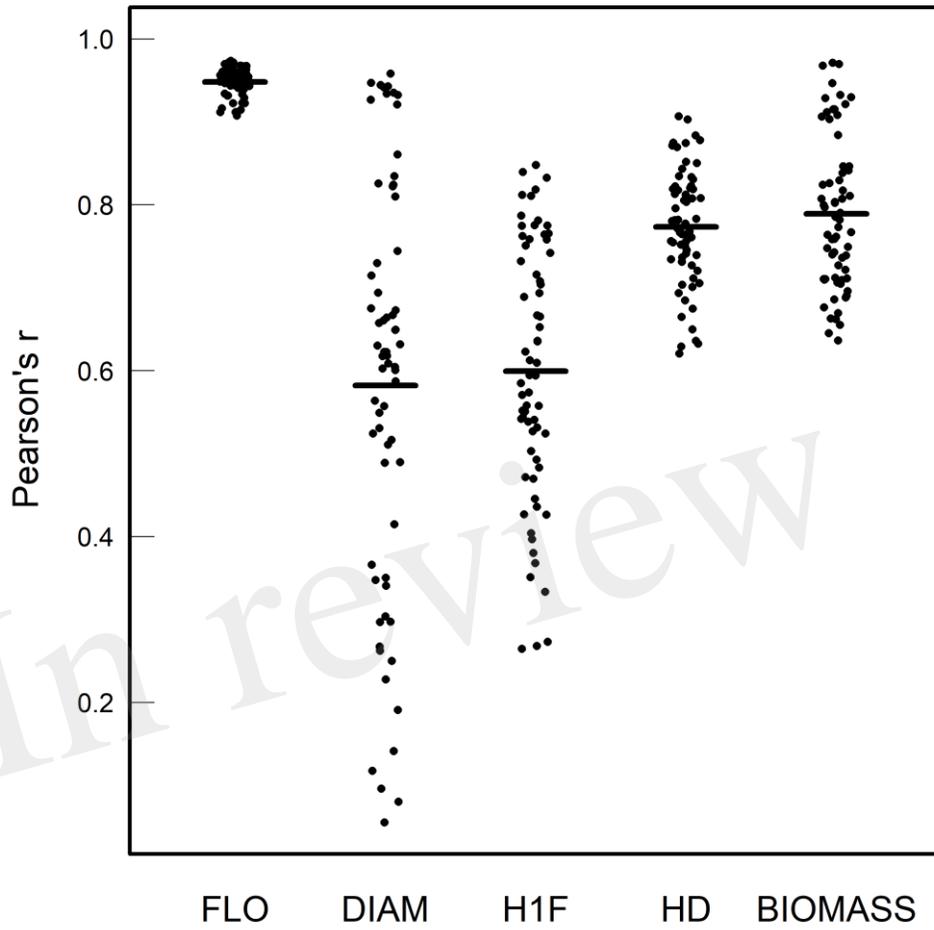
736 **Figure 1**



737

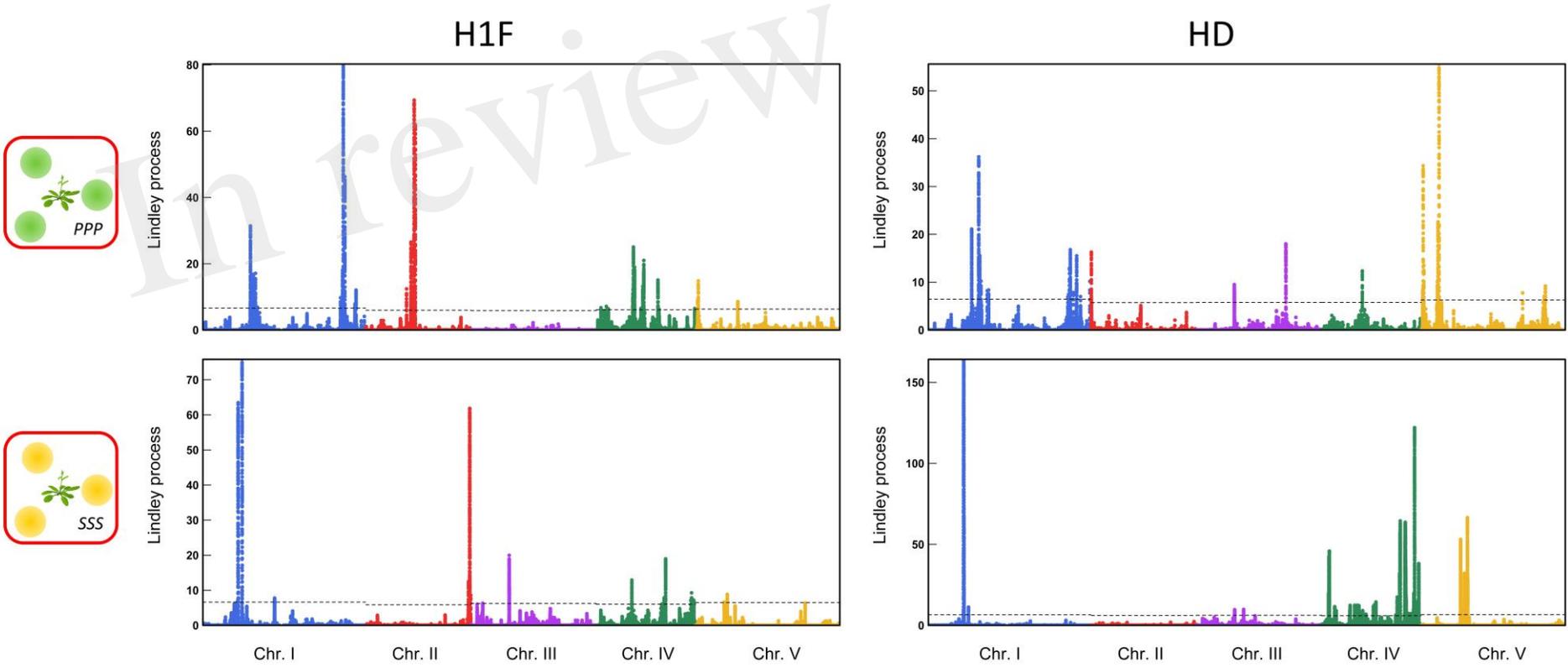
738 **Figure 2**

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740

741 **Figure 3**



742

746 **Figure 5**

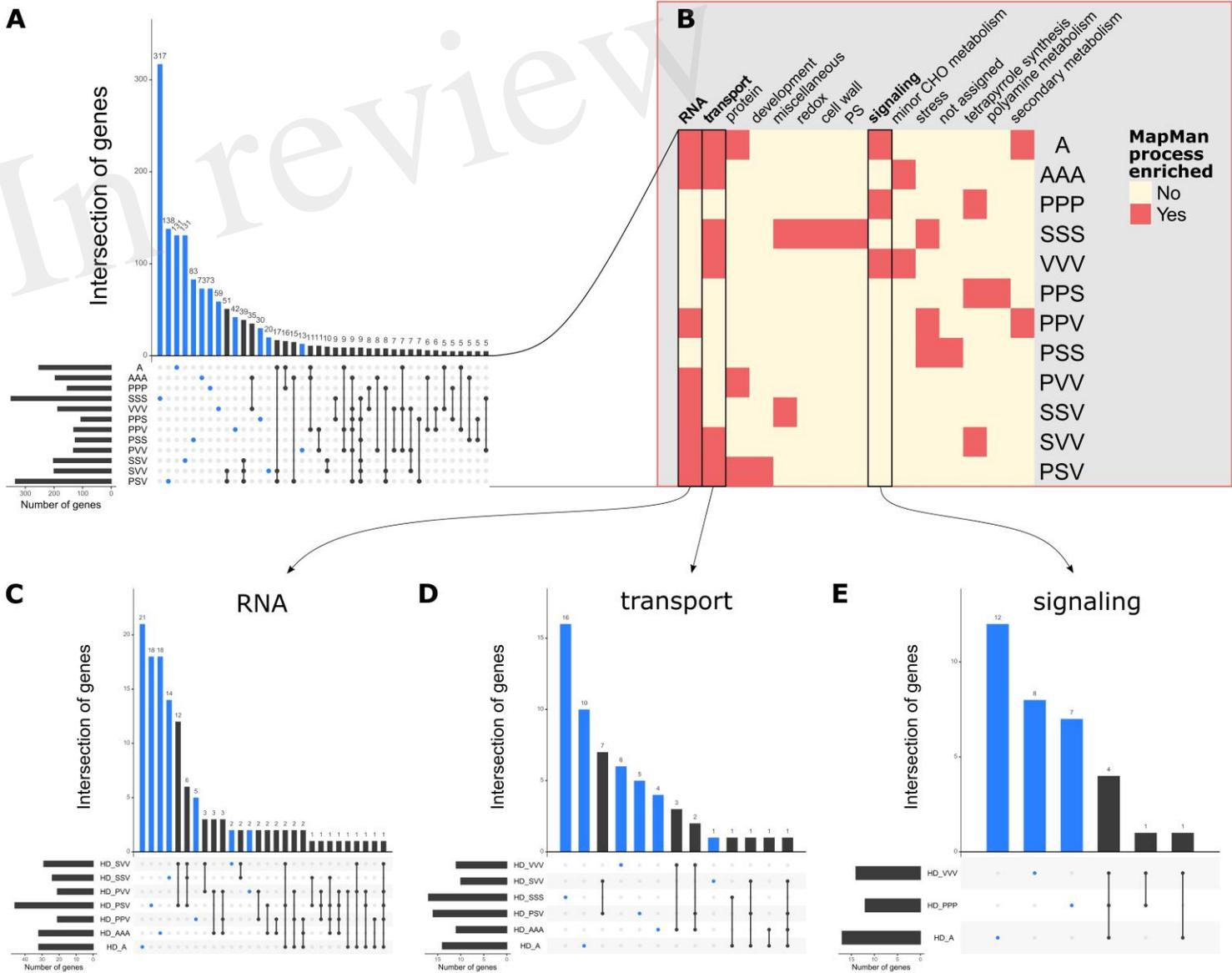


Figure 1.JPEG

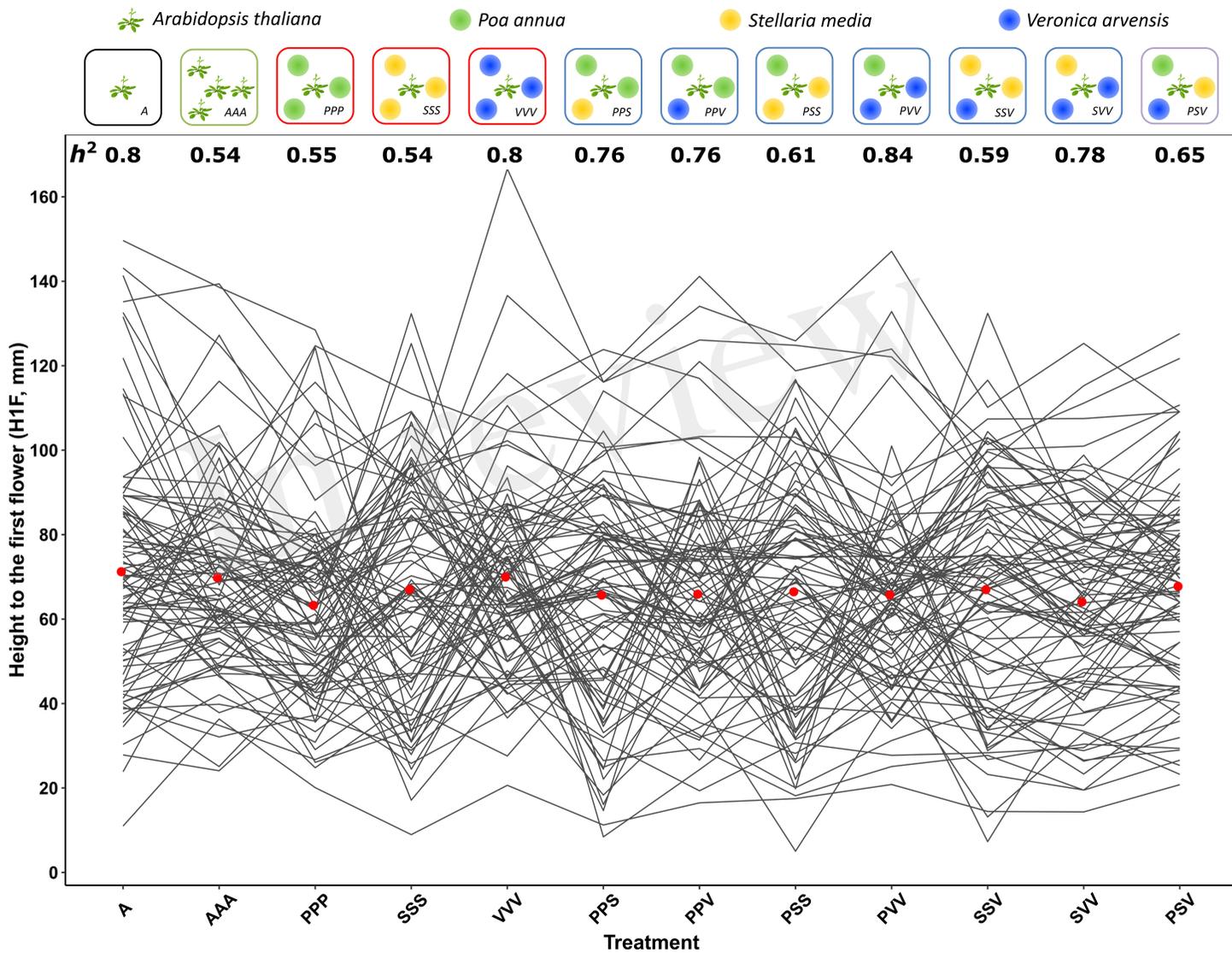


Figure 2.TIFF

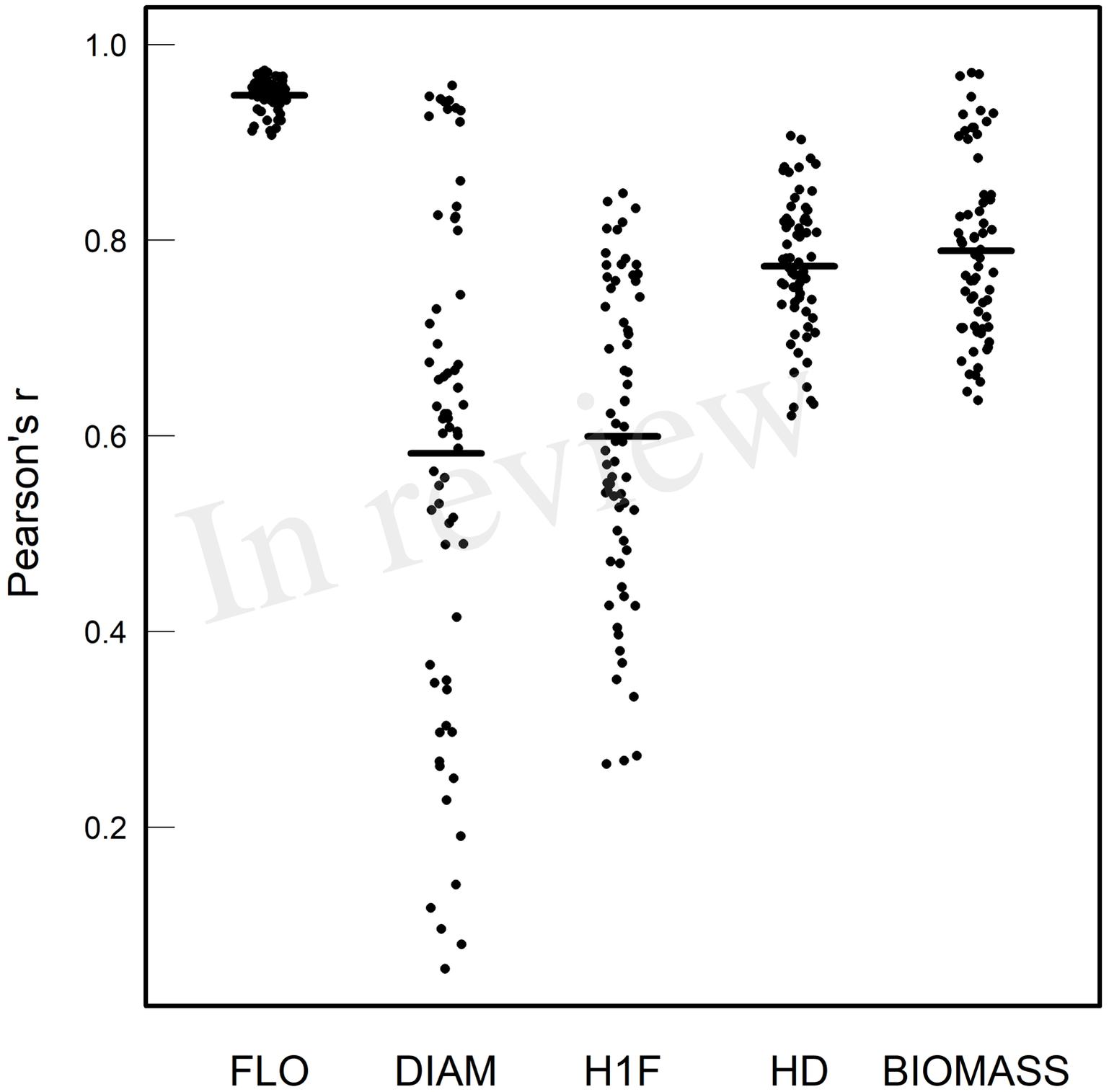


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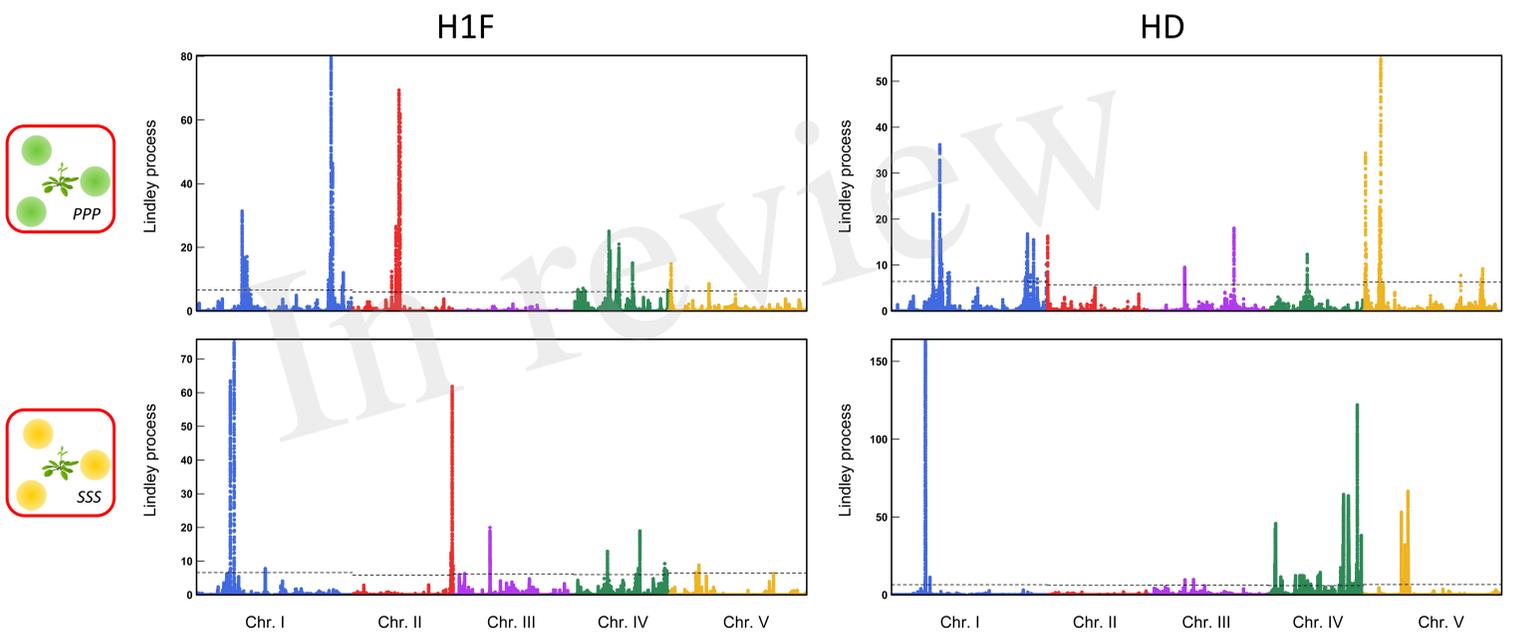


Figure 4.JPEG

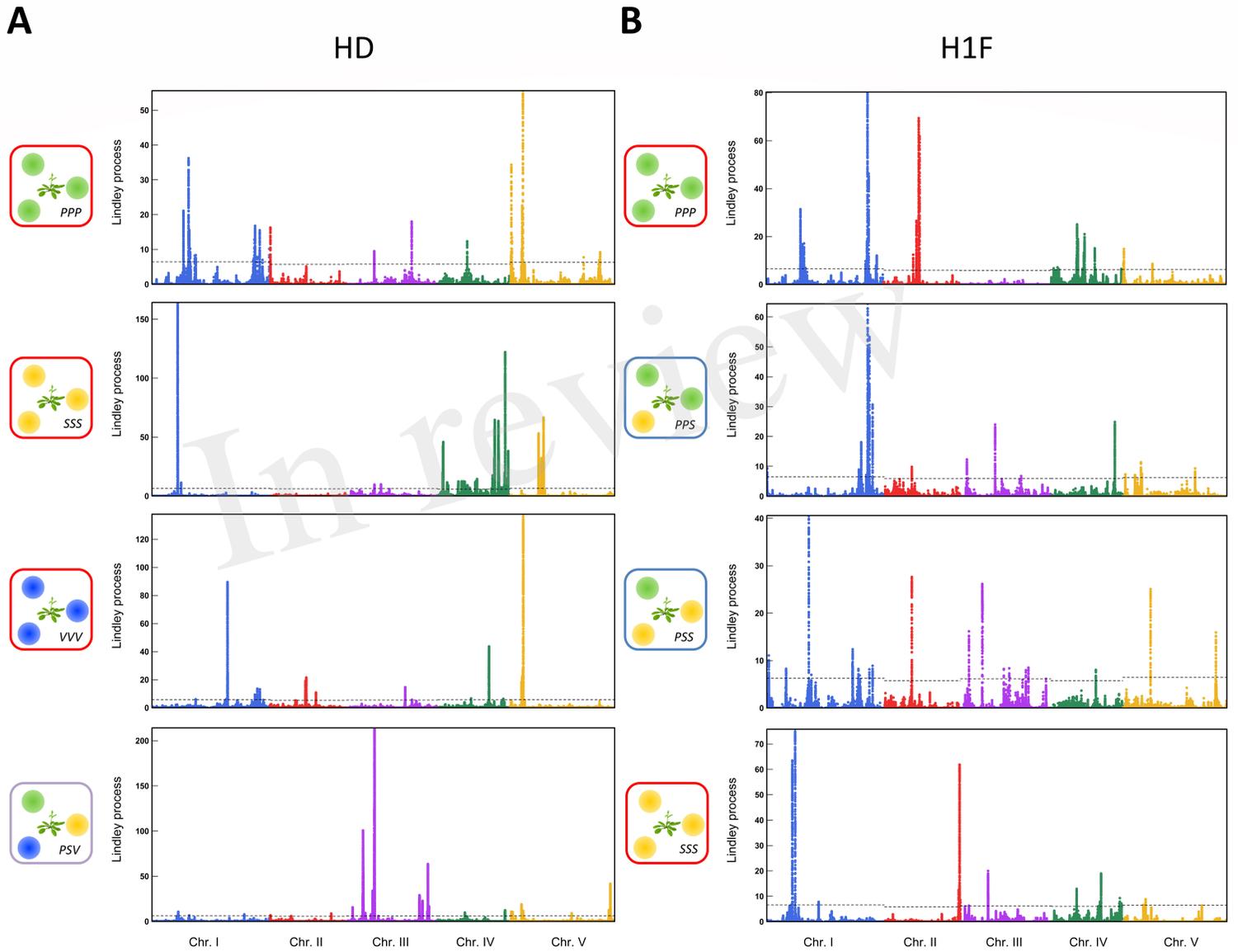


Figure 5.JPEG

