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1 # Asymmetric redundancy of *ZERZAUST* and *ZERZAUST HOMOLOG* in different
2 accessions of *Arabidopsis thaliana*

3

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31

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34

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35 **ABSTRACT**

36 Divergence among duplicate genes is one of the important sources of evolutionary
37 innovation. But, the contribution of duplicate divergence to variation in Arabidopsis
38 accessions is sparsely known. Recently, we studied the role of a cell wall localized protein,
39 ZERZAUST (ZET), in Landsberg *erecta* (L*er*) accession. Here, we present the study of *ZET*
40 in Columbia (Col) accession, which not only showed differential expression patterns in
41 comparison to L*er,* but also revealed its close homolog, *ZERZAUST HOMOLOG (ZETH).*
42 Although, genetic analysis implied redundancy, expression analysis revealed divergence,
43 with *ZETH* showing minimal expression in both Col and L*er*. In addition, *ZETH* shows
44 relatively higher expression levels in Col compared to L*er.* Our data also reveal
45 compensatory up-regulation of *ZETH* in Col, but not in L*er,* implying it is perhaps
46 dispensable in L*er*. However, a novel CRISPR/Cas9-induced *zeth* allele confirmed that *ZETH*
47 has residual activity in L*er.* The results provide genetic evidence for accession-specific
48 differences in compensation mechanism and asymmetric gene contribution. Thus, our work
49 reveals a novel example for how weakly expressed homologs contribute to diversity among
50 accessions.

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52 # INTRODUCTION

53 How genetic variation translates into phenotypic variation is of immense scientific interest
54 (Weigel 2012). Among others, gene duplication followed by functional divergence is an
55 important source of evolutionary complexity and innovation in multicellular organisms
56 (Ohno 1970; Lynch and Conery 2000; Lynch and Katju 2004). Many gene pairs after
57 duplication revert to single gene state but the ones that sustained undergo functionalization.
58 The Arabidopsis genome underwent several duplication events which resulted in large
59 number of homologous genes and regions across the genome (Blanc and Wolfe 2004;
60 Ambrosino *et al.* 2016; Panchy *et al.* 2016). The functional importance of homologs has been
61 demonstrated in various aspects of plant signaling and metabolism (Briggs *et al.* 2006). But,
62 whether and how the differentiation in duplicate gene expression contributes to accession
63 variation in Arabidopsis is not known.
64

65 Studies have shown that divergence of many duplicate genes occurs by expression divergence
66 among and within species (Gu *et al.* 2004; Li *et al.* 2005). This phenomenon expands gene
67 regulatory networks and contributes to physiological and morphological diversity (Carroll
68 2000; Lynch and Conery 2000; Gu *et al.* 2004; Rensing 2014). In Arabidopsis, about two-
69 thirds of duplicates were shown to exhibit expression divergence (Haberer *et al.* 2004). An
70 evolutionary study on gene duplication revealed that duplicate genes show a high degree of
71 variance in expression within species and suggested that this variation partly depends upon
72 the biological function of the gene involved (Kliebenstein 2008). Another study also found
73 high variance of duplicated gene expression between closely related *A. thaliana* and *A.*
74 *arenosa* (Ha *et al.* 2009).
75

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76 Functional redundancy among homologs is widespread in Arabidopsis, since several single
77 loss-of-function mutants lack phenotype (Briggs *et al.* 2006). Homologous genes can be
78 either fully or partially redundant. But, when two homologous genes show unequal genetic
79 redundancy, a mutation in one of them causes a phenotype and the phenotype is enhanced
80 when the other homolog is mutated as well. Interestingly, the defect in the other homolog
81 itself doesn't result in any phenotype on its own. For example, the receptor-like kinase gene
82 *BRASSINOSTEROID INSENSITIVE 1 (BRI1)* is accompanied by its close homolog *BRI1-*
83 *LIKE1 (BRL1)*. Although *brl1* lacks a mutant phenotype it enhances the severe dwarf
84 phenotype of *bri1* mutants (Caño-Delgado *et al.* 2004). This kind of unequal functional
85 redundancy can be explained by divergence in duplicate expression, however, their
86 perseverance in plant genome is under debate given the dispensable nature of the duplicate.
87

88 Genetic factors involved in plant morphogenesis will have crucial role in the differentiation
89 of various Arabidopsis accessions. Tissue morphogenesis in Arabidopsis requires the cell
90 wall-localized GPI-anchored β-1,3 glucanase ZERZAUST *(*ZET*)* (Vaddepalli *et al.* 2017).
91 *ZET* was initially identified as a genetic component of the *STRUBBELIG (SUB)* signaling
92 pathway along with QUIRKY, a C2 domain containing protein (Fulton *et al.* 2009). Absence
93 of *ZET* results in a so-called *strubbelig-like mutant* (*slm*) phenotype characterized by
94 abnormal integument initiation and outgrowth, aberrant floral organ and stem morphology,
95 reduced plant height and irregular leaf shape.

96 Our previous studies have shown that mutations in *SUB* and *QKY* in Col background result in
97 obvious *slm* mutant phenotypes (Fulton *et al.* 2009; Vaddepalli *et al.* 2011, 2014). But in the
98 current work, we discovered that *ZET* acts differently in Col accession due to the presence of
99 the close homolog *ZERZAUST HOMOLOG* (*ZETH*). Using genetic and gene expression tools
100 we show how *ZET* and *ZETH* diverged between the two common laboratory accessions Col

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101 and L*er* in terms of expression and function. Furthermore, we try to understand the
102 contribution of the weakly expressing redundant homolog on the morphological diversity of
103 accessions.

104 # RESULTS

105 **Molecular identification of *ZETH***

106 In L*er* background, *zet-1* carrying a loss-of-function mutation in *ZET* locus (At1g64760) (Fig.
107 1A,B), shows a strong *slm* mutant phenotype (Vaddepalli *et al.* 2017) (Fig. 1D,F). Except one
108 amino acid in the signal peptide, ZET shows no difference between Col and L*er*. We
109 investigated the functionality of *ZET* in Columbia accession by analyzing two available T-
110 DNA insertion lines (*zet-3* and *zet-4*) (Fig. 1A). We expected the T-DNA insertion in *zet*-4 to
111 cause a mutant phenotype as it is predicted to result in a truncated ZET protein (Fig. 1A,B).
112 But, the plants surprisingly failed to display the twisted morphology, characteristic of *slm*
113 mutants (Fig. S1B). We asked, if the observed accession-specific phenotypic differences
114 could relate to a close homolog of *ZET*. A BLAST search with the *ZET* coding sequence
115 revealed that *ZET* is most closely related to At2g19440 with 89 percent identity at the amino
116 acid level (Fig. S1A). We named this gene *ZERZAUST HOMOLOG* (*ZETH)*. *ZET* and *ZETH*
117 form a subclade within the larger β clade of β-1,3 glucanase (BG) genes, which comprises 11
118 members (Doxey *et al.* 2007; Gaudioso-Pedraza and Benitez-Alfonso 2014). Sequence-based
119 analysis of the evolutionary history revealed that ZET and ZETH duplication is specific to
120 species within the Arabidopsis lineage (Fig. S2).
121
122 To assess ZETH activity in Col, we investigated a T-DNA line (*zeth-1*), which is presumed to
123 carry a truncated protein (Fig. 1A,B). But, like *zet-4*, the *zeth-1* insertion line also failed to
124 show a mutant phenotype (Fig. S1C). However, the *zet-4* and *zeth-1* double mutant exhibited
125 a strong phenotype (Fig. 1G-I, S1D) suggesting that the two genes act redundantly. The

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126 double mutant resembled *zet-1* plants except for appearing less bushy but with exaggerated
127 twisting of flowers and increased sterility. Nevertheless, we could complement the double
128 mutant plants by introducing a construct encoding a translational fusion of ZET to the GFP
129 variant T-Sapphire driven by the endogenous *ZET* promoter (pZET::TS:ZET), ruling out the
130 contribution of any other background mutation (Fig. 1J). This construct was used in a
131 previous study to complement *zet-1* mutants in L*er* background (Vaddepalli *et al.* 2017).
132

133 **Accession-specific regulation of *ZET* and *ZETH* expression**

134 Next, we analyzed the expression pattern of *ZET* and *ZETH* in Col and L*er* accessions to
135 assess the cause for the mutant phenotype disparities between the two accessions. Our qPCR
136 data from various tissues revealed that these genes are co-expressed (Fig. 2A). Surprisingly,
137 we found much lower levels of *ZETH* transcripts in comparison to *ZET*. Moreover, *ZETH*
138 expression was even further reduced in L*er* where it was barely detectable in rosette leaves,
139 stems, or flowers. Additional qPCR tests revealed that *ZET* and *ZETH* expression levels in
140 seedlings and flowers undergo compensatory regulation in the *zeth-1* and *zet-4* mutants in
141 Col, respectively (Fig. 2B). This result provides evidence for redundant functions of *ZET* and
142 *ZETH* in the Col background and thus offers a convenient explanation for the lack of
143 phenotype in single mutants in this accession. Interestingly, this compensatory regulation
144 appears to be absent in flowers of L*er* accession since *ZETH* expression was not detectably
145 upregulated in *zet-1* mutant.
146

147 **L*er* carries a functional *ZETH* gene**

148 Despite minimal *ZETH* expression profiles in both accessions, appearance of prominent
149 phenotypes only in L*er,* when *zet* is mutated, can be attributed to any or all of the following
150 reasons. It could be because of the low expression of *ZETH*, the lack of compensatory

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151 mechanism, or the L*er* version of ZETH exhibiting a different amino acid composition when
152 compared to Col which might affect its activity. The L*er*/Col variants of ZET differ by only
153 one amino acid at position 3 in the predicted signal peptide (change from an asparagine to a
154 lysine) but there are nine amino acid differences between the L*er*/Col variants of ZETH (Fig
155 3A). We wanted to test if these changes affect the activity of ZETH in L*er*. For this purpose,
156 we replaced the *ZET* coding sequence 3' to the predicted signal peptide with the equivalent
157 L*er* or Col variants of *ZETH* sequence in our complementing pZET::TS:ZET reporter
158 (Vaddepalli *et al.* 2017). This resulted in *zet-1* plants transgenic for the L*er* or Col variants of
159 *ZETH,* under the control of the native *ZET* promoter (*pZET::TS:ZETHL/C zet-1*).
160 Interestingly, the T1 plants of the transgenic *zet-1* lines exhibited a wild-type phenotype with
161 both variants (Figures 3B-G) (80/80 (*TS:ZETHL*), 167/172 (*TS:ZETHC*)) indicating that the
162 accession-specific amino acid alterations do not affect ZETH function.
163
164 Although *ZETH* of L*er* is functional, its expression is quite weak indicating its functional
165 contribution is perhaps insignificant. But, *zet1 zeth-1* double mutants in Col accession exhibit
166 a stronger phenotype compared to *zet-1* (L*er*) (Fig. 1G-J). These interesting observations
167 prompted us to check whether *ZETH* has some residual activity in L*er* even though its
168 expression is very low. Using CRISPR\Cas9 technique (Wang *et al.* 2015) we generated a
169 mutation in the first exon of *ZETH* in the *zet-1* background. The novel allele *zeth-2* is
170 predicted to result in a truncated ZETH protein consisting of only the first 80 amino acids
171 (Fig. 1A,B). Surprisingly, *zet-1 zeth-2* double mutant plants in L*er* showed an exaggerated
172 *zet-1* phenotype and appeared closer to *zet-4 zeth-1* double mutants in the Columbia
173 background (Fig. 3H-J). The finding indicates that the weakly expressed *ZETH* in L*er*
174 exhibits residual activity, which is insufficient to fully substitute for the lack of *ZET*.
175

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176 ***ZETH* acts in a dose dependent manner**

177 Thus far, our results have established the functional role for the weakly expressed *ZETH* in
178 both Col and L*er* accessions, implying small amount of ZETH protein can have a noticeable
179 impact. Next, we asked if this gene is acting in a dose-dependent manner. For this purpose,
180 we checked the effect of *ZETH* gene copy number on *zet* mutant phenotype (Fig. 4).
181 Interestingly in the L*er* background, *zet-1 zeth-2/+* displayed an intermediate phenotype
182 between the single *zet-1* mutant and the double *zet-1 zeth-2* mutant. The leaf petioles are
183 somewhat elongated in *zet-1* with narrow blades. This phenotype got slightly enhanced in *zet-*
184 *1 zeth-2/+* background, whereas *zet-1 zeth-2* double mutants show further worsening of the
185 phenotype. The phenomenon of dosage-dependent enhancement of mutant phenotype was
186 also observed for floral organs and was particularly obvious for siliques depending on the
187 *ZETH* copy number. Surprisingly, in Columbia background the *zet-4 zeth-1/+* mutant showed
188 wild type morphology in all the organs tested except siliques which displayed shortening of
189 length with no twisting. Despite these peculiarities between accessions, the observations
190 indicate that mutation in *zeth* contributes to the overall exaggerated morphologies of double
191 mutants in a dose dependent manner. Our results also imply that the extent of the *ZETH*
192 effect on plant morphology is accession dependent.

193

194 **ERECTA influences the *zet-1* phenotype**

195 Col and L*er* accessions display obvious discrepancies in their phenotypic appearance. For
196 example, L*er* exhibits shorter stems and more compact inflorescences (Passardi *et al.* 2007).
197 The phenotypic differences could be ascribed to genetic variability between the accessions
198 and the contribution of accession-specific modifiers needs to be addressed. Major difference
199 between the Col and L*er* accessions is the lack of *ERECTA* (*ER*) in the L*er* background.
200 Previous work revealed that *SUB* and *QKY* show a synergistic interaction with *ERECTA* (*ER*)

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201 with respect to the control of plant height (Vaddepalli *et al.* 2011, 2014). We investigated the
202 phenotype of *zet-1* (L*er*) plants transformed with pKUT196, a plasmid carrying 9.3 kb of
203 Col-0 DNA spanning the entire genomic *ER* locus (Torii *et al.* 1996; Godiard *et al.* 2003).
204 Like other *slms, zet-1 ER* transgenic plants also exhibited ameliorated plant height and stem
205 twisting whereas aberrant floral organ phenotype appeared unaffected by the *ER* transgene
206 (Fig. 5). Our result exemplifies the influence of accession-specific modifiers on plant
207 morphology.
208

209 **DISCUSSION**

210 Numerous studies have shown the compensation of gene loss by duplicate genes implying
211 that close homologs give robustness to the plants against mutations (Hanada *et al.* 2009).
212 Studies have also shown stronger reduction in duplicate expression and this expression
213 divergence was noted as an important innovation for conservation of the duplicate gene
214 (Ganko *et al.* 2007; Panchy *et al.* 2016). Despite acknowledging this interesting pattern,
215 examples are missing that show functional relevance of gene duplicates, since retention of
216 almost identical duplicates goes against the evolutionary instability of genetic redundancy
217 (Lynch and Conery 2000). Here, our work reveals an unexpected variation of a weakly
218 expressed gene and its homolog between accessions. Our results imply that divergence in
219 duplicate expression may play a crucial role in accession-specific genetic adaptations.
220

221 Redundancy between duplicate genes by a compensation mechanism via feedback responsive
222 circuit serves as an advantage for biological systems to overcome stochastic fluctuations in
223 signaling pathways (Kafri *et al.* 2006, 2009). In the Col background, an overall higher
224 expression level of *ZETH*, in combination with a compensatory upregulation in *zet-4* mutants
225 seems to account for their wild-type appearance. Although this phenomenon is absent in L*er*,

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226 the residual expression of *ZETH* in L*er* seems to be above the threshold, otherwise the *zet-1*
227 *zeth-2* double mutant would have resembled the single mutant *zet-1*. Furthermore,
228 understated changes in expression pattern may further enhanced by tissue level sampling.
229 Although segregating mutants in Col background appear to be fine morphologically, we may
230 have missed subtler cellular phenotypes in our analysis. All the experiments were performed
231 in controlled lab conditions. There is also a possibility that *ZETH* may express at higher
232 levels under different conditions and reveal a novel function. Indeed, the role of SUB in
233 coordinating cell proliferation and differentiation during leaf development was revealed only
234 at high ambient temperature of 30 °C (Lin *et al.* 2012).
235
236 Initially, we assumed *ZETH* in L*er* background as a pseudogene, since a previous RNA-seq
237 analysis has found that *ZETH* transcripts were undetectable in young L*er* flowers (Jiao and
238 Meyerowitz 2010). Although our qRT-PCR results showed *ZETH* expression, the
239 functionality was still in question given the very low expression pattern in all the tissues
240 tested. But, surprisingly our results indicate that weakly expressed *ZETH* is functionally very
241 relevant. Our results also highlight the importance of gene specific analysis, since in large
242 scale studies differentiating between identical duplicate sequences is challenging. Thus, small
243 expression differences are often overlooked as the focus goes inadvertently on highly
244 expressed genes.
245
246 Previous evidence indicated that *slm*s may have additional functions apart from their role in
247 plant development (Fulton *et al.* 2009). Transcriptome analysis showed that several *ZET*
248 responsive genes are related to biotic and abiotic stress responses. It is intriguing to speculate
249 that the high expression of *ZET* might be attributed to its stress related functions since
250 minimal *ZETH* expression in Col was enough for wild type appearance of *zet-4* mutants.

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251 Future experiments could test this possibility by assessing if *zet* is more susceptible to stress
252 compared to *zeth*. Interestingly, *ZET* transcript level was shown to be altered in Arabidopsis
253 plants infected with *Fusarium oxysporum* (Fallath *et al.* 2017). Thus, further analysis of *ZET*
254 may reveal its potential role in adaptation, apart from morphogenesis.
255
256 Duplicate genes provide mutational robustness to living organisms. In yeast and
257 *Caenorhabditis elegans*, functional compensation by the duplicated gene displayed higher
258 robustness to gene perturbation than singletons (Gu *et al.* 2003; Conant and Wagner 2004).
259 But, *ZETH* showed a highly reduced expression pattern compared to *ZET*. Such a reduction in
260 expression was proposed to facilitate the retention of duplicates and the conservation of their
261 ancestral functions (Qian *et al.* 2010). In this scenario, loss of either of the duplicate genes
262 renders the total expression level lower than normal which would hamper the function. This
263 also inhibits functional divergence of duplicated genes and helps in rebalancing gene dosage
264 after duplication. Interestingly, with *ZET* this phenomenon was observed only in L*er,* where
265 mutating *ZET* was enough for the manifestation of phenotype, but not in the Col background.
266 These results indicate that the divergent behavior of duplicates may vary depending on the
267 accession and the specific gene pair under study. Since the *ER* locus was able to partially
268 alleviate *zet-1* mutant phenotype, the influence of accession-specific differences on gene
269 contribution needs to be considered. Further, it would be interesting to know if there exists a
270 correlation between accession-specific modifiers and expression divergence of certain
271 duplicates.
272
273 A study on homologs revealed significant diversity in expression pattern among different
274 accessions of Arabidopsis (Kliebenstein 2008). For instance, background specific regulation
275 and unequal genetic redundancy has been observed for *BRI1* (Caño-Delgado *et al.* 2004;

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276 Zhou *et al.* 2004). In another example, the sucrose transporter AtSUC1 was shown to have
277 differential tissue expression pattern depending on the accession it was tested (Feuerstein *et*
278 *al.* 2010). The observed accession-specific disparities in *ZETH* expression levels may relate
279 to differences in its *cis*-regulatory region, either caused by DNA polymorphisms, as was
280 found for the tomato *fw2.2*, rice *qSH1*, or Arabidopsis *FLOWERING LOCUS* (*FT*) loci
281 (Konishi *et al.* 2006; Cong *et al.* 2008; Schwartz *et al.* 2009; Liu *et al.* 2014), or by
282 epigenetic variation (Durand *et al.* 2012). Thus, our work provides an interesting example for
283 the diversification of cis and/or trans regulatory elements between two Arabidopsis
284 accessions. A correlation between duplicate divergence and evolution of cis-regulatory
285 elements and networks was observed (Arsovski *et al.* 2015). But, how the transcriptional
286 networks regulate the levels of the duplicated genes and their role in the context of evolution
287 is largely unexplored. Further studies, using the various Arabidopsis accessions available,
288 could help in understanding the role of expression divergence among duplicates in
289 differentiation of accessions.
290

291 # MATERIALS AND METHODS

292 **Plant work, Plant Genetics and Plant Transformation**

293 *Arabidopsis thaliana* (L.) Heynh. var. Columbia (Col-0) and var. Landsberg (*erecta* mutant)
294 (L*er*) were used as wild-type strains. Plants were grown as described earlier (Fulton *et al.*
295 2009). The *zet-1* mutant was described previously (Vaddepalli *et al.* 2017). T-DNA insertion
296 lines were received from the GABI-KAT (*zet-3*, GABI-KAT-460G06) (Kleinboelting *et al.*
297 2012) and Wisconsin collections (*zet-4*, WiscDsLoxHs057_03H; *zeth-1*,
298 WiscDsLoxHs066_12G) (Sussman *et al.* 2000). Plants were transformed with different
299 constructs using Agrobacterium strain GV3101/pMP90 (Koncz and Schell 1986) and the
300 floral dip method (Clough and Bent 1998). Transgenic T1 plants were selected on

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301 Hygromycin (20 µg/ml) or Glufosinate (Basta) (10 µg/ml) plates and transferred to soil for
302 further inspection.
303
304 **Recombinant DNA work**
305 For DNA and RNA work standard molecular biology techniques were used. PCR-fragments
306 used for cloning were obtained using Phusion high-fidelity DNA polymerase (New England
307 Biolabs, Frankfurt, Germany) or TaKaRa PrimeSTAR HS DNA polymerase (Lonza, Basel,
308 Switzerland). PCR fragments were subcloned into pLitmus 28i (NEB). All PCR-based
309 constructs were sequenced. Primer sequences used for cloning and qRT PCR in this work are
310 listed in S1 Table.
311
312 **Cloning**
313 Genomic fragments of *ZETH* were amplified from Col-0 and L*er* backgrounds using primers
314 ZETHCol_F/ZETHCol_R and ZETHL*er*_F/ZETHL*er*_R and sub cloned into pZET::TS:ZET
315 (Vaddepalli *et al.* 2017) using XmaI/BamHI restriction sites to obtain pZET::TS:ZETHCol
316 and pZET::TS:ZETHL*er* respectively. For CRISPR/cas9 ZETH construct, the egg cell-
317 specific promoter-controlled CRISPR/Cas9 system was used as described (Wang *et al.* 2015).
318 *zet-1* plants were transformed with the CRISPR/cas9 ZETH construct by floral dip method.
319 T1 plants were screened for exaggerated phenotype and *ZETH* locus was sequenced for
320 identifying the mutation.
321
322 **Quantitative RT-PCR analysis**
323 Tissue for quantitative real-time PCR (qPCR) was harvested from plants grown in long day
324 conditions. With minor changes, tissue collection, RNA extraction and quality control were
325 performed as described previously (Box *et al.* 2011). RT-PCR was performed on Biorad

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326 CFX96 by using iQ SYBR Green Supermix (Bio-Rad) according to the manufacturer's
327 recommendations. All expression data were normalized against reference genes At5g25760,
328 At4g33380, and At2g28390 by using the ΔΔ-Ct method (Czechowski 2007). Experiments
329 were performed in biological and technical triplicates.
330

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336 AUTHOR CONTRIBUTIONS

337 P.V. and K.S. designed the research. P.V and L.F. performed experiments. P.V. and K.S.
338 analyzed the data. P.V. wrote the paper.

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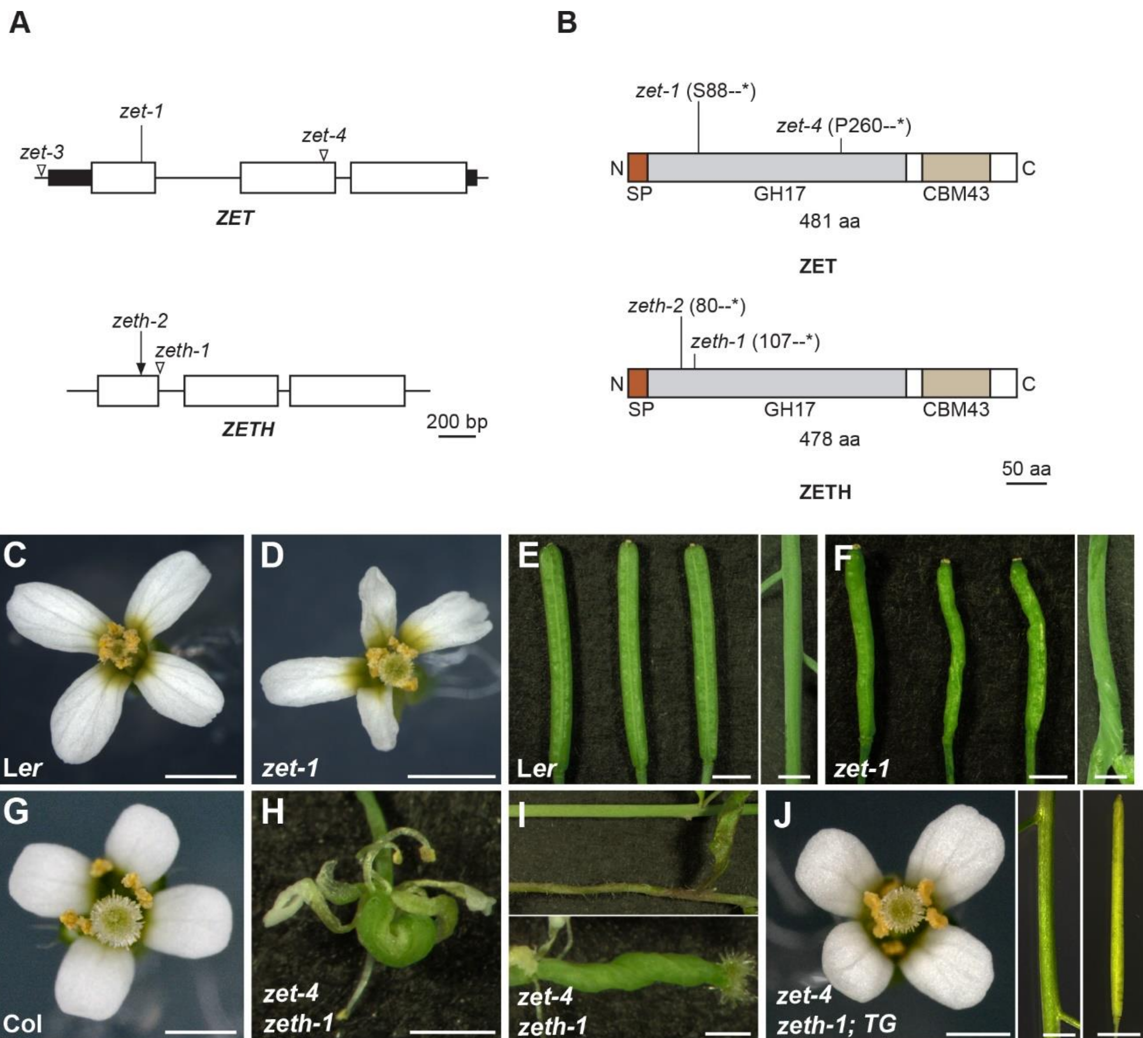
519 **FIGURES**



520

521 **Fig. 1. Molecular and genetic characterization of *ZET* and *ZETH*.** (A) Cartoon depicting
522 the genomic organization of *ZET* and *ZETH*. Horizontal lines represent introns. Filled
523 rectangles mark untranslated regions. The positions of EMS-, CRISPR-, and T-DNA-induced
524 mutations are denoted by lines, arrow, and open triangles, respectively. (B) Schematic view
525 of the predicted ZET protein. The signal peptide (SP), GH17 and CBM43/X8 domains are
526 highlighted. The position and effects of the *zet* mutations are indicated. Phenotypic analysis
527 (C-J). Comparison of (C, E) L*er*. (D, F) *zet-1,* (G) Col and (H, I) *zet-4 zeth-1*. Note the
528 aberrant flower (D, H), stem and silique (F, I) morphology of mutants. Mutant phenotype is
529 strong in *zet-4 zeth-1*double mutant (H, I). (J) *zet-4 zeth-1* double mutant phenotype is
530 complemented by *pZET::TS:ZET* (*TG*). Scale bars:1 mm

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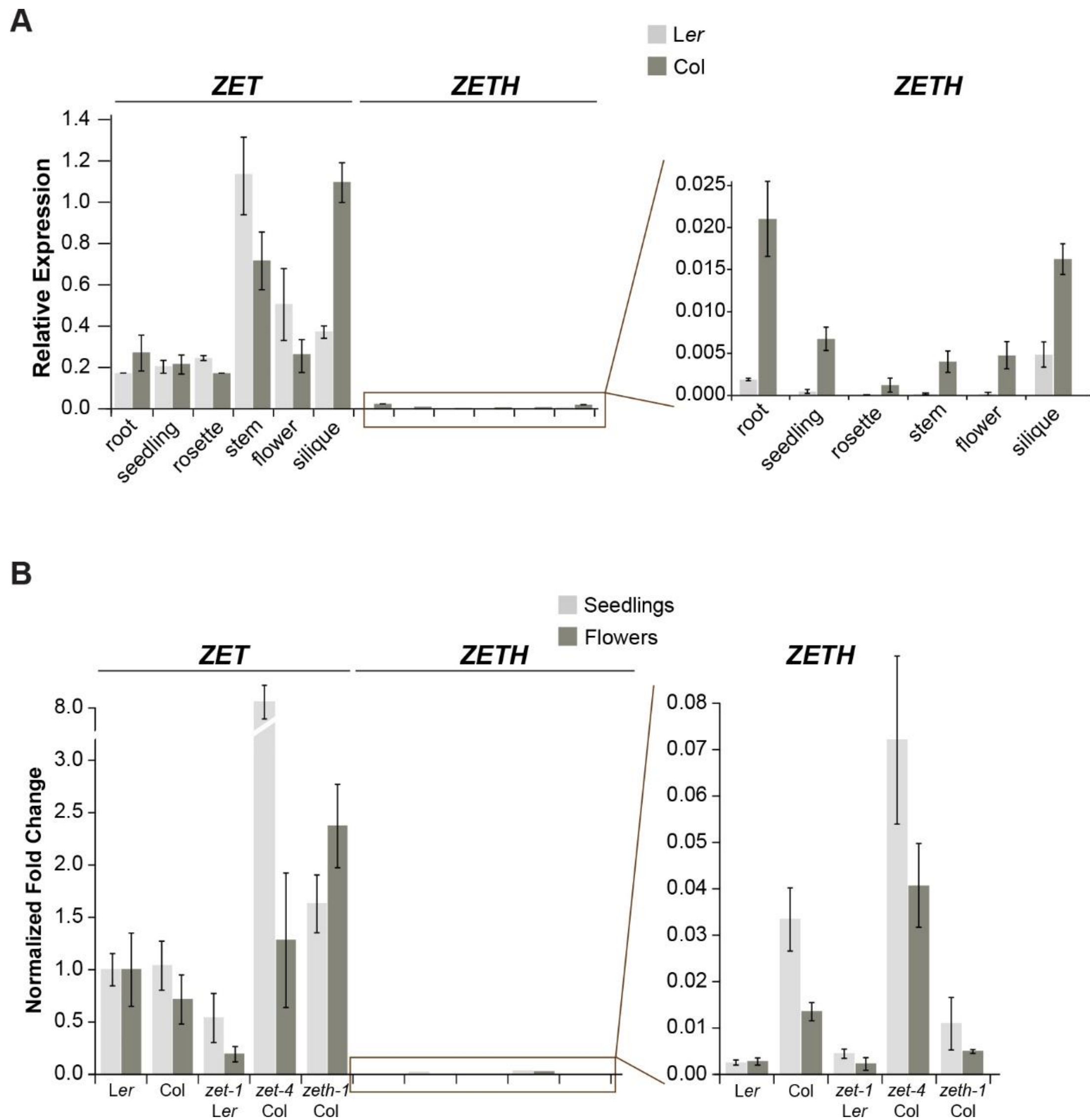

531

532 **Fig. 2. Expression analysis of *ZET* and *ZETH*.** (A) Tissue distribution of *ZET* and *ZETH*
533 transcript expression levels by qPCR. (n= 3 biological replicates). Means ± SEMs are
534 indicated. Age of plants: roots and seedlings, 10 days; rosette, 3 weeks; stem, flowers (stages
535 1-12) and siliques (stage 17), 5 weeks. (B) Comparison of *ZET* and *ZETH* mRNA levels in
536 seedlings and stage 1-12 flowers of indicated mutants by qPCR. (n= 3 biological replicates).
537 Means ± SEMs are indicated.

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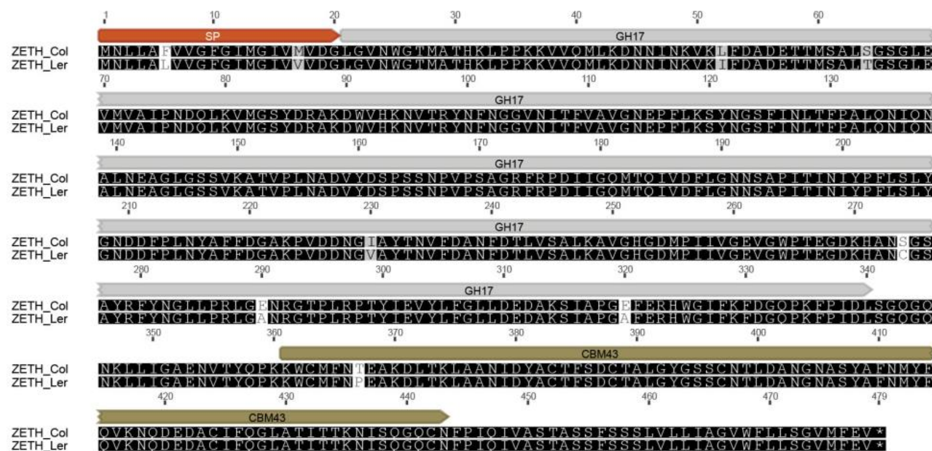

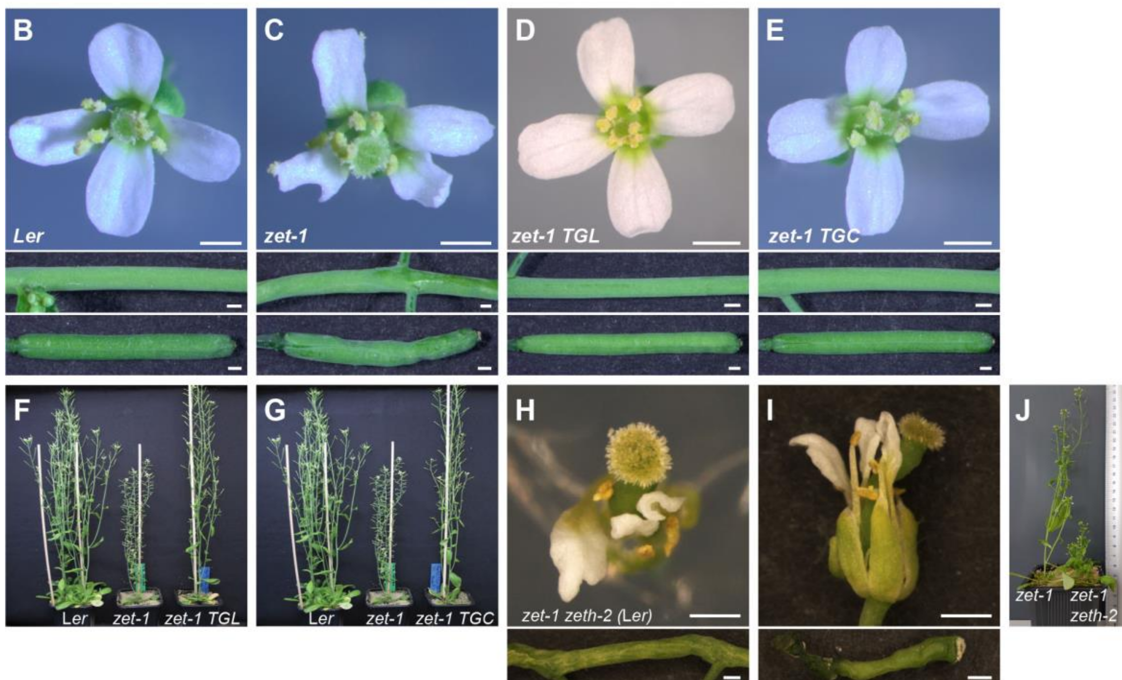

Fig. 3. *ZETH* in L*er* has residual function. (C) Alignment of ZETH amino acid sequences of Col and L*er*. (B-E) Complementation of *zet-1* phenotype by two TS:ZET reporter constructs. Upper panels: Stage 13 flower. Middle panel: siliques. Bottom panel: Stem. Genotypes are indicated. (C) Note aberrant floral morphology. Siliques and stems are twisted. (D, E) Note normal phenotype of a *zet-1* plant carrying either the L*er* or Col variant of *ZETH* under the control of the endogenous *ZET* (L*er*) promoter (TGL: *pZET::TS:ZETH*/L*er*, TGC: *pZET::TS:ZETH*/Col). (F, G) Whole-plant appearance. Genotypes are indicated. (H-J) Phenotype of *zet-1 zeth-2* (L*er*) mutant. Mutant phenotype is exaggerated. Scale bars: 0.5 mm.

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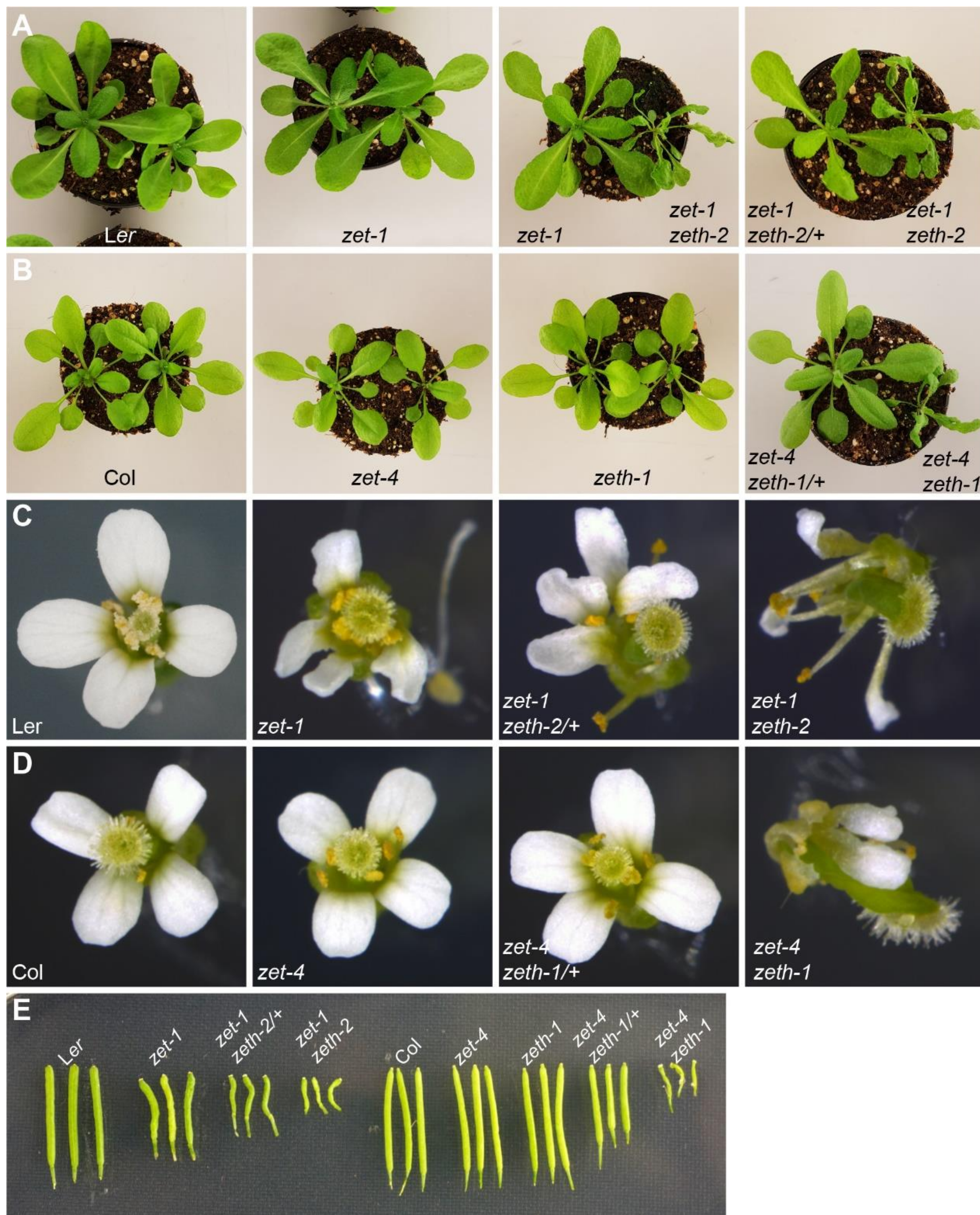

548

549 **Fig 4: *ZETH* acts in dosage dependent manner**

550 Genotypes are indicated. (A, B) Rosette leaves of three-week-old plants. (A) Notice slightly
551 elongated petiole and narrow leaf blade phenotype in *zet-1*, which gets enhanced in *zet-1*
552 *zeth-2/+* and *zet-1 zeth-2* mutants. (B) In Col background aberrant morphology is apparent
553 only in double mutants *zet-4 zeth-1*. (C, D) Flowers. (E) Siliques. Similar to leaves, twisting
554 morphology of flowers and siliques is exaggerated progressively in L*er* mutants, but in Col
555 only double mutant shows phenotype with the exception of siliques. Siliques of *zet-4 zeth-*
556 *1/+* in Col are shorter.

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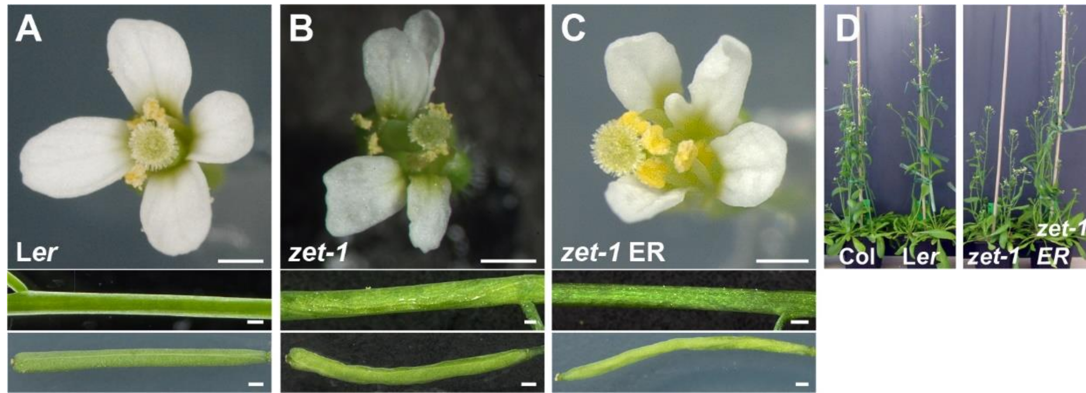
557 

558 **Fig. 5. The *zet-1* phenotype in the presence of functional *ERECTA* (*ER*).** (A-C)
559 Comparison of wild type, *zet-1* and *zet-1* plants transgenic for Col *ERECTA* Morphology of
560 flowers (upper panel), stems (central panel) and siliques (bottom panel). (A) Wild-type L*er*.
561 (B) *zet-1*. Note the aberrant flower and silique morphology. Stem twisting is mild. (C)
562 Transgenic *zet-1 ER*. Note the irregular flower and silique morphology. Stem morphology is
563 essentially normal. (D) Plant height comparisons of six-week-old *zet-1 ER* transgenic plants
564 in comparison to wild type and mutant reference lines. Note the rescue of plant height in *zet-1*
565 *ER* plants. Scale bars: (A-C) 0.5 mm

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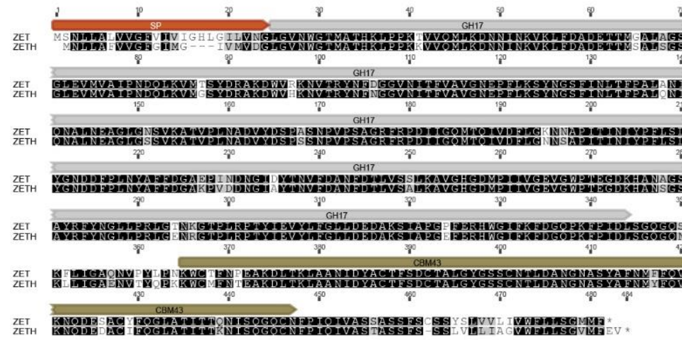

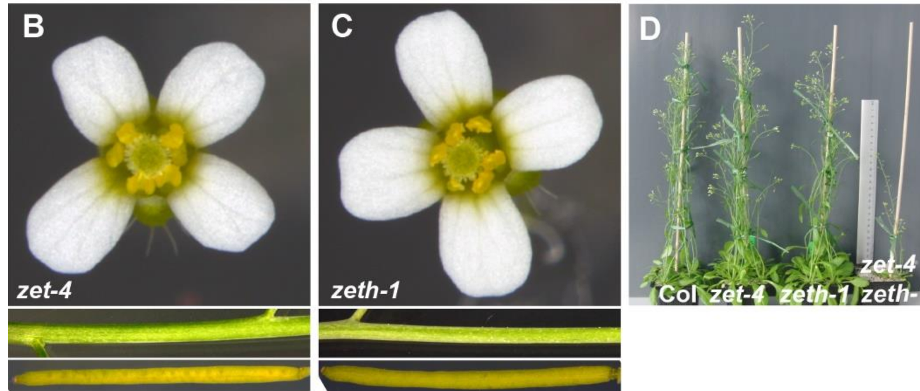

567

568 **Fig. S1.** A) Alignment of ZET and ZETH amino acid sequences. B) *zet-4.* C) *zeth-1*. Flower,
569 stem and silique morphology is essentially normal. (D) Whole-plant appearance. Genotypes
570 are indicated.

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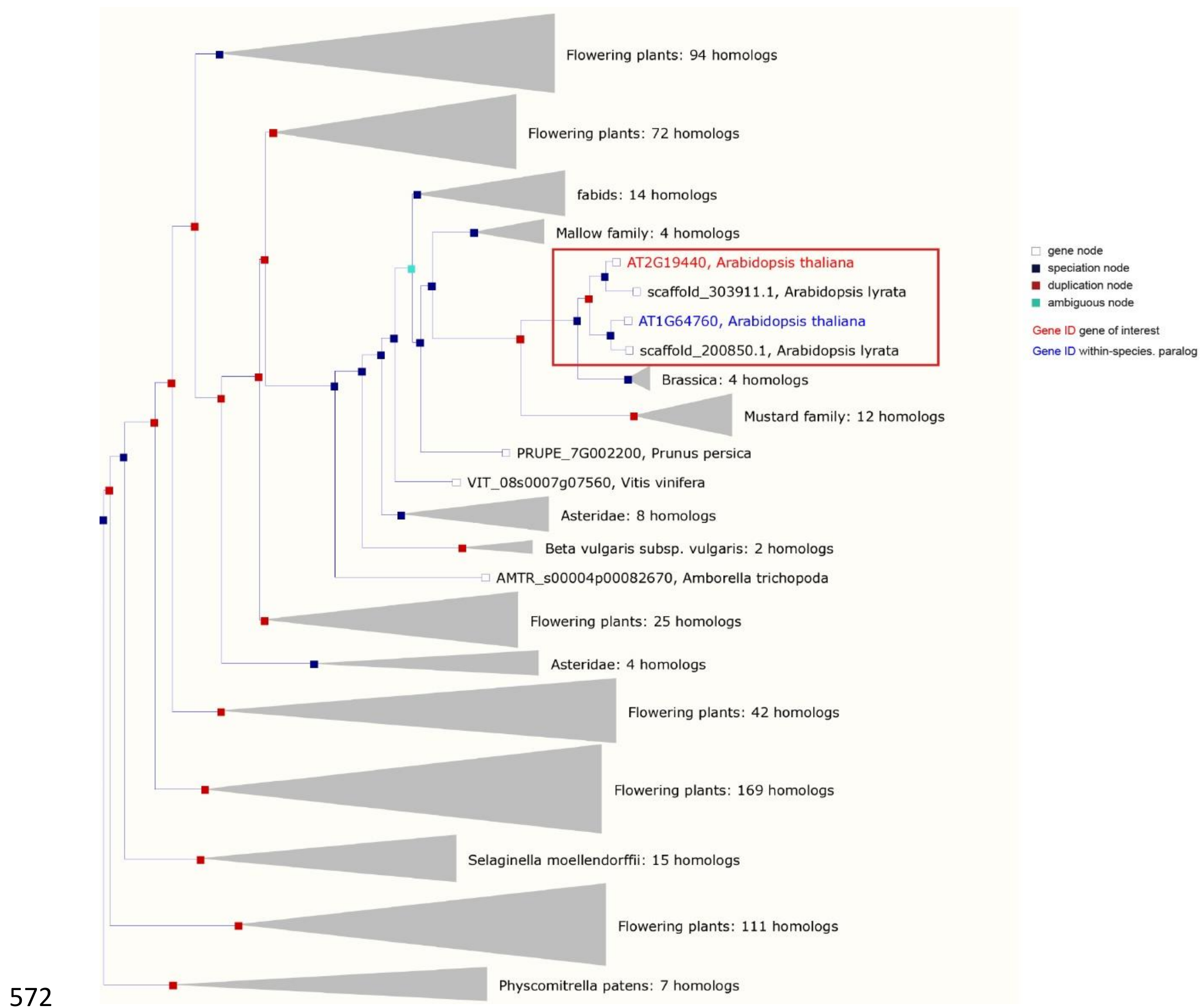

572

573 **Fig. S2. Evolutionary history of ZET (AT1G64760) and ZETH (AT2G19440).**

574 Phylogenetic tree showing the evolution of the ZET and its closest homolog (ZETH). The

575 tree shows that duplication is specific to Arabidopsis and it is not present in any other

576 Brassicaceae members. (Source: http://plants.ensembl.org)

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578 **Supplementary Table S1. Primers used in this study.**

Primer Name	**Sequence (5'-3')**
ZETHCol_F	ATCCCGGGCTAGGTGTCAACTGGGGAACAATG
ZETHCol_R	TAGGATCCCATATTATGCAATTTAATACATGG
ZETHL*er*_F/	ATCCCGGGCTAGGTGTGAACTGGGGAACAATG
ZETHL*er*_R	TAGGATCCATCACGTACATTAACTGCCGTTAG
ZETqRT_F	TCAAGATGAGAGTGCTTGCTATTTT
ZETqRT_R	CACAACCAAAGAATAAGACGAACAAGA
ZETHqRT_F	CCAGGATGAGGATGCTTGTATCTTC
ZETH qRT_R	TCCTGATAAGAGAAACCAAACTCCAGC
DT1-F0_ZETH	TGTAATGATCAGCTTAAGGTTGTTTTAGAGCTAGAAATAGC
DT2-R0_ZETH	AACAACCTTAAGCTGATCATTACAATCTCTTAGTCGACTCTAC
DT1-BsF_ZETH	ATATATGGTCTCGATTGTAATGATCAGCTTAAGGTTGTT
DT2-BsR_ZETH	ATTATTGGTCTCGAAACAACCTTAAGCTGATCATTACAA

579