

1 Transcriptomic Response to Nitrogen Availability Highlights Signatures of 2 Adaptive Plasticity During Tetraploid Wheat Domestication

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27

28 **Abstract**

29 The domestication of crops, with the development of the agroecosystems, is associated with
30 major environmental changes and represent a model to test the role of phenotypic plasticity.
31 Here we investigated 32 genotypes representing key stages of tetraploid wheat domestication.
32 We developed a dedicated pipeline combining RNA-Seq data, estimates of evolvability and
33 Q_{ST} to characterize the plasticity of gene expression and identify signatures of selection under
34 different nitrogen conditions. The analysis of gene expression diversity showed contrasting
35 results between primary and secondary domestication in relation to nitrogen availability.
36 Indeed, nitrogen triggered the expression of twice the number of genes in durum wheat
37 compared to emmer and wild emmer. Q_{ST} distributions and Q_{ST} - F_{ST} comparisons revealed
38 distinct selection signatures at each domestication stage. While primary domestication affected
39 the expression of genes involved in biotic interactions, secondary domestication was associated
40 with changes in expression of genes involved in metabolism of amino acids, particularly lysine.
41 Selection signatures were found also in differentially expressed genes, specifically involved in
42 nitrogen metabolism, such as *glutamate dehydrogenase*. Overall, our findings show that
43 nitrogen availability had a pivotal role during the domestication and adaptive responses of a
44 major food crop, with varying effects across different traits and growth conditions.

45

46 **Introduction**

47 Domestication influences the genetic diversity of animals and plants as they adapt to
48 agroecosystems, and undergo selection to meet human preferences and needs. This process is
49 typically associated with the genome-wide loss of nucleotide diversity due to the combined
50 consequences of selection and genetic drift, which is known as the domestication bottleneck.
51 The loss of genetic diversity has been documented in many domesticated species by comparing
52 them with wild relatives (Bitocchi et al., 2017). A parallel effect is the reprogramming of gene
53 expression and the loss of expression diversity, which was first reported in the common bean
54 (*Phaseolus vulgaris*) (Bellucci et al., 2014) and subsequently in other domesticated plants and
55 animals (Sauvage et al., 2017; Liu et al., 2019; Burgarella et al., 2021). Similar observations
56 have been reported at the level of metabolic diversity (Beleggia et al., 2016; Alseekh et al.,
57 2021).

58 Changes in nucleotide and gene expression diversity during the domestication of tetraploid
59 wheat (*Triticum turgidum* L., $2n = 4x = 28$; AABB genome) are not fully understood. Evidence

60 indicates that domestication occurred in two well-defined phases: Primary domestication from
61 wild emmer (*Triticum turgidum* ssp. *dicoccoides*) to emmer (*Triticum turgidum* ssp. *dicoccum*)
62 started ~12,000 years ago in the Fertile Crescent. This was followed by secondary
63 domestication from emmer to durum wheat (*Triticum turgidum* ssp. *durum*), which started
64 ~2,000 years ago in the Near East and gave rise to durum wheat, the most important form of
65 tetraploid wheat and currently the most widespread Mediterranean crop (Gioia et al., 2015;
66 Taranto et al., 2020).

67 The molecular mechanisms underlying phenotypic plasticity in crops (Laitinen and Nikoloski,
68 2019) and their wild relatives must be understood to address the challenges faced by modern
69 agriculture, including the overreliance on nitrogen (N) fertilizers to meet Sustainable
70 Development Goals (SDGs). N is an essential macronutrient whose availability is directly
71 linked to crop yield and grain quality (protein content) (Barneix, 2007; Howarth et al., 2008;
72 Laidò et al., 2013), but it is also harmful to people and nature. Indeed, excess of N from
73 agricultural sources is one of the major pollutant in fresh water (Bijay-Singh and Craswell,
74 2021). Understanding genetic variations in N acquisition, assimilation and metabolism can
75 therefore provide novel sustainable strategies for crop improvement (Plett et al., 2018;
76 Hawkesford and Griffiths, 2019). In tetraploid wheat, phenotypic differences related to N
77 availability primarily arose during secondary domestication rather than primary domestication
78 (Gioia et al., 2015), but the relationship between N metabolism and changes in gene expression
79 plasticity during domestication is unclear.

80 Here we analysed 32 wild emmer, emmer and durum wheat genotypes by RNA-Seq to
81 determine how contrasting differences in N availability shaped the nucleotide and gene
82 expression diversity of tetraploid wheat during primary and secondary domestication. Our
83 results provide insight into the pivotal role of N during the domestication and adaptive
84 plasticity of one of our major food crops.

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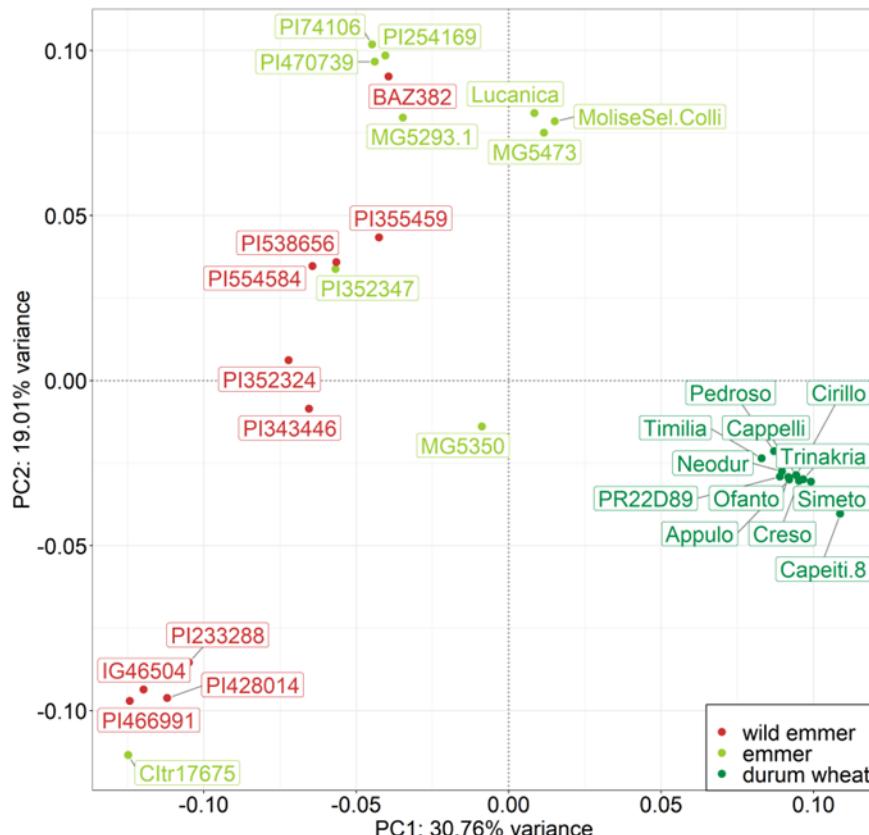
86 **Results and discussion**

87 **A greater loss of nucleotide diversity occurred during the secondary domestication of 88 tetraploid wheat**

89 We prepared 128 RNA-Seq libraries from 4-week-old leaves of 32 tetraploid wheat genotypes
90 representing *T. turgidum* ssp. *dicoccoides*, ssp. *dicoccum* and ssp. *durum* (Supplementary Table
91 S1). On average, 6.8 million of reads per genotype (Supplementary Table S1) were mapped to

92 the A and B reference subgenomes of bread wheat (Alaux et al., 2018). The mapping frequency
93 exceeded 85% for all the three subspecies and the fraction of reads mapping to gene regions
94 exceeded 72% (Supplementary Table S1).

95 Variant calling produced 800,996 high-quality single-nucleotide polymorphisms (SNPs). The
96 number of polymorphic sites was similar in wild emmer (617,128) and emmer (613,509), but
97 was much lower in durum wheat (425,513), confirming the higher genetic diversity of the wild
98 population. We identified 190,377 common SNPs shared by all three taxa. As expected, wild
99 emmer and emmer shared the highest percentage of SNPs (33%, 206,578). In contrast, durum
100 wheat shared only 11% (46,352) of its SNPs with wild emmer and 17% (71,147) with emmer.
101 SNPs principal component analysis (PCA) revealed the broad genetic structure of the three
102 wheat taxa (Figure 1) and confirmed that secondary domestication had a greater impact than
103 primary domestication in differentiating the durum wheat subspecies. The analysed 12 durum
104 wheat genotypes are genetically very similar, forming a dense cluster that is clearly
105 distinguishable from the wild emmer and emmer genotypes. In contrast, the wild emmer and
106 emmer genotypes were loosely clustered, indicating a greater genetic admixture. These results
107 are consistent with previous genetic studies on the origins of domesticated wheat and reflect
108 the multiple stages of domestication (Luo et al., 2007; Civáň et al., 2013; Oliveira et al., 2020),
109 and indicate that the used genotypes are representative.



110

111 **Figure 1: Principal component analysis of 32 wheat genotypes based on single-nucleotide**
112 **polymorphisms (SNPs).** The first two principal components (PC1 and PC2) are shown. The
113 three colors represent different taxa. Labels show the accession name of each genotype.

114

115 Nucleotide diversity estimates (Table 1) show the expected substantial loss of nucleotide
116 diversity during domestication. The average nucleotide diversity of durum wheat was ~35%
117 lower than domesticated emmer, which was in turn ~11% lower than wild emmer, highlighting
118 the greater impact of secondary domestication. When the cumulative effect of primary and
119 secondary domestication is taken into account, we observed a ~42% reduction in the nucleotide
120 diversity of durum wheat compared to its wild ancestor (Table 1).

121

122

123

124

	wild emmer	emmer	durum wheat	Loss of nucleotide diversity (%)		
				Lpd	Lsd	both
π	0.0050	0.0045	0.0029	11.4	34.6	42.1
θ	0.0047	0.0040	0.0029	15.3	27.2	38.3

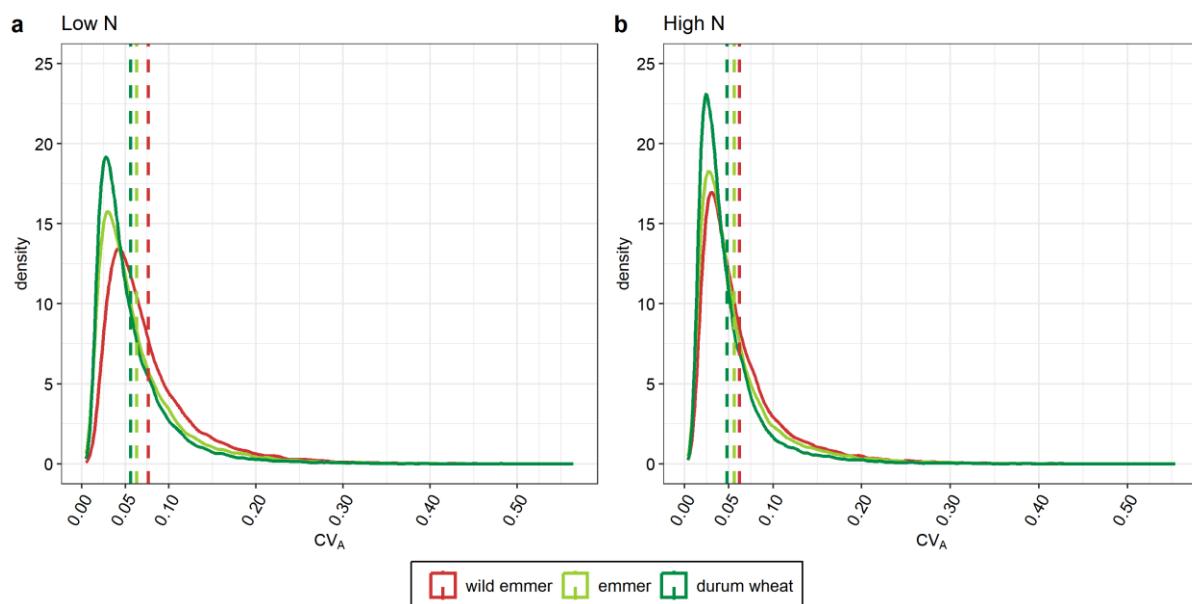
125 **Table 1: Nucleotide diversity estimates and diversity loss for the three wheat taxa.**

126 Diversity loss is shown during primary domestication (wild emmer to emmer, Lpd), secondary
127 domestication (emmer to durum wheat, Lsd) and both processes (wild emmer to durum wheat),
128 based on average π and θ values. The π and θ symbols represent averaged estimates of
129 nucleotide diversity.

130

131 **The variability of gene expression during domestication was influenced by N availability**

132 To quantify the diversity of gene expression in each subspecies, we calculated evolvability
133 scores under high and low N availability conditions. Evolvability was estimated using the
134 additive coefficient of variation (CV_A) in read counts (Supplementary Table S2). In contrast to
135 heritability, CV_A is a standardized measure of additive genetic variation that is not influenced
136 by other sources of variance (Houle, 1992; Hansen et al., 2011), and is therefore well suited for
137 comparative analysis (Garcia-Gonzalez et al., 2012). As for nucleotide diversity, we found that
138 the CV_A decreased during domestication under both N conditions; however, the mean CV_A of
139 all three subspecies was higher under low N conditions (Figure 2a,b; Table 2). High N
140 availability therefore appears to promote a more uniform gene expression pattern, whereas
141 higher variability is observed during N starvation. The association between domestication and
142 declining diversity in gene expression has also been reported in crops, such as: common bean
143 (Bellucci et al., 2014), tomato (Sauvage et al., 2017) and sorghum (Burgarella et al., 2021) as
144 well as domesticated animal species (Liu et al., 2019).



145

146 **Figure 2: Density plots of the additive coefficient of variation (CV_A) in the three wheat**
147 **taxa.** Comparison of the estimated density functions of the CV_A in gene expression, calculated
148 using all 32,358 genes. **a** High nitrogen conditions. **b** Low nitrogen conditions. Dashed lines
149 represent the averaged CV_A value, colored according to the different taxa.

150

151 We used the contrasting N conditions of our samples to examine whether the loss of expression
152 diversity is associated with the specific aspects of the cultivation environment, causing primary
153 and secondary domestication to have a significantly different impact. Under high N conditions,
154 we observed a ~9% loss in expression diversity in emmer compared to wild emmer (effect of
155 primary domestication) and a ~15% loss in durum wheat compared to emmer (effect of
156 secondary domestication). In contrast, these losses were ~18% and 11% under N starvation
157 conditions, revealing twice the loss of expression diversity during primary domestication, but
158 a lower value during secondary domestication (Table 2). All four values differed significantly
159 from each other (Mann–Whitney test, $p < 0.001$). The opposing expression diversity profiles
160 during domestication under high and low N conditions were observed not only for overall gene
161 expression, but also for the subgroup comprising all differentially expressed genes (DEGs) and
162 the subgroup comprising all unmodulated genes (Supplementary Table S3). The loss of
163 expression diversity among the DEGs due to primary domestication was ~9% and ~15% under
164 high and low N conditions, respectively, whereas the loss due to secondary domestication was
165 ~18% and ~14% under high and low N conditions, respectively (Supplementary Table S3).

166 The loss of expression diversity among the unmodulated genes was similar to the values for
167 overall gene expression (Supplementary Table S3).

	wild emmer	emmer	durum wheat	Loss of expression diversity (%)		
				Lpd	Lsd	both
CV _A high N	0.062	0.056	0.048	9.1	14.5	22.3
CV _A low N	0.076	0.063	0.056	17.6	11.1	26.7

168 **Table 2: Mean additive coefficient of variation (CV_A) in gene expression and loss of
169 expression diversity for the three wheat taxa.** Diversity loss is shown during primary
170 domestication (wild emmer to emmer, Lpd), secondary domestication (emmer to durum wheat,
171 Lsd) and both processes (wild emmer to durum wheat), based on averaged CV_A values
172 calculated for all 32,358 genes.

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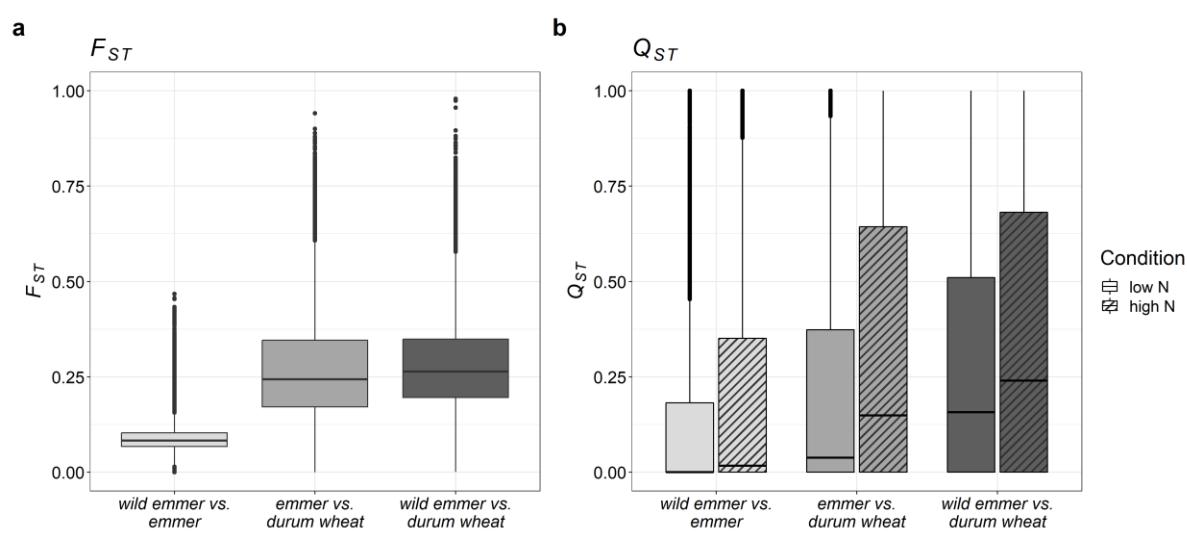
174 A phenotypic study of the same accessions used in the present work has already shown that
175 secondary domestication reduced the phenotypic diversity under high N conditions, but the
176 reduction was smaller and not significant under N starvation conditions (Gioia et al., 2015). In
177 the case of durum wheat, selection has apparently enhanced the growth response to N
178 availability, indicating a putative focus on improving N uptake and utilization efficiency. Our
179 expression diversity results indicate that selection has favored specific traits and thus led to a
180 more uniform set of cultivars, as also suggested in earlier study using morphological traits
181 (Gioia et al., 2015).

182

183 **Domestication and nitrogen availability shaped the divergence of tetraploid wheats**

184 Genetic differentiation among the three subspecies was estimated by calculating the pairwise
185 fixation index (F_{ST}) for every gene locus in our dataset. As shown in Figure 3a, the lowest
186 genetic differentiation was observed between wild emmer and emmer (mean $F_{ST} = 0.09$),
187 whereas much higher genetic differentiation was found between emmer and durum wheat
188 (mean $F_{ST} = 0.27$) and, similarly, between wild emmer and durum wheat (mean $F_{ST} = 0.28$).
189 These values align with earlier findings that examined broad collections of tetraploid wheat
190 accessions (Luo et al., 2007; Mazzucotelli et al., 2020), and provide additional evidence for the
191 representativeness of the genotypes used.

192 Divergence at the transcriptomic level was estimated by calculating Q_{ST} , the quantitative analog
193 of F_{ST} , taking N availability into account as an environmental variable. Under both N
194 conditions, we observed the same trend shown for F_{ST} (Figure 3b). Specifically, secondary
195 domestication had a stronger impact on differentiation (emmer vs durum wheat, mean $Q_{ST\ LN}$ =
196 0.23, mean $Q_{ST\ HN}$ = 0.33) than primary domestication (wild emmer vs emmer, mean $Q_{ST\ LN}$ =
197 0.16, mean $Q_{ST\ HN}$ = 0.23). Interestingly, the Q_{ST} distributions of every pairwise comparison
198 showed higher values under high N conditions compared to N starvation (Figure 3b),
199 suggesting that N availability during domestication significantly contributed to the
200 differentiation of gene expression in tetraploid wheats.



201
202 **Figure 3: F_{ST} and Q_{ST} distributions.** **a** Boxplots showing the gene locus F_{ST} distribution for
203 every subspecies pairwise comparison. **b** Boxplots showing the transcript Q_{ST} distribution for
204 every subspecies pairwise comparison under low nitrogen and high nitrogen conditions,
205 represented by empty and hatched grayscale bars, respectively.

206
207 The Q_{ST} distributions were used to perform a “selection scan”, seeking genes whose expression
208 was potentially under selection. Starting from 5,868 genes meeting the heritability criteria
209 ($H^2 \geq 0.7$ or $S \times N \geq 0.2$, that is the species \times environment variance component i.e., every species
210 subgroup \times N condition; Supplementary Figure S1), we retained 973 genes having Q_{ST} values
211 in the 5% right tail of the distributions. The Q_{ST} – F_{ST} comparison method (Leinonen et al., 2013)
212 was then used to confirm that the divergent expression (high Q_{ST} values) of the filtered genes
213 was caused by directional selection ($Q_{ST} > F_{ST}$) and not by genetic drift ($Q_{ST} \approx F_{ST}$) or stabilizing
214 selection ($Q_{ST} < F_{ST}$) (Leinonen et al., 2013). After removing F_{ST} values < 0.01 , we retained

215 967 genes satisfying the criterion $Q_{ST} > F_{ST}$, indicating that their expression was likely
216 subjected to directional selection in at least one of the evolutionary contexts examined herein
217 (i.e., primary and/or secondary domestication under high and/or low N availability conditions)
218 (Supplementary Table S4).

219 Gene Ontology (GO) enrichment analysis revealed that selection acted on distinct gene
220 categories during primary and secondary domestication (Supplementary Figure S2). During
221 primary domestication, we found categories associated with “defense-related programmed cell
222 death, modulated by biotic interactions”, indicating an enhanced plant hypersensitive response
223 to pathogens. This can be interpreted as a consequence of the transition from the natural
224 growing environment of the wild genotypes to agroecosystems characterized by high-density
225 domesticated crop monocultures. In this context, crops face higher disease pressure from crop-
226 specific pathogens (Savary et al., 2019) and therefore induce a hypersensitive response, which
227 can lead to programmed cell death and necrosis as a defense mechanism. It is important to note
228 that pathogen defense mechanisms in plants often overlap with the regulation of beneficial
229 symbiotic interactions, therefore, one expects a trade-off between traits associated with
230 symbiosis and innate immunity (Porter and Sachs, 2020). Moreover, domesticated crops are
231 less able to fully benefit from microbial interactions than their wild relatives, as observed in a
232 comparative study of bread wheat landraces as well as old and modern varieties (Valente et al.,
233 2023). One contributing factor is the widespread use of high-input agricultural practices,
234 because the availability of fertilizers reduces the need for plants to invest in symbiotic
235 relationships (Martín-Robles et al., 2018). Additionally, certain target traits in plant breeding,
236 such as phytohormones that regulate flowering time and plant height, can have unintended
237 effects on beneficial symbiosis due to pleiotropy (Sawers et al., 2018).

238 Among the genes found to be under selection during secondary domestication, we observed
239 the enrichment of categories associated with amino acid metabolism, particularly those related
240 to the “lysine catabolic process” (Supplementary Figure S2). This included genes encoding the
241 bifunctional enzyme lysine ketoglutarate reductase/saccharopine dehydrogenase (LKR/SDH).
242 This enzyme is ubiquitous in plants and animals, and represents the key step in lysine
243 catabolism via the saccharopine pathway (SACPATH). The structure and transcription of the
244 *LKR/SDH* gene has been studied in *T. durum* and compared with other plants, showing species-
245 dependent differences in expression levels including lineage-specific differences between
246 monocots and dicots (Anderson et al., 2010). Lysine is the first limiting essential amino acid
247 in cereal grains and its catabolic pathway has been targeted to increase the lysine content of

248 maize and rice seed (Houmard et al., 2007; Frizzi et al., 2008; Long et al., 2013). Generally,
249 the quantity of lysine-containing proteins in cereal seeds is much lower than that of storage
250 proteins devoid of lysine, such as prolamins (specifically gliadin in wheat). The SACPATH
251 seems to channel the lysine skeleton into the production of glutamic acid, which is a precursor
252 of proline, one of the most abundant amino acids in glutens (Arruda et al., 2000).

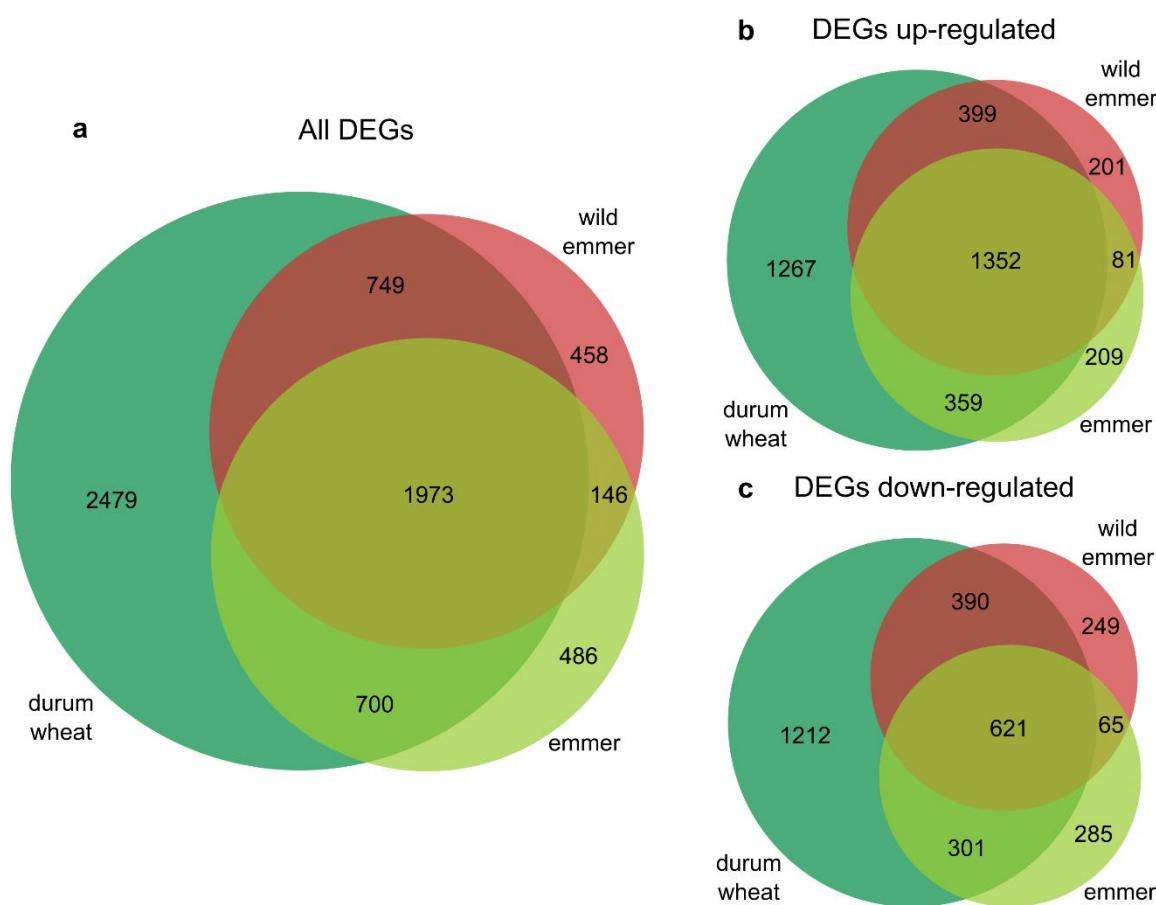
253 General changes in amino acid metabolism during domestication have been observed in other
254 crops based on nucleotide data, including sunflower (Chapman et al., 2008), maize (Swanson-
255 Wagner et al., 2012) and common bean (Bellucci et al., 2014). Evolutionary metabolomics has
256 also revealed signatures of selection affecting amino acid metabolism during secondary
257 domestication (Beleggia et al., 2016). In durum wheat, domestication was linked to the
258 selection of a specific protein composition and led to a notable decrease in the diversity of
259 gliadin and glutenin subunits, strongly correlating with grain yield and the technological
260 properties of gluten (Laidò et al., 2013). The analysis of spring wheat genotypes has shown
261 that the SACPATH is upregulated in response to drought stress, and is significantly more active
262 in drought-tolerant compared to drought-susceptible genotypes (Michaletti et al., 2018). This
263 may reflect the role of proline, which can be produced from this pathway, as a major constituent
264 of storage proteins and one of the main osmoprotectants produced as a response to stress (Kavi
265 Kishor et al., 2022). These findings suggest that selection for stress-tolerant genotypes as well
266 as seed protein composition during wheat domestication influenced the expression of
267 SACPATH genes.

268

269 **Changes in nitrogen availability trigger gene expression, resulting in a twofold increase**
270 **in the number of differentially expressed genes in durum wheat compared to emmer and**
271 **wild emmer wheat.**

272 We identified DEGs in each subspecies that discriminated between high N conditions and N
273 starvation using a stringent pipeline and strict thresholds (p-adjust < 0.001) to reduce the
274 number of false positives. We found 3,326 DEGs in wild emmer, 3,305 in emmer and 5,901 in
275 durum wheat, with more upregulated than downregulated genes in all three subspecies. Durum
276 wheat had the highest percentage of private DEGs (~42%, 2,479), whereas similar numbers
277 were found in wild emmer (~14%, 458) and emmer (~15%, 486). Wild emmer and emmer
278 shared ~23% (749) and ~21% (700), respectively, of their DEGs with durum wheat. The
279 percentage of DEGs shared only between wild emmer and emmer was 4% (146), but almost

280 60% of wild emmer and emmer DEGs and ~33% of durum wheat DEGs were shared by all
281 three taxa (Figure 4a). The proportions of private and shared DEGs were preserved when we
282 separated them into upregulated and downregulated subsets (Figure 4b,c). In all three taxa,
283 most DEGs were located on chromosomes 2A, 2B, 3A, 3B, 5A and 5B, each carrying > 7.5%
284 of the DEGs; in contrast, chromosomes 6A and 6B each contained only ~5% of the DEGs
285 Supplementary Figure S3.



286
287 **Figure 4: Differentially expressed genes (DEGs) when comparing high and low nitrogen
288 conditions within each subspecies.** Venn diagrams showing **a** Total set of DEGs; **b**
289 upregulated DEGs only; and **c** downregulated DEGs only.

290
291 GO enrichment analysis of the DEGs meeting the threshold FDR < 0.05 revealed 23 macro-
292 categories in wild emmer, 21 in emmer and 25 in durum wheat (Supplementary Figure S4).
293 The main differences between the three subspecies were observed for categories related to
294 “signaling”, “regulation of biological process”, “developmental process”, and “metabolic
295 process” Supplementary Table S5. We observed the uniform enrichment of GO categories

296 associated with upregulated genes in all three subspecies, including terms linked to N and
297 amino acid metabolism as well as carbon metabolism and photosynthesis (Supplementary
298 Table S5). In contrast, the enrichment of GO categories associated with downregulated genes
299 was more selective, with some GO categories related to N metabolism enriched only in durum
300 wheat, including GO:0006807 and GO:0034641 (N compound and cellular N compound
301 metabolic process, respectively) and GO:0006536 “glutamate metabolic process”
302 (Supplementary Table S5). Functional annotations of the most strongly modulated genes (top
303 5% $|\log_2\text{FC}|$ values) are reported in Supplementary Table S6.

304 Our data confirm, on a larger set of samples, earlier observations on the response of wheat to
305 N starvation based on transcriptomics and metabolomics data^{36–38}. These earlier studies
306 included one emmer and one durum wheat genotype also present in our sample set (Beleggia
307 et al., 2021), but also considered the durum wheat cultivar Svevo (Curci et al., 2017) and
308 various bread wheat cultivars (Sultana et al., 2020). As expected, genes involved in N
309 metabolism were modulated during N starvation. Among the key genes for N assimilation,
310 those encoding asparagine synthetase and nitrite reductase were upregulated in every taxon,
311 whereas those encoding glutamate carboxypeptidase and glutamate decarboxylase were
312 downregulated. We observed contrasting profiles for genes encoding ureide permease
313 (encoding a ureide transporter), which were strongly upregulated in all three subspecies in
314 response to N stress, whereas genes encoding nitrate transporters were strongly downregulated.
315 The modulated genes also included transporters of amino acids and other nutrients.

316 N starvation also influenced other metabolic pathways, revealing many further DEGs involved
317 in C metabolism, especially fatty acid metabolism, glycolysis, photosynthesis and the
318 tricarboxylic acid (TCA) cycle. About 10% of the highest-ranking DEGs represented
319 transcription factors and protein kinases. The most common functional category (accounting
320 for 17% of annotated DEGs) reflected the general stress response to N starvation, including the
321 mitigation of oxidative stress and detoxification. Examples included genes encoding
322 *cytochrome P450s*, *glutaredoxin family*, *glutathione S-transferases* and *peroxidases*
323 (Supplementary Table S6).

324 To compare gene expression between the three taxa while taking the environmental effects into
325 account, we also identified DEGs between each pair of subspecies under all N conditions.
326 Accordingly, we compared emmer *vs* wild emmer (primary domestication, high and low N),
327 durum wheat *vs* emmer (secondary domestication, high and low N) and durum wheat *vs* wild
328 emmer (cumulative effect, high and low N) (Supplementary Figure S5). The wild emmer *vs*

329 emmer comparison revealed few DEGs regardless of N availability (12 and 11 DEGs under
330 high and low N conditions, respectively), whereas the emmer *vs* durum wheat comparison
331 revealed 41 DEGs associated with high N and 29 associated with N starvation, and the wild
332 emmer *vs* durum wheat comparison revealed 46 DEGs associated with high N and only 10
333 associated with N starvation. These data indicate that the number of DEGs increases during
334 domestication but only when there is a sufficient N supply (Supplementary Figure S5).
335 Interestingly, there were more upregulated than downregulated genes in all pairwise
336 comparisons under high N conditions (~65%) but the proportion increased under N starvation
337 conditions, particularly for the comparison of wild emmer *vs* durum wheat (90%). The
338 preponderance of upregulated genes during domestication has also been observed in maize
339 (Lemmon et al., 2014), whereas domestication was shown to increase the proportion of
340 downregulated genes in common bean (Bellucci et al., 2014), egg-plant (Page et al., 2019) and
341 sorghum (Burgarella et al., 2021) landraces compared to wild relatives. The absence of
342 consistent patterns suggests that the evolution of domesticated phenotypes is driven by specific
343 processes that are unique to each crop.

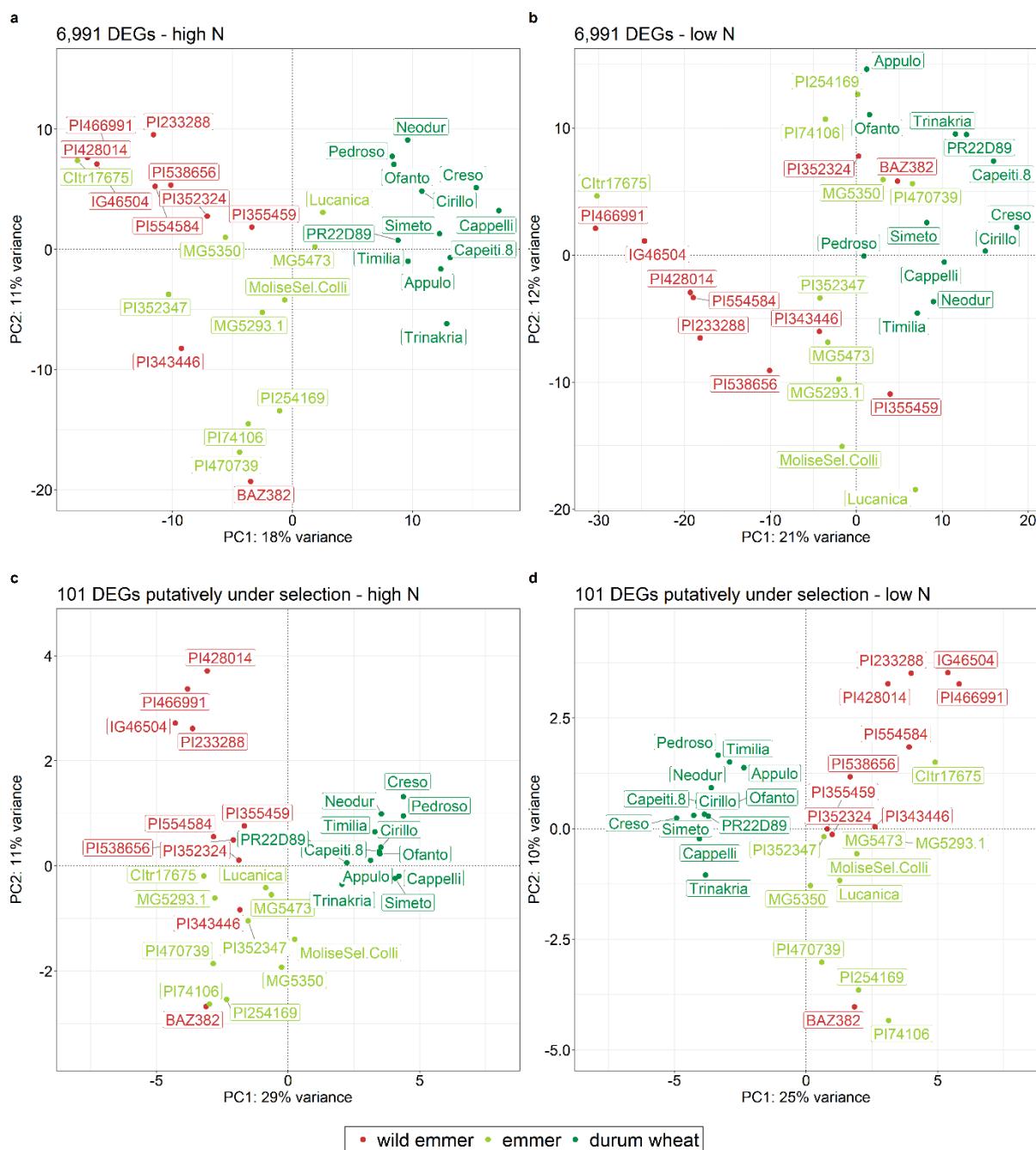
344 Among the 102 DEGs (Supplementary Table S7) found in at least one of the six pairwise
345 comparisons between subspecies, 35 were also found among DEGs identified between
346 contrasting N conditions and of which 24 were proposed to be under selection. Overall, six
347 genes were identified in all three experiments (i.e., differentially expressed between subspecies
348 and between contrasting N conditions, and showed evidence of selection).

349

350 **Selection shaped the expression profiles of genes modulated by nitrogen availability**

351 The 6,991 DEGs found in at least one species when comparing the contrasting N conditions
352 included 101 putatively under selection, which are candidates for the adaptive response to N
353 availability. We applied PCA to the normalized read counts in order to investigate if the
354 different genotype groups can be separated based on their gene expression. Initially we
355 incorporated all 6,991 DEGs (Figure 5a,b) before focusing on the subset of 101 DEGs that
356 were also putatively under selection (Figure 5c,d). When considering all DEGs, PC1 did not
357 completely separate the durum wheat genotypes from the other taxa, in contrast to the clear
358 separation observed for the SNP data (Figure 1), and this was particularly evident during N
359 starvation (Figure 5b). There was also a moderate degree of overlap between the wild emmer
360 and emmer genotypes along PC2. However, when we focused on the DEGs under selection,

361 PC1 separated the durum wheat genotypes into a densely clustered group (as observed for the
 362 SNP data) under both N conditions, and PC2 separated the wild emmer and emmer genotypes
 363 more clearly, especially under high N conditions (Figure 5c,d).



364
 365 **Figure 5: Principal component analysis of differentially expressed genes when comparing**
 366 **high and low nitrogen conditions within each subspecies. a,b Plots based on all 6,991 DEGs**
 367 **(not filtered): a high nitrogen conditions and b low nitrogen conditions. c,d Plots based on 101**
 368 **DEGs that are also putatively under selection: c high nitrogen conditions and d low nitrogen**

369 conditions. Samples are represented by taxa-based colored dots. Labels show the accession
370 name of each genotype.

371

372 The integration of selection signatures (based on Q_{ST}/F_{ST} values) and differential expression
373 analysis uncovered a set of 101 candidate genes that are interesting due to their potential roles
374 in the domestication and diversification of cultivated wheat, specifically in relation to N
375 availability. Functional annotation (Supplementary Table S8) revealed upregulated genes
376 involved in carbon (C) metabolism as well as some transcription factors and transporters, as
377 well as both upregulated and downregulated genes responsible for general stress responses and
378 N metabolism, specifically those encoding enzymes involved in amino acid metabolism, such
379 as methionine aminopeptidase, aspartokinase and glutamate dehydrogenase (GDH). The latter
380 is particularly noteworthy because, in addition to its modulation in response to different N
381 conditions and the presence of selection signatures, it was also upregulated in the comparison
382 between wild emmer and durum wheat under high N conditions. GDH is a key enzyme
383 involved in N metabolism and N/C balance (Miflin and Habash, 2002). This is supported by
384 the co-localization of quantitative trait loci for GDH activity and physiological traits associated
385 with the flag leaf lamina, such as soluble protein and amino acid content, as well as flag leaf
386 area and dry weight (Fontaine et al., 2009). Selection signatures were also identified in the
387 *GDH* gene when comparing landraces with old and modern durum wheat cultivars (Taranto et
388 al., 2020). Our results confirm that N metabolism has been a key driver during the evolutionary
389 history of wheat, particularly the central role of glutamate in the process of domestication. This
390 was also suggested by a combined transcriptomics and metabolomics study, showing that
391 glutamate and γ -aminobutyric acid (mainly synthetized from glutamate) are central to the
392 genotype-specific response of emmer and durum wheat to N starvation (Beleggia et al., 2021).

393

394 We have shown that significant changes occurred at the nucleotide and gene expression levels
395 during the domestication of tetraploid wheat, taking into account the environmental variable of
396 N availability. We confirmed that more nucleotide diversity has been lost during secondary
397 domestication compared to primary domestication, and revealed a parallel trend in the loss of
398 gene expression diversity associated to the domestication process, with a stronger effect due to
399 secondary domestication and unveil a parallel different impact of primary and secondary
400 domestication on the loss of expression diversity, which appears to be related to N availability

401 in the durum wheat selection environment. We present evidence that selection may have
402 operated in different directions during primary and secondary domestication, the former
403 involving changes related to biotic interactions and the latter related to amino acid metabolism.
404 By screening a large number of genotypes, we found a major transcriptional response in durum
405 wheat (compared to emmer and wild emmer) to changes in N availability. Finally, through the
406 innovative combination of RNA-Seq analysis and the estimate of quantitative genetics
407 parameters, we developed a pipeline to identify selection signatures and phenotypic plasticity
408 in gene expression data based on evolvability and Q_{ST}/F_{ST} scores. Our findings, elucidating the
409 role of N in tetraploid wheat domestication and adaptive response can guide the development
410 of innovative strategies for crop improvement, resource use efficiency, and environmental
411 sustainability.

412

413 **Materials and methods**

414 **Plant material and experimental design**

415 The study included 32 tetraploid wheat genotypes, comprising 10 accessions of wild emmer
416 (*T. turgidum* ssp. *dicoccoides*), 10 accessions of emmer (*T. turgidum* ssp. *dicoccum*), and 12
417 accessions of durum wheat (*T. turgidum* ssp. *durum*) (Supplementary Table S1). The samples
418 we analysed were part of a larger experiment, conducted in October 2012 and described
419 elsewhere (Gioia et al., 2015). Briefly, wheat genotypes were grown for 4 weeks under high
420 nitrogen (N+) and nitrogen starvation (N-) conditions in the Phytec Experimental Greenhouse
421 at the Institute of Biosciences and Geosciences (IBG-2), Plant Sciences Institute,
422 Forschungszentrum Jülich GmbH, Germany (50°54'36" N, 06°24'49" E). Seeds of uniform
423 size and mass were visually selected, surface sterilized (1% (w/v) NaClO for 15 min) and pre-
424 germinated. After germination, seedlings showing uniform growth (seminal root length, 1–2
425 cm) were transferred to soil-filled rhizoboxes, which were placed in the automated
426 GROWSCREEN-Rhizo phenotyping system available at IBG-2. We used a Type 0 manually
427 sieved peat soil (Nullerde Einheitserde; Balster Einheitserdewerk, Frondenberg, Germany),
428 which provided low nutrient availability (ammonium N and nitrate N concentrations of < 1.0
429 and < 1.0 mg l⁻¹, respectively). All plants were watered twice daily with 400 ml of tap water
430 and were supplied three times per week with 200 ml of modified Hoagland solution (Hoagland
431 and Arnon, 1950) with or without added N. For the N starvation conditions, KNO₃ and
432 Ca(NO₃)₂ were replaced with K₂SO₄ and CaCl₂·6(H₂O), respectively. The experiment was

433 carried out under natural lighting in the greenhouse, with an air temperature of 18–24 °C and
434 a relative humidity of 40–60%. For each N treatment, we used two replicates of each genotype
435 with two plants per replicate (four plants per genotype in total). After 4 weeks, leaves were
436 pooled from two plants of the same genotype growing in the same rhizobox. Accordingly, four
437 independent biological replicates (two replicates per N condition) were produced for each
438 genotype, with the exception of wild emmer IG 46504, PI 233288, PI 466991, PI 538656,
439 emmer MG 5293/1, and durum wheat Creso, Pedroso and Trinakria, for which only three
440 replicates were available, and emmer Molise Sel. Colli and durum wheat Simeto, for which
441 eight replicates were available. The tissues were immediately frozen in liquid N₂ and stored at
442 –80 °C. Further details of the experiment and growth conditions are provided elsewhere(Gioia
443 et al., 2015).

444 **RNA extraction and sequencing**

445 RNA was extracted from 100 mg of frozen ground leaves per replicate using the Spectrum
446 Plant Total RNA kit (Sigma-Aldrich, St Louis, MO, USA) followed by treatment with RNase-
447 free DNase using the On-Column DNase I Digestion Set (Sigma-Aldrich). RNA integrity and
448 purity were assessed by agarose gel electrophoresis and a Bioanalyzer 2100, respectively
449 (Agilent/Bonsai Technologies, Santa Clara, CA, USA). Only RNA samples with an RNA
450 integrity number > 8.0 were considered suitable for analysis.

451 Library construction and RNA sequencing were carried out using the Illumina mRNA-Seq
452 platform at the Montpellier Genomix sequencing facility (<http://www.mgx.cnrs.fr>) as
453 previously described (David et al., 2014). Briefly, RNA samples were processed using TruSeq
454 RNA sample preparation kits v2 (Illumina, San Diego, CA, USA). Libraries were quantified
455 by real-time PCR using the KAPA Library Quantification Kit for Illumina Sequencing
456 Platforms (Roche, Basel, Switzerland), followed by quality control using a DNA 100 Chip on
457 a Bioanalyzer 2100. Cluster generation and sequencing were carried out using the Illumina
458 HiSeq 2000 instrument and TruSeq PE Cluster Kit v3, following the Illumina
459 PE_Amp_Lin_Block_V8.0 recipe, and Illumina TruSeq PE Cluster v3-cBot-HS kits with the
460 2 × 100 cycles, paired-end, indexed protocol, respectively (David et al., 2014).

461 **RNA-Seq library processing and mapping**

462 We pre-processed 128 raw paired-end RNA-Seq libraries (David et al., 2014). Cutadapt
463 (Martin, 2011) was then used to remove adaptor sequences and trim the end of reads with low
464 quality scores (parameter -q 20) while keeping reads with a minimum length of 35 bp. Reads

465 with a mean quality score < 30 were discarded, and orphan reads (whose mates were discarded
466 in the previous filtering steps) were removed (David et al., 2014). The final quality of trimmed
467 and filtered reads was assessed using FastQC (Andrews, 2014).

468 The bread wheat (*Triticum aestivum* cv. Chinese Spring) genome assembly IWGSC RefSeq
469 v2.1, along with the corresponding genome annotation, were downloaded from the IWGSC
470 data repository hosted by URGI-INRAE (<https://wheat-urgi.versailles.inra.fr/>) and used as a
471 reference to map each cleaned library to the A and B sub-genomes. The bread wheat genome
472 was chosen deliberately to ensure the inclusion of an outgroup species that is closely related to
473 the subspecies in the panel. By doing so, we aimed to avoid bias that could arise from selecting
474 only one subspecies among our panel of accessions. We have confidence in this strategy
475 because the *T. aestivum* A and B subgenomes are derived from the tetraploid species included
476 in the study.

477 STAR v2.7.0e (Dobin et al., 2013) was used for read mapping with the --quantMode
478 TranscriptomeSAM and --quantTranscriptomeBan Singleend options. The output alignments
479 were translated into transcript coordinates (in addition to alignments in genomic coordinates),
480 allowing insertions, deletions and soft-clips in the transcriptomic alignments. The
481 transcriptomic alignments were used as inputs for salmon v1.6.0 (Patro et al., 2017) to quantify
482 gene expression. Raw read counts were computed for all genes in each sample and, to filter out
483 weakly-expressed transcripts, only genes with at least 1 count per million (CPM) in at least 10
484 samples (of the same subspecies) were retained. This was calculated separately in each of the
485 three subspecies and the raw counts of the filtered genes in each subspecies were then combined
486 for downstream analysis, for a total of 32,358 genes (Supplementary Table S2).

487 **Variant identification**

488 Variants were called by applying BCFtools v1.15 (previously SAMtools) (Danecek et al.,
489 2021) to the alignment bam files. The “*bcftools mpileup*” command was used to determine the
490 genotype likelihoods at each genomic position, with a minimum alignment quality of 20 and a
491 minimum base quality of 30. The actual calls were obtained using the “*bcftools call*” command.
492 The resulting VCF file was filtered using the “*bcftools view*” command, removing indels and
493 keeping only sites covered by at least three reads in all genotypes. Subsequently, only biallelic
494 SNPs with maximum values of 50% missingness and a 1% minor allele frequency were
495 retained. To identify private and shared SNPs among the different subspecies, every possible

496 comparison of the three subsampled VCF files (wild emmer, emmer and durum wheat) was
497 carried out using the “*bcftools isec*” command.

498 **Population genetics analysis**

499 Variants were filtered (one SNP per 500 kb) using the VCFtools *--thin 500000* option (v0.1.17)
500 (Danecek et al., 2011) and then converted into ped format with PLINK (v1.90p) (Purcell et al.,
501 2007). PLINK was also used to compute genetic distances between individuals with the --
502 *distance-matrix* flag. The output matrix was used as input for PCA with the *cmdscale* function
503 of R (v4.2.1) (R Core Team, 2022).

504 Genetic diversity statistics, including nucleotide diversity (π and θ) (Tajima, 1983; Watterson,
505 1975) were computed on the alignment bam files for each subspecies, from the folded site
506 frequency spectra using ANGSD(Korneliussen et al., 2014). First, the *doSaf* function was used
507 to estimate per-site allele frequencies (Saf) then *realSFS* was used to get the site frequency
508 spectra. The statistical loss of diversity (Vigouroux et al., 2002) was used to test the impact of
509 primary and secondary domestication on the molecular diversity of the three subspecies. For
510 primary domestication, the statistic was computed as $[1 - (x_{\text{emmer}}/x_{\text{wild}})]$, where x_{emmer} and x_{wild}
511 are the diversities in emmer and wild emmer, respectively, measured using π , θ and D. If x_{emmer}
512 was higher than x_{wild} , then the parameter was calculated as $[(x_{\text{wild}}/x_{\text{emmer}}) - 1]$. The loss of
513 diversity due to secondary domestication in durum wheat versus emmer was calculated as $[1 -$
514 $(x_{\text{durum}}/x_{\text{emmer}})]$, where x_{durum} and x_{emmer} are the diversities in durum wheat and emmer,
515 respectively. If x_{durum} was higher than x_{emmer} , then the parameter was calculated as
516 $[(x_{\text{emmer}}/x_{\text{durum}}) - 1]$.

517 We calculated F_{ST} for each pair of populations using ANGSD (Korneliussen et al., 2014). Saf
518 and 2D SFS were calculated as for nucleotide diversity, then the *fst index* function was used to
519 obtain the global estimate. To get an F_{ST} value for each gene in our dataset, we used the *fst*
520 *print* function, which prints the posterior expectation of genetic variance between populations
521 (called A), and total expected variance (called B) for every locus. We then computed the
522 weighted F_{ST} as the ratio of the summed As and summed Bs for every gene region, using an *ad*
523 *hoc* R script.

524 **Expression profiles, heritability and Q_{ST} analysis**

525 Raw read counts of the 32,358 genes were normalized using the *vst* method allowing the
526 additive coefficient of variation (CV_A) (standard deviation/mean) to be calculated for the two
527 N conditions in every subspecies, averaging the biological replicates of every genotype. The

528 statistical loss approach (Vigouroux et al., 2002) was then applied to test the loss of expression
529 diversity in the different groups, as previously reported (Bellucci et al., 2014). The statistical
530 significance of the differences between each CV_A value and the percentage loss of expression
531 diversity was determined using the Mann-Whitney test in R (v4.2.1) (R Core Team, 2022) with
532 the function *wilcox.test*.

533 To compute heritability, the raw counts of each subspecies under each condition were first
534 normalized using the trimmed mean M-values normalization method in the R package
535 edgeR(Robinson et al., 2010) and the voom normalization method in the R package
536 limma(Smyth, 2005). To determine the variance component of each factor and heritability, the
537 following model was considered:

538
$$Y_{ijkl} = S_i + G_{j(i)} + N_k + (S \times N)_{ik} + (G \times N)_{jk(i)} + \varepsilon_{l(ijk)},$$

539 where Y_{ijkl} is the normalized gene expression level, S_i is the species factor, $G_{j(i)}$ is the genotype
540 factor nested in species, N_k is the N level factor, $(S \times N)_{ik}$ is the interaction between species
541 and N levels, $(G \times N)_{jk(i)}$ is the interaction between genotypes and N levels, and $\varepsilon_{l(ijk)}$ is the
542 residual error. All factors were treated as random effects in the model except the intercept,
543 which was a fixed effect. The linear mixed models were fitted using the *lmer* function in R
544 package lme4 based on the normalized data of each transcript(Bates et al., 2015). The
545 heritability (H^2) was calculated as $H^2 = \frac{V_S + V_G}{V_A}$, where $V_A = V_S + V_G + V_N + \frac{V_{S \times N}}{n} + \frac{V_{G \times N}}{n} + \frac{V_\varepsilon}{n}$,
546 V_S is the variance of species, V_G is the variance of genotype, V_N is the variance of N level, $V_{S \times N}$
547 is the variance of species and N level interaction, $V_{G \times N}$ is the variance of genotype and N level
548 interaction, V_ε is the residual variance, and n is the number of N levels. $V_{S \times N}$ and $V_{G \times N}$ represent
549 the genotype \times environment interaction variance components at the species and genotype
550 (nested in species) levels, respectively.

551 Q_{ST} was calculated between pairs of the three subspecies under low and high N levels
552 separately. The wild emmer *vs* emmer comparison revealed the effects of primary
553 domestication, the emmer *vs* durum wheat comparison revealed the effects of secondary
554 domestication, and the wild emmer *vs* durum wheat comparison revealed the cumulative effect
555 of domestication. To this end, the model can be reduced to $Y_{ijl} = S_i + G_{j(i)} + \varepsilon_{l(ij)}$ at each N
556 level. The Q_{ST} value was calculated as $Q_{ST} = \frac{V_S}{V_S + V_G}$, the ratio of between-species and within-
557 species variance.

558 Q_{ST} distributions were used to perform a “selection scan” on a restricted number of genes. First,
559 genes were filtered for $H^2 \geq 0.7$ and, in order not to lose genes whose expression was strongly
560 influenced by N availability, also the species \times environment ($S \times N$) variance component was
561 evaluated (i.e., every species subgroup \times N condition), retaining those genes meeting the
562 threshold $S \times N \geq 0.2$ (Supplementary Figure S1). Successively, we obtained six different Q_{ST}
563 value distributions (Q_{ST} WILD EMMER VS EMMER, Q_{ST} EMMER VS DURUM WHEAT and Q_{ST} WILD EMMER VS
564 DURUM WHEAT, each for high and low N conditions) and we retained the 5% upper tail of every
565 distribution. Finally, we compared F_{ST} and Q_{ST} values for every gene, discarding F_{ST} values $<$
566 0.01. We confirmed that every retained gene satisfied the condition $Q_{ST} > F_{ST}$ allowing it to be
567 classed as undergoing directional selection.

568 **Differential expression analysis**

569 Differential gene expression was assessed by analysing the pre-processed raw count dataset
570 (32,358 genes). We identified DEGs by comparing (i) two conditions (i.e., high and low N
571 levels) within each subspecies, and (ii) pairs of the three subspecies under the same N levels,
572 which considered the genotypes nested in species. For the two scenarios, we used three
573 different approaches to detect DEGs: one linear model-based approach implemented in the R
574 package limma (Smyth, 2005), and two Poisson model-based approaches implemented in the
575 R packages edgeR (Robinson et al., 2010) and DESeq2 (Love et al., 2014). In all approaches,
576 the normalization of raw counts was applied by default in the package before differential
577 analysis. To reduce the number of false positives, the intersection of DEGs resulting from the
578 three approaches was retained (Zhang et al., 2014; Rapaport et al., 2013) and the significance
579 threshold was set to an adjusted p-value < 0.001 . The DEGs between high and low N levels in
580 at least one subspecies were used for PCA following the DESeq2 approach (Love et al., 2014),
581 first using all the DEGs, then repeating the analysis on the DEGs considered to be under
582 selection. At each step, counts were normalized using the *vst* method before the *plotPCA*
583 function was applied to define principal components 1 and 2 for the two N levels separately.

584 **GO enrichment analysis**

585 Enriched terms in the DEGs and genes under selection were identified using agriGO (v.2.0)
586 (Tian et al., 2017) with *T. aestivum* reference annotations and the following parameters:
587 hypergeometric test, multiple hypothesis test adjustment according to the Hochberg FDR
588 procedure at significance level < 0.05 and minimum number of mapping entries of 3.

589

590 **Data availability:** The raw sequence reads generated and analysed in this study have been
591 deposited in the Sequence Read Archive (SRA) of the National Center of Biotechnology
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593

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603 the RNA-Seq analysis. A.P., H.T. and Z.N. performed the bioinformatics analysis and analysed
604 the data. C.D.Q, A.R.L, M.R. provided technical support for RNA-Seq analysis. A.P., R.B.,
605 R.P. wrote the paper. Z.N., U.S., V.D.V., G.F., E.Bi., L.N., E.Be., N.P., P.D.V. reviewed and
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608

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610

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802 **Figure legends**

803 **Figure 1: Principal component analysis of 32 wheat genotypes based on single-nucleotide**
804 **polymorphisms (SNPs).** The first two principal components (PC1 and PC2) are shown. The
805 three colors represent different taxa. Labels show the accession name of each genotype.

806 **Figure 2: Density plots of the additive coefficient of variation (CV_A) in the three wheat**
807 **taxa.** Comparison of the estimated density functions of the CV_A in gene expression, calculated
808 using all 32,358 genes. **a** High nitrogen conditions. **b** Low nitrogen conditions. Dashed lines
809 represent the averaged CV_A value, colored according to the different taxa.

810 **Figure 3: *F_{ST}* and *Q_{ST}* distributions.** **a** Boxplots showing the gene locus *F_{ST}* distribution for
811 every subspecies pairwise comparison. **b** Boxplots showing the transcript *Q_{ST}* distribution for
812 every subspecies pairwise comparison under low nitrogen and high nitrogen conditions,
813 represented by empty and hatched grayscale bars, respectively.

814 **Figure 4: Differentially expressed genes (DEGs) when comparing high and low nitrogen**
815 **conditions within each subspecies.** Venn diagrams showing **a** Total set of DEGs; **b**
816 upregulated DEGs only; and **c** downregulated DEGs only.

817 **Figure 5: Principal component analysis of differentially expressed genes when comparing**
818 **high and low nitrogen conditions within each subspecies.** **a,b** Plots based on all 6,991 DEGs
819 (not filtered): **a** high nitrogen conditions and **b** low nitrogen conditions. **c,d** Plots based on 101
820 DEGs that are also putatively under selection: **c** high nitrogen conditions and **d** low nitrogen
821 conditions. Samples are represented by taxa-based colored dots. Labels show the accession
822 name of each genotype.

823

824 **Supplementary Materials**

825 **Supplementary Figure S1:** Workflow of gene expression selection scanning.

826 **Supplementary Figure S2:** GO categories of genes under selection.

827 **Supplementary Figure S3:** Genome-wide distribution of differentially expressed genes
828 (DEGs) in the comparison between contrasting nitrogen conditions within each subspecies.

829 **Supplementary Figure S4:** GO classification of differentially expressed genes (DEGs) in the
830 comparison between contrasting nitrogen conditions within each subspecies.

831 **Supplementary Figure S5:** Differentially expressed genes (DEGs) between subspecies.

832

833 **Supplementary Table S1:** List of the 128 samples and read mapping results.

834 **Supplementary Table S2:** Raw read counts of the 32,358 genes.

835 **Supplementary Table S3:** Mean CV_A in gene expression for the three wheat taxa and loss of
836 expression diversity. The loss of expression diversity is shown for two gene subgroups (6,991
837 DEGs and 25,367 non-DEGs).

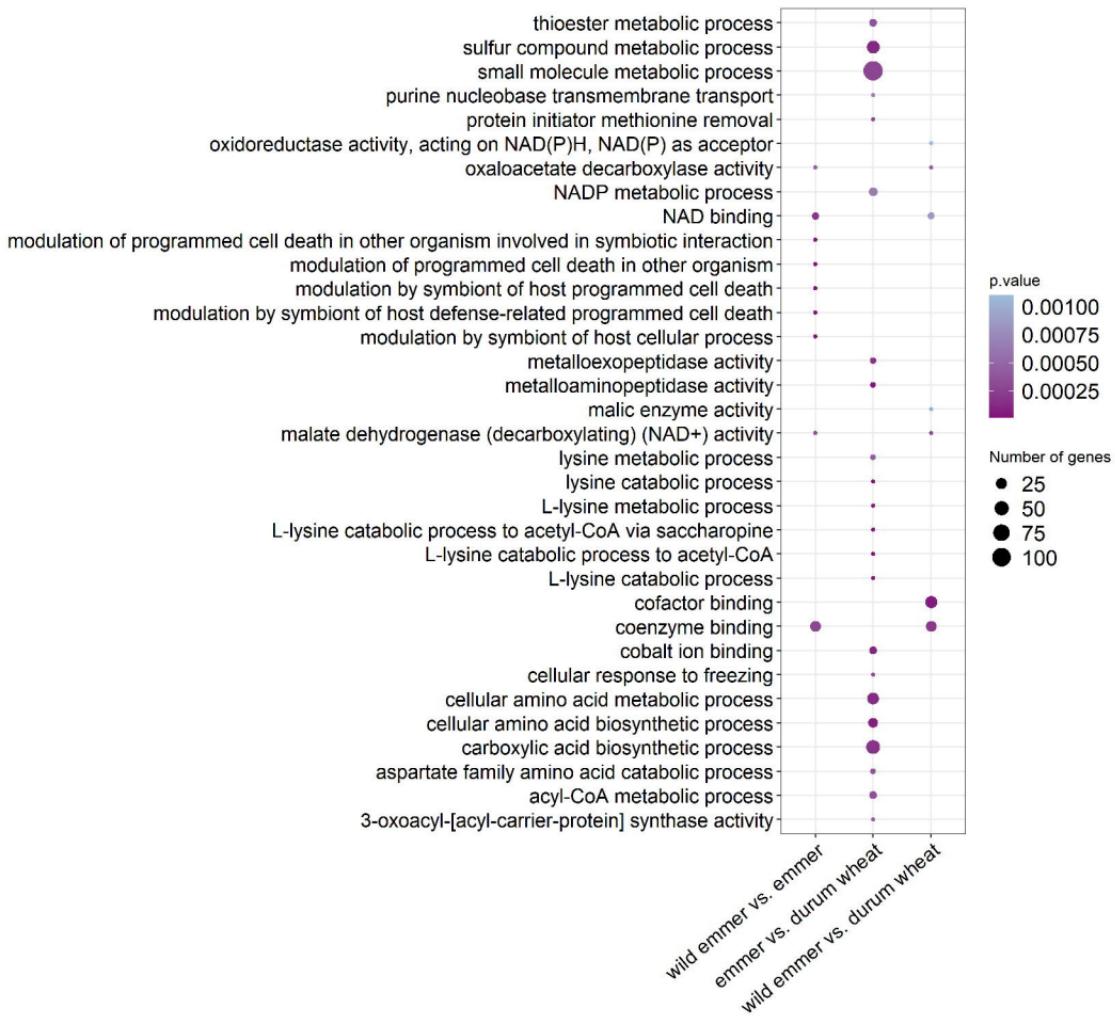
838 **Supplementary Table S4:** List of the 967 genes retained from the “selection scan”. Each gene
839 is accompanied by its functional annotation and the group in which the selection signal was
840 detected.

841 **Supplementary Table S5:** List of GO “Biological process” and “Molecular function”
842 subcategories for differentially expressed genes (DEGs). GO subcategories are shown for
843 upregulated and downregulated genes under different nitrogen conditions for each subspecies,
844 satisfying the criterion $p \leq 10^{-5}$.

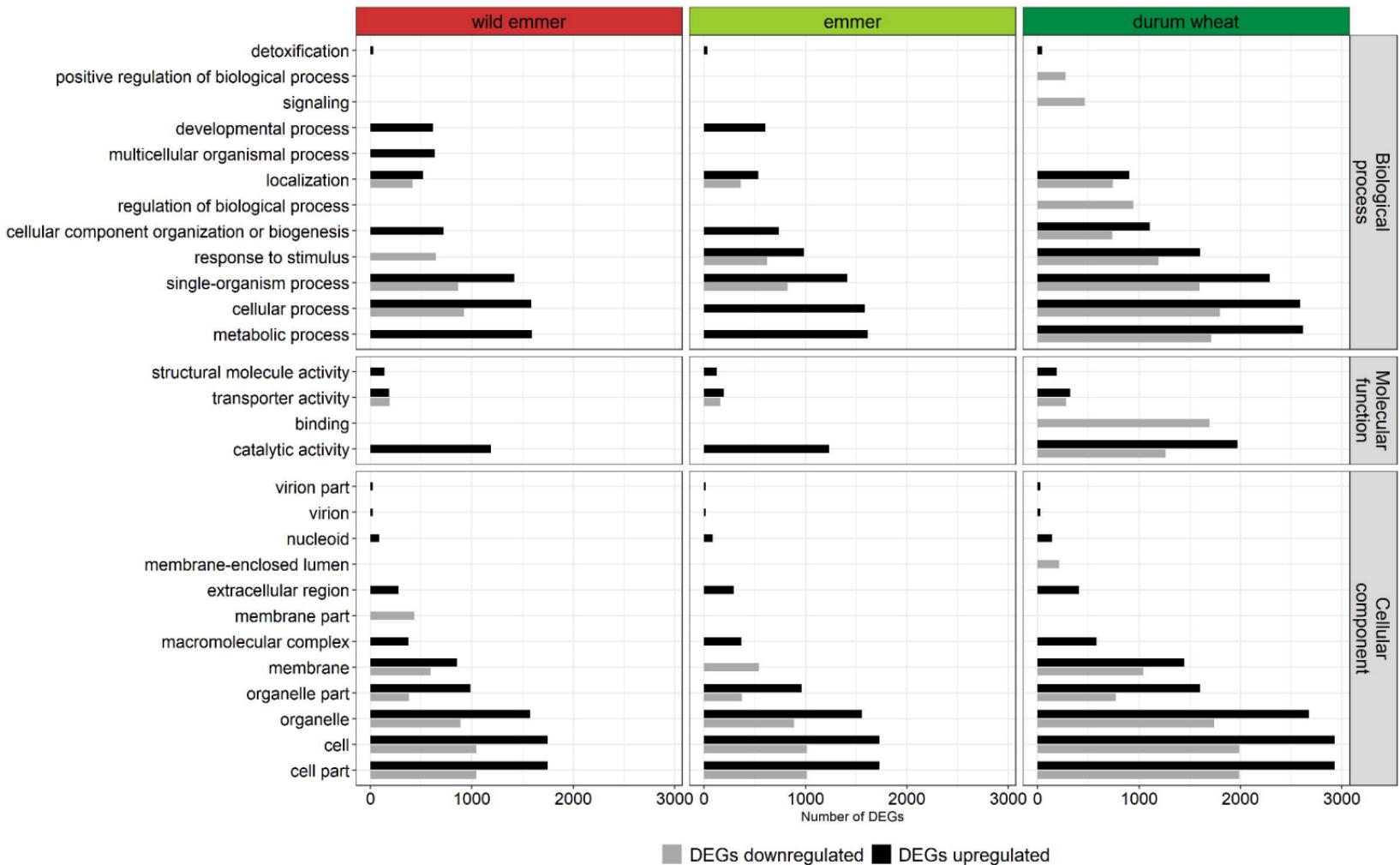
845 **Supplementary Table S6:** Functional annotations of the differentially expressed genes
846 (DEGs) between nitrogen conditions in each subspecies. Genes with the top 5% $|\log_2\text{FC}|$ values
847 are shown.

848 **Supplementary Table S7:** Functional annotations of the differentially expressed genes
849 (DEGs) between subspecies under all nitrogen conditions. The corresponding $\log_2\text{FC}$ values
850 are shown.

851 **Supplementary Table S8:** Functional annotation of the 101 genes selected by the integration
852 of selection signatures and differential expression analysis between nitrogen conditions.





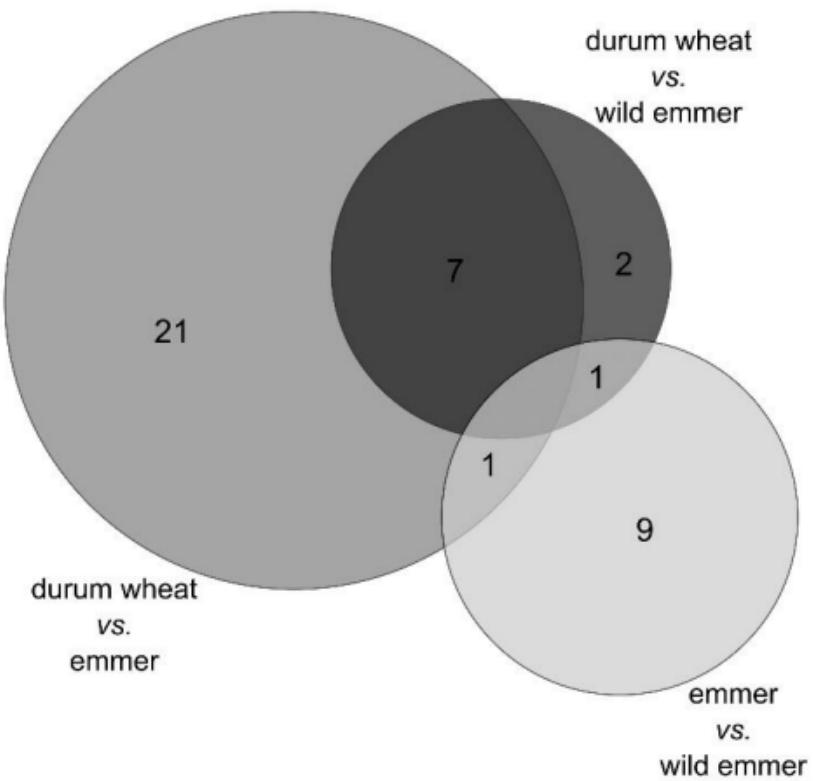


a

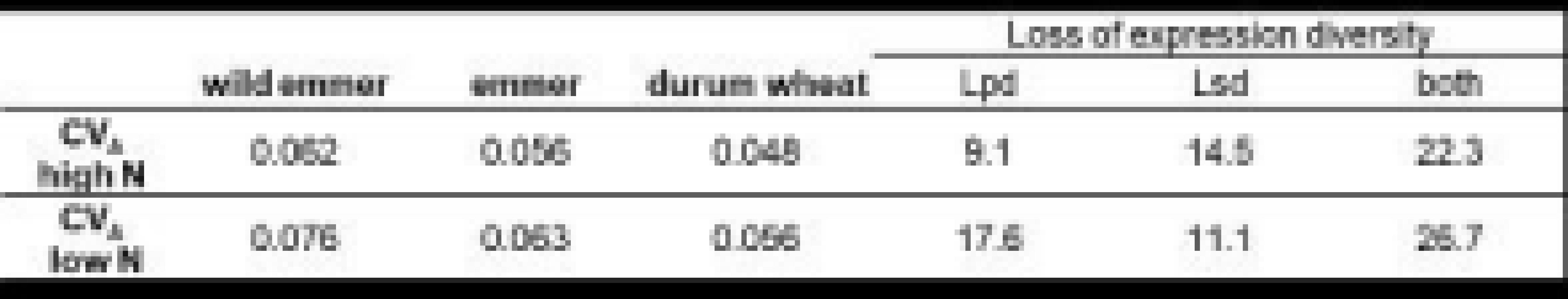
DEGs between species
in high N conditions

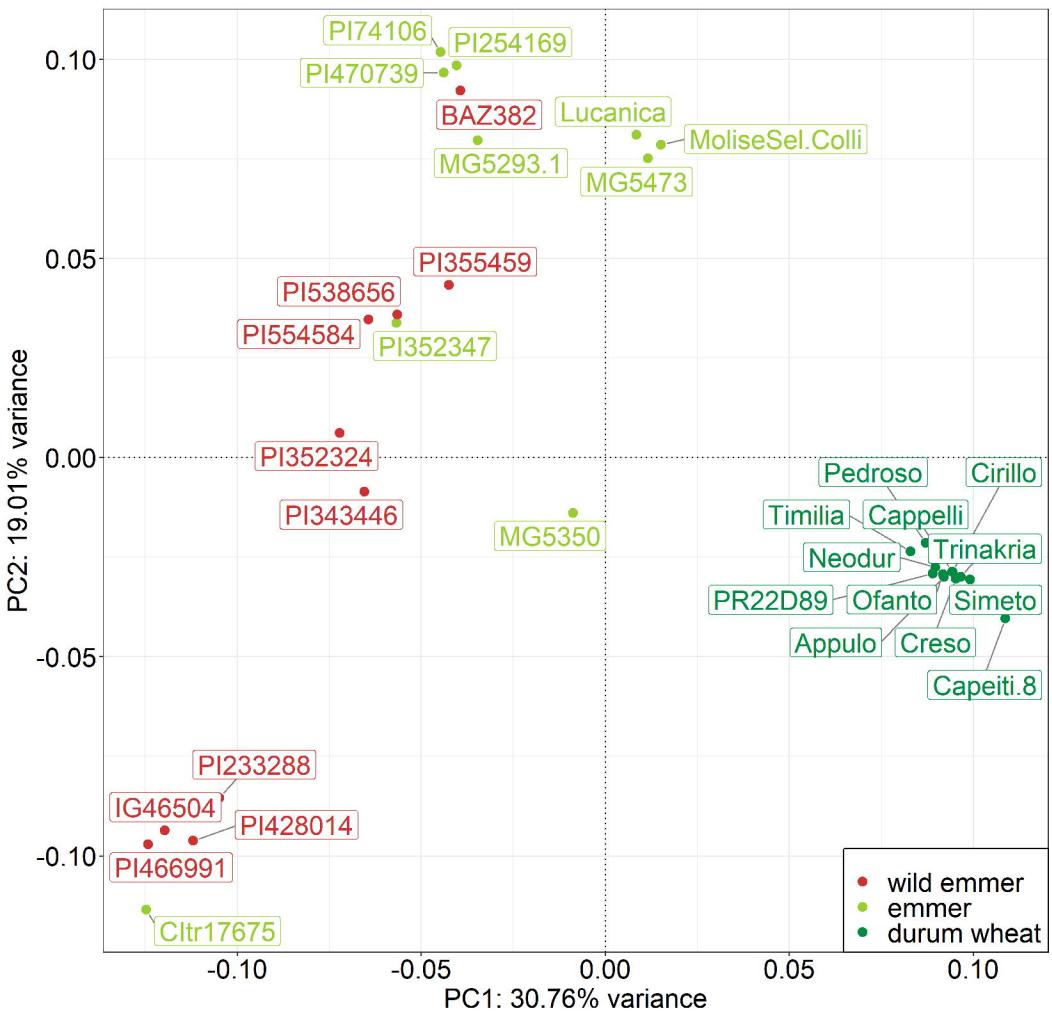
**b**

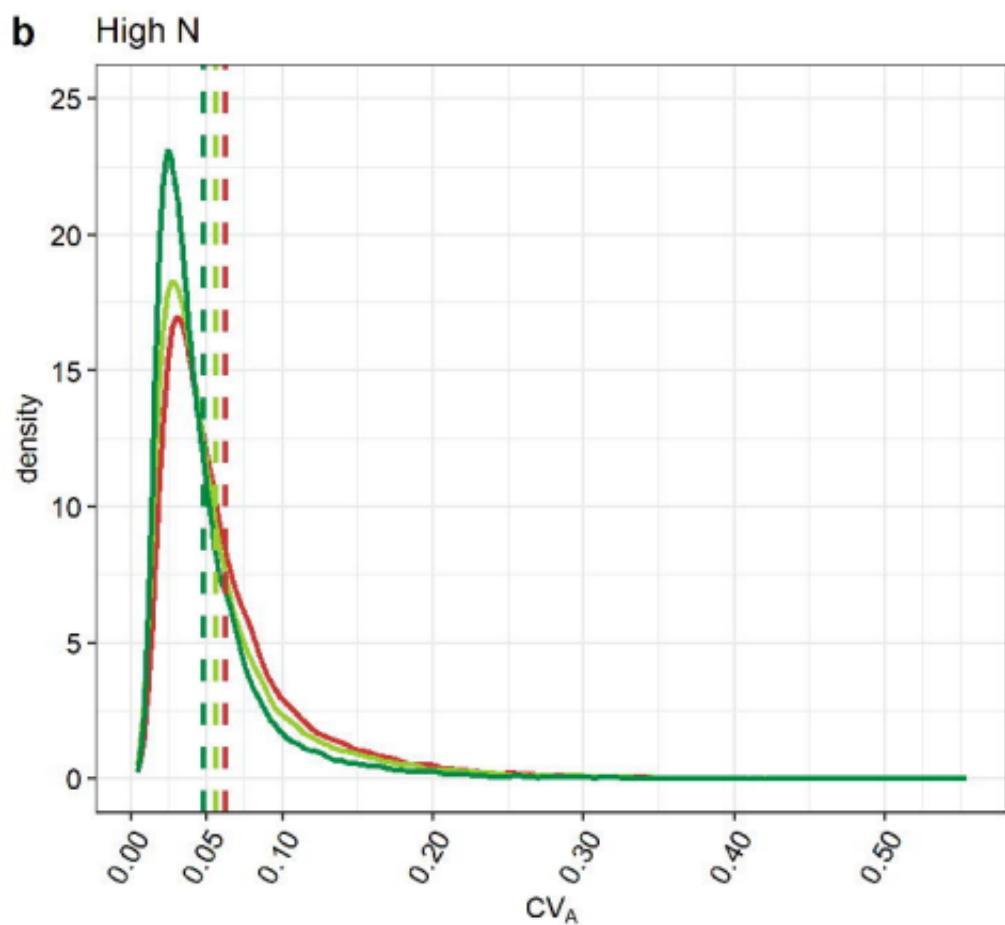
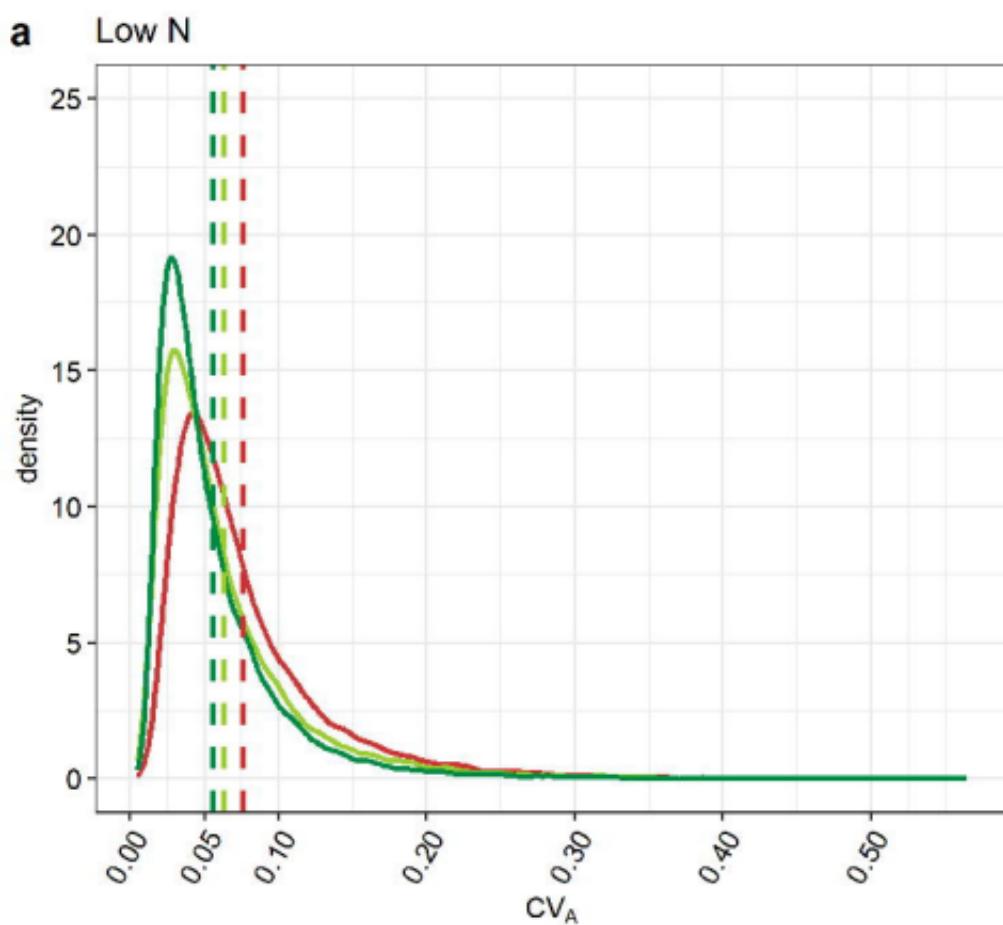
DEGs between species
in low N conditions



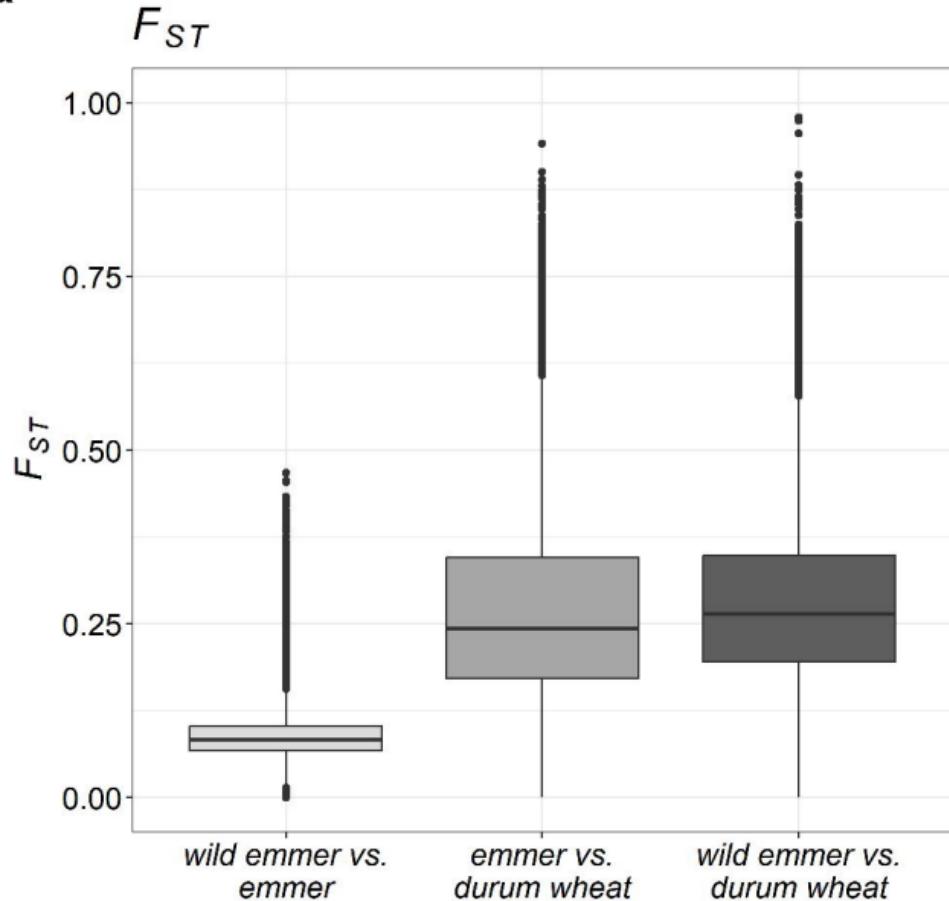
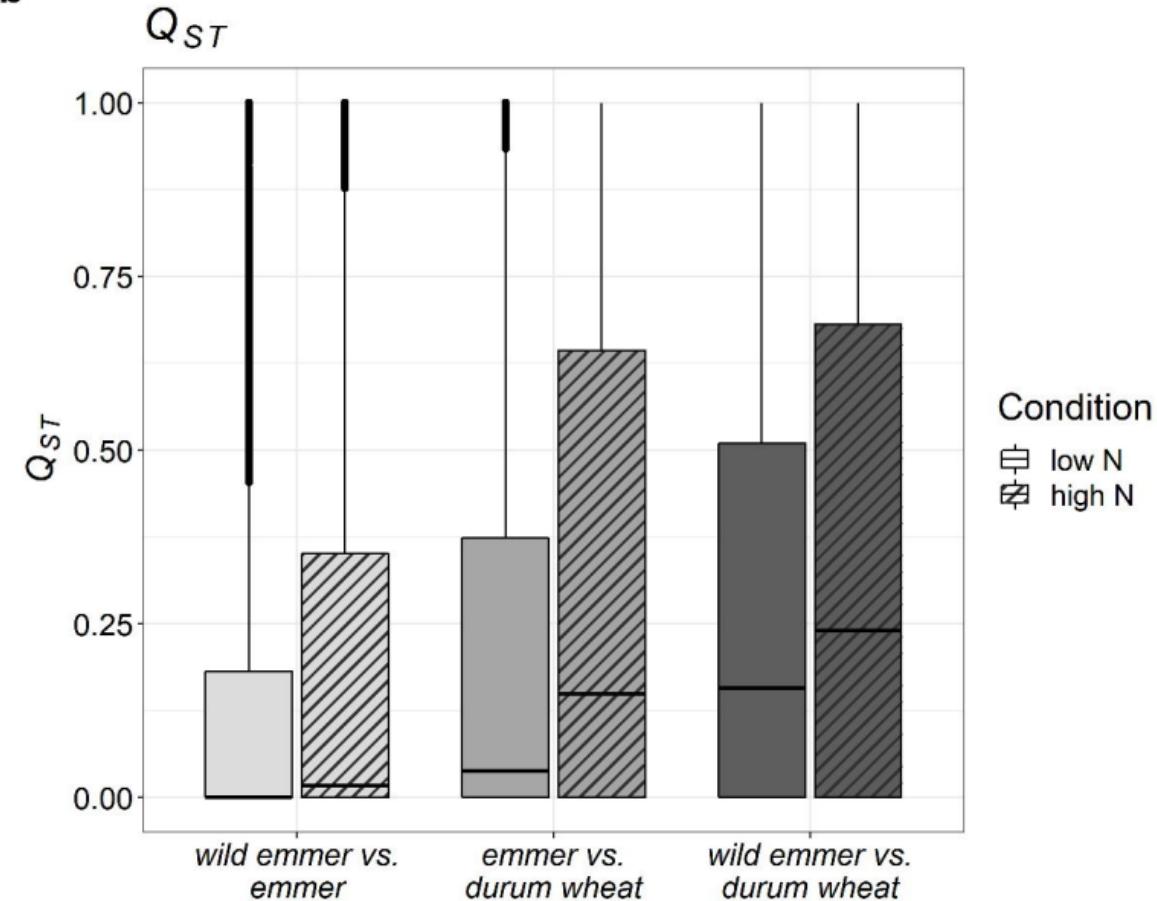
	Loss of <i>durum wheat</i> diversity						
	wild emmer	emmer	durum wheat	Lpd	Lsd	Lsd	both
π	0.0000	0.0040	0.0000	11.4	34.6	34.6	32.1
θ	0.0047	0.0040	0.0029	18.3	27.2	27.2	26.2
D	0.2613	0.4806	1.0843				

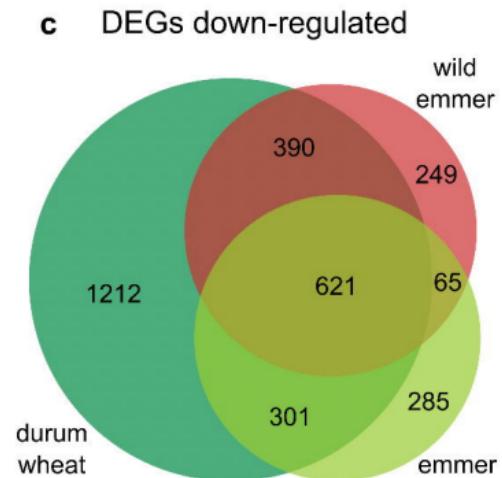
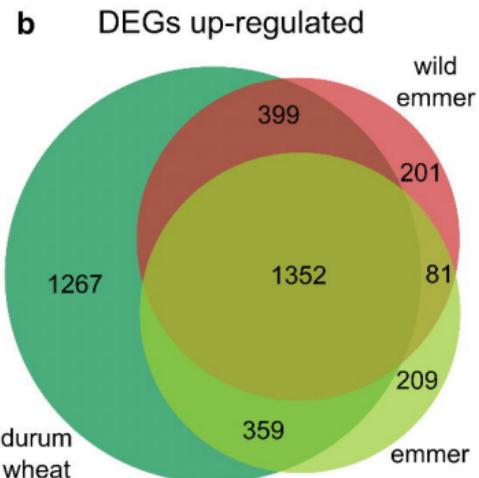
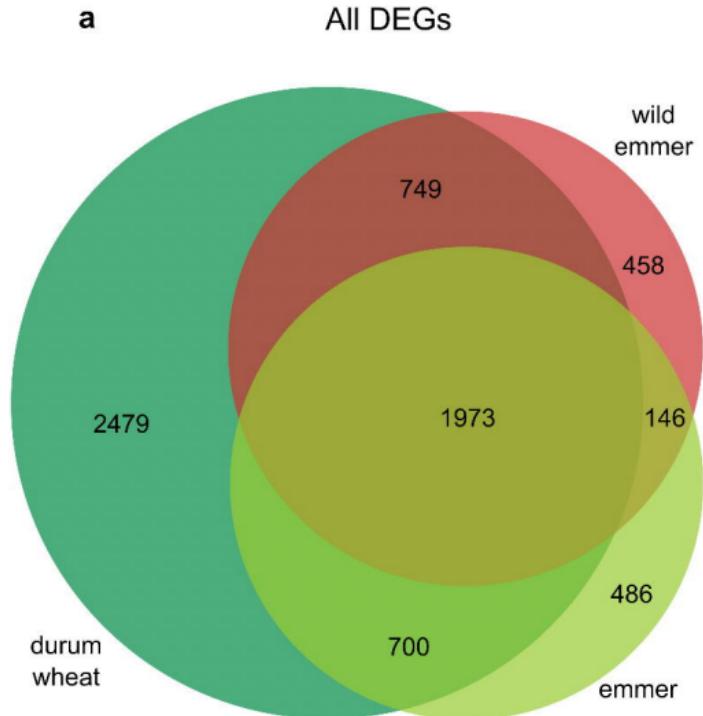


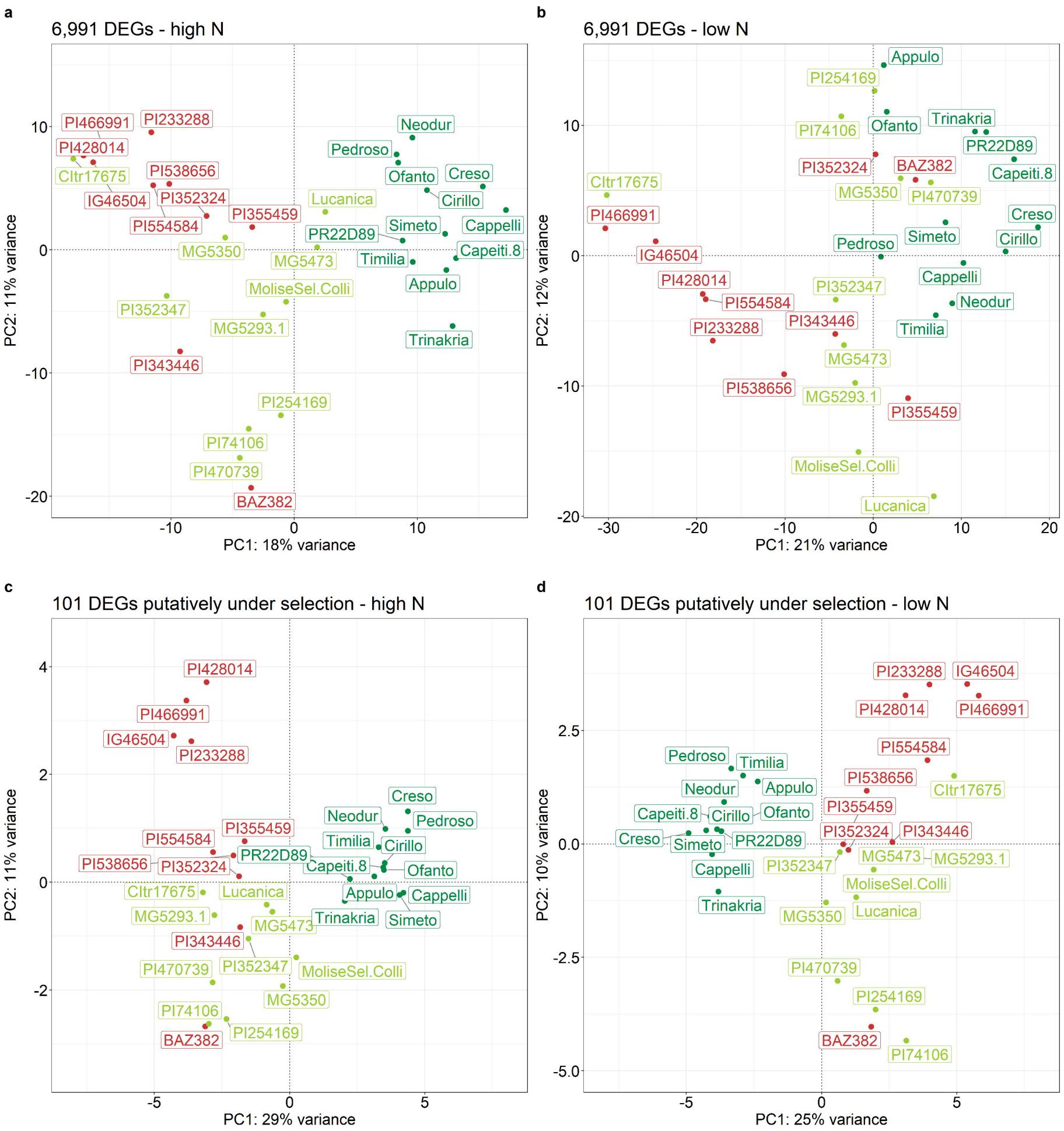


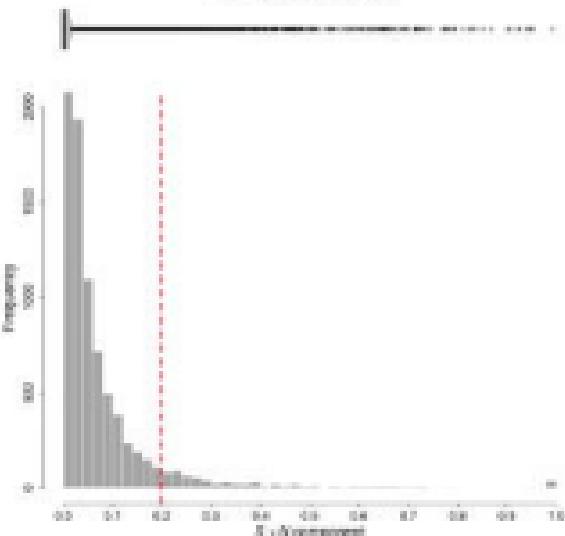
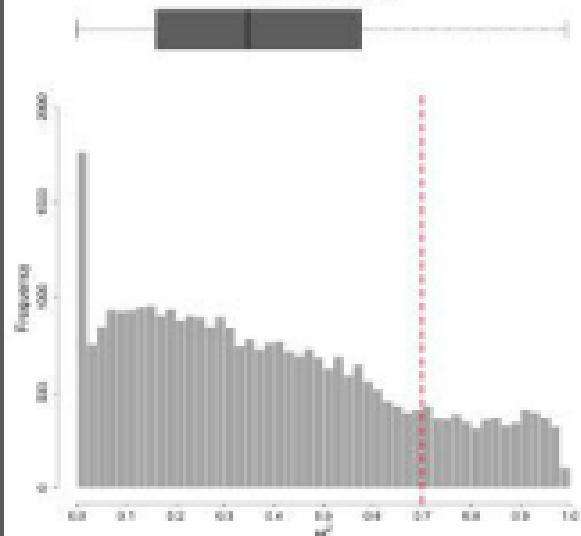


wild emmer emmer durum wheat

a**b**





a H^2 values distribution**b** $S \times N$ component distribution**c**

1

$H^2 \geq 0.7$
5,227 genes

$S \times N$ component ≥ 0.2
641 genes

2

Top 5% of every Q_{ST} distribution
6 groups with ~285 genes each. TOT. 973 GENES

3

$F_{ST} > 0.01$ and $Q_{ST} > F_{ST}$
967 genes