

# **Wild emmer introgressions alter root-to-shoot growth dynamics in response to water stress**

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1 **Abstract**

2 Water deficit is a major limiting factor for wheat (*Triticum* sp.) development and productivity.  
3 One approach to increase water stress adaptation in wheat is incorporating novel alleles from  
4 the drought-adapted wheat progenitor, wild emmer (*T. turgidum* ssp. *dicoccoides*). We explored  
5 this idea in the context of vegetative growth by examining the phenotypic consequence of a  
6 series of wild emmer (*acc.* Zavitan) introgressions into elite durum wheat (*cv.* Svevo) under  
7 water-limited conditions. Using image-based phenotyping we cataloged divergent (from  
8 Svevo) growth responses to water stress ranging from high plasticity to high stability among  
9 the introgression lines. We identified an introgression line (IL20) that exhibits a highly plastic  
10 response to water stress by shifting its root-to-shoot biomass ratio for detailed characterization.  
11 By combining genotypic information with root transcriptome analysis, we propose several  
12 candidate genes (including a root-specific kinase) that can confer the shoot-to-root carbon  
13 resource allocation in IL20 under water stress. Discovery of high plasticity trait in IL20 in  
14 response to water stress highlights the potential of wild introgressions for enhancing stress  
15 adaptation via mechanisms that may be absent or rare in elite breeding material.

16

## 17 INTRODUCTION

18 Wheat (*Triticum* sp.) is the most widely grown crop in the world, providing about one-fifth of  
19 the caloric intake by humans (FAOstat, 2017). To meet the food demand of the rising  
20 population, it is estimated that we will need at least a 60% increase in wheat production by 2050  
21 (Myers et al., 2017). This is expected to be a major challenge, especially under projected climate  
22 change scenarios, and associated increases in variability of precipitation and frequency and  
23 intensity of drought in many agricultural regions (Cook et al., 2018). Genetic improvement in  
24 wheat, coupled with better agronomic management, is a core component for addressing this  
25 challenge. Key targets for enhanced adaptability are increased biomass accumulation during  
26 vegetative growth, enhanced water-use-efficiency and stability of yield parameters during  
27 reproductive growth under water-limiting environments (Araus et al., 2002). These can be  
28 realized at the molecular, morphological, and physiological level (Gupta et al., 2020). Increased  
29 genetic variability in breeding programs, particularly when the introduced variants can be  
30 associated with improved adaptation to water stress, can be a valuable resource for developing  
31 climate resilient wheat cultivars for the future.

32 From a physiological perspective improved adaptation to water-limited environments,  
33 especially with mild-to-moderate water stress, is inherently linked to higher biomass  
34 accumulation, which is a function of photosynthetic capacity at the canopy level. Canopy  
35 photosynthesis typically translates into higher yield in many crops and environments (Zelitch,  
36 1982; Ashley and Boerma, 1989). Water stress results in a decline in turgor, cell division, and  
37 leaf growth, which also decreases the photosynthetic surface area and hence, overall  
38 photosynthetic capacity independent of the photosynthetic efficiency (Hsiao, 1973). Therefore,  
39 vegetative shoot growth in crops such as wheat can be considered an integrated trait on a  
40 temporal scale, with growth before reproductive transition impacting grain yields. Increasing  
41 genetic variation in wheat for rate of leaf area growth and duration of growth under water stress  
42 can be important yield determining trait under water stress (Richards, 2000). Capturing the  
43 phenotypic variation in shoot growth for genetic analysis requires accurate longitudinal  
44 measurements that are now feasible with non-destructive imaging platforms (Berger et al.,  
45 2010).

46 Relatively less is known about root responses to water stress due to inherently higher root  
47 plasticity and difficulty in accurately measuring their phenotypic traits. Wheat breeding is  
48 known to have reduced root size in modern varieties relative to wild ancestors or landraces in  
49 many environments (Waines and Ehdaie, 2007). This is likely due to higher photosynthetic cost  
50 of root growth and respiration that led to allelic enrichment that favours reduced carbon

51 allocation to roots when selections are made under optimal environments (Lambers and Atkin,  
52 1996). Under water stress, shoot growth is reduced as more carbon is allocated to roots, which  
53 results in higher root-to-shoot ratio (Correa et al., 2019). Deeper roots and more lateral root  
54 growth under water limited conditions enables plant access to more water during grain filling  
55 (Campos et al., 2004). The resulting greater stomatal conductance, cooler canopies and  
56 maintenance of physiological activity reduces grain yield losses in later developmental stages  
57 (Kirkegaard et al., 2007). Optimal root-to-shoot partitioning enables the balance between  
58 productivity and root water absorption (Voss-Fels et al., 2018). While the impact of the root-  
59 to-shoot allocation on drought tolerance and yield is likely to be context dependent, phenotypic  
60 plasticity in resource partitioning is an important trait to characterize from a genetic perspective  
61 for overall germplasm enhancement and may compensate for relatively lower allelic enrichment  
62 due to breeding selections made under optimal water conditions. The impact of root-to-shoot  
63 plasticity on grain yield will also depend on the seasonal precipitation profile and soil type  
64 among other factors.

65 One of the challenges with capturing the genetic variation underlying the plasticity in  
66 shoot and root growth in response to water stress is the temporal resolution needed for  
67 phenotyping a large number of accessions, making this intractable through manual, destructive  
68 measurements. High-throughput, image-based platforms can greatly improve the temporal  
69 resolution of phenotyping shoot responses to water stress across populations, although similar  
70 approaches for directly measuring roots responses are still limiting (Yang et al., 2020). Our  
71 ability to identify novel phenotypic responses and their genetic basis depends on the level of  
72 detectable phenotypic variation in the population and access to genomic resources. The range  
73 of phenotypic variation within a background or population can be increased significantly by  
74 introducing alleles from wild or related species [e.g. tomato (*Solanum lycopersicum*; Arms et  
75 al., 2015), barley (*Hordeum vulgare*; Baum et al., 2003) and rice (*Oryza sativa*; Tsujimura et  
76 al., 2019)]. Wild relatives, especially those adapted to semi-arid environments can be source of  
77 novel water stress responsive phenotypes that may be missing or rare in breeding germplasm.

78 In this study, we focused on wild emmer wheat [*T. turgidum* ssp. *dicoccoides* (Körn.)  
79 Thell.], the direct allotetraploid ( $2n = 4x = 28$ ; genome BBAA) progenitor of all domesticated  
80 wheats (McFadden and Sears, 1946). Wild emmer thrives across the Near Eastern Fertile  
81 Crescent in a wide eco-geographic amplitude and harbors a rich allelic repertoire for numerous  
82 agronomic traits, including drought tolerance (Peleg et al., 2005). Introgression of wild emmer  
83 alleles has been shown to impact wheat adaptation to water stress (Golan et al., 2018; Merchuk-  
84 Ovnat et al., 2017). We have used a set of wild emmer introgression lines (ILs) in an elite

85 tetraploid wheat background to discover novel phenotypic responses to water stress, using high  
86 temporal resolution imaging platform. We tested the hypothesis that introducing introgressions  
87 from wild emmer into durum wheat will expand the range of phenotypic responses to water  
88 stress with minimal loss in desirable agronomic traits of the elite cultivar. We identified two  
89 contrasting water stress response strategies, one where the phenotypic stability under water  
90 stress is observed and another that involves rapid change in carbon allocation relative to the  
91 elite cultivar. We characterized representative ILs for these two strategies for gaining further  
92 physiological insights. One of the ILs exhibiting a change in root-to-shoot ratio in response to  
93 water-stress was used for genetic and transcriptomic analysis to identify candidate genes  
94 localizing to the wild emmer introgressions. Our study highlights the potential of wild  
95 introgressions to promote various water stress responsive dynamics, as well as characterization  
96 of water stress adaptive mechanisms that can enhance climate resilience in wheat.

97

## 98 **RESULTS**

### 99 **Wild emmer introgressions confer divergent water stress responses**

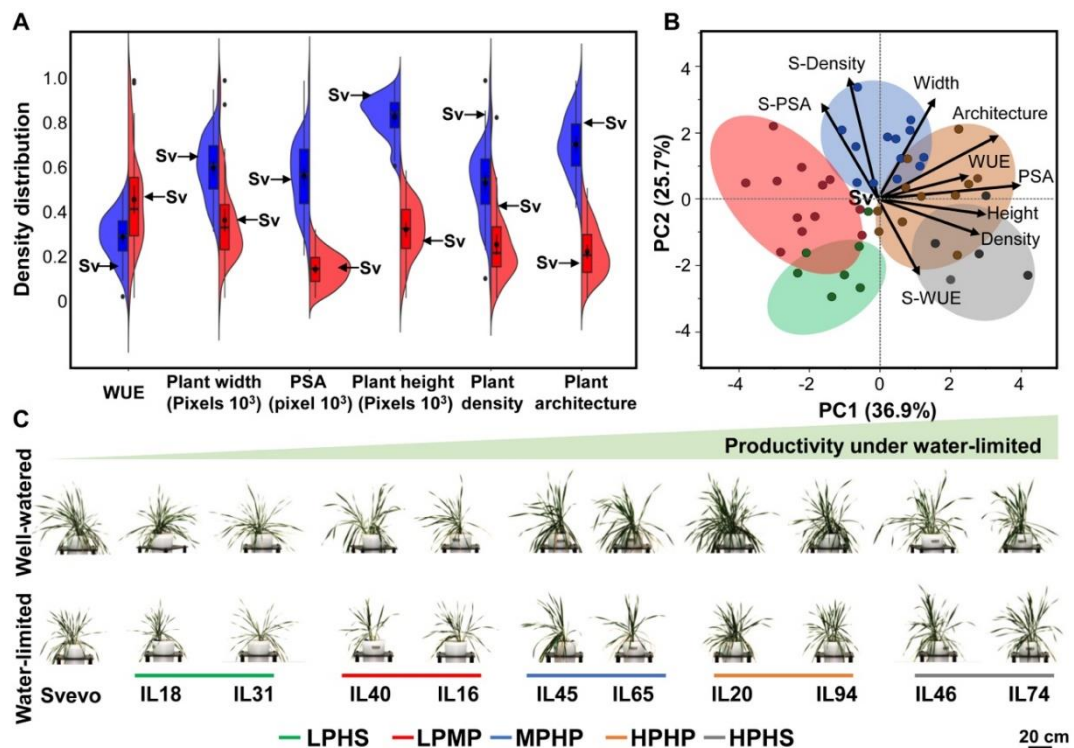
100 The goal of this study was to investigate if the introduction of small wild emmer introgressions  
101 can expand the range of phenotypic response to water stress in an elite wheat cultivar  
102 background. To accomplish this, we developed a set of introgression lines (ILs) using elite  
103 durum wheat cultivar, Svevo as the backcross parent, and Zavitan as the source of wild emmer  
104 introgressions (Avni et al., 2014). Zavitan is well-adapted to the semi-arid environment and has  
105 a sequenced and annotated genome thus making it more accessible for downstream genetic  
106 analysis compared to other wild emmer accessions (Avni et al., 2017). The subset of ILs  
107 selected for this study by eliminating the wild alleles for the *Reduced height (Rht)-B1b* gene  
108 and non-brittle spike (*TtBtr1*) genes. This set included 47 wild emmer ILs were genotyped using  
109 the 90K SNP chip, and poses 1.3-14.2% of the Zavitan genes per IL (Oren, 2020). To examine  
110 their phenotypic responses to water stress using an automated high-throughput, image-based  
111 phenotyping platform. These ILs were grown under well-watered (WW; 80% field capacity)  
112 and water-limited (WL; 30% field capacity). Starting 11 days after transplanting (DAT) for 35  
113 days of imaging, we collected five side-view images for each plant on each imaging day, to  
114 calculate the projected shoot area (PSA) from pixel counts and estimate daily shoot biomass  
115 accumulation as described in Campbell et al. (2015) and Knecht et al. (2016). The temporal  
116 PSA is a reliable proxy for shoot growth dynamics of the ILs and Svevo (Supplemental Fig.  
117 S1). We did not include Zavitan in this imaging experiment as it exhibits a weak growth habit  
118 when grown in pots under greenhouse conditions (observed during multiple seed increases).

119 Since retention of desirable agronomic traits was a key criterion for initial selection of ILs for  
120 this study, we deemed the elite parent, Svevo to be a more suitable control for comparing  
121 phenotypic responses under WL treatment. Our observation that the PSA-based growth curves  
122 for Svevo were similar to the median response of all ILs collectively during the experiment  
123 supports this rationale. This suggests that the ILs biomass accumulation (derived from PSA)  
124 segregated around Svevo performance (Supplemental Fig. S2A). Other morphological traits  
125 such as plant width and convex area (architecture) also exhibited a pattern similar to PSA  
126 (Supplemental Fig. S2B-E). Although, most of the ILs reached the target of 30% field capacity  
127 in the WL treatment after an average of 19 DAT (ranged from 14 to 24 DAT) (Supplemental  
128 Fig. S3), many exhibited significant differences in biomass accumulation as early as 10 DAT  
129 (collectively), indicating an early water stress response as well as divergence in shoot growth  
130 response among the ILs (Supplemental Fig. S4).

131 To examine the consequences of early water stress response, we plotted the density  
132 distribution of the ILs under WW and WL treatments for several morphological traits at 35  
133 DAT. The ILs exhibited a broad range for all the traits with Svevo positioned close to the  
134 average value for most traits (Fig. 1A). Overall, the ILs showed a strong reduction in PSA as  
135 evident from the separation of the distribution curves in response to water stress. A relatively  
136 lower separation between the WW and WL treatments was observed for additional  
137 morphological traits, such as plant width, WUE and density and. WUE was derived from daily  
138 increments in PSA and daily water use by the genotypes. The phenotypic distribution of plant  
139 height among the ILs under WL treatment varied more around the mean compared to the WW  
140 treatment. This was in contrast to the change in phenotypic distribution of PSA for the IL set,  
141 which varied less and was narrower under WL treatment. This could be due to plant height  
142 being one of the selection criteria for the ILs. Notably, the phenotypic range of WUE varied  
143 more under WL compared to the WW conditions.

144 Understanding the relationship between the morpho-physiological traits can provide  
145 better insights into the key determinants of expanded phenotypic range observed among the  
146 ILs. Therefore, we performed correlation analysis between these traits at 35 DAT  
147 (Supplemental Fig. S5 and Supplemental Table S1). PSA was positively correlated with all  
148 morphological traits suggesting that plant biomass and architecture are tightly associated  
149 regardless of water availability. Under WL conditions, PSA and plant density were positively  
150 correlated with WUE, suggesting that plant architecture can affect the WUE under stress. Water  
151 stress was relatively more consequential in altering the inverse correlation between height and  
152 width, density, and WUE relative to WW treatment (Supplemental Fig. S5B). To further

153 understand the response of plant phenotypic traits to WL treatment, we performed principal  
 154 component analysis (PCA) of the morpho-physiological traits under WL treatment as well as  
 155 in relative terms S (i.e., drought susceptibility index) (Fig. 1B). We define stress indices as a  
 156 plant's ability to maintain similar behavior under WL relative to its WW values for a given trait.  
 157 PCA identified three major PCs (Eigenvalues > 1.2) accounting collectively for 76% of the  
 158 phenotypic variance among the ILs (Supplemental Fig. S6). PC1 explained 36.9% of total  
 159 variation and related positively with PSA, plant height, plant architecture, WUE, and plant  
 160 density. PC2 explained 25.7% of the total variation and related positively with plant width, S-  
 161 PSA, and S-density and negatively with S-WUE. PC3 explained 13.4% of the total variation  
 162 and was positively related with WUE, S-PSA, and plant density. These results suggest that  
 163 higher biomass and greater plant density were positively correlated with higher WUE under  
 164 WL conditions. From the S-index perspective, high S-PSA which reflects significant biomass  
 165 reduction due to water stress correlated with low S-WUE and confirmed that without WUE  
 166 adaptation, plants will reduce their biomass gain under water stress.



167  
 168 **Figure 1.** Wild introgressions promote phenotypic diversity. (A) Density distribution of morpho-  
 169 physiological traits for 47 introgression lines (ILs) under well-watered (WW, blue) and water-limited (WL,  
 170 red). Water-use efficiency (WUE), plant width, projected shoot area (PSA), plant height, plant density and  
 171 plant architecture (convex area). The parental line Svevo (Sv) is marked with arrow. (B) Principal component  
 172 (PC) analysis of morpho-physiological traits under WL conditions and expressed as drought susceptibility  
 173 index (S). Biplot vectors are trait factor loadings for PC1 and PC2. The five clusters of stress responsiveness:  
 174 high productivity - high stability (HPHS; gray), high productivity - high plasticity (HPHP; Orange), moderate  
 175 productivity-high plasticity (MPHP; Blue), low productivity-moderate plasticity (LPMP; Red), low  
 176 productivity-high stability (LPHS; Green). (C) Representative images of ILs from each responsiveness  
 177 cluster under WW and WL treatments at 35 d after transplanting.

178           Next, we sought to categorize the observed phenotypic divergence among the ILs with  
179 the goal to obtain more genotypic specificity associated with the phenotypic patterns (Fig. 1C).  
180 For this we performed hierarchical clustering analysis of the morpho-physiological traits under  
181 WL treatment and derived stress index traits (Supplemental Fig. S7). Clustering analysis  
182 separated the ILs into five distinct clusters, which we are broadly describe as following: Cluster  
183 1 (high productivity and high stability, HPHS), Cluster 2 (high productivity and high plasticity,  
184 HPHP), Cluster 3 (moderate productivity and high plasticity, MPHP), Cluster 4 (low  
185 productivity and moderate plasticity, LPMP), and Cluster 5 (low productivity and high stability,  
186 LPHS). The productivity in the context of this analysis implies biomass accumulation under  
187 WL and plasticity is defined as the genotype's ability to exhibit a relatively rapid change in a  
188 phenotypic trait in response to water stress. Svevo resolved to Cluster 4 (LPMP), which is  
189 characterized by low PSA and WUE, and an intermediate response to water stress. The two  
190 most productive clusters (HP), Cluster 1 and Cluster 2 showed differential stress response as  
191 expressed in the drought susceptibility index. Cluster 1 exhibited low S-PSA values indicative  
192 of a smaller change between WW and WL treatments. Cluster 2 has the highest WUE under  
193 WL and relatively high values of S-PSA, resulting in high plasticity for these ILs. Several  
194 Cluster 3 ILs have relatively higher value for convex area and plant width without a  
195 compensating decline in height. This increase in width and/or convex area without a  
196 compensating decline in plant height is also evident for two ILs in Cluster 1. Our clustering  
197 analysis enabled us to resolve ILs into various phenotypic categories and also identify  
198 representative ILs for each of the cluster for detailed characterization.

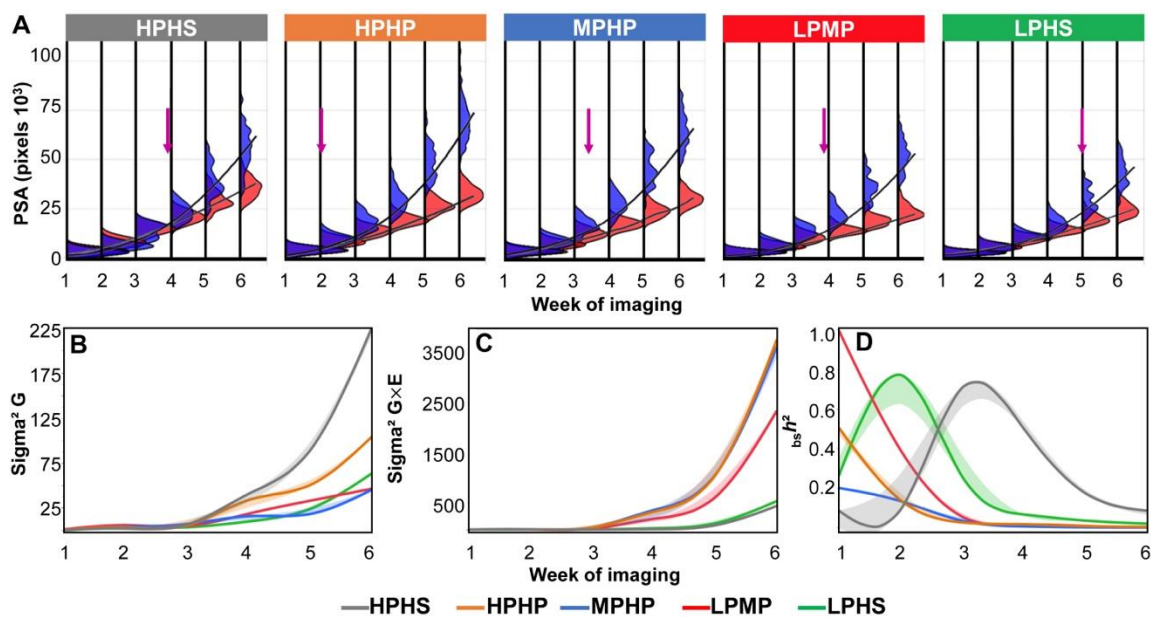
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### 200 **Water stress responsiveness classification based on temporal growth dynamics**

201 Although the clustering analysis using endpoint measurements of the ILs provides a useful  
202 perspective, the temporal dynamics for these traits that precede these phenotypic outcomes can  
203 enhance our understanding of water stress responses. For this, we mapped the overall  
204 trajectories and phenotypic distributions of these traits on a weekly basis (Fig. 2A). In general,  
205 all clusters exhibited higher biomass accumulation and higher coefficient of variance (CV)  
206 under WW relative to WL treatment (Fig. 2A; Supplemental Table S2). The PSA distributions  
207 under WW and WL treatments showed that the high stability (HS) clusters exhibited substantial  
208 overlap between the WW and WL curves in weeks 5 and 6. The point of significant response  
209 to water stress was determined when three contiguous days of significant ( $P \leq 0.05$ ) difference  
210 in growth between treatments was recorded, which ranged between 10 DAT (HPHP cluster) to  
211 26 DAT (HPHS cluster) (Fig. 2A; Supplemental Table S3). A similar pattern was found for



212 plant architecture and density (Supplemental Fig. S8). The parental line (Svevo; LPMP cluster)  
 213 expressed an intermediate response (17, 18, and 15 DAT for PSA, plant architecture, and  
 214 density, respectively; Supplemental Fig. S8). Although the MPHP cluster exhibits high biomass  
 215 accumulation under WW treatment, it was labeled as moderate productivity (MP) based on its  
 216 performance under WL treatment. The cluster's classification to productivity (i.e., HP, MP, and  
 217 LP) was found to be significantly different ( $P < 10^{-4}$ ) under WL. This analysis enabled us to  
 218 capture the temporal dynamics, which are typically challenging to determine without extensive  
 219 destructive sampling but important to examine the level of plasticity under varying  
 220 environmental conditions.



221  
 222 **Figure 2.** Longitudinal dynamics of the five responsiveness clusters. (A) Longitudinal frequency  
 223 distribution of biomass accumulation (projected shoot area; PSA) of each water stress responsive cluster  
 224 under well-watered (WW; blue) and water-limited (WL; red) treatments. The five clusters are: high  
 225 productivity-high stability (HPHS; gray), high productivity-high plasticity (HPHP; Orange), moderate  
 226 productivity-high plasticity (MPHP; Blue), low productivity-moderate plasticity (LPMP; Red), low  
 227 productivity-high stability (LPHS; Green). The time point of significant difference in response to water  
 228 stress is marked with an arrow ( $P \leq 0.05$ ). Longitudinal heritability components of (B) genetic ( $\text{Sigma}^2 G$ ),  
 229 (C) environmental interaction ( $\text{Sigma}^2 G \times E$ ), and (D) broad-sense heritability ( $_{bs}h^2$ ). Continues line  
 230 represent the smooth curve through the data and the shaded area represents the standard error of the smooth  
 231 curve.

232

### 233 Plant responsiveness clusters expressed in heritability dynamics

234 To dissect the genetic (G) and environmental (E) components of PSA underlying each  
 235 responsiveness cluster through studied developmental stages, we calculated broad-sense  
 236 heritability and its components. The HPHS cluster exhibited the highest genetic component  
 237 ( $\text{sigma}^2 G$ ), which increased with the progression of water stress duration (Fig. 2B). On the  
 238 other hand, HPHP and MPHP clusters had lower genetic components and the highest G×E  
 239 interaction ( $\text{Sigma}^2 G \times E$ ) (Fig. 2B, C). The broad-sense heritability dynamics ( $_{bs}h^2$ ) of PSA

240 showed clear separation into stability (LPHS and HPHS) and plasticity (LPMP, MPHP, and  
241 HPHP) (Fig. 2D). In general, the level of PSA  $_{bs}h^2$  decreased over time. Heritability dynamics  
242 of plant density showed a strong genetic component for HPHP and a high environmental effect  
243 for LPMP that increased over time. Plant architecture presented a high environmental effect on  
244 MPHP, causing low  $_{bs}h^2$  for this cluster (Supplemental Fig. S9). Overall, the heritability  
245 dynamics of the responsiveness cluster emphasized that stability and plasticity derived from  
246 both genetic and environmental effects within the IL panel.

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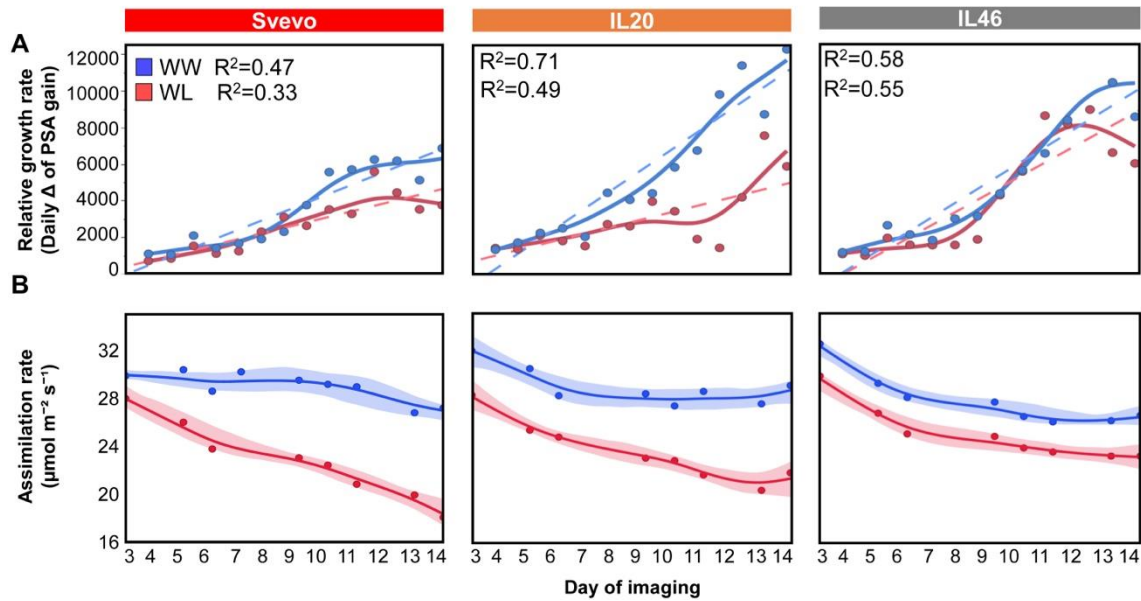
### 248 **IL20 exhibited higher assimilation rate under water-limited conditions**

249 We next focused on the phenotypic plasticity/stability under WL, by comparing two high  
250 productivity clusters HPHP and HPHS, represented by IL20 and IL46, respectively, for  
251 downstream physiological analysis. We targeted the temporal window during the early growth  
252 stages (15-19 Zadocks scale; Zadocks et al., 1974), and used the same experimental design that  
253 previously enabled us to categorize ILs based on growth rate and water stress response. Under  
254 WL treatment, the relative growth rate dynamics demonstrated the advantage of the two  
255 productive clusters as expressed in higher linear equation slope 452.3 for IL20 and 558.1 for  
256 IL46, compared to Svevo (284.45;  $P<0.005$ ) (Fig. 3A; Supplemental Table S4). Under WW  
257 treatment, only IL20 had higher slope compared to Svevo ( $P=0.001$ ). While IL46 maintained a  
258 similar linear equation slope under both water treatments (representing the high stability  
259 cluster), IL20 exhibited a significant change in the regression pattern, from 849.75 under WW  
260 to 452.32 under WL ( $P<10^{-3}$ ) (Fig. 3A) consistent with its selection based on high plasticity.

261 To complement the imaging of the two ILs and Svevo, we also measured gas exchange  
262 parameters over eight time points during the course of the experiment. Average assimilation  
263 rate (A) declined with the progression of water stress as expected, with Svevo exhibiting the  
264 most reduction (37.7%), whereas the high stability IL46 had only 22.9% reduction (Fig. 3B).  
265 Notably, IL20 exhibited the highest assimilation rate under WW treatment over time (30.19  
266  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ), whereas under WL both IL46 and IL20 exhibited similar A (25.35 and 24.26  
267  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , respectively), that was significantly higher than Svevo (22.66  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ;  
268  $P<0.047$ ; Supplemental Fig. S10). IL20 also maintained significantly higher stomatal  
269 conductance ( $g_s$ ) ( $P=0.013$ ) and transpiration rate (E) ( $P=0.024$ ) under WL relative to Svevo  
270 (Supplemental Fig. S11, Supplemental Table S5). Under WW treatment, both IL20 and IL46  
271 had higher ( $g_s$ ) compared to Svevo.

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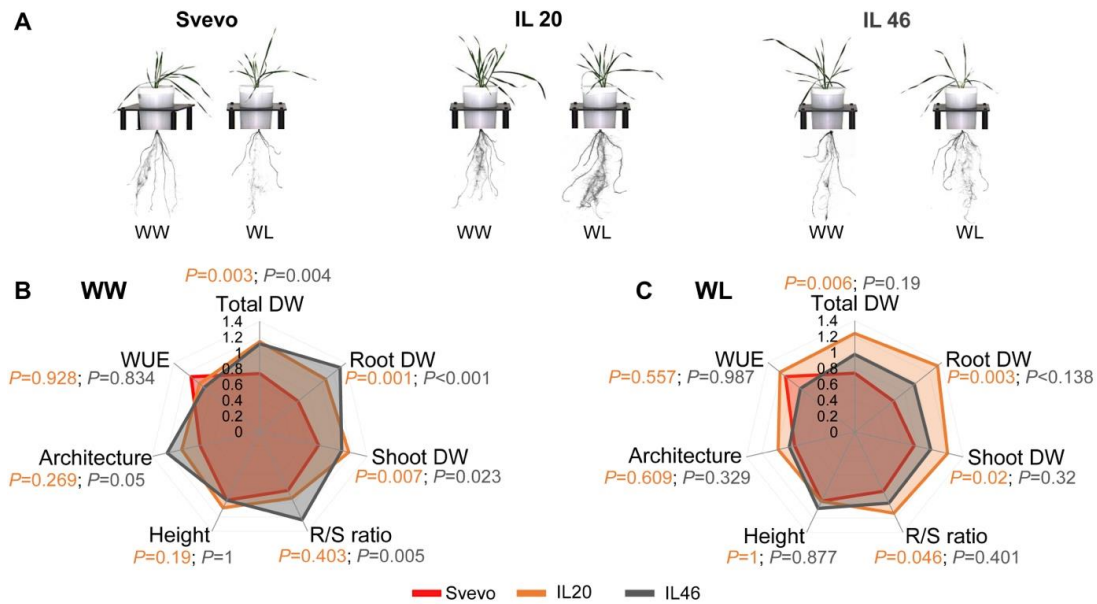
274 **Figure 3.** Longitudinal dynamics of Svevo, IL20 and IL46 for (A) relative growth rate, (B) net  
 275 assimilation rate under well-watered (WW; blue) and water-limited (WL; red) treatments. Dashed  
 276 lines represent the fitted linear growth of each genotype under specific water treatment. Markers  
 277 represents the genotypic mean under specific water treatment ( $n=4$ ). Continuous line represents the  
 278 smooth curve through the data and the shaded area represents the standard error of the smooth curve.  
 279

### 280 **IL20 exhibits higher root-to-shoot ratio under water stress**

281 Since IL20 maintained higher assimilation rate and stomatal conductance under WL treatment,  
 282 we investigated if this was related to improved water uptake due to differential root growth  
 283 response under water stress (Fig. 4A). We measured root dry weights from 21 DAT plants  
 284 grown in potted soil and found that both IL46 and IL20 had higher root biomass relative to  
 285 Svevo ( $P \leq 0.001$ ) under WW treatment. However, under WL treatment, Svevo root biomass  
 286 were significantly lower than IL20 ( $P=0.003$ ). Further, IL20 also exhibited a higher root-to-  
 287 shoot ratio when compared with Svevo under WL conditions ( $P=0.046$ ) (Fig. 4B, C;  
 288 Supplemental Table S6). Our data suggests that the root response of IL20 under water stress  
 289 diverges from Svevo. To explore this differential root response to water stress in a  
 290 developmental context, we performed a seedling stage assay using a paper roll set-up (Placido  
 291 et al., 2013). While the shoot length of IL20 and Svevo was similar under WW and WL  
 292 treatments, IL20 exhibited significantly higher root length throughout the experiment, with  
 293 10.3% longer roots at the end of the experiment (25.21 vs. 22.85 cm, for IL20 and Svevo,  
 294 respectively;  $P=0.006$ ) under WL. This advantage expressed in the higher (12.5%) root-to-  
 295 shoot ratio of IL20 compared with Svevo on the last day ( $P=0.001$ ; Supplemental Fig. S12A-  
 296 F). This suggests that the root growth dynamic of IL20 is different from Svevo even during  
 297 early seedling stage and more apparent under WL treatment and results in increase in the root-  
 298 to-shoot ratio. These results show that root biomass in later stages (19 in Zadocks scale) and

299 root length at the seedling stage (11 in Zadocks scale) have a similar response in IL20 under  
 300 WL treatment (Fig. 4, Supplemental Fig. S12).

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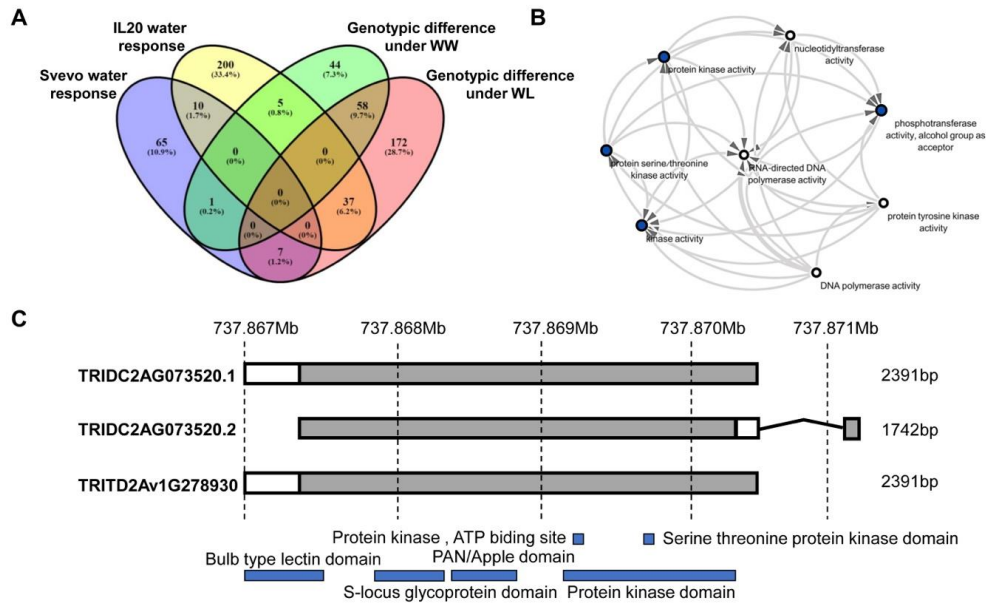
303 **Figure 4.** Morpho-physiological modification in response to water stress. (A) Representative image of  
 304 Svevo, IL20, and IL46 under well-watered (WW) and water-limited (WL) treatments 14 d after  
 305 transplanting. Radar charts comparing the phenotypic traits of Svevo (red), IL20 (orange), and IL46 (gray)  
 306 plants under (B) WW and (C) WL treatments. Values are means ( $n=4$ ). Total dry weight (Total DW),  
 307 water-use efficiency (WUE), plant architecture (convex area), plant height (height), root-to-shoot ratio  
 308 (R/S ratio), shoot DW, and root DW.

309

### 310 Transcriptome analysis of IL20 and Svevo roots in response to water stress

311 Given the differential root growth and the root-to-shoot ratio between Svevo and IL20 in the  
 312 seedling stage, we reasoned that the underlying gene(s) responsible for these phenotypes could  
 313 be the same, resulting in similar root-to-shoot ratio plasticity that was observed in later  
 314 vegetative stages. Therefore, we performed a transcriptome analysis on roots from the seedling  
 315 stage experiment to identify candidate genes that underlie the root-to-shoot plasticity  
 316 phenotype. Seedling root sampling for transcriptome is more precise as it limits gene transcript  
 317 changes caused by root damage that occurs with sampling roots from older plants growing in  
 318 soil or sand. We combined the transcriptome analysis with the genotypic data of IL20 and Svevo  
 319 to map the differentially expressed genes (DEGs) to specific introgressions. IL20 has six  
 320 introgressions from Zavitan, the wild emmer parent, distributed on five chromosomes (Table  
 321 S7), accounting for ~4.5% from the Zavitan genome (Avni et al., 2017). Based on public  
 322 annotations, a total of 651 genes from the homozygous regions (Supplemental Table S8) map  
 323 to these introgressions. Under WL treatment, when the root phenotype is most apparent, we  
 324 identified 599 DEGs (Fig. 5A) between Svevo and IL20, with 37 genes (6.17%) co-localizing

325 to the introgressions. Of these, 425 genes were down-regulated and 174 genes were up-  
 326 regulated in IL20 (Supplementary Table S9). Under WL treatment, 39.23% of the DEGs were  
 327 differently expressed between Svevo and IL20 (56 up- and 179 down-regulated), whereas only  
 328 11.35% were expressed differently under WW treatment.  
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**Figure 5.** Differently expressed genes (DEGs) comparison for IL20 and Svevo. **(A)** A four-way Venn diagram of DEGs among IL20 and Svevo under well-watered (WW) and water-limited (WL) treatments. **(B)** Network co-expression pattern of the DEGs associated with kinase activity. Node with blue color represents higher interaction. **(C)** Splice variation of TRIDC2AG073520 gene, a candidate for root phenotypes of IL20 that maps to the introgression. TRITD2Av1G278930 represent Svevo allele for the current gene.

### 338 Candidate genes associated with longer roots under water stress

339 We next examined the differentially abundant transcript(s) that localize to the introgressions in  
 340 IL20 and identified 17 DEGs under WW and 18 DEGs under WL treatments between IL20 and  
 341 Svevo. Two DEGs (TRIDC4AG049220 and TRIDC4AG049940) were found to express only  
 342 in IL20 in response to water stress. Given the IL20 root phenotype, we targeted root-related  
 343 DEGs from this set, which yielded five candidate genes (CG; Supplementary Table S10). The  
 344 criteria used to filter these five genes are based on literature searches of orthologs with root-  
 345 associated phenotypes. Three of these genes were up-regulated in IL20 under WL  
 346 (TRIDC4AG046080, TRIDC4AG048600, and TRIDC2AG073520), one gene was down-  
 347 regulated under WL (TRIDC4AG046660) and one gene (TRIDC4AG046110) showed up-  
 348 regulation under WW treatment only. Of these five genes, TRIDC4AG046080 is a low  
 349 confidence gene based on the annotation of the Zavitan genome. The remaining four genes  
 350 either have a SNP (TRIDC2AG073520, TRIDC4AG048600), or carry multiple polymorphisms

351 (TRIDC4AG046660), or were presence/absence variation between the Zavitan and Svevo  
352 genomes (TRIDC4AG046110) (Supplementary Table S10). TRIDC4AG046110 encodes a  
353 *FARI-RELATED SEQUENCE 4-like* isoform that is down-regulated in salt-susceptible sweet  
354 sorghum (*Sorghum bicolor*) roots (Yang et al., 2018). TRIDC4AG048600 is a *SIMILAR TO*  
355 *RCD ONE 1 (SROI)* gene. In *Arabidopsis (Arabidopsis thaliana)*, a double mutant of *AtSROI*  
356 exhibited shorter roots and a smaller cell division zone compared to wildtype plants (Teotia and  
357 Lamb, 2011). Sequence alignment of this gene against the Zavitan genome indicates a truncated  
358 protein in the Zavitan genome that may result in loss of function or a modified function.

359 The remaining three DEGs were associated with protein kinase function (Supplementary  
360 Table S10), where network analysis of molecular functions showed significant downstream  
361 transferase activity elements in various kinase activities (Fig. 5B). TRIDC4AG046080 is a  
362 homolog of a rice domain of the unknown function (*DUF581*) that, in *Arabidopsis*, was found  
363 to play a role in sucrose non-fermenting-related kinase (*SnRK1*) (Nietzsche et al., 2016).  
364 TRIDC4AG046660 is a Leucine-rich repeat receptor protein kinase (LRR-RLK) and  
365 TRIDC2AG073520 is a G-type lectin S-receptor-like serine/threonine-protein kinase (RLK).  
366 We examined the sequence of TRIDC2AG073520 in the Zavitan genome (Avni et al., 2017)  
367 and identified two splice variants on chromosome 2A, which are 2391bp and 1742bp for  
368 TRIDC2AG073520.1 and TRIDC2AG073520.2, respectively. In contrast, only a single variant  
369 (2391bp) was found in the tetraploid durum wheat (*cv. Svevo*) (Fig. 5C) as well as among 10  
370 hexaploid bread wheat cultivars genomes (Appels et al., 2018; Walkowiak et al., 2020). This  
371 CG was mapped in the expression atlas of the Zavitan tissue-specific gene and suggest that it  
372 could be the primary candidate gene associated with the root-to-shoot ratio difference exhibited  
373 by IL20 (Supplemental Fig. S13).

374

375

## 376 **DISCUSSION**

377 Wild plants have developed various reversible and non-reversible phenotypic plasticity  
378 strategies to cope with environmental uncertainty. Selection by humans, often under less  
379 variable environmental conditions has likely resulted in higher crop-plant phenotypic stability  
380 (Lopes et al., 2015). Consequently, many modern cultivars may have lost some of the fitness  
381 components needed for adapting to climate driven variation in many regions (Kissoudis et al.,  
382 2016). Wild ancestors of modern crops offer a promising source for genetic diversity and novel  
383 drought adaptive traits (Peleg et al., 2005; Golan et al., 2018).

384 The introgression of Zavitan alleles into a modern durum cultivar promoted higher  
385 phenotypic diversity under both WW and WL treatments, as expressed in plant architecture and  
386 biomass accumulation (Fig. 1). Although the IL panel was developed from a single wild emmer  
387 accession (Zavitan), yet it resulted in wide segregation of morpho-physiological traits (either  
388 positively or negatively). This accession originated from a habitat with high soil moisture  
389 fluctuations, due to a shallow brown basaltic soil type, which has been shown to promote  
390 diversity (Poot and Lambers, 2008; Peleg et al., 2008). This phenotypic variation is associated  
391 with the quantitative nature of these traits and the different combinations of wild and  
392 domesticated alleles. Interestingly, the mean biomass accumulation trajectory over time of the  
393 IL panel was similar to Svevo under both water treatments.

394 Water stress reduced biomass by around 50% (i.e., PSA) and altered plant architecture  
395 (i.e., convex area 12.5-48.5%) relative to the WW treatment (Supplementary Fig. S2), with both  
396 variables being positively associated with one another (Supplementary Fig. S5). Increased  
397 phenotypic variation in response to water stress was quantified by the calculation of drought  
398 susceptibility index (S-index). The combination of IL performance under WL with their S-  
399 indexes resulted in five distinct clusters of high phenotypic stability (HPS, LPS) and  
400 phenotypic plasticity (HPP, MPP, LPP). Phenotypic stability is often associated with  
401 small changes in plant performance in response to unfavorable conditions. Escape (i.e., rapid  
402 growth to avoid the stress) is a common strategy of wild plants in xeric habitats and has been  
403 repeatedly reported for many wild grasses such as emmer wheat (Peleg et al., 2005),  
404 *Brachypodium distachyon* (Opanowicz et al., 2008), and *Avena barbata* (Sherrard and  
405 Maherali, 2006). Accordingly, the two clusters exhibiting phenotypic stability had biomass  
406 reductions of only 45 and 40% for LPS and HPS, respectively. Interestingly, the LPS had  
407 characteristics of “small plants” (PSA, 50.4, and 27.8 kPixel for WW and WL, respectively),  
408 whereas HPS had high biomass under WW and the highest values among all clusters under  
409 WL (67.1 and 40.6 kPixel, respectively). These results suggest that the phenotypic stability  
410 strategy is not size-dependent, but rather an active mechanism that enables plants to cope with  
411 water stress.

412 Wild emmer wheat populations were found to harbor rich phenotypic diversity for  
413 drought-adaptive traits, which correspond with the wide inter-annual and seasonal fluctuations  
414 in soil moisture availability of the Mediterranean basin (Peleg et al., 2005). Accordingly, the  
415 phenotypic plasticity clusters exhibited a greater reduction in biomass accumulation (55 and  
416 56% for MPP and HPP, respectively). The HPP cluster had the highest biomass under

417 WW (PSA 81.8 kPixel); while under WL it exhibited more reduction, although biomass was  
418 still relatively high (36.4 kPixel) compared to all clusters.

419 Plant acclimation to water stress elicited physiological, morphological, and metabolic  
420 responses that occurred through coordinated spatio-temporal processes. These processes  
421 changed the physiological status of plants toward a new steady-state level that supports growth  
422 and fitness under unfavorable conditions. The temporal characterization of the responsiveness  
423 clusters showed that clusters with high phenotypic plasticity responded earlier (12, 8, and 10  
424 kPixel for LPMP, MPHP, and HPHP, respectively) than those in high stability clusters (20 and  
425 26 kPixel for LPHS and HPHS, respectively) (Fig. 2A). To understand the longitudinal genetic  
426 architecture of the responsiveness clusters, we calculated broad sense heritability ( $_{bs}h^2$ )  
427 dynamics. While the plasticity clusters exhibited a decrease in PSA  $_{bs}h^2$  over time as a  
428 consequence of high G×E interaction ( $\text{Sigma}^2 \text{G} \times \text{E}$ ) and low genetic component ( $\text{Sigma}^2 \text{G}$ ),  
429 the more phenotypically stable clusters showed increased heritability during early growth and  
430 decreased heritability at later stages, which corresponds to the delayed stress responses (Fig.  
431 3).

432 Plants exhibit morphological and physiological adjustments to maintain water status and  
433 carbon assimilation under water stress (Chaves et al., 2009). The two high productivity clusters  
434 (HPHS and HPHP) exhibit contrasting response mechanisms, with the plasticity cluster  
435 responding earlier (16, 17, and 8 d early for PSA, plant density and plant architecture,  
436 respectively; Fig. 2; Supplementary Fig. S8). Detailed characterization of representative  
437 accessions for these two clusters (represented by IL20 and IL46 for HPHP and HPHS,  
438 respectively) confirmed the earlier response of HPHP in terms of relative growth rate (Fig. 3A),  
439 thus suggesting size-independent plant responsiveness to water stress. Consistent with the  
440 growth phenotype, IL46 maintained similar photosynthetic and transpiration rates under WW  
441 and WL, while IL20 responded as early as day 12, limiting its assimilation rate. Notably, IL20  
442 had the highest photosynthetic rate under WW and exhibited a larger reduction under WL yet  
443 was able to maintain significantly higher assimilation rate than Svevo.

444 A fast stress responsiveness strategy may negatively affect carbon assimilation and  
445 growth; on the other hand, early acclimation can trigger a metabolic shift of carbon allocation  
446 to different plant organs (Rodrigues et al., 1993; Bohnert and Sheveleva, 1998). Thus, under  
447 water limitation, root-to-shoot ratio plasticity can mediate optimal resource partitioning  
448 between growth and development (Shipley and Meziane, 2002; Voss-Fels et al., 2017). Modern  
449 bread wheat cultivars have lower root-to-shoot ratios as compared to landraces (Siddique et al.,  
450 1990). Moreover, a comparison among wild emmer, domesticated emmer, and durum wheat



451 showed a trend of reduced root-to-shoot ratio during the initial domestication from wild to  
452 domesticated emmer, and during wheat evolution under domestication (Gioia et al., 2015;  
453 Roucou et al., 2018). Accordingly, the introgression of alleles from Zavitan in the background  
454 of the elite durum wheat cultivar significantly increased the root-to-shoot ratio (30%) under  
455 WL as compared with the parental line (Fig. 4C). Likewise, Merchuk-Ovnat et al. (2017)  
456 reported a higher root-to-shoot ratio in response to water stress from wild emmer (*acc.* G18-  
457 16) introgression in the background of elite bread wheat cultivar. Thus, introducing new genetic  
458 diversity for root-to-shoot ratio plasticity from wild progenitors will facilitate the resilience of  
459 modern wheat cultivars to the projected fluctuating water availability during the growing  
460 season.

461 The root system is the site of interactions with the rhizosphere; thus, root architectural  
462 plasticity (i.e., allocational, morphological, anatomical, or developmental) is a critical  
463 adaptation strategy to environmental cues (Rellán-Álvarez et al., 2016; Golan et al., 2018). To  
464 better understand the genetic mechanism associated with the increased root biomass of IL20,  
465 we analyzed transcriptome response of roots under water stress. In general, differential  
466 transcriptional response of IL20 WL was greater than Svevo (223 vs. 73 DEGs, respectively).  
467 This is consistent with a previous study where miRNA expression in the roots of two wild  
468 emmer accessions (TR39477 and TTD-22) were significantly higher compared with  
469 domesticated durum wheat (*cv.* Kızıltan) under water stress (Akpınar et al., 2015). These results  
470 emphasize the potential of higher plasticity in transcriptional response of wild relatives  
471 compared to the domesticated germplasm.

472 Downstream gene network analysis highlighted the key role of protein kinases as hubs of  
473 interaction (Fig. 5B). Three CGs (TRIDC4AG046080, TRIDC2AG073520, and  
474 TRIDC4AG046660) were found associated with protein kinase function that mediates plant  
475 hormone and nutrient signaling, and cell cycle regulation (Laurie and Halford, 2001; Virlet et  
476 al., 2017). TRIDC4AG046660 is a leucine-rich repeat receptor-like protein kinase (LRR-RLK).  
477 Mutants of this gene in *Arabidopsis* (*At2g33170*) controls root growth and are mediated by  
478 cytokinin (Colette et al., 2011). TRIDC4AG046080 (DUF581 in rice) interacts with *SnRK1* and  
479 regulated by hormones and differentially regulated by hormones and environmental signals  
480 (Nietzsche et al., 2016). Wheat mutants containing a conserved DUF581 domain revealed a  
481 salt-induced gene (TaSRHP). Early stages of salt stress typically have an osmotic stress  
482 component that is similar to water stress. Over-expression of this gene in wild-type *Arabidopsis*  
483 *thaliana* resulted in enhanced resistance to both salt and drought stresses (Hou et al., 2013).

484 TRIDC2AG073520 (TRITD2Av1G27893 in Svevo) is a G-type lectin S-receptor-like  
485 serine/threonine-protein kinase gene. The domesticated allele contains a nonsynonymous  
486 mutation expressed as an amino acid shift (isoleucine to threonine). This CG was significantly  
487 up-regulated under WL in IL20 (FC 2.29,  $P_{adj}=0.03$ ). In *Arabidopsis*, drought and salinity  
488 stress-induced up-regulation of the gene (Sun et al., 2013). Moreover, the gene was expressed  
489 specifically in root tissue from the early seedling stage to 50% of ear emergence (Supplemental  
490 Fig. S14; Ramírez-González et al., 2018). Genetic dissection showed that the genomic region  
491 of this gene overlaps with a QTL affecting lateral root number per primary root (Maccaferri et  
492 al., 2016).

493 Two splice variants of TRITD2Av1G278930 were identified in the wild emmer genome  
494 (TRIDC2AG073520.1 and TRIDC2AG073520.2) and these included several polymorphisms  
495 in each variant. The TRIDC2AG073520.1 variant is similar to the domesticated variant,  
496 although it contains a nonsynonymous SNP. This gene was compared to the wheat pan-genome  
497 (Walkowiak et al., 2020) and similar SNP was found in all genomes compare to Zavitan,  
498 suggesting variation between wild and domesticated wheats. The TRIDC2AG073520.2 variant  
499 is different in length and exon number; however, the domains remain similar to the  
500 domesticated variant and the additional exon does not encode for a specific known domain (Fig.  
501 5C). The underlying mechanism by which the identified splice variance and/or amino acid  
502 substitution affect wild emmer response to stress via longer root systems are yet to be  
503 discovered.

504

### 505 **Concluding remarks and future perspective**

506 In this study, we show that targeting small and hence more genetically tractable wild  
507 introgressions can still yield surprisingly divergent phenotypic responses to water stress even  
508 after selection of ILs that have agronomically viable phenology. Our detailed physiological  
509 characterization combined with temporal phenomics approaches provides novel insights into  
510 the divergent water stress response dynamics in an elite durum background. Although, we  
511 focused specifically on one IL for downstream characterization of its root-to-shoot plasticity  
512 response under water stress and candidate gene discovery, this work lays the foundation for  
513 characterization of additional lines from this IL panel and other introgressions in wheat.  
514 Collectively, our results suggest that incorporating the wild gene/alleles can enable greater  
515 phenotypic plasticity and has the potential to enhance environmental stress resilience.

516

517

## 518 MATERIAL AND METHODS

### 519 Plant material and experimental design

520 The wild emmer accession Zavitan was collected at the Zavitan nature reserve (32°56'24.52"N,  
521 35°42'10.56"E; 288.4 m above sea level), Israel and was selected as the donor parent due to its  
522 robust morphology and drought tolerance (Avni et al., 2014). Uniform seeds of 47 wild emmer  
523 wheat (*acc.* Zavitan) introgression lines (IL) in the background of elite durum wheat (*cv.* Svevo)  
524 and their recurrent parent were used for the current study. A recombinant inbred line population  
525 from cross between durum wheat (*cv.* Svevo) and wild emmer (*acc.* Zavitan) was previously  
526 developed (Avni et al., 2014). Adapted RILs (i.e. with the genetic composition of post-  
527 domestication alleles of dwarfing gene *Reduce height (Rht)-B1b* and non-brittle spike genes  
528 (*TiBtr1-A and TiBtr1-B*), were selected and backcrossed three time and selfed over three  
529 generations. The 47 ILs were genotyped (Infinium iSelect 90K SNP chip array; Wang et al.,  
530 2014), resulting is equal SNP distribution across the genomes. Altogether, the ILs population  
531 cover 99.1% of the wild emmer Zavitan genome (Oren, 2020). Detailed information for the ILs  
532 panel is provided in Supplementary Table S11. Seeds were surface disinfected (1% sodium  
533 hypochloric acid for 30 minutes) and placed in Petri dishes on moist germination paper (Anchor  
534 Paper Co., St. Paul, MN, USA) about 3 cm apart, at 24°C in the dark for 5 days. Three uniform  
535 seedlings from each line were transplanted to a single pot (2L, 45×19.5 cm) filled with 1.2 kg  
536 of Fafard germination soil (Sungro, Massachusetts, USA), with osmocote fertilizer and  
537 Micromax micronutrients. Six days after transplanting (DAT), plants were thinned to one plant  
538 per pot. Pots were placed on automated carriers in the greenhouse (22/16°C day/night) and  
539 watered daily to 80% field capacity until the beginning of the experiment (11 DAT), 2-4 in  
540 Zadocks scale (Zadocks et al., 1974). The growth stages were tracked until the tillering stage,  
541 Zadocks 29-33. Water stress was initiated from the first day of imaging and the WL treatment  
542 pots reached the target field capacity within 16-24 days with an average of 19 days after  
543 initiation of imaging. The daytime Photosynthetic Active Radiation (PAR) was supplemented  
544 with LED red/blue light lamps, with an intensity of 200  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . The experiment was  
545 conducted at the Nebraska Innovation Campus greenhouse, high-throughput plant phenotyping  
546 core facility (Scanalyzer 3D, LemnaTec GmbH, Aachen, Germany), University of Nebraska-  
547 Lincoln.

548 A two-way factorial complete randomized experimental design, with 47 ILs and the  
549 recurrent parent, Svevo, was conducted. There were two water treatments: well-watered  
550 (control, WW) at 80% field capacity (FC) and water-limited (WL) at 30% FC (Supplementary  
551 Fig. S3), with three replicates for each combination. As quality control, we used empty pots,

552 placed randomly in every second row. In total there were 296 pots. Plants were imaged daily  
553 for 35 days with visible Red, Green, and Blue (RGB) camera (Basler, Ahrensburg, Germany)  
554 taking 5 side-views (rotating 72°) and a single top-view. The image size was 2454×2056 pixels.  
555 After imaging, each pot was automatically weighed and watered to meet its calculated target  
556 weight. Greenhouse temperature kept at 22/16°C (day/night) during the experiment.

557 Based on the results of the first experiment, we selected two ILs (IL20, IL46) for detailed  
558 physiological characterization, alongside their parental line Svevo. A two-way factorial  
559 complete random design was conducted, with three genotypes, and two water treatments as  
560 described above, with four replicates for a total of 24 pots. The imaging started 7 DAT and  
561 imaging continued for 14 days.

562

### 563 **Image processing**

564 PhenoImage GUI software (<http://vis.unl.edu/~yu/Research.htm>) was used for image  
565 processing based on MATLAB (The Mathworks, Inc., Massachusetts, USA). The workflow  
566 consisted of three main steps: image cropping, plant segmentation, and attribute extraction. In  
567 brief, image cropping was used to remove the frame of the chamber, followed by a background  
568 removal step based on color differences. Plant segmentation was based on filtration of pixel  
569 intensity (i.e., distinguishing between plant and non-plant pixels). As a result, the software can  
570 give the plant dimension, pixel sum, image moment, and convex area. All raw RGB images  
571 were deposited in the CyVerse and can be accessed at <https://rb.gy/zvbief>. Image data were  
572 stored in the following data structure: pot number\_genotype\_water treatment\_replicate\_date/side  
573 view\_degree/image.png.

574

### 575 **Morpho-physiological trait characterization**

576 We extracted some of the key morphological traits derived from RGB images included, PSA,  
577 plant height and width, plant architecture (convex area), plant density, and water-use efficiency  
578 (WUE) at the final day of the experiment. *Plant height* and *plant width* were calculated from  
579 plant dimensions. *Plant architecture* (convex area) was calculated to predict plant architecture  
580 trajectory. *Density* was calculated based on the ratio between pixel sum and plant architecture.  
581 *Plant biomass* was calculated based on the projected shoot area (PSA) as described by  
582 (Campbell et al., 2015). On the last day of the experiment, a subset of 19 ILs harvested, oven-  
583 dried (80° C), and weighed to obtain shoot dry weight. Correlation analysis showed a high  
584 correlation between PSA and shoot dry weight ( $r=0.96$ ;  $P<10^{-4}$ ; Supplementary Fig. S14). The  
585 *relative growth rate* (RGR) was calculated by dividing daily pixel accumulation with pixel

586 numbers from the previous day. Daily *water-use efficiency* ( $WUE_t$ ) was calculated as described  
587 by Momen et al. (2019), where (t) represents the day.

$$588 \quad WUE_t = \frac{\Delta PSA \text{ (Pixels)}}{\Delta WU \text{ (ml)}}$$

589 where  $\Delta PSA$  is the daily PSA:

$$590 \quad \Delta PSA = PSA_{t-1} - PSA_t$$

591 and  $\Delta WU$  is the daily water used:

$$592 \quad \Delta WU = \text{Pot weight}_{t-1} - \text{Pot weight}_t$$

593

594 *Photosynthetic rate, transpiration rate, and stomatal conductance* were measured between 10  
595 and 22 DAT (Zadocks 15-19) using a portable infra-red gas analyzer (LI-6800XT; Li-Cor Inc.,  
596 Lincoln, NE, USA). Measurements were conducted at the mid-portion of the last fully expanded  
597 leaf from 9:00 to 13:00 ( $n=3$ ).

598 *Root biomass* was measured at 22 DAT from 2L pots (45×19.5 cm) filled with 1.2 kg of Fafard  
599 germination soil (Sungro, Massachusetts, USA). Root tissue was harvested ( $n=4$ ), washed and  
600 oven-dried (80° C) for 72h, and weighed to obtain root dry weight. *The root-to-shoot ratio* was  
601 calculated by dividing root dry weight with PSA (shoot dry weight).

602

### 603 **Characterization of root and shoot length**

604 Uniform seeds were germinated in a Petri dish on moist germination paper for 5d in the dark at  
605 22-25°C. Five seedlings of each genotype were placed on moist germination paper (25 × 38  
606 cm; Anchor Paper Co., St. Paul, MN, USA), about 5 cm apart, with the germ end facing down.  
607 The paper was covered with another sheet of moist germination paper and rolled to a final  
608 diameter of 3 cm. The bases of the rolls were placed on a 4L beaker in a darkened growth  
609 chamber at a temperature of 24C/16C, 15h/9h day/night, at 50-60% relative humidity. A two-  
610 way factorial design was used with two genotypes (Svevo and IL20) and two water  
611 availabilities: WW and WL, with 8 replicate for each combination (total of 32). Eight cigar rolls  
612 were placed in a container (4 L) where the well water treatment was refilled on daily basis to keep  
613 the availability of 100 ml of water. The water-limited treatment filled once with 20 ml and did  
614 not re-filled during the experiment, or 20 ml (without refilling) for WL. Each container was  
615 wrapped with plastic to prevent water evaporation. Shoot and root length were measured daily  
616 by scale, from 3 to 8 DAT (Zadocks 11).

617

618

## 619 **Statistical Analyses**

620 The JMP® ver. 15 statistical package (SAS Institute, Cary, NC, USA) was used for statistical  
621 analyses unless otherwise specified. The longitudinal response was fitted for genotypes  
622 (collectively or separately) under each water treatment. Analysis of Variance (ANOVA) was  
623 used to assess the possible effects of genotype (G), environment (E), and G×E interactions on  
624 morpho-physiological traits of genotypes. Frequency distribution was determined for all  
625 morpho-physiological traits on the last day. Components of descriptive statistics are graphically  
626 presented in box plot: median value (horizontal short line), quartile range (25 and 75%) and  
627 data range (vertical long line). Principle Component Analysis (PCA) was used to determine  
628 associations between traits. PCA was based on a correlation matrix and is presented as biplot  
629 ordinations of the ILs (PC scores). Three components were extracted using eigenvalues >1.2 to  
630 ensure the meaningful implementation of the data by each factor. An agglomerative hierarchical  
631 procedure with an incremental sum of squares grouping strategy was employed using Ward's  
632 method (Ward, 1963), for classification. Pearson correlation for all morpho-physiological traits  
633 was conducted for each water treatment. Drought-susceptibility index (S) was calculated  
634 according to Fischer and Maurer (1978):

$$635 \quad S = \frac{1 - Y_{WL}/Y_{WW}}{1 - X_{WL}/X_{WW}}$$

636 where  $Y_{WL}$  and  $Y_{WW}$  are the mean phenotypic values of a certain genotype under the respective  
637 treatments, and  $X_{WL}$  and  $X_{WW}$  are the mean performances of all genotypes. Morpho-  
638 physiological correlation matrix and Density distribution were plotted with R software (RStudio  
639 Team, 2015).

640

## 641 **Broad-sense heritability dynamics**

642 Broad-sense heritability ( ${}_b h^2$ ) and its components, genetic component ( $\sigma_g^2$ ), and G×E  
643 interaction ( $\sigma_{g \times e}^2$ ), were calculated for each day of imaging across the two water treatments  
644 using ANOVA-based variance components:

$$645 \quad h^2 = \sigma_g^2 / \sigma_g^2 + \sigma_{g \times e}^2 / e,$$

646 where  $\sigma_g^2 = [(MS_{IL} - MS_{IL \times e})/e]$ ,  $\sigma_{g \times e}^2 = MS_{IL \times e}$ ,  $e$  is the number of water treatments and  
647 MS is the mean square.

648

649

650 **RNA extraction and sequencing**

651 Root tissues were collected daily from 8 to 11 days after germination (Zadocks 11) and frozen  
652 in liquid nitrogen until RNA extraction. RNA was extracted using the plant/fungi total RNA  
653 purification kit (Norgen Biotek Corp., Canada) with on-column DNase treatment (Qiagen,  
654 Germany). Sample contamination and RNA integrity were assessed using the Nan D-1000  
655 spectrophotometer (Thermo Fisher Scientific). Based on the physiological analysis, we selected  
656 samples from day six for RNAseq, with two repeats for each combination (total 8). Single-end  
657 (150bp) bar-coded cDNA libraries were prepared for sequencing on the Illumina HiSeq2000  
658 sequencer (NGS Core, Nebraska Medical Center Omaha, USA).

659

660 **Accession Number**

661 Raw sequencing files of mRNA sequencing are available at the short read archive of the  
662 National Center for Biotechnology Information (<https://www.ncbi.nlm.nih.gov>) under  
663 accession number GSE163450.

664

665 **Data processing and analysis**

666 FastQ quality of each sample was manually inspected using FastQC  
667 (<http://www.bioinformatics.babraham.ac.uk/projects/fastqc>). Barcode removal, filtering, and  
668 trimming of low-quality reads were executed using the command line tools Trimmomatic  
669 (Bolger et al., 2014). Each RNA-seq read was trimmed to make sure the average quality score  
670 exceeded 30 and has a minimum length of 70bp. Sequences were aligned to the available Svevo  
671 and Zavitan reference genomes (Avni et al., 2017; Maccaferri et al., 2019). Using TopHat  
672 (Trapnell et al., 2009), allowing for up to 2 bp mismatches per read. Reads mapped to multiple  
673 genomic locations were removed. Numbers of reads per gene were counted by the software tool  
674 of HTSeq-count using corresponding rice gene annotations and the “union” resolution mode  
675 was used (<http://www-huber.embl.de/users/anders/HTSeq>). Differential expression analysis of  
676 count data and data visualization were conducted with the DESeq2 package (Love et al., 2014).  
677 To detect significant DEGs, a 5% false discovery rate (FDR) correction for multiple  
678 comparisons was determined (Benjamini and Hochberg, 1995), and a minimal  $|0.5| \log_2FC$   
679 threshold was applied. Venn diagrams were created with  
680 <http://bioinformatics.psb.ugent.be/webtools/Venn>. Gene ontology, Singular Enrichment  
681 Analysis (SEA), and Parametric Analysis of DEGs set Enrichment for biological processes and  
682 pathways was conducted with AgriGO (<http://systemsbiology.cau.edu.cn/agriGOv2>; Tian et  
683 al., 2017).

684

## 685 **Gene ontology network**

686 Biological processes and molecular function networks were established using the DEGs GO  
687 terms with REVIGO software (<http://revigo.irb.hr>); this summarizes lists of GO terms using a  
688 clustering algorithm that relies on semantic similarity measures (Supek et al., 2011). The  
689 analysis outputs were transferred to the Cytoscape software (<https://cytoscape.org>), which  
690 served as a network biology analysis and visualization tool (Otasek et al., 2019).

691

## 692 **Genetic analysis of candidate DEGs**

693 Candidate genes were analyzed on the wheat efp browser for expression in different tissues and  
694 phenological stages ([http://bar.utoronto.ca/efp\\_wheat/cgi-bin/efpWeb.cgi](http://bar.utoronto.ca/efp_wheat/cgi-bin/efpWeb.cgi); Ramírez-González  
695 et al., 2018). Gene sequences were compared with the publically available genome of Svevo  
696 [https://wheat.pw.usda.gov/GG3/genome\\_browser](https://wheat.pw.usda.gov/GG3/genome_browser) and compared to Zavitan gene sequences  
697 with a blast against the Zavitan genome [https://wheat.pw.usda.gov/cgi-](https://wheat.pw.usda.gov/cgi-bin/seqserve/blast_wheat.cgi)  
698 [bin/seqserve/blast\\_wheat.cgi](https://wheat.pw.usda.gov/cgi-bin/seqserve/blast_wheat.cgi). Differences in splice variance number of candidate genes were  
699 perceived from the blast on the GrainGenes website [https://wheat.pw.usda.gov/cgi-](https://wheat.pw.usda.gov/cgi-bin/seqserve/blast_wheat.cgi)  
700 [bin/seqserve/blast\\_wheat.cgi](https://wheat.pw.usda.gov/cgi-bin/seqserve/blast_wheat.cgi). DNA translation to amino acids was done with the free online  
701 software <https://web.expasy.org/translate>

702

## 703 **Supplemental Data**

704 The following supplemental materials are available.

705 **Supplemental Table S1.** Correlations between morpho-physiological traits under well-  
706 watered and water-limited treatments.

707 **Supplemental Table S2.** Longitudinal coefficient of variance for PSA.

708 **Supplemental Table S3.** Comparison of PSA, plant architecture and plant architecture  
709 density under two water treatments for each cluster.

710 **Supplemental Table S4.** Regression equation of relative growth rate.

711 **Supplemental Table S5.** Comparisons of A, T, and *gsw* between Svevo, IL20, and IL46  
712 under two water treatments throughout the experiment.

713 **Supplemental Table S6.** Comparisons of morpho-physiological traits between Svevo, IL20,  
714 and IL46 under two water treatments.

715 **Supplemental Table S7.** Physical location of wild emmer introgressions of IL20 on the  
716 Zavitan genome.

717 **Supplemental Table S8.** Gene annotation within IL20 introgressions.



- 718 **Supplemental Table S9.** Significant differentially expressed genes.
- 719 **Supplemental Table S10.** Root-related candidate genes.
- 720 **Supplemental Table S11.** List of ILs and their chromosomal introgressions.
- 721 **Supplemental Figure S1.** Correlation between projected shoot area (PSA) and shoot dry  
722 weight.
- 723 **Supplemental Figure S2.** Longitudinal dynamics of morpho-physiological traits under  
724 contrasting water treatment.
- 725 **Supplemental Figure S3.** Experimental design.
- 726 **Supplemental Figure S4.** Plant projected shoot area (PSA) dynamics of introgression lines  
727 and Svevo under well-watered and water-limited treatments.
- 728 **Supplemental Figure S5.** Correlation matrix between morpho-physiological traits under  
729 well-watered and water-limited treatments.
- 730 **Supplemental Figure S6.** Principal component analysis of morpho-physiological traits.
- 731 **Supplemental Figure S7.** Hierarchical clustering of morpho-physiological traits under water-  
732 limited and in terms of susceptibility index and clusters expression  
733 pattern.
- 734 **Supplemental Figure S8.** Longitudinal dynamic of plant architecture and density.
- 735 **Supplemental Figure S9.** Longitudinal heritability of plant density and architecture.
- 736 **Supplemental Figure S10.** Longitudinal dynamics of Svevo, IL20 and IL46 assimilation rate  
737 under well-watered and water-limited treatments.
- 738 **Supplemental Figure S11.** Longitudinal dynamics for stomatal conductance and transpiration  
739 rate under well-watered and water-limited treatments.
- 740 **Supplemental Figure S12.** Longitudinal dynamics of the root-to-shoot ratio under contrasting  
741 water treatment.
- 742 **Supplemental Figure S13.** Heat map of candidate genes from Zavitan expression atlas and  
743 read count of the candidate gene TRIDC2AG073520 at different  
744 developmental stages
- 745 **Supplemental Figure S14.** Expression atlas of TRIDC2AG073520 in the wheat efp browser.
- 746

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## 756 LITERATURE CITED

757 **Akpinar BA, Kantar M, Budak H** (2015) Root precursors of microRNAs in wild emmer and  
758 modern wheats show major differences in response to drought stress. *Funct Integr*  
759 *Genomics* **15**: 587–598

760 **Appels R, Eversole K, Feuillet C, et al.** (2018) Shifting the limits in wheat research and  
761 breeding using a fully annotated reference genome. *Science* **361**: eaar7191

762 **Araus JL, Slafer GA, Reynolds MP, Royo C** (2002) Plant breeding and drought in C<sub>3</sub> cereals:  
763 what should we breed for? *Ann Bot* **7**: 925-940

764 **Arms EM, Bloom AJ, St. Clair DA** (2015) High-resolution mapping of a major effect QTL  
765 from wild tomato *Solanum habrochaites* that influences water relations under root chilling.  
766 *Theor Appl Genet* **128**: 1713–1724

767 **Ashley DA, Boerma HR** (1989) Canopy photosynthesis and its association with seed yield in  
768 advanced generations of a soybean cross. *Crop Sci* **29**: 1042–1045

769 **Avni R, Nave M, Barad O, et al.** (2017) Wild emmer genome architecture and diversity  
770 elucidate wheat evolution and domestication. *Science* **357**: 93–97

771 **Avni R, Nave M, Eilam T, Sela H, Alekperov C, Peleg Z, Dvorak J, Korol A, Distelfeld A**  
772 (2014) Ultra-dense genetic map of durum wheat × wild emmer wheat developed using the  
773 90K iSelect SNP genotyping assay. *Mol Breed* **34**: 1549–1562

774 **Baum M, Grando S, Backes G, Jahoor A, Sabbagh A, Ceccarelli S** (2003) QTLs for  
775 agronomic traits in the Mediterranean environment identified in recombinant inbred lines  
776 of the cross “Arta” × *H. spontaneum* 41-1. *Theor Appl Genet* **107**: 1215–1225

777 **Benjamini Y, Hochberg Y** (1995) Controlling the false discovery rate: A practical and  
778 powerful approach to multiple testing. *J R Stat Soc Ser B* **57**: 289–300

779 **Berger B, Parent B, Tester M** (2010) High-throughput shoot imaging to study drought  
780 responses. *J Exp Bot* **61**: 3519–3528

781 **Bohnert HJ, Sheveleva E** (1998) Plant stress adaptations-making metabolism move. *Curr Opin*  
782 *Plant Biol* **1**: 267–274

783 **Campbell MT, Knecht AC, Berger B, Brien CJ, Wang D, Walia H** (2015) Integrating  
784 image-based phenomics and association analysis to dissect the genetic architecture of

- 785 temporal salinity responses in rice. *Plant Physiol* **168**: 1476–1489
- 786 **Campos H, Cooper M, Habben JE, Edmeades GO, Schussler JR** (2004) Improving drought  
787 tolerance in maize: a view from industry. *Field Crops Res* **90**: 19-34
- 788 **Chaves MM, Flexas J, Pinheiro C** (2009) Photosynthesis under drought and salt stress:  
789 Regulation mechanisms from whole plant to cell. *Ann Bot* **103**: 551–560
- 790 **Cook, BI, Mankin, JS., Anchukaitis, KJ** (2018). Climate change and drought: From past to  
791 future. *Curr Clim Change Rep* **4**: 164-179
- 792 **Correa J, Postma JA, Watt M, Wojciechowski T** (2019). Soil compaction and the  
793 architectural plasticity of root systems. *J Exp Bot* **70**: 6019-6034
- 794 **Fischer RA, Maurer R** (1978) Drought resistance in spring wheat cultivars. I. Grain yield  
795 responses. *Aust J Agric Res* **29**: 897–912
- 796 **Food and Agriculture Organization of the United Nations** (2017) The future of food and  
797 agriculture: Trends and challenges. Food Agric Organ United Nations.
- 798 **Gioia T, Nagel KA, Beleggia R, Fragasso M, Ficco DBM, Pieruschka R, De Vita P, Fiorani  
799 F, Papa R** (2015) Impact of domestication on the phenotypic architecture of durum wheat  
800 under contrasting nitrogen fertilization. *J Exp Bot* **66**: 5519–5530
- 801 **Golan G, Hendel E, Méndez Espitia GE, Schwartz N, Peleg Z** (2018) Activation of seminal  
802 root primordia during wheat domestication reveals underlying mechanisms of plant  
803 resilience. *Plant Cell Environ* **41**: 755–766
- 804 **Gupta A, Rico-Medina A, Caño-Delgado AI** (2020) The physiology of plant responses to  
805 drought. *Science* **368**: 266–269
- 806 **Hou X, Liang Y, He X, Shen Y, Huang Z** (2013) A novel ABA-responsive TaSRHP Gene  
807 from wheat contributes to enhanced resistance to salt stress in *Arabidopsis thaliana*. *Plant*  
808 *Mol Biol Report* **31**: 791–801
- 809 **Hsiao TC** (1973) Plant responses to water stress. *Annu. Rev. Plant Physiol* **24**: 519-570.
- 810 **Kirkegaard JA, Lilley JM, Graham JM** (2007) Impact of subsoil water use on wheat yield.  
811 *J Agric Res* **58**: 303-315
- 812 **Kissoudis C, van de Wiel C, Visser RGF, van der Linden G** (2016) Future-proof crops:  
813 challenges and strategies for climate resilience improvement. *Curr Opin Plant Biol* **30**: 47-  
814 56
- 815 **Knecht AC, Campbell MT, Caprez A, Swanson DR, Walia H** (2016) Image Harvest: An  
816 open-source platform for high-throughput plant image processing and analysis. *J Exp Bot*  
817 **67**: 3587–3599

- 818 **Lambers H, Atkin OK, Millenaar FF** (2002) Respiratory patterns in roots in relation to their  
819 functioning *In* Y Waisel, A Eshel, U Kafkaki, eds, *Plant Roots: The Hidden Half.*,  
820 Fourth edition. Marcel Dekker, Inc, New York, pp 521-552
- 821 **Laurie S, Halford NG** (2001) The role of protein kinases in the regulation of plant growth and  
822 development. *Plant Growth Regul* **34**: 253–265
- 823 **Lopes MS, El-Basyoni I, Baenziger PS, et al.** (2015) Exploiting genetic diversity from  
824 landraces in wheat breeding for adaptation to climate change. *J Exp Bot* **66**: 3477–3486
- 825 **Love MI, Huber W, Anders S** (2014) Moderated estimation of fold change and dispersion for  
826 RNA-seq data with DESeq2. *Genome Biol* **15**: 550
- 827 **Maccaferri M, El-Feki W, Nazemi G, Salvi S, Canè MA, Colalongo MC, Stefanelli S,**  
828 **Tuberosa R** (2016) Prioritizing quantitative trait loci for root system architecture in  
829 tetraploid wheat. *J Exp Bot* **67**: 1161–1178
- 830 **Maccaferri M, Harris NS, Twardziok SO, et al.** (2019) Durum wheat genome highlights past  
831 domestication signatures and future improvement targets. *Nat Genet* **51**: 885–895
- 832 **McFadden ES, Sears ER** (1946). The origin of *Triticum spelta* and its free-threshing hexaploid  
833 relatives. *J Hered* **37**: 81-89
- 834 **Merchuk-Ovnat L, Fahima T, Ephrath JE, Krugman T, Saranga Y** (2017) Ancestral QTL  
835 alleles from wild emmer wheat enhance root development under drought in modern wheat.  
836 *Front Plant Sci* **8**: 1–12
- 837 **Momen M, Campbell MT, Walia H, Morota G** (2019) Utilizing trait networks and structural  
838 equation models as tools to interpret multi-trait genome-wide association studies. *Plant*  
839 *Methods* **15**: 107
- 840 **Myers SS, Smith MR, Guth S, Golden CD, Vaitla B, Mueller ND, Dangour AD, Huybers**  
841 **P** (2017) Climate change and global food systems: Potential impacts on food security and  
842 undernutrition. *Annu Rev Public Health* **38**: 259–277
- 843 **Nietzsche M, Landgraf R, Tohge T, Börnke F** (2016) A protein-protein interaction network  
844 linking the energy-sensor kinase SnRK1 to multiple signaling pathways in *Arabidopsis*  
845 *thaliana*. *Curr Plant Biol* **5**: 36–44
- 846 **Opanowicz M, Vain P, Draper J, Parker D, Doonan JH** (2008) *Brachypodium distachyon*:  
847 making hay with a wild grass. *Trends Plant Sci* **13**: 172–177
- 848 **Oren L** (2020) Genetic dissection of polygenic traits from wild emmer wheat using Near  
849 isogenic line (NIL) population. M.Sc. Thesis submitted to School of Plant Sciences and  
850 Food Security, Tel Aviv University, Israel
- 851 **Otasek D, Morris JH, Bouças J, Pico AR, Demchak B** (2019) Cytoscape automation:

- 852       empowering workflow-based network analysis. *Genome Biol* **20**: 1–15
- 853 **Peleg Z, Fahima T, Abbo S, Krugman T, Nevo E, Yakir D, Saranga Y** (2005) Genetic  
854       diversity for drought resistance in wild emmer wheat and its ecogeographical associations.  
855       *Plant Cell Environ* **28**: 176–191
- 856 **Peleg Z, Saranga Y, Krugman T, Abbo S, Nevo E, Fahima T** (2008) Allelic diversity  
857       associated with aridity gradient in wild emmer wheat populations. *Plant Cell Environ* **31**:  
858       39–49
- 859 **Placido DF, Campbell MT, Folsom JJ, Cui X, Kruger GR, Baenziger PS, Walia H** (2013)  
860       Introgression of novel traits from a wild wheat relative improves drought adaptation in  
861       wheat. *Plant Physiol* **161**: 1806–1819
- 862 **Poot P, Lambers H** (2008) Shallow-soil endemics: Adaptive advantages and constraints of a  
863       specialized root-system morphology. *New Phytol* **178**: 371–381
- 864 **Ramírez-González RH, Borrill P, Lang D, et al.** (2018) The transcriptional landscape of  
865       polyploid wheat. *Science* **361**: eaar6089
- 866 **Rellán-Álvarez R, Lobet G, Dinnyen JR** (2016) Environmental control of root system  
867       biology. *Annu Rev Plant Biol* **67**: 619–642
- 868 **Richards RA** (2000) Selectable traits to increase crop photosynthesis and yield of grain crops.  
869       *J. Exp. Bot* **51**: 447–458
- 870 **Rodrigues M, Chaves M, Wendler R, David M, Quick W, Leegood R, Stitt M, Pereira J**  
871       (1993) Osmotic adjustment in water stressed grapevine leaves in relation to carbon  
872       assimilation. *Funct Plant Biol* **20**: 309–321
- 873 **Roucou A, Violle C, Fort F, Roumet P, Ecartot M, Vile D** (2018) Shifts in plant functional  
874       strategies over the course of wheat domestication. *J Appl Ecol* **55**: 25–37
- 875 **Sherrard ME, Maherali H** (2006) the adaptive significance of drought escape in *Avena*  
876       *barbata*, an annual grass. *Evolution* **60**: 2478
- 877 **Shipley B, Meziane D** (2002) The balanced-growth hypothesis and the allometry of leaf and  
878       root biomass allocation. *Funct Ecol* **16**: 326–331
- 879 **Siddique KHM, Tennant D, Perry MW, Belford RK** (1990) Water use and water use  
880       efficiency of old and modern wheat cultivars in a Mediterranean-type environment. *Aus J*  
881       *Agric Res* **41**: 431–447
- 882 **Sun XL, Yu QY, Tang LL, Ji W, Bai X, Cai H, Liu XF, Ding XD, Zhu YM** (2013) *GsSRK*,  
883       a G-type lectin S-receptor-like serine/threonine protein kinase, is a positive regulator of  
884       plant tolerance to salt stress. *J Plant Physiol* **170**: 505–515
- 885 **Supek F, Bošnjak M, Škunca N, Šmuc T** (2011) Revigo summarizes and visualizes long lists

- 886 of gene ontology terms. PLoS One **6**: e21800
- 887 **Teotia S, Lamb RS** (2011) RCD1 and SRO1 are necessary to maintain meristematic fate in  
888 Arabidopsis thaliana. J Exp Bot **62**: 1271–1284
- 889 **Tian T, Liu Y, Yan H, You Q, Yi X, Du Z, Xu W, Su Z** (2017) AgriGO v2.0: A GO analysis  
890 toolkit for the agricultural community, 2017 update. Nucleic Acids Res **45**: W122–W129
- 891 **Tsujimura Y, Sugiyama S, Otsuka K, Htun TM, Numaguchi K, Castillo C, Akagi T, Ishii  
892 T, Ishikawa R** (2019) Detection of a novel locus involved in non-seed-shattering  
893 behaviour of Japonica rice cultivar, *Oryza sativa* ‘Nipponbare.’ Theor Appl Genet **132**:  
894 2615–2623
- 895 **Trapnell C, Pachter L, Salzberg SL** (2009). TopHat: discovering splice junctions with RNA-  
896 Seq. Bioinformatics **25**: 1105-1111
- 897 **Virlet N, Sabermanesh K, Sadeghi-Tehran P, Hawkesford MJ** (2017) Field Scanalyzer: An  
898 automated robotic field phenotyping platform for detailed crop monitoring. Funct Plant  
899 Biol **44**: 143–153
- 900 **Voss-Fels KP, Snowdon RJ, Hickey LT** (2018) Designer roots for future crops. Trends Plant  
901 Sci **23**: 957-960
- 902 **Voss-Fels KP, Robinson H, Mudge SR, et al.** (2017) *VERNALIZATION1* modulates root  
903 system architecture in wheat and barley. Mol Plant **11**: 226-229
- 904 **Waines JG, Ehdaie B** (2007) Domestication and crop physiology: Roots of Green-Revolution  
905 wheat. Ann Bot **100**: 991-998
- 906 **Walkowiak S, Gao L, Monat C, et al.** (2020) Multiple wheat genomes reveal global variation  
907 in modern breeding. Nature **588**: 277–283
- 908 **Ward Jr, Joe H, Hook, ME** (1963). Application of an hierarchical grouping procedure to a  
909 problem of grouping profiles. Educ Psychol Meas **23**: 69-81
- 910 **Yang W, Feng H, Zhang X, Zhang J, Doonan JH, Batchelor WD, Xiong L, Yan J** (2020)  
911 Crop phenomics and high-throughput phenotyping: Past decades, current challenges, and  
912 future perspectives. Mol Plant **13**: 187–214
- 913 **Yang Z, Zheng H, Wei X, Song J, Wang B, Sui N** (2018) Transcriptome analysis of sweet  
914 Sorghum inbred lines differing in salt tolerance provides novel insights into salt exclusion  
915 by roots. Plant Soil **430**: 423–439
- 916 **Zadoks JC, Chang TT, Konzak CF** (1974) A decimal code for the growth stages of cereals.  
917 Weed Res **14**: 415-421
- 918 **Zelitch I** (1982) The close relationship between net photosynthesis and crop yield. Bioscience  
919 **32**: 796-802