Les impacts de la sélection artificielle sur l’espace phénotypique des espèces cultivées

Présentation générale

Ce premier chapitre concerne l'ensemble des études réalisées sur le blé dur en conditions extérieures. À partir d'une **série de domestication du blé dur**, les principaux objectifs de ce premier chapitres sont : i) analyser les compromis écophysiologiques chez une espèce cultivée, ii) élargir le syndrome de domestication du blé dur et iii) étudier les changements dans la stratégie d'utilisation des ressources au cours de la domestication.

Avec l'expérimentation 1, 13 **traits fonctionnels** ont été étudiés sur trois sous-espèces représentatives de la domestication du blé dur (voir la partie Matériel et Méthodes pour plus d'informations). Leur étude a illustré des différences dans la morphologie et l'architecture des **systèmes aériens et souterrains** entre les quatre groupes étudiés. De plus, une étude approfondie des réseaux possibles entre ces 13 traits a mis en évidence des changements dans leurs relations au cours de la domestication. Les différences physiologiques liées à ce résultat nous ont permis de conclure sur un changement dans la **stratégie fonctionnelle de la plante** pour l'utilisation des ressources : les formes modernes ont une **stratégie plus « acquisitive des ressources »** que les formes ancestrales. La caractérisation du phénotype des espèces cultivées, qui s'appuie sur l'étude de traits fonctionnels végétatifs, suggère un syndrome de domestication plus complet. Nos résultats illustrent l'intérêt d'une telle approche pour élargir le **syndrome de domestication des espèces cultivées**.

Les résultats obtenus avec cette étude sont présentés sous la forme d'un article scientifique.

Shifts in plant functional strategies over the course of wheat domestication

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Keywords: agroecology, domestication syndrome, ecophysiological trade-off, functional coordination, human selection, phenotypic integration, phenotypic space, plant economics spectrum, plant functional strategies, plant functional trait.

Introduction

The domestication of plants has led to spectacular phenotypic modifications in the goal of yield improvement (Zeder et al., 2006; Meyer et al., 2012). Such domestication syndromes previously described (e.g., Fuller et al., 2014) are however incomplete given that the change in plant strategies has never been fully explored (Milla et al., 2015). Today, it is crucial to understand how cultivated plants are adapted to novel environments (Guarda et al., 2004; Denison, 2012) through the analysis of phenotypic traits not directly targeted by human selection (Denison et al., 2003; Martin and Isaac, 2015b; Milla et al., 2014, 2015). This should reveal hidden ecophysiological and biophysical constraints within the phenotype, help identify plant breeding limitations, and explore the unintended consequences of crop domestication (Meyer et al., 2012; Milla et al., 2015). In an agroecological context, identifying potential ecophysiological shifts over the course of domestication is particularly promising. First, this can help target ‘lost’ traits and trait combinations that allowed wild ancestors to be adapted to ‘low-input’ environments characterized by a lower amount of fertilization and/or pesticides (Wood et al., 2015). Second, this can emphasize within-crop potential phenotypic variability (Prieto et al., 2017), the raw material for the conception of crop mixtures that could be designed to maximize the diversity of traits related to resource-use complementarity (Litrico and Violle, 2015). Indeed, a key postulate of functional ecology is that plant functional traits are proxies for species’ niche axes and the ability of species (or genotypes) to capture and use resources (McGill et al., 2006; Violle et al., 2007; Violle and Jiang, 2009). As a consequence, natural ecosystems composed of species characterized by contrasting plant functional traits and strategies should maximize several functions including biomass production (Gross et al., 2017). If this is the case in cultivated systems too, it could be possible in those systems to favour yield production as well as other ecosystem services while limiting fertilization through the optimization of crop resource use by complementarity mechanisms among varieties displaying contrasted values for pivotal functional traits (Barot et al., 2017). This is one of the foundation stones of agroecology (Malézieux et al., 1999). In this article, analyzing a unique domestication historical series of tetraploid wheat (*Triticum turgidum L.*), we highlight how revisiting plant domestication through bridging concepts from both agronomy and ecology can be used to further advance the emerging field of agroecology.

During the process of plant domestication, selection pressure have impacted vegetative growth and reproductive organs of a large range of crop species in the same way (the so-called ‘domestication syndrome’: Hammer, 1984). This is reflected by typical phenotypical responses (Donald and Hamblin, 1983) such as: loss of dormancy, increase in seed size, modification of seed dispersal mode and apical dominance among other responses (Meyer et al., 2012). Until recently, geneticists and plant breeders believed that only a few genes were involved in this domestication syndrome. However, recent papers on different species (Swanson-Wagner et al., 2012; Bellucci et al., 2014; Beleggia et al., 2016) suggested that this syndrome has significantly affected the whole-plant functioning as well as a larger part of the genome. Thus the traits targeted by artificial selection may be more strongly correlated with other plant traits than previously thought, and considering whole-plant phenotypic response to domestication becomes a critical avenue for both theoretical and applied questions.

In ecology, comparative approaches based on the screening of several plant functional traits measured on the phenotype (Keddy, 1992; Violle et al., 2007) have allowed the identification of some key fundamental biophysical and ecophysiological laws of phenotypic diversification that seem to hold for a wide range of wild species (Westoby et al., 2002; Westoby and Wright, 2006; Shipley, 2007; Reich 2014; Garnier et al., 2016; Shipley et al., 2016). In particular, ecological strategies of wild plant species appeared to fall into a global universal economics spectrum (Reich et al., 1992; Westoby et al., 2002) where the position of each species could be explained by their ability to cope with their environment in terms of resource availability, occurrence of perturbations and biotic interactions, as well as constraints related to inherent covariations among traits. The ‘Leaf Economics Spectrum’ (LES) (Reich et al., 1997; Wright et al., 2004) is a particularly well studied ecophysiological trade-off. It describes resource economics strategies and associated construction costs that appear at the leaf level (Tosens et al., 2016). A notable illustration of the LES is the negative relationship between photosynthesis rate and leaf lifespan highlighted across species worldwide (Reich et al., 1992; Wright et al., 2004). This suggests that a leaf cannot simultaneously exhibit high metabolic rate and high structural defense, the latter being exhibited by denser tissues and a longer lifespan. In short, there should be a slow-fast growth-related trade-off in plants occurring at the organ level too, notably at the leaf level (Reich, 2014). Beyond the leaf scale that has been well explored in functional ecology, the exploration of root physiological and morphological traits– even if still overlooked – tends to similarly highlight the existence of a ‘Root Economics Spectrum’ that constraints resource foraging by roots and plant functioning more broadly (e.g., Ho et al., 2005; Mommer and Weemstra, 2012; Roumet et al., 2016; Wang et al., 2016). Finally, plant functional ecology theory suggests a coordination of above- and below-ground functioning which should summarize optimization of resource economy at the whole-plant level (Liu et al., 2010; Freschet et al., 2010; Freschet et al., 2013; Messier et al., 2017) but also trade-offs between key functions such as resource acquisition and plant defense (Reich et al., 2003; Maire et al., 2009; Grubb, 2016). Overall, if those tradeoffs reflect structural constraints for the plant phenotype, they may be minimally modifiable by artificial selection. In that case, they could represent a strong limitation for the diversification of crop species over the course of domestication and for the improvement of yield-related traits in plant breeding programs (Milla et al., 2015).

In this study, we analyse the domestication history of tetraploid wheat through the lens of the theoretical corpus of trait-based ecology. Martin and Isaac (2015) called for a systematic characterization of crop phenotypes through the analysis of four main dimensions of functional trait variation: leaf, root, whole-plant, and reproductive traits. Given that the reproductive syndrome of domestication is well known in tetraploid wheat (Nesbitt, 1996), we therefore focus on the vegetative phenotype by measuring a set of root, leaf, and whole-plant traits (Table 1) associated with resource-use strategies and by analyzing their coordination within the phenotype. The domestication of wild emmer (*Triticum turgidum* ssp. *dicoccoides)* began in the mountains of the Fertile Crescent between the 8th and the 10th millennium BC. Firstly it led to a non-brittle hulled sub-species (*T. t.* ssp. *dicoccum*) which was one of the first cereals domesticated (Nesbitt, 1996). Then human-driven mutations at multiple loci, in particular at Tg – tenacious glume – and Q loci (Simonetti et al., 1999; Simons et al., 2006; Peleg et al., 2011), gave rise to traits such as soft glumes, non-hulled grains improving threshing efficiency (Tzarfati et al., 2013) and facilitating its widespread cultivation. This phenotypic change combined with hybridization between different forms (Matsuoka, 2011) led to free-threshing sub-species (including *T. t.* ssp. *durum*). Hulled grains and free threshing forms (from 7th and 3rd millenium BP respectively) played a crucial role in the development of Mediterranean civilizations. They became widely spread with the early agricultural movements leading to agriculture systems centralized around tetraploid wheat. Nowadays, elite durum varieties and landraces grown in different environments are of major importance for grain production in the Mediterranean basin (Araus et al., 2007). In this study, we took advantage of a unique collection of genotypes belonging to three main sub-species representative of tetraploid wheat domestication to experimentally examine changes in plant functional strategies over the course of this domestication history. First, we examined whether there had been a shift towards resource acquisition strategies due to the cultivation of modern varieties in high-input (water and fertilization) conditions. Second, we examined whether functional coordination and trade-offs are sensitive to human selection. We hypothesised that those biophysical constraints should still hold in both ancient and modern varieties, while above- and belowground plant functioning may be decoupled in modern varieties due to potential adaptation to agricultural intensification. Finally, we discuss our results in the context of agroecology where novel plant functional strategies need to be explored for the design of crop systems where resource-use complementary could be promoted.

TABLE 1 List of morphological and physiological traits studied, their abbreviations, and units. Following the organization proposed by Garnier et al. (2017), traits are grouped per organ (leaf or root) or whole-plant level.

Trait	Abbreviation	Units
Leaf traits		
Maximum net photosynthetic rate	A_{mass}	nmol g^{-1} s^{-1}
Leaf mass per area	LMA	g m^{-2}
Leaf area	LA	m^2
Leaf nitrogen content	LNC	%
Leaf longevity	LL	°Cd
Root traits		
Crown root angle	RA	°
Mean root diameter	RD	mm
Percent of fine roots	PFR	%
Specific root length	SRL	m g^{-1}
Whole-plant traits		
Plant height	H	cm
Plant diameter	PD	cm
Plant dry mass	PDM	g
Root-to-shoot ratio	RSR	

Materials and methods

Plant material

We selected 40 genotypes from the domestication history of tetraploid wheat *Triticum turgidum* (Table S1, Supporting information). The history was split into three domestication groups corresponding to wild, primitive, and free threshing sub-species of tetraploid wheat domestication. The latter was split into two sub-groups according to the pre and post green revolution periods. To select these genotypes, a “core collection” was constructed by maximizing the genetic diversity of each group based on 21 non-linked microsatellite markers mapped on 14 *durum* chromosomes (Röder et al., 1998). From this collection, we used: ten wild emmer genotypes (*T. turgidum* ssp. *dicoccoides*, DD), ten emmer primitive domestic genotypes (*T. turgidum* ssp. *dicoccum*, DC), and 20 durum wheat genotypes (*T. turgidum ssp. durum*). The durum wheat genotypes were split into two groups: the first (landrace) consists of 10 lines issued from landraces (ten genotypes) (DL hereafter) and the second one consists of 10 elite varieties registered in Europe after the Green Revolution (from 1970's to 1990's) (DE hereafter). The genotypes came from seed collections maintained in different stock centers (Table S1). For each genotype, seeds came from successive self-fertilizations realized in common gardens to limit residual heterozygosis and to ensure that the material is genetically fixed. Seeds were provided by INRA UMR AGAP in Montpellier.

Experimental design

We set up the experiment at the INRA-SupAgro campus (France, 43° 37' 02''N, 3° 51' 18'' E) for a duration of 90 days in outdoor conditions (minimal temperature = 2 °C and maximal temperature = 19 °C). Plants were grown in 9-L pots (17.5 cm diameter and 38.5 cm depth) filled with 2 mm sieved topsoil taken from a natural grassland (France, 44° 26' 25'' N, 3° 55' 58'' E; 0.994 g N kg^{-1} soil, 4.17 g K kg^{-1} soil and 0.166 g P kg^{-1} soil). To synchronize germination, we put seeds into Petri dishes for three days at 4 °C, and four days at ambient temperature. After germination, two seedlings were transplanted (at 2 cm depth) in each pot and thinned to one plant per pot at the first leaf stage. We performed a randomized complete block design using five blocks, where each genotype was represented by one plant in each block. To avoid water stress, plants were watered once or three times per week with 150 mL of tap water, according to climatic conditions.

One genotype from *T. turgidum* ssp. *dicoccoides* did not grow up during the experiment; we eliminated it from the next measurements (Table S1).

Plant trait measurements

Leaf longevity calculated using thermal time (LL, °Cd) was estimated during vegetative growth from daily observations of the fourth leaf produced on each plant, from its appearance until its complete senescence. The sum of degree-days (° Cd) was calculated using a 0 °C base temperature and from appearance to complete senescence based on cumulated daily mean temperatures from the meteorological station located near the common garden.

We determined maximal photosynthetic rate (A_{max}, nmol CO_2 s^{-1}) at vegetative stage on a fully expanded leaf (the seventh or eighth leaf produced) by using a portable photosynthesis system with a leaf assimilation chamber (CIRAS, PP systems). Measurements were performed at constant relative humidity (80%), ambient temperature (25 °C), constant $[CO_2]_{atm}$ (400 ppm), and at saturating light (PAR = 1500 µmol s^{-1} m^{-2}). Leaf nitrogen content (LNC, %) was determined non-destructively on the same leaf using near infrared spectroscopy (NIRS) with a portable near-infrared spectrometer (Fieldspec 2500, Analytical Spectral Devices (ASD) Inc., Boulder, Colorado, USA) used with the Leaf Clip and a white background panel for the measurement of reflectance, and using a calibration previously performed on a large range of wheat varieties (Ecarnot et al., 2013). For each leaf, we recorded two spectra on the ad-axial leaf surface at one and two thirds of the leaf length. The mean of these two spectra was used to estimate LNC.

Just before harvest, 90 days after sowing, we measured plant height (H, cm). At the same time, maximum plant diameter (PD, cm) was estimated as the length between leaves diametrically opposed. The leaves used for gas exchange measurements were collected and rehydrated in tap water at 4 °C during 24 hours. Then, we scanned them (150 dpi) and leaf area (LA, m^2) was determined by image analysis (ImageJ, U.S. National Institutes of Health, Bethesda, MD, USA). Each leaf was then dried at 60 °C during 72 h to determine leaf dry weight. We calculated leaf mass per area (LMA, g m^{-2}) as the ratio of leaf dry weight to leaf area, and we used it to calculate the maximal photosynthetic rate per unit of leaf dry mass as A_{mass} (nmol CO_2 g^{-1} s^{-1}). Remaining aboveground material was oven-dried for 72 h at 60 °C to determine shoot dry weight (SDW, g).

At harvest, above- and belowground parts were separated. We determined root crown angle as the angle between roots and the soil surface; horizontal was considered as 0° and vertical as 90°. The root system of each plant was cleaned with tap water. We selected two seminal roots, i.e. roots coming directly from the embryo (Richard et al., 2015) from each plant. We focused on seminal roots because they are primarily involved in adaptation to environmental changes in wheat (Richard et al., 2015; Richards and Passioura, 1981). Another advantage for a comparative screening approach is that seminal roots are produced immediately after germination and have therefore the same age at harvest for all the genotypes. Roots were scanned (600 dpi) to measure mean root diameter (RD, mm), root length and mean percent of fine roots (diameter < 0.2 mm; PFR, %) using WinRHIZO Pro (version 2009; Regent Instrument, Quebec, Canada). Dry weight of these roots was determined after drying 60 °C during 72 h. We calculated specific root length (SRL, m g^{-1}) as the root length divided by the root dry weight. Total root dry weight was determined after drying at 60 °C during 72 h (RDW, g). We calculated total plant dry mass (PDM, g) by cumulating above-and belowground dry biomass. The root-to-shoot ratio (RSR) was also calculated.

Statistical analyses

We performed a principal component analysis (PCA) on genotype-level mean trait values to explore relationships between traits and to represent genotypes of different sub-species groups in a reduced dimensional space. We used the *varimax* function to improve the interpretation of the resulted PC axes. Statistical significance of multivariate differences between sub-species was tested using the Monte-Carlo permutation test [*randtest.between* function in R/*ade4* package, $n = 999$ (Dray and Dufour, 2017)].

For each trait, we performed a linear mixed model to test the main effects of sub-species, genotypes and block, where sub-species and block were treated as fixed factors and genotype effect as a random factor nested within the *ith* sub-species. Statistically significant differences between sub-species were determined from pairwise comparisons based on Tukey-adjusted least-squares means at a probability level of $P \leq 0.05$.

To characterize trait-trait relationships, we calculated Spearman's correlation coefficients for each sub-species using individual data. Based on those correlations, a network of trait relationships was drawn for each sub-species using the *network* R package. We considered a probability level of $P \leq 0.05$.

All statistical analyses were performed with the R software (R Core development team, 2017) using appropriate packages.

Results

Investigating a multi-trait multi-organ domestication syndrome

A principal component analysis was performed to analyse the pattern of trait covariations among the 39 studied genotypes. The first three principal components (PC) explained 34%, 21%, and 11% of the total variance, respectively (Fig. 1; see also Table S2). Leaf, root and whole-plant traits contributed uniformly to PC axes. PC1 opposed genotypes with high leaf longevity (LL) and root-to-shoot ratio (RSR) to genotypes characterized by high leaf nitrogen content (LNC), leaf area (LA) and plant height (H) (Fig. 1a). Root morphological traits, *i.e.* root diameter (RD) opposed to both specific root length (SRL) and percent fine root (PFR), contributed to PC1. Leaf morpho-physiological traits, *i.e.* leaf mass per area (LMA) negatively correlated to maximum net photosynthetic rate (A_{mass}), and total plant dry mass (PDM) had also a strong contribution to PC2. Plant diameter (PD) was at an intermediate position between the two first PCs.

The projection of the genotypes in the PCA (Fig. 1b) showed that genotypes from ancestral (*T. turgidum* ssp. *dicoccoides*), first domesticated (*T. turgidum* ssp. *dicoccum*) and more recent (*T. turgidum* ssp. *durum*) sub-species were significantly discriminated in the first factorial plane ($P < 0.001$; permutation tests of between-sub-species PCA). However, high variability between genotypes was still distinguishable as indicated by the distance of the genotypes from the centroid of each sub-species group. This variability was mainly organized along an orthogonal axis to that defined by the alignment of the sub-species' centroids.

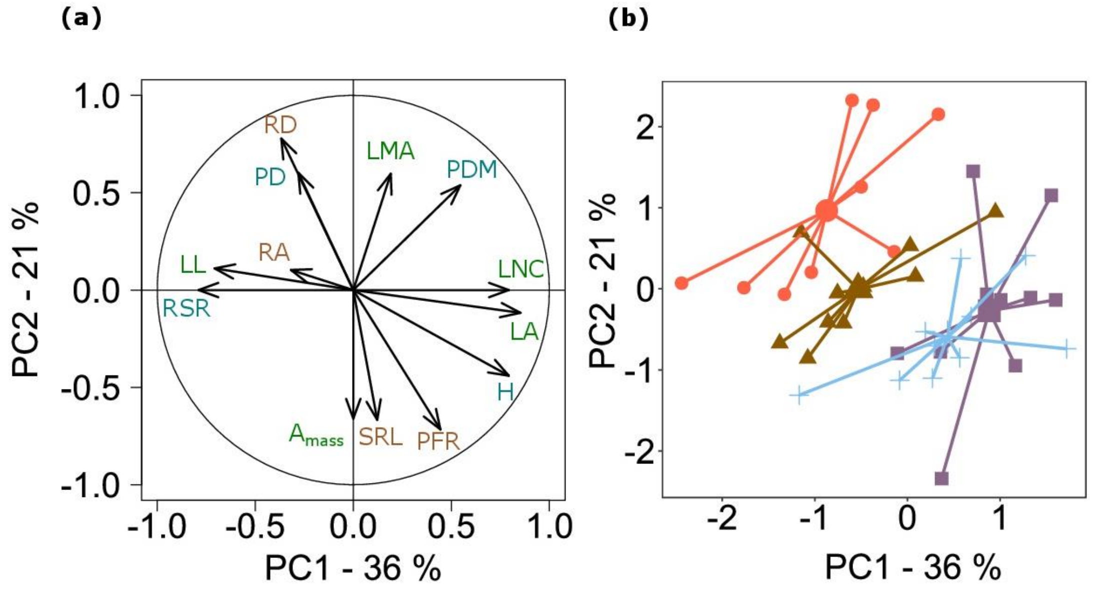


FIGURE 1 Results of the Principal Component Analysis (PCA) (first two axes represented) using leaf, root and whole-traits of the 39 studied wheat genotypes. (a) Representation of the leaf (green), root (brown) and whole-plant (blue) traits in the PC1-PC2 plan and (b) representation of the genotypes (n = 39) and the barycenters for each of the four sub-species, in the same factorial plan. *T. turgidum* ssp. *dicoccoides* genotypes are represented by red circles, *T. turgidum* ssp. *dicoccum* by brown triangles, *T. turgidum* ssp. *durum* (landrace) by purple squares and *T. turgidum* ssp. *durum* (elite) by blue crosses. A_{mass}: maximal photosynthetic capacity (nmol g^{-1} s^{-1}); LA: leaf area (m^2); LMA: leaf mass per area (g m^{-2}); LL: leaf longevity (°Cd); LNC: leaf nitrogen content (%); RA: crown root angle (°); D: mean root diameter (mm); SRL: specific root length (m g^{-1}); PFR: percent of fine roots (%); H: plant height (cm); PD: plant diameter (cm); PDM: plant dry mass (g); and RSR: root-to-shoot ratio.

Trait-by-trait variation over the course of domestication

Significant differences in A_{mass}, LA, LL and LNC were found across and within the four sub-species (Table 2; Fig. S1a-d). Specifically, A_{mass} was significantly higher in *T. turgidum* ssp. *durum* elites (DE) than in primitive sub-species (*T. turgidum* ssp. *dicoccoides,* DD, and *T. turgidum* ssp. *dicoccum*, DC) (Fig. 2a), whereas LL showed the reverse trend (Fig. 2c). LA was significantly higher in *T. turgidum* ssp. *durum* but with a significant decrease after the Green Revolution (Fig. 2b). LNC tended to be higher in *T. turgidum* ssp. *durum* sub-species compared to wild and primitive sub-species but the difference was only significant between *T. turgidum* ssp. *durum* landraces (DL) compared to *T. turgidum* ssp. *dicoccoides* (Fig. 2d). No significant differences between sub-species were found for LMA (Fig. 2e). As expected, the whole series of domestication was located in the "efficient resources acquisition" part of the worldwide Leaf Economics Spectrum identified for wild species (Fig. S4).

TABLE 2 Changes in functional traits during the domestication of tetraploid wheat. For each trait, P-values are provided for sub-species and genotypes nested within sub-species effects in linear mixed-effect models. The block effect was not significant for all traits except leaf area (LA; *P* = 0.007, and is not presented). For abbreviations, see Table 1.

Trait	Subspecies	Genotypes
Leaf		
A_{mass}	0.01	0.002
LA	<0.0001	<0.0001
LMA	0.4	<0.0001
LL	0.009	<0.0001
LNC	0.001	<0.0001
Root		
RA	0.03	0.2
RD	0.001	0.08
SRL	0.09	0.2
PFR	<0.0001	1
Whole-plant		
H	<0.0001	<0.0001
PD	<0.0001	<0.0001
PDM	0.63	<0.0001
RSR	0.005	<0.0001

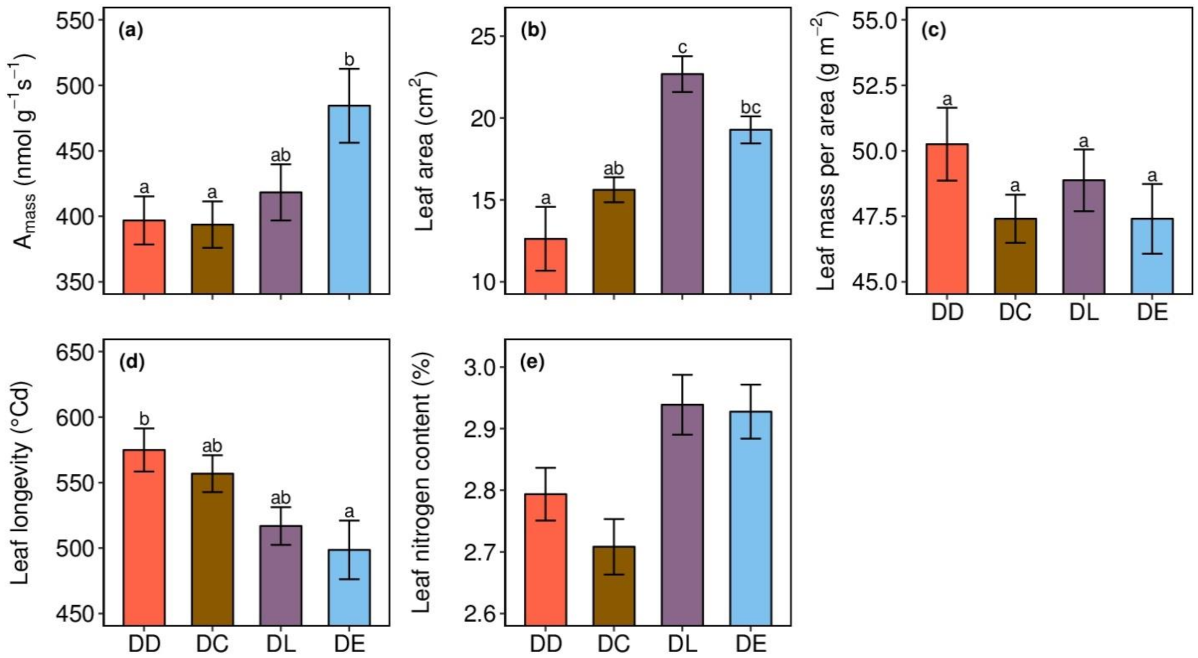


FIGURE 2 Variation in leaf trait values between the studied sub-species of tetraploid wheat. Five leaf traits were studied: (a) maximum net photosynthetic rate, (b) leaf area, (c) leaf longevity, (d) nitrogen content per unit of leaf dry mass and (e) leaf mass per area. Bars and error bars represent means ± SEM (n = 9-10 genotypes) for each sub-species group coloured differently and ranked according to their dates of apparition during the domestication of durum wheat (DD: *T. turgidum ssp. dicoccoides* in red, DC: *T. turgidum ssp. dicoccum* in brown, DL*: T. turgidum ssp. durum* landrace in purple, DE*:* T. *turgidum ssp. durum* elite in blue). Different letters above bars indicate significant differences at $P < 0.05$ using Tukey's multiple comparison test.

Root crown angle (RA), RD and PFR differed significantly between sub-species (Fig. 3a-c) and between genotypes within sub-species for RA (Table 2; Fig. S2a). RA was lower in *T. turgidum* ssp. *durum* elites than in *T. turgidum* ssp. *dicoccoides* (Fig. 3a). *T. turgidum ssp. durum* landraces had lower mean diameter and higher percentage of fine root than *T. turgidum* ssp. *dicoccoides* and *T. turgidum* ssp. *dicoccum* genotypes, while *T. turgidum* ssp. *durum* elites had intermediate values between *T. turgidum* ssp. *dicoccum* and *T. turgidum ssp. durum* landraces but differed significantly from *T. turgidum* ssp. *dicoccoides* (Fig. 3b-c). SRL tended to increase during domestication but the differences between sub-species were not significant (Fig. 3d).

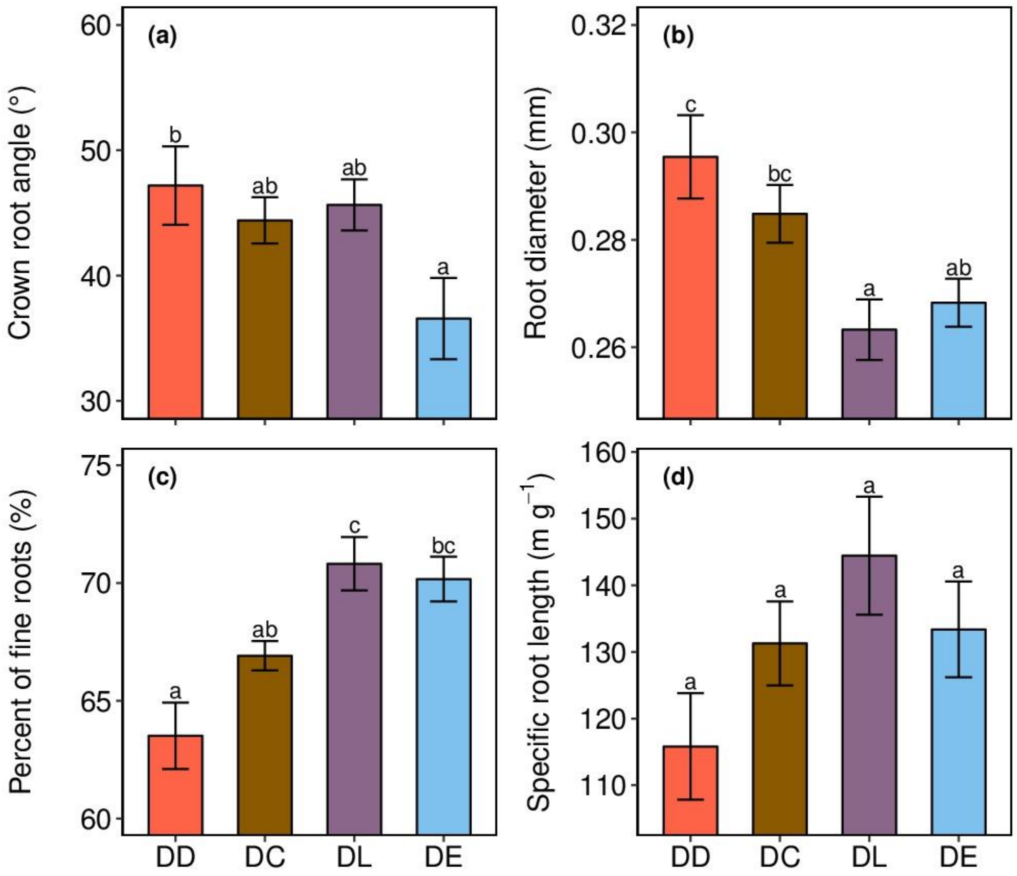


FIGURE 3 Variation in root trait values between the studied sub-species of tetraploid wheat. Four root traits were studied: (a) crown root angle, (b) root diameter, (c) percent of fine roots, (d) specific root length. Bars and error bars represent means ± SEM (n = 9-10) for each sub-species group colored differently and ranked according to their dates of apparition during the domestication of wheat durum (DD: *T. turgidum* ssp. *dicoccoides* in red, DC: *T. turgidum* ssp. *dicoccum* in brown, DL*: T. turgidum* ssp. *durum* landrace in purple, DE*:* T. *turgidum* ssp. *durum* elite in blue). Different letters above bars indicate significant differences at $P < 0.05$, using Tukey's multiple comparison test.

Vegetative height (H) was significantly higher in *T. turgidum* ssp. *durum* landraces and elites compared to *T. turgidum* ssp. *dicoccoides* and *dicoccum* (Fig. 4a). In accordance with dwarf gene introgression, H decreased between *T. turgidum* ssp. *durum* landraces and elites (Fig. S3a). Similarly, PD strongly decreased between *T. turgidum* ssp. *dicoccoides* and other sub-species (Fig. 4b; Fig. S3b). PDM did not change significantly during domestication (Table 2; Fig. 4c). RSR was significantly lower for *T. turgidum* ssp. *durum* landraces than *T. turgidum* ssp. *dicoccoides* and *T. turgidum* ssp. *dicoccum* while *T. turgidum* ssp. *durum* elites had an intermediate mean value (Fig. 4d).

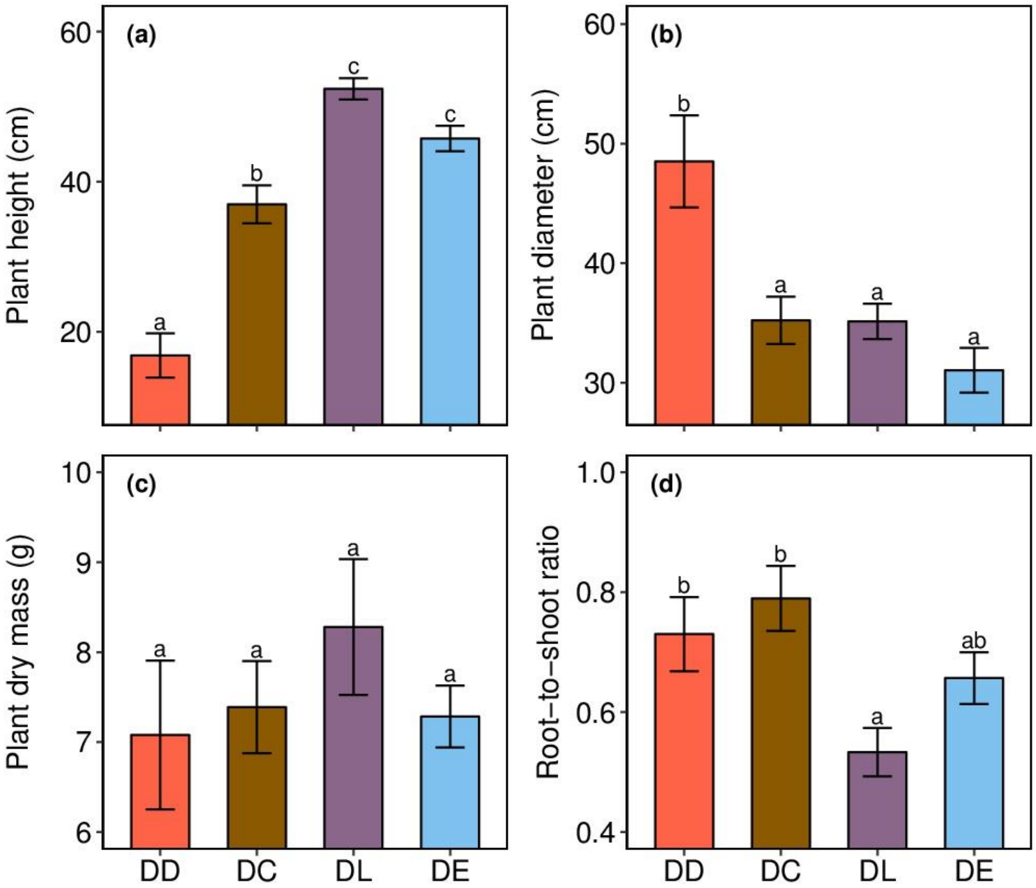


FIGURE 4 Variation in whole-plant trait values between the studied sub-species of tetraploid wheat. Four whole-plant traits were studied: (a) plant height, (b) plant diameter, (c) plant dry mass and (d) root-to-shoot ratio. Bars and error bars represent means ± SEM (n = 9-10) for each sub-species group coloured differently and ranked according to their dates of apparition during the domestication of durum wheat (DD: *T. turgidum ssp. dicoccoides* in red, DC: *T. turgidum ssp. dicoccum* in brown, DL*: T. turgidum ssp. durum* landrace in purple, DE*:* T. *turgidum ssp. durum* elite in blue). Different letters above bars indicate significant differences at $P < 0.05$, using Tukey's multiple comparison test.

Shifts in trait-trait relationships during domestication

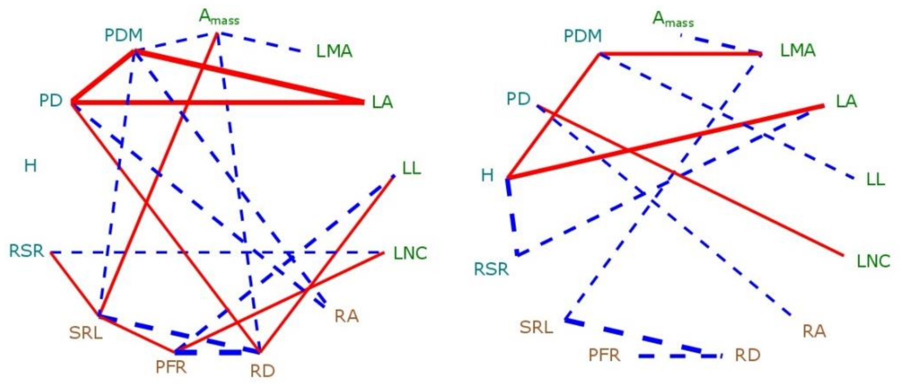
Contrasted patterns of within-subspecies trait-trait relationships were observed (Fig. 5). The wild *T. turgidum* ssp. *dicoccoides* had the highest degree of significant links between traits, with 2.92 significant links by trait on average. Genotypes from this sub-species displayed strong links between above- and below-ground traits (Fig. 5a), notably traits from the Root Economics Spectrum (RD, PFR and SRL). In addition, traits from the Leaf Economics Spectrum (LNC and LL) were significantly related with two root traits (PFR and RD). Both sets of traits were related with PFR but with opposite correlation signs. PDM was negatively correlated with SRL, RA and A_{mass} and positively with individual LA and plant diameter (PD). Surprisingly, H was not correlated to other traits.

With 12 significant correlations, the primitive domestic lines of *T. turgidum* ssp. *dicoccum* expressed the lowest number of links between traits (1.85 significant links by trait on average; Fig. 5b). This sub-species displayed only one relationship between above- and below-ground traits, LMA and SRL being negatively correlated. PDM was negatively correlated with LL and positively with LMA and H. Moreover, H was positively correlated with LA and negatively with RSR, respectively.

T. turgidum ssp. *durum* landraces displayed more significant correlations than its domesticated ancestor with 2.0 significant correlations by trait on average (Fig. 5c). Two links between above- and below-ground traits were observed with a significant positive correlation between A_{mass} and the PFR and a negative one between the PFR and PDM. H was correlated positively with PDM, LA, LNC and negatively with RA and RSR (Fig. 5c).

After the Green Revolution, *T. turgidum* ssp. *durum* elites showed a modification of the correlation pattern in comparison to the landrace ones (Fig. 5d). They displayed a similar mean number of significant correlations by trait as *T. turgidum* ssp. *dicoccum* lines (2.15). They did not show any relationship between Root Economics Spectrum traits and other traits except RA, and the correlation between LMA and RA was the only significant correlation between above- and below-ground traits. H was still correlated positively with PDM and LNC and negatively with PD and RSR. The latter was also negatively related to LA and positively to A_{mass} (Fig. 5d).

(a) *T. turgidum* ssp. *dicoccoides* **(b)** *T. turgidum* ssp. *dicoccum*



(c) *T. turgidum* ssp. *durum* (Landrace) **(d)** *T. turgidum* ssp. *durum* (Elite)

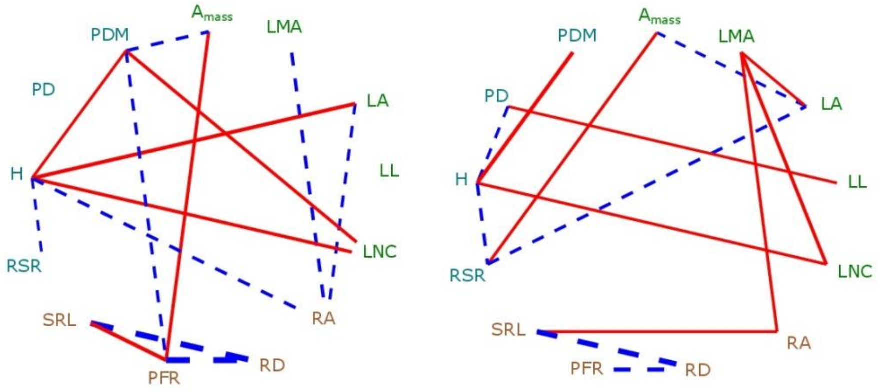


FIGURE 5 Correlograms based on Spearman scores using leaf, root and whole-plant traits per wheat sub-species: (a) *T. turgidum* ssp. *dicoccoides*; (b) T. *turgidum* ssp. *dicoccum*; (c) *T. turgidum* ssp. *durum* (landrace)*;* (d) *T. turgidum durum* (elite). See Table 1 for abbreviations of leaf (green), root (brown) and whole-plant (blue) traits. Only significant ($P \leq 0.05$) positive (red lines) and negative (blue and dashed lines) correlations are presented. Thickness of a line relates to the strength of the correlation.

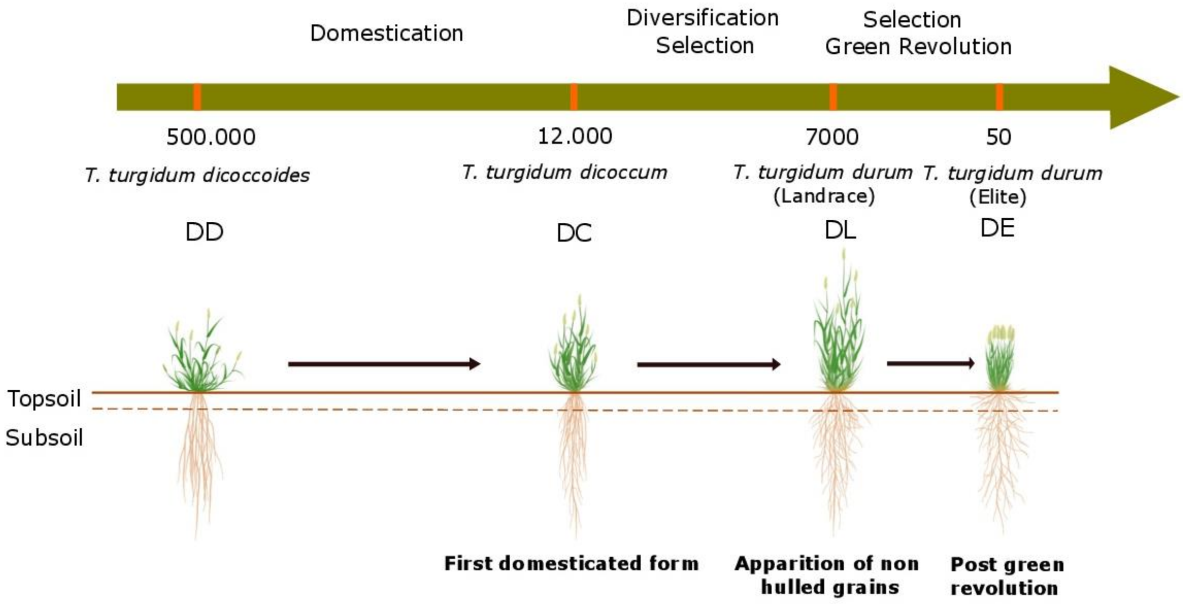


FIGURE 6 Historical timeline of the series of tetraploid wheat domestication and schematic representation of the 'vegetative domestication syndrome' identified in this study. Approximate dates as well as events of appearance for each sub-species are indicated. Wild form, *T. turgidum* ssp. *dicoccoides*, appeared *ca*. 500 000 years ago in the Fertile Crescent. The first domesticated form, *T. turgidum* ssp. *dicoccum*, appeared 12 000 years ago. During human migration towards the Mediterranean, wheat lost the "hulled grain" characteristic and a new sub-species appeared 7 000 years ago: *T. turgidum* ssp. *durum*. With the Green Revolution and associated phenotypic modifications such as plant size, *T. turgidum* ssp. *durum* genotypes can be distinguished between landrace group (before Green Revolution) and elite group (after Green Revolution). The latter regroups genotypes that have been selected for 50 years by breeders. Drawings are interpretations based on findings from this study as well as based on previous descriptions in the literature (for: number of inflorescences, rooting depth and reproductive plant height).

Discussion

Broadening the domestication syndrome

Here we used a unique series of domestication in tetraploid wheat to explore changes in resource-use strategies during the domestication process. We highlighted remarkable shifts in above- and below-ground traits towards a greater ability of modern varieties to acquire rapidly and efficiently soil resources (see Fig. 6 for a synthesis). Such changes have been poorly explored so far because crop syndrome of domestication was mostly described by modifications of reproductive organs (Charmet, 2011; Fuller et al., 2014).

Strikingly, without focusing on any of the reproductive traits previously highlighted as the cornerstone of the domestication syndrome in cereals, and in wheat in particular, the phenotypic spaces of the sub-species studied diverged strongly when considering a new set of traits that characterize the vegetative functioning. This result suggests that the domestication syndrome is certainly more complex than the ones previously discussed (Zeder et al., 2006; Meyer et al., 2012). One explanation is that selection targeted traits of agronomical interest but may have also generated indirect selection of a larger set of traits (Milla et al., 2015). Indirect selection may result from biophysical constraints linking traits together, from genes with pleiotropic effects, or from natural selection through effects of (uncontrolled) biotic and abiotic environmental changes associated with management practices. In addition, the abiotic environment (e.g., soil, climate) where crops developed may have changed over time due to human migration from the centres of crop origin (Diamond, 2002), which represents another complex form of selection pressures on a myriad of traits. Altogether, unknown and potentially undesirable traits or combinations of traits selected during domestication have to be added to the long list of factors that still limit yield improvement in cereals and other cultivated species (Ray et al., 2012).

Plant functional strategies and agricultural intensification

We examined the variation of individual vegetative traits at the leaf, root and whole-plant levels. We found major shifts in both above- and belowground traits over the course of domestication.

The significantly higher rate of photosynthesis of the most recent ('elite') genotypes contradicts previous findings that suggested a lack of impact of domestication on photosynthesis (Gifford and Evans, 1981). Interestingly, this trend paralleled an increase in leaf area and nitrogen content and a decrease in leaf longevity. This change echoes the leaf economics spectrum (LES) (Wright et al., 2004) that describes physiological tradeoffs at the leaf level across wild species using the same set of traits. Domestication may thus have increased metabolic rates and nutrient content in the leaves at the expense of structural defense and longer leaf lifespan (Kikuzawa and Lechowicz, 2011).

Plant height first increased during wheat domestication. This change, progressive from *T. turgidum* ssp. *dicoccoides* to *T. turgidum* ssp. *durum* (genotypes before the Green Revolution), is then interrupted for *T. turgidum* ssp. *durum* (elite), i.e. post-Green Revolution. This results from the introgression of dwarf genes into durum wheat genome (Russell, 2013).

This phenotypic trajectory reflects a shift in selection drivers during wheat domestication. Both artificial and natural selections have first promoted competitive strategies and thus taller plants (Violle et al., 2009). Then, crop density after the Green Revolution and the arm race towards taller plants that efficiently compete for light was less beneficial for crop assemblages as a whole, as compared to directional selection towards smaller individual plants (Donald and Hamblin, 1983). Meanwhile, we observed a decrease in biomass allocated to roots in favor of shoot parts over the course of domestication. We found no further evidence in the literature regarding the influence of the introgression of dwarf genes on the root-to-shoot ratio after the Green Revolution but our findings may stimulate other works on this aspect.

At the root level, the most recent genotypes were characterized by a smaller crown root angle and a decrease in root diameter corresponding to a more horizontal root system with finer roots. Domestication may have favoured an increase in horizontal fine roots to enhance an intensive exploration of the soil top layers by roots (Andresen et al., 2016; Olmo et al., 2016). This seems particularly adapted to enhance the capacity of genotypes to acquire efficiently the large amount of nutrients (in particular immobile resources such as phosphorus) that can be found in high-fertilized cropping systems since the Green Revolution (Zhu et al., 2005; Lynch, 2007; Lynch and Brown, 2012; Passot et al., 2016).

Overall, changes in plant strategy discovered here, are in agreement with an adaptation to resource-rich environments: over the course of domestication, all leaf, root and whole-plant traits reflect a global shift in plant strategy to become more resource acquiring.

Functional coordination and trade-offs in the context of domestication and plant breeding

While we qualitatively highlighted that phenotypic changes emerged along a principal axis related to the leaf economics spectrum (LES) (see above), we did not find the significant leaf trait-trait relationships (e.g. negative relationship between leaf lifespan and photosynthetic rate: Fig. S4) that define the LES (Donovan et al., 2014; Martin et al., 2017; Niinemets, 2015; Wright et al., 2004). The comparison between intra- vs. interspecific LES remains scarce in the literature (e.g., Vasseur et al., 2012; Blonder et al., 2013; Prieto et al., 2017). Two reasons may explain the lack of significant relationships in wheat. First, most traits might have reached a physiological optimum thanks to artificial selection such that no tradeoff emerges anymore. Second, the range of trait values (e.g., leaf lifespan) might be too restricted to generate significant trait-trait relationships (Funk and Cornwell, 2013). We encourage future studies to deeply explore within-species LES using a large set of genotypes, notably crop genotypes.

We showed changes in patterns of trait-trait relationships during domestication. Wild ancestors displayed more correlations, particularly between root and leaf traits, than the domesticated forms. This result suggests that domestication and breeding may have consciously or unconsciously decoupled the different aspects of resource capture and use in modern varieties (Geng et al., 2014; Cheng et al., 2016). On the contrary, plant height which was not related to the global strategy of the wild ancestor, was related to traits from the leaf economics spectrum and biomass production, from the first step of the domestication (*T. turgidum* ssp. *dicoccum*) to recent 'elite' lines. This demonstrates the importance of this trait for the fitness of domesticated plants grown in productive monoculture fields where crop density is high and competition for light intense (Cousens, Barnett, and Barry 2003). For *T. turgidum* ssp. *durum* before Green Revolution, which is the tallest group within the tetraploid wheat domestication history (Motzo and Giunta, 2007), individual plants also displayed smaller root system angles, a possible way to improve their anchorage. Conversely, the other below-ground traits were not related to the whole-plant strategy. Altogether, we suspect that, at its early stages, domestication uncoupled above- and below-ground traits in response to changes in crop density and fertilization regimes, thus favouring (i) high stature, competitive ability and light-use efficiency, and (ii) shallow root systems that are efficient to quickly acquire a large amount of nutrients (Gioia et al., 2015). However, the inherent maintenance of some functional coordination and trade-offs between plant organs suggests that selection of traits could have provoked selection of parallel traits.

Implications for an agroecological rethinking of cereal cultures

Agriculture is facing three major interconnected economical, societal and environmental challenges: (i) increasing agricultural yields to feed the global population, (ii) reducing the amount of inputs (fertilization, water, pesticides) to meet agri-environmental recommendations, and (iii) improving the sustainability of cultivated systems (Dale et al., 2013; Schulte et al., 2014). Those challenges drove the emergence of long-standing and contemporary themes in agroecological science (Dalgaard et al., 2003; Wezel et al., 2009). As such, there is a growing consensus that a global reconsideration of agricultural systems is necessary to move from post Green Revolution 'simplified' agricultural systems to practices that optimize the use of genetic and phenotypic diversity (Malézieux et al., 1999; Garnier and

Navas, 2012; Litrico and Violle, 2015; Barot et al., 2017). Recently, Prieto et al. (2015) experimentally demonstrated the positive role of species and genotypic diversity for biomass production and yield stability in grassland systems. In cereals, intercropping systems where they are mixed with other species of agronomic interest (e.g., legumes) are a demonstration of the benefit of biodiversity for crop sustainability (Pretty and Bharucha, 2014; Brooker et al., 2015). Crop mixtures, i.e. field-level mixtures of several varieties of a given species, are under study, too (Litrico and Violle, 2015; Barot et al., 2017). In this context, our study can help target the traits and genotypes of interest. Indeed, the design of crop mixtures and intercropping systems largely lays on resource-use complementary mechanisms and the choice of species and varieties displaying contrasting functional strategies (Litrico and Violle, 2015; Bedoussac and Justes, 2011).

Introduction of diversity in fields, although appealing, is challenging (Chateil et al., 2013; Gaba et al., 2015; Malézieux et al., 2009; Thomas et al., 2015). This has stimulated a renewal of research on the optimization of crop mixtures in recent years thanks to the input of ecological mechanisms (in particular resource-use complementarity) that are expected to favour resource-use efficiency at the crop stand level. As a prerequisite, this approach requires the existence and understanding of variability in the traits of interest in crop collections (Litrico and Violle, 2015; Prieto et al., 2017). Interestingly, here we found within- and between-subspecies phenotypic diversity for traits related to resource capture and use. Even if phenotypes of modern wheat varieties have converged towards a unique resource-use strategy due to homogenization and artificiality (high levels of inputs and farmer's controls on plants) of the intensive cropping systems, phenotypic diversity still exists (see the dispersion around the centroid of each group in Figure 1). Therefore, it may be possible to mix elite varieties based on this diversity even if root trait variability (e.g., root angle or specific root length) is much smaller than size-related trait variability. The lack of variability of root strategies is an issue given their tight link with soil resource acquisition. A screening of root traits for a larger panel of elite genotypes seems necessary to pursue this question. The comparison of ancestral and elite varieties shows higher phenotypic diversity, notably for root traits. With shallower and thinner root systems, elites are efficient to quickly acquire a large amount of nutrients concentrated in topsoil provided by fertilization (Garnett et al., 2009). But, contrary to ancestors, their root system is not efficient to acquire deep stored water in case of water-limited conditions (Wasson et al., 2012). The phenotypic diversity of root systems illustrated the putatively contrasted nutrient and water acquisition strategies between ancestors and elites. Mixing ancestral and elite varieties is obviously too complicated for technical reasons of field management. However, going back to ancestral forms to ‘pick up’ some ‘native’ traits and trait combinations fitted to more stressful conditions (e.g. nutrient starvation or water limitation) may be the key (e.g. Dawson et al., 2013; Allaby et al., 2015). In this context, a genetic approach – coupled to an experimental approach with e.g., varying nutrient or water availability – would help identify genes implicated behind those traits and highlight some future improvements of plant breeding (Golan et al., 2015) and the design of crop mixture and intercropping systems.

Authors' Contributions

All the authors designed the study; A.R. compiled and analysed the data collected by the co-authors; all the authors wrote the manuscript. All the authors gave their final approval for publication.

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Supporting Information

The following Supporting Information is available for this article:

Supporting Table S1 List of the 40 tetraploid wheat genotypes used in this study.

Supporting Table S2 Standardized loadings of the PCA presented in the main text.

Supporting Fig. S1 Variation in leaf traits among genotypes of tetraploid wheat.

Supporting Fig. S2 Variation in root traits among genotypes of tetraploid wheat.

Supporting Fig. S3 Variation in whole-plant traits among genotypes of tetraploid wheat.

Supporting Fig. S4 Projection of the studied collection of tetraploid wheat domestication in the leaf longevity-net photosynthetic rate relationship.

SUPPORTING TABLE S1 List of the 40 tetraploid wheat genotypes used in this study. The genotype 46310 from *T. turgidum ssp. dicoccoides* did not grow.

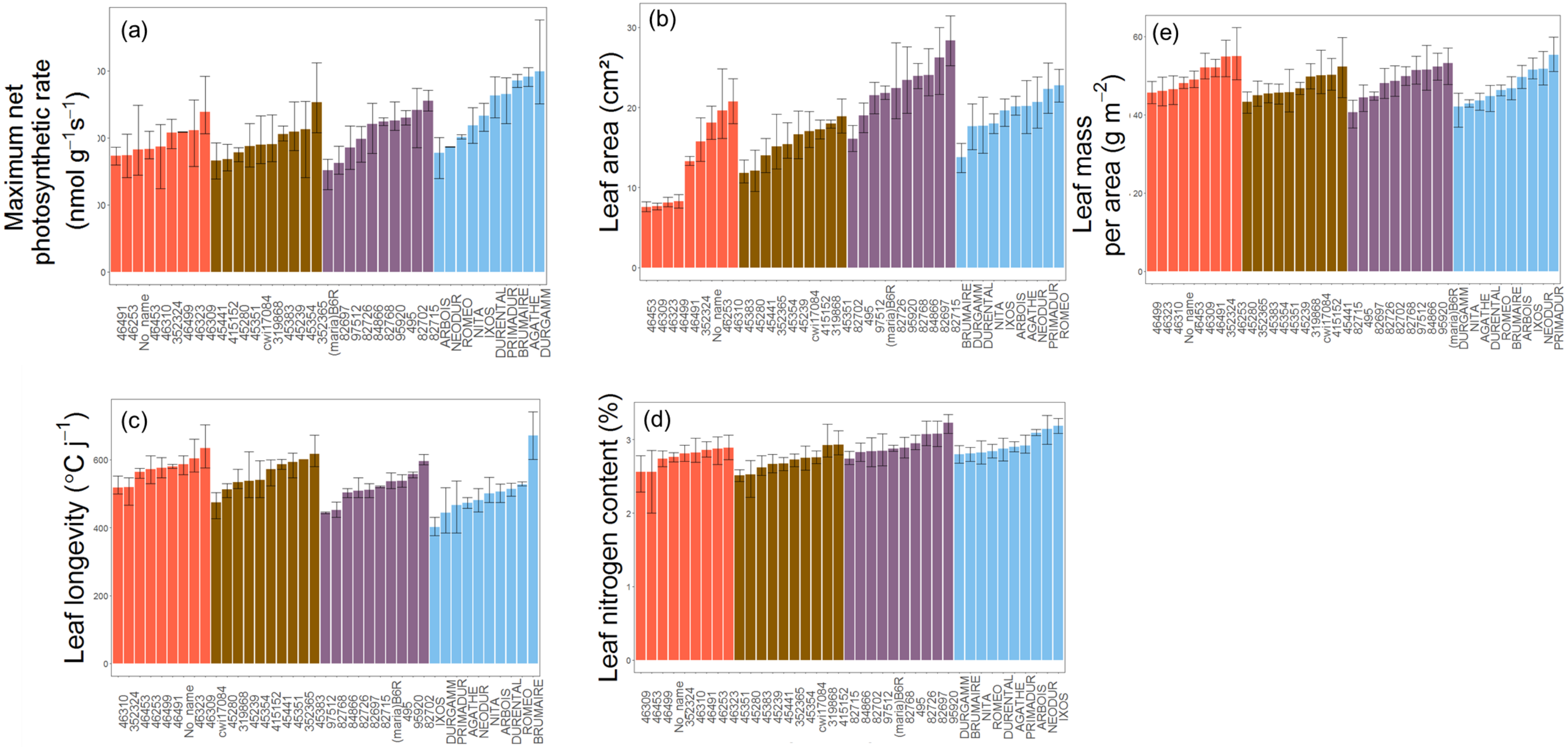
Subspecies	N	Collection
T. turgidum ssp. *dicoccoides*	46499	ICARDA
(ancestral form)	46491	ICARDA
	46323	ICARDA
	46309	ICARDA
	46310	ICARDA
	46501	ICARDA
	46453	ICARDA
	46253	ICARDA
	467014	USDA
	352324	USDA
T. turgidum ssp. *dicoccum*		
(first domesticated form)	45383	ICARDA
	45351	ICARDA
	45239	ICARDA
	45354	ICARDA
	45280	ICARDA
	45441	ICARDA
	352365	USDA
	319868	USDA
	cwi17084	CIMMYT
	415152	USDA
T. turgidum ssp. *durum* (Landrace)		
(durum wheat before GR)	495	Montpellier
	84866	ICARDA
	97512	ICARDA
	95920	ICARDA
	82697	ICARDA
	82702	ICARDA
	82768	ICARDA
	82715	ICARDA
	82726	ICARDA
	(maria)B6R	580
T. turgidum ssp. *durum* (Elite)		
(durum wheat after GR)	AGATHE	GEVES
	BRUMAIRE	GEVES
	DURGAMM	GEVES
	NITA	GEVES
	ROMEO	GEVES
	PRIMADUR	GEVES
	ARBOIS	GEVES

	NEODUR	GEVES
	IXOS	GEVES
	DURENTAL	GEVES

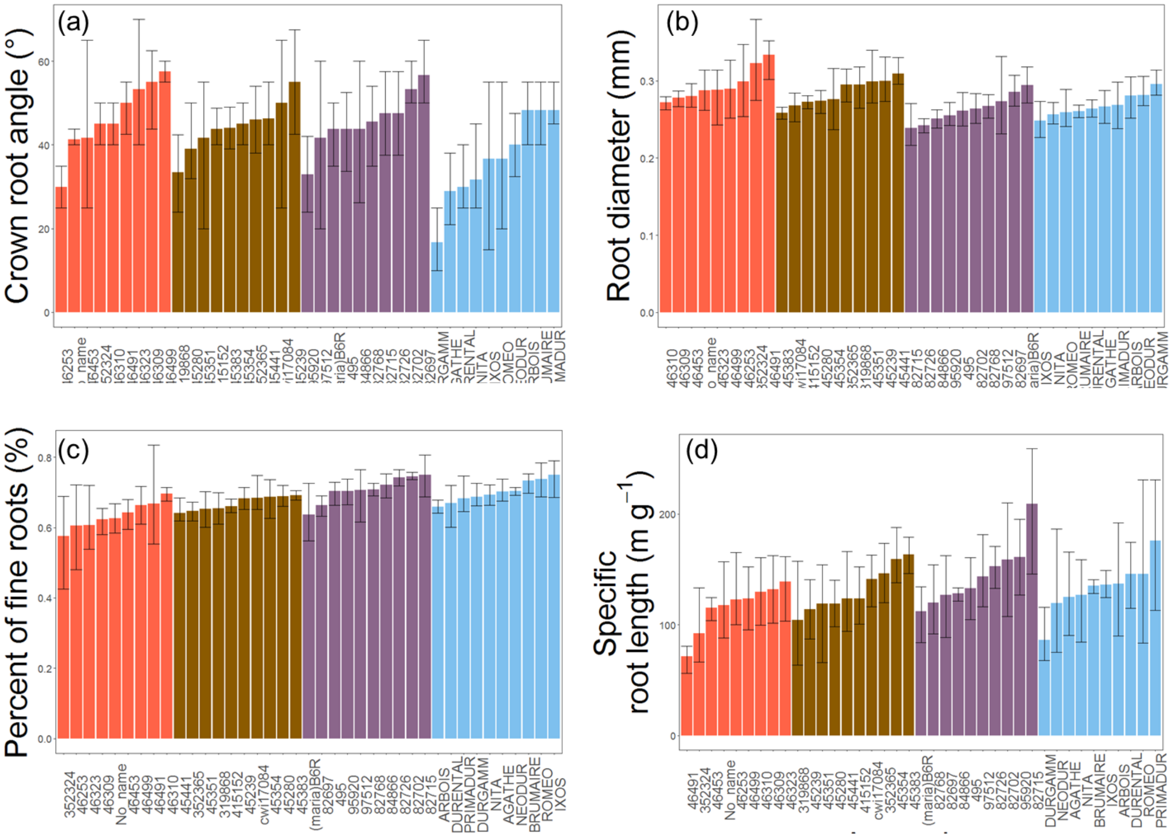
ICARDA (International Center for Agricultural Research in the Dry Areas) Beirut, Lebanon
USDA-ARS (US Department of Agriculture) National Small Grains, wheat collection, USA
CIMMYT (International Maize and Wheat Improvement Center), Mexico
GEVES (French Group for the Study and Control of Varieties and Seeds), France
INRA (French National Institute of Agricultural Research) wheat collection, Montpellier

SUPPORTING TABLE S2 Standardized loadings of the PCA presented in the main text (first three axes), for each functional trait, based upon correlation matrix. For abbreviations, see Table 1.

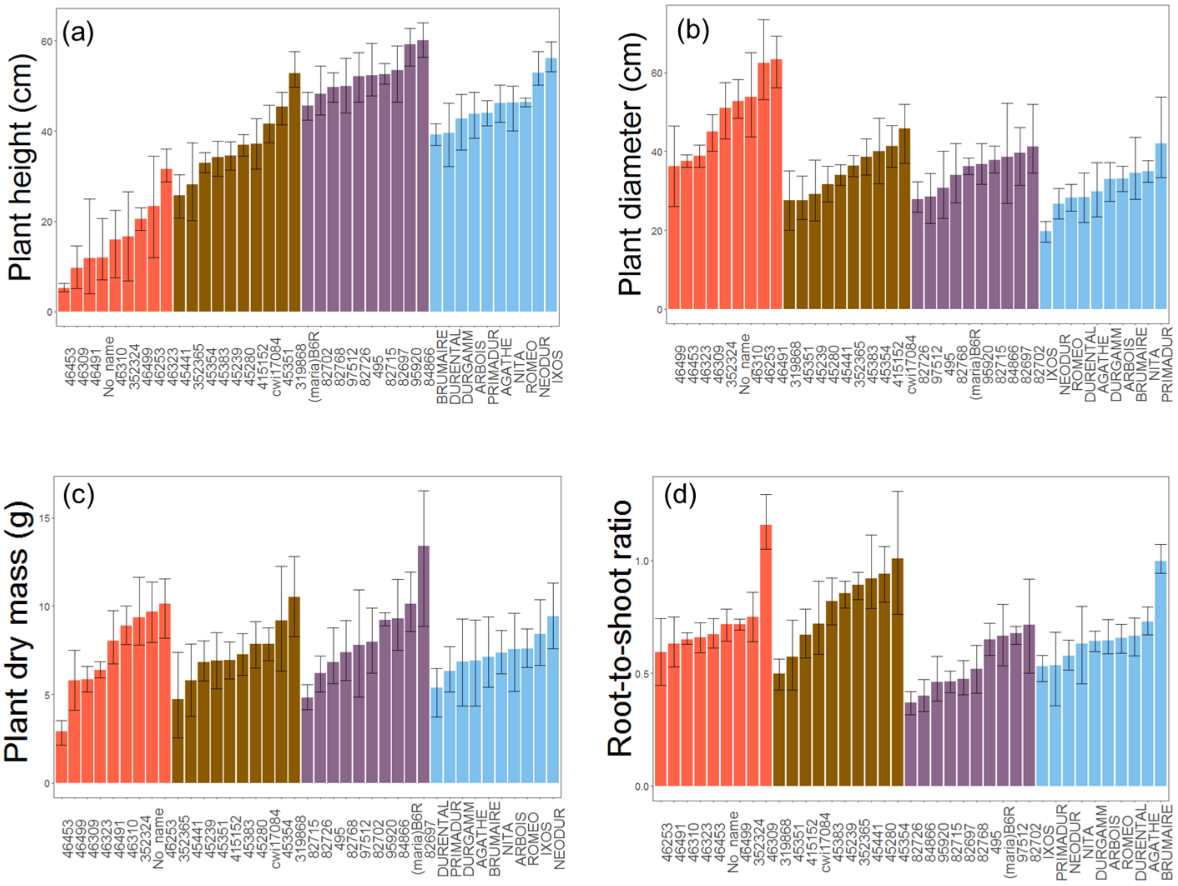
Traits	PC1	PC2	PC3
Leaf			
Amass	-0.22	0.51	-0.66
LA	0.85	0.28	-0.07
LNC	0.74	0.09	-0.03
LL	-0.59	0.03	0.36
LMA	0.20	-0.21	0.18
Root			
RA	-0.28	0.20	0.82
RD	-0.23	-0.83	0.10
PFR	0.31	0.66	-0.08
SRL	0.04	0.91	0.12
Whole-plant			
H	0.67	0.37	-0.04
PD	-0.05	-0.19	0.09
RSR	-0.77	0.13	0.06
PDM	0.72	-0.36	0.28



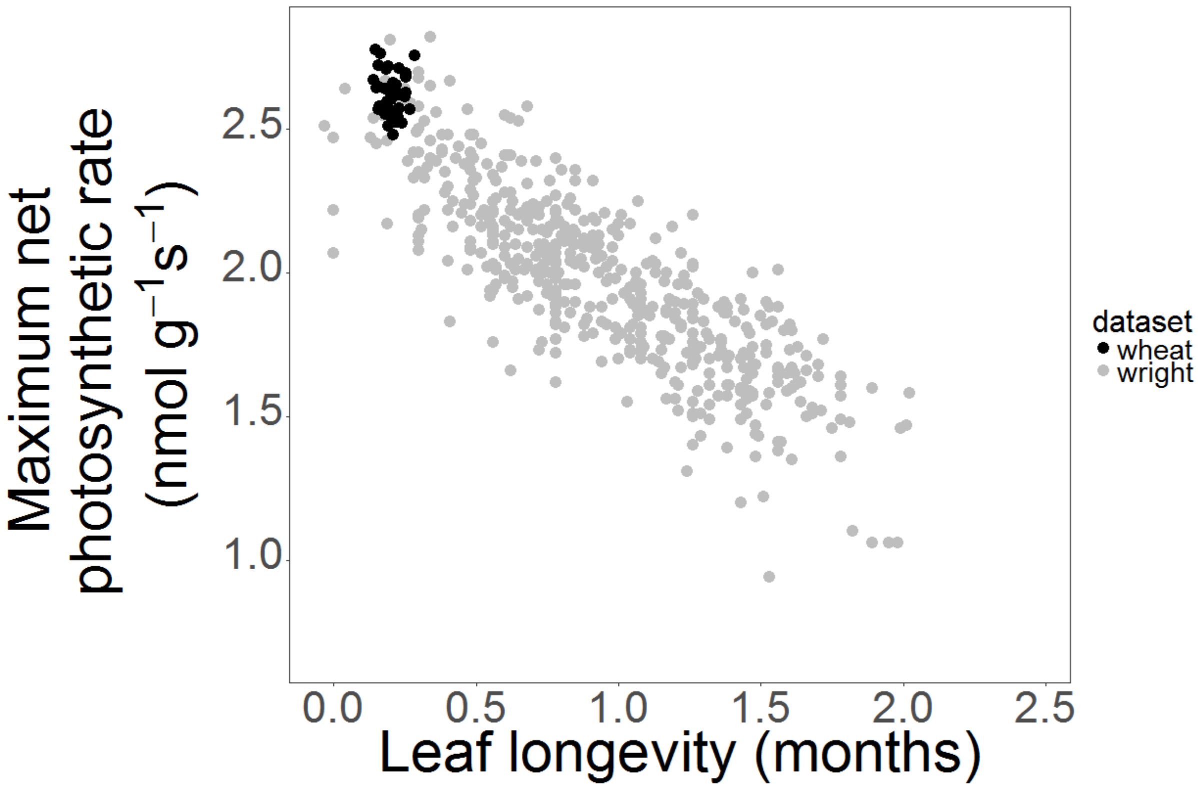
SUPPORTING FIG. S1 Variation in leaf traits among genotypes of tetraploid wheat. (a) Maximum net photosynthetic rate, (b) leaf area, (c) leaf longevity, (d) leaf nitrogen content, and (e) leaf mass per area. Genotypes are split into four groups corresponding to the three sub-species and distinction between *T. turgidum* ssp. *durum* (landrace) and (elite). Groups are ranked according to their dates of apparition during the domestication of tetraploid wheat and colored differently (*T. turgidum* ssp. *dicoccoides* in red, *T. turgidum* ssp. *dicoccum* in brown, *T. turgidum* ssp. *durum* landrace in purple and *T. turgidum* ssp. *durum* elite in blue). Within groups, genotypes are ranked according to their values for the functional trait under scrutiny. Bars and error bars represent means ± SEM (n =3 or 5).



SUPPORTING FIG. S2 Variation in root traits among genotypes of tetraploid wheat. (a) Crown root angle, (b) root diameter, (c) percent of fine roots, and (d) specific root length. Genotypes are split into four groups corresponding to the three sub-species and distinction between *T. turgidum ssp. durum* (landrace) and (elite). Groups are ranked according to their dates of apparition during the domestication of tetraploid wheat and colored differently (*T. turgidum* ssp. *dicoccoides* in red, *T. turgidum* ssp. *dicoccum* in brown, *T. turgidum* ssp. *durum* landrace in purple and *T. turgidum* ssp. *durum* elite in blue). Within groups, genotypes are ranked according to their values for the functional trait under scrutiny. Bars and error bars represent means ± SEM (n =3 or 5).



SUPPORTING FIG. S3 Variation in whole-plant traits among genotypes of tetraploid wheat. (a) Plant height, (b) plant diameter, (c) plant dry biomass, (d) root-to-shoot ratio. Genotypes are split into four groups corresponding to the three sub-species and distinction between *T. turgidum* ssp. *durum* (landrace) and (elite). Groups are ranked according to their dates of apparition during the domestication of tetraploid wheat and colored differently (*T. turgidum* ssp. *dicoccoides* in red, *T. turgidum* ssp. *dicoccum* in brown, *T. turgidum* ssp. *durum* landrace in purple and *T. turgidum* ssp. *durum* elite in blue). Within groups, genotypes are ranked according to their values for the functional trait under scrutiny. Bars and error bars represent means ± SEM (n = 5).



SUPPORTING FIG. S4 Projection of the studied collection of tetraploid wheat domestication in the leaf longevity-net photosynthetic rate relationship, a central trade-off of the Leaf Economics Spectrum (LES). Grey points represent mean trait values for individual wild species (data from Wright et al., 2004; n = 2548), and black points represent the mean trait values of each genotype of the tetraploid wheat domestication collection from this study (n = 39).