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RESEARCH PAPER

Impact of domestication on the phenotypic architecture of durum wheat under contrasting nitrogen fertilization

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Abstract

The process of domestication has led to dramatic morphological and physiological changes in crop species due to adaptation to cultivation and to the needs of farmers. To investigate the phenotypic architecture of shoot- and root-related traits and quantify the impact of primary and secondary domestication, we examined a collection of 36 wheat genotypes under optimal and nitrogen-starvation conditions. These represented three taxa that correspond to key steps in the recent evolution of tetraploid wheat (i.e. wild emmer, emmer, and durum wheat). Overall, nitrogen starvation reduced the shoot growth of all genotypes, while it induced the opposite effect on root traits, quantified using the automated phenotyping platform GROWSCREEN-Rhizo. We observed an overall increase in all of the shoot and root growth traits from wild emmer to durum wheat, while emmer was generally very similar to wild emmer but intermediate between these two subspecies. While the differences in phenotypic diversity due to the effects of primary domestication were not significant, the secondary domestication transition from emmer to durum wheat was marked by a large and significant decrease in the coefficient of additive genetic variation. In particular, this reduction was very strong under the optimal condition and less intense under nitrogen starvation. Moreover, although under the optimal condition both root and shoot traits showed significantly reduced diversity due to secondary domestication, under nitrogen starvation the reduced diversity was significant only for shoot traits. Overall, a considerable amount of phenotypic variation was observed in wild emmer and emmer, which could be exploited for the development of pre-breeding strategies.

Key words: Image analysis, nitrogen fertilization, phenotyping, pre-breeding, root, shoot, *Triticum* spp.

Introduction

Since the work of Darwin (1875), the process of crop domestication has been seen as a change in relatively few major morphological, yield, and quality traits (e.g. the domestication syndrome). More recently, several studies based on

Abbreviations: ΔCV_A, loss of phenotypic diversity; CV_A, coefficient of additive genetic variation; DAS, days after sowing; ETR_{max}, maximum electron transport rate; Fv/Fm, potential photosynthetic efficiency; LRL, length of (visible) lateral root; PPFD, photosynthetic photon flux density; $PPFD_{sat}$, saturating photosynthetic photon flux density; PRL, length of (visible) primary root; PS, Photosystem; RDW, dry root biomass; RGRr, root relative growth rate; RGRs, shoot relative growth rate; RSD, root system depth; RSW, root system width; SFW, fresh shoot biomass; SPAD, leaf chlorophyll content; TLA, total leaf area; TLN, total leaf number; TRL, total length of all (visible) root.

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the analysis of nucleotide variation and molecular profiling have suggested that the phenotypic changes associated with the process of domestication and breeding were much more extended than previously believed (Bellucci *et al.*, 2014). The evolution of crop plants from wild to domesticated crops and then the changes imposed with modern plant breeding have been characterized by sharp changes in the agro-ecosystems, along with major changes in soil structure and fertility through the human interventions of ploughing and fertilization of the soil. These environmental changes might have favoured the adaptation of crop plants to the novel environments, with possible modifications to their root system and to their physiological responses to the level of soil fertility, and in particular to nitrogen availability. With few exceptions (e.g. Grando and Ceccarelli, 1995, for barley), previous analyses of cereal domestication have only considered the above-ground plant organs, while root traits have largely been neglected because of technical limitations in the often inaccurate and time-consuming methods of phenotyping root traits (Smit *et al.*, 2000; Waines and Ehdaie, 2007; Lynch and Brown, 2012; Fiorani and Schurr, 2013).

Tetraploid wheat domestication took place ~12 000 years ago in the Fertile Crescent. The wild progenitor has been identified as the wild emmer *Triticum turgidum* ssp. *dicoccoides*, which gave rise to the first domesticated tetraploid wheat, emmer (*Triticum turgidum* ssp. *dicoccum*) (Tanno and Willcox, 2006; Zohary *et al.*, 2012). About 2000 years after this event, human migration and the spread of agriculture from this region to and throughout Europe and Asia led to expansion of the cultivation of emmer. During the same period, durum wheat (*Triticum turgidum* ssp. *durum*) appeared in the Near East, and by the second millennium BC, this had gradually replaced its ancestor, emmer, to become the major cultivated form of tetraploid wheat (Maier, 1996; Nesbitt and Samuel, 1998; Zohary *et al.*, 2012). Thus, considering that domestication is an ongoing process that also includes modern plant breeding, in tetraploid wheat, we can consider here a primary domestication (from wild emmer to domesticated emmer) and a secondary domestication (from emmer to naked forms and durum wheat).

Wild and domesticated tetraploid wheat (emmer and durum wheat) differ according to several phenotypic changes, which have included loss of spike shattering, loss of tough glumes, increased seed size, reduced number of tillers, more erect growth, and reduced seed dormancy. Genetic analysis of various domestication-associated traits has detected genomic regions that are subjected to selection, and has also led to identification of the major genes that regulate these traits (Dubcovsky and Dvorak, 2007; Haudry *et al.*, 2007; Laidò *et al.*, 2013, 2014).

It has been well documented that tetraploid wheat genotypes can differ in root characteristics, such as root length density and root biomass (O'Toole and Bland, 1987; Hoad *et al.*, 2001; Liao *et al.*, 2004; Manschadi *et al.*, 2006; Waines and Ehdaie, 2007; Sanguineti *et al.*, 2007; Nakhforoosh *et al.*, 2014; Russo *et al.*, 2014). However, no information is available on the effects of primary domestication and subsequent secondary domestication on the phenotypic architecture of the root systems through direct comparisons of wild emmer with emmer, and with modern durum wheat. Wild emmer and emmer might have crucial roles in breeding programmes, because of their wide genetic variation for important agronomic and adaptive traits (Reynolds *et al.*, 2007; Laidò *et al.*, 2013, 2014). For this reason, exploration of the phenotypic architecture of different tetraploid wheat is an important step towards efficient exploitation of exotic germplasm for wheat breeding.

New developments in plant phenotyping technologies have drastically increased the possibility of non-invasive measurements of different plant traits, and of characterization of phenotypes in a high-throughput mode (Pieruschka and Poorter, 2012; Cobb *et al.*, 2013; Fiorani and Schurr, 2013). The use of soil-filled rhizoboxes with transparent plates, in combination with imaging technologies, has been suggested as a non-destructive approach to simultaneously characterize root and shoot growth that can fill the gap that exists between laboratory and field methods for root and shoot observations (Nagel *et al.*, 2012).

The aim of the present study was to characterize the phenotypic architecture of shoot- and root-related traits in tetraploid domesticated emmer and durum wheat, in relation to wild emmer, their wild relative. We thus investigated the below-ground and above-ground growth traits under contrasting conditions of nitrogen availability, to quantify the impact of primary domestication (from wild emmer to emmer) and secondary domestication (from emmer to durum wheat) on the phenotypic diversity of plants at an early stage of development.

Materials and methods

Plant materials

Thirty-six genotypes that represent three stages in tetraploid wheat evolution (i.e. the domestication groups) were used in this study, which comprised 12 wild emmer lines (*T. turgidum* ssp. *dicoccoides*; called 'wild emmer' in this study), 12 emmer primitive domestic lines (*T. turgidum* ssp. *dicoccum*; called 'emmer' in this study), and 12 modern durum wheat varieties (*T. turgidum* ssp. *durum*; called 'durum wheat' in this study) (Table 1; Laidò *et al.*, 2013). The samples were defined to be representative of the three groups, and the available information derived from previous studies (Laidò *et al.*, 2013, 2014) related to their genetic diversity (molecular and phenotypic traits), pedigree information (durum wheat), and geographical origin (wild and domesticated emmer) were used as the sampling criteria. The durum wheat varieties are modern cultivars, most of which are Italian materials that are representative of durum wheat breeding over the last 100 years. The accessions of emmer and wild emmer were collected from regions covering the most cultivated areas and from their natural distribution. All of the genotypes used here were evaluated according to morphological and biochemical traits during the growing season of 2008–2009, and by molecular simple sequence repeat and diversity array technology markers (Laidò *et al.*, 2013). The collection is stored at the Centro di Ricerca per la Cerealicoltura (CRA-CER) in Foggia, Italy.

Experimental design

The 36 wheat genotypes, 12 for each group, were grown in October 2012 for 4 weeks under optimal and under nitrogen-starvation conditions in the PhyTec Experimental Greenhouse at the Institute

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Table 1. *List of the 36 tetraploid wheat genotypes used in this study*

Subspecies	Accession no.	Origin
T. turgidum ssp. *dicoccoides* (wild emmer)	BAZ 382[a]	–
	PI 233288[b]	Israel
	PI 428014[b]	Israel
	PI 466991[b]	Israel
	IG 46504[c]	Syria, May Saloun
	PI 538656[b]	Turkey
	PI 554584[b]	Turkey
	PI 343446[b]	Israel
	PI 481539[b]	Israel
	PI 352324[b]	Lebanon
	PI 355459[b]	Armenia
	PI 470944[b]	Syria, Al Qunaytirah
T. turgidum ssp. *dicoccum* (domesticated emmer)	MG 5473[d]	Spain
	Farvento[e]	Italy
	Lucanica[e]	Italy
	Molise Sel. Colli[e]	Italy
	MG 5350[d]	Ethiopia
	MG 5344/1[d]	Ethiopia
	MG 5293/1[d]	Italy
	PI 74106[b]	-
	CItr 17675[b]	Lebanon
	PI 254169[b]	Iran
	PI 352347[b]	Palestine
	PI 470739[b]	Turkey
T. turgidum ssp. *durum* (durum wheat)	Appulo[e]	Italy
	Capeiti-8[e]	Italy
	Cappelli[e]	Italy
	Cirillo[e]	Italy
	Creso[e]	Italy
	Neodur[e]	France
	Ofanto[e]	Italy
	Pedroso[e]	Spain
	PR22D89[e]	Italy
	Simeto[e]	Italy
	Timilia[e]	Italy
	Trinakria[e]	Italy

[a]Wheat collection of the Genebank of IPK Gatersleben, Germany.
[b]USDA-ARS National Small Grains, wheat collection, USA.
[c]ICARDA Beirut, Lebanon.
[d]National Research Council, CNR, Bari Wheat collection, Italy.
[e]Pure lines derived from the local population collected directly or from commercial varieties conserved by CRA-CER Foggia, Italy.

of Biosciences and Geosciences (IBG-2): Plant Sciences Institute, Forschungszentrum Jülich GmbH, Germany (50°54'36''N, 06°24'49''E). Plants were grown in rhizoboxes and the experiment layout was a split-plot design with two replications—the main plot nitrogen treatment and the subplot genotype. In each rhizobox, two genotypes of the same group were grown with four plants, two per genotype. Each genotype was replicated twice and the replication consisted of two plants growing in the same rhizobox. Overall, 72 rhizoboxes were used (two nitrogen treatments×three wheat groups×12 genotypes per group×two replicates). The same experiment was repeated as a replication in time in December 2012. Overall, four replicates per genotype were used.

Before sowing, the mean seed weight for each genotype was estimated by weighing 10 lots of 20 seeds for each of the 36 genotypes. For each genotype, grains of uniform size were visually selected, surface sterilized, and pre-germinated, before being transplanted into the soil-filled rhizoboxes. The grains were surface sterilized in 1% NaClO (w/v) for 15 min and rinsed 10 times with deionized water. They were then placed in rows on wet filter paper (Whatman 3MM) in Petri dishes, with the grain oriented vertically so that the radicles faced downwards. The Petri dishes were sealed with Parafilm to avoid evaporation and to ensure sufficient moisture during germination, and treated at 20–22 °C for 2 d in the dark, to promote and synchronize germination. After germination, seedlings that showed uniform growth (seminal root length, 1–2 cm) were transplanted (at 2 cm depth) into the rhizoboxes (90 × 70 × 5 cm), which were placed into the automated GROWSCREEN-Rhizo phenotyping system (Nagel *et al.*, 2012). The rhizoboxes were filled to a volume of ~18 l with 'Typ 0' manually sieved peat soil (Nullerde Einheitserde; Balster Einheitserdewerk, Fröndenberg, Germany), which provided low nutrient availability, with a pH of 6.1, and the available phosphate, potassium, magnesium, ammonium nitrogen, and nitrate nitrogen concentrations of 7.0, 15.0, 98.0, <1.0, and <1.0 mg l^{-1}, respectively. The inclination angle of the rhizoboxes was adjusted to 43°, with the transparent Plexiglas plate of the rhizoboxes facing downwards to prevent exposing the roots to light (for details, see Nagel *et al.*, 2012).

A preliminary experiment showed that the portion of the root system that was visible through the transparent Plexiglass/soil surface facing downwards was representative of the total root system (r^2=0.91 for barley and wheat plants; Nagel *et al.*, 2012; Nagel *et al.*, unpublished data). This confirmed that, also in wheat, the non-destructive analysis of the root length at the rhizobox surface could be a measure of the effect on total root length (Nagel *et al.*, 2012).

All plants were watered regularly twice a day with 400 ml of tap water and supplied three times per week with 200 ml of modified Hoagland solution with or without added nitrogen (stock solution: 5 mM KNO_3, 5 mM $Ca(NO_3)_2$, 2 mM $MgSO_4$, 1 mM KH_2PO_4, plus trace elements; Hoagland and Arnon, 1950). For the nitrogen-starvation solutions, 1 mM KNO_3 and 5 mM $Ca(NO_3)_2$ were replaced by 2.5 mM K_2SO_4 and 5 mM $CaCl_26(H_2O)$, respectively. The experiments were carried out under natural lighting in a greenhouse, with the air temperature kept between 18 and 24 °C, and the relative humidity between 40 and 60%.

Quantification of phenotypic traits

The GROWSCREEN-Rhizo phenotyping system was equipped with RGB cameras to acquire a side view of the shoot (Nagel *et al.*, 2012). However, this set-up was developed to image individual plants at the centre of each rhizobox. In these experiments, because more than one plant was sown in each rhizobox, the degree of overlap between leaves of adjacent plants would not allow an accurate estimation of the projected area with the camera geometry used. For this reason, twice per week, the length and width of each of the leaves were measured manually using a ruler, and the leaf areas were then calculated according to the following equation:

$$\text{Leaf area} = \text{leaf length} \times \text{maximum width} \times k \qquad (1)$$

where k is the shape factor, which is 0.858 for wheat leaves (Kalra and Dhiman, 1977; Masle and Passioura, 1987; Liu *et al.*, 2002). The total leaf area (TLA) of each plant was calculated by adding up the areas of all of the leaves. In addition, the total number of leaves (TLN) was counted per plant.

Root system architecture parameters such as visible primary root length (PRL), visible lateral root length (LRL), total root length (TRL) of all of the visible roots, root system depth (RSD), and root system width (RSW) were quantified every day during the experiments, using the automated phenotyping

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system GROWSCREEN-Rhizo and the image-based software tool GROWSCREEN-Root (Nagel *et al.*, 2012). When the first genotype reached the bottom of the rhizobox (day 17), four time points were chosen (days 6, 9, 13, and 17) to perform the root measurements and to create the growth dynamics curves.

At the end of the experiment, at 28 d after sowing (DAS) (Zadoks stage 14–18 for optimal nitrogen; Zadoks stage 12–14 for nitrogen starvation; Zadoks *et al.*, 1974), the wheat plants were harvested and the above-ground biomass was determined (shoot fresh weight; SFW). The root systems were carefully washed and subsequently oven dried (Heraeus UT 6760; Thermo Scientific Heraeus, Langenselbold, Germany) at 70 °C for at least 48 h to determine the root biomass (root dry weight; RDW). To compare the relationships between root and shoot growth, the ratio between the total root length of all of the visible roots and the total leaf area (TRL:TLA) was also calculated for the two treatments. An overview of the trait definitions is given in Table 2.

For both leaves and roots, the relative growth rates (shoot relative growth rate, RGR_s, $cm^2\ d^{-1}$; root relative growth rate, RGR_r, $cm\ d^{-1}$) were calculated according to the following:

$$\mathrm{RGR} = 1/t \times \ln\left(\mathrm{A2}/\mathrm{A1}\right) \quad (2)$$

where A1 and A2 are the TLA or TRL at times 1 and 2, respectively, and t is the number of days between times 1 and 2. In addition, the percentage reductions in the responses to nitrogen starvation were estimated by $[(T_{N+} - T_{N-})\times 100/(T_{N+})]$, where T_{N+} and T_{N-} are the average trait performances of a genotype under the optimal and nitrogen-starvation conditions, respectively. From these data, radar charts were generated using the radar-chart option in Excel.

At 27 DAS, the chlorophyll content (SPAD units) was estimated with a SPAD-502 chlorophyll meter (Minolta Corp., Ramsey, NJ, USA). Three measurements per plant were taken at random locations in the middle of the second and third leaves, and the mean value was used for the analysis.

Using a pulse-amplitude-modulated fluorometer (PAM-2000; Walz, Effeltrich, Germany), the maximum quantum yield of photosystem (PS) II [or the potential photosynthetic efficiency (Fv/Fm)] was determined for plant grown under the optimal and nitrogen-starvation conditions. Fv/Fm was measured after dark adaptation of the middle sections of the second leaves with a leaf clip for at least 30 min prior to a saturating light pulse. The wheat second leaves were exposed to eight incremental steps of photosynthetic photon flux density (PPFD) that were pre-programmed into the PAM fluorometer, with each step lasting 10 s. The electron transport rate (ETR) was then estimated by according to $ETR=\Delta F/Fm' \times PPFD \times 0.5 \times 0.84$, with $\Delta F/Fm'$ as the quantum yield of light-adapted leaves, 0.84 as an estimate of the absorbed PPFD, and 0.5 to account for the partitioning of the light absorption between PSI and PSII (Genty *et al.*, 1989). The ETR values were plotted against PPFD, and the maximum electron transport rate (ETR_{max}), which represents the photosynthetic capacity, was determined by regression analysis and curve fitting using a single exponential function (Rascher *et al.*, 2000), as provided by Sigma Plot Version 11.0, from Systat Software (San Jose, CA, USA):

$$f(x) = a\left(1 - e^{-bx}\right) \quad (3)$$

From the results of Eqn (3), the cardinal points were determined with $a=ETR_{max}$, and the saturating photosynthetic photon flux density ($PPFD_{sat}$) was reached at 0.9 ETR_{max} (Rascher *et al.*, 2000).

Statistical analysis

Statistical analyses of the combined data from the two experiments were performed with JMP®, version 8 (SAS Institute, Cary, NC, USA). The mean value of the two plants per genotype planted in each rhizobox was obtained and used to perform the following statistical analysis. Descriptive statistical parameters were calculated for all of the phenotypic traits for the three wheat groups (wild emmer, emmer, and durum wheat) under the optimal and nitrogen-starvation treatments. The coefficient of additive genetic variation (CV_A) was expressed as:

$$\mathrm{CV_A} = \frac{\sqrt{V_A}}{\bar{X}} \quad (4)$$

where V_A is the additive genetic variance and $\bar{X}$ is the phenotypic mean of the trait, which was used as the comparable measure for unit-free evaluation of phenotypic diversity as between treatments,

Table 2. *Morphological and physiological traits scored in this study*

Trait	Acronym	Description
Shoot		
Total leaf number	TLN	Manually counted
Total leaf area (cm^2)	TLA	Calculated on all the leaf as leaf length×maximum width×0.858 (Kalra and Dhiman, 1976)
Shoot fresh weight (g)	SFW	Measured 28 DAS
Shoot relative growth rate ($cm^2\ d^{-1}$)	RGR_s	Calculated from the TLA
Root		
Primary visible root length (cm)	PRL	Length of visible seminal roots
Lateral visible root length (cm)	LRL	Length of visible lateral roots
Total visible root length (cm)	TRL	Total length of all visible roots
Visible root system depth (cm)	RSD	Maximal vertical depth of a visible root system
Visible root system width (cm)	RSW	Maximal horizontal distribution of the visible root system
Root dry weight (g)	RDW	Measured 28 d from sowing after oven dried at 70 °C for ~48 h
Root relative growth rate ($cm\ d^{-1}$)	RGR_r	Calculated on the TRL
Root:shoot		
Total visible root length:total leaf area ($cm\ cm^{-2}$)	TRL:TLA	Ratio between total visible root length and total leaf area
Photosynthesis		
Leaf chlorophyll content (SPAD units)	SPAD	Leaf chlorophyll content measured 27 DAS on first and third leaf
Maximum electron transport rate (μmol)	ETR_{max}	Electrons $m^{-2}\ s^{-1}$, maximum electron transport rate measured 27 DAS
Saturating photosynthetic photon flux (μmol)	$PPFD_{sat}$	Photons $m^{-2}\ s^{-1}$, saturating photosynthetic photon flux density measured 27 DAS
Potential photosynthetic efficiency	Fv/Fm	Potential photosynthetic efficiency measured 27 DAS

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between groups, and within treatments (Houle, 1992). Unlike heritability, CV_A is a measure of additive genetic variation that is standardized by the trait mean and is therefore independent of other sources of variance (Houle, 1992; Hansen *et al.*, 2011). It is precisely these properties that make CV_A suitable for comparative purposes (Garcia-Gonzalez *et al.*, 2012). Hence, in this study, we used CV_A to compare the patterns of genetic variation.

To measure the loss of phenotypic diversity due to the primary domestication process in the domestic emmer versus wild emmer, the statistic $\Delta CV_{Apd}=1-(CV_{Aemmer}/CV_{Awild})$ was used, where CV_{Aemmer} and CV_{Awild} are the $CV_{Avalues}$ for emmer and wild emmer, respectively; if CV_{Aemmer} was higher than CV_{Awild}, then the parameter was calculated as $\Delta CV_{Apd}=(CV_{Awild}/CV_{Aemmer})-1$. The ΔCV_A parameter ranged from –1 to 1, where a negative value indicated no loss of CV_A diversity and a positive value indicated loss of CV_A diversity. This statistics is analogous to that proposed by Vigouroux *et al.* (2002) for the relative loss of molecular diversity and was used by Bellucci *et al.* (2014) for gene expression diversity. After primary domestication, the durum wheat was subject to additional selective events during the evolution of landraces and modern breeding. The loss of phenotypic diversity that occurred during the secondary domestication process in durum wheat versus emmer was calculated as $\Delta CV_{Asd}=1-(CV_{Adurum}/CV_{Aemmer})$, where CV_{Adurum} and CV_{Aemmer} are the CV_A values in the durum wheat and emmer, respectively; if CV_{Adurum} was higher than CV_{Aemmer}, then the parameter was calculated as $\Delta CV_{Asd}=(CV_{Aemmer}/CV_{Adurum})-1$. The differences between the distributions of the CV_A were evaluated statistically using Wilcoxon signed ranked tests for paired data.

Combined analysis of variance was carried out to test the main effects (domestication groups, treatments) and the interaction effects, according to the linear mixed model defined by:

$$P_{ijk}=\mu+G_i+T_j+A(G_i)_k+G_i\times T_j+\varepsilon_{ijk} \quad (5)$$

where μ is the general mean, G_i is the fixed effect of the ith domestication group (i=wild emmer, emmer, or durum wheat), T_j is the fixed effect of the jth treatment (j=the optimal and nitrogen-starvation conditions), $A(G_i)_k$ is the random effect of the genotypes (which represents the unit of replication) nested within the ith domestication group, $G_i\times T_j$ is the interaction between the ith domestication group and the jth treatment, and ε_{ijk} is the error of P_{ijk}. Mean discrimination was performed by applying Tukey's test, and statistically significant differences were determine at a probability level of $P\leq 0.05$.

A multivariate statistical approach, i.e. principal component analysis, was applied to the shoot and root data to determine the overall shoot and root system distinctiveness among the groups and treatments, and to investigate the relationships between the traits. Moreover, Pearson's partial correlation coefficients were performed between seed weight and root and shoot measurements, to determine whether variation in seed weight had a role in root and shoot development.

Results

When plotted on a logarithmic scale, the relationship between the shoot and root biomass was linear across both experiments and nitrogen treatments (Fig. 1). Despite slight differences in the intercepts of these relationships, the slopes were strikingly parallel, which showed that the root:shoot ratio responded in the same manner across the two experiments. Thereafter, the data from the two experiments were combined and considered as a replication in time for further analysis.

In summary, as can be seen from Table 3, the effects of the nitrogen treatments were significant for all of the traits, with the exceptions of Fv/Fm and RGR_r. The genetic differences between the domestication groups were significant for most of the traits analysed, including all four of the shoot traits and five of the root traits (PRL, TRL, RSD, RSW, and RDW), and, considering the photosynthesis-related traits, only for SPAD (Table 3). The interactions between treatments were highly significant in three cases: for two shoot traits

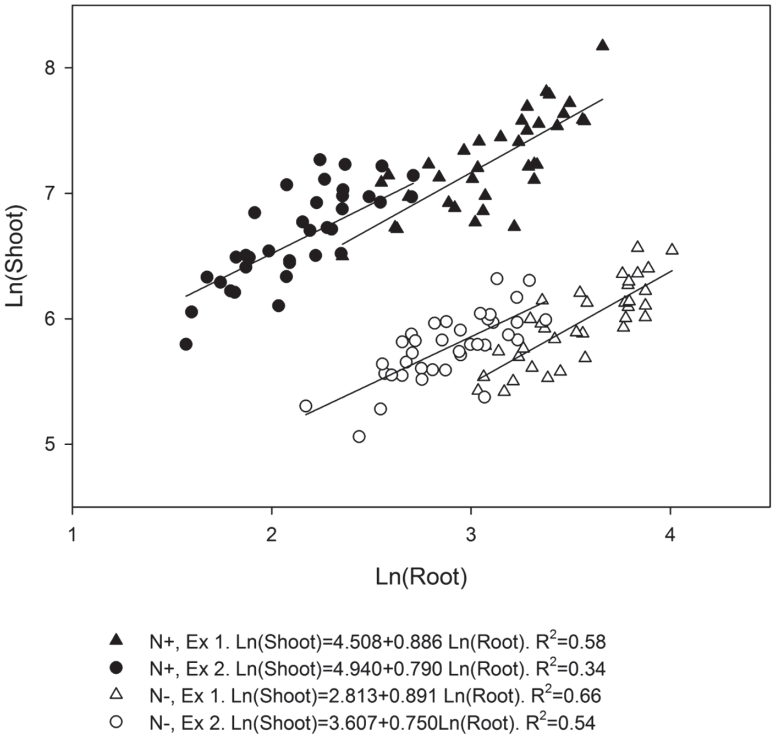

Fig. 1. Scatter plots showing the ln-transformed root biomass versus the ln-transformed shoot biomass for the two experiments (Ex 1, Ex 2) and treatments (optimal nitrogen, N+; nitrogen starved, N–). Each symbol represents a mean for a single genotype over four biological replicates.

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Table 3. *Means and analysis of variance for the wheat domestication groups grown under the optimal nitrogen (N+) and nitrogen starvation (N−) conditions*

Data shown are for 12 genotypes per group, with four replicates, as means over two experiments. Different superscript letters represent significant differences among the domestication groups within the treatments (Tukey's HSD, $P<0.05$). For abbreviations, see text and Table 2. SFW and RDW were measured at 28 DAS, SPAD, ETR_{max}, $PPFD_{sat}$, and Fv/Fm at 27 DAS, and all of the other traits at 17 DAS.

Treatment group	Shoot				Root						Root:shoot	Photosynthesis			
	TLN (*n*)	TLA (cm^2)	SFW (g)	RGR_s (% d^{-1})	PRL (cm)	LRL (cm)	TRL (cm)	RSD (cm)	RSW (cm)	RDW (g)	RGR_r (% d^{-1})	TRL:TLA ($cm\ cm^{-2}$)	SPAD (unit)	ETR_{max} (μmol $m^{-2}\ s^{-1}$)	$PPFD_{sat}$ (μmol $m^{-2}\ s^{-1}$)	Fv/Fm
N+ Wild	2.54^{bc}	14.68^{bc}	0.91^{b}	11.52^{ab}	101.86^{c}	6.07^{b}	107.93^{c}	43.12^{cd}	14.67^{c}	0.016^{de}	9.55^{a}	7.50^{b}	32.58^{b}	114.78^{a}	311.03^{a}	0.71^{a}
Emmer	2.81^{ab}	16.75^{b}	1.09^{b}	10.16^{b}	94.46^{c}	5.80^{b}	100.25^{c}	40.20^{d}	17.07^{bc}	0.015^{e}	9.43^{a}	6.06^{b}	31.84^{b}	124.09^{a}	326.84^{a}	0.77^{a}
Durum	3.06^{a}	22.56^{a}	1.65^{a}	12.85^{a}	112.76^{bc}	10.75^{a}	123.51^{bc}	47.87^{c}	20.56^{ab}	0.021^{cd}	10.41^{a}	5.46^{b}	36.26^{a}	129.72^{a}	324.09^{a}	0.77^{a}
N- Wild	2.09^{e}	9.57^{d}	0.30^{c}	6.60^{c}	137.92^{b}	4.44^{b}	142.36^{b}	58.75^{b}	18.36^{abc}	0.024^{bc}	10.54^{a}	15.41^{a}	24.65^{c}	59.81^{b}	175.71^{b}	0.77^{a}
Emmer	2.16^{de}	10.49^{d}	0.37^{c}	6.19^{c}	146.58^{ab}	5.08^{b}	151.66^{ab}	56.53^{b}	21.02^{ab}	0.028^{ab}	10.35^{a}	14.84^{a}	24.18^{c}	55.44^{b}	168.52^{b}	0.77^{a}
Durum	2.42^{cd}	12.08^{cd}	0.48^{c}	7.56^{c}	178.24^{a}	5.30^{b}	183.53^{a}	66.83^{a}	21.27^{a}	0.033^{a}	8.48^{a}	15.81^{a}	27.39^{c}	59.72^{b}	170.22^{b}	0.75^{a}
Treatment	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.001	<0.0001	<0.0001	0.0007	<0.0001	0.9906	<0.0001	<0.0001	<0.0001	<0.0001	0.6607
Group	<0.0001	<0.0001	<0.0001	0.0048	0.0401	0.0517	0.0278	<0.0001	0.0029	0.0004	0.6600	0.4681	0.0010	0.8539	0.9907	0.4749
TxG	0.1891	<0.0001	<0.0001	0.4637	0.0046	0.0264	0.0175	0.1313	0.1572	0.0172	0.0100	0.0590	0.3777	0.4494	0.7526	0.4148

(TLA and SFW) and one root trait (PRL). In four cases, the interactions were marginally significant (LRL, TRL, RDW, and RGR_r; Table 3).

Nitrogen effects

The strong effects of the nitrogen treatments on the phenotypic responses of all of the genotypes are shown in Fig. 2, where the two main principal components are presented. Indeed, all of the samples were subdivided by the first principal component, which explained 49% of the phenotypic variance in two highly differentiated groups that corresponded to the two nitrogen treatments. A further 21, 9, and 7% of the total variation was explained by the second, third, and fourth principal components, respectively. The others components were <5%. From visual observation, the plants under the optimal nitrogen treatment appeared healthy, while those under the nitrogen-starvation treatment showed chlorosis symptoms on the leaves.

Overall, the nitrogen starvation induced reductions in growth for shoot-related traits, while the opposite effect was seen (a growth increase) for root-related traits, with the exception of LRL (Fig. 3). At harvest (28 DAS), nitrogen starvation had decreased SFW by a mean of 68%, while it increased RDW by 66% (Fig. 3). Similar decreases were obtained for TLN and TLA with nitrogen starvation, with an overall genotype reduction at 17 DAS of 21 and 40%, respectively. At 17 DAS, again for the combined genotypes, nitrogen starvation increased PRL by 50%, TRL by 44%, RSD by 39%, and RSW by 17% compared with the optimal nitrogen treatment (Fig. 3). In contrast to the other root traits, under nitrogen starvation LRL was significantly lower (by 31%) compared with the combined LRL of roots grown under the optimal condition. At 17 DAS, nitrogen starvation led to a strong and statistically significant increase in root:shoot ratio across the combined genotypes, measured as TRL:TLA, which was up to 248% (Fig. 3, Table 3).

Considering the photosynthesis-related traits, for the combined genotypes, SPAD was significantly reduced under nitrogen starvation, by 24%, compared with the optimal nitrogen condition. Similarly, the light-adapted fluorescence parameters ETR_{max} and $PPFD_{sat}$ of the leaves were significantly reduced by nitrogen starvation. At 27 DAS, ETR_{max} and $PPFD_{sat}$ of the seedlings under nitrogen starvation were significantly lower than for the optimal nitrogen seedlings (54 and 48%, respectively; $P<0.001$). Conversely, the nitrogen starvation treatment did not affect the Fv/Fm ratio, which measures the intrinsic efficiency of PSII photochemistry in the dark-adapted state (Fig. 3, Table 3).

Primary domestication and secondary domestication effects

Significant phenotypic variations were observed among the domestication groups (wild emmer, emmer, and durum wheat) for most of the analysed traits (Table 3). Considering the traits for which the effects of the domestication groups were significant, an overall increase was observed for all of the shoot and root growth traits from the wild emmer to the durum wheat, with the emmer generally very similar to the

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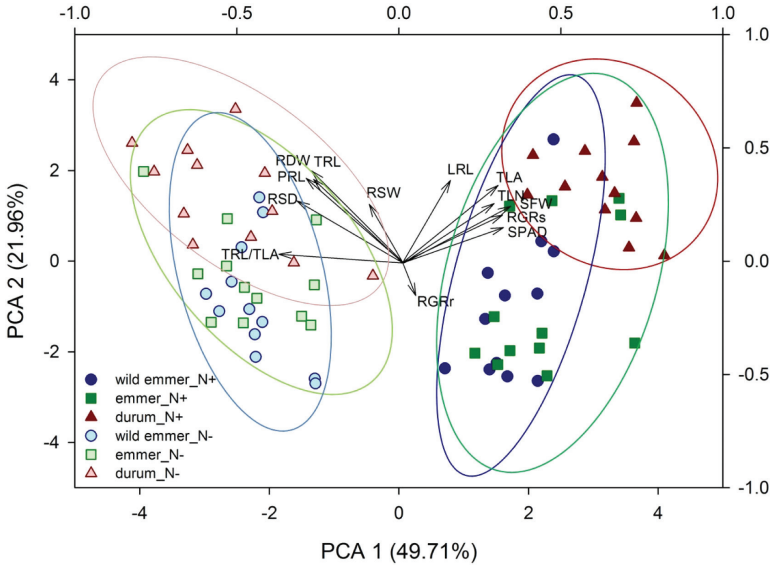

Fig. 2. Principal component analysis (PCA) of the morphological and physiological data obtained from wheat plants grown under optimal nitrogen (N+) and nitrogen-starvation (N–) conditions. Data shown are for 12 genotypes per group, with four replicates, as means over the two experiments. Arrows indicate the loadings for each trait along the first two components, which comprised 72% of the total genetic variation for 13 traits (for abbreviations, see text and Table 2).

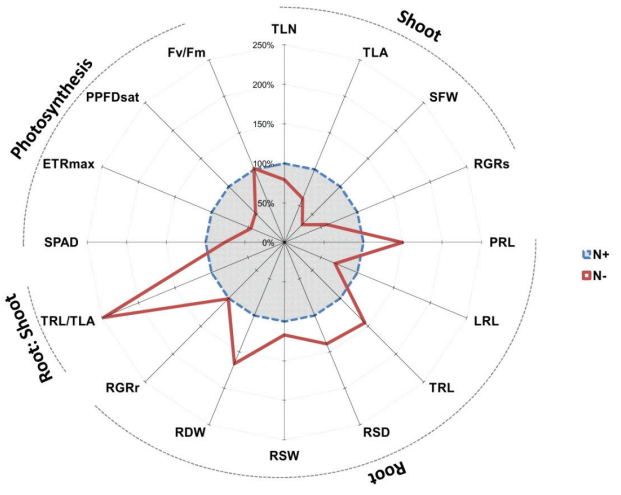

Fig. 3. Effects of optimal nitrogen (N+) and nitrogen-starvation (N–) conditions on the 36 wheat genotypes analysed in this study. Treatment effects are visualized for comparison by plotting the relative responses of each morphological and physiological trait in a radar chart, where each trait is represented by a spoke of a wheel (for abbreviations, see text and Table 2). The size of each parameter for the optimal nitrogen conditions was set to 100% (grey central area), and the changes in the traits induced by nitrogen starvation are given as red lines departing from the 100% circle depicted as a blue dotted line. SFW and RDW were measured at 28 DAS, SPAD, ETR_{max}, $PPFD_{sat}$, and Fv/Fm at 27 DAS, and all of the other traits at 17 DAS.

wild emmer, or intermediate between these two subspecies (Table 3). Indeed, while no significant differences between the wild emmer and emmer could be found for both optimal nitrogen and nitrogen starvation, in several cases durum wheat showed enhanced growth compared with wild emmer, and for a few of the traits, also with emmer. For shoot traits (TLA and SFW), under optimal nitrogen, wild emmer and emmer showed significantly lower growth compared with durum wheat, while under nitrogen starvation no differences were observed among these wheat groups. For PRL, under optimal nitrogen there were no differences between the wheat groups, while under nitrogen starvation the wild emmer showed significantly lower PRL than the durum wheat, with the emmer being intermediate and not significantly different from either the wild emmer or the durum wheat.

The shoot and root growth dynamics measured over 17 d of vegetative growth showed differences over time among the treatments and domestication groups that were comparable to those described above for the final shoot and root traits (Fig. 4). For most traits, the treatment and domestication effect increased over time (Fig. 4), but for the lateral root development under optimal nitrogen, durum wheat showed accelerated growth of laterals after 13 DAS in comparison with wild emmer and emmer.

Effects of domestication phenotypic diversity under the contrasting nitrogen treatments

The CV_A for all of the traits measured ranged from 2 to 58% under optimal nitrogen, and from 2 to 66% under nitrogen starvation (Table 4). The highest CV_A values were obtained for LRL for both nitrogen treatments (58 and 64%, respectively), while the lowest was observed for Fv/Fm (2% under both nitrogen treatments). Overall, under optimal nitrogen, durum wheat showed less variation, as measured by the CV_A, than the wild emmer and emmer (Wilcoxon rank-sum test: $P<0.05$), although there were no significant differences observed under nitrogen starvation (Table 4). While the differences in the phenotypic diversity due to the effects of primary domestication (i.e. wild emmer versus emmer) were small and never significant, for secondary domestication (i.e. emmer versus durum wheat), the transition from emmer to durum wheat was marked by a large and significant decrease in CV_A. In particular, this reduction was 3.4-fold greater under optimal nitrogen compared with nitrogen starvation, where the

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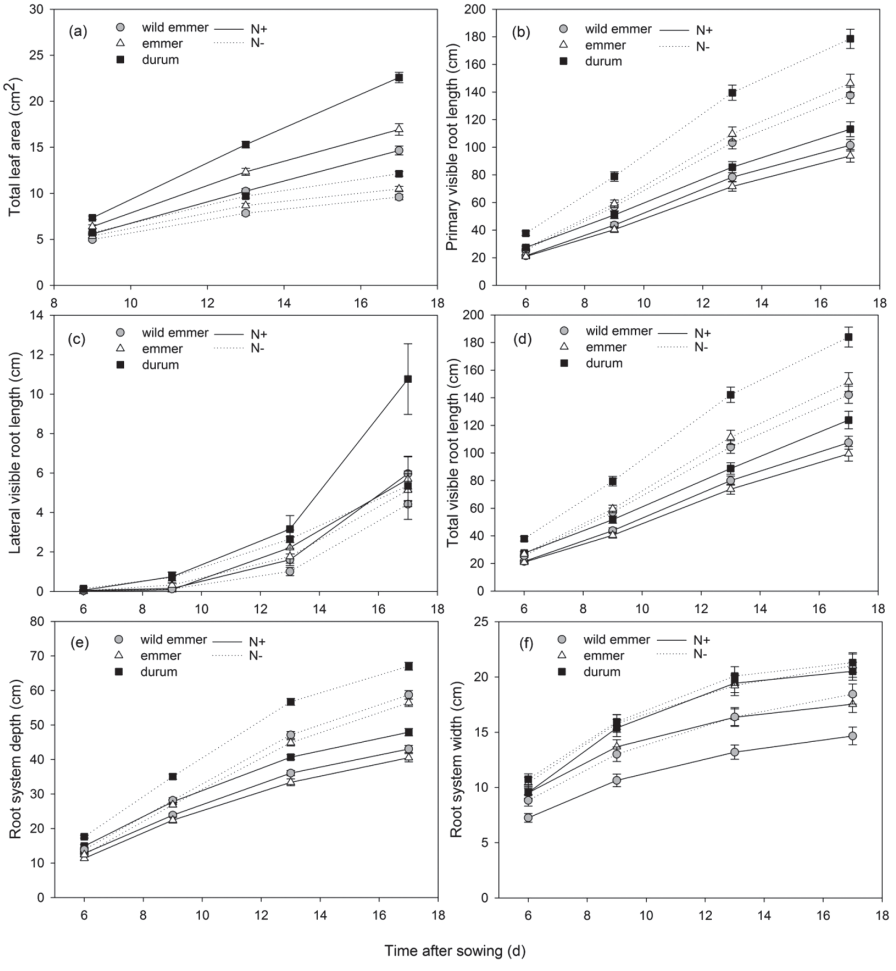

Fig. 4. Effects of domestication on shoot and root growth dynamics under optimal nitrogen (N+) and nitrogen-starvation (N–) conditions for development of projected TLA (a), PRL (b), LRL (c), TRL (d), RSD (e), and RSW (f). Each value represents the mean±standard error.

7% reduction was not statistically significant (Wilcoxon rank-sum test: $P>0.05$; Table 5).

When considering shoot-related and root-related traits separately, under optimal nitrogen condition, these shoot and root traits showed significantly reduced diversity during secondary domestication (ΔCV_{Asd} of 51 and 23%, respectively), while under nitrogen starvation the reduced diversity was significant only for the shoot traits (ΔCV_{Asd} of 23%, Wilcoxon rank-sum test: $P<0.05$; Table 5).The mean seed weight (±standard error) of the three domestication groups was 40.02 (±0.84) mg per seed for wild emmer, 41.68 (±0.67) mg per seed for emmer, and 62.75 (±0.52) mg per seed for durum. Taking into account this result, and to determine whether the variation in seed weight has a role in root and shoot development, correlations between seed weight and root traits were also carried out (Supplementary Table S1, available at *JXB* online). The correlations between seed weight and root traits (LRL, PRL, TRL, RSD, RSW, and RDW) of the seedlings were small and negligible under both optimal and nitrogen-starvation treatments. This result is in agreement with previous reports that have suggested that seed size has a negligible influence on root length in wheat (Sanguineti *et al.*, 2007). Additionally, Sanguineti *et al.* (2007) showed only a very few chromosome regions that influenced both seed weight and root traits, thus indicating a prevailingly independent genetic basis for root traits and seed weight. Moderately high coefficients of correlation were found only for shoot traits (TLN, TLA, and SFW).

Discussion

In the current study, the phenotypic diversity of tetraploid wheat domestication was analysed under contrasting levels of nitrogen fertilization, to determine the changes that occurred during primary and secondary domestication evolutionary processes, including modern breeding. Previous investigations of phenotypic diversity in wheat have typically focused on the above-ground plant traits, or have considered subspecies of tetraploid wheat separately. There has been little (if any) characterization of the root phenotypic architecture of emmer and durum wheat compared with their wild emmer tetraploid progenitors. Wild emmer and emmer are recognized as sources of potential traits to be introduced into current wheat breeding programmes (Reynolds *et al.*, 2007; Laidò *et al.*, 2014). Therefore, in combination with a deep genomic characterization, the phenotypic diversity of wild emmer and emmer needs to be described and measured, in terms of different environmental conditions and using a

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Table 4. *CV_A values of the measured traits in the wheat domestication groups under optimal nitrogen and nitrogen-starvation conditions*

Trait		CV_A							
		Optimal nitrogen				Nitrogen starvation			
		Wild emmer	Emmer	Durum	Overall	Wild emmer	Emmer	Durum	Overall
Shoot	TLN	0.14	0.10	0.04	0.10	0.06	0.09	0.14	0.10
	TLA	0.23	0.25	0.14	0.21	0.23	0.16	0.13	0.17
	SFW	0.27	0.28	0.18	0.24	0.22	0.22	0.14	0.19
	RGRs	0.21	0.29	0.10	0.20	0.24	0.26	0.15	0.22
Root	PRL	0.23	0.21	0.23	0.22	0.23	0.20	0.22	0.21
	LRL	0.60	0.71	0.43	0.58	0.64	0.61	0.66	0.64
	TRL	0.25	0.21	0.22	0.23	0.23	0.20	0.21	0.21
	RSD	0.12	0.10	0.10	0.11	0.07	0.08	0.09	0.08
	RSW	0.21	0.22	0.18	0.21	0.19	0.20	0.16	0.18
	RDW	0.29	0.30	0.12	0.24	0.19	0.21	0.11	0.17
	RGRr	0.22	0.19	0.21	0.21	0.23	0.20	0.23	0.22
Root:shoot	TRL:TLA	0.15	0.23	0.27	0.22	0.12	0.16	0.29	0.19
Photosynthesis	SPAD	0.09	0.06	0.07	0.07	0.12	0.14	0.08	0.11
	ETR_{max}	0.56	0.36	0.33	0.42	0.35	0.56	0.47	0.46
	$PPFD_{sat}$	0.48	0.34	0.31	0.38	0.35	0.48	0.40	0.41
	Fv/Fm	0.01	0.03	0.01	0.02	0.02	0.01	0.02	0.02
Overall	Mean	0.25	0.24	0.18	0.23	0.22	0.24	0.22	0.22

Table 5. *Loss of phenotypic diversity for shoot- and root-related traits during the primary domestication (ΔCV_{Apd}) and secondary domestication (ΔCV_{Asd}) processes, under optimal nitrogen and nitrogen starvation treatments*

Treatment	Trait	ΔCV_A (%)	
		ΔCV_{Apd}	ΔCV_{Asd}
Optimal nitrogen	All	5	24[a]
	Shoot	–8	51[a]
	Root	–1	23[a]
Nitrogen starvation	All	–7	7
	Shoot	4	23[a]
	Root	5	1

[a]$P<0.05$ by Wilcoxon rank-sum test (two-sided alternative).

wide range of phenotyping approaches, from morphology to molecular phenotyping (e.g. metabolomics), if these are to be used effectively in breeding and management programmes for wheat.

Phenotypic variation in relation to nitrogen supply

Overall, nitrogen starvation induced reductions in the growth parameters of the shoot-related traits for all of the genotypes analysed. In turn, this effect was linked to both a decreased rate of leaf emergence and reduced leaf elongation rates compared with the optimal nitrogen level. Effects of growth-limiting nitrogen availability on traits relating to shoot growth and development have been observed in previous studies (Waring *et al.*, 1985; McDonald, 1992). However, in contrast to the shoot traits, nitrogen starvation increased all of the quantified root-related traits except for LRL, with a resulting strong increase in the root:shoot ratio under nitrogen starvation.

On average, the root biomass was more affected by nitrogen starvation than the root length. Changes in specific root length under stress conditions can be due to either lower mean root diameter or root tissue density, or to the formation of aerenchyma. Aerenchyma formation in the root cortex can decrease root tissue density, and increase specific root length (Zhu *et al.*, 2010). In particular, induction of root aerenchyma has been proposed to increase plant performance and to improve carbon economy under nitrogen stress (Zhu *et al.*, 2010; Saengwilai *et al.*, 2014).

According to functional biomass equilibrium, in limiting environments, plants allocate more biomass to roots when the factor limiting the growth is below ground (e.g. water, nutrient shortage), to potentially enhance the uptake of that limiting factor (Poorter *et al.*, 2012). The root is devoted to mineral nutrient acquisition, and it is the first organ that senses and signals mineral starvation. Passioura (1983) provided substantial evidence that the assimilation product consumed by the roots is twice that of the shoots. Thus, crops with lower root:shoot ratios, which will partition more dry matter to the above-ground biomass, have higher grain yields (Zhang *et al.*, 2009). Here, we showed that optimal nitrogen treatment reduced the root:shoot ratio, which suggests that a suitable nitrogen supply can accelerate growth and development of above-ground parts, which is beneficial for producing high-yielding wheat. However, under nitrogen-starvation conditions, growth was reduced in the above-ground parts of the wheat plants, while growth of the roots increased. Similarly, greater root:shoot ratios have been reported in wheat genotypes in response to a low nitrogen supply (Welbank *et al.*, 1973; Barraclough *et al.*, 1989).

Another general response of wheat roots to nitrogen starvation is an increase in rooting depth through the allocation of more carbon assimilates to the roots and the promotion root

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elongation, so that the roots can capture the downward-moving nitrates in the soil (Scheible *et al.*, 1997; Remans *et al.*, 2006). In the present study, nitrogen starvation enhanced the root elongation of seminal roots and especially enhanced the rooting depth, while it decreased the lateral root development to some extent. This mechanism has been observed to varying degrees in a wide range of plant species, and it serves as an important step in the ability of the plant to compete with its neighbours for a limited supply of nutrients (Ericsson, 1995). Recently, a model analysis suggested that a relative low density of lateral roots is advantageous for nitrogen uptake efficiency (Postma *et al.*, 2014). This increase in nitrogen uptake efficiency is mostly explained by decreased root competition and increased soil exploration, and partly by steeper rooting, when the density of lateral root branching decreases (Postma *et al.*, 2014).

Primary domestication and secondary domestication

Our data indicated that, for all of the traits analysed, domesticated emmer did not show significant differences from wild emmer, which suggests that primary domestication did not affect shoot and root traits under either optimal nitrogen or nitrogen-starvation conditions. The significant group effects shown by the considered traits suggest that the secondary domestication process has modified the wheat plant architecture markedly, while the primary domestication effects were marginal.

The absence of any primary domestication effects was also suggested by the similar levels of diversity for all of the phenotypic traits measured according to the CV_A. This appears to be different from what was found for seminal roots of barley, where primary domestication effects were observed and wild barley showed shorter roots compared with landraces (Grando and Ceccarelli, 1995). In contrast to primary domestication, strong signals due to the secondary domestication effects of selection were seen for most of the analysed traits. This pattern can be explained by invoking the effects of selection during the development of durum wheat and in modern wheat breeding, by considering that all of the durum wheat genotypes are cultivars that were released after the beginning of the last century (i.e. the first durum variety Cappelli, which was released by Nazzareno Strampelli in 1915; Laidò *et al.*, 2013). The role of selection is also indicated by reductions in the phenotypic diversity due to the development of durum wheat cultivars (i.e. secondary domestication). Selection appears to have acted towards the general enhancement in growth responsiveness to nitrogen availability in durum wheat, which reflects the breeding process under greater soil fertility. Interestingly, the significant treatment-by-group effects suggest that durum wheat responds strongly to high nitrogen by developing an increased leaf area and increasing shoot biomass. Also, an increased leaf area together with enhanced photosynthetic capacity from wild emmer to durum wheat might indicate improved nitrogen-uptake efficiency in durum wheat. To evaluate this hypothesis, the nitrogen efficiency needs to be analysed. However, the observed differences in shoot biomass can also be partially associated with a larger seed size or with the shorter growth cycle of durum wheat compared with emmer and wild emmer (Laidò *et al.*, 2013).

Thus, overall, our data indicated that secondary domestication episodes of breeding have greatly affected the plant architecture, in terms of both shoots and roots, which has promoted the ability of the plants to increase their growth under high nitrogen fertilization, and, to a lesser extent, to increase the above-ground growth also under low nitrogen availability. In contrast, tetraploid wheat primary domestication did not substantially influence plant growth, at least in the very early stages, as seen here for both the optimal nitrogen and the nitrogen-starvation regimes. Patterns of phenotypic variation can change throughout the developmental stages of individual plants. A further analysis of the phenotypic consequences of the genetic changes that occurred during primary domestication and secondary domestication breeding episodes needs to be conducted on different growth stages and also under field conditions, to fully determine their effects on plant adaptation and yield under different soil and crop management conditions.

The wild emmer, emmer and durum wheat genotypes included in the present study varied markedly and consistently in their total above-ground traits. In contrast, the differences in root traits among the three domestication groups, if any, were relatively small and inconsistent. However, when we examined the CV_A, we found that the three groups had different CV_A values for both shoot and root traits, with wild emmer and emmer showing the greatest CV_A. These results indicate that there is considerable genetic variation in the studied phenotypic traits in wild and landrace germplasm, which can potentially be exploited for the development of pre-breeding programmes. Similar to other studies, our findings support the view that there is a need for conservation evaluation and use of wild progenitors and landrace populations for further crop improvements. Indeed, the occurrence of higher genetic diversity for phenotypic traits suggests that wild germplasm and landraces have strong potential to harbour many genes that might be the targets of selection to modify the phenotypic architecture of improved varieties.

Supplementary data

Supplementary data are available at *JXB* online.

Supplementary Table S1. Pearson correlation coefficients between seed weight and root and shoot traits in wheat domestication groups.

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