Differential plasticity to water and nutrients between crops and their wild progenitors

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A B S T R A C T

Crop domestication has resulted in relevant phenotypic divergences between crop plants and wild progenitors, but domestication effects on phenotypic plasticity are poorly known. We grew plants of domesticated and wild progenitor accessions of seven taxonomically-diverse crops in three experimental glasshouse treatments differing in soil water and nutrient availabilities, and measured growth, morphological and physiological traits that are key in plant responses to water and nutrient limitations. We compared growth performance, trait means, and norms of reaction between domestication statuses and crop species. Phenotypes were significantly affected by the experimental treatments. Under low water and low nutrient availabilities, plants decreased specific leaf area to produce smaller and thicker leaves, and decreased stomatal conductance. Plasticity to changes in water availability was more pronounced than to varying nutrient supply. Domesticated and wild accessions significantly differed in plasticity patterns to limiting water for several key traits (maximum height, total leaf area, plant-level photosynthetic rate and growth performance traits). Our results thus provide initial insight into generalized domestication effects on phenotypic plasticity. Importantly, crop plants outperformed wild plants in favorable conditions, but suffered a higher loss of performance under water stress, which contrasts with studies suggesting that selection for high performance in optimum conditions might also improve it in stressful environments. We propose that the greater phenotypic homeostasis of the wild progenitors may be a target for future breeding focused on selection for adaptive norms of reaction in heterogeneous environments.

1. Introduction

The domestication of wild plants has resulted in remarkable phenotypic differences between current crop cultivars and their progenitors. Evolution under cultivation involved changes in plant physiology, morphology and reproduction (see e.g. Fuller, 2007; Gepts, 2010; Meyer et al., 2012; Meyer and Purugganan, 2013; Milla et al., 2014, 2015; Preece et al., 2017; Turcotte et al., 2017). Domestication bottlenecks might also result in increased phenotypic uniformity (Meyer and Purugganan, 2013), which results in constant phenotypes in different environments (Makumburage and Stapleton, 2011). Phenotypic constancy might benefit agricultural performance (Makumburage and Stapleton, 2011). However, constancy also constrains the ability of crop plants to adjust to environmental heterogeneity if the optimum phenotype varies across environments, i.e. if a plastic phenotype is advantageous (de Visser et al., 2003). Although our understanding on how plant phenotypes change after domestication has increased over the last decades, the effects of domestication on phenotypic plasticity remain scarcely explored.

Plants respond to environmental conditions by altering their phenotypes. Although once regarded as environmental noise, it is now clear that (adaptive) phenotypic plasticity involves adjustments to express functionally appropriate phenotypes in contrasting conditions (Sultan, 1995, 2003). The importance of phenotypic plasticity for the adaptation of crop plants to environmental heterogeneity was recognized early (Bradshaw, 1965). Bradshaw noted that yield stability across environments could be driven by plasticity of traits underlying yield. Accordingly, when environmental conditions are variable, functional adaptive plasticity contributes to fitness (yield) homeostasis, and to the maintenance of genetic diversity (Byers, 2005; Matesanz et al., 2010; Nicotra et al., 2010).

Despite its relevance, plasticity and genotype by environment interactions have traditionally been perceived as an obstacle to produce crops with constant phenotypes in alternative environments (Bloomfield et al., 2013; Blum, 2005; Sadras et al., 2009). Indeed, increased phenotypic robustness, i.e. decreased phenotypic plasticity, has been a target of breeding (de Visser et al., 2003; Makumburage and Stapleton, 2011; Semchenko and Zobel, 2005, but see Khush, 2001).

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Recently, however, several authors have highlighted the importance of considering the benefits of adaptive plasticity while breeding, in part due to the pressing need to understand crop responses to increased human-created environmental variability (Larson et al., 2014; Mercer and Perales, 2010; Nicotra and Davidson, 2010; Sadras and Denison, 2016; Sadras and Richards, 2014). Understanding how plasticity has evolved during domestication provides necessary baseline knowledge in this context (Larson et al., 2014).

The onset of domestication is often accompanied by strong genetic bottlenecks that can reduce genetic diversity (Doebley et al., 2006; Gepts, 2010; Meyer and Purugganan, 2013). Plasticity is a trait subject to evolutionary change and, as such, requires the presence of genetic variation to evolve. Therefore, genetic bottlenecks might have resulted in decreased plasticity of crops if genetic variation for plasticity was impoverished during domestication (see e.g. Gallardo et al., 1996; Mack, 1996; Piperno et al., 2015). Alternatively, increased diversity linked to later crop diversification (Meyer and Purugganan, 2013) may provide opportunities for the evolution of plasticity in crops. Furthermore, plasticity may have evolved during domestication as a by-product of trait selection, if trait means and their plasticities are genetically correlated (Hoffmann and Merila, 1999; McKey et al., 2012; Sadras and Denison, 2016; Scheiner and Lyman, 1989). To date, the few single-species studies comparing norms of reaction of crop domesticates and their wild progenitors have not provided a clear or robust pattern on the effects of domestication on phenotypic plasticity (Niénard et al., 2013; Schlichting and Levin, 1988; Shimizu et al., 2010).

In this study, we assessed changes in phenotypic plasticity after the domestication of seven taxonomically diverse crops. To study plasticity, the most customary and robust approach is to conduct experiments aimed at depicting the norms of reaction of plant traits. In such experiments, replicates of different genotypes are grown in a set of contrasting, ecologically relevant environments (Pigliucci, 2001; Sultan, 2011). We implemented a norm-of-reaction approach where we compared trait means and trait variation of crop domesticates and their wild progenitors across experimental treatments reflecting variations in two environmental factors key in agricultural environments (water and nutrient supply). We measured morphology and physiology traits related to plant response to water and nutrient limitations, and growth performance. We hypothesize that evolution under cultivation has altered phenotypic plasticity for key functional traits of crop plants. Specifically, we addressed the following questions: i) do crop domesticates and their wild progenitors show similar functional plasticity in response to nutrient and water availabilities; ii) is there evidence of changes in plasticity during domestication? and iii) is the evolution of trait means coupled to the evolution of norms of reaction?

2. Materials and methods

2.1. Study system and experimental treatments

We selected seven taxonomically diverse herbaceous crops for this study: chard (Beta vulgaris L.), cabbage (Brassica oleracea DC.), sunflower (Helianthus annuus L.), tomato (Solanum lycopersicum L.), durum wheat (Triticum durum Desf.), maize (Zea mays L.), and pea (Pisum sativum L.). This sample includes a group of crops of diverse geographic origin and for which the onset of domestication dates back 10000-2000 yr (Hancock, 2012; Sauer, 1993; Smith, 2008). For each crop species, we obtained seed lots of two accessions: one of a cultivated variety of the crop (domesticated, D) and one of its recognized wild progenitor (W; see Table 1 for accessions identifiers and seed donors). In February 2014, ca. 100 seeds of each accession were set to germinate in sterilized Petri dishes soaked in distilled water and maintained at 4 °C. Plants from all accessions included in the experiment germinated within 15 days after sowing. When the radicle emerged from the testa, 42–54 germinated seeds of each accession, except for the pea domesticated accession (see Table S1), were transplanted to single-plant pots and were randomly assigned to each of three treatments (see below). The size of the experiment was 644 plants. See table S1 in Supplementary information for number of replicates per accession and treatment.

Plants were grown for eight wk. (March to May 2014) in three treatments differing in soil water and nutrient availability: a high-resource (Control) treatment, a moisture-limited (Low Water) treatment, and a nutrient-limited (Low Nutrients) treatment. All pots (1.4 L; 10 × 10 × 17 cm) were filled with sand and supplemented with slow release fertilizer (Basacote Plus 6 M, Compo). Pots in the Control and Low Water treatments were supplemented with 11.2 g of fertilizer (8 g/L), whereas pots in the Low Nutrient treatment received 2.8 g (2 g/L). These amounts of fertilizer were set to grow plants under high and low nutrient availability conditions, respectively (Day et al., 2003). Soil moisture was maintained in the different treatments by supplying tap water through sprinklers. Pots in the Control and Low Nutrient treatments were watered 3–4 times per week, to maintain soil water content at 90–100% of field water holding capacity (16.57% ± 0.35 SWC, based on soil samples from individual pots measured at two time points during the experiment). Pots in the Low Water treatment were initially watered in the same fashion to ensure plant establishment after transplant, and watering was progressively reduced over three weeks. After this period, plants in this treatment were watered once or twice per week, achieving average moisture levels of ~40% of field capacity (6.84% ± 1.14). The Low Water and Low Nutrient treatments thus represent substantial environmental stresses compared to the favorable Control, and were designed to reflect the spatial and temporal environmental heterogeneity inherent to both natural and agricultural environments. The implementation of the experimental treatments guaranteed that all plants within a treatment were subjected to the same growing conditions. All pots of the experiment were arranged in two contiguous glasshouse benches at Universidad Rey Juan Carlos (Móstoles-Madrid, central Spain), and received full sun (mean midday photosynthetically active radiation [PAR] ∼1100 µmol m−2 s−1, measured in three consecutive clear days of the experiment).

2.2. Data collection

2.2.1. Physiology traits

Physiological measurements were taken on a subsample of 4–6 individuals per accession and per experimental treatment (N = 212). Data were collected between 10 and 13 h during three homogeneously sunny days (28th–30th April 2014; cloud cover below 10% in all days). Intrinsic photosynthetic rate (µmol CO2 m−2 s−1) and stomatal conductance (mol H2O m−2 s−1) were measured in one fully-expanded leaf per plant using a LiCor 6400 infrared gas analyzer with built-in LED light source and CO2 mixer (LI-COR, Lincoln, NE, USA), using the leaf chamber fluorometer. Measurements were taken using a reference [CO2] of 400 ppm, PPFD of 1200 µmol m−2 s−1 (set to mimic light levels in the glasshouse), and gas flow of 500 µmol s−1. Relative humidity was kept constant and close to ambient conditions (humidity range: 20–40%); air temperature ranged from 20 to 30 °C. Measurements were logged only when the stability criteria were met (Licor 6400 User’s Manual, Li-COR Inc.). When the leaf did not completely cover the chamber, leaf tracings were scanned and leaf area was calculated using ImageJ software (http://imagej.nih.gov/ij/). Intrinsic water use efficiency (IWUE, µmol CO2 mol−1 H2O) was calculated as the ratio of photosynthetic rate to stomatal conductance.

2.2.2. Morphology and allocation traits

Plant height (cm) was measured for all plants three times (2nd, 16th and 23rd April) throughout the experiment (N = 644). Maximum height was plant height (cm) at harvest time. After eight weeks of growth, the aboveground parts of 9–12 plants per accession (except for Pisum D (3–8 replicates), and Beta W and Zea W in the Low Water treatment (6 replicates); N = 426 plants total) were harvested during five consecutive days. We set our harvest time (eight wk.) so that all plants in...
the experiment had undergone significant vegetative growth, but prior to any of them switching to reproductive development. Harvest was thus conducted when the first flower buds or signals of bolting were observed in the earliest flowering species (wild and domesticated accessions of tomato and wheat). All replicates of each domesticate-wild pair were harvested on the same day. Before harvesting, leaf area (cm²) was measured as the one-side area of a fresh leaf lamina per individual, scanned at 400 dpi resolution, and analyzed using ImageJ software. Leaf thickness (mm) was measured in the same leaf using a dial thickness gauge (Mitutoyo Co., Aurora, IL, USA). These leaves were scanned at 400 dpi resolution, and analyzed using ImageJ software. Leaf density (g cm⁻³) was calculated as leaf mass/leaf area x leaf thickness. All remaining aboveground tissues were harvested, separated and bagged, oven-dried as described above, and weighed to determine leaf (all leaf laminae) and stem (petioles and stems) biomass. The ratio of total leaf laminae mass to mass of petioles plus stems (leaf to stem mass ratio, unitless) was computed as a proxy for proportional investment in leaf blades versus structural support. Whole-plant total estimated leaf area (TELA, cm²) was calculated as SLA x total leaf laminae mass of each individual plant. Plant-level instantaneous photosynthetic rate (μmol CO₂ s⁻¹) was computed by multiplying the arithmetic mean of the photosynthetic rate of each accession and treatment by individual TELA. Root systems were manually washed and a subsample of roots < 2 mm in diameter was collected and scanned in Petri dishes filled with water. Root subsamples were then bagged, oven-dried and weighed. Scanned root images were converted to binary black and white and analyzed with WinRhizo (WinRHIZO Pro, Regent Instruments Inc., Quebec, Canada) to determine root length. Specific root length was calculated as the ratio of root length to its mass (mg⁻¹).

2.2.2. Performance traits

Absolute growth rate was calculated as the increase in height per unit time [AGR = (h₂-h₁)/(t₂-t₁)], where h₁ and h₂ are plant height at four (t₁) and six weeks (t₂) in treatment, cm day⁻¹]. Relative growth rate for the same time periods was calculated as the increase in plant height per unit time, but accounting for initial size [RGR = (ln h₂-ln h₁)/(t₂-t₁), cm⁻¹ day⁻¹]. Aboveground biomass per plant (g) was computed as the sum of all aerial plant fractions at harvest time.

2.3. Data analyses

True outliers were identified using box plots stratified per trait, treatment and accession, and were categorized as such if data points exceeded lower or upper generous line bounds (extending 3*IQR range out of Q1 or Q3) of the box plot. A total of 12 plants were removed. Afterwards, data were squared-root or log-transformed to meet assumptions of statistical models conducted afterwards. In order to assess plasticity to water and nutrient availabilities separately, the database was rearranged in two datasets: data from Control and Low Water treatments, and data from Control and Low Nutrient treatments. To assess the plastic response to either water or nutrient limitations, general linear models (glm) were performed for each individual trait, with Domestication status (D or W), Crop Identity (tomato, sunflower, maize, durum wheat, cabbage, chard and pea), and Treatment (Control vs. Low Nutrient or Control vs. Low Water), plus all possible interactions, as fixed factors. A significant main treatment effect was taken as evidence for plasticity in the trait. A significant main effect of Crop ID and Domestication status indicated overall mean differences among crop species and domestication statuses, respectively, in functional traits. A significant Domestication × Treatment interaction indicated differences in plasticity between crop domesticates and their wild relatives, i.e. non-parallel norms of reaction for a given trait. The Treatment × Crop ID interaction indicated differential overall responses among crop species to the treatments, i.e. crops species have different abilities to express plasticity. A significant Crop ID × Domestication status term indicates that potential differences between domesticates and wild accessions vary across crops. Finally, the triple Treatment × Crop ID × Domestication interaction indicates an idiosyncratic response, where wild and domesticated accessions within each crop show differential responses to the treatments. Traits showing significant differences in their means but similar plasticity between domesticated and wild plants or those showing differential plasticity but similar trait means are taken as evidence of independent evolution of trait means and plasticity.

When the Domestication or Domestication × Treatment terms were significant, individual analyses were performed to test for the fixed effect of Domestication status and Crop ID (and their interaction) within each treatment. Additionally, to assess the magnitude of the plastic response, unbiased Cohen’s d were computed for each trait as the difference between trait means in each treatment divided by the pooled standard deviation (Cohen, 1988). All the analyses were performed in Statistica 8.0 (Tulsa, OK, USA).

3. Results

Phenotypic expression was significantly affected by environmental treatment, both for varying nutrient and water availabilities.
Table 2  
Plasticity to nutrient availability. Effects of nutrient treatment (Low Nutrient vs. Control), Domestication status (domesticated vs. wild), Crop identity (maize, wheat, tomato, sunflower, cabbage, chard and pea), and their interactions on functional traits. Statistical significance of each term in the model was assessed using Type III tests. Adjusted model R², F-ratios, degrees of freedom (df) and significance levels are shown. Bold figures are significant at P < 0.10; * P < 0.05, ** P < 0.01, *** P < 0.001. Residual degrees of freedom are 424, 269 and 115 for growth, morphological and physiological traits, respectively.

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<tbody>
<tr>
<td>Absolute growth rate</td>
<td>0.79</td>
<td>23.51***</td>
<td>41.27***</td>
<td>252.72***</td>
<td>4.88***</td>
<td>10.41***</td>
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<tr>
<td>Relative growth rate</td>
<td>0.79</td>
<td>82.14***</td>
<td>46.27***</td>
<td>161.90***</td>
<td>16.04***</td>
<td>40.90***</td>
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<tr>
<td>Aboveground biomass</td>
<td>0.63</td>
<td>51.29***</td>
<td>50.09***</td>
<td>52.63***</td>
<td>0.82</td>
<td>5.15***</td>
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<tr>
<td>Leaf area</td>
<td>0.87</td>
<td>84.37***</td>
<td>223.19***</td>
<td>250.61***</td>
<td>0.23</td>
<td>4.54***</td>
</tr>
<tr>
<td>Leaf thickness</td>
<td>0.81</td>
<td>44.85***</td>
<td>26.25***</td>
<td>148.94***</td>
<td>0.21</td>
<td>13.29***</td>
</tr>
<tr>
<td>Leaf density</td>
<td>0.71</td>
<td>0.38</td>
<td>12.10***</td>
<td>104.16***</td>
<td>0.67</td>
<td>3.60***</td>
</tr>
<tr>
<td>Maximum Height</td>
<td>0.90</td>
<td>22.13***</td>
<td>89.56***</td>
<td>622.24***</td>
<td>0.00</td>
<td>7.64***</td>
</tr>
<tr>
<td>TELA</td>
<td>0.57</td>
<td>97.78***</td>
<td>10.99***</td>
<td>38.58***</td>
<td>0.05</td>
<td>3.64***</td>
</tr>
<tr>
<td>Leaf-stem mass ratio</td>
<td>0.86</td>
<td>7.01**</td>
<td>86.27***</td>
<td>150.15**</td>
<td>1.46</td>
<td>4.91***</td>
</tr>
<tr>
<td>SLA</td>
<td>0.52</td>
<td>35.14***</td>
<td>0.2</td>
<td>29.75**</td>
<td>0.00</td>
<td>3.03**</td>
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<tr>
<td>SRL</td>
<td>0.48</td>
<td>0.06</td>
<td>0.12</td>
<td>36.96***</td>
<td>0.00</td>
<td>4.19***</td>
</tr>
<tr>
<td>Photosynthetic rate</td>
<td>0.35</td>
<td>46.45**</td>
<td>0.263</td>
<td>4.15***</td>
<td>0.11</td>
<td>1.18</td>
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<tr>
<td>Stomatal conductance</td>
<td>0.41</td>
<td>11.37**</td>
<td>0.376</td>
<td>15.23***</td>
<td>0.06</td>
<td>0.91</td>
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<tr>
<td>Water use efficiency</td>
<td>0.56</td>
<td>0.305</td>
<td>0.651</td>
<td>32.85***</td>
<td>0.05</td>
<td>1.08</td>
</tr>
<tr>
<td>Plant-level photosynthetic</td>
<td>0.76</td>
<td>385.42**</td>
<td>22.46***</td>
<td>56.14***</td>
<td>0.19</td>
<td>9.39**</td>
</tr>
</tbody>
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(Tables 2 and 3; most Treatment effects significant at P < 0.001; Fig. S1). Except for three traits, treatment effect sizes (Cohen’s d) were larger for the response to low water conditions than to nutrient conditions (Fig. 1; see also mean squares of the Treatment effect in Table S2). For several key traits, these patterns of plastic response varied significantly among crop domesticates and their wild progenitors (Tables 2 and 3; note Domestication × Treatment interaction). The response of domesticated and wild accessions to the experimental treatments was similar across crop species (generally non-significant triple interaction; Tables 2 and 3; Figs. S2 and S3). Crop species differed largely in their phenotypic expression (Tables 2 and 3, significant Crop ID main effects).

3.1. Performance traits

Relative growth rate, absolute growth rate and aboveground biomass decreased in the Low Nutrient (by 38%, 12% and 52%, respectively) and Low Water treatments (by 40%, 31% and 54%, respectively) compared to the favorable conditions of the Control (Fig. 2). Except for RGR, the plastic response to nutrient variability was similar for domesticated and wild accessions (Table 2). In contrast, domesticated plants showed a larger reduction in RGR, AGR and aboveground biomass than wild plants as a response to moisture limitations (Table 3). As a result, performance did not differ between domesticated and wild accessions in the Low Water treatment (Figs. 2 and 3, Table S3). Crop species widely differed in their growth traits means (significant Crop ID main effect) and in their plasticity (significant Treatment × Crop ID interaction), but response patterns within domestication statuses were similar across crops (Figs. 3, S2 and S3).

3.2. Morphology and allocation traits

All morphological and allocation traits were affected by the experimental treatments, except for leaf density and SRL in response to nutrient limitation (Tables 2 and 3; Fig. 4). Plants from domesticated and wild accessions reduced maximum height similarly as a response to nutrient limitation (5% and 8% average decrease for wild and domesticates, respectively; Fig. 4, Table 2). That response was larger when
plants were water-limited, and differed significantly between domesticates (33% reduction in height) and wild relatives (19%; significant Domestication × Treatment interaction; Fig. 4, Table 3). As a result of this interaction, average differences in height between domesticated and wild plants (significant main Domestication effect; Tables 2 and 3) were less pronounced in the Low Water treatment (Fig. 4; Table S3).

Plants produced significantly smaller and thicker leaves in the Low Nutrient (28% reduction in size and 15% increase in thickness) and the Low Water treatments (29% and 5%, respectively) compared to the Control. For these traits, domesticated and wild accessions showed similar plasticity patterns, but trait means differed consistently between domestication statuses across treatments: domesticated plants had larger but thinner leaves in all conditions (Figs. 3 middle, 4 a and b; Table S3). Leaf density, although similar across treatments, was also generally higher for domesticated plants (Fig. 4g). Specific leaf area decreased as a response to nutrient (13%) and water-limiting (13%)

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**Fig. 1.** Effect size of the experimental treatments over trait expression. Dots and error bars are D-Cohen and 95% confidence intervals, averaged for the several crop species and domestication statuses included in the study. Plasticity to water and nutrients refers to the plastic response between the favorable Control and the Low Water and Low Nutrient treatments, respectively.

**Fig. 2.** Plant performance in the three treatments of the experiment, averaged across crops. Plots are norms of reaction of growth rates and final plant biomass to nutrient and water treatments for domesticated and wild accessions. Least square means ± 1 se. averaged for the seven species included in the study are shown. Different colors of the norm of reaction indicate a significant Treatment × Domestication interaction, i.e. domesticated and wild accessions differ in their plasticity; a) aboveground biomass, b) absolute growth rate and c) relative growth rate. Significant differences between domestication statuses within treatments are indicated by asterisks (see Table S3 for results of the models). The effect of treatment was significant in all cases (Tables 2 and 3). Note that the Y axis is scaled to 5–95% of data range.
conditions (Fig. 4e). Means as well as response patterns for this trait were similar between plants from domesticated and wild accessions (Tables 2 and 3).

Total leaf area (TELA) was sharply reduced in response to nutrient (46%) and water limitation (41%; Fig. 4c). TELA differences between domestication statuses varied from one treatment to another. Although domesticated plants had higher TELA in both Control and nutrient-limited conditions, the differential plasticity to water conditions between domesticated and wild plants led to equivalent TELA in the Low Water treatment (Fig. 4c; Table S3). Allocation to stem biomass moderately decreased from the Control to the Low Water (8%) and Low Nutrient treatments (22%, Fig. 4d). Plants from wild accessions allocated more biomass to leaves than those from domesticated accessions, and this was consistent across treatments, although the difference was more pronounced in the Low Water treatment (Table 3; Table S3). Indistinguishable trait means and plasticity for domesticated and wild accessions were also found for specific root length. Again, crop species widely differed in their trait means and their plasticity, but response patterns within domestication statuses were similar across crops (Figs. S2 and S3).

### 3.3. Physiology traits

Physiology traits differed among treatments, although the response to water stress was more pronounced (Tables 2 and 3; Figs. 1 and 5). Plants significantly reduced photosynthetic rate and stomatal conductance when nutrients and water were limiting (Fig. 5). Plants from wild and domesticated accessions responded similarly to nutrient limitations (30% vs. 32% reduction in photosynthetic rate and 23% vs. 26% reduction in conductance for domesticate and wild plants, respectively). However, plants from wild accessions were able to maintain significantly higher stomatal conductance (77% vs. 64% reduction in domesticate and wild plants, respectively) and marginally higher photosynthetic rate (73% vs. 60%) than domesticate plants as a
response to moisture limitations. Similarly, differences in plasticity between domesticated and wild plants led to marginally higher whole-plant photosynthetic rate of plants from wild accessions in the Low Water treatment (Table 3; Figs. 3 and 5; Table S3). The proportionally higher reduction in photosynthetic rate compared to that in stomatal conductance led to no significant differences in water use efficiency across treatments (Fig. 5). Crops species differed in the means of their physiological traits but generally showed a similar degree of plasticity (Fig. S3).

4. Discussion

Plants of our seven crops responded plastically to the experimental limitation of water and nutrient supplies. However, the extent of the plastic response, particularly to varying water conditions, differed between domesticated and wild accessions for key functional traits such as plant height, total leaf area and plant-level photosynthetic rate, as well as for all performance traits. Our study therefore provides the first evidence of changes in phenotypic plasticity during domestication for

Fig. 4. Norms of reaction of morphology and allocation traits to nutrient and water treatments for domesticated (black) and wild (white) accessions. Least square means ± 1 se, averaged for the seven species included in the study are shown. Different colors of the norm of reaction indicate a significant Treatment × Domestication interaction, i.e. domesticated and wild accessions differ in their plasticity. a) leaf area, b) leaf thickness, c) leaf density, d) maximum height, e) total estimated leaf area (TELA), f) leaf to stem mass ratio, g) specific leaf area and h) specific root length. Significant differences between domestication statues within treatments are indicated by asterisks (see Table S3 for results of the models). The effect of treatment was significant in all cases except for leaf density and SRL in response to nutrient variability. Note that the Y axis is scaled to 5-95% of data range.
Despite pronounced among-crop differences in trait means, and in the magnitude of plasticity, the effect of domestication on plasticity was similar across crops, which suggests that evolution under cultivation generally alters norms of reaction. Furthermore, our results show that the higher performance (i.e. growth and biomass production) of crops compared to wild plants in optimum conditions is not associated with higher performance in stressful environments, which may have important consequences for yield stability and breeding under environmentally-variable conditions (Mercer and Perales, 2010; Sadras and Denison, 2016).

4.1. Effects of domestication on phenotypic plasticity

Increased phenotypic uniformity – or robustness – is a common target of breeding programs, since it may decrease yield losses associated to among-plant variation in relatively constant environments (de Visser et al., 2003; Des Marais et al., 2013 Makumburage and Stapleton, 2011). Indeed, canalization, i.e. the reduced expression of phenotypic trait variation, of various phenotypic traits related to plant morphology and architecture has been described for several crops (Bloomfield et al., 2014; Ménard et al., 2013; Piperno et al., 2015). In our study, only leaf density remained canalized across all three environments. Plasticity was ubiquitous, and was highest for physiology traits (effect sizes \( d > 1 \)), consistent with their assumed high environmental liability and reversibility, and intermediate (effect sizes \( d < 1 \)) for growth, morphology and allocation traits (see e.g. Agrawal et al., 2008; Des Marais et al., 2013; Freschet et al., 2015; Nicotra et al., 2007; Poorter et al., 2010). Our results show that, although the magnitude of plasticity varied among traits, domestication has not resulted in a generalized loss of plasticity to diverse water and nutrient supplies. These results also agree with previous studies showing that several crops retain functional plasticity despite strong directional selection during the domestication process (see e.g. Bingham and Bengough, 2003; Ménard et al., 2013; Sadras et al., 2016, 2009; Yamori et al., 2010).

Despite their shared ability to respond plastically, domesticated and wild accessions significantly differed in plasticity patterns for several traits. In general, the direction of the plastic response (i.e., whether trait values decreased or increased across treatments) was common to all accessions. However, there was significant variation in the intensity of the response, particularly to varying water conditions. Domesticated and wild accessions showed non-parallel norms of reaction for all performance traits and for several key morphology and physiology traits. Crop plants were able to capitalize on the benign conditions of the control treatment, outperforming wild plants in terms of growth rates and carbon and biomass gain, which is consistent with crop evolution in high-resource agricultural environments (McKey et al., 2012; Milla et al., 2014). However, the domestication effect disappeared or even reversed in the low water treatment, where performance was mainly indistinguishable between domesticated plants and their wild counterparts. This master of some scenario (sensu Richards et al., 2006), where crops show higher responsiveness in favorable conditions and a large performance loss in stressful conditions, can have important implications. Provided the direct impact of size- and growth-related traits on crop yield in annuals (Weiner and Freckleton, 2010), our results show that the evolution of higher crop performance (and yield) in optimum conditions has not resulted in higher performance in water-limited conditions compared to wild plants. This is in conflict with other studies suggesting that selection for higher performance in optimum conditions may also improve yield in stressful environments (Araus et al., 2002; Blum, 2005; del Pozo et al., 2016; Sadras and Richards, 2014). Furthermore, the lower phenotypic homeostasis for performance traits of domesticated plants can compromise their response to increased environmental heterogeneity associated to climate change (D’Odorico and Bhattachan, 2012; Gao et al., 2006). Indeed, it has been proposed that higher environmental variation may select for higher functional plasticity (Alpert and Simms, 2002; Matesanz et al., 2010), and a few studies have experimentally confirmed this expectation (e.g. Lazaro-Nogal et al., 2015). In this context, our results suggest that wild relatives of common crops may be useful genetic resources for breeding strategies focused on promoting adaptive norms of reaction (Hajjar and Hodgkin, 2007; Milla et al., 2017, 2015; Nicotra et al., 2010).
Irrespective of changes in plasticity, domestication also had a significant effect on trait means across treatments. For several growth and functional traits, domestication led to the evolution of trait means without associated changes in plasticity patterns, particularly in response to nutrient variability. For example, leaf traits such as leaf area and density were consistently higher in domesticated plants across environments (see Fig. 4), with parallel norms of reaction to their wild relatives. These results confirm that, during domestication, trait plasticity can evolve independently of the evolution of the trait mean, and concur with other studies discussing potential constraints to the independent variation between them (Hoffmann and Merilä, 1999; Sadras et al., 2016; Scheiner and Lyman, 1989). For instance, Shimizu et al. (2010), in a study of plant architecture and biomass traits of wild and cultivated rice accessions, identified quantitative trait locus (QTLs) that controlled phenotypic plasticity without affecting phenotypic means. Importantly, our results open new research avenues for breeding programs targeting precise combinations of trait means and plasticities (Sadras et al., 2016; Sadras and Richards, 2014).

Previous work has extensively addressed the effects of domestication on functional traits, including resource-use, stomatal and yield-related traits. Only a few studies, however, have investigated the effects of domestication on phenotypic plasticity, mainly focusing on the molecular mechanisms underlying the plastic response ( Lukens and Doebley, 1999; Shimizu et al., 2010). The norm-of-reaction approach, where plasticity patterns of domesticated accessions and their wild relatives are compared, has seldom been used, and has always focused on individual case studies (Peleg et al., 2005; Schlichting and Levin, 1988; Shimizu et al., 2010). For instance, Gallardo et al. (1996) found a lower level of developmental plasticity in roots of wild lettuce compared to cultivated lettuce as a response to soil moisture heterogeneity. Conversely, Ménard et al. (2013) found that domestication has no effect on growth form plasticity to contrasting habitat conditions in domesticated and wild manioc. Altogether, these results are diverse and of limited generality due to the biological features of each crop and the idiosyncrasy of each domestication process. Our results thus provide evidence of a multi-crop change in plasticity during domestication, and constitute a first step forward towards a general understanding of domestication effects on phenotypic plasticity.

4.2. Differential plasticity to variations in water and in nutrient availabilities

Substantial differences in growth rates and plant biomass confirm that the Low Nutrient and Low Water treatments effectively limited plant performance. In general, the direction of plasticity was similar to varying nutrient and water conditions. However, for ~80% of the traits, the size of the treatment effect was larger as a response to water deficit, compared to nutrient limitation. The Low Nutrient treatment represented a fourfold reduction in the nutrient supply compared to a nearly twofold reduction in water availability. Thus, our results suggest that stress type rather than stress quantity played a more significant role in eliciting the plastic response in our study (see also Funk et al., 2007; Roy et al., 2012; Volis et al., 2002). The relatively stronger effect of variation in water availability may have implications for breeding if heterogeneity in water conditions is more likely to occur than nutrient variations in agricultural environments (Howden et al., 2007).

In addition to the passive response that reflects resource limitation (e.g. reduced growth when water and nutrients were limiting; Nicotra et al., 2010; Sultan, 2000), plants responded actively by adjusting their phenotype to nutrient and water shortage. For instance, when soil water and/or nutrients were limiting, plants from all accessions decreased specific leaf area to produce smaller and thicker leaves, and decreased stomatal conductance to avoid water loss. These morphological and physiological adjustments have repeatedly been interpreted as signatures of adaptive plasticity to environmental variation (see e.g. Bell and Sultan, 1999; Sultan and Bazzaz, 1993). Based on such ecophysiological expectations and on the large phenotypic differences in growth across environments, we suggest that part of the observed plasticity was undoubtedly adaptive, which contributes to the currently-limited evidence of the expression of adaptive plasticity in crops (Sadras et al., 2009; Yan et al., 2012). However, we have not formally tested the adaptive value of plasticity in purely agricultural terms, i.e. whether plastic changes were correlated with crop yield, which remains as a prominent research gap.

4.3. Strengths and limitations of the study

We conducted the first assessment of the effects of domestication on plasticity using multiple crops simultaneously. Furthermore, the use of diverse axes of resource variation allowed comparisons of the plastic response to changes in two environmental factors key in agricultural environments (water and nutrient supply). Accordingly, the evaluation of multiple functional traits related to growth, morphology and allocation provided a thorough examination of the effects of domestication, both on trait means and on their plasticity. The choice of multiple species and environments inevitably resulted in the reduction of the number of accessions evaluated per crop. However, it has been recognized that increasing the number of species when testing for the existence of general patterns is more effective than increasing replication within species (van Kleunen et al., 2014). Future studies aimed at testing our findings should nonetheless include more (or different) crops, and higher intraspecific variation within crops. Furthermore, experimental designs should include multifactorial environments to assess plasticity – not only of functional traits but also of fitness components – in more realistic settings, and should include an assessment of the adaptive value of plasticity and their role to modulate yield and yield stability.

Author contributions

SM and RM conceived and designed the experiment. SM and RM collected the data; SM analyzed the data and wrote the manuscript, with input from RM.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.envexpbot.2017.10.014.

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62
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