

## RESEARCH PAPER

# Limited evolutionary divergence of seedlings after the domestication of plant species

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**ABSTRACT**

The most vulnerable stage in the life of plants is the seedling. The transition from wild to agricultural land that plants experienced during and after domestication implied a noticeable change in the seedlings' environment. Building on current knowledge of seedling ecology, and on previous studies of cassava, we hypothesise that cultivation should have promoted epigeal germination of seedlings, and more exposed and photosynthetic cotyledons. To test this hypothesis, we phenotyped seedling morpho-functional traits in a set of domesticated and wild progenitor accessions of 20 Eudicot herbaceous crop species. Qualitative traits like epi- versus hypogeal germination, leafy versus storage type of cotyledons, or crypto- versus phanerocotyledonar germination, remained conserved during the domestication of all 20 species. Lengths of hypocotyls and epicotyls, of cotyledon petioles, and indices of cotyledon exposure to the aboveground environment changed during evolution under cultivation. However, those changes occurred in diverse directions, depending on the crop species. No common seedling phenotypic convergence in response to domestication was thus detected among the group of species studied here. Also, none of the 20 crops evolved in accordance with our initial hypothesis. Our results reject the idea that strong selective filters exerted unconsciously by artificial selection should have resulted in generalised channelling of seedling morphology towards more productive and more herbivore risky phenotypes. This result opens up unexplored opportunities for directional breeding of seedling traits.

**INTRODUCTION**

The domestication of certain wild plants altered the evolutionary trajectories of both humans and crop domesticates (Hancock 2004). For plants, a number of generalised phenotypic divergences between current domesticates and their wild ancestors reflect this sharp evolutionary change. This has been termed the domestication syndrome (Hammer 1984). Traits ascribed to the domestication syndrome are commonly of direct relevance to human use, such as loss of seed dispersal mechanisms, phenological synchronisation or gigantism in harvestable organs (Fuller 2007). However, we know comparatively little about how domestication has impacted the evolution of traits of only indirect agricultural interest, even if their impact over plant fitness is well known (McKey *et al.* 2012). In this context, we ignore whether and how the functional morphology of seedlings, as a trait that is most relevant for plant performance in agricultural lands, has evolved during domestication and further cultivation of our herbaceous crops.

Seedling strategy is a fundamental part of plant life history, relevant to regeneration components of fitness in the wild (Kitajima & Fenner 2000). After germination, seedlings can display diverse functional morphologies. These are manifest through cotyledon traits, biomass allocation patterns and phenological strategies that indicate regeneration niches (Lloret *et al.* 1999; Kitajima & Fenner 2000; Ibarra-Manríquez *et al.* 2001). For example, in Neotropical communities, species tend to display leafy, nude and aboveground cotyledons (Ibarra-

Manríquez *et al.* 2001). This relates to prevalent selection pressures in the Neotropics, where a head start and high early relative growth rates are adaptive in crowded plant communities. Another example relates seed size and cotyledon type with tolerance to burial. Reserve types of cotyledons and large seeds tend to tolerate burial more effectively, which makes those traits more abundant among species typical of deeper soils or late successional stages (Kitajima & Fenner 2000). Domestication took wild plants from certain wild habitats into agricultural ecosystems, where plants were selected for generations under widely different environmental conditions (Denison 2012). It is thus reasonable to expect that the seedling strategy that was more suitable in the wild should be different to that which is better adapted to croplands.

Little is known about the evolutionary divergences that cultivation may have exerted over the functional morphology of seedlings. We are aware of only one previous study that investigated shifts in seedling strategy as a consequence of plant domestication (Pujol *et al.* 2005a). Pujol *et al.* (2005a) studied non-progenitor wild relatives, wild progenitors and cultivated varieties of *Manihot esculenta* (cassava). It was found that the transition from wild to cultivated cassava meant that seedlings evolved longer hypocotyls and epigeal germination. Also, cotyledons shifted to being leafy, emerged out of the testa and were displayed further away from the plant axis by means of longer cotyledon petioles (Pujol *et al.* 2005a). This is a remarkable finding for several reasons. First, seedling qualitative traits tend to be evolutionarily conserved, it is rare for polymorphisms to

exist within a single genus (Garwood 1995). Second, the evolutionary change of the cassava seedlings is a clear result of shifting selection pressures during the transition from wild to artificial environments. In their natural environment, the wild relatives of cassava face tougher abiotic pressures and more unpredictable disturbance regimes than in agricultural lands. This makes cotyledons and their axillary meristems fitter belowground, to avoid and/or be able to recover from potential fire or herbivore disturbances. On the other hand, the more productive, albeit riskier, aboveground, leafy seedling phenotype is at an adaptive advantage for volunteer seedlings aiming to make it to the next generation under artificial selection in fertile, human-protected and fire-predictable environments (Pujol *et al.* 2005a).

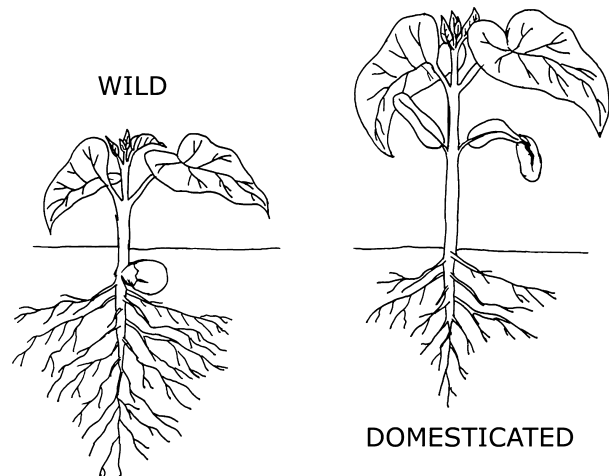
Although each domestication process has its own historical, ecological and geographic peculiarities, the findings of Pujol *et al.* (2005a) may well indicate a plausible working hypothesis for the generalised effects of domestication on the functional morphology of seedlings. All domestication processes have probably involved a transition from resource-poor to resource-rich environments, irrespective of crop identity (Denison 2012). Moreover, in artificial environments humans have partly taken care of plant protection against herbivores and pathogens (Rosenthal & Dirzo 1997). In fact, a number of case studies have shown that herbivore and/or pathogen resistance mechanisms were frequently lost or down-regulated during domestication (Mondolot *et al.* 2008; Archetti 2009; Dávila-Flores *et al.* 2013). Also, most importantly, disturbance regimes and resource availability become more predictable in agricultural lands. This scenario should relax selection for survival and promote selection for high early growth in seedlings (Kitajima 1994; Veneklaas & Poorter 1998; Poorter 1999). There are additional reasons to expect that seedling strategy has changed as a result of artificial selection. For example, increased selection for fast growth might have impacted seedling biomass allocation patterns. High seedling relative growth rate (RGR) frequently relies on allocating a high proportion of biomass to leaves (Poorter & Lambers 1991). Also, more abundant and predictable water and nutrient supplies in croplands should select for shallow and relatively small root systems. Taken together, these mechanisms may have resulted in noticeable impacts on the way seedlings allot assimilates amongst plant parts.

Here, we hypothesised that evolution under cultivation should have resulted in (i) a more epigeal, exposed and leafy strategy for displaying cotyledons; and (ii) reduced biomass allocation of seedlings to roots (Fig. 1). Our hypotheses are based on results from the cassava studies, on ecological patterns of biomass allocation in seedlings and on expectations of relaxation of herbivore pressures under artificial selection. To test these hypotheses, we obtained seeds from domesticated and wild representatives of a uniquely large group of 20 phylogenetically diverse eudicot crops. Seedlings were grown under common garden conditions. Their functional morphology was then phenotyped and compared among crop species and domestication statuses.

## MATERIALS AND METHODS

### Study system and seedling growth procedures

Our study target is a set of 20 taxonomically diverse eudicot herbaceous crops (Table S1). Monocots were excluded because



**Fig. 1.** Hypothesised evolution of seedling morphology following plant domestication (see Introduction). Wild progenitors (left) were predicted to have reserve, belowground cotyledons that do not emerge out of the testa during germination (CHR morphotype). Domesticated accessions (right) were hypothesised to have photosynthetic, aboveground cotyledons that emerge out of the testa during germination (PEL morphotype). Also, domesticates were predicted to allocate less biomass to roots, to elongate hypocotyls further and to display cotyledons in longer petioles. Drawing by Nieves Martín-Robles.

the focal hypotheses of the study are concerned with changes in the placement and morphology of cotyledons. Monocot coleoptiles, *i.e.* the developmental analogues to eudicot cotyledons, are small and membranous and probably not subject to the relevant herbivory or resource use selective pressures of the kind hypothesised here.

For each crop, we obtained seed lots of two accessions: one representative of a modern, domesticated stage of the species; and a second from its most likely wild progenitor (see Table S1 for accession identifiers, seed donors and literature source for wild progenitor assignment). This collection of seeds was grown under a common garden regime in 2012. The seeds were set to germinate in dark, cold growth chambers and, when radicle emergence could be observed, ten to 15 seedlings were taken to containers and placed in a greenhouse (Universidad Rey Juan Carlos, Móstoles-Madrid, Spain, 40°18'48" N–3°52'57" W). Long containers were purpose-built to allow seedling roots to grow for several weeks before reaching the end of the containers and starting to deform, and to allow the lower part of the container to be visually inspected for the presence of rootlets. To achieve this, 42-cm long black round plastic cylinders were placed inside 25-cm long Jumbo Rootainers (Haxnicks, Wiltshire, UK), resulting in a final container with a rooting volume of 42-cm deep × ~50-cm<sup>2</sup> wide. The lower part of these cases was removable, and thus able to be inspected without root or substrate disturbance, and was checked every other day to assess the rooting depth of the deepest visible rootlet. The substrate used was pure sand, to allow complete recovery of all fine roots at harvest time. The pots were fertirrigated twice a week with a complete nutrient solution to allow regular development in the sandy substrate. The plants were watered in the greenhouse at dawn and/or sunset through regular automatic water sprinkling, and as needed to maintain them under optimal growth conditions. Pairs of wild–domesticated accessions of each crop were grown sequentially throughout 2012, matching the most appro-

priate time of the year for the performance of each species, although always concurrently, and at the same spatial location for both accessions of each crop.

### Seedling harvest, processing and measurements

When the earliest seedlings to reach the bottom of the container were detected, the whole set of individuals belonging to a crop, including wilds and domesticates, were dug up and harvested. Five to ten (median nine, see Table S2) healthy and properly developed individuals were harvested per accession. Before harvest, the stem of each seedling was marked at soil surface level. Afterwards, plants were carefully dug up and the whole root system was recovered. The following morphometric measurements were then taken with a micrometer to the nearest mm: (i) total shoot length, as the distance from the soil surface level mark to the upper true leaf node (mm); (ii) hypocotyl length, taken as the distance from the cotyledonar node downwards to the first root ramification (mm, Hypoc onwards). Note that, strictly, the hypocotyl goes from the cotyledonar node down to the collet, which is sometimes visible as an external line or a swollen area. Since the demarcation of common criteria for defining the collet in such a wide set of species could be problematic, we opted for a functional definition of the lower limit of the hypocotyl, namely the distance to first lateral root ramification. (iii) Epicotyl length, taken as the distance from the cotyledonar node upwards to the first true leaf node (mm, Epic onwards); and (iv) cotyledon petiole length, for petiolate cotyledons (mm, Cot Pet onwards). From the above measurements, three ratios were calculated: the hypocotylar index (HCI; length of the hypocotyl as a proportion of total shoot length), the cotyledon petiole index (CPI; mean length of the two cotyledon petioles in relation to total shoot length), and the cotyledon exposure index (CEI) as the sum of the two indices, which thus indicates the height at which the cotyledons are placed relative to the total height of the seedling's shoot (Pujol *et al.* 2005a).

Also, the behaviour of cotyledons during germination was qualitatively characterised following Pérez-Harguindeguy *et al.* (2013). To assess the exposure of cotyledons to the external environment, each seedling was classified as either cryptocotyledonar (cotyledons remaining within the split testa) or phanerocotyledonar (cotyledons emerging from the testa). Seedlings were also classified according to whether cotyledons remained belowground (hypogeal) or emerged aboveground (epigeal). Additionally, seedlings were tagged as either leafy or reserve cotyledonar types, depending on the functional specialisation of cotyledons towards resource storage or early photosynthesis. After morphological measurements, seedlings were sorted into above- and belowground parts, and placed in a drying oven at 65 °C for 3 days. Dry weight of samples was measured with a microbalance to the nearest µg (MT XP6, Mettler-Toledo, Westerville, OH, USA). Total seedling dry weight (g, Size hereon) was used as a measure of plant size. Root to shoot dry weight ratio (R:S) was taken as a proxy of relative allocation to below- and aboveground functions.

### Statistics

Qualitative variables showed no variance within accessions and no change between domestication statuses (see Table 1). There-

**Table 1.** Exposure, position and form of cotyledons during the germination of seedlings of wild and domesticated representatives of each crop.

crop	wild	domesticated
<i>Amaranthus</i>	P-E-L	P-E-L
<i>Beta</i>	P-E-L	P-E-L
<i>Brassica</i>	P-E-L	P-E-L
<i>Capsicum</i>	P-E-L	P-E-L
<i>Cicer</i>	C-H-R	C-H-R
<i>Cynara</i>	P-E-L	P-E-L
<i>Eruca</i>	P-E-L	P-E-L
<i>Glycine</i>	P-E-L	P-E-L
<i>Gossypium</i>	P-E-L	P-E-L
<i>Helianthus</i>	P-E-L	P-E-L
<i>Lathyrus</i>	C-H-R	C-H-R
<i>Lens</i>	C-H-R	C-H-R
<i>Linum</i>	P-E-L	P-E-L
<i>Lupinus</i>	P-E-L	P-E-L
<i>Solanum</i>	P-E-L	P-E-L
<i>Medicago</i>	P-E-L	P-E-L
<i>Pisum</i>	C-H-R	C-H-R
<i>Spinacea</i>	P-E-L	P-E-L
<i>Trifolium</i>	P-E-L	P-E-L
<i>Vicia</i>	C-H-R	C-H-R

Data are displayed as Exposure–Position–Form for each accession. Exposure can be Phanero cotyledonar (P) or Cryptocotyledonar (C). Position can be Epigeal (E) or Hypogeal (H) germination. Form can be Reserve (R) or Leafy (L) type of cotyledon. See Materials and Methods for more details on the variables. See Table S1 for details on accession identifiers and wild and domesticated taxonomic identity for each crop. No within-accession variability was observed for these qualitative traits.

fore, the analyses described below refer only to quantitative variables.

Variation in seedling phenotypes among crops and domestication statuses was analysed through multivariate (whole phenotype) and univariate (trait-wise) techniques. For the multivariate level of analysis, a permutational MANOVA model was built to ascertain the contribution of crop identity and domestication status to explain variation in overall seedling morphology between individuals (Anderson & Ter Braak 2003). This approach was chosen rather than a traditional MANOVA because it does not make distributional assumptions, and several of the variables deviated from normal distribution. A multivariate matrix containing Size, log(R:S), Epic, Hypo, log(HCI), log(CPI) and log(CEI) scores of each individual was used as the dependent variables matrix. This matrix was standardised by column totals, and its derived Euclidean distance matrix was further computed. Domestication status (wild *versus* domesticated) was introduced into the model as fixed effect explanatory factor, whereas random intercept and slope terms were allowed based on Crop ID (*e.g.* *Vicia*). Significance of effects was computed through Monte Carlo permutation tests (4999 randomisations). Permutational MANOVA *s* were carried out using the PERMANOVA+ module for the PRIMER software (PRIMER-E, Plymouth, UK).

Trait-wise differences between crops and domestication statuses were analysed using univariate generalised linear mixed models (GLMM). Eight GLMM were performed, one for each seedling trait. Explanatory factors were all introduced as described above for PERMANOVA analyses, and Poisson family

distribution was specified for response variables. The models were fitted through maximum likelihood (ML), as advised for generalised mixed modelling (Zuur *et al.* 2009); this was taken as the full model for model comparisons (see below). To evaluate the performance of random and fixed effect predictors to account for variation in our responses, we adopted a model selection procedure. This was done to avoid common weaknesses of hypothesis testing approaches when applied to mixed modelling (Zuur *et al.* 2009). We generated five models for each response variable: (i) a null model with a constant as predictor; (ii) a fixed effects model with domestication status as fixed predictor; (iii) a model with only an intercept random effect of Crop ID as predictor; (iv) a model with domestication and random Crop ID intercept as predictors; and (v) a full model including the random Crop ID slope term (see above). The Akaike information criterion (AIC) was obtained from each model, and the amount of information recovered (*i.e.* decrease in AIC) with respect to the null model was taken as an estimate of the explanatory ability of the predictors composing each model. Models 1 and 2 were generated using the *glm* function of the stats library of R 2.7.0 software (R Development Core Team 2008). Models 3–5 were obtained from the *glmer* function of the lme4 package (Bates 2010). Both functions use the same ML estimating procedure, and the same error distribution was specified. Thus, AIC scores are directly comparable among *glm* and *glmer*.

We also ran a principal components analysis (PCA) to synthetically visualise seedling trait divergences following domestication for the various crops used in this study. To this end, we first calculated, separately for each crop, the difference between its domesticated and its wild progenitor average score of each seedling trait ( $\Delta\text{trait}_{D-W}$ ). Then, differences were z-standardised ( $\Delta\text{trait}_{stD-W}$ ). A PCA was run out of this matrix of standardised within-crop differences in seedling traits. PCA analyses were carried out using Canoco 4.5 (Ter Braak & Smilauer 1998).

## RESULTS

Most wild progenitors of the crops considered here displayed epigeal germination, and had leafy cotyledons that emerged out of the testa during early development. However, five of the progenitors of legume crops retained the storage cotyledons belowground and inside the testa during germination (Table 1). No crop species showed a response to domestication in qualitative seedling traits. Moreover, in no case did qualitative traits differ between individual seedlings of a given accession.

Results of PERMANOVA showed that quantitative traits of seedlings, taken together in a single multivariate analysis, were significantly different among crop species, but poorly divergent among domestication statuses within a given crop (Table 2). Yet, the random slope term Domestication | Crop ID was statistically significant in PERMANOVA models. This means that the seedling phenotype of some species reacted to domestication in different directions to that of other species, even if domestication status was relatively unimportant when compared to crop identity.

When examined trait-wise, we observed that quantitative traits related to seedling size, to aboveground allocation and to exposure of cotyledons reacted differently to domestication, and generally in diverse ways depending on the crop species

**Table 2.** Summary statistics of PERMANOVA multivariate analysis for crop identity and domestication status effects and their interaction on a distance matrix built from the seedling traits measured (Size, R:S, Cot Pet, Epic, Hypoc, HCl, CPI and CEI. See legend of Fig. 2 for details of variables).

source of variation	df	MS	pseudo-F	P (from 4999 permutations)
Crop ID	19	21,509	146.07	<b>0.0002</b>
Domestic	1	887.04	0.55	0.5864
Domestic   Crop ID	19	1761.20	11.96	<b>0.0002</b>
Residual	293	147.25		

Crop ID = Crop identity; Domestic = Domestication status (wild versus domesticated). Values of  $P < 0.05$  are shown in bold. The model explained ca. 90% of total variance in the seedling traits distance matrix.

(Table 3, Fig. 2, Figure S1). Seedlings of domesticated accessions were generally larger than those of their wild counterparts (Table 3, Fig. 2, Figure S1). For all other quantitative traits, the effect of domestication status *per se* was unimportant. The percentage of information gained in models when adding the term domestication status ranged from only 0% to 2%, depending on individual traits (Table 3). However, it is remarkable that the full model was consistently the best model, based on AIC selection criteria, irrespective of the trait under consideration (Table 3). This means that including the random slope term Domestication | Crop ID always improved the model, even if crop identity was the most relevant predictor of each seedling quantitative trait. Thus, overall, individual traits changed during each specific domestication process, but in inconsistent and idiosyncratic ways depending on the crop species (Figure S1). This is congruent with multivariate PERMANOVA analyses.

As a whole, the results indicate that the response of quantitative seedling traits to domestication was diverse. In Fig. 3 we illustrate this point through factor analysis of the standardised difference between domesticated and wild accessions of each crop ( $\Delta\text{trait}_{stD-W}$ ). We found that, when domestication implied increases in seedling size and in the length of cotyledon petioles, plants tended to decrease allocation to roots (Fig. 3). Also, increased elongation of epicotyls tended to co-vary negatively with more elongated hypocotyls and cotyledon exposure metrics (Fig. 3). Our set of 20 crops showed diverse eigenvalues over the two first axes of ordination. First, a group of crops displayed no remarkable differences between domesticated and wild accessions; crops representative of this group include fodder legumes or *Cynara* and *Beta*. Second, several species decreased the length of cotyledon petioles and allocated more biomass to roots when domesticated, *e.g.* *Eruca*, *Capsicum* or *Spinacea*. Third, domestication of species such as *Vicia* or *Solanum* resulted in longer cotyledon petioles, but decreased allocation of biomass to roots. Fourth, other species, such as *Linum*, decreased hypocotyl length during domestication. Finally, domestication of *Helianthus* stretched hypocotyls and reduced epicotyl length.

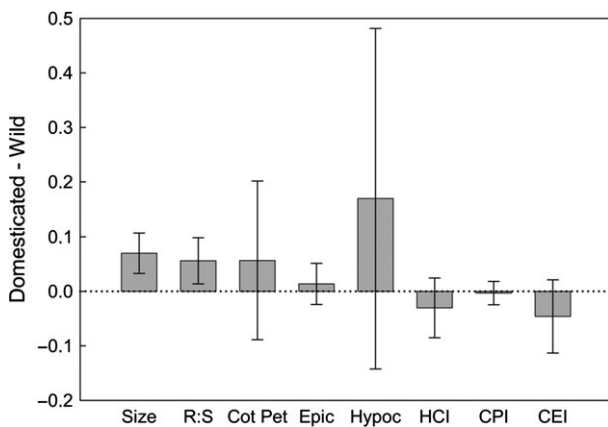
## DISCUSSION

None of the 20 evolutionary divergences examined here were in accordance with our hypothesised response of seedlings to domestication and further selection (Fig. 1). None of the

**Table 3.** Results of univariate generalised linear mixed models (GLMM) for crop identity and domestication status effects on the seedling traits measured (Size, R:S, Cot Pet, Epic, Hypoc, HCI, CPI and CEI. See legend of Fig. 2 for variables).

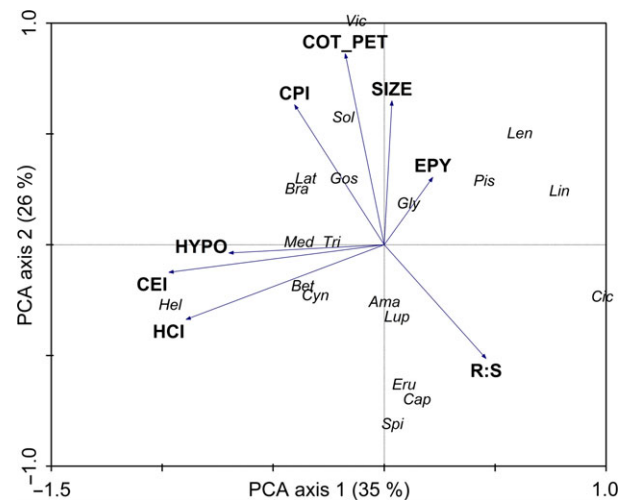
model	size		R:S		Cot Pet		Epic	
	AIC	%info	AIC	%info	AIC	%info	AIC	%info
$y \sim \text{Domestic} + \text{Crop ID} + \text{Domestic} \mid \text{Crop ID} + \varepsilon$ (full)	<b>63,170,222</b>	91	<b>127,548,293</b>	63	<b>10,320</b>	94	<b>3,541,402</b>	92
$y \sim \text{Domestic} + \text{Crop ID} + \varepsilon$	166,457,402	75	190,202,195	45	19,311	88	8,619,583	81
$y \sim \text{Crop ID} + \varepsilon$	239,736,339	65	191,370,672	44	19,322	88	8,938,079	80
$y \sim \text{Domestic} + \varepsilon$	608,836,659	10	340,821,963	1	165,137	0	44,767,151	1
$y \sim 1 + \varepsilon$ (null)	676,316,463	–	342,967,650	–	165,147	–	45,016,498	–
model	Hypoc		HCI		CPI		CEI	
	AIC	%info	AIC	%info	AIC	%info	AIC	%info
$y \sim \text{Domestic} + \text{Crop ID} + \text{Domestic} \mid \text{Crop ID} + \varepsilon$ (full)	<b>467,916</b>	92	<b>114,902</b>	88	<b>19,922</b>	92	<b>111,959</b>	88
$y \sim \text{Domestic} + \text{Crop ID} + \varepsilon$	1,020,455	82	198,358	79	25,568	90	189,421	79
$y \sim \text{Crop ID} + \varepsilon$	1,107,122	80	199,051	79	27,868	89	191,514	79
$y \sim \text{Domestic} + \varepsilon$	5,506,654	2	964,313	0	242,391	1	894,538	0
$y \sim 1 + \varepsilon$ (null)	5,597,882	–	964,891	–	243,679	–	895,989	–

To compare the explanatory ability of each predictor, a series of five models are shown for each response variable. AIC is shown for each model, together with its percentage decrease with respect to the null model. %info =  $(\text{AIC}_{\text{null}} - \text{AIC}_i) / \text{AIC}_{\text{null}}$ . Crop ID = Crop identity; Domestic = Domestication status (wild versus domesticated). Degrees of freedom were 1 for Crop ID, 19 for Domestic, 19 for Domestic | Crop ID and 293 for Residual sources of variation. Bold type indicates best fitting model.



**Fig. 2.** Within-crop differences (domesticated–wild ancestor) for the average of the 20 crops in the eight seedling traits measured. Mean differences are estimates of GLM models for the effect of domestication status (see Materials and Methods). Error bars are  $\pm 1$  SE, computed from the magnitude (SD) of the crop identity effect in GLM models. Size is total seedling dry weight (g). R:S is mass-based root to shoot ratio. Cot Pet is cotyledon petiole length (dm). Epic and Hypoc are epicotyl and hypocotyl lengths, in cm and dm, respectively. HCI, CPI and CEI are hypocotyledon, cotyledon petiole and cotyledon exposure indices (see Materials and Methods). Crop identity was collapsed for simplicity, but crop-wise plots are available in Figure S1. See Table 3 for statistics.

crop species evolved qualitative shifts in seedling traits as a consequence of artificial selection. Also, even if seedling quantitative traits changed during most of the domestication processes considered here, this occurred in diverse directions, depending on the identity of crop species. Therefore, our results provide no support for the idea of generalised convergent evolution of seedling morphologies to better cope with increased habitat productivity, or with decreased and/or more predictable disturbance regimes during domestication and further improvement. It is also pertinent to acknowledge



**Fig. 3.** Results of a PCA of within-crop differences in quantitative seedling traits. PCA biplot showing the loadings of traits and eigenvalues of the several crops for the two main axes of the PCA. Standardised differences within crops (domesticated–wild ancestor), rather than accession-specific means, were used to exclude the overriding effect of crop identity on building the ordination scheme (see Table 2) and thus highlight the effect of domestication, if any. Abbreviations of traits as in Fig. 2.

that our broad-scale multi-species approach compromises detailed survey within each crop. Thus, drawing conclusions as regards the behaviour of each specific crop should be done with care, particularly for quantitative traits. Below we discuss the implications of our results and revisit the assumptions of our initial hypotheses. Also, we suggest that the absence of a channelled seedling phenotypic change during crop evolution provides opportunities for future directional breeding to enhance the contribution of seedling quantitative traits to optimise plant performance in croplands.

### Conserved evolution of qualitative seedling traits under artificial selection

The seedling is the most vulnerable life-history stage (Harper 1977). Seedlings are thus subjected to strong selective pressures in the wild, and seedling phenotypes should reflect a tight coupling with prevalent biotic and abiotic conditions. In spite of this, seedling qualitative traits tend to be evolutionary conserved (Ibarra-Manríquez *et al.* 2001). Qualitative seedling traits are seldom variable within a given species [but see cassava in Pujol *et al.* (2005a)] and very rarely polymorphic, even within plant genera (Essig 1987; Garwood & Tebbs 1995). In accordance with this body of literature, our qualitative data did not react to domestication and also showed a high degree of phylogenetic structure, although we did observe some degree of polymorphism within specific lineages. For instance, most wild legumes had reserve and underground cotyledons, which have remained invariable during evolution under cultivation (Table 1). However, species such as wild lupin, lucerne and clover had aboveground and leafy cotyledons. This indicates some degree of evolvable seedling qualitative traits, even if at a very broad taxonomic scale. All other eudicot lineages studied here had photosynthetic cotyledons, which were also conserved after domestication. Therefore, we did not find the intra-crop evolutionary lability of qualitative seedling traits reported for cassava (Pujol *et al.* 2005a) in any of the crops examined here. More specifically, no single wild progenitor displayed the evolutionary trajectory previously found in wild cassavas following domestication (Pujol *et al.* 2005a). Cassava seedlings were indeed polymorphic in terms of germination types, both within and among species, which makes this biological group particularly suitable for divergence under selective pressures (Pujol *et al.* 2005a). One possible explanation for the patterns observed in cassava is that seedling traits in that species were circumstantially linked to wider suites of traits directly selected by humans, instead of having evolved as adaptive reactions to changing selective pressures upon seedling establishment.

In conclusion, qualitative traits of the seedling phenotype tend to be highly conserved, at least at the taxonomic scale where crop breeding operates. Therefore, it seems improbable that breeding can provide generalised changes in qualitative traits that result in seedlings better adapted to the cropland environment.

### Diverse evolutionary trajectories of quantitative seedling traits after domestication

In contrast, quantitative seedling traits reacted to artificial selection in most of the crops studied here. However, that response was highly diverse among crops and never congruent with our initial expectation of phenotypic divergence in response to domestication. This result calls for a re-evaluation of the assumptions of our hypothesis.

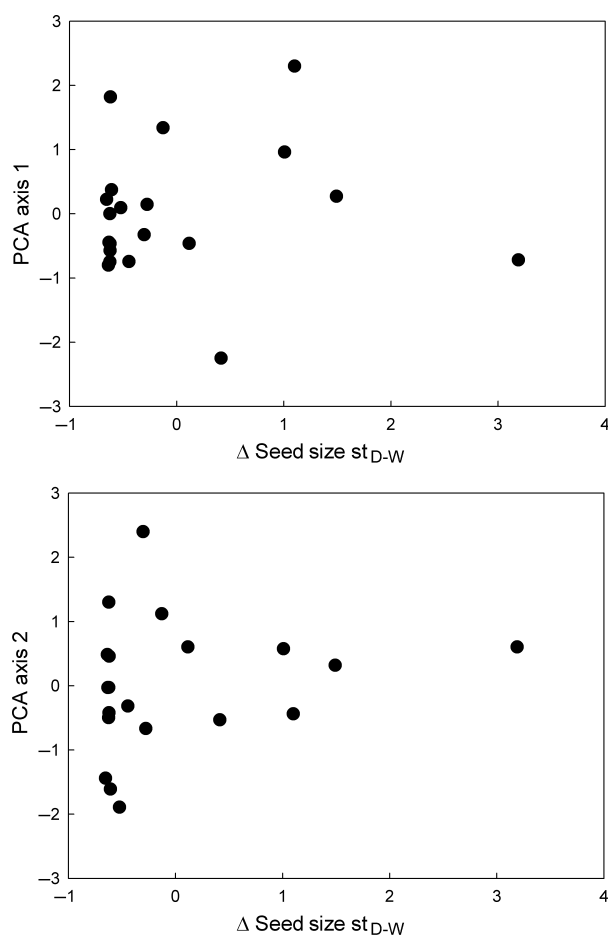
We assumed that habitat productivity should have necessarily increased while transiting to agricultural environments. This was the case for cassava: cassava wild progenitors live in savanna habitats (Allem *et al.* 2001). In savannas, CHR seedling morphology could reduce sensitivity to fire, drought and low nutrient availability, as predominant selective factors in that environment (Elias & Mckey 2000;

Oliveira & Marquis 2002). Domestication of cassava eliminated unpredictability in disturbance regimes, increased soil nutrient availability and selected for high growth rate seedlings (Pujol *et al.* 2005b). The wild progenitors of the set of species investigated in our study, however, have diverse ecologies and habitat affinities. For instance, wild progenitors of lupin tend to inhabit ruderal habitats of the western Mediterranean Basin, whereas ancestors of chickpea or lentil commonly occur on limestone outcrops from eastern Mediterranean areas (Ladizinsky *et al.* 1984; Berger *et al.* 2008). *Solanum pimpinellifolium*, the closest wild relative of the cultivated tomato, thrives in semi-arid habitats of the Peruvian Pacific area, while *Gossypium hirsutum* is described as a tropical species (Sauer 1993). Therefore our assumption that wild progenitors would consistently inhabit less productive habitats than their cultivated relatives across such a diverse set of species might require formal testing. Moreover, even if current fertility levels are consistently higher in croplands (Denison 2012), this does not necessarily extrapolate to the several millennia that most herbaceous crops have spent thriving in agricultural lands. Biomass accumulation in wild grasslands might have provided similar fertility levels to those that occurred in early-cultivated lands where biomass was removed annually, and the capacity to replace fertility might have been lower than expected from our current-day experience of agriculture.

Similarly, the assumption that herbivory is more relevant as a selection agent in the wild than in agricultural lands might need revision. Pesticides and chemical protection have seriously decreased the activity of herbivores in croplands since the second half of the 20th century (Kingsbury 2009). However, this is certainly a short evolutionary time when compared to the several millennia that most of the crops studied here have been raised under artificial selection. During those earlier stages of domestication and improvement, crop protection might not have been very efficient. A combination of human intervention and plant investment in chemical defences might have been necessary to preserve crop biomass, which was probably more nutritious, abundant and attractive to herbivores than that of the surrounding wild marginal lands. Also, higher biodiversity in marginal lands might have promoted the existence of complex mechanisms of herbivore control, which ameliorate the impacts of herbivory on primary producers. There is solid empirical evidence to back the idea that herbivores are less abundant in highly diverse terrestrial ecosystems (Haddad *et al.* 2009). Moreover, recent experiments comparing the preferences of insect herbivores and phloem feeders between crops and their wild progenitors have not yielded clear cut results amongst herbaceous crops (Turcotte unpublished). In summary, selection for highly productive, and consequently herbivore-exposed, seedling phenotypes might not have been as directional as previously hypothesised in the Introduction. Contingencies associated with diverse domestication histories, ecologies and geographies of each crop may have prevailed. More intensive research into each of the species considered here should advance our understanding in this regard.

Alternative explanations to account for the high degree of crop dependency encountered here might include coordinated evolution with other traits that are known to be different

between crops and their wild progenitors. Of direct relevance in this regard, most domestication processes have involved increases in seed size (Kluyver *et al.* 2014). Larger seeds may have counterbalanced selective pressures, thus favouring the evolution of more exposed leafy type cotyledons in agricultural environments. For example, larger seeds tend to belong to hypogeal species (Fenner & Thompson 2005). Seed size is also generally related to seedling vigour and seedling establishment success in the wild (Westoby *et al.* 1996; Turnbull *et al.* 2008), but see (Hanley *et al.* 2007). Moreover, larger seeds in crops might have evolved in part due to deeper seed burial by farmers, when compared to other dispersal mechanisms (Fuller & Allaby 2009). However, a recent study failed to find evidence in support of increased seed size during domestication as a mechanism to better cope with deeper burial in a diverse set of grain legume species (Kluyver *et al.* 2014). Furthermore, the degree of divergence in seed size did not explain variance in quantitative seedling traits in our collection of domestication processes (Fig. 4). Therefore, even if deeper burial promotes changes in hypocotyl and epicotyl elongation rates (Kitajima & Fenner 2000), direct links between larger seeds, increased burial depth



**Fig. 4.** Relationship between changes in seed size and seedling traits during crop evolution. Z-standardised differences in seed mass (g) between domesticated and wild accessions of the 20 species studied here ( $\Delta$  Seed Size st<sub>D-W</sub>) and summary variables of the evolutionary trajectories of quantitative seedling traits during domestication (PCA axes 1 and 2 of Fig. 3). No simple regression model fitted either of the two scatter plots. Seed mass data taken from Milla *et al.* (unpublished).

by farmers and evolution of seedling functional morphology under domestication look untenable at our current state of knowledge. However, we cannot discard that interactions with other plant traits may have synergised with, or counterbalanced, selective pressures shaping the evolution of seedling strategy under domestication. For example, we did not examine putative shifts in chemical and physical herbivory defence mechanisms (Barton & Hanley 2013). Those may have more closely indicated changes in herbivore pressure during domestication, and thus override the adaptive relevance of morphological traits that affect not only herbivore response but also a wider array of plant functions. This might be the case for several of the seedling traits put forward in Pujol *et al.* (2005a), which may not have reacted to disturbance as hypothesised. For instance, the growth response to artificial defoliation of cotyledons of pea (hypogeal) and sunflower (epigeal) seedlings is similar (Hanley *et al.* 2004). This sheds some additional doubt on the precise adaptive value in croplands of shifts in cotyledon position.

In summary, domestication effects over seedling phenotypes were small. Even when quantitative traits changed under cultivation, this occurred in diverse directions that were inconsistent with hypotheses of evolutionary convergence. This does not downgrade the importance of seedling traits in croplands. On the contrary, we know that seedling traits are functionally relevant in a number of ways. The strategic display of the embryonic leaves is known to affect productive performance (Kitajima & Fenner 2000). Moreover, early competition with neighbouring plants is highly dependent on a head start on the part of the competitive winner (Weiner 1990). In this context, we know that herbaceous plants have evolved into more aggressive competitors for light during crop evolution (Milla *et al.* unpublished). This has consequences for early outperformance of weeds, which is a desirable property in crop plant populations (Weiner *et al.* 2010) but may also signify diminishing returns from productive tissues (Falster & Westoby 2003; Milla *et al.* unpublished). Seedling and adult competitive and productive traits may not be properly coupled to deliver optimal performance of crops in agricultural lands. The fact that unconscious selection did not impact seedling morphology in a consistent manner provides open ground for directional breeding. Qualitative traits seem too evolutionarily conserved to be disrupted during domestication (but see cassava in Pujol *et al.* 2005a) but quantitative traits look sufficiently labile. Ideotypic seedling morphology could be devised and natural variation within crop gene pools surveyed. For example, morphotypes that provide a head start for effectively outcompeting weeds and increase crop productivity, but become less aggressive aboveground competitors later in ontogeny, could be a sensible target.

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## SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

**Table S1.** Common name, taxonomic and domestication status and seed origin information for each accession of the 20

domesticated–wild ancestor pairs used in this study.

**Table S2.** Arithmetic mean, SD and number of replicates of the eight seedling morphology traits for the domesticated (D) and wild ancestor (W) accessions of each crop species.

**Figure S1.** Bisector plots of seedling traits.

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