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Differential patterns of within- and between-population genetically-based trait variation in *Lupinus angustifolius* L.

Running title: Within- and between-population traits variation in *Lupinus angustifolius* L.

*For correspondence. E-mail cristinapoyatosfernandez@gmail.com

Authors: Cristina Poyatos[1,*], Sandra Sacristán-Bajo[1], Pablo Tabarés[1], Samuel Prieto-Benítez[2], María Luisa Rubio Teso[1]; Elena Torres[3], Javier Morente-López[1,4], Carlos Lara-Romero[1], José María Iriondo[1], Alfredo García Fernández[1]

Affiliations:

[1]Grupo de Ecología Evolutiva (ECOEVO). Área de Biodiversidad y Conservación, Departamento de Biología y Geología, Universidad Rey Juan Carlos-ESCET, Tulipán s/n. 28933 Móstoles, Madrid (Spain).

[2]Ecotoxicology of Air Pollution, CIEMAT. Avda. Complutense, 40. 28040, Madrid (Spain).

[3]Departamento de Biotecnología-Biología Vegetal, Universidad Politécnica de Madrid, Av. Puerta de Hierro 2-4, 28040, Madrid (Spain).

[4]Grupo de Ecología y Evolución en Islas, Instituto de Productos Naturales y Agrobiología (IPNA-CSIC), Avda. Astrofísico Francisco Sánchez 3, 38206, San Cristóbal de La Laguna, Santa Cruz de Tenerife (Spain).

ABSTRACT

Background and aims. Within-population genetic and phenotypic variation play a key role in the development of adaptive responses to environmental change. Between-population variation is also an essential element to assess the evolutionary potential of species in response to changes in environmental conditions. In this context, common garden experiments are a useful tool to separate the genetic and environmental components of phenotypic variation. We aimed to assess within and between-population phenotypic variation of *Lupinus angustifolius* L. in terms of its evolutionary potential to adapt to ongoing climate change.

Methods. We evaluated populations' phenotypic variation of foliar, phenological and reproductive traits with a common garden experiment. Patterns of functional trait variation were assessed with 1) mixed models analyses and coefficients of variation (CV) with confidence intervals; 2) principal component analyses (PCA); and 3) correlations between pairs of traits. Analyses were performed at the population level (four populations) and at the latitude level (grouping pairs of populations located in two latitudinal ranges).

Key results. Phenotypic variation had a significant genetic component associated with a latitudinal pattern. 1) Mixed models found lower SLA, advanced flowering phenology and lower seed production of heavier seeds in southern populations, whereas CV analyses showed lower within-latitude variation especially in phenological and reproductive traits at the southern populations. 2) PCAs showed a clearer differentiation of phenotypic variation between latitudes than between populations. 3) Correlation analyses showed a greater number of significant correlations between traits in southern populations (25 *vs.* 13).

Conclusions. Between-population phenotypic variation was determined by contrasting temperature and drought at different latitude and elevation. Southern populations had differential trait values compatible with adaptations to high temperatures and drought.

Moreover, they had lower within-population variation and greater number of trait correlations probably as a result of these limiting conditions, making them more vulnerable to climate change.

Key words: *Lupinus angustifolius*, traits, intraspecific variation, population variation, evolutionary potential, latitude, climate change.

INTRODUCTION

Phenotype trait expression is the result of a complex set of traits that is determined by the interaction of the genetic component and the environment (Murren, 2012). Climate change triggers abrupt environmental changes prompting the need for a trait expression change in a quick adaptive process (Nagahama *et al.*, 2018; Cruz-Maldonado *et al.*, 2021). In this context, many species are threatened because their evolutionary processes take place more slowly than environmental changes due to climate change (Botkin *et al.*, 2007). Therefore, understanding the change in phenotypic trait variation along environmental gradients is necessary to develop strategies aimed at the conservation, restoration and management of populations (Mousavi-Derazmahalleh *et al.*, 2018), since species success depends in great part on their intraspecific trait variation (McGill *et al.*, 2006; Pescador *et al.*, 2015; Halbritter *et al.*, 2018; Funk *et al.*, 2019). For this reason, the number of studies that have monitored intraspecific trait variation have multiplied in the last few years (e.g., Carvalho *et al.*, 2020; Westerband *et al.*, 2020; Kühn *et al.*, 2021; Martin and Isaac, 2021).

Intraspecific trait variation has its basis in individual variation and in the traits' variability expression. It can be evaluated from the genetic variation and phenotypic plasticity perspectives (Violle *et al.*, 2012), two qualities that largely determine plants' persistence in a climate change scenario (Funk *et al.*, 2019; Westerband *et al.*, 2021) by maintaining population fitness in environments with great climatic variation (Matesanz *et al.*, 2010; Welles and Funk, 2021).

Many studies have used common garden experiments to distinguish the genetic or phenotypic plasticity origin of such variation (e.g., Lara-Romero *et al.*, 2014; Moncalvillo *et al.*, 2019; Matesanz *et al.*, 2020).

Species' phenotypic variation can be decomposed in two different sources of variability: within- and between-populations. Within-population phenotypic variation is an element that determines population success because its genetic component allows evolution by natural selection (Bolnick *et al.*, 2011; Westerband *et al.*, 2021). High within-population adaptive genetic variation may favour rapid evolutionary responses that can mitigate climate change effects and limit extinction rates (Sgrò *et al.*, 2011). However, in highly stressful conditions, within-population variation may be reduced due to the presence of powerful environmental filters that act above the level of the individual (Pescador *et al.*, 2015; Luo *et al.*, 2016; Helsen *et al.*, 2020). These abiotic filters can generate more coordinated phenotypes by increasing the number of correlations between traits (Carvalho *et al.*, 2020). This scenario limits natural selection performance and favours compensation between traits to maintain phenotype viability (Milla and Reich, 2011). On the other hand, between-population variation includes differences between populations that can be explained by the evolutionary history of populations, comprising isolation or adaptation processes, neutral genetic mechanisms, demographic bottlenecks and biotic and abiotic filters (Ohsawa and Ide, 2008). In this work, we have highlighted the role of latitude and elevation as shapers of abiotic filters. Latitude and elevation model the distribution of many climatic variables generating environmental gradients that may operate at a reduced spatial distance. For instance, increasing latitude is usually associated with lower temperatures, greater precipitation, and lower daily solar radiation (Li *et al.*, 1998). Similarly, increasing elevation is usually associated with lower temperatures and greater precipitation, but also to higher radiation exposure and snow accumulation (Körner, 2007). In this way, they influence traits' phenotypic expression (Hulshof *et al.*, 2013) and plants'

responses, shaping scenarios in which natural selection can play a relevant role in generating local adaptations (Ohsawa and Ide, 2008; Halbritter *et al.*, 2018).

Drought and rising temperatures can produce different responses according to the species considered. Annual species usually develop a drought escape strategy (Welles and Funk, 2021) that affects traits phenotypic variation. In this way, some foliar traits (e.g. specific leaf area or SLA and leaf dry matter content or LDMC) have been reported to be highly influenced by these climatic conditions (Yulin and Zhang, 2005; Hulshof *et al.*, 2013). In warmer environments, leaves tend to reduce evapotranspiration and to promote carbon assimilation (lower SLA and higher LDMC values) (Welles and Funk, 2021). In this sense, they can grow faster to (i) advance their phenology (e.g. earlier flowering onset) and (ii) to ensure their seeds contain the necessary assimilates to germinate (e.g. heavier seeds) (Rubio de Casas *et al.*, 2017; Anstett *et al.*, 2021; Welles and Funk, 2021; Matesanz *et al.*, 2022).

Here, we evaluated the within- and between-populations phenotypic variation of *Lupinus angustifolius* L. (Fabaceae) regarding foliar, phenological and reproductive traits using a common garden approach. To do so, we used two pairs of *L. angustifolius* populations selected according to their latitudinal divergence and marked climatic/environmental differences (northern *vs.* southern populations). The main objective of this study was to assess the genetically based phenotypic variation of *L. angustifolius* populations in terms of their evolutionary potential to respond to environmental threats derived from climate change.

In this context, we addressed the following hypotheses:

1) We consider that between-population phenotypic variation will mostly originate from divergent selection processes resulting from the contrasting temperature and water availability conditions caused by differences in latitude and elevation. Therefore, we expect between-population phenotypic variation to be greater at the latitudinal level (northern *vs*. southern

populations) than at the within-latitude level (differences between populations at a given latitude) and that the trait values obtained at each location will be associated with adaptations to high temperature and water availability constraints.

2) Southern latitude populations of *L. angustifolius*, occurring at higher temperatures and aridity, will experience more intense selective pressures (i.e., drought tolerance, early flowering onset, etc.). We expect southern populations to have a greater number of significant correlations between traits and less variation within and between populations compared to northern populations, since the intensity of selective pressures has been positively associated with phenotypic integration (i.e., the correlation of multiple functionally related traits) (Pigliucci, 2003; Carvalho *et al.*, 2020).

3) Because higher selective pressures concomitant to southern populations are associated with specific limitations (i.e., high temperature, drought), the effect of these pressures will be more marked in functional traits more closely associated to these limitations. Therefore, we expect that between-population phenotypic variation at the contrasting latitudes will differ depending on the group of traits analysed (foliar, phenological and reproductive). Specifically, we anticipate that warmer environment populations will reduce water loss by lowering SLA and increasing LDMC values. In addition, we expect that they will advance the flowering phenology and produce larger seeds to guarantee their viability as part of an escape strategy to drought.

MATERIAL AND METHODS

Species and study site

Lupinus angustifolius is an annual self-pollinated herbaceous plant distributed across the Mediterranean Basin, occurring preferably in well-drained sandy acid soils, including disturbed or abandoned areas (Castroviejo and Pascual, 1999; Talhinhas *et al.*, 2006). This legume has

palmate leaves with narrow leaflets and its racemose inflorescence contains bluish-purple flowers. Under natural conditions, *L. angustifolius* flowers tend almost exclusively to self-pollinate, as self-pollination takes place in early stages of development when the flower is still closed (Kazimierska and Kazimierski, 2002). Its legume fruits have variable seed content, commonly between three and six (Castroviejo and Pascual, 1999). The seed presents a hard cover that allows it to remain dormant until environmental conditions are adequate (Berger *et al.*, 2017). The flowering season takes place between March and August (Castroviejo and Pascual, 1999).

The western half of the Iberian Peninsula is considered the genetic diversity centre of the species and it is where this species is most widely distributed (Mousavi-Derazmahalleh *et al.*, 2018). In 2016, two pairs of *L. angustifolius* populations occurring in the Iberian Peninsula were selected according to their latitudinal divergence, showing marked differences in temperature and precipitation (Table 1). Two of them, Zafrón (hereafter, FRO) and Zarapicos (hereafter, PIC) are located in Central Spain in Salamanca province, whereas the other two, La Garranchosa (hereafter, GAR) and Rivera de la Lanchita (hereafter, RIV) are located in southwestern Spain in Badajoz province. The latter two populations are located at lower latitude and elevation than the former. They also experience higher temperatures and lower water availability (Table 1). The populations were selected over others that were visited because they all had hundreds of individuals suggesting more stable population dynamics. In each population, 100 mother plants were haphazardly selected and their seeds were collected separately. The experiment developed in the present work used individuals descended from those originally sampled in 2016, since this work is part of a more extensive project (EVA project, AdAptA-lab, Rey Juan Carlos University, Móstoles, Madrid, http://adapta-lab.com/eva/).

Comentado [1]: ¿Por qué lo pusimos con mayúscula? ¿en inglés se pone así o lo corregimos y ponemos en minúscula?

Common garden experiment

A common garden experiment was established in randomised blocks at the Rey Juan Carlos University (Móstoles, Madrid, CULTIVE laboratory https://urjc-cultive.webnode.es/; Table 1) to minimise the effect of environmental conditions and assess the genetic component in phenotypic trait expression. In November 2016 three seeds per each sampled mother plant of the species were subjected to manual mechanical scarification with a nail cutter, sown in a six-litre (L) pot with a 1:1 mix of commercial substrate and sand, and placed in a greenhouse irrigated by nebulisation. After germination and seedling emergence, the largest seedling was selected, and the other two were clipped. In March 2017 *L. angustifolius* individuals were moved to the acclimation area outside the greenhouse and grown with drip irrigation until plant senescence (June 2017). For two additional seasons (2018 and 2019), a new generation of *L. angustifolius* was grown in the common garden experiment using seeds obtained by natural self-pollination. Each season followed the same schedule: sowing in November, moving to outside facilities in March and plant senescence in June. After the first generation 25% of genotypes in each population were randomly selected and maintained with three siblings per genotype. When we mention "season", we are alluding to the period between November and June, that is, from the time we started working with the seeds until we collected the fruits.

Trait measurements

Twelve functional traits were measured for *L. angustifolius* individuals during the 2018-2019 season (Supplementary data Table S1) to avoid the variation associated with maternal effects in the first two seasons (2016-2017 and 2017/2018). In November 2018, seeds were weighed prior to sowing, and, once sown, the pots were monitored daily in November and December 2018 to record the date of emergence of the individuals and obtain seed germination time. In March 2019, we recorded the date when the first flower began to bloom for each plant. Thus, we defined flowering onset as the number of days from the date of sowing to the date of appearance of the first flower. The recording of flowering end dates and leaf collection to

measure foliar traits began in April 2019. Finally, the fruits produced by each plant were collected in May 2019 to count the number of seeds per fruit.

- Foliar traits

Six foliar traits were obtained by performing calculations and direct measurements on leaves: 1) sum of leaf area (FA), 2) specific leaf area (SLA), 3) relative water content (RWC), 4) leaf dry matter content (LDMC), 5) nitrogen content (N) and 6) phosphorus content (P). As the *Lupinus angustifolius* leaf is palmately compound (with five leaflets that are attached in a single point at the end of the petiole), these traits were measured by collecting the central leaflet of eight randomly chosen leaves per individual. FA was measured by scanning the leaflets (CanoScan LiDE 210; Canon; Japan, Tokio) and adding the leaflet areas obtained with ImageJ software (Abramoff *et al.*, 2004). SLA resulted from the ratio of FA and dry mass (measured in grams as the sum of the dry mass of the eight leaflets). The leaflets were dried in an oven at 60ºC for at least 72 hours (Digiheat-TFT; SELECTA; Spain, La Rioja). RWC was obtained as a percentage resulting from the following formula: [(Mf-Md)/(Ms-Md)] x 100, where Mf, Md and Ms are the fresh mass, dry mass and saturated mass of the leaflets sampled measured in grams (g), respectively. Dry mass was measured with an XPR microbalance (Mettler-Toledo; Greifensee, Switzerland), whereas fresh and saturated mass were measured with an analytical balance of KERN ABJ-ABS series (Solent Scale; Chichester, United Kingdom). LDMC is the percentage obtained from the ratio of dry and fresh mass of the leaflets. Finally, we performed the digestion of 50 milligrams (mg) of leaf sample according to the Kjeldahl method (Radojevic and Bashkin, 2015). Subsequently, Skalar protocols were used to obtain total nitrogen and phosphorus content (Skalar San++ autoanalyzer; Skalar, Netherlands). These analyses were performed at the Rey Juan Carlos University (Móstoles, Madrid, NUTRILAB laboratory https://nutrilab-urjc.es).

- Phenological traits

Four phenological traits were recorded: 1) seed germination time (Germination time), 2) flowering onset (Onset), 3) flowering end (End), and 4) flowering duration (Duration). Germination time was defined as the days elapsed from seed sowing to seed emergence. Onset and End were calculated as the days elapsed from the date of seed emergence to the appearance of the first open flower (Matesanz *et al.*, 2020), and the date the plant stopped producing new flowers, respectively. Duration was calculated as the days elapsed from flowering onset to flowering end.

- Reproductive traits

Two reproductive traits were measured: 1) germinated seed weight (Seed mass) and 2) average number of seeds per fruit (Seeds per fruit). Each seed mass was individually weighed in mg using an XPR microbalance (Mettler-Toledo; Greifensee, Switzerland). Seeds per fruit was obtained from 15 fruits randomly obtained from each individual (Supplementary data Table S1). These traits were measured as they are known to influence *L. angustifolius* reproductive success in water stressed environments (Helsen *et al.*, 2020).

Statistical analyses

All statistical analyses were performed using the R statistical environment (version 4.0.3) (R Core Team, 2020). To analyse the data at different spatial levels (population and latitudinal region), *L. angustifolius* populations were classified according to their latitude as "north" (FRO and PIC) or "south" (GAR and RIV) (Table 1).

First, trait responses were analyzed using Linear Mixed Models (LMM) and Generalized Mixed Models (GLMM) at both spatial levels (population and latitudinal region). For this approach, we use the glmer and lmer functions within the *lme4* package version 1.1-31 (Bates *et al.*, 2022). We defined LMM for continuous response variables (i.e., continuous traits: FA, SLA, RWC,

LDMC, N, P, Seed mass and Seeds per fruit) and GLMM for discrete response variables (i.e., discrete traits: Germination time, Onset, End and Duration). We used a Gaussian distribution for continuous traits and a Poisson distribution for discrete traits. In all models, mother and block variables were introduced as random variables to control the variance due to maternal effects and the variance associated with position within the common garden experiment. At the population level, we included the variable population as a fixed variable and, at the latitudinal level, we nested the variable population inside the fixed variable latitude. The significance of fixed variables was determined by a type-II Wald chi-square statistic test. The significance test was obtained from the anova function within the *car* package version 3.1-1 (Fox and Weisberg, 2019). To study the existence of significant differences in trait values between population pairs, we then performed a *post hoc* analysis using the Bonferroni adjustment with pairwise.t.test function in *pairwiseCI* package version 0.1-27 (Schaarschmidt and Gerhard, 2019).

Within and between population variation of each trait was estimated by calculating its coefficient of variation (CV). The 95% confidence intervals of the coefficients of variation were calculated using the cv_versatile function of the R package *cvcqv* version 1.0.0 (Beigy, 2019). Comparison of CV confidence intervals between populations was performed using the inference described by Cumming (2009). Then, CV was represented by violin plots applying the vioplot function in *vioplot* R package version 0.3.7 (Adler *et al.*, 2020).

To visualize patterns of similarity between sample units at the population and latitude levels we performed principal component analyses (PCA) (Abdi and Williams, 2010) using the PCA function of the *FactoMineR* package version 2.4 (Lê *et al.*, 2008) which automatically standardise the variables. Leaf nitrogen and phosphorus content were not included in this analysis due to insufficient sample size. PCA results were represented with the *factoextra* package version 1.0.3 (Kassambara and Mundt, 2016).

Finally, Spearman correlation matrices were obtained with the R package *Hmisc* version 4.6-0 (Harrell Jr and Dupont, 2020) for all pairs of traits at the different study levels, and then represented using R packages *igraph* version 1.2.7 (Csardi and Nepusz, 2006) and *ggraph* version 2.0.5 (Pedersen, 2021). In addition, correlation matrices between populations or between latitudes were compared using the cortest.mat function of the *psych* R package version 2.1.9 (Revelle, 2021) which develops a chi-square (χ^2) test considering the differences between all possible pairs of correlations. This test is generated against the null hypothesis of no differences between the correlation matrices (Carvalho *et al.*, 2020).

RESULTS

Within- and between-population variation and latitude differentiation

Mean values differed in a greater number of traits at the population level (nine) than at the latitudinal level (seven) (Table 2). Both levels showed significant differences for FA, SLA, Seed mass, Onset, End and Duration (Table 2), but population level also showed significant differences for RWC, LDMC and Seeds per fruit (Table 2). Models detailed information is provided in Supplementary data (Tables S3, S4, S5 and S6). Differences between populations were greater between populations at different latitudes than between populations at the same latitude (Supplementary data Table S7). In particular, RIV was the southern population with the greatest number of significant differences in trait means with northern latitude populations (Supplementary data Table S7). Between populations of the same latitude, FRO and PIC populations significatly differed in FA, LDMC and Seeds per fruit, whereas GAR and RIV populations differed in SLA, RWC, LDMC, Onset, Duration and Seed mass traits (Supplementary data Table S7 and Figs. S1 and S2).

The first two principal components of the PCAs explained 48.40% of the variation in *L. angustifolius* (Fig. 1 A). PC1 axis had an important contribution from Onset, End and Seed

mass, whereas, in the PC2 axis, LDMC, Germination time and SLA were important traits (Fig. 1 A; Supplementary data Table S8). In general terms, *L. angustifolius* individuals had similar traits according to their population of origin (Fig. 1 B). This is supported by the significant differences obtained at the population level for most of the studied traits (Table 2). Within-population phenotypic variation differed depending on the studied trait (Table 3). Thus, the phenotypic variation of five of the study traits was higher in FRO and PIC populations, whereas in four other traits, GAR was the population with the greatest within-population variation. Finally, population RIV had the least within-population variation in most of the traits (Table 3, Fig. 1 B).

From a latitudinal perspective, southern individuals were more clustered to each other than northern ones (Fig. 1 C). When we grouped *L. angustifolius* populations in northern and southern latitudes, we found fewer significant differences between the mean values of all traits, i.e., nine traits with significant differences at the population level *versus* seven at the latitude level (Table 2). Thus, northern latitude populations showed larger FA, finer leaves (higher SLA) and higher P (Table 2; Supplementary data Table S2 and Fig. S3). The CV of FA was significantly higher at the northern latitude, while RWC showed a significantly higher CV at the southern latitude (Supplementary data Table S7).

Flowering onset and end dates were later in the northern latitude populations, and duration was shorter (FRO and PIC, Tables 2; Supplementary data Table S2 and Fig. S4). Onset, End and Duration showed significantly greater within-latitude variation in the northern latitude populations, while Germination time showed greater variation at the southern latitude (Supplementary data Table S2). Seeds from the northern latitude populations had lower mass. Variation in Seed mass and Seeds per fruit was also significantly greater in the northern latitude populations (Supplementary data Table S2 and Fig. S4).

Correlation patterns between traits

All trait correlation matrices were significantly different from one another, both at the population and latitudinal levels. A more complex correlation network was observed in GAR and RIV (with 15 and 23 significant correlations, respectively) than in FRO and PIC (with 11 and 13 significant correlations, respectively) (Fig. 2A, Supplementary data Tables S9 and S10). FRO, GAR and RIV populations presented significant correlations between the three trait blocks (foliar, phenological, and reproductive), whereas PIC presented significant correlations only between phenological and foliar traits (Fig. 2A, Supplementary data Tables S9 and S10). The southern and northern latitudes presented 25 and 13 significant trait correlations, respectively, nine of which were common to both latitudes (Fig. 2B, Supplementary data Table S11). Additionally, the number of significant correlations between reproductive traits was greater in the southern latitude populations (five in the southern latitude *versus* one in the northern latitude) (Fig. 2B, Supplementary data Table S11).

DISCUSSION

Common garden experiments allow the evaluation of phenotypic traits of plants from different origins under a common and homogeneous environment, reducing to negligible the effect of the environmental component in the expression of the phenotypes (de Villemereuil *et al.*, 2016). Therefore, the differences observed in the phenotypic values can be attributed to genetic variation. This way, our common garden experiment provided an assessment of the genetic basis of the within- and between-population phenotypic variation in the studied *Lupinus angustifolius* populations, as well as some insight on their evolutionary potential to respond to climate change (Lara-Romero *et al.*, 2014). The results of the PCAs (Fig. 1 B and C) in combination with the CVs values at both study levels (Table 3, Supplementary data Table S2) showed a larger phenotypic variation when the populations from different latitudes were

compared. Futhermore, several of the trait values obtained at each location (Table 2 and 3, Supplementary data Table S2) can be associated with adaptations to high temperature and water availability constraints, supporting, thus, our first hypothesis. The results obtained also supported our second hypothesis with lower phenotypic variation in most traits in the southern latitude populations (Supplementary data Table S2) and greater number of significant correlations between traits (Supplementary data Tables S9, S10 and S11). Finally, as predicted in our third hypothesis, between-population phenotypic variation differed depending on the group of traits analysed. We expected a more marked differential pattern on foliar traits as they are known to be closely related to the drought and high temperature environmental filters that mainly affect the southern populations. However, differences in phenological traits were, in this case, also very determinant allowing southern populations to develop an escape strategy to drought.

Greater phenotypic variation at the latitudinal level

The distribution of individuals in the PCAs allowed us to identify a greater dispersion of phenotypes at the latitudinal level (Fig. 1B and 1C). This is supported by the knowledge that environmental filters at local levels (population level) favor similar phenotypes (Gaüzère *et al.*, 2022), i.e., lead to a reduction of phenotypic variation. Combining this observation with the CVs detected at both levels (population and latitudinal), allowed us to conclude that there is greater phenotypic variation at the latitudinal level than between populations (Tables 3, Supplementary data Table S2). For example, the clustering of RIV and GAR slightly diluted the lower CV values of the RIV population for some of the traits (e.g., FA, SLA, RWC, LDMC, N, P, Germination time, Duration, Seed mass and Seeds per fruit). As we predicted, phenotypic variation was greater on the latitudinal level for some of the traits. Thus, this pattern of phenotypic variation at the latitudinal level indicated that latitude acts as a potential environmental filter (Hulshof *et al.*, 2013). As Ohsawa and Ide (2007) suggest, this may be due

to the greater environmental differences existing at this scale, favouring more contrasted between-population variation.

Greater number of significant correlations between traits and less variation within and between populations in southern populations

Southern populations were more limited in their phenotypic variation probably due to the presence of more restrictive abiotic filters (e.g., more intense drought and aridity), supporting the observations of Anderegg *et al*. (2020) and Carvalho *et al*. (2020). We noted this in the lower phenotypic variation found in many traits (lower CV) at the southern latitude (e.g., FA, N, Onset, End, Duration, Seed mass and Seeds per fruit) (Supplementary data Table S2). Thus, higher temperatures and lower water availability generated more challenging environmental conditions reducing the number of available phenotypes (and, thus, genotypes) (Sgrò *et al*., 2011; Pescador *et al*., 2015; Luo *et al*., 2016; Carvalho *et al*., 2020; Helsen *et al*., 2020) in *L. angustifolius* southern populations.

The increase in the number of correlations between traits found in *L. angustifolius* southern populations (Fig. 2), in addition to lower phenotypic variation, presents a more complicated situation for these populations. The combination of these two elements generates more coordinated phenotypes, resulting in the process of phenotypic integration (Carvalho *et al*., 2020). The occurrence of these trade-offs may discard phenotypes that could favour the survival of *L. angustifolius* southern populations under changing environmental conditions. This ultimately could put populations at risk (Anstett *et al*., 2021).

For future studies, it would be interesting to add other species to integrate characteristics of the species that are known to affect phenotypic variation (e.g., life cycle and reproductive strategy). In this sense, *L. angustifolius* is an annual species with an autogamous reproductive strategy (Castroviejo and Pascual, 1999). An annual strategy triggers a higher number of correlations

and constrictions between traits (Carvalho *et al.*, 2020) by undergoing faster evolution (with greater loss of genetic variation) (Anstett *et al.*, 2021). The autogamous character contributes to reducing population genetic variation, favouring population isolation and local adaptation events.

Phenotypic variation varies according to the group of traits studied

Our third hypothesis was supported by the differences found in phenotypic variation according to the trait groups formed (foliar, phenological and reproductive). This underlines the importance of studying a variety of traits when assessing the evolutionary potential of a species and, therefore, its capacity to respond to various threats (Westerband *et al.*, 2021).

- Foliar traits

The lower SLA values (a consequence of lower FA) obtained in the southern latitude populations (Supplementary data Table S2) could be a strategy aimed at reducing leaf evapotranspiration in warmer and drier environments (Pescador *et al.*, 2015; Matesanz *et al.*, 2020). Thus, water constraints seem to play a fundamental role in some foliar traits at southern latitudes (Pescador *et al.*, 2015). In northern latitudes the response is likely to be different. Summer drought is less intense in northern latitudes of *L. angustifolius* (FRO and PIC) where annual temperatures are lower (Table 1). This allows *L. angustifolius* individuals to extend their capture of resources (higher SLA) and develop a strategy of rapid biomass production (Yulin and Zhang, 2005). At the same time, lower LDMC values would be expected in northern *L. angustifolius* populations since SLA and LDMC are known to be negatively correlated. However, the relationship between SLA and LDMC weakens when nutrient limitation becomes the main determining factor (Yulin and Zhang, 2005).

- Phenological traits

Phenological trait responses to an environmental change are essential to ensure population viability (Nagahama *et al.*, 2018). When such responses are triggered in the flowering period, their reproductive success may be affected (Nagahama *et al.*, 2018; Buonaiuto and Wolkovich, 2020). Flowering period is a complex process that depends on multiple factors defined by the environment itself. These could be summarized as (i) cold temperatures from autumn to late winter, (ii) warm temperatures from late winter to early spring, and (iii) photoperiod (Ettinger *et al.*, 2020). In this study, flowering phenology was much earlier in southern latitude populations of *L. angustifolius* (GAR and RIV) than in northern populations (FRO and PIC) (Table 3). We infer that southern populations of *L. angustifolius* have developed a drought escape strategy by expressing an earlier flowering onset under environmental conditions that do not allow them to extend their life cycle (Anstett *et al.*, 2021; Welles and Funk, 2021; Matesanz *et al.*, 2022). For this reason, within the same project, a field experiment has been developed for verifying the results obtained in this section (Sacristán-Bajo, under preparation). With this experiment, we could specify that this pattern favours GAR and RIV reproductive success in a warmer environment with lower water availability (Matesanz *et al.*, 2020). Southern latitude populations of *L. angustifolius* showed greater variation in germination time according to their CVs. This provides a greater response capacity to adjust to different and unpredictable environmental conditions (Sgrò *et al.*, 2011). Thus, seeds can remain dormant to germinate when conditions become favourable (Rubio de Casas *et al.*, 2017).

- Reproductive traits

L. angustifolius reproductive traits showed marked patterns between latitudes. Southern populations GAR and RIV had lower seed production, but the seeds were heavier. This could be interpreted as an adaptation to drought (Rubio de Casas *et al.*, 2017; Matesanz *et al.*, 2020). Greater seed mass is related to a strategy focused on ensuring individual survival until it can reach reproductive maturity in stressful environments (Helsen *et al.*, 2020). However, this

reduces the growing season and the maturation time to seed and fruit (Primack, 1987). Reducing seed maturation time triggers a trade-off, reducing the number of seeds produced (Helsen *et al.*, 2020).

CONCLUSION

The genetic basis of phenotypic variation studied in the present work allows us to assess the potential of *Lupinus angustifolius* to generate an adaptive response to climate change. Moreover, the evaluation of within- and between-population variation in different functional traits was highly relevant for identifying the different environmental conditions that shape variation in the phenotypic traits of populations and the potential of populations to deal with environmental changes. In this context, populations with lower within-population variation and more coordinated phenotypes (GAR and RIV) may be more vulnerable to swift environmental changes because it reduces their ability to adapt. The assessment of different groups of functional traits showed that phenological traits were the most determinant for phenotypic expression in *L. angustifolius*. The reproductive system and life cycle of *Lupinus angustifolius* conditioned the traits pattern, as well as the latitudinal and altitudinal differences of the populations studied. Finally, this work highlights the role of the study of within- and between-populations traits variation in predicting populations' ability to face climate change.

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LITERATURE CITED

Abdi H, Williams LJ. 2010. Principal component analysis. *WIREs Computational Statistics* 2 : 433-459.

Abràmoff MD, Magalhães PJ, Ram SJ. 2004. Image processing with ImageJ. *Biophotonics International* 11 : 36-42.

Adler D, Kelly ST. 2020. *vioplot: violin plot.* R package version 0.3.7.

Anderegg LDL, Loy X, Markham IP, *et al*. 2020. Aridity drives coordinated trait shifts but not decreased trait variance across the geographic range of eight Australian trees. *New Phytologist Foundation* 229 : 1375-1387.

Anstett DN, Branch HA, Angert AL. 2021. Regional differences in rapid evolution during severe drought. *Evolution Letters* 5 : 130-142.

Bates D, Maechler M, Bolker B, *et al*. 2022. *Linear Mixed-Effects Models using 'Eigen' and S4*. R package version 1.1-31.

Beigy M. 2019. *cvcqv: Coefficient of Variation (CV) with Confidence Intervals (CI).* R package version 1.0.0.

Berger JD, Shrestha D, Ludwig C. 2017. Reproductive strategies in mediterranean legumes: trade-offs between phenology, seed size and vigor within and between wild and domesticated *Lupinus* species collected along aridity gradients. *Frontiers in Plant Science* 8 : 1-16.

Bolnick DI, Amarasekare P, Araújo MS, *et al*. 2011. Why intraspecific trait variation matters in community ecology. *Trends in Ecology and Evolution* 26 : 183-192.

Botkin DB, Saxe H, Araújo MB, *et al*. 2007. Forecasting the effects of global warming on biodiversity. *BioScience* 57 : 227-236.

Buonaiuto DM, Wolkovich EM. 2020. Differences between flower and leaf phenological responses to environmental variation drive shifts in spring phenological sequences of temperate woody plants. *Journal of Ecology* 109 : 2922-2933.

Carvalho B, Bastias CC, Escudero A, Valladares F, Benavides R. 2020. Intraspecific perspective of phenotypic coordination of functional traits in Scots pine. *PLOS ONE* 2 : 1-15.

Castroviejo S, Pascual H. 1999. *Lupinus* L. In: Talavera S, Aedo C, Castroviejo S, *et al*., eds. *Flora iberica. Plantas vasculares de la Península Ibérica e Islas Baleares.* Madrid: Real Jardín Botánico CSIC, 251-260.

Cruz-Maldonado N, Weemstra M, Jiménez L, *et al*. 2021. Aboveground-trait variations in 11 (sub)alpine plants along a 1000-m elevation gradient in tropical Mexico. *Alpine Botany* 131 : 187-200.

Csárdi G, Nepusz T. 2006. "The igraph software package for complex network research." *InterJournal*, *Complex Systems* 1695 : 1-9.

Cumming G. 2009. Inference by eye: Reading the overlap of independent confidence intervals. *Statistics in Medicine* 28 : 205-220.

De Villemereuil P, Gaggiotti OE, Mouterde M, Till-Bottraud I. 2016. Common garden experiments in the genomic era: new perspectives and opportunities. *Heredity* 116 : 249-254.

Ettinger AK, Chamberlain CJ, Morales-Castilla I, *et al*. 2020. Winter temperatures predominate in spring phenological responses to warming. *Nature Climate Change* 10 : 1137-1142.

Fox J, Weisberg S. 2019. *An R Companion to Applied Regression*. R package, version 3.1-1.

Funk WC, Forester BR, Converse SJ, Darst C, Morey S. 2019. Improving conservation policy with genomics: a guide to integrating adaptive potential into U.S. Endangered Species Act decisions for conservation practitioners and geneticists. *Conservation Genetics* 20 : 115-134.

Gaüzère P, Blonder B, Denelle P, *et al*. 2022. The functional trait distinctiveness of plant species is scale dependent. *Ecography*. 2023 : 1-15.

Halbritter AH, Fior S, Keller I, *et al*. 2018. Trait differentiation and adaptation of plants along elevation gradients. *Journal of Evolutionary Biology*. 31 : 784-800.

Harrell Jr FE, Dupont C. 2020. *Package "Hmisc" for R: Harrell Miscellaneous*. R package, version 4.6-0.

Helsen K, Prasad Acharya K, Jessen Graae B, *et al*. 2020. Earlier onset of flowering and increased reproductive allocation of an annual invasive plant in the north of its novel range. *Annals of Botany* 126 : 1005-1016.

Hulshof CM, Violle C, Spasojevic MJ, *et al*. 2013. Intra-specific and inter-specific variation in specific leaf area reveal the importance of abiotic and biotic drivers of species diversity across elevation and latitude. *Journal of Vegetation Science* 24 : 921-931.

Kassambara A, Mundt F. 2016. *Package "factoextra" for R: Extract and Visualize the Results of Multivariate Data Analyses*. R package, version 1.0.3.

Kazimierska EM, Kazimierski T. 2002. Biology of flowering, embryological and caryological peculiarities. In: Kurlovich, B.S. (ed.) *Lupins. Geography, classification, genetic resources and breeding*. St. Petersburg OY International North Express, 205-239.

Körner C. 2007. The use of "altitude" in ecological research. *Trends in Ecology and Evolution*. 22 : 569–574.

Kühn P, Backes AR, Römermann C, Bruelheide H, Haider S. 2021. Contrasting patterns of intraspecific trait variability in native and non-native plant species along an elevational gradient on Tenerife, Canary Islands. *Annals of Botany* 127 : 565-576.

Lara-Romero C, García-Camacho R, Escudero A, Iriondo JM. 2014. Genetic variation in flowering phenology and reproductive performance in a mediterranean high-mountain specialist, *Armeria caespitosa* (Plumbaginaceae). *Botanical Journal of the Linnean Society* 176 : 384-395.

Lê S, Josse J, Husson F. 2008. FactoMineR: A package for multivariate analysis. *Journal of Statistical Software* 25 : 1–18.

Li B, Suzuki J-I, Hara T. 1998. Latitudinal variation in plant size and relative growth rate in *Arabidopsis thaliana*. *Oecologia* 115: 293-301.

Luo Y-H, Liu J, Tan S-L, *et al*. 2016. Trait-based community assembly along an elevational gradient in subalpine forests: quantifying the roles of environmental factors in inter- and intraspecific variability. *PLoS ONE* 5 : 1-20.

Martin AR, Isaac ME. 2021. The leaf economics spectrum's morning coffee: plant size-dependent changes in leaf traits and reproductive onset in a perennial tree crop. *Annals of Botany* 127 : 483-493.

Matesanz S, Gianoli E, Valladares F. 2010. Global change and the evolution of phenotypic plasticity in plants. *Annals of the New York Academy of Sciences* 1 : 35-55.

Matesanz S, Ramos-Muñoz M, Moncalvillo B, *et al*. 2020. Plasticity to drought and ecotypic differentiation in populations of a crop wild relative. *AoB PLANTS* 12 : 1-13.

Matesanz S, Ramos-Muñoz M, Rubio Teso ML, Iriondo JM. 2022. Effects of parental drought on offspring fitness vary among populations of a crop wild relative. *The Royal Society Publishing* 289 : 1-11.

McGill BJ, Enquist BJ, Weiher E, Westoby M. 2006. Rebuilding community ecology from functional traits. *Trends in Ecology and Evolution* 21 : 178-185.

Milla R, Reich PB. 2011. Multi-trait interactions, not phylogeny, fine-tune leaf size reduction with increasing altitude. *Annals of Botany* 107 : 455-465.

Moncalvillo B, Méndez M, Iriondo JM. 2019. Ecotypic differentiation reveals seed colour-related alkaloid content in crop wild relative. *Plant Biology* 21 : 942-950.

Mousavi-Derazmahalleh M, Bayer PE, Nevado B, *et al*. 2018. Exploring the genetic and adaptive diversity of pan-Mediterranean crop wild relative: narrow-leafed lupin. *Theoretical and Applied Genetics* 131: 887-901.

Murren CJ. 2012. The integrated phenotype. *Integrative and Comparative Biology* 52 : 64-76.

Nagahama A, Kubota Y, Satake A. 2018. Climate warming shortens flowering duration: a comprehensive assessment of plant phenological responses based on gene expression analyses and mathematical modeling. *Ecological Research* 33 : 1059-1068.

Ohsawa T, Ide Y. 2008. Global patterns of genetic variation in plant species along vertical and horizontal gradients on mountains. *Global Ecology and Biogeography* 17 : 152-163.

Pedersen TL. 2021. *Package "ggraph" for R: An implementation of grammar of graphics and networks*. R package, version 2.0.5.

Pescador DS, De Bello F, Valladares F, Escudero A. 2015. Plant trait variation along an altitudinal gradient in mediterranean high mountain grasslands: controlling the species turnover effect. *PLoS ONE* 3 : 1-16.

Pigliucci M. 2003. Phenotypic integration: studying the ecology and evolution of complex phenotypes. *Ecology Letters* 6 : 265-272.

Primack RB. 1987. Relationships among flowers, fruits and seeds. *Annual Review of Ecology and Systematics* 18 : 409-430.

Radojevic M, Bashkin VN. 2015. *Practical environmental analysis*, 2nd edn. Cambridge: RSC Publishing.

Revelle W. 2021. *psych: Procedures for Psychological, Psychometric, and Personality Research*. R package version 2.1.9.

R Core Team. 2020. *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org.

Rubio de Casas R, Willis CG, Pearse WD, Baskin CC, Baskin JM, Cavender-Bares J. 2017. Global biogeography of seed dormancy is determined by seasonality and seed size: a case study in the legumes. *New Phytologist* 214 : 1527-1536.

Sacristán-Bajo S, García-Fernández A, Lara-Romero C, *et al*. 2023. Population origin determines the adaptive potential for the advancement of flowering onset in *Lupinus angustifolius* L. (Fabaceae). *Evolutionary Applications* 16 : 62-73.

Schaarschmidt F, Gerhard D. 2019. *Confidence Intervals for Two Sample Comparisons*. R package version 0.1-27.

Sgrò CM, Lowe AJ, Hoffmann AA. 2011. Building evolutionary resilience for conserving biodiversity under climate change. *Evolutionary Applications* 4 : 326-337.

Talhinhas P, Leitao J, Neves-Martins J. 2006. Collection of *Lupinus angustifolius* L. germplasm and characterisation of morphological and molecular diversity. *Genetic Resources and Crop Evolution* 53 : 563-578.

Violle C, Enquist BJ, McGill BJ, *et al*. 2012. The return of the variance: intraspecific variability in community ecology. *Trends in Ecology and Evolution* 27 : 244-252.

Welles SR, Funk JL. 2021. Patterns of intraspecific trait variation along an aridity gradient suggest both drought tolerance strategies in an invasive herb. *Annals of Botany* 127 : 461-471.

Westerband A, Knight TM, Barton KE. 2020. Intraspecific trait variation and reversals of trait strategies across key climate gradients in native Hawaiian plants and non-native invaders. *Annals of Botany* 127 : 553-564.

Westerband AC, Funk JL, Barton KE. 2021. Review: Intraspecific trait variation in plants: a renewed focus on ecological processes. *Annals of Botany* 127 : 397-410.

Yulin L, Zhang TH. 2005. Specific leaf area and leaf dry matter content of plants growing in sand dunes. *Botanical Bulletin* 46 : 127-134.

Fig. 1. Principal component analyses of *Lupinus angustifolius*. The x axis (PC1) represents principal component one, while the y axis (PC2) the principal component two. Variable contributions in the construction of the principal components (A1 and A2) are represented with a colour scale from orange (high contribution) to cyan (low contribution). In each axis (A1 and

A2), percentage of variance explained by each principal component is indicated. Principal component analyses at the population level (B1 and B2) and latitudinal level (C1 and C2) represent individuals by points with different colors and shapes depending on the population (B1 and B2) or latitude (C1 and C2) to which they belong.

Fig. 2. Network of significant correlations of *Lupinus angustifolius* populations (A) and latitudes (B). Arrows symbolize the type of significant correlation obtained: continuous line = positive correlation; dashed line = negative correlation. The size of the arrows indicates the value of the correlation: correlation values between 0/0.29 and 0/-0.29, 0.5-point size; values between 0.3/0.49 and -0.3/-0.49, 1-point size; and values 0.5 and above and equal or below -0.5, 1.5-point size. Foliar traits are represented by green: sum of leaf area (FA), relative water content (RWC), specific leaf area (SLA), leaf dry matter content (LDMC), phosphorus (P) and nitrogen content (N); phenological traits by pink: seed germination time (Germination time), flowering onset, end and duration (Onset, End and Duration); and reproductive traits by blue: germinated seed weight (Seed mass) and average seeds per fruit (Seeds per fruit).

Table 1. Geographic coordinates and climatic variables corresponding to the studied populations of *Lupinus angustifolius*. The climatic variables are represented as mean annual temperature (T) measured in degrees Celsius, and May-July precipitation (P) measured in millimeters; elevation is measured in meters. Latitude to which each population belongs (north

or south) is also denoted. Climatic data of *L. angustifolius* populations belong to the 1985-2015 time series and common garden data located in Móstoles (Madrid) belong to the 2017-2020 time series. Data were obtained from Sacristán-Bajo *et al.*, (2023).

Localities (population)	Latitude	Geographic coordinates	Elevation	T	P
Zafrón (FRO)	North	41.024192N; 6.028155W	840	12.4	92
Zarapicos (PIC)	North	41.004358N; 5.813066W	820	12.6	89
Rivera de la Lanchita (RIV)	South	38.351586N; 6.576084W	352	16.8	61
La Garranchosa (GAR)	South	38.325735N; 6.433735W	422	16.5	64
Common garden (Móstoles) 2017		40.334615N; 3.882168W	690	15.1	72
Common garden (Móstoles) 2018				14.6	95
Common garden (Móstoles) 2019				14.8	16
Common garden (Móstoles) 2020				15.1	69

Table 2. Chi-square statistic (X^2), degrees of freedom (Df) and p-value of type II Wald chi squared tests for all GLMM and LMM generated on population and latitude level. Significant values are indicated by the p-value when <0.05 = *, <0.01 = ** and <0.0001= ***.

Fixed effects	X^2	Df	p-value	Fixed effects	X^2	Df	p-value
FA				**Seed mass**			

Population	57.30	3	***	Population	296	3	***
Latitude	4.98	1	*	Latitude	98.52	1	***
SLA				**Seeds per fruit**			
Population	75.39	3	***	Population	168.52	3	***
Latitude	4.97	1	*	Latitude	2.23	1	0.14
RWC				**Onset**			
Population	12.71	3	**	Population	173.71	3	***
Latitude	1.30	1	0.25	Latitude	73.94	1	***
LDMC				**End**			
Population	57.25	3	***	Population	34.53	3	***
Latitude	3.22	1	0.07	Latitude	33.62	1	***
N				**Duration**			
Population	0.72	3	0.87	Population	102.53	3	***
Latitude	0.05	1	0.83	Latitude	8.99	1	**
P				**Germination time**			
Population	7.83	3	0.05	Population	3.38	3	0.34
Latitude	3.99	1	*	Latitude	2.19	1	0.14

Table 3. Mean, standard deviation (SD) and coefficients of variation (CV) with 95% confidence intervals for leaf traits: 1) sum of leaf area (FA), 2) specific leaf area (SLA), 3) relative water content (RWC), 4) leaf dry matter content (LDMC), 5) nitrogen (N) and 6) phosphorus (P) content; phenological traits: 1) seed germination time (Germination time), 2) flowering onset (Onset), 3) end (End) and 4) duration (Duration); and reproductive traits: 1) weight of

germinated seed (Seed mass) and 2) average of seeds per fruit (Seeds per fruit), from *Lupinus angustifolius* populations (FRO, PIC, GAR and RIV).

	Mean ± SD				CV			
	FRO	PIC	GAR	RIV	FRO	PIC	GAR	RIV
FA (cm^2)	14.39 ± 3.10	16.81 ± 4.79	13.15 ± 2.61	11.93 ± 2.24	21.47 (17.57; 26.42)	28.42 (23; 33.95)	19.79 (17.10; 23.05)	18.69 (16.13; 21.52)
SLA (cm^2/g)	211.31 ±32.78	216.42 ± 31.63	198.30 ± 30.17	174.27 ± 27.08	15.46 (12.85; 18.33)	14.56 (12.29; 17.25)	15.17 (12.49; 18.30)	15.49 (13.14; 18.21)
RWC (%)	84.91 ± 4.29	83.82 ± 3.92	84.66 ± 5.98	86.80 ± 4.31	5.04 (4.28; 5.87)	4.66 (3.83; 5.60)	7.04 (5.87; 8.42)	4.95 (4.02; 6.04)
LDMC (%)	15.28 ± 1.07	14.69 ± 1.11	13.76 ± 1.25	14.57 ± 0.92	6.99 (5.94; 8.22)	7.49 (5.62; 9.46)	9.03 (7.25; 10.81)	6.30 (5.16; 7.47)
N (mg/g)	50.61 ± 13.67	50.71 ± 13.48	52.85 ± 14.64	49.70 ± 10.71	26.76 (21.50; 33.86)	26.32 (17.12; 36.38)	36.69 (17.48; 54.20)	21.33 (14.80; 29.79)
P (mg/g)	4.88 ± 1.16	4.57 ± 0.96	4.60 ± 1.05	3.81 ± 0.78	23.52 (16.50; 32.03)	20.69 (12.39; 30.48)	33.01 (11.71; 49.61)	20.17 (15.93; 25.79)
Germination time (days)	9 ± 2	9 ± 2	9 ± 3	9 ± 2	22 (17; 27)	20 (17; 23)	28 (23; 34)	20 (17; 24)
Onset (days)	116 ± 8	118 ± 10	103 ± 5	99 ± 6	7 (5; 9)	8 (7; 10)	5 (4; 6)	6 (5; 7)
End (days)	165 ± 6	165 ± 6	155 ± 4	157 ± 5	4 (3; 5)	4 (3; 6)	3 (2; 3)	3 (3; 4)
Duration (days)	47 ± 9	47 ± 8	51 ± 5	57 ± 6	19 (14; 23)	17 (14; 20)	10 (8; 13)	10 (7; 12)
Seed mass (mg)	112.60 ± 18.25	106.56 ± 21.32	163.83 ± 23.48	154.99 ± 17.95	16.15 (13.24; 19.61)	19.94 (15.93; 24.35)	14.29 (12.64; 15.98)	11.55 (9.48; 13.49)
Seeds per fruit	5.75 ± 0.32	5.19 ± 0.42	5 ± 0.32	5.07 ± 0.29	5.47 (4.42; 6.63)	7.97 (6.49; 9.63)	6.39 (4.70; 8.07)	5.79 (4.47; 7.19)