# Ex situ cultivation impacts on plant traits and drought stress response in a multi-species experiment

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## Abstract

Ex situ collections of wild plants of conservation value are supposed to preserve the phenotypic variability of their wild source populations as well as their plastic responses to environmental stress. However, genetic erosion and adaptive evolutionary changes during cultivation are likely to impose strong constraints to those aims. To date, is it not known whether cultivation affects plant trait variability and stress response of species in botanic garden collections. We studied the effects of cultivation on the trait expression, genetic trait variability, and drought stress response in 12 plant species cultivated in Meise Botanic Garden, Belgium. We found that cultivation increased germination rate and leaf length across all species, while it decreased flowering and delayed the beginning of the flowering period in six drought-tolerant species. We furthermore found indications that plant response to drought was reduced by cultivation in some performance variables, while mortality due to drought slightly increased with cultivation. In three out of 10 traits measured, genetic variability decreased with increasing cultivation time in the botanic garden indicating a loss of evolutionary potential. Our results suggest that the preservation of the phenotypic status and evolutionary potential in ex situ collections is a challenging task and that the application of up-to-date protocols is decisive to achieve meaningful conservation collections.

Key words: ex situ conservation, botanic gardens, trait variability, evolutionary potential, unconscious selection, adaptation

## 1. Introduction

Preserving threatened plant species ex situ (i.e. outside their natural habitats) has grown to a fundamental pillar in global conservation efforts (Donaldson, 2009; Maunder et al., 2004; Mounce et al., 2017). In this context, botanic garden collections of seeds and living plants of rare and endangered plants have become a more and more important resource and now 40 % of the world's threatened species are somehow backed-up in botanic gardens (Mounce et al., 2017). This demonstrates the potential of botanic garden's ex situ collections in preventing the extinction of endangered plants, and even a resource of returning species extinct in the wild (Abeli et al., 2019).

However, ex situ collections of plants in botanic gardens have also been associated with several problematic processes, i.e. genetic erosion, inbreeding depression or adaptation to the artificial cultivation conditions (Ensslin et al., 2015; Ensslin and Godefroid, 2019; Havens et al., 2004; Schoen and Brown, 2001). These concerns arise from the fact that most populations ex situ are very small, increasing adverse effects of genetic drift and the likelihood of inbreeding (Ellstrand and Elam, 1993). Moreover, as plants in ex situ collections are grown out of their natural conditions, selection pressures are expected to change considerably, increasing the risk of a genetic adaptation to cultivation and a loss of adaptations to the wild conditions over time (Ensslin et al., 2015; Husband and Campbell, 2004). In general, the combination of stochastic processes (genetic and demographic changes due to small population sizes) and selection pressures (relaxed selection pressures and unconscious selection by gardeners) may result in a reduction of genetic variability (genetic erosion) and a differentiation away from their original wild population (Ensslin et al., 2015).

Genetic variability of the plant's phenotype is a crucial feature of a population as it is what natural selection acts upon (Falconer and Mackay, 1996; Vitt and Havens, 2004). Hence, variation in phenotypic traits in a population can be directly connected to its adaptability to cope with environmental changes (Lande and Shannon, 1996; Shaw and Etterson, 2012; Vitt and Havens, 2004). In ex situ populations, these back-up populations are expected to preserve all evolutionary relevant variability, and thereby the potential to evolve to environmental changes. It has been shown in several studies that plants cultivated ex situ can be genetically depauperate and may not represent the genetic diversity of their wild populations (Brütting et al., 2013; Christe et al., 2014; Rucińska and Puchalski, 2011). However, whether this also translates into a change in morphological traits or a reduction of trait variability has not been studied. Neutral genetic markers used to infer genetic diversity of a population (i.e. microsatellites, AFLPs or RAPDs) do not necessarily reflect the variability in morphological characters and therefore may not be an appropriate assessment of morphological genetic diversity (Reed and Frankham, 2001; Vitt and Havens, 2004). Only very few studies so far investigated shifts in phenotypic traits resulting from ex situ cultivation. Those studies found an increase in the germination rate, advanced phenological timing and a change in plant architecture (Ensslin et al., 2018, 2015; Enßlin et al., 2011; Espeland et al., 2017; Rauschkolb et al., 2019). These studies suggest that phenological (including germination) and life-history traits associated with plant fitness might be most affected by evolutionary changes due to cultivation. However, it has not been studied whether this could translate into reduced evolutionary potential or a change in stress response. In recent years, the plant growth strategy has received considerable interest due to its link to plant's responses to environmental change (Funk et al., 2017). Specific leaf area (SLA) is an important trait for distinguishing fast-growing from slow-growing individuals and can describe the effects of plant domestication (Matesanz and Milla, 2018). Whether plants in ex situ collections show a shift towards a more fast-growing strategy (Matesanz and Milla, 2018), has not been studied yet.

A population's evolutionary potential is composed of the genetic variation in quantitative traits as well as the plants' response to stresses (i.e. phenotypic plasticity; Hoffmann & Sgrò 2011; Franks, Weber & Aitken 2014). If plastic responses are costly, the selection will remove them if they are not needed anymore (DeWitt et al., 1998). In the relaxed selection of an ex situ environment, stresses such as lack of water and nutrient availability or competition are typically reduced (Ensslin et al., 2015; Havens et al., 2004). Hence, there is concern that the reduced environmental stresses may result in a loss of specific adaptations to those stresses. As the magnitude of adaptation to a new environment depends on the difference between the previous and the new habitat (Husband and Campbell, 2004), species from more stressful habitats (e.g. dry and nutrient-poor grasslands) may be more prone to genetic changes than species from mild or stable habitats, which may manifest itself in a stronger change in their responses to stress when cultivated.

In this study, we measured plant traits and their responses to drought stress in 12 plant species that varied in years in cultivation from 1 to 30 years from the Meise Botanic Garden, Belgium. We hypothesized that the longer ex situ cultivation will result in a change in trait means, a reduction in trait variability and a reduced response to drought stress. Specifically, we were interested in the following questions: 1. Do cultivated plants show a shift in traits associated with phenology, fitness, performance and growth-strategy traits compared to wild-collected plants? 2. Do cultivated plants show reduced responses to drought compared to wild-collected plants, and is the change in response greater in drought-tolerant than in drought-intolerant species? 3. Do cultivated plants have a reduced genetic variability in traits compared to wild-collected plants?

## 2. Methods

### 2.1 Species selection

We conducted a multi-species common garden experiment with 12 herbaceous plant species of the European flora (Table 1). All species are common species in Belgium, meaning that they are not threatened and most of them widely distributed (van der Meijden et al., 2016). Seeds of the 12 species were collected in 2015 from the garden beds of Meise Botanic Garden and wild populations close to the original collection site. In the wild populations, seeds were collected randomly from 7-10 individuals across the whole population. Plants were sampled several meters apart to ensure that we collected from separate genets. Garden populations were less extensive, so it was not possible to maintain a distance of several meters between plants, but we were able to sample 8-10 individuals, unless the population was so small that all plants had to be sampled (e.g., in *Helianthemum nummularium*, *Bromus erectus* and *Brachypodium pinnatum*, Table 1). The cultivated species had been grown in the beds of the botanic garden between 1 and 30 years (Table 1). Due to logistical reasons and the disappearance of the some of the original populations, the wild populations did not exactly match the populations originally sampled for the ex situ collections. Most of the populations however, came from the same bio-climatic region (within 1 and 53 km), except for three species, which originally came from France and were substituted from a greater distance (300-400 km) from the wild population. Because the species differ naturally in their sensitivity to drought, we used their Ellenberg values (Ellenberg et al., 1992) to discriminate drought-tolerant (Ellenberg F ≤3) from drought-intolerant (Ellenberg F > 3) species (Table 1).

### 2.2 Germination and common garden experiment

Seeds of all species were germinated in March 2016 in Petri dishes filled with 1% agar (10g/l). Species that required cold stratification had been pre-placed in a fridge at 5°C for two months (also on agar). Seeds of *Trifolium pratense* and *Helianthemum nummularium* were scarified prior to germination with a scalpel. For the germination, we placed all petri dishes into incubation chambers (LMS Cooled Incubator A280; LMS Ltd, Sevenoaks, UK) and recorded germination twice a week. Germination treatments (temperature and day length) for each species followed the recommendations of the Seed Information Database (Royal Botanic Gardens Kew, 2017). In April 2016, we planted the seedlings into 0.8 litre pots filled with a mixture of standard potting soil and sand (3:1), and placed in an open tunnel greenhouse (4 m width, 2.2 m high, 21 m long) on the compound of Meise BG (see Ensslin & Godefroid, 2019). Temperatures in the greenhouse ranged between 10 and 30 °C over the measurement period (recorded with a temperature logger in the greenhouse, Model EL-USB 2, Dataq Instruments Inc, USA). We recorded the date of planting and the size of each plant at the start as a covariate for the analyses. We randomly placed the plants into four blocks, of which two blocks were treated with drought treatments in July and August 2016. Blocks and individual positions within the blocks were randomized twice each season. We measured the plants across two years in 2016 and 2017. In 2016, we measured plant height, number of flowering stems, length of the longest leaf (hereafter referred to as 'leaf length') and total aboveground biomass at the end of the first season. In 2017, we measured survival, number of flowering stems, leaf length, SLA and biomass at the end of the second season. SLA was measured as the area of three freshly cut and scanned leaves, divided by their dry weight. After scanning, leaves were dried at 29 °C for three days in a drying oven and the weight measured with a precision scale. We used the programme imageJ to determine the leaf area from scanned leaves (https://imagej.net). Furthermore, we recorded the beginning of flowering period in every individual in both years, and the duration of flower production (i.e. the time that open flowers were visible on the plant) in 2017. We recorded flowering as a binary variable (yes or no) throughout the experiment. Because only four of the 12 species flowered in the first year, we merged the variables related to the flowering, i.e. flowering (yes/no), beginning of the flowering period and number of flowering stems from both years, while all other variables were analysed separately in the two years (Table 2). This means that flowering meant whether each individual flowered within the duration of the whole experiment (two years), and the beginning of the flowering period was counted from the day when each individual was potted until it produced the first flower. The number of flowering stems was averaged across both years if species flowered in both years.

### 2.3 Drought treatment

In summer 2016, we subjected half of the plants to two periods of intensive drought. The first drought treatment started on the 12$^{th}$ of June and lasted until the 30$^{th}$ of June; while the second drought treatment started on the 11$^{th}$ of July and lasted until the 20$^{th}$ of July. In both drought treatments, plants subjected to drought were not watered until they showed strong signs of suffering (wilting of leaves). This meant that within one treatment, we only gave enough water to the suffering plants to prevent their death, but left the ones that did not show signs of suffering untouched. The drought treatments were stopped after 10 and 19 days, respectively, in order not to risk the complete death of the more drought-sensitive species. The control plants were watered either every day or two times per week depending on the season to prevent a shortage of water availability. Drought stress response was analysed as the difference in measured traits between plants under drought compared to control plants. An increase in drought stress response means a greater difference between drought and control plants.

### 2.4 Quantitative genetic design

We used a maternal half-sib design in our experiment meaning that all individuals had a known mother (maternal lineage), but an unknown father. Genetic variation in quantitative traits can be inferred from maternal lineage as genetically controlled traits are significantly more similar when they are from individuals of the same maternal lineage, than when they are taken randomly (Falconer and Mackay, 1996). In the analyses, we used the maternal lineage as an indication of genetic variation in traits (Ensslin and Fischer, 2015; Falconer and Mackay, 1996). We grew four individuals per maternal lineage, for each treatment and species combination. The only exception was the wild origin seed of *Bromus erectus*, which could not be collected separately by maternal lineage (Table 1) and therefore, did not contribute to the maternal lineage effect. In this species, we planted 25 wild and 24 garden origin individuals per treatment. Due to mortality of young seedlings in some species, this resulted in 1451 individuals for the experiment of the possible 1650 (Table A1 in the appendix). To further test genetic trait variability between garden-cultivated and wild plants across all species, we calculated standardized coefficients of variation across maternal lineages with all continuous traits and analysed those in separate mixed effect models as described below (Table 3).

## 2.5 Statistical analyses

We used linear and generalized linear mixed models (Bates et al., 2015) to test whether cultivated plants differed in morphological traits and their response to drought stress compared to wild plants. To explore this, we fitted the full models including main effects and interactions and determined significance by omitting one factor from the model and comparing the two models with a likelihood ratio test. Significances of all predictors were retained in the model and used for a subsequent FDR-Analysis (graphically sharpened method) to account for multiple testing (Pike, 2011).Because our plant species had been cultivated for very different periods in the botanic garden (Table 1), we used the time the plants had spent in cultivation (in years) as continuous explanatory variable. Wild collected plants were defined as having zero cultivation time. Moreover, we included species´ drought-tolerance (Ellenberg values) and drought treatment as explanatory variables. We tested these variables and all their interactions on the continuous plant traits (plant performance, phenology and growth strategy) and binary response traits (survival, flowering). We included species identity, maternal lineage and block as random variables, while all others as fixed. For the species identity effect, we accounted for a random intercept and a random slope (Drought/Species) in our model assuming trait means as well as their response to drought varied among species. We also tried to fit a random slope for the maternal lineage, but these models did not converge. We therefore included the random intercept (maternal lineage) in the final model. We included two covariates, the starting plant size to account for the maternal environment and the geographic distance between wild and original source population to account for the difference in wild and original population identity (see section 2.1). We discuss effects only across all 12 species in order to minimize a potential confounding effect of cultivation time and population identity. Some variables were log- or square root transformed to meet assumptions of normality and heteroscedasticity (Table 2).

We used the effects package (Fox, 2003) to display regression lines and confidence intervals (CI). Partial residuals were fitted with the remef package (Hohenstein and Kliegl, 2020). As CIs made by the effects package may not always be reliable, we calculated credible intervals (a Bayesian analogue of confidence intervals) for a subsample of the tests with the sim function of the arm package (Gelman and Yu-Sung, 2018). As the credible intervals were almost indistinguishable from the CIs, we decided to keep the CIs for simplicity. All analyses were performed with R version 3.4.2 (R Core Team, 2020).

## 3. Results

### 3.1 Trait means and drought stress response

We found significant effects of drought-tolerance, drought treatment and cultivation and their interactions in all response variables except for the duration of flowering and the biomass in the second year (Table 2).

Drought treatment and drought-tolerance: The drought treatment affected all species negatively in three performance traits (survival, plant height, number of flowering stems) and reduced flowering (Survival: $\chi^2$=4.5, P=0.046; height: $\chi^2$=5.1, P=0.036; flower stems: Chi$^2$=6.4, P=0.01; Fig. A1a-d, Appendix). However, drought-tolerant species responded much less to the drought treatment in terms of biomass and flowering than drought-intolerant species (Drought x drought-tolerant interaction, biomass: $\chi^2$= 5.4, P=0.032; flowering: $\chi^2$=5.5, P=0.034; Fig A2a-b, Appendix). One year after the drought, drought-intolerant species, which had experienced drought, re-grew stronger (leaf length), while drought-tolerant species produced slightly smaller leafs than their control plants ($\chi^2$=4.7, P=0.034; Fig A2c, Appendix).

Ex situ cultivation: Cultivation time affected the species' trait means in eight out of 12 cases, but this often depended on the other factors (Table 2). Germination rate as well as leaf length increased with increasing cultivation time in the garden, while flowering was reduced (Germination: $\chi^2$=26.8, P<0001, leaf length: $\chi^2$=8.5, P=0.009, flowering: $\chi^2$=8.3, P=0.01 Fig. 1a-c). While for drought-tolerant species, cultivation in the garden delayed the beginning of the flowering period, reduced the number of flowering stems and slightly increased SLA, this was not the case for drought-intolerant species, where the flowering time and SLA remained unaffected and stem number even increased (Drought-tolerance x cultivation time interaction, flowering start: $\chi^2$=24.5, P<0.001, flowering stems: $\chi^2$=8.5, P=0.009, SLA: $\chi^2$=5.1, P=0.034; Fig. 2a-c). The effect of cultivation time also depended on the drought treatment of the plants (Table 2). Here, cultivation resulted in a higher mortality under drought (Drought x cultivation time interaction, $\chi^2$=4.3, P=0.049; Fig. 2d). However, plants that were cultivated for longer times slightly decreased in leaf length and in SLA when they had experienced drought, while this was not the case for the control plants (Leaf length: $\chi^2$=5.9, P=0.034, SLA: $\chi^2$=4.7, P=0.042; Fig. 2e-f).

For the plant height, drought-intolerant species responded less to drought the longer they were cultivated, while it was opposite for drought-tolerant species (Fig. A3, Appendix). However, only one drought-tolerant species (*Origanum vulgare*) produced measurable tillers in the first year (only rosettes for all other drought-tolerant species), so for the drought-tolerant species, this result may also be a population identity effect (see section 2.5).

### 3.2 Trait variability

We found genetic variation in quantitative traits in 10 out of 12 traits studied (Maternal lineage effect; Table 2). We found an effect of cultivation time on trait variability in three out of the 12 analysed traits. In all these traits, i.e. the germination rate, the leaf length and the aboveground biomass in the first year, the coefficient of variation decreased with increasing cultivation time (Germination: $\chi^2$=8.6, P=0.022, leaf length: $\chi^2$= 11.3, P=0.006, biomass: $\chi^2$=10.4, P=0.001; Fig. 1d-f). The variability in leaf length increased also with the drought treatment, but only for the drought-intolerant species (Drought-tolerance x drought interaction, $\chi^2$=6.8, P= 0.043; Fig A4, Appendix).

## 4. Discussion

Cultivation of plants in botanic gardens may influence the nature and variability of traits via stochastic effects and selection during the cultivation process, including loss of selective advantages to environmental stressors. We found indications that not only traits had changed during cultivation, but also trait variability and the response of the plants to drought stress, i.e. phenotypic plasticity.

### 4.1 Changes in trait means and drought stress response

Altered environmental conditions and management practices have been considered as key drivers of a rapid evolutionary response in cultivated species (Ensslin et al., 2015; Husband and Campbell, 2004). Known as the domestication syndrome (Hammer, 1984), this involves genetic changes in trait means towards an increased plant vigour, the homogenisation of traits (reduction of trait variability) and the reduction of costly adaptations to natural abiotic stressors (Milla et al., 2015). While those changes directly or indirectly contributed to the creation of crop species and hence to the aim of better making use of the plants, they would mostly be unwanted and potentially detrimental in the context of conservation (Havens *et al.* 2004; Ensslin & Godefroid 2019, but see Chivers et al., 2016).

Potential changes due to the cultivation of wild plants have received increasing interest in recent years (Basey et al., 2015; Ensslin et al., 2015; Espeland et al., 2017), but in-depth studies are still rare. In our study of 12 common herbaceous species from a Belgian botanic garden, we found indications that garden-cultivation resulted in an increase in germination rate, a decrease in flowering probability, and for drought-tolerant species also a delay in flowering time. The increase of germination rate in botanic garden plants has been shown and discussed in several studies before (Ensslin et al., 2018; Enßlin et al., 2011; Rauschkolb et al., 2019) and has also been found in commercially produced seeds for grassland restoration and medicinal plants (Qu et al., 2005; Schröder and Prasse, 2013).

The decrease in flowering probability and the delay in flowering observed in our study are at first glimpse puzzling as we could have expected acceleration of phenology and life cycle together with the increase in germination rate (Donohue, 2002). However, later flowering of botanic garden cultivated plants has also been found by Rauschkolb *et al.*, (2019), who explained it with a potential relaxation of the flowering threshold due to missing competition under garden conditions. Flowering threshold sizes are highly heritable and can evolve as a consequence of habitat stability (Wesselingh et al., 1997). As all species of our experiment were cultivated in ordinary cultivation beds with no competition and good nutrient provision, an evolution of increased threshold size in the garden collections seems a possible explanation for the observed pattern. However, a study with seed of the same origin as the longest cultivated species in our experiment (*Digitalis lutea*), showed an earlier flowering in the garden plants (T. Sandner, unpublished data). This indicates that selection on earlier or later flowering may depend on the cultivation, seed collection and regeneration method of the species in the garden (Ensslin and Godefroid, 2019). In general, our study shows that phenology is a sensitive trait, which may undergo strong and rapid shifts in ex situ collections. Interestingly, ex situ cultivation did not affect aboveground biomass production in our experiment. Similarly, Ensslin et al., (2015) and Rauschkolb et al., (2019) did not find significant differences in biomass production between ex situ and wild plants, while they did, like us, find changes in traits referring to phenology and plant architecture (e.g. number of flowering stems).

Aboveground biomass is a rather broad trait that encompasses productivity and summarizes many fine-tuned traits of plant architecture, life cycle strategy and resource use. We assume that changes due to ex situ cultivation may happen more quickly in narrow-defined traits (because of direct selection on them) and may take more time in more broad traits such as biomass.

Changes in trait means varied by drought-tolerance of the species. Delay in the beginning of the flowering period and the increase in SLA in drought-tolerant species point to a change in the growth strategy towards increased resource acquisition (as shown for an increase in SLA, see e.g Milla and Matesanz, 2017) and increase in threshold size of the flowering rosette (see discussion above). A fertilization treatment would give more insights here. An experiment with a dry grassland plant in Germany, Enßlin *et al.*, (2011) found that garden-cultivated plants responded to fertilization by producing one big flowering stem rather than the several smaller ones found in wild plants. Adaptive changes in species due to cultivation are expected to increase with the relative difference between the new ex situ environment and the old natural environment (Husband and Campbell, 2004). As we assume that the change in environment when growing the plants in the garden beds was greater in drought-tolerant than in drought-intolerant species, it might explain their greater changes in leaf and phenological traits during cultivation. The number of flowering stems slightly decreased in drought-tolerant species during cultivation (as also found in other studies, see Ensslin et al., 2015; Enßlin et al., 2011), but increased in drought-intolerant species. As there were only six species in each group and flowering probability was not very high in some species, longer studies with more habitat generalists and specialists could give further insights into this matter.

We found that the effect of cultivation on plant traits was often dependent on the drought treatment. Here, cultivation resulted in both an increase and decrease of response to drought, depending on the trait in question. For instance, there was an increase in mortality when cultivated plants experienced drought, but those who survived also showed a reduced response performance-related traits (plant height, leaf length, SLA). As the plants in the botanic garden beds are usually watered when drought stress occurs, they do not experience strong selection as in nature. If drought responses are costly, e.g. producing stress-resistant enzymes or proteins, or by altering the architecture of the plant (DeWitt et al., 1998; van Kleunen and Fischer, 2007), then those responses could be selected against in the optimized conditions of a garden. Moreover, as most of the populations in the gardens are very small, stress responses can be lost by demographic stochasticity and random genetic drift (Ellstrand and Elam, 1993), or changed by inbreeding (Sandner and Matthies, 2018). We interpret our results that garden-cultivated plants may have reduced some of their natural responses to drought, such as to reduce transpiration by growing smaller and producing smaller leaves. The increased mortality of cultivated plants under drought may have negative consequences if these plants were used in reintroductions.

We note that the drought treatment may have been perceived differently by drought-tolerant and intolerant species, as shown by the stronger biomass reduction in drought-intolerant species with drought (Fig. A2a, Appendix). However, this potential difference in stress perception did not influence the cultivation effects on plant response to drought, as shown by the non-significant three-way interactions of drought-tolerance, drought treatment and cultivation (Table 2, see section 3.1 for the plant height effect). While we are confident that our results show real changes in the response to drought stress during cultivation, the effect sizes (i.e. magnitude of the change in response) were rather small. Field transplantation studies with ex situ cultivated material would be needed to test the consequences of those changes under natural conditions.

## 4.2 Genetic variation in traits during cultivation

The domestication syndrome predicts a reduction in variability, particularly in phenological traits, as a consequence of selection by agricultural growth and harvesting methods (Meyer et al., 2012). Similar predictions can be made from stochastic processes, which may also result in a reduction in phenotypic trait variability and hence, evolutionary potential (Vitt and Havens, 2004). In our analyses with 12 species, we found a reduction in trait variability in three performance traits (germination rate, biomass, leaf length). Genetic variation in traits is considered important for adaptation to rapid changes in environmental and biotic conditions (Jump et al., 2009). The reduction of variability could compromise the ability of cultivated species to adapt to rapid changes in their natural habitat. To our knowledge, this is the first study that demonstrates a reduction of evolutionary potential, a real concern in ex situ-cultivated plants.

We were surprised to find only a weak impact of the drought treatment on the trait variability as it may be expected to increase under stress (Stanton et al., 2000) and hence, a reduction of variability could have been more strongly visible in drought-stressed plants. While we found this for one trait (leaf length first year), it was only true for drought-intolerant species and did not change with cultivation time. As the drought-tolerant species were potentially less stressed by our drought treatment (see discussion above in 4.1), further studies with more and different kinds of stresses are needed to reveal how trait variability and stress interact in ex situ collections.

We acknowledge that the species in our experiment were common plants in Belgium and were not collected with a conservation purpose for cultivation in the botanic garden. Therefore, those collections were not sampled with a particular protocol and may not have been treated with special care in the garden to prevent genetic changes. Moreover, we do not know the number of generations that the plants have passed in the ex situ environment and as our plants were all perennials, there is a possibility that some individuals still represent the first generation planted in the garden (especially the shortly cultivated species). We hence cannot clearly say to which degree adaptive evolution or genetic drift by founder effects were responsible for the observed effects. Also maternal effects might have influenced our results as we used the F1 generation for our study, especially in the germination where we did not account for it. However, several other studies showed that ex situ cultivation can result in genetic changes in phenotypic traits within only very few generations (Ensslin et al., 2015; Espeland et al., 2017; Nagel et al., 2019; Rauschkolb et al., 2019). We therefore believe that the patterns observed in our study reflect well the risks that are threatening the genetic and phenotypic representation of conservation collections of endangered plants.

## 4.3 Conclusions

We conclude that changes in traits, a reduction of trait variability and drought stress response are realistic concerns in ex situ collections, especially when species are cultivated over longer time scales. Our results moreover indicate that drought-tolerant species respond differently to cultivation than drought-intolerant ones, with some hints to an increased resource acquisition strategy in drought-tolerant species. Hence, we reinforce our call to cultivate conservation-dedicated collections with a long-term perspective (in contrast to a short-term multiplication for direct reintroduction measures) in

near-to-nature habitat rather than under optimal growth conditions (Ensslin and Godefroid, 2019).

We found a reduced trait variability and a slight reduction of response to drought stress in cultivated plant species, indicating a reduced evolutionary potential in the ex situ collections. This could compromise their ability to survive and evolve under natural conditions, particularly with current rapid change of climate and land use. We therefore strongly recommend that the cultivation of wild plant species follows the latest protocols to avoid the above-mentioned risks. While those protocols are readily available (Basey et al., 2015; Ensslin and Godefroid, 2019; Maschinski and Albrecht, 2017), they still need to be tested for their effectiveness. A number of botanic gardens are already offering specific trainings in how to support rare and endangered species (Ensslin and Godefroid, 2019; Sharrock and Chavez, 2013). We suggest that all institutions dealing with ex situ conservation have their gardeners follow a training in conservation horticulture that addresses the implications of genetic and evolutionary processes on their collections.

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608 Table 1 : Overview of the species used in the experiment, the plant family they belong to, the
609 number of mother plants from which the seeds were collected (ML = Maternal lineages; G/W
610 stands for garden and wild origin), and the time (years) the garden plants had been cultivated at
611 Meise Botanic Garden. Drought-tol = drought-tolerant (Yes when F ≤ 3, No when F > 3, according
612 to Ellenberg et al., 1992).

| Species | Family | ML (G/W) | Cult. time | Drought-tol |
|---|---|---|---|---|
| *Brachypodium pinnatum* | Poaceae | 6/10 | 17 | Yes |
| *Bromus erectus* | Poaceae | 4/1 | 15 | Yes |
| *Dianthus carthusianorum* | Caryophyllacae | 8/8 | 1 | Yes |
| *Digitalis lutea* | Plantanginaceae | 10/9 | 30 | No |
| *Geum urbanum* | Rosaceae | 10/10 | 19 | No |
| *Globularia bisnagarica* | Plantaginaceae | 10/10 | 10 | Yes |
| *Helianthemum nummularium* | Cistaceae | 3/7 | 20 | Yes |
| *Linaria vulgaris* | Plantaginaceae | 8/10 | 10 | No |
| *Origanum vulgare* | Lamiacea | 9/10 | 20 | Yes |
| *Pulicaria dysenterica* | Asteraceae | 10/8 | 15 | No |
| *Teucrium scorodonia* | Lamiaceae | 10/10 | 20 | No |
| *Trifolium pratense* | Fabaceae | 10/10 | 1 | No |

613

614

Table 2 : Overview of the glmm and lmm models testing the effects of drought-tolerant and drought-intolerant species type (Drought-tol), drought treatment (Drought), cultivation time (Cult. Time) and all their interactions on 12 performance and growth variables in a multi-species common garden experiment. The variables: geographic distance (Geogr dist) and size of the plants at the start of the experiment (Startsize) were treated as co-variables. Transformation indicates when a transformation of a dependent variable was necessary to fit model assumptions. Dependent variables : Germination rate (Germ), beginning of the flowering period (Fl start), leaf length (Leaf l), plant height (Height), aboveground biomass (Biom), survival, flowering, flowering duration (Fl dur), specific leaf area (SLA), number of flowering stems (Fl stems). / = Random effects. N= sample size for each trait measurement. Significant effects are bold faced. P-values were corrected for false discovery rates using the method proposed by Pike (2011).

| Year of measurement | 2016 | both | 2016 | 2016 | 2016 | both | both | 2017 | 2017 | 2017 | both | 2017 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Trait | Germ | Fl start | Leaf l | Height | Biom | Survival | Flowering | Fl dur | Leaf l | SLA | Fl stems | Biom |
| Transformation | - | - | sqrt | - | sqrt | glmer | glmer | sqrt | log+1 | log+1 | sqrt | sqrt |
| Sample size (N) | 410 | 591 | 1429 | 634 | 1350 | 1447 | 1447 | 328 | 1205 | 1200 | 617 | 1176 |
| Geogr dist | 0.111 | 0.412 | 0.465 | **0.034** | 0.272 | 0.166 | 0.388 | 0.085 | 0.141 | 0.290 | **0.037** | **0.044** |
| Startsize | **-** | 0.037 | **0.000** | **0.001** | **0.000** | 0.430 | **0.002** | 0.096 | **0.006** | 0.430 | 0.128 | **0.000** |
| Drought-tol | **0.045** | **0.020** | 0.436 | **0.034** | **0.027** | 0.360 | **0.037** | 0.456 | 0.228 | **0.017** | 0.465 | 0.070 |
| Drought | - | 0.238 | 0.194 | **0.036** | 0.096 | **0.046** | **0.030** | 0.432 | 0.431 | **0.046** | **0.011** | 0.178 |
| Cult. time | **0.000** | **0.000** | **0.009** | 0.440 | 0.510 | 0.135 | **0.010** | 0.465 | 0.225 | 0.456 | 0.128 | 0.298 |
| Drought x Cult. time | - | 0.111 | 0.238 | 0.418 | 0.418 | **0.049** | 0.349 | 0.430 | **0.034** | **0.042** | 0.096 | 0.426 |
| Drought-tol x Drought | - | 0.272 | 0.070 | 0.415 | **0.034** | 0.054 | **0.034** | 0.436 | **0.034** | 0.456 | 0.085 | 0.070 |
| Drought-tol x Cult. time | 0.096 | **0.000** | 0.418 | 0.298 | 0.298 | 0.381 | 0.363 | 0.190 | 0.272 | **0.034** | **0.009** | 0.314 |
| Drought-tol x Drought x Cult. time | - | 0.125 | 0.358 | **0.013** | 0.326 | 0.418 | 0.230 | 0.430 | 0.194 | 0.430 | 0.456 | 0.266 |
| 1/Block | - | - | 0.090 | 0.076 | 0.076 | 0.272 | 0.456 | - | **0.019** | 0.096 | 0.497 | **0.036** |
| 1/Species | **0.000** | **-** | | - | - | - | **0.000** | **0.000** | **-** | - | **0.000** | **-** |
| 1/Maternal lineage | **0.000** | **0.000** | **0.000** | **0.001** | **0.000** | 0.418 | **0.000** | 0.272 | **0.000** | **0.000** | **0.000** | **0.000** |
| Drought/Species | | 0.128 | **0.000** | **0.000** | **0.000** | **0.000** | **-** | - | **0.034** | **0.020** | **-** | **0.042** |

Table 3 : Overview of the glmm and lmm models testing the effects of drought-tolerant and drought-intolerant species type (Drought-tol), drought treatment (Drought), cultivation time (Cult. Time) and all their interactions on the coefficient of variation (CV) of 12 performance and growth variables in a multi-species common garden experiment. The variables: geographic distance (Geogr dist) and size of the plants at the start of the experiment (Startsize) were treated as co-variables. Transformation indicates when a transformation of a dependent variable was necessary to fit model assumptions. Dependent variables: Germination rate (Germination), beginning of the flowering period (Fl start), leaf length (Leaf l), plant height (Height), aboveground biomass (Biom), flowering duration (Fl dur), specific leaf area (SLA), number of flowering stems (Fl stems). / = Random effects. N= sample size for each trait measurement. Significant effects are bold faced. P-values were corrected for false discovery rates using the method proposed by Pike (2011).

| Year of measurement | 2016 | both | 2016 | 2016 | 2016 | 2017 | 2017 | 2017 | both | 2017 |
|---|---|---|---|---|---|---|---|---|---|---|
| CV of Trait | Germination | Fl start | Leaf l | Height | Biom | Fl dur | Leaf l | SLA | Fl stems | Biom |
| Transformation | sqrt | sqrt | sqrt | sqrt | sqrt | - | - | - | - | - |
| Sample size (N) | 193 | 160 | 363 | 183 | 356 | 84 | 321 | 321 | 166 | 322 |
| Geogr Dist | 0.427 | 0.200 | 0.728 | **0.039** | 0.783 | 0.092 | 0.427 | 0.448 | 0.223 | 0.162 |
| Drought-tol | 0.525 | 0.723 | 0.724 | 0.092 | 0.385 | 0.723 | 0.728 | 0.728 | 0.110 | 0.456 |
| Drought | - | 0.728 | 0.299 | 0.000 | 0.724 | 0.581 | 0.728 | 0.581 | 0.783 | 0.728 |
| Cult. time | **0.022** | 0.728 | **0.006** | 0.385 | **0.001** | 0.427 | 0.724 | 0.783 | 0.623 | 0.378 |
| Drought x Cult. time | - | 0.514 | 0.092 | 0.162 | 0.728 | 0.579 | 0.724 | 0.630 | 0.724 | 0.630 |
| Drought-tol x Drought | - | 0.724 | **0.043** | 0.783 | 0.581 | 0.728 | 0.484 | 0.728 | 0.728 | 0.092 |
| Drought-tol x Cult. time | 0.783 | 0.783 | 0.514 | 0.120 | 0.448 | 0.756 | 0.120 | 0.385 | 0.378 | 0.724 |
| Drought-tol x Drought x Cult. time | - | 0.350 | 0.783 | 0.043 | 0.728 | 0.728 | 0.378 | 0.514 | 0.484 | 0.427 |
| 1/Species | **0.000** | **0.000** | **0.000** | 0.728 | **0.000** | 0.783 | **0.023** | **0.000** | **0.000** | 0.043 |

636 Figure legends

637

638 Figure 1 : Influence of cultivation time (years) on trait means (a-c) and trait variability (d-f) of 12 ex-
639 situ cultivated and wild-collected species in a multi-species experiment at Meise Botanic Garden,
640 Belgium. (a) Germination rate (P<0.001), (b) leaf length of the first year (P=0.009) and (c) flowering
641 (P=0.01). Trait variability was measured as the coefficient of variation (CV, see section 2.5). (d) CV
642 of germination rate (P=0.022), (e) CV of leaf length of the first year (P=0.006) and (f) CV of the
643 aboveground biomass in the first year (P=0.001). The graphs show regression lines (solid), partial
644 residuals (points, except c) and confidence intervals (dotted lines; see section 2.5).

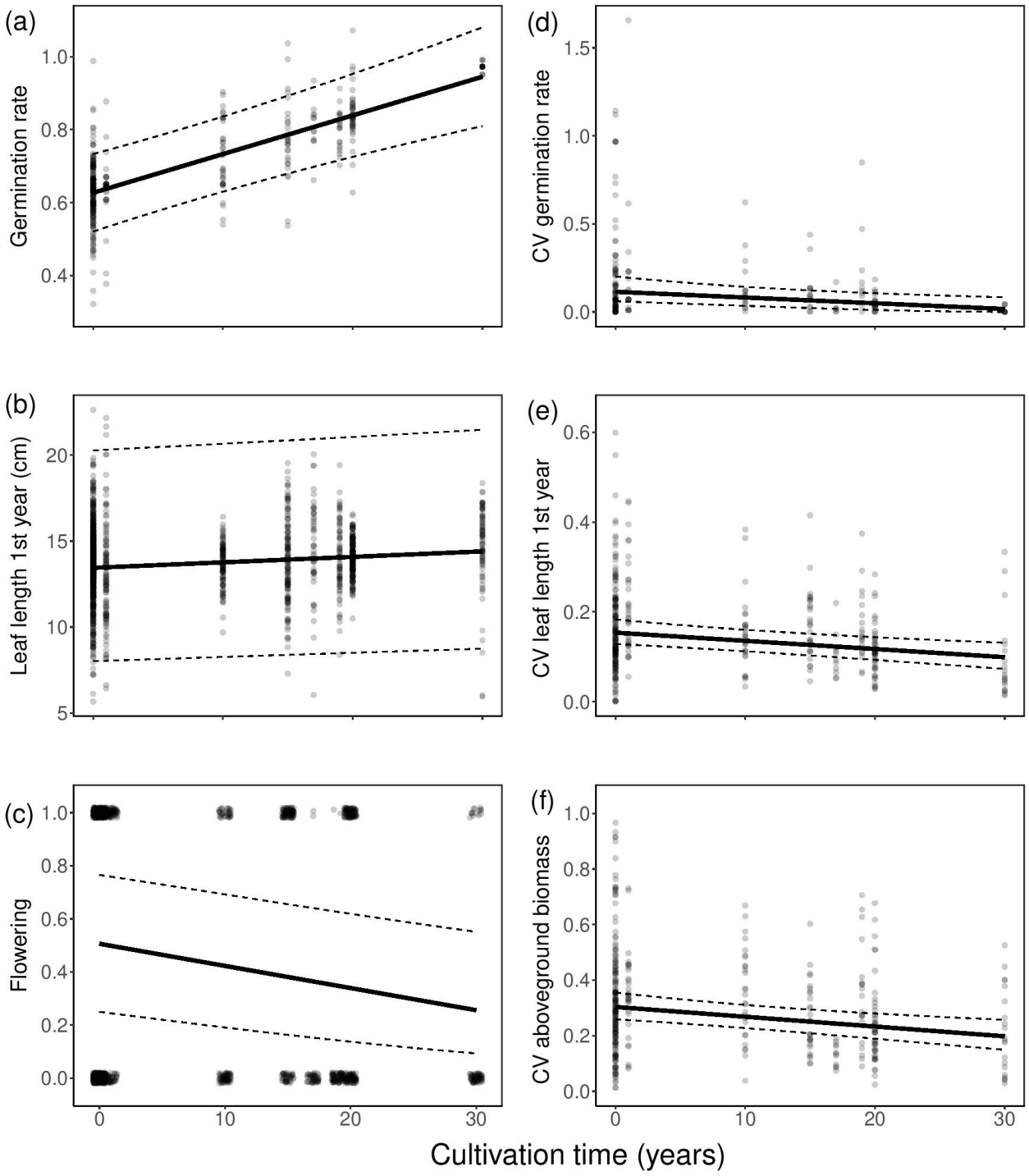
645

646 Figure 2 : Interaction plots of the effects of cultivation time (years) and drought tolerant (blue)
647 versus drought intolerant (orange) species (a-c), as well as drought stressed (brown) and control
648 (green) plants (d-f) on survival, phenology and performance of 12 ex-situ-cultivated and wild-
649 collected species in a multi-species experiment at Meise Botanic Garden, Belgium. (a) Day of first
650 flowering (P<0.001), (b) number of flowering stems (P=0.009) and (c) specific leaf area (P=0.034).
651 (d) Survival (0.049), (e) leaf length of the 2$^{nd}$ year (P=0.034) and (f) specific leaf area (P=0.043).
652 The graphs show regression lines (solid), partial residuals (points, except d) and confidence
653 intervals (dotted lines; see section 2.5). The specific leaf area (c, f) is plotted on a log scale to
654 increase readability of the graph due to high spread of the data.

655

656

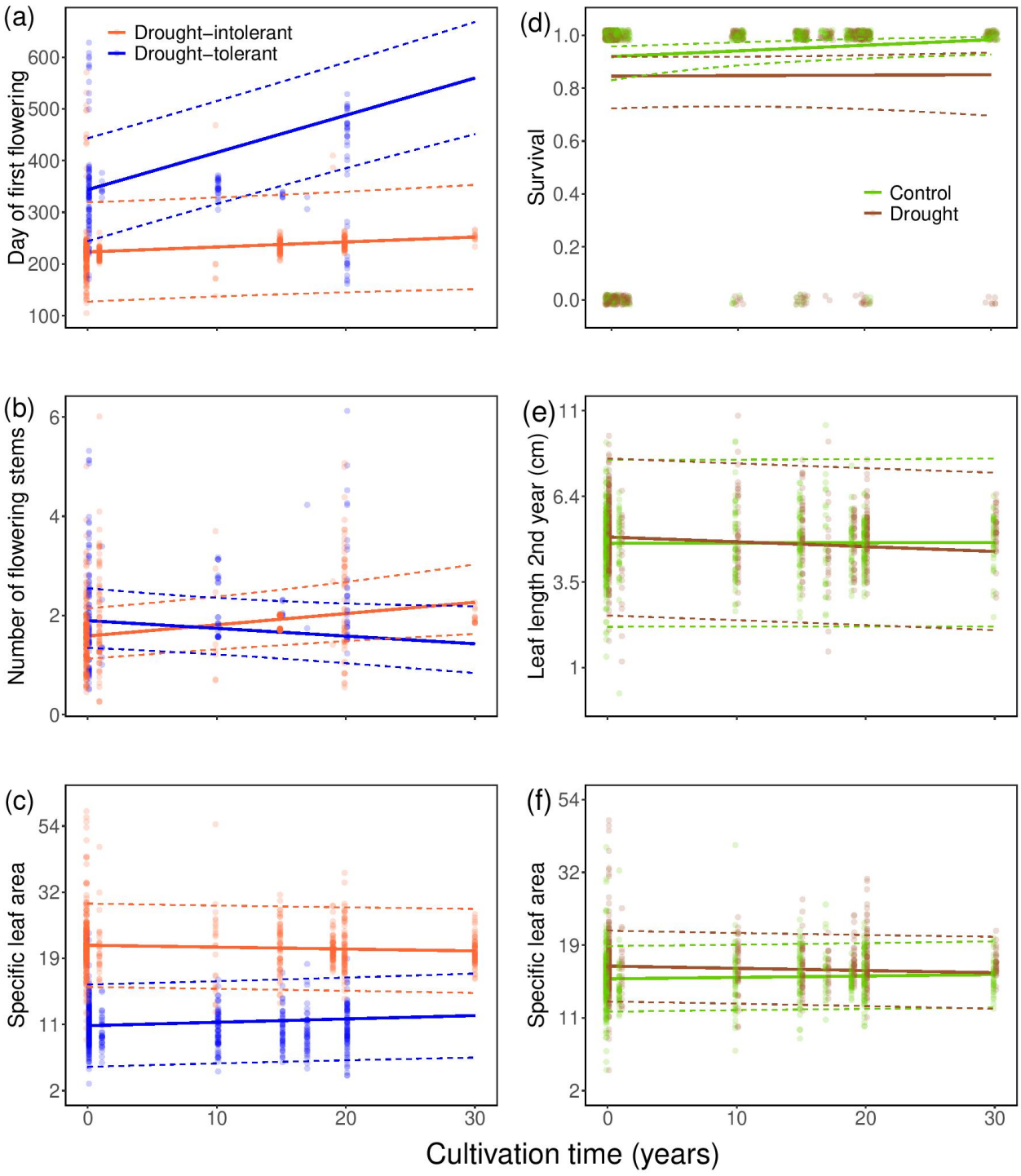
657 Fig1




658

659

660 Fig 2


(a)
Drought−intolerant
Drought−tolerant
Day of first flowering
600
500
400
300
200
100
(d)
Survival
1.0
0.8
0.6
0.4
0.2
0.0
Control
Drought
(b)
Number of flowering stems
6
4
2
0
(e)
Leaf length 2nd year (cm)
11
6.4
3.5
1
(c)
Specific leaf area
54
32
19
11
2
(f)
Specific leaf area
54
32
19
11
2
0
10
20
30
Cultivation time (years)

661