Developmental stability, canalization and phenotypic plasticity in annual herbaceous species under different biotic and abiotic conditions

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Research Article

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Abstract

The relationships among developmental stability, canalization and phenotypic plasticity have not been well understood. Inconsistent conclusions from different studies suggested the complexity of their associations, probably depending on specific traits, environmental contexts and plant growth stages. To address this issue, we conducted three experiments (EXP I ~ III) with several annual herbaceous species, to investigate the relationships among leaf (or cotyledon) developmental stability, canalization and plasticity and their variations with different biotic and abiotic environmental conditions and plant growth stages, with comparisons among different species at their early growth stage. We analyzed variations in mean trait value, lamina fluctuating asymmetry (FA), coefficient of variation (CV) and plasticity ($RDPI_s$) and their correlations for lamina size (LS) of individual plants, for LS, petiole length (PL) and petiole angle (PA) of different plant layers in *Abutilon theophrasti* at three densities in infertile and fertile (or only fertile) soil conditions at three (or two) stages, and for cotyledon size (CS) of five species in contrasting light conditions and seeding depths. High vs. low density decreased LS (with negative $RDPI_s$), FA indexes and CVs, either for individual plants or different layers, especially in fertile soil. Shading was more likely to increase CS (except for *A. therophrasti*) and FA and decrease CV; deep seeding increased CS of some species in full light, but decreased CS and FA of other species in shading (except for *A. therophrasti*). FA indexes more likely had positive correlations with mean value, CV and $RDPI_s$ of traits; correlations between CV and $RDPI_s$ can be positive, negative or insignificant. Correlations among the three variables were more likely positive or insignificant for traits of LS, CS and PL, but more likely negative or insignificant for PA. High density and infertile soil may favor more positive over negative correlations among variables. Results suggested higher levels of lamina FA more likely indicate higher growth rates of plants or modules. Developmental stability was more likely to have positive correlations with canalization, and negative correlations with plasticity, indicating certain common mechanisms associated with them. Environmental stresses can lead to greater phenotypic variations at different levels, facilitating cooperation between the three processes in dealing with environmental challenges.

Introduction

Phenotypic plasticity, canalization and developmental stability are three most common sources of phenotypic variation (Palmer 1996; Wagner et al. 1997). How do they interact to generate phenotypic diversity has become a key focus of ecological evolutionary developmental biology ('eco-evo-devo') (Pfennig 2016). Phenotypic plasticity is a shift in phenotype due to changes in environments (Schlichting 1986; Schlichting & Pigliucci 1998), which is fundamentally evaluated as the difference in a given trait due to environmental effects in spite of many plasticity indexes ($RDPI_s$ adopted in this study) (Valladares et al. 2006). Developmental canalization or robustness, indicates the ability of a genotype to produce consistent phenotypes regardless of environmental and genetic variabilities (Waddington 1942), in which environmental canalization indicates the insensitivity of a phenotype to environmental variation or any form of robustness against non-heritable perturbations (Flatt 2005; Wagner et al. 1997). The level of canalization is evaluated with coefficient of variation in traits (CV) in a population (Woods et al. 1999). Environmental canalization and phenotypic plasticity, as opposing to each other, both describe the degree of sensitivity of a phenotype to the environmental variation (Flatt 2005). A closely related and partly overlapping concept with micro-environmental canalization is developmental stability, defined as the tendency of traits to resist the effect of developmental errors (Debat & David 2001; Nijhout & Davidowitz 2003; Palmer & Strobeck 1986; Waddington 1957). It is evaluated using fluctuating asymmetry (FA) – random deviation from perfect bilateral symmetry (Møller & Swaddle 1997; Van Valen 1962).

Most authors consider the three processes separately based on historical definitions, seldom investigate them simultaneously in a single study. In spite of increasing interests it has incurred recently, there have been no clear conclusions on the relationships between the three processes (Debat & David 2001; Flatt 2005). For example, developmental stability and canalization are believed to have independent or at least partially different (Debat et al. 2000; Debat et al. 2009; Milton et al. 2003; Rego et al. 2006) or partly overlapping underlying mechanisms (Breno et al. 2011; Debat et al. 2009; Lazić et al. 2016; Reale & Roff 2003; Willmore et al. 2005). And plasticity and developmental stability may have correspondence (Clarke 1998;

Willmore et al. 2005; Woods et al. 1999) or not (Debat et al. 2000; Milton et al. 2003). Nevertheless, developmental stability, canalization and plasticity should all play crucial roles in species survival and adaptation to environments (Draghi 2020; Hallgrimsson et al. 2019; Kawano 2020), and there should be certain correlations among them (Scheiner et al. 1991). The discrepancy in results of different studies suggested the complexity of their relationships, which may depend on many factors such as specific traits, environmental contexts and growth stages (Mustafić & Freund 2013; Takahashi 2019; Woods et al. 1999).

Most relevant studies often use animals as study objects. However, plants may be better materials as they more closely rely on the three processes in face of environmental variabilities. We also need to conduct field experiments under more realistic environmental conditions with combined several stressors to investigate the relationships among developmental stability, canalization and plasticity(Gutiérrez 2020). Here we implemented three experiments (EXP I~III) with several annual herbaceous species under field conditions, to better understand the relationships among developmental stability, canalization and plasticity in their leaf or cotyledon traits under multiple abiotic and biotic environmental conditions at different stages. Specific questions are: 1) will the increase of density affect lamina FA, CV and $RDPI_s$ in traits for individual plants or different layers? 2) are there any correlations among the three variables in response to density? 3) do their responses and correlations vary with different soil conditions or growth stages? 4) will these variables and their correlations differ in response to light conditions or seeding depth for cotyledon traits of different species?

Materials And Methods

Study site and species

The three experiments were conducted between 2007 and 2009 at the Pasture Ecological Research Station of Northeast Normal University, Changling, Jilin province, China (123°44 E, 44°40 N). In EXP I and II, we used an annual weed of *Abutilon theophrasti* Meic. (Malvaceae) as study species. *A. theophrasti* is an old-field annual weedy species that is typically found in open fields and waste places, where it grows to a height of up to 1-1.5 m (Gleason & Cronquist 1991). *A. theophrasti* displayed intermediate plasticity (McConnaughay & Bazzaz 1992), it colonizes relatively nutrient-rich habitats and grows rapidly, reaching reproductive maturity within 90 days, and complete its life cycles in about five months (McConnaughay & Coleman 1999). Seeds of *A. therophrasti* were collected from local wild populations near the research station in the late August of 2006 and were dry stored at -4^{o}C. In EXP III, we used another four annual weed species including *Lpomoea purpurea* L. Roth., *Convolvulus arvensis* L., *Carpesium abrotanoides* L. and *Xanthum mongolicum* Kitag in addition to *A. theophrasti* Meic., which are all common wild plant species that widely spread over the natural habitats and frequently-disturbed fields. Seeds of all species were collected in late August of 2008, from local wild populations in the nearby of research station and were dry stored at -4^{o}C.

Experimental design

EXP I – The experiment used a split plot design, with infertile and fertile soil conditions assigned as two whole plots, each of which was divided into nine sub-plots (2 × 3 m), with three plant density (13.4, 36 and 121 plants·m^{-2}) and three blocks randomly distributed. Seeds were sown at three densities on June 7, 2007. When grew to 30, 50 and 70 days after seedling emergence, plants were harvested. At each of three stages, five to six individuals were randomly chosen from each plot, making the maximum total of 6 replicates × 3 blocks × 3 densities × 2 soils × 3 stages = 324 sampling. For each individual, lamina size (width) and lamina FA were measured for all leaves on its main stem.

EXP II – A completely randomized block design was implemented with three plant density treatments (13.4, 36 and 121 plants·m^{-2}) and three blocks randomly distributed into nine plots (2 × 3 m) in fertile soil. Seeds were sown on June 7, 2007. Plants were harvested at 50 and 70 days of growth after seedling emergence. At each stage, five to six individuals were randomly chosen from each plot, making the maximum total of 6 individuals × 3 replicates × 3 densities × 2 stages = 108 sampling. Each individual plant was divided into different layers of 10cm length at 50 d and of 20cm length at 70 d. For

each individual, lamina size (width), lamina FA, petiole length and petiole angle (the angle between petiole and main stem) were measured for leaves on the main stem in each plant layer.

EXP III – A split plot design was applied, with light conditions as the main factor, seeding depth and species as sub-factors. The whole field was divided into two major plots (2 × 2 m), subjected to full light and shade respectively. Within each plot, seeds were sown in lines at 10 cm inter-distance and two depth of 0.5 cm and 2.0 cm. Each line was assigned to one species and one seeding depth, lines with different seeding depths and species randomly distributed. Shading treatment was set up by covering one of the major plots with plastic sheets of 25% light transmittance. Seeds were sown on June, 7, 2009. Most of seeds emerged four days after sowing. Seedlings were harvested at the stage of three to four leaves fully developed, 10-25 replicates were sampled for each species in each treatment, with the maximum total of 25 individuals × 5 species × 2 seeding depth × 2 light conditions = 500 samplings. Lamina width and FA of each cotyledon was measured for all individuals.

Soil and density treatments

Infertile soil conditions were set up with the soil (aeolian sandy soil, pH = 8.3) of the experimental field at the station that had been utilized repeatedly every year. The soil nutrient contents were organic C 3.1 $mg \cdot kg^{-1}$, available N 21.0 $mg \cdot kg^{-1}$, available P 1.1 $mg \cdot kg^{-1}$ during the growth season of 2007 (Zhao et al. 2010). Good soil conditions were created by covering 5-10 cm virgin soil (meadow soil, pH = 8.2) transported from the nearby meadow in the north of the research station that had never been cultivated before. Main nutrients of the soil were: organic C 18.7 $mg \cdot kg^{-1}$, available N 47.5 $mg \cdot kg^{-1}$, available P 4.0 $mg \cdot kg^{-1}$ during the growth season of 2007 (Zhang 2013).

Low, medium and high densities were set up by sowing seeds at three inter-planting distances of 30, 20 and 10 cm, to reach the target plant densities of 13.4, 36 and 121 $plants \cdot m^{-2}$ respectively. Most seeds emerged four days later after sowing. Seedlings were thinned to the target densities when they reached four-leaf stage. Plots were hand weeded when necessary and watered regularly with irrigation pipes before experiencing drought.

Statistical analyses

The width of right and left sides of each lamina was measured twice with a digital caliper for calculation of lamina fluctuating asymmetry (FA). We used almost all kinds of indexes (FA_1-FA_8 and FA_{10}) to calculate lamina FA as an estimate of developmental stability (Palmer 1994; Palmer & Strobeck 2003). ANOVA analyses showed similar results for most of the indexes. FA_1 and FA_2 are indexes with and without effects of leaf size respectively, and FA_{10} is the only index with measurement error variance partitioned out of the total between-sides variance (Palmer 1994) and was calculated with the corrected formula. Thus we adopted three indexes of FA_1, FA_2 and FA_{10}, with formulas as follows (Palmer 1994; Palmer & Strobeck 2003):

$$FA_1 = \sum \left| R - L \right| / n$$

$$FA_2 = \sum [(R - L)/S]/n$$

$$FA_{10} = 0.798 \times \sqrt{s^2} \quad [s^2 = (MS_{sj} - MS_m)/M]$$

where R and L were the width of right and left sides of a lamina, n was the total number of laminas, and S (represented lamina size) was calculated by $(R+L)/2$.

Developmental canalization in a given trait were calculated with coefficient of variation (CV, the standard deviation divided by mean value of the trait), which was divided into among-leaf and among individual CVs (LF-CV and IN-CV). Plasticity in traits was calculated with the formula of simplified Relative Distance Plasticity Index ($RDPI_s$)(Valladares et al. 2006):

$RDPI_s = (X - Y)/(X + Y)$

where X was the adjusted mean trait value of plants at high or medium density, and Y was the adjusted mean value at medium or low density. Therefore, there were three kinds of plasticity, including plasticity in response to high vs. medium density (H-M $RDPI_s$), high vs. low density (H-L $RDPI_s$) and medium vs. low density (M-L $RDPI_s$). To compare the degrees of plasticity, we also calculated the absolute values of $RDPI_s$ (ABS $RDPI_s$) in trait for each case, in correspondence to the relative plasticity (REL $RDPI_s$) for all the above mentions of plasticity.

All variables for traits were used in statistics (Table 1), and the original data was log-transformed, petiole angles were square root-transformed, before any analysis to minimize variance heterogeneity. All analyses were conducted using SAS statistical software (SAS Institute 9.0 Inc. 2002). Three-way ANOVA was performed for overall effects of growth stage, soil conditions, population density and their interactions on all variables of lamina size in EXP I, for effects of growth stage, population density, plant layer and their interactions on variables of lamina size, petiole length and petiole angle in EXP II, and for effects of light conditions, seeding depth and species and their interactions on variables of cotyledon size in EXP III (Table 2). Then we used one-way ANOVA for effects of density on all variables in each soil conditions at each stage and across all soils and stages in EXP I, one-way ANOVA for differences among layers in traits at each density and one-way ANCOVA for effects of density on traits for each layer with plant total biomass as a covariate in EXP II, and one-way ANOVA for effects of light, seeding depth or species within each of other treatments combined. In ANCOVA, whenever total biomass explained significant variation in a layer-specific trait in response to density, the trait was said to exhibit apparent plasticity (McConnaughay & Coleman 1999; Weiner 2004; Wright & McConnaughay 2002). By contrast, any variation in trait expression that was independent of total biomass (plant size) was considered as an indication of true plasticity (Weiner 2004). Multiple comparisons used LSD method in General Linear Model (GLM) program. For each treatment and across all treatments in the three experiments, correlations among all variables were analyzed with PROC CORR, producing Pearson Correlation Coefficients and Partial Pearson Correlation Coefficients for all relationships, with LS or CS as covariate in partial correlation analyses.

Results

Variation in variables

EXP I – Soil condition, growth stage and population density and their interactions had significant effects on lamina size (LS) and lamina FA indexes, soil conditions and growth stage and their interaction affected the LF-CV of lamina size, while soil conditions and population density and their interaction affected its IN-CV, with little effects on its plasticity (RDPI) (Table 3). High density reduced lamina size in both soil conditions at almost all stages ($P < 0.01$; Fig. 1). In fertile soil, high density decreased FA indexes at 50 d and 70 d of plant growth ($P < 0.01$), decreased LF-CV only at 70 d ($P = 0.025$), increased IN-CV significantly especially at 50 d and 70 d ($P < 0.01$); little variation due to density was found in infertile soil.

EXP II – Growth stage, population density and architecture layer had significant effects on FA_1 and FA_{10}, stage and density affected IN-CV of petiole angle (PA), $RDPI_s$ of petiole length (PL) and angle, while layer effects were significant for IN-CV of lamina size (LS), petiole length and $RDPI_s$ of LS; interactions between factors were more significant for trait $RDPI_s$ (Table 4). Across both stages, mean values of LS and PL of most layers and their differences among layers decreased with higher densities ($P < 0.05$); medium and high densities also decreased some layers of PA, lamina FA_1 and FA_2 ($P < 0.05$), and high density relative to low density decreased FA_{10} of L2-L5 ($P < 0.05$; Fig. 2). On the other hand, density had no significant effects on IN-CV and $RDPI_s$ in traits of individual layers (Fig. 3). Across all traits, high and medium densities relative to low density reduced differences among layers in IN-CV at 50 d ($P < 0.05$), but did not at 70 d. PL of all layers were the most plastic of all traits at 70 d ($P < 0.01$). IN-CV increased with higher layers for LS and PL ($P < 0.05$), but not for PA; and this trend was alleviated with greater densities. The degree of $RDPI_s$ in all traits decreased with higher layers, especially at higher densities at 50 d ($P < 0.05$), and effects of layers were only significant for LS at 70 d ($P < 0.05$).

EXP III – All factors and their interactions were significant for mean cotyledon size (CS), and light, interactions between light conditions and species and between seeding depth and species were significant for IN-CV (Table 5). Responses of variables to shading were highly idiosyncratic for different species: compared to full light, shading increased CS of *Lpomoea purpurea*, *Convolvulus arvensis*, and *Carpesium abrotanoides* (with positive $RDPI_s$s, $P < 0.05$) and IN-CV of *C. abrotanoides* ($P = 0.018$) for both seeding depths, increased FA_1 of *Lpomoea* ($P = 0.010$) and FA_2 of *C. abrotanoides* ($P < 0.01$) at shallow depth, but decreased CS of *Abutilon therophrasti* (negative $RDPI_s$, $P < 0.001$) at shallow depth and IN-CV of *L. purpurea* and *A. therophrasti* ($P = 0.013$) across both seeding depths (Fig. 4). In response to seeding depth, deep burial increased CS in full light for *L. purpurea* ($P = 0.027$) and *Xanthum mongolicum* ($P < 0.001$) and in shading for *A. therophrasti* (positive $RDPI_s$s), increased IN-CV in both light conditions for *C. arvensis* ($P = 0.015$); but decreased CS and FA_1 for *L. purpurea* ($P < 0.001$ and $P = 0.006$) and CS and FA_2 for *C. abrotanoides* ($P = 0.022$ and $P < 0.01$) in shading (negative $RDPI_s$s), decreased CS in full light for *Abutilon* ($P < 0.001$).

Correlations among variables

For all three experiments, there were positive, negative and no correlations among variables (Table 6). Across all kinds of relationships, positive correlations were in greater amount than negative ones in EXP II and EXP III, while negative correlations were more than positive ones in EXP I. FA indexes more likely had positive correlations than negative ones with other variables except for LF-CV (more negative or insignificant correlations). LF-CV had no correlations with other variables except having more negative vs. positive correlations with mean lamina size. Correlations between trait $RDPI_s$ and IN-CV were negative or insignificant in EXP I, but more positive than negative in EXP II.

EXP I – Correlations among variables varied with densities and soil conditions (Table 7, 8). LS had more negative vs. positive correlations with FA indexes at low density in infertile soil, but more positive vs. negative correlations with them at all densities in fertile soil (Table 7). Correlations between FA and LF-CV were negative or insignificant at low density and medium densities, but positive or insignificant at high density at 30 d. LS had negative correlations with its IN-CV and $RDPI_s$ across all treatments (Table 8). $RDPI_s$ positively correlated with FA in infertile soil, while negatively correlated with IN-CV in fertile soil.

EXP II – Across both stages and all densities, correlations of mean trait values with their IN-CV, $RDPI_s$ and lamina FA indexes were more likely positive or insignificant at 50 d, but more likely negative or insignificant at 70 d for LS; and these correlations were also more likely negative or insignificant for PL, but more likely positive or insignificant correlations for PA (Table 9). Correlations among lamina FA, and trait IN-CV and $RDPI_s$, were mainly positive or insignificant for LS and PL, and correlations between lamina FA and trait $RDPI_s$ were mainly negative or insignificant for PA (Table 10).

EXP III – Across all treatments, CS had negative correlations with FA_1, positive correlations with FA_2; IN-CV positively correlated with FA_1 and FA_2 (Table 11, 12). Plasticity in response to shading (S-F $RDPI_s$) had positive correlations with FA_{10} in shading only, and had no correlations with IN-CV (Table 12).

Discussion

Responses of different variables

Our results showed *Abutilon theophrasti* adopted two strategies simultaneously in dealing with the increase of density: a strategy of vertical growth suggested by the decreased lamina size, petiole length and angle by high density at whole-plant level or for most layers, and a strategy of shade avoidance suggested by the canalized performance in the top layer of traits over densities in EXP I and II (Gruntman et al. 2017). Correspondingly, reduced cotyledon size by shading vs. full light at shallow depth revealed its lower tolerance for shade, compared to the other species in EXP III. By contrast, *Lpomoea purpurea*, *Convolvulus arvensis*, and *Carpesium abrotanoides* had increased cotyledon size in response to shading at both

seeding depths, suggesting their greater tolerance for shade (Zheng 2011). Similarly, deep seeding can also induce adaptive responses in cotyledon to maximize the growth potential of seedlings in spite of the probability of reduced seedling emergence (Chen & Maun 1999; Seiwa et al. 2002; Tobe et al. 2005). However, effects of both shading and deep depth led to reduction or no change in cotyledon size of species except *A. theophrasti*, suggesting the greater capacity of *A. theophrasti* to tolerate and actively respond to multiple stresses than the other species.

Stresses may trigger the developmental instability of an organism (Clarke et al. 1992; Gonzalez et al. 2014; Hagen et al. 2008; Møller 1998; Parsons 1990; Woods et al. 1999). However, FA may be an unreliable indicator of environmental stresses (Abeli et al. 2016; Palmer 1994a; Palmer & Strobeck 2003). This may be because the response of FA to stress is often trait-dependent (Woods et al. 1999), and the relationships between developmental stability and environmental conditions are often complicated and not simply in correspondence (Bonduriansky 2009). We found high vs. low density reduced lamina FA indexes of *A. theophrasti*, consistent with other results (Black-Samuelsson & Andersson 2003; Cuevas-Reyes et al. 2011; Kruuk et al. 2003). Meanwhile, shade increased cotyledon FA of *L. purpurea*, while deep seeding depth decreased FA of *L. purpurea* and *C. abrotanoides*. An alternative explanation is that favorable environments allow faster growth of plants or modules, prompting higher developmental instability and FA levels (Hodge 2004; Martel et al. 1999; Morris et al. 2012). And asymmetry could increase with leaf size because larger leaves require more resources to be produced (Møller & Eriksson 1994). Higher FA has been found in more favorable conditions, like higher nutrient availability (Milligan et al. 2008), less polluted soil (Velickovic & Perisic 2006), or water supplementation (Fair & Breshears 2005). Therefore, developmental instability should not be harmful, and the state of being instable simply suggested the organisms or modules are in the state of fast growing (Morris et al. 2012), or beneficial effects of favorable environments. However, studies have shown that animals prefer to choose the spouses or foods with fitness-related traits of lower levels of FA (Møller & Eriksson 1994; Møller & Thornhill 1998). It may because high levels of FA did not necessarily point to lower fitness, but a stage of rapid growing thus being immature of the target animals or plants, whereas those in a relatively stable developmental state are more suitable for mating or digestion.

As an indicator of variation among leaves within an individual, LF-CV should be equivalent to FA as an indicator of developmental stability, thus showing similar responses to density as FA. Meanwhile, high density increased or did not affect among-individual CV (IN-CV), and increased the degree of plasticity (ABS $RDPI_s$) in traits, while shade decreased IN-CV of cotyledon size of *L. purpurea* and *A. theophrasti*, and increased that of *C. abrotanoides*. It suggested the complexity of the response of IN-CV to density, biased by many unknown factors. However, as petiole length decreased, and petiole angle, lamina FA, trait IN-CV and $RDPI_s$ increased with higher plant layers, implying modules with greater FA and variations among individuals are less likely to produce passive responses to density.

Correlations among variables

By analyzing relationships among FA, CV and $RDPI_s$ across different stages, treatments, plant layers, or different species, we showed mixed results for each kind of correlations, suggesting the complexity of these relationships, with some consistent aspects: (1) FA indexes more likely positive correlated with mean values of a trait and its IN-CV and $RDPI_s$; (2) there were more negative than positive correlations between LF-CV and mean value of a trait, LF-CV and FA, and mixed correlations between IN-CV and $RDPI_s$ as well as little correlations between LF-CV and $RDPI_s$ and between LF-CV and IN-CV; (3) correlations among FA, IN-CV and $RDPI_s$ were more likely positive than negative for lamina size, cotyledon size and petiole length, but less likely for petiole angle; (4) environmental stresses may favor positive relationships among variables, for instance: correlations between LF-CV and FA were more likely negative vs. positive at lower densities, and more positive vs. negative at high density; there were negative correlations between $RDPI_s$ and IN-CV in fertile soil, but positive correlations between FA and $RDPI_s$ in infertile soil.

Arguments have persisted for the relationships among developmental stability, canalization and plasticity. However, results of EXP II in this study showed clear more positive than negative correlations between FA and CV, accordant with other results

(Clarke 1998; Hallgrímsson et al. 2002; Klingenberg & McIntyre 1998; Kok et al. 2019; Lazić et al. 2015, 2016; Takahashi et al. 2010; Willmore et al. 2005). Both developmental stability and canalization emerge as by-products of regulatory complexity and redundancy in developmental systems and share overlapping developmental bases (Siegal & Bergman 2002). We also found FA was more likely to have positive correlations with RDPIs in all the three experiments, consistent with others (Baranov et al. 2019; Gómez-Robles et al. 2013; Leung et al. 2017; Tonsor et al. 2013). Although LF-CV or IN-CV had more negative correlations with both FA and $RDPI_s$ in EXP I, but IN-CV had more positive ones with them in EXP II. This may be to a large extent because variations among leaves or individuals in traits tended to decline or change little over time, while FA and $RDPI_s$ tended to increase with leaf size (EXP I); and these variables had highly consistent variations with different plant layers (EXP II).

We believed that there should be a synergistic effect in variations of FA, CV and $RDPI_s$ (Nishizaki et al. 2015), though depending on different circumstances. Robustness and stability are never perfect as they are maintained dynamically, and can be readily perturbed by both genetic and environmental factors. The tight rope between stability and change sways easily and through the release of genetic variation, may be an important enabler of rapid phenotypic evolution (Nijhout & Reed 2014). In this process, cryptic or hidden genetic variation (genetic variation that does not translate into phenotypic variation) might be uncovered (i.e. phenotypically expressed) (Gibson & Dworkin 2004), and then might be selected on (Braendle & Félix 2009). Therefore the cooperation between plasticity and canalization may lead to more-canalized phenotype in some circumstances, or more-plastic phenotype due to uncovered cryptic genetic variation in other circumstances (Gibson & Dworkin 2004). But both the response and the robustness to a novel environment can be compatible in a certain degree of phenotypic variations due to noise, and the highest adaptability is achieved at a certain noise level where the gene expression dynamics are near the critical state to lose the robustness (Kaneko 2012). Environmental canalization at one level of organization may explain plasticity at another, and vice versa (Del Giudice et al. 2018; McDonald et al. 2018).

More positive than negative correlations among FA, CV and $RDPI_s$ with higher densities and infertile soil suggested environmental stresses might have uncovered the cryptic variations at both phenotypic and genetic levels (Nijhout & Reed 2014; Snell-Rood et al. 2015), leading to higher FA, greater phenotypic variations and stronger ability to avoid passive phenotypic responses to environmental stresses. And environmental factors may play a coordinated role in promoting the cooperation between plasticity, canalization and stability (Baranov et al. 2019), which should be beneficial for dealing with stresses. Elgart et al. (2015) showed how stress mediates tuning of developmental robustness and plasticity: when the stress is mild enough, plastic changes in some processes may prevent drastic changes in more robust traits; when the stress is sufficiently severe, this buffering may no longer be able to prevent such overt changes, leading to phenotypic variability subjected to selection that might assist survival at the population level.

Conclusions

Our results showed *Abutilon theophrasti* adopted both strategies of vertical growth and shade avoidance in response to increased density, with greater capacity to tolerate and actively respond to multiple stresses than the other species. Higher FA was more likely an indicator of a fast-growing state of plants or modules, which was associated with variations among individuals, and the ability to improve plastic responses. This was supported by more positive than negative correlations between developmental instability, canalization and plasticity, indicating some common mechanisms for them and their cooperation in dealing with environmental stresses.

Declarations

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Compliance with ethical standards

Conflict of interest

No conflict of interests have been declared.

Authors' contributions

Both authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Shu Wang. The first draft of the manuscript was written by Shu Wang and both authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Appendix

See Appendix Tables S1-S6.

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Tables

Table 1. Trait variables with their units, abbreviations, measured stages and individual values for correlation analyses in the three experiments of this study.

Experiment	Species	Trait	Unit	Abbrev.	Stage	Individual values
EXP I (Infertile and fertile soils)	*Abutilon*	Lamina size	mm	LS	30 d, 50 d, 70 d	Stage
		Lamina fluctuating asymmetry	mm	FA	30 d, 50 d, 70 d	Stage
		Among-leaf coefficient of variation	-	LF-CV	30 d, 50 d, 70 d	Stage
		Among-individual coefficient of variation	-	IN-CV	30 d, 50 d, 70 d	Stage
		Plasticity in response to high vs. medium density	-	H-M $RDPI_s$	30 d, 50 d, 70 d	Stage
		Plasticity in response to high vs. low density	-	H-L $RDPI_s$	30 d, 50 d, 70 d	Stage
		Plasticity in response to medium vs. low density	-	M-L $RDPI_s$	30 d, 50 d, 70 d	Stage
		Relative plasticity	-	REL $RDPI_s$	30 d, 50 d, 70 d	Stage
		Absolute plasticity	-	ABS $RDPI_s$	30 d, 50 d, 70 d	Stage
EXP II (Fertile soil)	*Abutilon*	Lamina size	mm	LS	50 d, 70 d	Layer
		Petiole length	cm	PL	50 d, 70 d	Layer
		Petiole angle	o	PA	50 d, 70 d	Layer
		Lamina fluctuating asymmetry	mm	FA	50 d, 70 d	Layer
		Among-individual coefficient of variation	-	IN-CV	50 d, 70 d	Layer
		Plasticity in response to high vs. medium density	-	H-M $RDPI_s$	50 d, 70 d	Layer
		Plasticity in response to high vs. low density	-	H-L $RDPI_s$	50 d, 70 d	Layer
		Plasticity in response to medium vs. low density	-	M-L $RDPI_s$	50 d, 70 d	Layer
EXP III	*Abutilon* *Convolvulus* *Lpomoea* *Carpesium* *Xanthum*	Cotyledon size	mm	CS	-	Species
		Cotyledon fluctuating asymmetry		FA	-	Species
		Among-individual coefficient of variation	-	IN-CV	-	Species
		Plasticity in response to shade vs. full light	-	S-F $RDPI_s$	-	Species
		Plasticity in response to deep vs. shallow depth	-	D-S $RDPI_s$	-	Species

Table 2. The methods of analyses, effect factors, measured traits and their variables used in the three experiments of this study. Abbreviations for all variables are in Table 1.

Experiment	Method	Effects factor	Trait	Variable
EXP I	Three-way, one-way ANOVA; PROC CORR	Soil conditions, density, growth stage	Lamina size	Mean value
				FA_1, FA_2, FA_{10}
				LF-CV
				IN-CV
				$RDPI_s$
EXP II	Three-way, one-way ANOVA and ANCOVA; PROC CORR	Density, layer, growth stage	Lamina size	Mean value
			Petiole length	FA_1, FA_2, FA_{10}
			Petiole angle	IN-CV
				H-M $RDPI_s$
				H-L $RDPI_s$
				M-L $RDPI_s$
EXP III	Three-way, one-way ANOVA; PROC CORR	Light conditions, seeding depth, species	Cotyledon size	Mean value
				FA_1, FA_2, FA_{10}
				IN-CV
				S-F $RDPI_s$
				D-S $RDPI_s$

Table 3. EXP I: *F*-values for three-way ANOVA on all variables with soil conditions (SC), growth stage (GS), population density (PD) and their interactions as effects. * P < 0.10, ** P < 0.05. *** P < 0.01.

Source of variation	Df	Log_{10} (LS)	FA_1	FA_2	FA_{10}	LF-CV	IN-CV	REL $RDPI_s$	ABS $RDPI_s$
SC	1	54.90***	5.73*	14.82***	66.82***	5.05*	13.84*	3.20	4.45
GS	2	2320.60***	339.73***	88.84***	254.44***	58.35***	2.10	3.28	5.87
PD	2	59.68***	12.35***	0.38	14.51**	1.01	8.80*	4.65	3.03
SC × GS	2	175.36***	9.66***	25.47***	23.21***	90.76***	0.24	0.36	0.53
SC × PD	2	5.00**	6.18**	0.54	4.97**	2.16	14.97*	0.49	1.00
GS × PD	4	3.76**	3.95**	1.51	5.33***	0.55	5.18	0.52	1.24

Table 4. EXP II: *F*-values for three-way ANOVA on lamina FA (FA_1, FA_2 and FA_{10}), among-individual coefficient of variation (IN-CV) and relative plasticity (REL $RDPI_s$) of leaf traits, with growth stage (GS), population density (PD), layer (LA) and their interactions as effects. * P < 0.10, ** P < 0.05. *** P < 0.01.

Source of variation	Df	FA			IN-CV			REL $RDPI_s$		
		FA_1	FA_2	FA_{10}	LS	PL	PA	LS	PL	PA
GS	1	65.69***	34.6***	24.87***	0.28	0.87	40.71***	1.16	120.46***	35.10***
PD	2	12.80**	1.27	8.07**	0.34	2.58	6.68*	13.43**	54.61***	7.11*
LA	6	11.71***	12.15***	6.20**	3.92*	26.65***	2.64	7.90**	3.15	2.59
GS × PD	2	0.76	2.52	0.74	0.13	2.23	1.89	0.29	8.35*	7.06*
GS × LA	5	2.63	4.43*	1.43	0.22	3.50*	2.46	2.09	7.07**	2.07
PD × LA	11	1.23	0.86	0.84	1.05	0.69	1.27	1.33	4.72*	1.01

Table 5. EXP III: *F*-values for three-way ANOVA on cotyledon size (CS), cotyledon FA (FA_1, FA_2 and FA_{10}) and among-individual coefficient of variation (IN-CV) with light, seeding depth, species and their interactions as effects. * p < 0.10, ** p < 0.05. *** p < 0.01.

Source of variation	Df	Log_{10} (CS)	FA_1	FA_2	FA_{10}	IN-CV
Light	1	800.11***	0.87	0.05	0.00	13.24*
Depth	1	96.40***	0.39	0.00	0.00	2.03
Species	4	4310.65***	20.11*	7.64	4.80	8.83
Light × Depth	1	9.83**	3.54	4.84	0.17	0.01
Light × Species	3	36.55***	0.37	1.27	0.05	23.34*
Depth × Species	4	2.40*	0.49	0.44	0.13	11.22*

Table 6. Total amounts of significant correlations among variables calculated according to Table S1-S6. “+” indicates positive correlations, “-” indicates negative correlations. The number in bracket indicates the total amount of times that the variable appeared in correlations in all treatments.

EXP	Variable	Mean value	FA	LF-CV	IN-CV
EXP I	FA (54)	**8+/4-**			
	LF-CV (18)	0+**/3-**	**1+/5-**		
	IN-CV (5)	0+/0-	0+/0-	0+/0-	0+/0-
	$RDPI_s$ (10)	0+**/4-**	**3+**/0-	0+/0-	0+/**2-**
EXP II	FA (54)	**15+/4-**			
	IN-CV (18)	**3+/7-**	**6+/2-**		
	$RDPI_s$ (36)	**7+/5-**	**26+/6-**		**4+/1-**
EXP III	FA (12)	**4+/2-**			
	IN-CV (4)	**4+**/0-	**8+**/0-		
	$RDPI_s$ (4)	0+/0-	**1+**/0-		0+/0-

Figures

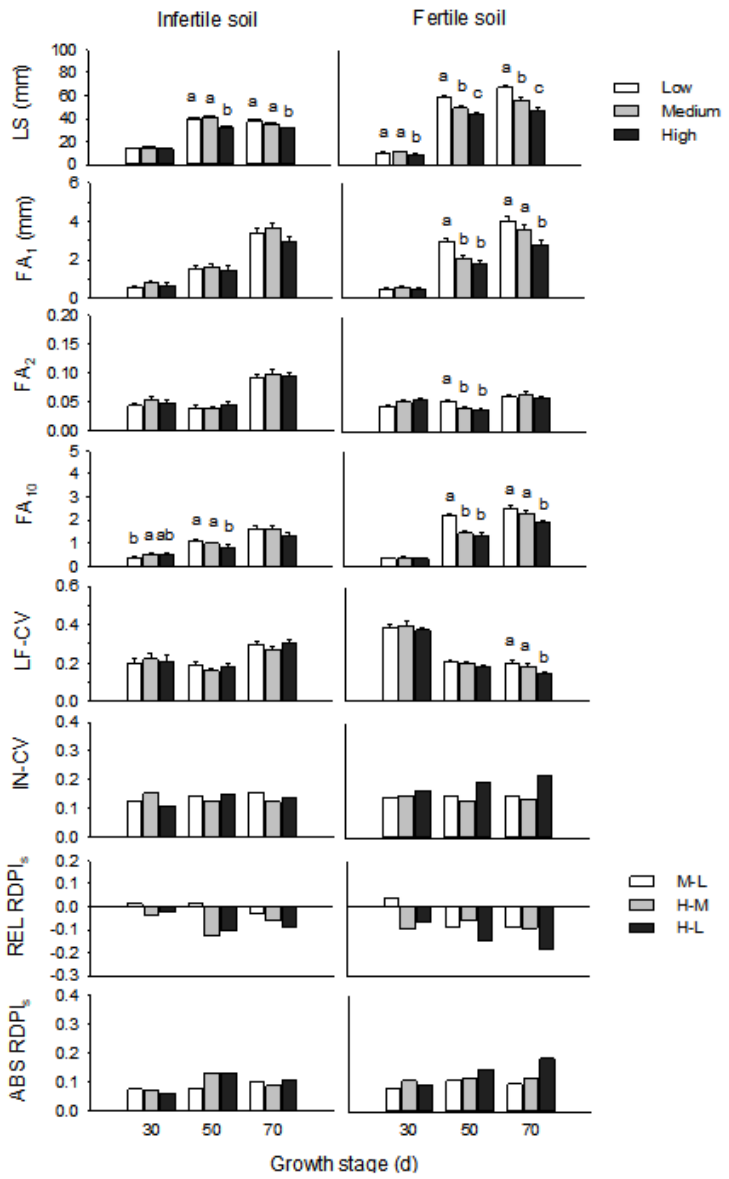

Figure 1

EXP I: Mean values of lamina size (LS), FA indexes, CVs and RDPIs of LS in response to density under infertile and fertile soil conditions at three stages. Different letters denote significant differences between density treatments within each soil condition and growth stage ($P < 0.05$). Abbreviations for all traits are in Table 1.

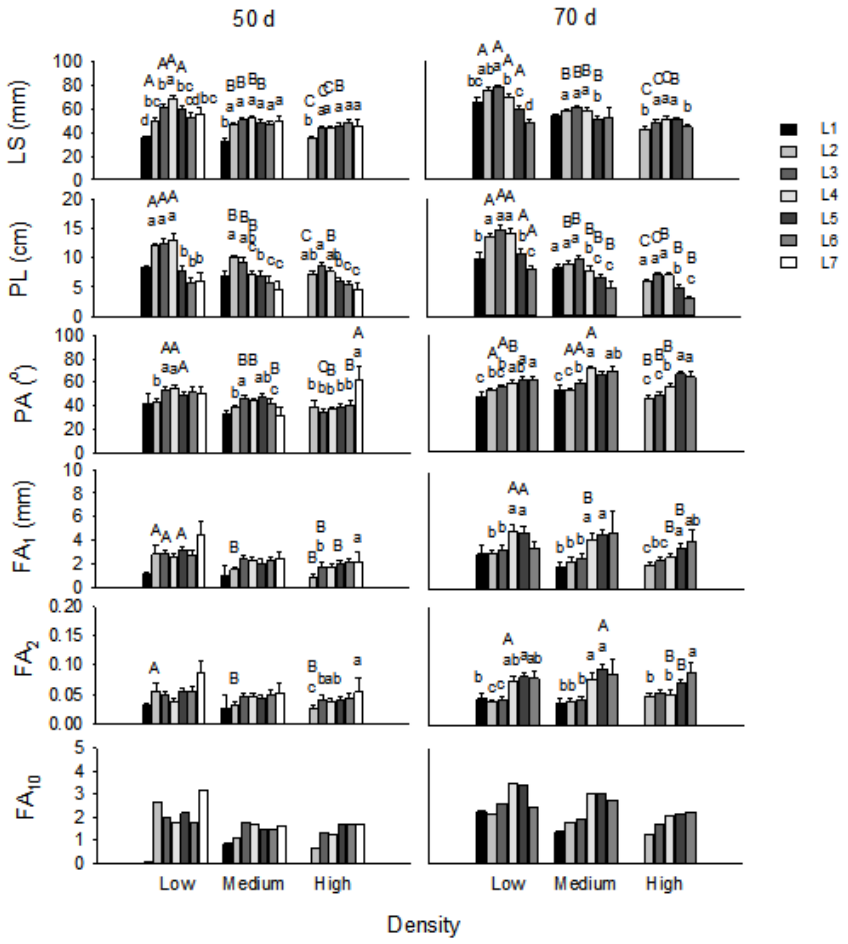

Figure 2

EXP II: Mean values of the three leaf traits and lamina FA indexes in L1~L7 vertical layers in response to density at 50 d and 70 d. Different letters in lowercase denote significant differences between density treatments, and different letters in uppercase denote significant differences between different layers ($P < 0.05$). Abbreviations for all traits are in Table 1.

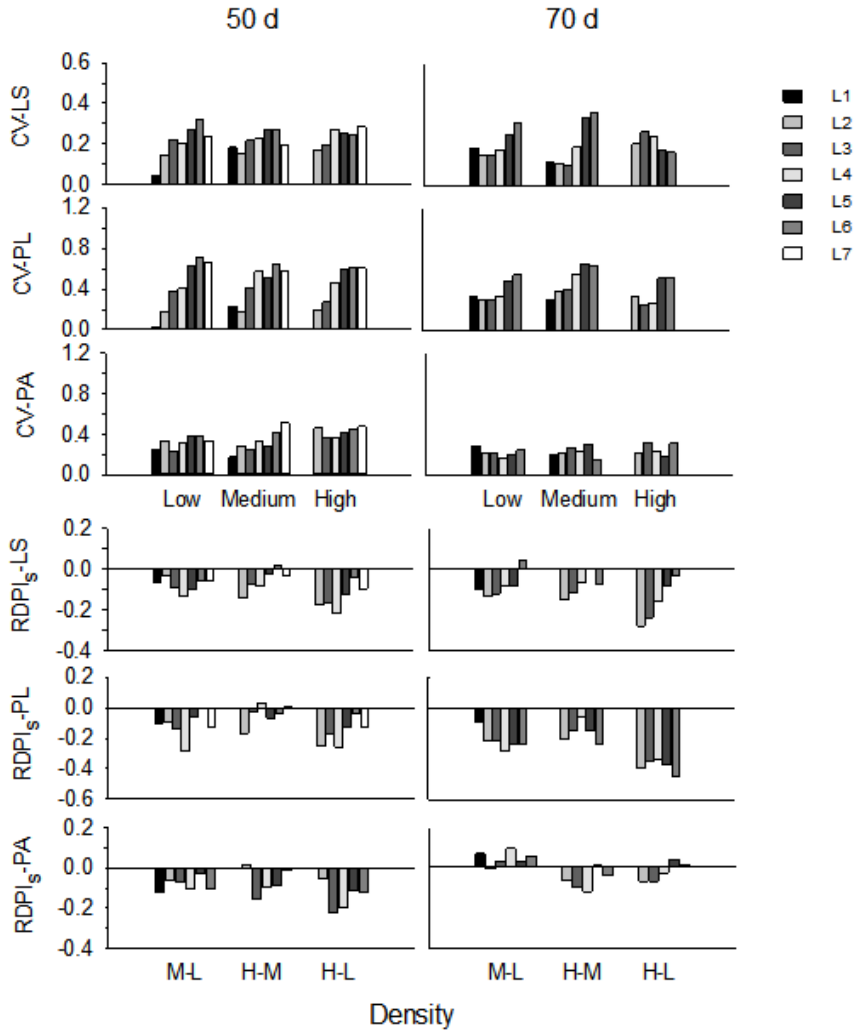

Figure 3

EXP II: IN-CV and RDPIs of traits in L1~L7 vertical layers in response to density for Abutilon theophras at 50 d and 70 d. Abbreviations for all traits are in Table 1.

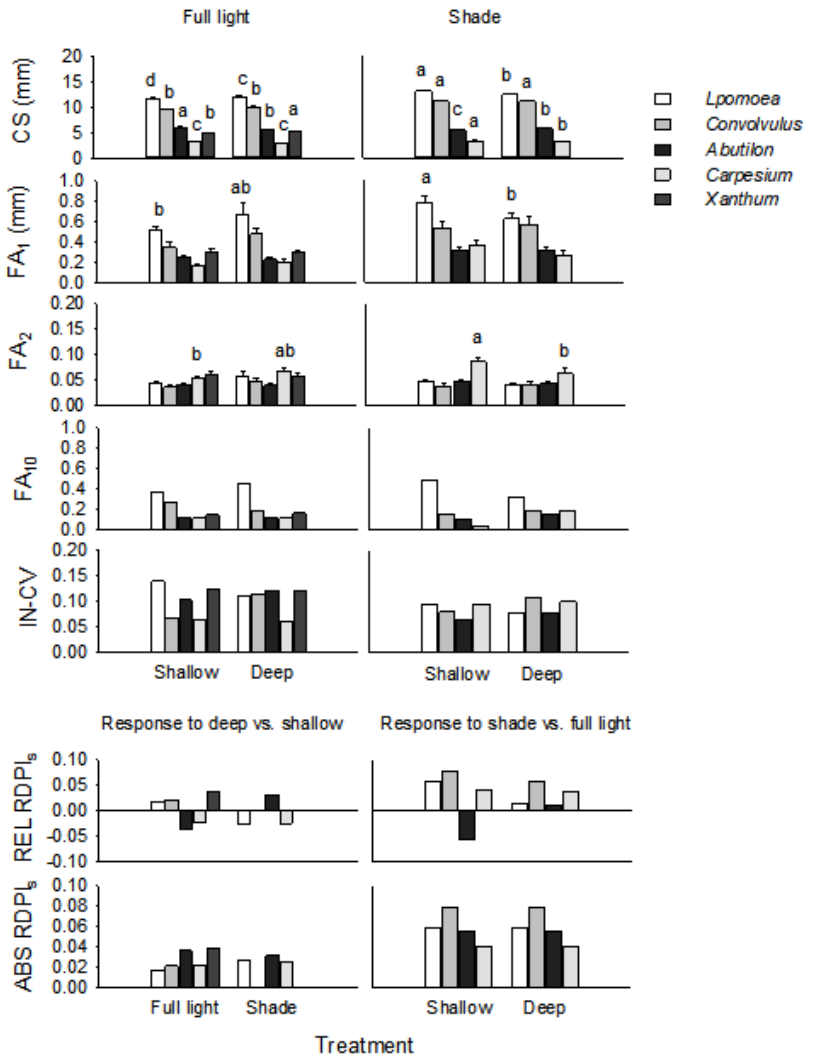

Figure 4

EXP III: Mean values of cotyledon size (CS), cotyledon FA indexes, and IN-CV and RDPIs of CS in responses to light conditions and seeding depth for Lpomoea purpurea, Convolvulus arvensis, Abutilon theophrasti, Carpesium abrotanoides and Xanthum mongolicum. Different letters denote significant differences between treatments within each species ($P < 0.05$). Abbreviations for all traits are in Table 1.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- AppendixPDfacvp2021.2PE.pdf