Associations between leaf developmental stability, canalization and phenotypic plasticity in an architectural perspective

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Research Article

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Abstract

The associations among developmental stability, canalization and phenotypic plasticity have received increasingly more attention, yet with rare direct evidence. Architectural analysis may provide a more powerful approach to addressing this issue. To investigate the relationships among the three mechanisms in architectural perspective, we subjected plants of *Abutilon theophrasti* to three densities, measured and calculated fluctuating asymmetry (FA), coefficients of variation (CV) and plasticity (PI) of three leaf traits, to analyze the correlations among these variables. As density increased, mean leaf size, petiole length and angle of most layers and mean leaf FA of some layers decreased (at both stages), CV of petiole angle increased (at day 50), and PI of petiole length and angle across all layers decreased (at day 70); leaf FA and CV of traits generally increased with higher layers at all densities. At both stages, there were more positive correlations between FA and CV at lower vs. high densities; at day 50, little correlation of plasticity with FA or CV was found; at day 70, more positive correlations between FA and PI occurred for response to high vs. low density than for response to medium vs. low density, and more positive correlations between CV and PI occurred at lower vs. high densities. Results suggested that developmental instability, decreased canalization and plasticity can be cooperative and the relationships between decreased canalization and plasticity are more likely to be positive if decreased canalization is due to vibrant growth rather than stressful effects. The relationships of plasticity with developmental instability differed from its relationship with decreased canalization in the way of variation. Decreased canalization should be more beneficial for possible plasticity in the future, while canalization may be the outcome of already-expressed plasticity.

Introduction

Plants are able to cope with environmental changes via phenotypic variations. The variations in phenotype usually result from two antagonistic processes: one is genetic, developmental and environmental effects, and the other is regulatory mechanisms for or against these effects. Three major regulatory mechanisms frequently investigated include developmental stability, canalization and phenotypic plasticity (Debat and David 2001), which have been often studied separately in respective systems (see Table S1 for their detailed introductions). Despite this, they may not necessarily evolve independently from each other, and the associations among them have received increasingly more attention since two decades ago (Debat and David 2001; Tucić et al. 2018) .

Studies on associations among developmental stability, canalization and phenotypic plasticity have mostly focused on animals and produced inconsistent conclusions (Debat and David 2001; Flatt 2005): the mechanisms for developmental stability and canalization may be independent or at least partly related (Debat et al. 2000; Debat et al. 2009; Lazić et al. 2016); plasticity and developmental stability may have correspondence or not (Debat et al. 2009; Richard et al. 1999; Willmore et al. 2005). One reason might be the lack of direct evidence for these relationships, especially in plants (Wang and Zhou 2021a, 2022b; Wang and Zhou 2022c). Plants with repetitively generated modules over their entire lifetime (de Kroon et al. 2005), exactly provide ideal materials for investigating this issue by architectural approach (Barthélémy and Caraglio 2007; de Kroon et al. 2005). The architectural variations of plants are considered to play important roles in dealing environmental challenges (Sarlikioti et al. 2011; Teichmann and Muhr 2015). In the perspective of plant architecture, we are able to analyze the correlations among developmental stability, canalization and plasticity across leaves of different positions.

The disputes on associations between developmental stability, canalization and phenotypic plasticity should fundamentally be ascribed to the complexity of these relationships in nature (Wang and Zhou 2021a), since most of their correlations can be insignificant in response to increased density (Wang and Zhou 2022c), or other abiotic factors (Wang and Zhou 2022b). Because relationships among these mechanisms may include both positive and negative correlations, and the overall results can be affected by the strength of environmental stresses (Wang and Zhou 2022c). To provide further evidence for this prediction, specific studies are needed on analyzing these relationships along the gradients of environmental conditions. Increased population density can induce significant variations in traits not only at whole-plant level, but at

modular levels and architectural levels. The architectural approach by analyzing correlations between variables across modules of different positions, may be a more powerful way in investigating the relationships among developmental stability, canalization and phenotypic plasticity. Growth stage not only affects competition strength via effects on plant size (Wang and Zhou 2021b), but also affects architectural plasticity of plants in response to density (Wang and Zhou 2022a). Taking effects of growth stage into account may provide another chance to prove how correlations among variables can be affected by the intensity of competition.

Here in a field experiment with *Abutilon theophrasti*, we subjected plants to three densities, measured and calculated mean value, FA, CV and PI of leaf traits in each vertical layers (with 10 or 20 cm inter-distance) for all individuals at two growth stages, to analyze correlations among these variables in response to increased density. Specifically, we asked the following questions: 1) does the increased density affect leaf FA or CV, or PI in all architectural layers? 2) are there any correlations among leaf FA, CV and PI across different layers? And 3) whether and how will these correlations respond to density at each growth stage?

Materials and Methods

Study species

Abutilon theophrasti Medicus (Malvaceae), an annual herbaceous species, is native to China and India but now spreads worldwide (Fig. S1). It grows rapidly to a height of up to 1-1.5 m (Gleason and Cronquist 1991), reaching reproductive maturity within 90 days, and completes life cycle in about five months (McConnaughay and Coleman 1999). It colonizes relatively nutrient-rich habitats, with substantial plasticity in allocation, morphology and architecture in response to varying increased population density (Wang et al. 2017, 2021; Wang and Zhou 2021a, 2022a) and detectable correlations among leaf developmental stability, canalization and plasticity (Wang and Zhou 2021a; Wang and Zhou 2022c).

Experimental design

The experiment was conducted in a research-special field at the Pasture Ecological Research Station of Northeast Normal University, Changling, Jilin province, China (123°44 E, 44°40 N) in 2007. The soil (aeolian sandy soil, pH = 8.3) of the experimental field was a little infertile because of frequent utilization annually, thus we covered the field with a layer of 5–10 cm virgin soil (meadow soil, pH = 8.2) transported from the nearby meadow (with no cultivation history) in the north of the research station, to improve the soil quality (Wang and Zhou 2021b). A completely randomized design was implemented with three density treatments and three replicate plots randomly distributed into nine plots of 2 × 3 m in size. 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 12.8, 27.5 and 108.5 plants·m^{-2} respectively. Seeds were sown on June 7, 2007, and most seeds emerged four days later after sowing. Then seedlings were thinned to the target densities when they reached four-leaf stage. Plots were hand weeded and watered regularly before experiencing drought.

Data collection

Plants were sampled at day 50 and 70 of growth after seedling emergence. At each sampling, five to six individuals were randomly chosen from each plot, making the maximum total of 6 individuals × 3 plots × 3 densities × 2 stages = 108 sampling. Each individual was divided into different architectural layers every 10 cm (day 50) or 20 cm (day 70) vertically from bottom to top. Samples from different layers, density treatments and plots were mixed together, and measured in a random sequence. For each layer per individual, we measured all the leaves on the main stem immediately after sampling when they were fresh. For each leaf, we measured the width of right and left halves (from the midrib to the margin) at the widest point of the leaf (perpendicular to the midrib) with a digital caliper (Wilsey et al. 1998). The width of each side was measured twice successively and immediately after each other. For each leaf, we also calculated the leaf size (LS) as the average width of right and left sides (Palmer and Strobeck 1986; Wilsey et al. 1998), and measured the length and angle (the angle between the petiole and the main stem) of each petiole.

Calculation of CV and PI

Canalization was evaluated by coefficient of variation (CV, the standard deviation divided by mean value of the trait) among individuals per layer for leaf size, petiole length and angle. For each trait, there were 18 individual values at most for each layer in a total of 5–7 layers.

Plasticity was calculated by simplified Relative Distance Plasticity Index (Valladares et al. 2006) for each of leaf size, petiole length and angle, with the abbreviated form of PI and the formula as:

$PI = (X - Y)/(X + Y)$ (1–1)

where X was the adjusted mean trait value at high or medium density, and Y was the adjusted mean value at low density. We calculated the plasticity both in response to high vs. low density (PI_{HL}) and the plasticity in response to medium vs. low density (PI_{ML}). Adjusted mean trait values were produced from one-way ANCOVA on original mean values, with density as effect and plant size (total mass) as a covariate.

Evaluation of FA

We compared various conventional indexes (FA_1-FA_8 and FA_{10}) in calculating the fluctuating asymmetry (FA) in leaf width, to identify the ones with the highest explanatory powers for our study design (Supplementary Table S2). Different indexes showed little response or similar trends in response to density or architectural layer (Tables S3-S5), thus we adopted FA_1, FA_2 (with and without effects of leaf size respectively) and FA_{10} (the only index with measurement error variance partitioned out of the total between-sides variance) in analyses, with the formula as (Palmer 1994; Palmer and Strobeck 2003):

$FA_1 = \sum |R - L| / n\,(2 - 1)$

$FA_2 = \sum (|R - L| / LS) / n\,(2-2)$

$FA_{10} = 0.798 \times \sqrt{(MS_{sj} - MS_m)} / M\,(2-3)$

where R and L were the widths of right and left sides of a leaf respectively, n was the total number of leaves, and LS (leaf size) was calculated by $(R + L)/2$, MS_{sj} was the mean squares of side × individual interaction, MS_m was the mean squares of measurement error, M was the number of replicate measurements per side, from a side × individual ANOVA on untransformed replicate measurements of R and L.

We measured skew (γ_1) and kurtosis (γ_2) to evaluate whether the leaf asymmetry deviated from normality. To detect the presence of antisymmetry, kurtosis (γ_2) was tested with a t-test of the null hypothesis $H_0: \gamma_2 = 0$, where a significant negative γ_2 indicates possible antisymmetry (Cowart and Graham 1999; Palmer 1994). To test the presence of directional asymmetry, we used two methods: 1) testing $(R - L)$ against 0 with one-sample t-test (the hypothesis $H_0: \gamma_1 = 0$); and 2) testing whether the difference between sides (mean squares for side effect [MS_s]) is greater than nondirectional asymmetry (mean squares for side × individual interaction [MS_{si}]) with factorial ANOVA (Palmer 1994; Wilsey et al. 1998). For layer, density and stage combination, two sets of samples (at day 50) showed leptokurtosis, indicating antiasymmetry; but only one set of samples showed left-dominant directional asymmetry (Tables S6 and S7). In addition, seven sets of samples at day 50 and ten sets at day 70 also showed greater mean difference between sides (MS_s) than between-sides variation (MS_{si}; Tables S8 and S9), indicating directional asymmetry. We regressed $|R - L|$ on LS for all the leaves of individuals per layer at each density and stage to determine the size-dependence of leaf asymmetry, and found several cases of leaf asymmetry were size-dependent (Tables S6 and S7). We also evaluated whether the between-sides variation is significantly larger than the measurement error (MS_m) in factorial ANOVA (Palmer 1994). The MS_m values for all cases were lower than MS_{si} values (Tables S8 and S9).

Statistical analysis

Mean value (MV), CV and PI of leaf size, petiole length and angle and leaf FA were used in statistics. 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 Incorporation 2002). Three-way ANOVA was performed for effects of growth stage, population density, plant layer and their interactions on all variables. Then we used one-way ANOVA for differences among layers for all variables at each density and one-way ANCOVA for effects of density on all variables for each layer or across all layers, with plant total biomass as a covariate. Multiple comparisons used LSD (Least Significant Difference) method in General Linear Model (GLM) program. For each of the three leaf traits at each density and stage, correlations among MV, FA (only results with FA_2 were presented due to similar results for different FA indexes), CV and PI across all layers were analyzed with PROC CORR, producing Pearson Correlation Coefficients (PCC) for all correlations and Partial Pearson Correlation Coefficients (PPCC) for correlations among FA, CV and PI, with mean trait value in control in partial correlation analyses.

Results

Growth stage and population density had significant effects on fluctuating asymmetry (FA) and plasticity (PI) of leaf traits for most cases; architectural layer effects were significant for FA and coefficient of variation (CV) of leaf traits for most cases; interactions between any two of the three factors (stage, density and layer) were most significant for PI of petiole length (Table 1). Across both stages, leaf traits of most or some layers and FA_1 and FA_2 of some layers decreased with higher densities (LSD, $P < 0.05$; Fig. 1). Across all layers, high relative to low density increased CV of petiole angle at 50 d ($P = 0.047$; Fig. 2e). Plasticity in response to high vs. low density (PI_{HL}) was lower than plasticity in response to medium vs. low density (PI_{ML}) for leaf size at 50 d (Fig. 3a), and for petiole length and angle at 70 d ($P < 0.05$; Fig. 3d, f).

For both stages, with higher layers, the leaf size of plants at low density first increased then decreased, the trend of which became less pronounced at medium and high densities ($P < 0.05$; Fig. 1a, b); the decrease of petiole length with higher layers was also more significant as density increased ($P < 0.05$; Fig. 1c, d); the increase of petiole angle and FA indexes with higher layers was more significant at 70 d than at 50 d for plants at all densities ($P < 0.05$; Fig. 1e-l). The increase with higher layers was also found in CV of leaf size and petiole length (Fig. 2a-d), and PI_{HL} of leaf size (Fig. 3a, b); as density increased, the differences among layers tended to magnify for PI of leaf size (Fig. 2a, b), but tended to diminish for CV of leaf size (Fig. 3a, b).

For all three leaf traits, correlations for mean value (MV) with other variables were either positive, negative or insignificant, but there were much more positive than negative correlations among FA, CV and PI (Tables 2 and 3). MV more likely had negative or insignificant correlations with FA for petiole length, but more likely had positive or insignificant correlations with FA for leaf size and petiole angle, whereas more likely had positive (50 d) or negative (70 d) correlations with CV at low and high vs. medium density (Table 2). But FA and CV mainly had positive correlations, more positive at low and medium vs. high densities. The correlations between MV and PI were more positive or less negative with higher densities, and PI had more positive than negative correlations with FA and CV, and positive correlations increased with higher densities at day 50, increased with lower densities at day 70 (Table 3).

Discussion

Plant response to density

-Developmental stability

Fluctuating asymmetry (FA) has been commonly used as an indicator of developmental stability (DS) or instability (DI) of organisms, which it is predicted to increase with environmental stress (Hagen et al. 2008; Møller 1998). Our results showed that in response to increased density, mean values of leaf traits in most layers decreased, indicating adverse effects of increased density, and FA decreased for some layers, consistent with another study of ours (Wang and Zhou 2022c). It suggested more-favorable environments, like higher nutrient availability (Milligan et al. 2008), less polluted soil (Velickovic and Perisic 2006), or water supplementation (Fair and Breshears 2005), may also allow faster growth of plants or modules, leading to higher DI and FA levels (Martel et al. 1999; Morris et al. 2012). Therefore higher FA may not be harmful, but indicate the plants in a sparse population are growing faster than those in a dense population (Morris et al. 2012; Wang and Zhou 2022c). Despite this, leaf FA of only one or two layers decreased with higher densities in this study, suggesting responses of different layers varied, depending on their growth state. For plants at day 50, the leaves in lower layers may grow faster than higher layers, leading to more pronounced responses to density; whereas the leaves in middle layers grew fastest at day 70, thus being more responsive to density. Therefore, not only the growth state reflected by leaf FA can be affected by environmental stress, but also the growth state can affect whether leaf FA responds to stress. The modules in faster growth may be more likely to respond to stress.

-Canalization

Unlike leaf FA, coefficient of variation (CV) of petiole angle for all layers increased with higher densities, especially for those in lower layers, consistent with our other results(Wang and Zhou 2022c). This should be because increased density resulted in greater variation in plant size, leading to increased variation in leaf traits, and increased CV with higher layers for plants at all densities. Compared to those in lower layers, leaf traits in higher layers had higher levels of CV across all densities, probably because modules in higher layers are more viable than lower layers. Plants may invest increasingly greater proportion of energy into leaf growth in higher layers over time (Wang and Zhou 2022a), and slight differences in growth rates between individuals can be translated into large variations in higher layers of leaves if they grew fast. Therefore, the increased CV with higher layers differed from its increase with higher densities: the former indicated that leaves were growing faster in higher vs. lower layers, similar to the increase of FA; while the latter indicated adverse environmental effects. Therefore, decreased canalization can either be advantageous or disadvantageous, depending on specific circumstances (Wang and Zhou 2021a), whereas developmental instability suggested better growth state (Wang and Zhou 2022c).

Correlations among different mechanisms

There can be certain relationships between the mechanisms of developmental stability (FA), canalization (CV) and phenotypic plasticity (PI), and thus they can explain each other (Del Giudice et al. 2018; McDonald et al. 2018), especially when their correlations follow some patterns or rules (Wang and Zhou 2022c). Furthermore, it is believed that the relationships among these mechanisms should be complex (Wang and Zhou 2022b), involving both positive and negative correlations, and the overall results can be positive, negative or non-significant, hinging on specific circumstances (Wang and Zhou 2022c). Our results showed a lot more positive correlations among FA, CV and PI than negative ones, indicating cooperative relationships among developmental instability, decreased canalization and increased plasticity (Debat et al. 2009; Lazić et al. 2016; Tonsor et al. 2013; Tucić et al. 2018). This may be because for an individual plant, different variables of leaf traits are more likely to follow the same pattern in terms of among-layer variations, leading to more positive relationships between these variables.

-Correlation between developmental stability and canalization

Our results that leaf fluctuating asymmetry (FA) in some layers decreased and coefficient of variation (CV) in petiole angle increased with higher densities, suggested the connections between lower FA and higher CV in response to increased density. However, we showed that within each density, FA and CV positively correlated in most cases. Other studies either predict the two variables are related or not (Debat et al. 2000; Debat et al. 2009; Lazić et al. 2016), but rare direct evidence for their correlations have been found. Based on the pure positive correlations between FA and CV in our results, we believe that

developmental instability and decreased canalization more likely have co-operative relationships. However, decreased canalization can either reflect greater growth potential or stressful effects (Wang and Zhou 2021a; Wang and Zhou 2022c). The decrease of canalization with higher layers should be due to more vibrant growth for leaves in higher layers, while its decrease with greater densities should be due to the increase of small plants in dense population (Wang and Zhou 2022c). Therefore, if higher developmental instability usually indicates an advantage, decreased canalization can be disadvantageous or advantageous. When the decreased canalization with higher layers is advantageous, it is more likely to have positive correlations with developmental instability (Kawecki 2000; Wang and Zhou 2021a).

-Correlations of plasticity with developmental stability and canalization

All the three mechanisms of developmental stability, canalization and plasticity should reflect the capacity of developmental system to regulate and adjust phenotypic expression (Siegal and Bergman 2002), there is a high probability that they are inter-related inherently, although they have been mostly investigated and interpreted in different study systems (Debat and David 2001). Increased developmental instability and inter-individual variability can be beneficial and facilitate adaptive responses in traits, leading to positive correlations with each other and with plasticity (Wang and Zhou 2022c). In spite of this, there should be some differences when interpreting the variations in developmental instability and canalization and their relationships with plasticity. For developmental instability, higher level may indicate faster growth or better (or less adverse) environmental conditions, and moderate stress (e.g. weak competition) is more likely to induces higher FA, suggesting its beneficial effects thus facilitating growth rate, while intense stress (e.g. strong competition) tends to decrease it, suggesting adverse effects (Wang and Zhou 2022c). However, decreased canalization (higher inter-individual variation) can either reflect the state of fast growth or beneficial environmental effects, or adverse effects of environmental stress (Wang and Zhou 2021a). This may explain why we found only positive correlations between FA and PI (Wang and Zhou 2022b), but both positive and negative correlations between CV and PI (Wang and Zhou 2022c).

It is also reported that the relationships between developmental instability, inter-individual variability and plasticity are complex and depend on the strength of environmental stresses, and positive correlations are more likely to occur in more favorable conditions, while negative correlations will increase and counteract positive ones under more stressful conditions (Wang and Zhou 2022c). In this study, we found that in spite of both being positive, developmental instability and inter-individual variability (decreased canalization) differed in how their relationships with plasticity vary with environmental conditions: the positive correlations between developmental instability and plasticity tended to occur when plastic response was stronger, like response to high vs. low density; whereas positive correlations between inter-individual variability and plasticity more likely occurred at low and medium than high densities. These suggested that the correlation between developmental instability and plasticity more relies on the strength of plastic response, while the correlation between inter-individual variability and plasticity more relies on the nature of environmental conditions. Higher inter-individual variability will be more beneficial for the ability of plasticity to produce more active responses (increase) or less passive responses (decrease), under environmental conditions not inducing plasticity. When exposed to the conditions inducing plastic responses, such positive relationship may weaken because of phenotypic convergence of different individuals due to environmental effects. These results revealed that intra-individual variability or decreased canalization are beneficial for plants dealing with possible environmental variations via adaptive plastic responses in the future, while decreased intra-individual variation or canalization may result from the expression of plastic responses.

Conclusions

Our results showed decreased fluctuating asymmetry (FA), coefficient of variation (CV) and plasticity (PI) for all architectural layers with higher densities, but increased FA and CV with higher layers. Leaf FA of only one or two layers decreased with higher densities, suggesting the growth state of leaves not only affects FA levels, but also affects whether the response of leaf FA to stress: the modules growing faster more likely respond to stress by increased FA. Besides, the increase of CV with

higher layers differed from its increase with higher densities: the former indicates better growth of leaves in higher vs. lower layers, while the latter indicated adverse density effects.

More positive correlations among FA, CV and PI than negative ones in this study indicated relationships among developmental instability, decreased canalization and increased plasticity can be cooperative when the increase of inter-individual variation is beneficial. The relationships of plasticity with developmental instability and decreased canalization differed in the way of variation: plasticity will have more positive correlations with developmental instability when stronger plastic responses are induced, and with decreased canalization in the environments not inducing plastic responses. Intra-individual variability or decreased canalization should be beneficial for future plastic responses, while decreased intra-individual variation may be the outcome of expressed plastic responses. Investigation on the associations among these mechanisms may not only better our understanding on the interactions between developmental process and environmental influences, but also promote reconsiderations on these mechanisms themselves respectively. Architectural analysis should be a useful and powerful approach in detecting the correlations among these variables.

Declarations

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Compliance with ethical standards

Data availability

Data are available via the Dryad Digital Repository:

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Conflict of interest

No conflict of interests have been declared.

Author contributions

Both authors contributed to the study conception and design. Shu Wang conducted the experiment, collected the data and performed statistical analyses. The first draft of the manuscript was written and edited by Shu Wang. All authors read and approved the final manuscript.

Supplementary information

The online version contains supplementary material available at

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Tables

Table 1 *F*-values for three-way ANOVA on mean leaf FA (FA_1, FA_2 and FA_{10}), coefficient of variation (CV) and plasticity (PI) for leaf size (LS), petiole length (PL), petiole angle (PA) with growth stage (GS), population density (PD), architectural layer (AL) and their interactions as effects. * $P < 0.10$, ** $P < 0.05$. *** $P < 0.01$.

Source of variation	Df	FA			CV			PI		
		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*
AL	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 × AL	5	2.63	4.43*	1.43	0.22	3.50*	2.46	2.09	7.07**	2.07
PD × AL	11	1.23	0.86	0.84	1.05	0.69	1.27	1.33	4.72*	1.01

Table 2 Pearson Correlation Coefficients (PCC) for correlations of mean trait value (MV) with leaf FA (FA_2) and mean coefficient of variation (CV) and Partial Pearson Correlation Coefficients (PPCC) for correlations between FA and CV for all layers of leaf size (LS), petiole length (PL) and angle (PA) for plants at low (L), medium (M) and high (H) densities and at day 50 and 70. The values in bold font indicate significant PPC or PPCC at the level of $P < 0.10$ (*), $P < 0.05$ (**) and $P < 0.01$ (***).

			Day 50		Day 70	
Variable	Density	Trait	FA	CV	FA	CV
MV	L	LS	0.470	**0.823****	-0.720	**-0.973****
		PL	-0.118	-0.078	-0.466	**-0.808***
		PA	0.241	0.108	**0.832****	-0.533
	M	LS	**0.831****	0.402	-0.464	-0.728
		PL	-0.263	-0.622	**-0.733***	-0.717
		PA	**0.846****	0.571	**0.913****	-0.069
	H	LS	**0.974*****	0.778	-0.032	0.293
		PL	-0.248	-0.684	**-0.914****	**0.879****
		PA	-0.194	**0.818***	**0.829***	-0.255
FA	L	LS		**0.768****		**0.821****
		PL		**0.750****		**0.828****
		PA		0.573		-0.319
	M	LS		**0.772***		**0.932*****
		PL		**0.887****		**0.976*****
		PA		**0.845****		0.049
	H	LS		0.680		-0.786
		PL		0.751		**0.854***
		PA		-0.443		0.186

Table 3 Pearson Correlation Coefficients (PCC) or Partial Pearson Correlation Coefficients (PPCC) for correlations of plasticity (PI) in response to medium vs. low density (PI_{ML}), high vs. Medium density (PI_{HM}) and high vs. low density (PI_{HL}) with mean trait value (MV), leaf FA (FA_2) and coefficient of variation (CV) for all layers of leaf size (LS), petiole length (PL), petiole angle (PA) for plants at low (L), medium (M) and high (H) densities and at day 50 and 70. The values in bold font indicate significant PPC or PPCC at the level of $P < 0.10$ (*), $P < 0.05$ (**) and $P < 0.01$ (***).

			Day 50			Day 70		
Density	Type	Trait	MV	FA	CV	MV	FA	CV
L	PI_{ML}	LS	-0.662	0.287	-0.273	**-0.943****	0.697	**0.889****
		PL	**-0.787***	0.467	0.033	-0.278	-0.584	-0.268
		PA	0.084	0.714	0.380	0.058	0.452	-0.060
	PI_{HL}	LS	-0.557	0.585	0.756	**-0.926****	**0.937****	**0.962*****
		PL	**-0.927****	0.466	0.789	**0.827***	-0.137	-0.620
		PA	**-0.907****	0.671	0.727	**0.938****	**0.919****	0.143
M	PI_{ML}	LS	-0.414	-0.461	-0.473	-0.599	0.633	**0.796***
		PL	-0.255	0.053	-0.130	0.263	-0.721	**-0.795***
		PA	0.607	0.207	0.126	0.552	0.279	-0.319
H	PI_{HL}	LS	0.560	0.470	0.153	0.294	**0.908****	-0.707
		PL	-0.724	0.688	0.626	**0.908****	-0.305	-0.701
		PA	**0.842***	-0.624	**0.913****	**0.990*****	0.804	-0.362

Figures

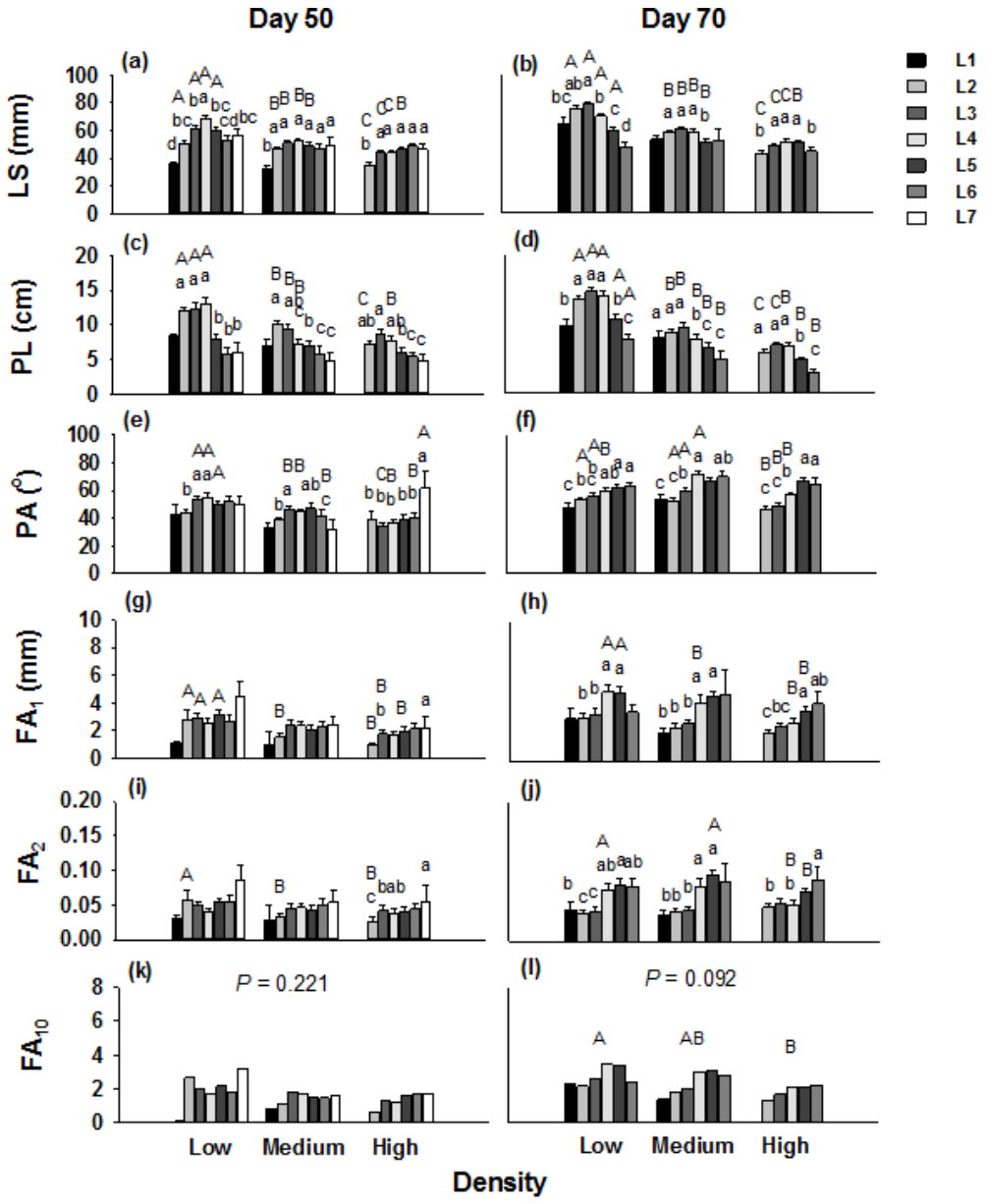

Figure 1

Mean values of leaf size (LS; a, b), petiole length (PL; c, d) and petiole angle (PA; e, f) and leaf fluctuating asymmetry (FA_1, FA_2 and FA_{10}; g-l) in vertical layers of L1~L7 in response to density for plants at day 50 (a, c, e, g, i, k) and 70 (b, d, f, h, j, l) of plant growth. Different letters in lowercase denote significant differences between density treatments, and different letters in uppercase denote significant differences between different layers ($P < 0.05$); P-values indicate the significance for differences among densities across all layers for FA_{10}.

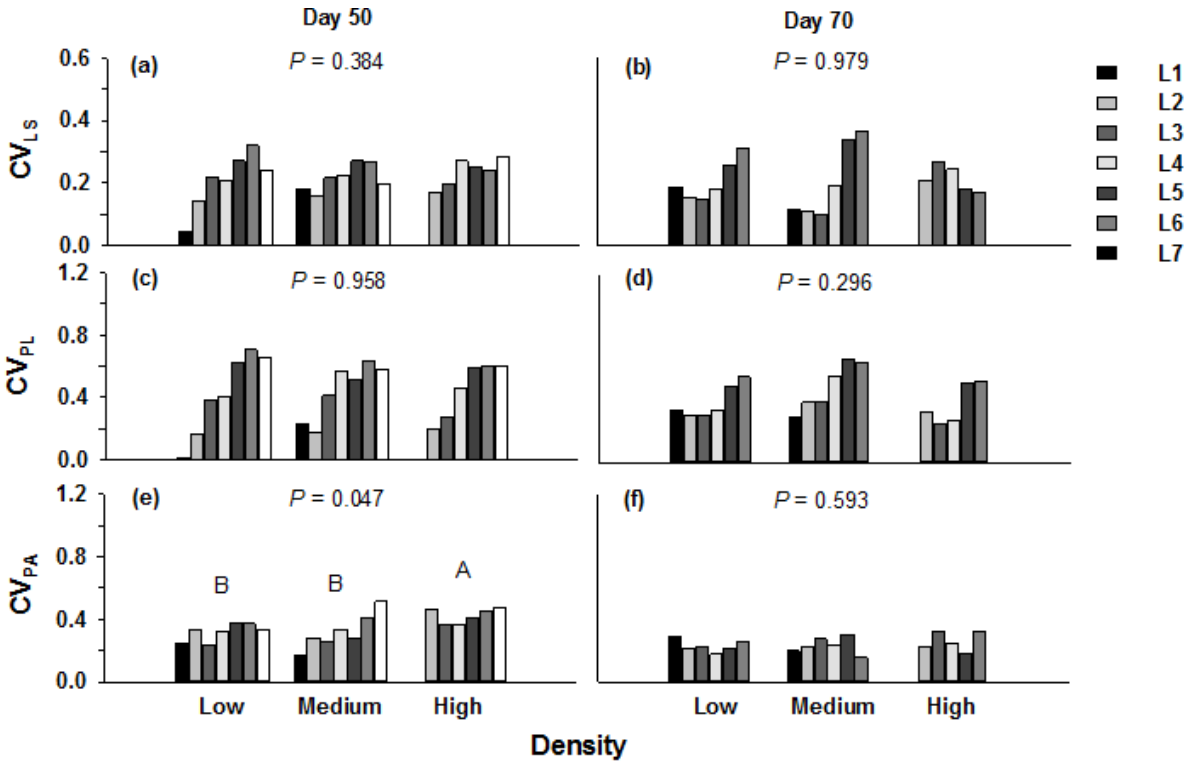

Figure 2

Coefficients of variation in leaf size (CV_{LS}; a, b), petiole length (CV_{PL}; c, d) and petiole angle (CV_{PA}; e, f) in vertical layers of L1~L7 in response to density for plants at day 50 (a, c, e) and 70 (b, d, f) of plant growth. Different uppercase letters and *P*-values indicate differences among densities across all layers and their significance.

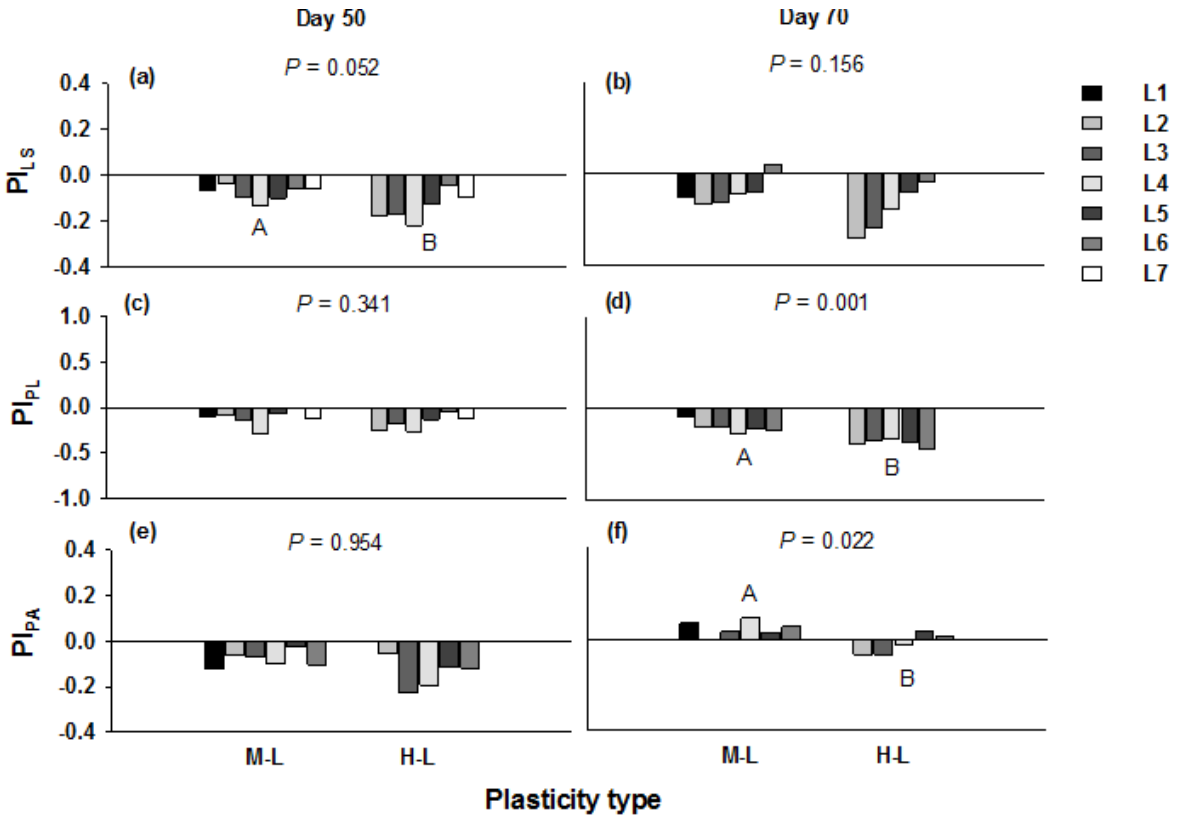

Figure 3

Plasticity of leaf size (PI_{LS}; a, b), petiole length (PI_{PL}; c, d) and petiole angle (PI_{PA}; e, f) in vertical layers of L1~L7 in response to medium vs. low density (M-L) and high vs. low density (H-L) for plants at day 50 (a, c, e) and 70 (b, d, f) of plant growth. Different uppercase letters and *P*-values indicate differences among densities across all layers and their significance.

Supplementary Files

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