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Research Article

Insights into the Differences in Leaf Functional Traits of Three Varieties of *Osmanthus fragrans* with Different Flower Colors

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Abstract

Leaf functional traits are adaptations that facilitate plants to grow in a wide range of environmental conditions. This study aims to gain insights into the differences in leaf functional traits of three varieties of *Osmanthus fragrans* with different flower colors (i.e., *O. fragrans* var. *thunbergii*, *O. fragrans* var. *latifolius*, and *O. fragrans* var. *aurantiacus*). Leaf length, leaf width, and single-leaf wet and dry weights decreased significantly in the following order: *O. fragrans* var. *thunbergii*, *O. fragrans* var. *latifolius*, and *O. fragrans* var. *aurantiacus*. Specific Leaf Area (SLA) of *O. fragrans* var. *aurantiacus* was also significantly higher than those of *O. fragrans* var. *thunbergii* and *O. fragrans* var. *latifolius*. This finding indicates that leaf construction cost of *O. fragrans* var. *aurantiacus* was significantly lower than those of *O. fragrans* var. *thunbergii* and *O. fragrans* var. *latifolius* to pay an utmost effort to increase light capture and use efficiency because of its low leaf size. Thus, one of the reasons causing the difference in flower colors of the three varieties of *O. fragrans* may be the difference in leaf functional traits among the three varieties of *O. fragrans* because most (or even all) of the compounds which form different colors in flower originates the leaves function (i.e. photosynthesis) and difference in leaf functional traits can trigger pronounced effects on the absorption and utilization of light as well the metabolic components.

Keywords: Leaf functional traits; Specific leaf area; Flower color; *O. fragrans* var. *thunbergii*; *O. fragrans* var. *latifolius*; *O. fragrans* var. *aurantiacus*

Introduction

The leaves can make plants gain resources (especially the acquisition of light) [1,2] for their growth and development [3]. Thus, the response of leaf functional traits [leaf functional traits are the leaf core attributes closely related to the colonization, survival, growth, and mortality of plants. These core attributes can significantly affect ecosystem functions and reflect the response of plants to environmental change [4]. To the adjustment in environmental factors could enable plants to acclimatize multiple environmental conditions and thereby be used as a proxy for a successful ecological strategy of plants because leaves are exposed and sensitive to external environments [5-8]. As one of the most important leaf functional traits, Specific Leaf Area (SLA, defined as investment per unit of light capture surface deployed) can be used to describe the resource-use strategy of plant species [5,9-11], i.e, SLA controls and maintains a balance between light capture and leaf construction cost [9]. Generally, a plant species with a high SLA typically has a higher growth rate, more rapid turnover of leaf material, and shorter lifespans than those species with low SLA [5,10-12]. Then again, leaf size (indicated by leaf length and leaf width), leaf shape index (calculated as the ratio of leaf length to leaf width), leaf chlorophyll and Nitrogen (N) concentrations, leaf thickness, single-leaf wet and dry weights, and leaf moisture are also crucial indices of leaf functional traits because those indices are also used as acceptable indicators of resource-use strategy of plants [5,7-9,13].

Osmanthus fragrans, which originated in China, has been widely used as an ornamental tree around the world currently, especially in China. At present, this species has many cultivars, and three of the most cultivated varieties in landscaping are *O. fragrans* var. *thunbergii* (yellow or golden flower), *O. fragrans* var. *latifolius* (white or ivory-white flower), and *O. fragrans* var. *aurantiacus* (red or orange-red flower) in China. The three varieties of *O. fragrans* are evergreen shrubs or small trees of the Oleaceae family. The flowering period of the three varieties of *O. fragrans* is usually the Mid Autumn Festival. Meanwhile, the three varieties of *O. fragrans* with different flower colors were present together in same sites. Because co-occurring species suffer from similar environmental selection pressures (i.e., habitat filtering), thus, the differences in leaf functional traits among those species are believed to be closely related to their successful ecological strategy. Thus, determination of the differences in leaf functional traits among the three varieties of *O. fragrans* with different flower colors is essential in illuminating the mechanism underlying their successful ecological strategy during their life history.

This study aimed to gain insights into the differences in leaf functional traits of the three varieties of *O. fragrans* with different flower colors. The leaf functional traits (i.e., leaf size, leaf shape index, leaf chlorophyll and N concentrations, SLA, leaf thickness, single-leaf wet and dry weights, and leaf moisture) of the three varieties of *O. fragrans* with different flower colors were assessed to gain insights into their ecological strategy because these indices can be used as indicators of resource-use strategy of plants [5,9,11-14].

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Table 1: Differences in leaf functional traits among the three varieties of *O. fragrans* with different flower colors. Data with different letters in a vertical row indicate a significant difference (P < 0.05). "ns" means not significant difference (P > 0.05).

Plant species	LL	LW	LSI	LCC	LNC	SLA	LT	SLWW	SLDW	LM
O. fragrans var. *thunbergii*	11.923±0.293a	4.688±0.113a	2.551±0.047ns	48.245±0.845ns	3.941±0.058ab	69.904±1.722b	0.071±0.002b	1.083±0.033a	0.596±0.022a	44.661±1.578ns
O. fragrans var. *latifolius*	10.887±0.235b	4.338±0.072b	2.514±0.051ns	47.783±0.617ns	3.912±0.040b	72.358±1.546b	0.073±0.002ab	0.983±0.028a	0.508±0.020b	48.100±1.703ns
O.fragrans var. *aurantiaus*	9.497±0.217c	3.835±0.071c	2.478±0.039ns	50.114±0.588ns	4.078±0.042a	98.246±3.028a	0.079±0.002a	0.768±0.028b	0.402±0.013c	47.397±0.684ns

Abbreviations: LL, Leaf Length (cm); LW, Leaf Width (cm); LSI, Leaf Shape Index; LCC, Leaf Chlorophyll Concentration (SPAD); LNC, Leaf N Concentration (mg g-1); SLA, Specific Leaf Area (cm^2 g-1); LT, Leaf Thickness (mm); SLWW, Single-Leaf Wet Weight (g); SLDW, Single-Leaf Dry Weight (g); LM, Leaf Moisture (%).

Table 2: Differences in plasticity indices of leaf functional traits of the three varieties of *O. fragrans* with different flower colors.

Plant species	LL	LW	LSI	LCC	LNC	SLA	LT	SLWW	SLDW	LM
O. fragrans var. *thunbergii*	0.357	0.328	0.270	0.236	0.199	0.331	0.361	0.381	0.455	0.657
O. fragrans var. *latifolius*	0.298	0.255	0.231	0.188	0.159	0.288	0.412	0.321	0.399	0.419
O. fragrans var. *aurantiacus*	0.294	0.321	0.251	0.157	0.145	0.371	0.261	0.478	0.424	0.300

Abbreviations have the same meanings as described in Table 1.

Materials and Methods

Experimental design

Leaves samples of the three varieties of *O. fragrans* with different flower colors (*O. fragrans* var. *thunbergii*, yellow or golden flower; *O. fragrans* var. *latifolius*, white or ivory-white flower; *O. fragrans* var. *aurantiacus*, red or orange-red flower) were collected during their florescence in Zhenjiang, China (32°20'N, 119°51'E) in mid October 2015. The sampling area has a subtropical humid climate. The annual mean temperature of the area is approximately 15.6 °C, and its monthly mean temperature reaches a maximum of 25 °C in July and decreases to a minimum of -1°C in January. The annual precipitation is approximately 1088 mm, and the rainy season comes in June and July. Most of samples of the three varieties of *O. fragrans* were present together in same sampled sites. A total of 20 plant samples for one variety of *O. fragrans* were collected from open areas randomly in gardens. Five fully expanded and intact leaves of one plant sample were selected randomly to determine their leaf functional traits.

Determination of leaf functional traits

Leaf shape index was calculated as the ratio of leaf length to the corresponding leaf width [7,8,14,15]. The leaf length is the maximum value along the midrib, while the width is the maximum value perpendicular to the midrib [14]. Leaf length and leaf width were measured using a ruler [7,8].

The relative chlorophyll and N concentrations in the leaves were estimated with a hand-held plant nutrient meter (TYS-3N, China). TYS-3N was used to calculate the index in "SPAD units" based on absorbance at 650 nm and 940 nm. SLA was computed using the ratio of the leaf area to the corresponding leaf dry weight (cm^2 g-1) according to previous studies [7,8,10]. Leaf thickness was calculated through the overlap of five leaves using a Vernier caliper with an accuracy of 0.01 mm [7,8]. Leaf moisture was calculated by subtracting the leaf dry weight from the leaf wet weight; the difference was then divided by the leaf wet weight [7,8]. Single-leaf wet weight was determined using an electronic balance. Single-leaf dry weight was obtained by initially subjecting the samples to oven-dried at 60 °C for 24 h to achieve a constant weight; final single-leaf dry weight was then determined using an electronic balance with an accuracy of 0.001 g [7,8].

Plasticity index [the index ranged from zero (no plasticity) to one (maximum plasticity)] of characteristics of the varieties of *O. fragrans* were calculated to characterize their phenotypic plasticity according to previously described methods [16,17].

Statistical analysis

Data were verified to determine the deviations from normality and homogeneity of variance before data analysis. Differences among various dependent variables were assessed using analysis of variance. Statistically significant differences were set at P values equal to or lower than 0.05. Patterns among various dependent variables were determined by correlation analysis using IBM SPSS Statistics (version 22.0).

Results

Differences in leaf functional traits among the three varieties of *O. fragrans*

Leaf length, leaf width, and single-leaf wet and dry weights were in the order of *O. fragrans* var. *thunbergii* > *O. fragrans* var. *latifolius* > *O. fragrans* var. *aurantiacus* (Table 1, P < 0.05). SLA of *O. fragrans* var. *aurantiacus* was significantly higher than those of *O. fragrans* var. *thunbergii* and *O. fragrans* var. *latifolius* (Table 1, P < 0.05). Leaf N concentration of *O. fragrans* var. *aurantiacus* was significantly higher than that of *O. fragrans* var. *latifolius* (Table 1, P < 0.05) but not *O. fragrans* var. *thunbergii* (Table 1, P > 0.05). Leaf thickness of *O. fragrans* var. *aurantiacus* was significantly higher than that of *O. fragrans* var. *thunbergii* (Table 1, P < 0.05) but not *O. fragrans* var. *latifolius* (Table 1, P > 0.05). No significant difference was found in leaf shape index, leaf chlorophyll concentration, and leaf moisture among the three varieties of *O. fragrans* (Table 1, P > 0.05).

Differences in plasticity indices of leaf functional traits among the three varieties of *O. fragrans*

The plasticity indices of leaf length, leaf chlorophyll and N concentrations, and leaf moisture decreased in the following order: *O. fragrans* var. *thunbergii*, *O. fragrans* var. *latifolius*, and *O. fragrans* var. *aurantiacus* (Table 2). The plasticity indices of leaf width, leaf shape index, SLA, and single-leaf wet and dry weights of *O. fragrans* var. *latifolius* were lower than those of *O. fragrans* var. *thunbergii* and *O. fragrans* var. *aurantiacus* (Table 2). The plasticity index of leaf

Table 3: Relationship among leaf functional traits of the three varieties of *O. fragrans* with different flower colors. *, ** and *** indicates significant differences at 0.05, 0.01, and 0.001 probability level, respectively. P values equal to or lower than 0.05 are in bold face print.

		LL	LW	LSI	LCC	LNC	SLA	LT	SLWW	SLDW	LM
LL	r	1.000	0.819***	0.491***	-0.067	-0.074	-0.391**	-0.465***	0.686***	0.712***	-0.213
	P		**<0.001**	**<0.001**	0.610	0.573	**0.002**	**<0.001**	**<0.001**	**<0.001**	0.102
LW	r		1.000	-0.094	-0.054	-0.080	-0.407**	-0.384**	0.680***	0.618***	-0.046
	P			0.474	0.682	0.543	**0.001**	**0.002**	**<0.001**	**<0.001**	0.726
LSI	r			1.000	-0.056	-0.029	-0.049	-0.232	0.163	0.281*	-0.267*
	P				0.673	0.824	0.709	0.075	0.214	**0.030**	**0.039**
LCC	r				1.000	0.957***	0.234	0.012	-0.191	-0.077	-0.162
	P					**<0.001**	0.073	0.928	0.144	0.559	0.215
LNC	r					1.000	0.238	0.031	-0.240	-0.122	-0.160
	P						0.068	0.814	0.064	0.353	0.222
SLA	r						1.000	0.114	-0.597***	-0.615***	0.177
	P							0.385	**<0.001**	**<0.001**	0.177
LT	r							1.000	-0.197	-0.256*	0.177
	P								0.132	**0.049**	0.175
SLWW	r								1.000	0.813***	0.103
	P									**<0.001**	0.433
SLDW	r									1.000	-0.492***
	P										**<0.001**
LM	r										1.000
	P										

Abbreviations have the same meanings as described in Table 1.

thickness of *O. fragrans* var. *aurantiacus* was minimal among the three varieties of *O. fragrans* (Table 2).

Relationship among leaf functional traits of the three varieties of *O. fragrans*

Correlation patterns among leaf functional traits of all the three varieties of *O. fragrans* were observed (Table 3). In particular, leaf length was positively correlated with leaf width, leaf shape index, and single-leaf wet and dry weights (Table 3, $P < 0.001$) but was negatively correlated with SLA and leaf thickness (Table 3, $P < 0.01$). Leaf width was positively correlated with single-leaf wet and dry weights (Table 3, $P < 0.001$) but was negatively correlated with SLA and leaf thickness (Table 3, $P < 0.01$). Leaf shape index was positively correlated with single-leaf dry weight (Table 3, $P < 0.05$) but was negatively correlated with leaf moisture (Table 3, $P < 0.05$). Leaf chlorophyll concentration was positively correlated with leaf N concentration (Table 3, $P < 0.001$). SLA was negatively correlated with single-leaf wet and dry weights (Table 3, $P < 0.001$). Leaf thickness was negatively correlated with single-leaf dry weight (Table 3, $P < 0.05$). Single-leaf dry weight was negatively correlated with leaf moisture (Table 3, $P < 0.001$).

Discussion

As three varieties of *O. fragrans* with different flower colors, *O. fragrans* var. *thunbergii*, *O. fragrans* var. *latifolius*, and *O. fragrans* var. *aurantiacus* display similar leaf functional traits in this study, such as leaf shape index, leaf chlorophyll concentration, and leaf moisture (Table 1). This may be due to that the three varieties belong to the same plant *O. fragrans*. Thus, the three varieties of *O. fragrans* exhibit the same characteristics. However, significant differences in some leaf functional traits among the three varieties of *O. fragrans* were also detected. In particular, leaf length, leaf width, and single-leaf wet and dry weights decreased significantly in the following order: *O. fragrans* var. *thunbergii*, *O. fragrans* var. *latifolius*, and *O. fragrans* var. *aurantiacus* (Table 1). Meanwhile, SLA of *O. fragrans* var. *aurantiacus* was also significantly higher than those of *O. fragrans* var. *thunbergii* and *O. fragrans* var. *latifolius* (Table 1). This implied that the material investment per unit area and per lamina of *O. fragrans* var. *aurantiacus* leaves was significantly lower than those of *O. fragrans* var. *thunbergii* and *O. fragrans* var. *latifolius* leaves to pay an utmost effort to increase light capture and use efficiency because of its low leaf size. This may also signal that leaf functional traits even vary among different varieties because they may suffer diverse environmental selection pressures (such as light and/or soil physicochemical properties) although most of samples of the three varieties of *O. fragrans* were present together in same sampled sites. Thus, one of the reasons causing the difference in flower colors of the three varieties of *O. fragrans* may be the difference in leaf functional traits among the three varieties of *O. fragrans* because most (or even all) of the compounds which form different colors in flower originates the leaves function (i.e. photosynthesis) and difference in leaf functional traits can trigger pronounced effects on the absorption and utilization of light, which is one of the most important ecological factors that affect plant growth [1,2], as well the metabolic components. To infer, the successfully ecological strategy of plants with different varieties of *O. fragrans* is to obtain an optimal trade-off between resource capture and conservation for them to gain more living resources and then gain a growth advantage.

Any functional trait that can enable plants to occupy a wide variety of environmental conditions and then contribute competitive advantage to a species in its habitat will be under natural selection pressure and may evolve. Hence, the phenotypic plasticity of the functional traits of species should be a probable target for selection [5,18]. The higher range of phenotypic plasticity of plants for any functional traits may play an essential role in their successful strategy [5,18]. This study showed that the plasticity indices of most leaf functional traits (except SLA and single-leaf wet weight) of *O. fragrans* var. *thunbergii* were higher than those of *O. fragrans* var. *latifolius* and *O. fragrans* var. *aurantiacus* (Table 1). While, the plasticity indices of SLA and single-leaf wet weight of *O. fragrans* var. *aurantiacus* were higher than those of *O. fragrans* var. *thunbergii* and *O. fragrans* var. *latifolius* (Table 1). We think that the higher range of phenotypic plasticity of leaf size, leaf shape index, leaf chlorophyll and N concentrations of *O. fragrans* var. *thunbergii* may gain an advantage in increasing resource (especially light) capture and use efficiency and the higher range of phenotypic plasticity of leaf thickness, single-leaf dry weight, and leaf moisture of *O. fragrans* var. *thunbergii* may help to obtain a more efficient control of water loss and nutrient deprivation. Meanwhile, the higher range of phenotypic plasticity of SLA and single-leaf wet weight of *O. fragrans* var. *aurantiacus* may enable it achieve an advantage in resource capture and growth rate. This may also imply that *O. fragrans* var. *aurantiacus* can possess the lower cost of leaf construction than the other two varieties of *O. fragrans* mainly via the enhanced phenotypic plasticity of SLA and single-leaf wet weight to adapt the changing environment.

Normally, leaves with higher SLAs pay less allocation of biomass into leaf construction to achieve high resource acquisition and use efficiency, and thereby exhibit lower leaf thickness and single-leaf wet and dry weights but higher leaf size and leaf moisture [5,11,18]. However, the result of this study indicates that SLA was negatively correlated with leaf size, leaf thickness and single-leaf wet and dry weights. This result is inconsistent with the findings of previous studies [5,11,18]. The negative relationship between SLA and leaf size, leaf thickness and single-leaf wet and dry weights may be attributed to the fact that species with larger leaves have diminishing returns on the biomass they invest in light capture and run-away selection for species with smaller leaves [19]. This revealed that leaves with higher size did not always appropriate low resource allocation on leaf construction and possess low SLA. Previous studies also have reported some inconsistent results of the correlations between leaf size and SLA, including positive [20,21], negative [8,19], unrelated [7,22], and/or variable among habitats [23]. This shows that there is species specificity for the relationship among leaf functional traits.

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