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Comparison of morphological and physiological characteristics in two phenotypes of a rare and endangered plant, *Begonia fimbristipula* Hance

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Abstract

The rare and endangered plant, *Begonia fimbristipula*, shows red and green phenotypes, differentiated by a coloration of the abaxial leaf surface. In this study, we compared morphological and physiological traits of both phenotypes. The results showed that the red phenotype contained a significantly higher chlorophyll content, closer arrangement of chloroplasts, and a more developed grana. In addition, the red phenotype transferred significantly more light energy into the electron transport during the photoreaction. Similarly, the maximum photosynthetic rate, instantaneous water-use and light-use efficiencies of the red *B. fimbristipula* were all significantly higher than those of the green individuals. The differentiation between these two phenotypes could be caused by their different survival strategies under the same conditions; epigenetic variations may be in some correlation with this kind of phenotype plasticity. Red *B. fimbristipula* has an advantage in resource acquisition and utilization and possesses a better self-protection mechanism against changes in environmental conditions, therefore, it might adapt better to global climate change compared to the green phenotype. Further studies on the possible epigenetic regulation of those phenotypic differentiations are needed.

Additional key words: anatomy; epigenetic; macronutrient; morphology; pigment.

Introduction

Plant phenotypes result from the interaction between genotype and environment. The phenotypes' different morphological or physiological characteristics form when they adjust to environmental stimuli (Bradshaw 1965). One genotype may show phenotypic plasticity by producing more than one phenotype when exposed to different environments (Davidson *et al*. 2011, Si *et al*. 2014). Studies on the phenotype and phenotypic plasticity of plants lead to new insight into plant adaptions to a specific environmental stimuli and corresponding mechanisms (Moriuchi and Winn 2005).

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Abbreviations: AQY – apparent quantum yield; Car – carotenoids; Chl – chlorophyll; F_0 – minimal fluorescence yield of the dark-adapted state; F_0' – minimal fluorescence yield of the light-adapted state; F_m – maximal fluorescence yield of the dark-adapted state; F_m' – maximal fluorescence yield of the light-adapted state; F_s – steady-state fluorescence yield; F_v/F_m – maximal quantum yield of PSII photochemistry; ILUE – instantaneous light-use efficiency; IWUE – instantaneous water-use efficiency; LA – leaf area; LCP – light-compensation point; LSP – light-saturation point; NPQ – nonphotochemical quenching; P_{max} – light-saturated net photosynthetic rate; P_N – net photosynthetic rate; q_P – photochemical fluorescence quenching coefficient; R_D – respiration rate; SEM – scanning electron microscope; SLA – specific leaf area.
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Research comparing morphological and physiological traits between phenotypes has been performed in a single plant with different leaf types and/or different plants of the same species that display phenotypic or ecotypic differentiation (Clausen *et al*. 1940, Titus and Sullivan 2003, Deng *et al*. 2012b, Kurepin *et al*. 2012). Differentiation of these traits are induced by environmental change or resource constraints in general, *e.g*., the leaves exhibit morphological and anatomical characteristics, such as stem elongation, larger specific leaf area, thinner leaf thickness, lower stomatal frequency, and regulation of the size, shape, and number of chloroplasts in order to adapt to conditions of low sunlight or water availability (Wells and Pigliucci 2000, Deng *et al*. 2012b, Kurepin *et al*. 2012). Such alterations allow the plant species to modify its physiological characteristics such as light compensation and saturation point or pigment content when habitat conditions change (Dalling *et al*. 2001, Nicotra *et al*. 2010, Deng *et al*. 2012a). Although the outcomes of such plasticity vary, the mechanisms can generally occur *via* gene expression or hormonal regulation when the plant receives appropriate environmental signals (Sultan 2000, Schlichting and Smith 2002). Thus, epigenetics has been used widely for the explanation of phenotypic plasticity across organisms (Mattick 2001, Boyko and Kovalchuk 2008). Such a modification (*e.g*., DNA methylation, histone modifications, and chromatin remodeling) can occur in response to environmental signals by up- or downregulation of gene expression without changing original DNA sequence (Wolffe and Matzke 1999, Zilberman and Henikoff 2005, Henderson and Jacobsen 2007, Chinnusamy and Zhu 2009, Li *et al*. 2014).

Begonia fimbristipula is a stemless perennial herb endemic to China (Fig. 1), belonging to the Begoniaceae family. It grows in warm, moist conditions and is found mainly in shady crevices of cliffs (Gu 1999, Shao *et al*. 2012). Both bulb and leaf tissues of *B. fimbristipula* can be used in traditional medicine. In addition, some soft drinks are made from *B. fimbristipula*, which taste slightly sour but delicious (Zhang and Li 1986, Xu *et al*. 2000). Besides containing a variety of medicinal and nutritive components, *B. fimbristipula* is also rich in red pigment, which has been suggested as a potential raw material for extraction of a natural pigment. Over the past two decades, *B. fimbristipula* has been assessed as an endangered species at low risk, due to overharvesting, habitat fragmentation, and a low reproductive rate (Xing 2005). Wild *B. fimbristipula* differentiates into two phenotypes, one having red leaves and the other green leaves, often co-occurring in one population. The ratio of two phenotypes is about 1:1 (Shao *et al*. 2013), and the difference between them is mainly reflected in its leaf blade underside color (Fig. 1). This perennial plant is aestivates, *i.e*., dormant in summer. Generally, each individual has about 2–3 leaves. Individual plants produce only one type of leaf at any given time and do not switch between the phenotypes in different years (Shao *et al.* 2013). Research on the anthocyanin content of leaves has demonstrated that its biosynthesis is controlled by the expression of structural and regulatory genes; *i.e*., the up- or downregulation of these genes is influenced by the accumulation of anthocyanin, with a subsequent effect on the colour of leaves, flower, fruits, and seeds (Holton and Cornish 1995, Kim *et al*. 2007). Currently, research on *B. fimbristipula* is mostly focused on tissue culture and pigment extraction, but little on the ecological differences between the two phenotypes.

In this study, we compared morphological and physiological traits, pigmentation, and macronutrient contents of red and green *B. fimbristipula*. Our objective was to provide a starting point toward a better understanding of how *B. fimbristipula* phenotypes with the same genetic origin adapt to their environment at the physiological and morphological levels. Specifically, we addressed the following questions: (*1*) What are the morphological differences between the two phenotypes? (*2*) What are the differences in physiological traits between the two phenotypes? (*3*) What are the differences in pigment and macronutrient contents between the two phenotypes? The results should improve our understanding of the ecology of *B. fimbristipula*, and could provide data-based framework for assessing the quality of *B. fimbristipula* used in traditional Chinese medicine.

Materials and methods

Study site is located in Dinghushan Nature Reserve (23°09'21"–23°11'30"N, 112°30'39"–112°33'41"E), Zhaoqing City, Guangdong Province, South China. The climate is typical lower subtropical monsoon. The mean annual temperature and precipitation of Dinghushan are 21.3°C and 1,927.3 mm, respectively, with a wet season from April to September and a dry season from October to next March. Selected individuals from the natural population of *B. fimbristipula* are located on moist cliff faces in the coniferous and broad-leaved mixed forest.

Plant material: *Begonia fimbristipula* is an aestivation plant, thus, all experiments were carried out during the period from 4–8 April 2014, when the individuals reached maximum of nutritional growth. Three populations were randomly chosen for this study, and three rigorous red and green *B. fimbristipula* individuals were randomly selected within each of these populations in order to measure the following parameters: light-response curves of photosynthesis, chlorophyll (Chl) fluorescence parameters, stomatal density, chloroplast ultrastructure, and pigment contents. After the light-response curves of photosynthesis and Chl fluorescence parameters were measured, the leaves were hand-harvested and cleaned to examine physiological traits, such as stomatal density, chloroplast ultrastructure, and pigment content, among others.

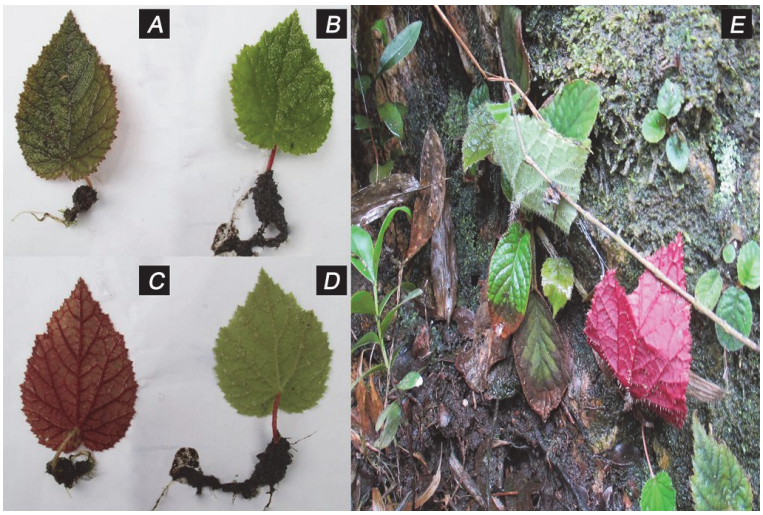

Fig. 1. The adaxial leaf side of the wild red (*A*) and green (*B*) *Begonia fimbristipula* phenotypes; the abaxial leaf side of red (*C*) and green (*D*) phenotypes. *(E)* red and green phenotypes in wild.

Anatomical and morphological traits: Photographs of nine fully expanded leaves were obtained using a digital camera (*IXUS 245, Cannon,* Japan) in order to calculate the leaf area (LA, cm^2) with *Image-J* software (Liu and Guan 2012). These leaves were oven-dried (65°C) to a constant mass and weighed, then the specific leaf area (SLA) was calculated as the ratio of leaf area to leaf dry mass [$cm^2\ g^{-1}$(DM)].

Leaf transverse section was identified by semithin sections. Samples from each of the nine leaves consisted of 0.5 × 1 cm blocks cut from the middle of each leaf and fixed with 4% glutaraldehyde mixed with 1% osmic acid buffer for 12 h at 4°C. The samples were then dehydrated with a series of alcohol mixtures, treated with epoxy propane and embedded in *EP 812* resin and sliced with a section-cutter (*LKB 118000*, *LKB*, Japan). The sections of leaf samples were examined and photographed with a microscope (*AX70*, *Olympus*, Tokyo, Japan).

Leaf epidermal structure was observed by scanning electron microscope (SEM; *JSM-6360LV*, *JEOL*, Japan). Additional 0.5 × 1 cm samples from each of the nine leaves were cut from the middle of each leaf and fixed with 4% glutaraldehyde for 12 h at 4°C, rinsed with 0.1 mol L^{-1} phosphate-saline buffer, dehydrated through an increasing alcohol series, then placed in a freeze-drying device (*JFD-310*, *JEOL*, Japan) after replacing the alcohol with tertiary butanol. The samples were vacuum dried before being coated with a sputter coater (*JFC-1600*, *JEOL*, Japan). They were subsequently observed and photographed with SEM (Donato and de Morretes 2013).

Chloroplast ultrastructure: The chloroplast ultrastructure of the nine leaves was observed with a transmission electron microscopy (*JEM-1010*, *JEOL*, Japan). Samples (0.5 × 1 cm) from the center of laminas were fixed with 4% glutaraldehyde, post-fixed with osmium acid, then dehydrated with a series of alcohol mixtures. Small pieces were embedded in *EP 812* resin, and the embedded samples were sectioned with an ultramicrotome (*ULPRACUT EXINXIN, Leica*, German) into slices (60 nm), and stained for detailed observation (Kordyum and Klimenko 2013).

Photosynthetic light-response curve: Three individuals each of red and green *B. fimbristipula* were randomly selected from three populations (n = 9). A photosynthetic light-response curve was measured under constant conditions (CO_2 concentration of 400 µmol $µmol^{-1}$; temperature of 25°C) with a portable photosynthesis system (*LI 6400*; *LI-COR*, Lincoln, NE, USA) on a sunny day. The photosynthetic capacity was measured at PPFD of 1,400; 1,200; 1,000; 800, 500, 300, 150, 100, 50, 30, 15, and 0 µmol $m^{-2}\ s^{-1}$. The light-saturated net photosynthetic rate (P_{max}), respiration rate (R_D), apparent quantum yield (AQY), light-compensation point (LCP), light-saturation point (LSP), instantaneous water-use efficiency (IWUE = P_N/E), and instantaneous light-use efficiency (ILUE = P_N/PAR) were calculated by using the nonrectangular hyperbola model of photosynthesis (Nijs *et al*. 1997, Ye and Yu 2008, Liu and Guan 2012).

Chl fluorescence: Chl fluorescence parameters of PSII were measured by a portable fluorescence spectrometer (*PAM-2100*, *Heinz Walz*, Effeltrich, Germany); parameters included the minimal fluorescence yield of the dark-adapted state (F_0), maximal fluorescence yield of the dark-adapted state (F_m), minimal fluorescence yield of the light-adapted state (F_0'), maximal fluorescence yield of the light-adapted state (F_m'), and steady-state fluorescence yield (F_s). The leaves were dark-adapted for 30 min in leaf clamps before measurement. F_0 and F_m were measured in the early morning before dawn, while other parameters were measured between 8:30–11:00 (Li *et al.* 2015). F_0 was measured under a light intensity of 0.5 µmol(photon) $m^{-2}\ s^{-1}$ and F_m was induced by 0.8-s pulse of saturating light (2,700 µmol $m^{-2}\ s^{-1}$ PPFD). The leaves were continuously irradiated with an actinic light of 138 µmol(photon) $m^{-2}\ s^{-1}$ for measurement of fluorescence

quenching components. F_m' was determined when the leaves were imposed to a second saturating pulse, and then the steady-state fluorescence F_s was recorded within 5 min. To measure F_0', the leaves were irradiated by a weak 5-s far-red light. The maximum quantum yield of PSII photochemistry ($F_v/F_m = (F_m - F_0)/F_m$), photochemical quenching coefficient [$q_P = (F_m' - F_s)/(F_m' - F_0')$], and nonphotochemical quenching [$NPQ = (F_m - F_m')/F_m'$] were calculated (Souza *et al*. 2004).

Leaf pigment content: Samples from the same nine leaves that had been tested for photosynthesis were used for measurement of the pigment content. To determine Chl and carotenoid (Car) contents, leaf discs (0.6 cm in diameter) were immersed in 80% acetone in a dark and cool place for five days. The light absorption of the extracted solution was measured at 663, 645, and 440 nm with an UV-visible spectrophotometer (*UV-3802*, *Unico,* China), and then the content of Chl *a*, Chl *b*, and Car was calculated (Lin *et al*. 1984).

To determine a proanthocyanidin content, the leaves were cut into pieces, and then extracted with pure methanol for 2 h using an ultrasonic bath at 40°C. The extraction solution was cooled and filtered (0.45 μm), then evaporated under vacuum using a rotavapor. Fifty milligrams of the extract was dissolved in 25 mL of distilled water. Then, 20 mL of ferric sulphate solution (77 mg of $FeSO_4 \cdot 7\ H_2O$ in 500 mL of HCl: *n*-buthanol = 2:3) was added to 2 mL of this solution and incubated at 95°C for 1 h. The light absorption of the extracted solution was measured at 540 nm using an UV-visible spectrophotometer (*UV-3802*, *Unico,* China), and the content of proanthocyanidins was calculated (Škerget *et al*. 2005).

In order to determine the anthocyanin content, the leaves were cut into small pieces, then extracted with methanol and HCl (99:1, v/v) at 4°C in the dark for five days. Absorption was measured at 530 and 650 nm with an UV-visible spectrophotometer (*UV-3802*, *Unico,* China), and then the content of anthocyanin was calculated (Murray and Hackett 1991, Reddy *et al*. 1995).

Macronutrient analysis: The leaf material was oven-dried (65°C) to a constant mass, then milled for determining carbon (C), nitrogen (N), and phosphorus (P) concentrations. The C concentration was determined by the potassium dichromate method (Dong 1996). After acid digestion, concentrations of N and P were determined by colorimetric analysis with an autoanalyzer (*H1-QC8000, Lachat,* USA) (Parkinson and Allen 1975). All the data were collected from five replicate leaves.

Statistical analysis: We performed *t*-tests (paired samples, two-tailed) to examine the differences between red and green *B. fimbristipula*. Differences at $p \leq 0.05$ were considered statistically significant. All statistical tests were performed using *SPSS 18.0* for *Windows* (*SPSS*, Chicago, IL, USA). Light-response curves were fitted using the nonlinear regression method (*SigmaPlot 10.0*, *Systat Software*, San Jose, CA), from which the P_{max}, R_D, AQY, LCP, LSP, ILUE, and IWUE were derived.

Results

Morphological and anatomical structure and chloroplast ultrastructure traits: Comparison of leaf morphological characteristics between the red and green phenotypes showed that there was no significant difference in LA, while the SLA of the green phenotype was significantly larger than that of the red phenotype (Table 1).

The thicknesses of the leaf, spongy tissue, and palisade of the green phenotype were significantly larger than that of the red phenotype (Table 1), but the palisade to spongy ratio did not differ. The leaf transverse section of the two genotypes showed clear differentiation in abaxial epidermis, palisade parenchyma, spongy parenchyma, and adaxial epidermis (Fig. 2). The mesophyll cells of red phenotype in spongy parenchyma were closely arranged, while in the green phenotype, they were loosely arranged and the cell clearances of palisade parenchyma were larger.

SEM micrographs showed that the adaxial and abaxial epidermic trichome density, as well as stomatal density of the red phenotype, were all significantly greater than that of the green phenotype (Table 1, Fig. 3). The stomata were found only on the abaxial side, not on the adaxial side. Epidermal structure of the red leaves was extremely uneven, with the edge of each adaxial epidermal cell sunken to form an intermediate convex structure (Fig. 3*A*,*E*,*G*), and an obvious depression of abaxial epidermal cells (Fig. 3*C*). In contrast, one part of the adaxial epidermal cells of the green leaves was smoothly upheaved (Fig. 3*F*), while the other parts were sunken similar to the red leaves (Fig. 3*F*,*H*); meanwhile, the abaxial epidermal cells were slightly concaved (Fig. 3*D*).

Ultrastructure analysis of chloroplast indicated that the number of chloroplasts within a cell of the green phenotype was larger than that of the red phenotype, but with a looser arrangement (Table 1, Fig. 4*A*,*B*). The chloroplasts of the red leaves were significantly greater than that of green leaves, which were arranged more compactly and closer to each other (Table 1, Fig. 4*A*,*C*,*D*). Concerning grana (Table 1, Fig. 4*E*,*F*), there was no significant difference in a grana thickness between the two phenotypes, but the red type had a significantly larger lamellae per granum and specific lamellae per μm compared with the green types, and the red type lamellae were well organized and aligned tightly.

Table 1. Comparisons in morphological, anatomical, and ultrastructural characteristics between red and green *Begonia fimbristipula*. Values are the means ± SE; $n = 9$ for leaf area and specific leaf area; leaf anatomical characteristics, for grana thickness, lamellae per granum and specific lamellae per μm. Means with *the same letter* are not significantly different ($p>0.05$).

	Parameter	Red	Green
Morphological characteristics	Leaf area [cm^2]	272.61 ± 21.32[a]	240.8 ± 19.28[a]
	Specific leaf area [$cm^2\ g^{-1}$]	69.81 ± 1.54[b]	104.84 ± 2.71[a]
Anatomical characteristics	Leaf thickness [μm]	169.76 ± 4.65[b]	247.36 ± 9.15[a]
	Adaxial epidermis thickness [μm]	51.9 ± 2.61[a]	50.55 ± 2.73[a]
	Palisade thickness [μm)	38.08 ± 0.97[b]	49.72 ± 1.47[a]
	Spongy thickness [μm]	29.32 ± 0.98[b]	41.86 ± 2.8[a]
	Palisade/spongy ratio	1.31 ± 0.05[a]	1.24 ± 0.12[a]
	Stomatal density [mm^{-2}]	47.92 ± 1.48[a]	29.07 ± 1.67[b]
	Adaxial epidermic trichome density [mm^{-2}]	6.10 ± 0.01[a]	2.82 ± 0.12[b]
	Abaxial epidermic trichome density [mm^{-2}]	3.39 ± 0.08[a]	1.07 ± 0.06[b]
Chloroplast ultrastructure	Chloroplast number	6.83 ± 0.47[b]	8.11 ± 0.45[a]
	Chloroplast size [μm^{-2}]	30.04 ± 1.63[a]	15.41 ± 1.21[b]
	Grana thickness [nm]	419.63 ± 44.1[a]	321.68 ± 20.59[a]
	Lamellae per granum	22.33 ± 2.29[a]	12.24 ± 1.09[b]
	Specific lamellae per μm	54.36 ± 1.27[a]	43.4 ± 4.51[b]

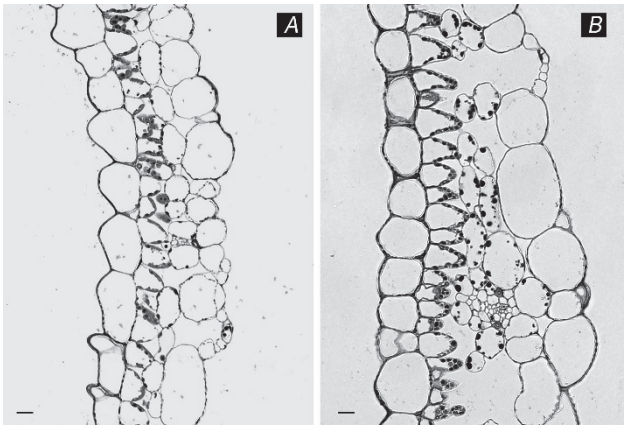

Fig. 2. Leaf anatomical structure of red (*A*) and green (*B*) *Begonia fimbristipula*. Bar – 20 μm.

Photosynthesis and Chl fluorescence: P_N of both phenotypes increased with increasing PPFD. The LSP was reached at approximately 550 μmol(photon) $m^{-2}\ s^{-1}$, then slowly declined with increasing PPFD, due to the effect of photoinhibition (Fig. 5). According to the parameters from the light-response curves (Table 2), P_{max}, IWUE, and ILUE of the red phenotype were significantly greater than those of the green phenotype, while the AQY of the red phenotype was lower than that of the green phenotype. The red phenotype had slightly higher R_D, LCP, and LSP, but the differences were not significant.

The Chl fluorescence parameters showed that F_v/F_m and NPQ did not differ significantly between the two phenotypes (Table 2) but the red phenotype had a significantly higher q_P.

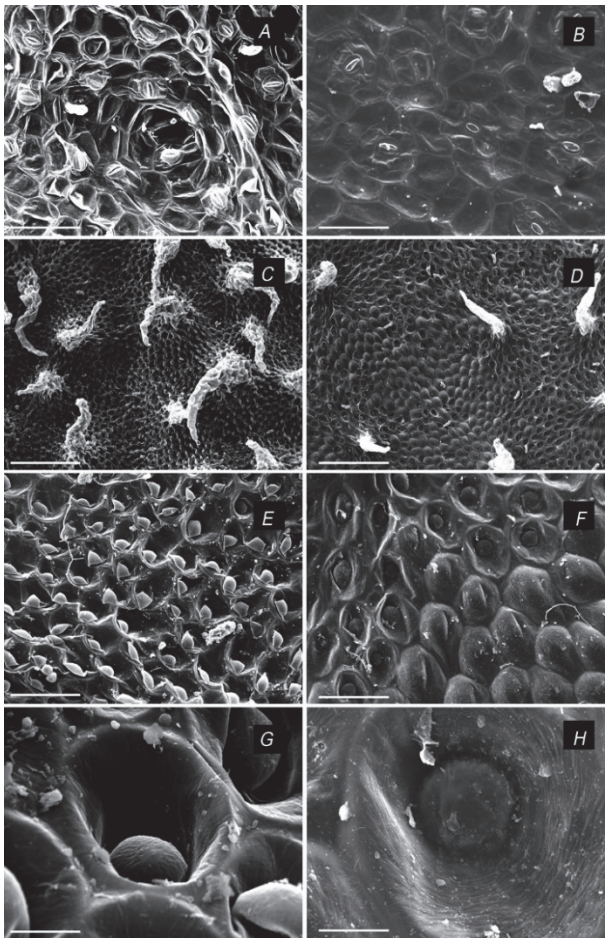

Fig. 3. SEM photomicrographs of leaf surfaces. Stomatal density of red (*A*) and green (*B*) phenotypes, bar – 100 μm; epidermic trichome density of red (*C*) and green (*D*) phenotypes, bar – 500 μm; the cell of adaxial epidermis of red (*E*, *G*) and green (*F*, *H*) phenotypes; bar – 100 μm for *E* and *F*, bar – 10 μm for *G,H*.

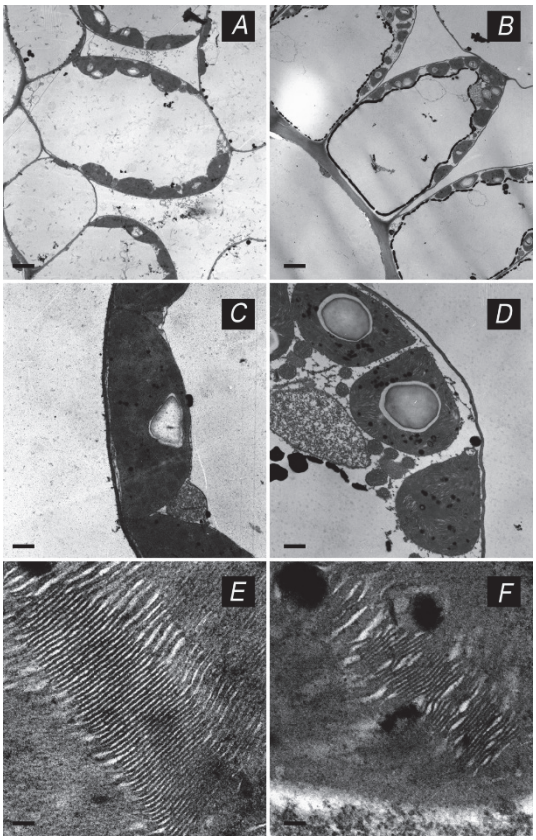

Fig. 4. Ultrastructure traits of chloroplasts in leaves of red and green *Begonia fimbristipula*. Mesophyll cell of red (*A*) and green (*B*) phenotypes; bar – 5 μm. Chloroplasts of red (*C*) and green (*D*) phenotypes, bar – 1 μm. Grana lamellae of red (*E*) and green (*F*) phenotypes, bar – 100 nm.

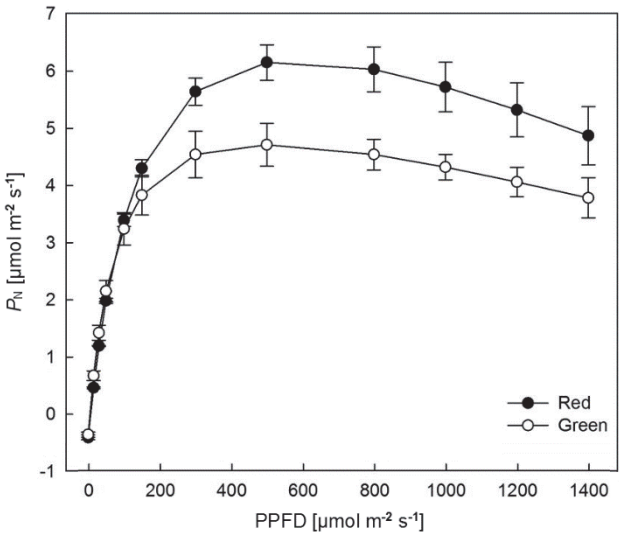

Fig. 5. Light-response curves of red and green *Begonia fimbristipula*. Values (means ± SD) are from nine leaves of red and green phenotypes.

Pigment and macronutrient contents: Chl *a* and Chl *b* contents of the red leaves were significantly higher than that of the green leaves (Table 3), but there was no difference in the content of Car. The anthocyanin content in the leaves of *B. fimbristipula* was significantly different between the red and green phenotypes; the green phenotype only possessed about 1.29% of anthocyanin in the red leaves, but the red phenotype had the markedly higher proanthocyanidin content (about 41 times) than that found in the green phenotype.

Quantification of the macronutrient contents showed that the C and N contents of *B. fimbristipula* were relatively high, while the P content was very low (Table 3). Although no difference in the C and P contents were found between the two phenotypes, the red phenotype had a significantly higher index of the N content, the N/P ratio.

Table 2. Comparisons in parameters from light-response curves, and chlorophyll fluorescence parameters between red and green *Begonia fimbristipula*. Values are the mean ± SE; $n = 9$. Means with *the same letter* are not significantly different ($p>0.05$). AQY – apparent quantum yield; F_v/F_m – maximal quantum yield of PSII photochemistry; ILUE – instantaneous light-use efficiency; IWUE – instantaneous water use efficiency; LCP – light-compensation point; LSP – light-saturation point; NPQ – nonphotochemical quenching; P_{max} – light-saturated net photosynthetic rate; q_P – photochemical fluorescence quenching; R_D – respiration rate.

Parameter	Red	Green
P_{max} [μmol m^{-2} s^{-1}]	6.18 ± 0.41^a	4.72 ± 0.26^b
R_D [μmol m^{-2} s^{-1}]	0.42 ± 0.05^a	0.38 ± 0.03^a
AQY [mol mol^{-1}]	0.06 ± 0.01^b	0.08 ± 0.01^a
LCP [μmol m^{-2} s^{-1}]	6.83 ± 0.63^a	4.87 ± 0.5^a
LSP [μmol m^{-2} s^{-1}]	587.81 ± 40.66^a	526.00 ± 51.96^a
ILUE [μmol mmol^{-1}]	6.21 ± 1.08^a	4.78 ± 0.53^b
IWUE [μmol mmol^{-1}]	17.00 ± 0.69^a	13.85 ± 0.65^b
F_v/F_m	0.79 ± 0.01^a	0.81 ± 0.01^a
q_P	0.50 ± 0.02^a	0.38 ± 0.02^b
NPQ	2.15 ± 0.21^a	2.17 ± 0.21^a

Table 3. Comparison in pigment and macronutrient contents in leaves between red and green *Begonia fimbristipula*. Values are the mean ± SE ($n = 9$ and $n = 5$ for the pigment and macronutrient content, respectively). Means with *the same letter* are not significantly different ($p>0.05$). Car – carotenoids; Chl – chlorophyll.

Parameters	Red	Green
Chl *a* [μg cm^{-2}]	18.29 ± 1.47^a	14.91 ± 0.64^b
Chl *b* [μg cm^{-2}]	8.64 ± 0.50^a	6.06 ± 0.08^b
Car [μg cm^{-2}]	3.61 ± 0.31^a	3.82 ± 0.32^a
Anthocyanin [μmol cm^{-2}]	0.0340 ± 0.0026^a	0.0002 ± 0.00004^b
Proanthocyanidins [mg cm^{-2}]	0.0057 ± 0.0006^a	0.0010 ± 0.0002^b
C [mg g^{-1}]	460.1 ± 15.8^a	403.2 ± 10.2^a
N [mg g^{-1}]	29.92 ± 0.44^a	23.51 ± 0.46^b
P [mg g^{-1}]	1.01 ± 0.03^a	0.92 ± 0.02^a
N/P	29.57 ± 0.82^a	25.61 ± 0.43^b

Discussion

Our research on the morphological structure, ultrastructure, and photosynthetic system parameters demonstrated that the red and green phenotypes both possessed typical shade-adaption characteristics, such as the larger LA and SLA, the thicker chloroplast grana, the lower LCP, LSP, and R_D, which are consistent with the other studies on plants grown under low irradiance (*e.g*., Dalling *et al*. 2001, Landhäusser and Lieffers 2001, Schulze *et al*. 2002, Joesting *et al*. 2009). Meanwhile, the N/P ratio of both phenotypes was greater than 16, suggesting that the growth of *B. fimbristipula* was limited by P (Koerselman and Meuleman 1996, Tessier and Raynal 2003).

Nevertheless, some important differences needed more attention. With respect to the leaf morphological and anatomical structure, the thinner leaf of the red phenotype showed more compactly arranged cells and smaller SLA than that of leaves in the green phenotype. Significant differences in these traits showed the two phenotypes of *B. fimbristipula* appeared to have different morphological and anatomical mechanisms for the shade adaptation (Abraham *et al*. 2014). With respect to the epidermal structure of leaves, stomata determine gas exchange and the water-transpiration rate of plants; thus the higher stomatal density in the red phenotype might contribute to water-use efficiency and regulation of gas exchange with a significantly higher IWUE and P_{max} (Sun *et al*. 2014), and the higher epidermal trichome density of red phenotype can reduce water evaporation. Compared with the green type, with more uneven epidermal structure, these traits may improve the drought and cold-tolerance capacity of the red phenotype (Moreira *et al*. 2012, Donato and Morretes 2013).

Modulating the number and size of chloroplasts is an important shade-adaptation strategy in plants (Deng *et al*. 2012a, Kordyum and Klimenko 2013). The red phenotype possessed much larger and more tightly arranged chloroplasts, while the green phenotype showed a greater number of chloroplasts, which further implied a different response to shade. The development of thylakoids plays a key role in photosynthesis since they are the site where photoreaction takes place (Allen and Forsberg 2001). Compared to the loosely arranged lamellae of the green phenotype, the much more tightly stacked grana and a higher number of grana lamellae in the red phenotype could result in more photosynthetic pigments and thus a significantly higher P_{max} and ILUE (Deng *et al*. 2012b, Shao *et al*. 2014), since the photosynthetic pigments in chloroplasts are vital for photosynthetic light energy capture, conversion, and dissipation.

The two phenotypes showed no difference in F_v/F_m, which represents the maximal light-harvesting capacity of PSII, suggesting that the two phenotypes did not differed in the potential capacity of photosynthesis (Kitajima and Butler 1975, Demmig-Adams and Adams 2006). However, the significantly higher q_P of the red phenotype implies that more energy was used in the electron transport during the photoreaction stage than that in the green type under the same conditions (Maxwell and Johnson 2000, Deng *et al*. 2012b). The large amount of anthocyanins in the leaves of the red phenotype might cause the red color of the blade. Lee and Collins (2001) reported that anthocyanins were commonly produced in abaxial surfaces of leaves of understory plants, and phylogeny influenced the distribution of anthocyanins in the epidermis and mesophyll of expanding leaves and the palisade parenchyma during senescence. Many *Begonia* species, including our study subject, are understory plants, and known to produce red leaves. Previous studies on anthocyanins also demonstrated that anthocyanins can significantly affect plant responses to environmental stress, protect organs and substances involved in photosynthesis processes, relieve photo-oxidation damage to leaves. Therefore, anthocyanins can greatly improve viability and resistance of plants (Lee 2002, Gould 2004, Hughes 2009, Pourcel 2013). Meanwhile, there is also evidence that anthocyanins are connected to the plant resistance to herbivorous insects (Coley and Aide 1989, Lee and Gould 2002, Cooney 2012). This trait is prominent in the performance of red leaves, since the red leaves are rarely eaten by insects in the field, while most of the green leaves are consumed during the same period. The phenotype differentiation of *B. fimbristipula* was a reflection of phenotype plasticity: the red phenotype modified its leaves, chloroplast and thylakoid structure in order to improve its P_{max}, ILUE and IWUE, while the structure and abundant anthocyanins contributed to its selfprotection from the biotic or abiotic stress. All these traits helped improve adaptation to the living conditions of *B. fimbristipula*.

Although gene expression or hormonal regulation may account for the phenotype differentiation, the exact mechanisms are still poorly known. Since the red and green *B. fimbristipula* occur in one population, we have got a better opportunity to understand their differentiation by epigenetic modification, including light, temperature, and nutrient stress regulating gene expression during anthocyanin biosynthesis (Yuan *et al*. 2009, Nicotra *et al*. 2010). Research on tissues of *B. fimbristipula* cultured under controlled conditions may further explain the epigenetic mechanisms for phenotypic differentiation (Zhang *et al*. 1985, Shao *et al*. 2012). The offsprings from tissue culture based on plants of wild red *B. fimbristipula* show some differences in their phenotype, *e.g*., the green phenotype, red phenotype, and a phenotype between the red and green leaf colors. Kubis *et al*. (2003) reported no difference in genetic constitution between oil plants derived from seeds and tissue culture, but some differences in a level of DNA methylation generated. More evidence on the relationship between the epigenetic regulation and the phenotype of tissue culture seedlings may reveal the

inner mechanism for phenotype differentiation such that in *B. fimbristipula*.

As it is difficult for plants to escape from their environments, where they currently grow, they must passively adapt to changing or even unfavourable conditions; therefore evolution can occur in the course of their long-term adaptation (Allis *et al*. 2007). *B. fimbristipula* usually grows in cliffs at relatively high altitude, where light, temperature, and other abiotic factors show a large daily variation. The differentiation produced the heritable red phenotype, which showed more flexibility to adapt to environmental changes. Subsequently, in the long run, the red *B. fimbristipula* could better maintain and multiply its populations during the long-term acclimation to abiotic environmental and biological factors.

Conclusion: Our results elucidated that both red and green phenotype exhibited similar shade-plant characteristics, but remarkable differences existed in their morphological structure, physiological traits, pigment and nutrient contents. These differences helped the red phenotype to utilize resource more effectively than the green phenotype under the same conditions. The red phenotype, with its higher tolerance to environmental conditions and higher acclimation ability, may help survive the the species under global climate change. The differentiated traits of red and green *B. fimbristipula* may also suggest the relevance of epigenetic modification without genetic change when facing environmental changes. Thus, there is an urgent need to focus research on the epigenetic aspects of plant biology in order to explore differentiation mechanisms of *B. fimbristipula*. Such work can also provide empirical and theoretical support for artificial cultivation and for better understanding survival strategies in other rare or endangered plants threatened by climate change.

References

Abraham E.M., Kyriazopoulos A.P., Parissi Z.M.: Growth, dry matter production, phenotypic plasticity, and nutritive value of three natural populations of *Dactylis glomerata* L. under various shading treatments. – Agroforest. Syst. **88**: 287-299, 2014.

Allen J.F., Forsberg J.: Molecular recognition in thylakoid structure and function. – Trends Plant Sci. **6**: 317-326, 2001.

Allis C.D., Jenuwein T., Reinberg D. *et al*. (ed.): Epigenetics. Pp. 408. Cold Spring Harbor Lab. Press, New York 2007.

Boyko A., Kovalchuk I.: Epigenetic control of plant stress. – Environ. Mol. Mutagen. **49**: 61-72, 2008.

Bradshaw A.D.: Evolutionary significance of phenotypic plasticity in plants. – Adv. Genet. **13**: 115-155, 1965.

Chinnusamy V., Zhu J.K.: Epigenetic regulation of stress responses in plants. – Curr. Opin. Plant. Biol. **12**: 133-139, 2009.

Clausen J., Keck D., Hiesey W.M.: Experimental Studies on the Nature of Species. I. Effect of Varied Environments on Western North American Plants. Pp. 132. Carnegie Inst. Washington, Washington DC 1940.

Coley P.D., Aide T.M.: Red coloration of tropical young leaves: a possible antifungal defence? – J. Trop. Ecol. **5**: 293-300, 1989.

Cooney L.J., van Klink J.W., Hughes N.M. *et al*.: Red leaf margins indicate increased polygodial content and function as visual signals to reduce herbivory in *Pseudowintera colorata*. – New Phytol. **194**: 488-497, 2012.

Dalling J.W., Winter K., Nason J.D. *et al*.: The unusual life history of *Alesis blackiana*: a shade-persistent pioneer tree? – Ecology **82**: 933-945, 2001.

Davidson A.M., Jennions M., Nicotra A.B.: Do invasive species show higher phenotypic plasticity than native species and, if so, is it adaptive? A meta-analysis. – Ecol. Lett. **14**: 419-431, 2011.

Demmig-Adams B., Adams W.W.: Photoprotection in an ecological context: the remarkable complexity of thermal energy dissipation. – New Phytol. **172**: 11-21, 2006.

Deng Y., Li C., Shao Q. *et al*.: Differential responses of double petal and multi petal jasmine to shading: I. Photosynthetic characteristics and chloroplast ultrastructure. – Plant Physiol. Bioch. **55**: 93-102, 2012a.

Deng Y., Shao Q., Li C. *et al*.: Differential responses of double petal and multi petal jasmine to shading: II. Morphology, anatomy and physiology. – Sci. Hortic.-Amsterdam **144**: 19-28, 2012b.

Donato A.M., de Morretes B.L.: *Plinia edulis* – leaf architecture and scanning electron micrographs. – Braz. J. Pharmacogn. **23**: 410-418, 2013.

Dong M.: [Survey, Observation and Analysis of Terrestrial Biocommunities]. Pp. 38. Chinese Standard Press, Beijing 1996. [In Chinese]

Gould K.S.: Nature's Swiss army knife: The diverse protective roles of anthocyanins in leaves. – J. Biomed. Biotechnol. **5**: 314-320, 2004.

Gu C.Z.: [Flora of China]. Pp. 194. Science Press, Beijing 1999. [In Chinese]

Henderson I.R., Jacobsen S.E.: Epigenetic inheritance in plants. – Nature **447**: 418-424, 2007.

Holton T.A., Cornish E.C.: Genetics and biochemistry of anthocyanin biosynthesis. – Plant Cell **7**: 1071-1083, 1995.

Hughes N. M.: The photoprotective role of anthocyanin pigment in leaf tissues. – Photosynthetica **46**: 637-640, 2009.

Joesting H.M., McCarthy B.C., Brown K.J.: Determining the shade tolerance of American chestnut using morphological and physiological leaf parameters. – Forest. Ecol. Manage. **257**: 280-286, 2009.

Kim B.G., Kim J.H., Min S.Y. *et al*.: Anthocyanin content in rice is related to expression levels of anthocyanin biosynthetic genes. – J. Plant Biol. **50**: 156-160, 2007.

Kitajima M., Butler W.L.: Quenching of chlorophyll fluorescence and primary photochemistry in chloroplasts by dibromothymoquinone. – Biochim. Biophys. Acta **376**: 105-115, 1975.

Koerselman W., Meuleman A.F.M.: The vegetation N:P ratio: a new tool to detect the nature of nutrient limitation. – J. Appl. Ecol. **33**: 1441-1450, 1996.

Kordyum E., Klimenko E.: Chloroplast ultrastructure and chlorophyll performance in the leaves of heterophyllous *Nuphar lutea* (L.) Smith plants. – Aquat. Bot. **110**: 84-91, 2013.

Kubis S.E., Castilho A.M., Vershinin A.V. *et al*.: Retroelements, transposons and methylation status in the genome of oil palm (*Elaeis guineensis*) and the relationship to somaclonal

variation. – Plant. Mol. Biol. **52**: 69-79, 2003.
Kurepin L.V., Farrow S., Walton L.J. *et al*.: Phenotypic plasticity of sun and shade ecotypes of *Stellaria longipes* in response to light quality signaling: Cytokinins. – Environ. Exp. Bot. **84**: 25-32, 2012.
Landhäusser S.M., Lieffers V.J.: Photosynthesis and carbon allocation of six boreal tree species grown in understory and open conditions. – Tree Physiol. **21**: 243-250, 2001.
Lee D.W.: Anthocyanins in leaves: distribution, phylogeny and development. – Adv. Bot. Res. **37**: 37-53, 2002.
Lee D.W., Collins T.M.: Phylogenetic and ontogenetic influences on the distribution of anthocyanins and betacyanins in leaves of tropical plants. – Int. J. Plant Sci. **162**: 1141-1153, 2001.
Lee D.W., Gould K.S.: Anthocyanins in leaves and other vegetative organs: An introduction. – Adv. Bot. Res. **37**: 1-16, 2002.
Li Q.Z., Li C.W., Yang T.W.: [Stress response and memory mediated by DNA methylation in plants.] – Plant Physiol. J. **50**: 725-734, 2014. [In Chinese]
Li H., Zhang G.C., Xie H.C., *et al*.: The effects of the phenol concentrations on photosynthetic parameters of *Salix babylonica* L. – Photosynthetica **53**: 430-435, 2015.
Lin Z.F., Li S.S., Lin G.Z., *et al*.: Superoxide dismutase activity and lipid peroxidation in relation to senescence of rice leaves. – Acta. Bot. Sin. **26**: 605-615, 1984.
Liu N., Guan L.: Linkages between woody plant proliferation dynamics and plant physiological traits in southwestern North America. – J. Plant. Ecol. **5**: 407-416, 2012.
Mattick J.S.: Non-coding RNAs: the architects of eukaryotic complexity. – EMBO Rep. **2**: 986-991, 2001.
Maxwell K., Johnson G.N.: Chlorophyll fluorescence – a practical guide. – J. Exp. Bot. **51**: 659-668, 2000.
Moreira N.S., Nascimento L.B.S., Leal-Casta M.V. *et al*.: Comparative anatomy of leaves of *Kalanchoe pinnata* and *K. crenata* in sun and shade conditions, as a support for their identification. – Braz. J. Pharmacogn. **22**: 929-936, 2012.
Moriuchi K.S., Winn A.A.: Relationships among growth, development and plastic response to environment quality in a perennial plant. – New Phytol. **166**: 149-158, 2005.
Murray J.R., Hackett W.P.: Dihydroflavonol reductase activity in relation to differential anthocyanin accumulation in juvenile and mature phase *Hedera helix* L. – Plant Physiol. **97**: 343-351, 1991.
Nicotra A.B. Atkin O.K., Bonser S.P. *et al*.: Plant phenotypic plasticity in a changing climate. – Trends Plant Sci. **15**: 684-692, 2010.
Nijs I., Ferris R., Blum H.: Stomatal regulation in a changing climate: a field study using free air temperature increase (FATI) and free air CO_2 enrichment (FACE). – Plant Cell. Environ. **20**: 1041-1050, 1997.
Parkinson J.A., Allen S.E.: A wet oxidation procedure suitable for the determination of nitrogen and mineral nutrients in biological materials. – Commun. Soil Sci. Plant **6**: 1-11. 1975.
Pourcel L., Irani N.G., Koo A.J.K. *et al*.: A chemical complementation approach reveals genes and interactions of flavonoids with other pathways. – Plant J. **74**: 383-397, 2013.
Reddy V.S., Dash S., Reddy A.R.: Anthocyanin pathway in rice (*Oryza sativa* L.): identification of a mutant showing dominant inhibition of anthocyanins in leaf and accumulation of proanthocyanins in pericarp. – Theor. Appl. Genet. **91**: 301-312, 1995.
Schlichting C.D., Smith H.: Phenotypic plasticity: linking molecular mechanisms with evolutionary outcomes. – Evol. Ecol. **16**: 189-211, 2002.
Schulze E.D., Beck E., Müller-Hohenstein K.: Plant Ecology. Pp. 118. Springer-Verlag, Berlin 2002.
Shao L., Liang K., Cheng X.W. *et al*.: [Effect of sucrose on growth of tissue culture seedling and anthocyanin accumulation in *Begonia fimbristipula* Hance on Dinghushan Mountain]. – Chin. Agr. Sci. Bull. **28**: 242-246, 2012. [In Chinese]
Shao L., Liang K., Cheng X.W., *et al*.: [Natural growth characteristics of *Begonia fimbristipula* Hance population in different habitats of Dinghu Mountain]. – Guangdong Agr. Sci. **40**: 42-46, 2013. [In Chinese]
Shao Q.S., Wang H.Z., Guo H.P. *et al*.: Effects of shade treatments on photosynthetic characteristics, chloroplast ultrastructure, and physiology of *Anoectochilus roxburghii*. – PLOS One. **9**: e85996, 2014.
Si C.C., Dai Z.C., Lin Y. *et al*.: Local adaptation and phenotypic plasticity both occurred in *Wedelia trilobata* invasion across a tropical island. – Biol. Invasions **16**: 2323-2337, 2014.
Škerget M., Kotnik P., Hadolin M. *et al*.: Phenols, proanthocyanidins, flavones and flavonols in some plant materials and their antioxidant activities. – Food. Chem. **89**: 191-198, 2005.
Souza R.P., Machado E.C., Silva J.A.B. *et al*.: Photosynthetic gas exchange, chlorophyll fluorescence and some associated metabolic changes in cowpea (*Vigna unguiculata*) during water stress and recovery. – Environ. Exp. Bot. **51**: 45-56, 2004.
Sultan S.E.: Phenotypic plasticity for plant development, function and life history. – Trends Plant Sci. **5**: 537-542, 2000.
Sun Y.Q., Yan F., Cui X.Y. *et al*.: Plasticity in stomatal size and density of potato leaves under different irrigation and phosphorus regimes. – J. Plant Physiol. **171**: 1248-1255, 2014.
Tessier J.T., Raynal D.J.: Use of nitrogen to phosphorus ratios in plant tissue as an indicator of nutrient limitation and nitrogen saturation. – J. Appl. Ecol. **40**: 523-534, 2003.
Titus J., Sullivan P.G.: Heterophylly in the yellow waterlily, *Nuphar variegate* (Nymphaeaceae): effects of [CO_2], natural sediment type, and water depth. – Am. J. Bot. **88**: 1469-1478, 2001.
Wells C.L., Pigliucci M.: Adaptive phenotypic plasticity: the case of heterophylly in aquatic plants. – Perspect. Plant Ecol. **3**: 1-18, 2000.
Wolffe A.P., Matzke M.A.: Epigenetics: regulation through repression. – Science **286**: 481-486, 1999.
Xing F.W.: [Rare plants of China]. Pp. 129. Hunan Educ. Publ. House, Changsha 2005. [In Chinese]
Xu X., Lu Z., Luo Y.F.: [Determination of trace elements in different graded leaves in *Begonia fimbristipula* Hance]. – Guangdong. Trace. Elem. Sci. **4**: 55-57, 2000. [In Chinese]
Ye Z.P., Yu Q.: A coupled model of stomatal conductance and photosynthesis for winter wheat. – Photosynthetica **46**: 637-640, 2008.
Yuan Y.Y., Chiu L.W., Li L.: Transcriptional regulation of anthocyanin biosynthesis in red cabbage. – Planta **230**: 1141-1153, 2009.
Zhang L.Y., Li G.G.: [The factors affecting anthocyanin content in plantlets of *Begonia fimbristipula* in vitro II]. – Acta. Bot. Yunnanica **8**: 60-66, 1986. [In Chinese]
Zhang L.Y., Li G.G., Guo J.Y.: [Some factors affecting the anthocyanin content in plantlets of *Begonia fimbristipula* in vitro I]. – Acta Bot. Yunnanica **7**: 195-202, 1985. [In Chinese]
Zilberman D., Henikoff S.: Epigenetic inheritance in *Arabidopsis*: selective silence. – Curr. Opin. Genet. Dev. **15**: 557-562, 2005