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Effects of hybridization on the determinants of photosynthetic capacity in *Buddleja* F1 hybrids

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Abstract

Photosynthetic capacity plays an important role in plant vegetative growth, and is often regarded as a key determinant of adaptability in plant species, including the hybrids. Interspecific hybridizations are common and can easily occur in *Buddleja*. The F1 hybrid investigated in this study is a newly discovered interspecific hybrid between *B. crispa* and *B. officinalis*, and it was found in the Sino-Himalayan region. In this study, the morphological traits, the stoichiometric characteristics and the gas-exchange traits in F1 hybrids and their parents were measured under the cultivation conditions. *Buddleja* F1 hybrids showed the high leaf mass per area, which was similar to *B. officinalis*. Although F1 hybrids presented a low light-saturated net photosynthetic rate, they did not reduced carbon cost by increasing the specific leaf area or decreasing the leaf dry mass per unit area. Compared to the parental species, F1 hybrids had low leaf C:N and C:P ratios, C concentration, as well as photosynthetic nitrogen-use efficiency. However, they had a great respiration efficiency through a markedly reduced rate of respiration. Furthermore, F1 hybrids showed similar photochemical efficiency to *B. officinalis*, which was significantly higher than that in *B. crispa*. These findings suggest that the F1 hybrids in our study show a high similarity to their parental species in the leaf economic spectrum and photosynthetic capacity.

Additional key words: chlorophyll fluorescence; effective quantum yield of PSII; light-saturated net photosynthetic rate; maximum photochemical efficiency of PSII; nonmetric multi-dimensional scaling.

Introduction

Natural hybridization and gene introgression are widespread in angiosperms. Hybridization is a potentially creative evolutionary process and can generate adaptive variation (Martin *et al*. 2006, Ma *et al*. 2010, 2014). Species-specific morphological or physiological traits can help plant species to adapt to different ecological environments (Moriuchi and Winn 2005).

Natural hybrids usually present unique combinations of morphological characters that can differ from those of their parents (Ma *et al*. 2010, 2014). These hybrids often have morphologies that are intermediate between those of their parents, and the hybrids may also have different ecological fitness (Martin *et al*. 2006, Ma *et al*. 2010, 2014). For example, certain hybrids of *Cucumis* species showed divergent phenotypes from the parents in response to low-light conditions (Yu *et al*. 2015). Furthermore, hybrids of *Sphagneticola trilobata* × *calendulacea* had invasive potentials that lay between the invasive potentials of the parents in shade and open environments (Li *et al*. 2016).

Hybrid plants may also form extreme phenotypes (transgressive segregation), producing new hybrid traits that can allow individuals to occupy ecological niches that neither of the parents can populate, rather than occupying

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Abbreviations: AQY – apparent quantum yield; Chl – chlorophyll; ETR – electron transport rate; F_m – maximum fluorescence yield in dark-adapted state; F_m' – maximum fluorescence yield in light-adapted state; F_0 – minimum fluorescence yield in dark-adapted state; F_0' – minimum fluorescence yield in light-adapted state; F_s – steady-state fluorescence yield; F_v/F_m – maximum photochemical efficiency of PSII; F_v'/F_m' – photochemical efficiency of PSII in the light; IRGA – infrared gas analyzer; LA – leaf area; LCP – light-compensation point; LDMC – leaf dry mass content; LES – leaf economic spectrum; LMA – leaf mass per area; LSP – light-saturation point; NMDS – non-metric multidimensional scaling; NPQ – nonphotochemical quenching; PCA – principal component analysis; P_N – net photosynthetic rate; P_{Nmax} – light-saturated net photosynthetic rate; PNUE – photosynthetic nitrogen-use efficiency; q_p – photochemical quenching; R_D – rate of respiration; R_E – respiration efficiency; SLA – specific leaf area; Φ_{PSII} – effective quantum yield of photosystem II.

Acknowledgements: We gained permission to collect and conserve F1 hybrids and their parental species in the experimental base of Honghe University, and each species was identified by Dr. Rongli Liao. Vouchers specimens of each species were deposited at the Herbarium of the College of Life Science and Technology, Honghe University. We thank Fuqiong Bai and Yanmei Luo for their help measuring the photosynthetic and chlorophyll fluorescence attributes at Honghe University. This work was supported by the Natural Science Foundation of China (Grant No. NSFC – 31660111) and Honghe University (Grant No. XJ14Z01, XJ15B17, and XJ16B05). The funding bodies were not involved in the design of the study, collection, analysis or interpretation of data, or in the writing of the manuscript.

niches that overlap with those of the parent species (Goulet *et al*. 2017). Thus, studies on the physiological and biochemical characters of plants, in particular those of hybrids, can give new insights into adaptation, and evolution of plant species.

Photosynthesis (the process by which plants and other organisms convert light energy into chemical energy), is a complex biophysical and chemical process (Douthe *et al*. 2018). In most plants, photosynthesis occurs in the leaves, which are in these cases specialized for photosynthesis (Douthe *et al*. 2018), and which are sensitive to environmental changes (Esau 1977). Moreover, chloroplasts, which are present in different concentrations in different angiosperm species, are the organelles responsible for the process of photosynthesis, and can influence the rates of photosynthesis in these plant species (Mathur *et al*. 2018). Effective photosynthesis is closely related to plant growth and increased carbohydrate accumulation (Geiger and Servaites 1994). Furthermore, high photosynthetic capacity, which has been used as a key determinant of plant adaptability, often results in strong vegetative growth (Fullana-Pericàs *et al*. 2017, Cao *et al*. 2018).

However, plant growth is not only closely related to photosynthetic capacity, but is also limited by nutrient stoichiometry [*e.g.*, nitrogen (N) and phosphorus (P) concentrations] in living cells (Güsewell 2004, Niklas *et al*. 2005, Sardans and Peñuelas 2012). Leaf N is closely related to photosynthetic capacity, while P predominately drives the generation and maintenance of proteins (Tian *et al*. 2018). In vascular plants, the plant growth rate is not only limited by the concentrations of leaf N and P, but also their ratios to each other (Güsewell 2004, Niklas *et al*. 2005, Tian *et al*. 2018). The N:P ratio < 14 usually indicates N limitation, while the ratio > 16 suggests P limitation (Koerselman and Meuleman 1996, Güsewell 2004).

Buddleja (Scrophulariaceae) species often present diverse phenotypes because of polyploidy or hybridization (Norman 2000, Chen *et al*. 2007, Liao *et al*. 2015). Polyploidy is recognized as a powerful force in phenotype diversification, and may be one of the most important mechanisms in plant evolution and rapid speciation (Li *et al*. 1996, Chen *et al*. 2007). However, interspecific hybridizations between *Buddleja* species are common and can easily occur (Norman 2000, Chen *et al*. 2007, Liao *et al*. 2015). For example, Liao *et al*. (2015) identified the natural hybridization and asymmetric introgression between *B. crispa* Benth. and *B. officinalis* Maxim. in Yunnan province. This is one of the most recently identified natural hybridizations in *Buddleja* (Liao *et al*. 2015) and is therefore an interesting model to use in the study of interspecific hybridization effects and adaptive mechanisms in *Buddleja*.

In the fields, *B. crispa* and *B. officinalis* and their F1 hybrids show different morphological traits, especially in leaf morphology. *Buddleja crispa* usually has cordate stipules, and the petiole is often winged. Leaf blades are ovate to triangular with white tomentum (Li and Leeuwenberg 1996). The leaves of *B. officinalis* are ovate to elliptic and yellow-tomentose on the lower leaf epidermis, and they usually lack stipules (Li and Leeuwenberg 1996, Liao *et al*. 2015). In the case of F1 hybrids, they present cordate stipules leaves, and the petiole is often winged (personal observation). Moreover, the leaves have ovate to elliptic and densely white-tomentose on both surfaces of young leaves, or on the lower leaf surface in mature leaves (Liao *et al*. 2015). Interestingly, the hybrids prefer slightly different habitats to their parent species, though the habitats of parental species and hybrids overlap in the fields. In the natural hybrid zones (*e.g.*, Xishan Mountain, Yimen, Cangshan Mountain), *B. crispa* prefers dry cliffs with more rocks and *B. officinalis* prefers open lands. But, the F1 hybrids prefer scrubland or understory environments (personal observation).

Asian *Buddleja* taxa are thought to have originated in the Sino-Himalayan region, and over 85% of the Asian *Buddleja* species have a distribution in this area (Li *et al*. 1996, Norman 2000, Nie *et al*. 2005). Until recently, though researchers have focused on the pharmaceutical value, pollination ecology and genetic diversity of *Buddleja* species, the studies on plant physioecological properties and adaptation were limited (Feng *et al.* 2007). Because most of a plant's energy and carbohydrates necessary for growth are produced by photosynthesis, photosynthetic capacity is an important determinant of plant adaptability, and photosynthetic characteristics are frequently used as indicators of the adaptability and resistance of plants (Fullana-Pericàs *et al*. 2017). To better understand the physiological performances of *Buddleja* F1 hybrids, in the present study, we explore the effects of hybridization on the leaf economic spectrum (LES) and photochemical characteristics in F1 hybrids and their parents when cultivated under common garden conditions.

Materials and methods

Plant material: In this study, the parental species *Buddleja crispa* and *B. officinalis*, and their F1 hybrids [marked and used in the experiment of Liao *et al*. (2015)] were transplanted into the experimental base at Honghe University between 25–30 August, 2018 (Fig. 1). Five individuals of each parental taxon and five F1 hybrids were introduced from Xishan Mountain (N24°57'52.59", E102°37'33.58"; 1,985 m a.s.l.). The plants were cultivated at Honghe University in an environment that was relatively moist and shaded, which is closer to the natural habitat of the F1 hybrids than to that of either parent. After cultivation for six months at Honghe University, photosynthetic characteristics were evaluated. This work was carried out between 21 February–28 March, 2019. The mature leaves from annual shoots were marked and used for testing.

Leaf trait: All selected mature leaves were submerged in water in the dark for ~ 12 h. Each leaf was then dried and weighed to give the leaf saturated fresh mass, following Shah *et al.* (2017). Six replicates for each taxon were performed. Fresh leaves were also scanned, and digitized by using *Image J*, and leaf area (LA) was calculated.

All the leaves were then marked and oven-dried at 105°C for 5 min, then dried to constant mass at 70°C over

Fig. 1. Plant habitats in the fields (*A*,*E*,*I*), plant growth under cultivation (*B*,*F*,*J*), leaf morphologies (*C*,*G*,*K*), and leaf cross-sections (*D*,*H*,*L*) of *Buddleja crispa* (*A–D*), F1 hybrids (*E–H*), and *Buddleja officinalis* (*I–L*).

three days following Shah *et al.* (2017). The dry mass of each leaf was then separately recorded. Specific leaf area (SLA), leaf mass per area (LMA), and leaf dry mass content (LDMC) were further calculated (Poorter and Remkes 1990, Reich *et al*. 1991, Witkowski and Lamont 1991, Vile *et al*. 2005).

Chlorophyll (Chl) content: Chl was extracted by using N,N-dimethylformamide following Inskeep and Bloom (1985). An appropriate sample of material (0.5 cm^2, fresh mass) of each species was punched from fresh leaves (avoiding major leaf veins where possible) through a circular leaf disk puncher (5 mm in diameter). Six 0.5-cm^2 leaf disks from six different leaves from each taxon were sampled, and immersed in N,N-dimethylformamide for ~ 12 h in the dark. The samples were then centrifuged at 1,000 rpm for 10 min, the supernatants were collected and the absorption values were recorded using an ultraviolet-visible spectrophotometer (*UV 5100B*, *Shanghai Metash Instrument Co.*, *Ltd.*, Shanghai, China) at both 664.5 and 647 nm. Three repetitions were made for each sample. For each taxon, the experiment was replicated three times, giving a total of 3 × 3 = 9 samples from each taxon. Chl concentration was then calculated as follows (Inskeep and Bloom 1985): Chl a = 12.70 $A_{664.5}$ – 2.79 A_{647}, Chl b = 20.70 A_{647} – 4.62 $A_{664.5}$, total Chl = 17.90 A_{647} + 8.08 $A_{664.5}$.

Leaf C, N, and P stoichiometry: Five dried leaves from different samples were selected in each taxon and ground to fine power and passed through a 0.15-mm sieve for C, N, and P analysis. The C concentration of the plant samples was analyzed using the Walkley-Black modified acid-dichromate $FeSO_4$ titration method (Fan *et al*. 2015). The total N content of each of the plant samples was determined following micro-Kjeldahl digestion using a flow injection autoanalyzer (*Smartchem 200*, *AMS*, Italy). The total P content was analyzed colorimetrically (*Smartchem 200*, *AMS*, Italy) using the ammonium molybdate method (Fan *et al*. 2015). Three repetitions were made for each sample. For each taxon, the experiment was replicated three times, giving a total of 3 × 3 = 9 samples from each taxon. Plant C, N, and P contents were expressed as g kg^{-1} on a dry mass basis. Stoichiometric ratios in leaves were also calculated on a mass basis.

Leaf gas exchange: PAR–net photosynthetic rate (P_N) response curves were evaluated between 8:30 and 11:30 h using a *Li-6400XT* (*Li-Cor Inc.*, USA) portable photosynthesis infrared gas analyzer (IRGA) system. The environmental CO_2 concentration was used and a 6 (2 × 3 cm)-cm^2 cuvette was fitted with a red-blue light source (*6400-02B*). Different PPFD gradients, including 2,000; 1,800; 1,500; 1,200; 800, 500, 200, 100, 80, 50, 20, 10, 0 μmol(photon) m^{-2} s^{-1} were designed. The 7–8th mature leaves from the tops of twigs were used, and five repetitions were done for each taxon.

The Chl fluorescence parameters were recorded by using a *Li-6400XT* (*Li-Cor Inc.*, USA) portable photosynthesis system with a fluorescent leaf chamber. After dark-adaption for 30 min, F_0 and F_m were recorded. The following day (9:00–11:00 h), the Chl a fluorescence traits of the same leaf were further evaluated. An actinic light pulse [1,200 μmol(photon) m^{-2} s^{-1}] was first applied, and the maximum fluorescence yield (F_m') was recorded under a saturating pulse of 5,000 μmol(photon) m^{-2} s^{-1}. The minimum fluorescence yield in the light-adapted state (F_0'), the maximum fluorescence yield in the light-adapted state (F_m'), and the steady-state fluorescence yield (F_s) were recorded.

Following this, the maximum photochemical efficiency of PSII [F_v/F_m; $F_v/F_m = (F_m - F_0)/F_m$], the photochemical efficiency of PSII in the light (F_v'/F_m'), the electron transport rate (ETR), the effective quantum yield [Φ_{PSII}; $\Phi_{PSII} = (F_m' - F_s)/F_m'$)], and the photochemical [q_P; $q_P = 1 - (F_s - F_0')/(F_m' - F_0')$], and nonphotochemical quenching [NPQ; $NPQ = (F_m - F_m')/F_m'$] were calculated (Redondo-Gómez *et al.* 2006, Ware *et al.* 2015, Lima *et al.* 2018).

Data analysis: The parameters of the light-response curve, including the light-saturated net photosynthetic rate (P_{Nmax}), the apparent quantum yield (AQY; slope of the linear portion of the light response curve), the light-compensation point (LCP), the light-saturation point (LSP), and the rate of respiration (R_D) were fitted to the curve using a modified model with nonrectangular hyperbola following Ye (2007).

Then, respiration efficiency (R_E) was evaluated following Feng *et al.* (2007). It was calculated as $R_E = P_{Nmax}/R_D$, where P_{Nmax} is the maximum photosynthetic rate of the plant species, and R_D is the dark respiration rate.

Additionally, the photosynthetic nitrogen-use efficiency (PNUE) was evaluated following Poorter and Evans (1998). It was calculated as $PNUE = P_{Nmax}/(1/14N_{mass} \times LMA)$, where P_{Nmax} is the maximum photosynthetic rate of the plant species, N_{mass} is the leaf nitrogen content per unit mass, and LMA is the leaf dry mass per unit area.

To characterize leaf trait dissimilarities between the three *Buddleja* taxa, a non-metric multidimensional scaling (NMDS) ordination based on a matrix of Chord distances was performed in *PAST* (*version 2.0*). The dissimilarity of leaf traits was further quantified through one-way *SIMPER* analysis in *PAST*. The contributions of each studied parameter to the total variations from each species were estimated by using principal component analysis (PCA) in *PAST version 2.0*. A variance–covariance matrix of parameters was used. Prior to NMDS and PCA analysis, all the data were standardized by using $\log_{10}$-transformation.

One-way analysis of variance (*ANOVA*) was performed to explore differences between the hybrids and parental species. *PAST version 2.0* was used for the statistical analysis. Specific differences between pairs of means were calculated using *Duncan*'s Multiple Range Tests with $P<0.05$. Figures were generated in *SigmaPlot version 14.0* (*Systat Software*, San Jose, CA), and were adjusted and assembled using *Adobe Illustrator CS4* (*Adobe Systems*, San Jose, CA).

Results

Plant growth: All the *Buddleja* individuals in our experiment performed well six months after transplantation (Fig. 1*B*,*F*,*J*). Both *B. crispa* and *B. officinalis* and their F1 hybrids presented similar LA, ranging from 93.21 ± 4.38 to 109.15 ± 8.97 cm^2 with no significant differences (Table 1). F1 hybrids showed high LDMC (0.25 ± 0.05 g g^{-1}), and no significant differences were found compared to their parental species. But, there were significant differences between the two parental species (Table 1). F1 hybrids also showed equal levels of SLA and LMA to *B. officinalis* with no significant differences (Table 1). However, *B. crispa* had the largest SLA (19.89 ± 0.80 m^2 kg^{-1}) and the lowest LMA (50.71 ± 2.18 g m^{-2}; Table 1).

Chl content: The Chl contents of the two parental *Buddleja* species and their F1 hybrids are presented in Table 1. F1 hybrids presented the largest Chl *a* and total Chl contents (8.02 ± 0.26 and 10.41 ± 0.56 mg g^{-1}, respectively) with significant differences (Table 1). *Buddleja crispa* had the lowest Chl *a*, Chl *b*, and total Chl concentrations (6.28 ± 0.23, 1.74 ± 0.11, and 8.02 ± 0.34 mg g^{-1}; Table 1). However, *B. crispa* and the F1 hybrids showed the largest Chl *a*/*b* ratios with no significant differences, 3.61 ± 0.11 and 3.72 ± 0.09, respectively (Table 1). *Buddleja officinalis* had the lowest Chl *a*/*b* ratio, 0.93 ± 0.24 (Table 1).

Leaf C, N, P stoichiometry: There were significant differences of leaf C, N, and P stoichiometry between the studied taxa (Fig. 2). F1 hybrids presented the lowest foliar C concentration (606.00 ± 3.46 mg g^{-1}) compared to the parental species (644.00 ± 2.00–706.00 ± 2.00 mg·g^{-1}; Fig. 2*A*). However, they accumulated relatively higher concentrations of foliar N and P, 29.68 ± 0.03 and 1.58 ± 0.00 mg g^{-1}, respectively (Fig. 2*B*,*C*). Meanwhile, F1 hybrids showed the lowest C:N and C:P ratios, 20.42 ± 0.14 and 381.76 ± 1.90, respectively (Fig. 2*D*,*E*). *Buddleja officinalis* showed the largest C:N and C:P ratios, 31.46 ± 0.12 and 653.05 ± 7.87, respectively (Fig. 2*D*,*E*). In addition, both parental *Buddleja* species and their F1 hybrids showed large N:P ratios (16.63 ± 0.04 to 20.67 ± 0.09) with significant differences (Fig. 2*F*).

Photosynthetic response to light: All the three *Buddleja* taxa showed the similar LSP [963.83 ± 81.23–1,098.89 ± 27.77 μmol(photon) m^{-2} s^{-1}] and LCP [23.31 ± 1.32–24.82 ± 2.21 μmol(photon) m^{-2} s^{-1}] with no significant differences (Table 2). However, F1 hybrids showed lower AQY [0.06 ± 0.001 μmol(CO$_2$) μmol^{-1}(photon)], P_{Nmax} [9.19 ± 0.41 μmol(CO$_2$) m^{-2} s^{-1}], and R_D [1.14 ± 0.05

Table 1. Leaf growth characteristics (mean ± SE, *n* = 6) of *Buddleja* F1 hybrids and their parental species. *Different letters* indicate significant differences between different taxa according to LSD tests ($p<0.05$). LA – leaf area, SLA – specific leaf area, LDMC – leaf dry mass content, LMA – leaf mass per area, Chl – chlorophyll.

Species	LA [cm^2]	SLA [m^2 kg^{-1}]	LDMC [g g^{-1}]	LMA [g m^{-2}]	Chl *a* [mg g^{-1}(FM)]	Chl *b* [mg g^{-1}(FM)]	Total Chl [mg g^{-1}(FM)]	Chl *a*/*b*
B. crispa	109.15 ± 8.97^a	19.89 ± 0.80^a	0.18 ± 0.01^b	50.71 ± 2.18^b	6.28 ± 0.23^b	1.74 ± 0.11^b	8.02 ± 0.34^b	3.61 ± 0.11^a
F1 hybrids	103.51 ± 9.93^a	15.67 ± 0.96^b	0.25 ± 0.05^{ab}	64.89 ± 3.55^a	8.02 ± 0.26^a	2.16 ± 0.10^a	10.41 ± 0.56^a	3.72 ± 0.09^a
B. officinalis	93.21 ± 4.38^a	14.46 ± 1.41^b	0.32 ± 0.02^a	72.20 ± 6.29^a	2.87 ± 0.30^c	3.33 ± 0.49^a	6.20± 0.39^b	0.93 ± 0.24^b

μmol(CO_2) m^{-2} s^{-1}] than their parental species (Table 2).

All the three *Buddleja* taxa showed similar R_E, ranging from 5.85 ± 0.57 to 8.06 ± 0.09 with no significant differences (Table 2). Additionally, F1 hybrids showed the lowest PNUE, 67.98 ± 4.15 μmol(CO_2) g(N)$^{-1}$ s^{-1}. The parental species *B. crispa* showed the largest PNUE [134.46 ± 5.44 μmol(CO_2) g(N)$^{-1}$ s^{-1}], and *B. officinalis* [104.67 ± 10.21 μmol(CO_2) g(N)$^{-1}$ s^{-1}] was the second highest (Fig. 3). Moreover, there were significant differences between all studied *Buddleja* taxa (Fig. 3).

Chl *a* fluorescence: All studied *Buddleja* F1 hybrids and their parental species showed high F_v/F_m ratios, ranging from 0.83 ± 0.004 to 0.85 ± 0.004 (Fig. 4*A*). F1 hybrids

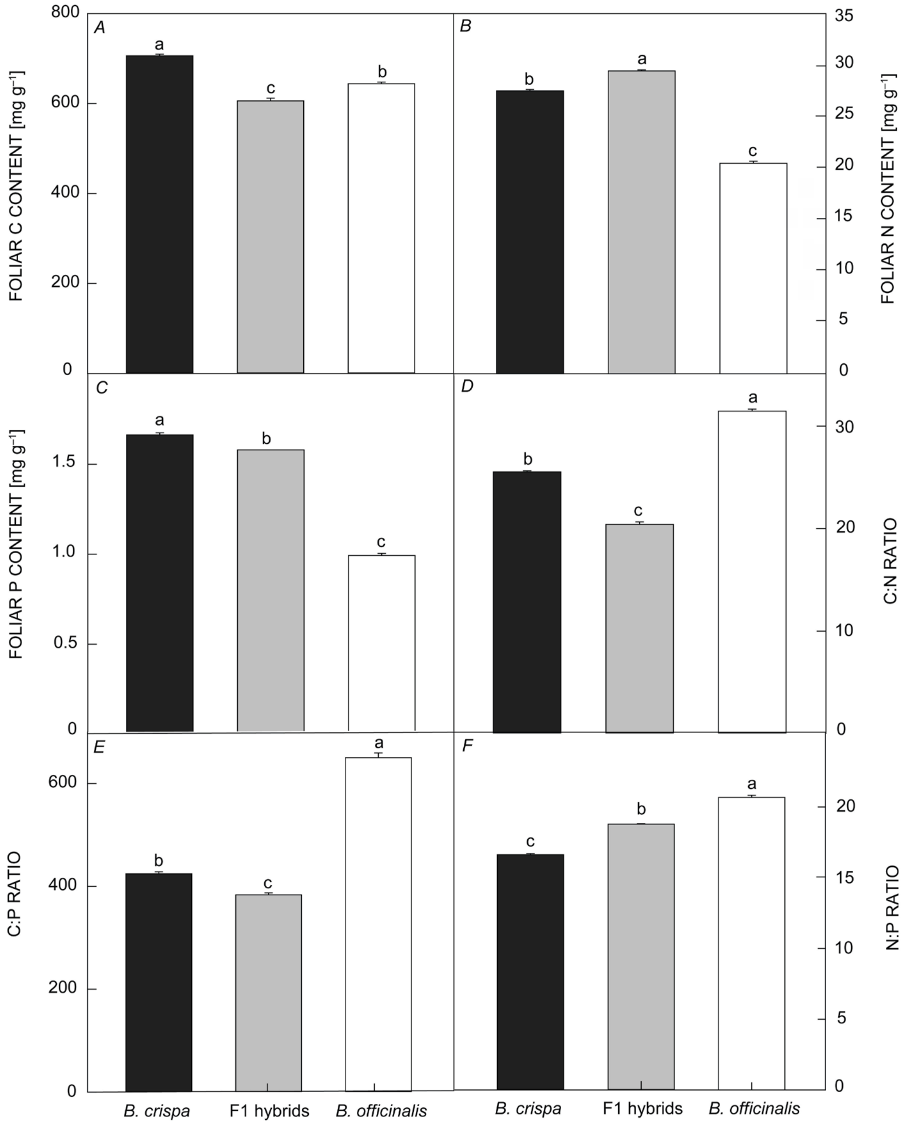

Fig. 2. Leaf C, N, P stoichiometry of *Buddleja* F1 hybrids and their parental species. (*A*) Foliar C concentration; (*B*) foliar N concentration; (*C*) foliar P concentration; (*D*) C:N ratio; (*E*) C:P ratio; (*F*) N:P ratio. Values are represented as the mean ± SE (n = 9). *Different letters* indicate significant differences between different taxa according to LSD test (p<0.05).

Table 2. Fitting parameters of PAR–P_N response curves based on a modified model with nonrectangular hyperbola (mean ± SE, n = 5) of *Buddleja* F1 hybrids and their parental species. AQY – apparent quantum yield, P_{Nmax} – light-saturated net photosynthetic rate, LSP – light-saturation point, LCP – light-compensation point, R_D – rate of respiration, R_E – respiration efficiency.

Species	AQY [μmol(CO_2) μmol^{-1}(photon)]	P_{Nmax} [μmol(CO_2) m^{-2} s^{-1}]	LSP [μmol(photon) m^{-2} s^{-1}]	LCP [μmol(photon) m^{-2} s^{-1}]	R_D [μmol(CO_2) m^{-2} s^{-1}]	R_E
B. crispa	0.08 ± 0.01^a	13.32 ± 0.43^a	1,098.89 ± 27.77^a	24.82 ± 2.21^a	1.79 ± 0.25^a	7.92 ± 0.83^a
F1 hybrids	0.06 ± 0.00^b	9.19 ± 0.41^b	1,050.61 ± 58.72^a	23.31 ± 1.32^a	1.14 ± 0.05^b	8.06 ± 0.09^a
B. officinalis	0.08 ± 0.01^a	10.59 ± 0.81^b	963.83 ± 81.23^a	24.73 ± 2.92^a	1.83 ± 0.16^a	5.85 ± 0.57^a

presented significantly higher F_v/F_m, q_P, NPQ, Φ_{PSII}, and ETR than that of *B. crispa* with significant differences (Fig. 4*A*,*C*–*F*). But, they showed similar photochemical efficiency compared to the parental species *B. officinalis* with no significant differences (Fig. 4).

Interspecific differentiation: The NMDS ordination produced a low stress value (0.075), illustrating interspecific differentiation in leaf traits among the three *Buddleja* taxa in our experiment. The NMDS biplot suggested that the two parental *Buddleja* species and the F1 hybrids each form discrete clusters with a low intraspecific differentiation (Fig. 5*A*). *Buddleja officinalis* showed the largest intraspecific differentiations, *B. cripsa* the second largest (Fig. 5*A*). Additionally, one-way *SIMPER* results suggested that there was a low interspecific dissimilarity (8.54) between F1 hybrids and *B. crispa*, and the dissimilarity value of 10.82 between the F1 hybrids and *B. officinalis*. The overall average similarity between the studied *Buddleja* taxa was up to 90.92.

The contribution of each leaf parameter to the total interspecific variation was evaluated through PCA (Fig. 5*B*). According to the results of the PCA, PCA 1 and 2 explained 56.29 and 29.90% of the total variations, respectively (Fig. 5*B*). Chl fluorescence parameters (*e.g.*, q_P, NPQ, Φ_{PSII}, and ETR), Chl *a*, Chl *b*, Chl *a*/*b*, LDMC, P content, R_D, and PNUE contributed greatly to the variations of the parental species *B. crispa* and *B. officinalis* compared to their F1 hybrids (Fig. 5*B*). In PCA 1, Chl fluorescence parameters (*e.g.*, q_P, Φ_{PSII}, and ETR), R_D, and PNUE contributed greatly to F1 hybrids, *B. officinalis* and *B. crispa*, respectively (Fig. 5*B*). In PCA 2, Chl *a*/*b*, Chl *a*, and P content contributed greatly to *B. crispa*; Chl *b* and LDMC contributed greatly to *B. officinalis*; Chl *a*/*b*, Chl *a*, and Chl fluorescence parameters (*e.g.*, q_P, Φ_{PSII}, and ETR) contributed greatly to F1 hybrids (Fig. 5*B*).

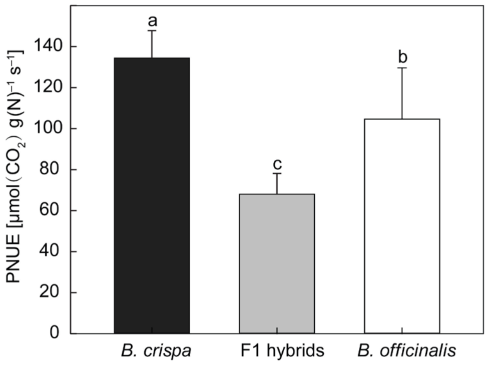

Fig. 3. Photosynthetic nitrogen-use efficiency (PNUE) of *Buddleja* F1 hybrids and their parental species. Values are represented as the mean ± SE (n = 6). *Different letters* indicate significant differences between different taxa according to LSD test (p<0.05).

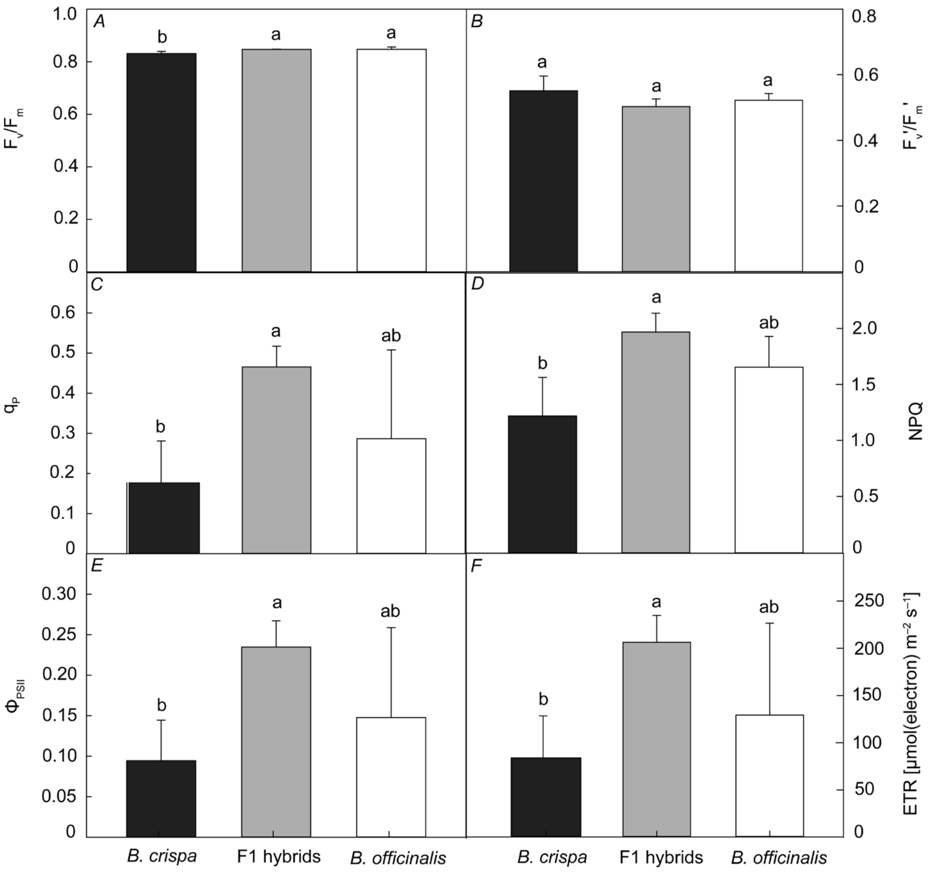

Fig. 4. Chlorophyll *a* fluorescence characteristics of *Buddleja* F1 hybrids and their parental species. (*A*) maximum photochemical efficiency of PSII, F_v/F_m; (*B*) photochemical efficiency of PSII in the light, F_v'/F_m'; (*C*) photochemical quenching, q_P; (*D*) non-photochemical quenching, NPQ; (*E*) effective quantum yield, Φ_{PSII}; (*F*) electron transport rate, ETR. Values are represented as the mean ± SE (n = 5). *Different letters* indicate significant differences between different taxa according to LSD test (p<0.05).

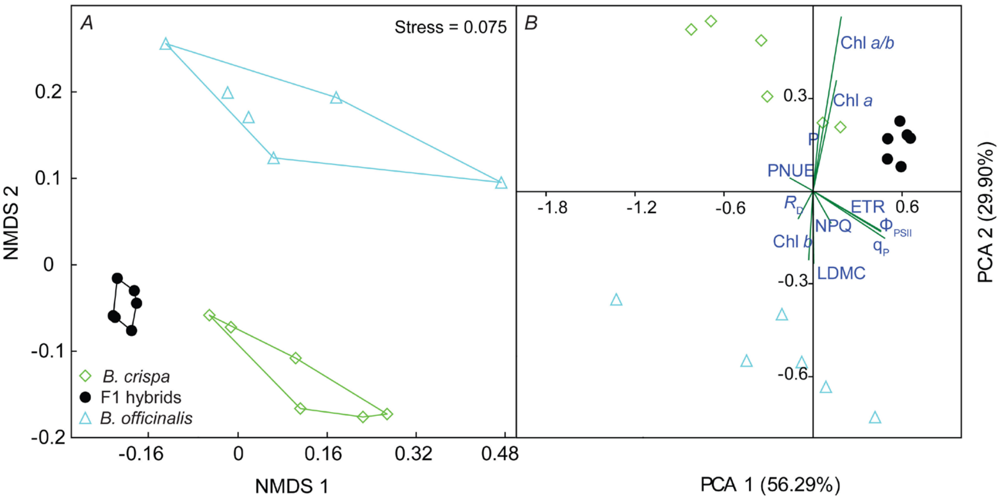

Fig. 5. Differentiation of leaf traits in *Buddleja* F1 hybrids and their parental species. (*A*) non-metric multidimensional scaling (NMDS) biplot based on Chord distances; (*B*) principal component analysis (PCA) biplot. The figure presents only parameters whose loading values are greater than 0.10 and 0.20 in PCA 1 and PCA 2, respectively. All data were $\log_{10}$-transformed. ETR – electron transport rate, Chl – chlorophyll, LDMC – leaf dry mass content), NPQ – nonphotochemical quenching, q_P – photochemical quenching, PNUE – photosynthetic nitrogen-use efficiency, R_D – rate of respiration, Φ_{PSII} – effective quantum yield of photosystem II.

Discussion

Hybridization can have immediate phenotypic consequences and may lead to local adaption through hybrid vigor in the fields (Goulet *et al.* 2007). Although natural hybridization is widespread in angiosperms, it commonly results in a Chl deficiency (Ma *et al.* 2010). The deficiency in Chl *b*, or low relative amounts of Chl *b*, not only affects the biosynthesis of Chl (Shimazaki *et al.* 2007, Ma *et al.* 2010, Terfa *et al.* 2013), but also disturbs the balance between light harvesting and photosynthesis through increasing the Chl *a*/*b* ratio (Anderson *et al.* 1988). In the present study, *Buddleja* F1 hybrids showed 90.92 of the overall similarity among the morphological and physiochemical traits to their parental species (Fig. 5). They not only showed a typical hybrid vigor with greater Chl *a*, Chl *b*, and total Chl *a*/*b* compared to their parental species (Table 1), but also demonstrated a powerful photosynthetic capacity, which is similar to *B. officinalis*, but lower than that in *B. crispa* species.

The leaf economics spectrum (LES) represents a suite of inter-correlated leaf traits concerning construction costs per unit leaf area (*e.g.*, LMA), nutrient concentrations and rates of carbon fixation (*i.e.*, stoichiometric characteristics; Wright *et al.* 2004, Onoda *et al.* 2017). It captures most of the interspecific variations in key traits concerning nutrient use and carbon fixation (Wright *et al.* 2004, Tian *et al.* 2018). Therein, LMA is an important component underlying the leaf economics spectrum (Onoda *et al.* 2011). A large LMA usually represents not only the high-density tissues (Witkowski and Lamont 1991), but also high N allocation to structural (cell-wall) biomass, because cell walls constitute 18–70% of leaf dry mass (Wright and Cannon 2001, Onoda *et al.* 2017). Moreover, it is closely correlated with plant photosynthetic capacity (Onoda *et al.* 2017). An increasing investment to cell walls in leaf mass may decrease the efficiency of photosynthesis through reduced CO_2 diffusion and/or a lower fraction of N in photosynthetic proteins (Terashima *et al.* 2011, Onoda *et al.* 2017).

In the present study, F1 hybrids (64.89 ± 3.55 g m^{-2}) presented similar LMA to *B. officinalis* (72.20 ± 6.29 g m^{-2}), but the LMA was significantly larger than that of *B. crispa* (50.71 ± 2.18 g m^{-2}; Table 1). This suggested that the F1 hybrids in our study allocated a low proportion of their N to photosynthetic components as *B. officinalis*. This was consistent with our results that the F1 hybrids had the lowest PNUE [67.98 ± 4.15 µmol(CO_2) g(N)$^{-1}$ s^{-1}; Fig. 3]. Onoda *et al.* (2004) found that there was a negative correlation between the proportion of N allocation to cell walls and leaf PNUE, which has been used in the determination of the efficiency of N-use resulting in growth (Garnier *et al.* 1995, Poorter and Evans 1998, Wright and Cannon 2001). At the same time, leaves with large LMA usually tend to have strong physical strength and longer leaf lifespans (Wright *et al.* 2004, Onoda *et al.* 2011). Guidi *et al.* (2016) also found that the trade-off between N allocation to cell walls and to Rubisco correlates well with the life spans in different plant leaves. Thus, the F1 hybrids in our study may acquire higher photosynthetic returns over long and sustained periods than do their parental species.

Plant growth is usually accompanied by increases in carbon fixation and nutrient accumulation. Stoichiometric flexibility, which reflects plant intrinsic physiological adjustment of the C:N:P ratios could improve performance in response to environmental variations (Zhang *et al.* 2013). Under rapid growth, elevated demands for increased allocation of resources to P-rich ribosomal RNA may decrease C:N, C:P, and N:P ratios (Elser *et al.* 2000, 2003). In the present study, F1 hybrids showed low C:N and C:P ratios compared to their parental species (Fig. 2*D*,*E*). All three *Buddleja* taxa studied here may present P limitation with the high N:P ratios, which were higher than 16.00 (Fig. 2*F*). Additionally, all three *Buddleja* taxa showed the high concentrations of foliar C, ranging from 606.00 ± 3.46 to 706.00 ± 2.00 mg g^{-1}. Thus, F1 hybrids and their parental species performed well under rapid growth conditions.

Although F1 hybrids showed lower light-capturing capacity with low P_{Nmax} (Table 2), they did not reduce carbon cost by increasing the specific leaf area and decreasing leaf dry mass per unit area (Table 1). On the contrary, the hybrids significantly reduced their R_D values, keeping a similar R_E level to their parental species (Table 2). Moreover, they presented the similar LSP [963.83 ± 81.23–1,098.89 ± 27.77 μmol(photon) m^{-2} s^{-1}] and LCP [23.31 ± 1.32–24.82 ± 2.21 μmol(photon) m^{-2} s^{-1}] to their parental species with no significant differences (Table 2). All these results suggest that these *Buddleja* F1 hybrids have similar environmental adaptive capacity compared to their parental species in the fields.

Chl *a* fluorescence provides an insight into the plant growth performance and the status of leaf photosynthetic apparatus (Guidi *et al.* 2016, Kalaji *et al.* 2016). The F_v/F_m ratio, which is highly correlated with light dose (Oliveira and Peñuelas 2005), has always been a sensitive indicator for evaluating photoinhibition on PSII (Figueroa *et al.* 1997, Sheng *et al.* 2008), with optimal values of around 0.83 for most angiosperms (Björkman and Demmig 1987). In our experiment, all our *Buddleja* plants showed high maximum quantum yields, with high F_v/F_m ratios (> 0.83) (Fig. 4). However, F1 hybrids and *B. officinalis* showed similar levels of NPQ, and these were higher than that of *B. crispa* (Fig. 4*D*), which implies greater heat dissipation of excessive excitation energy (Kalaji *et al.* 2016). Thus, these results suggested that the F1 hybrids studied here and the parental species *B. officinalis* showed a similar light tolerance with similar photosynthetic capacity.

Chl fluorescence competes for excitation energy with photochemical processes, especially photosynthesis (Guidi *et al.* 2016). It can efficiently reflect the leaf photochemical efficiency and growth adaptability of plants to species-specific environments (Kalaji *et al.* 2016). The parameters ETR and Φ_{PSII} capacity can often account for plant health and performance (Sheng *et al.* 2008, Kalaji *et al.* 2016). Carvalho *et al.* (2001) found that the level of q_P can indicate electron transport activity in PSII. In the present study, F1 hybrids showed similar q_P, Φ_{PSII}, and ETR to *B. officinalis*, but higher levels than those in *B. crispa* (Fig. 4*C*,*E*,*F*). Chl fluorescence may be less influenced by instantaneous changes in microclimate, and it can reflect the predominant photochemical status of leaf or plants (Oliveira and Peñuelas 2005). Thus, our results suggest that F1 hybrids in our study showed similar photochemical efficiency and growth adaptability to *B. officinalis*, but that these values were higher than those in *B. crispa* species.

In conclusion, the *Buddleja* F1 hybrids in our study showed very similar LES and photosynthetic capacity compared to their parental species, especially to *B. officinalis*. Although the hybrid phenotypes may lie between the values of that phenotype in either parents, as did LA, SLA, LDMC, LMA, Chl *b*, P_{Nmax}, LSP, N:P, and F_v/F_m (Tables 1,2; Figs. 2,4), other phenotypic values of the hybrids (total Chl, Chl *a*/*b*, AQY, R_D, C content, C:N, C:P, R_E, PNUE, F_v'/F_m', q_P, NPQ, Φ_{PSII}, and ETR) are outside the ranges of those in the parental species (Tables 1,2; Figs. 2–4). Chlorophyll *a* fluorescence parameters (*e.g.*, q_P, NPQ, Φ_{PSII}, and ETR), Chl *a*, Chl *b*, Chl *a*/*b*, R_D, and PNUE contributed greatly to the variations between F1 hybrids and their parental species (Fig. 5*B*), and successfully explained the photosynthetic capacity of F1 hybrids.

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