DOI: 10.32615/ps.2019.024 PHOTOSYNTHETICA 57 (1): 217-225, 2019

Morphological, anatomical and physiological traits of *Euryodendron excelsum* as affected by conservation translocation (augmentation *vs*. conservation introduction) in South China

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Abstract

Euryodendron excelsum is a rare and endangered evergreen tree in South China. We conducted two experimental translocations (augmentation and conservation introduction) on this species and assessed morphological, anatomical and physiological traits of leaves after translocation. The introduction plants showed smaller specific leaf area, less developed palisade tissues, smaller palisade/spongy tissue ratio, stomata density and anthocyanin content, lower values of maximal quantum yield of PSII photochemistry, photochemical quenching coefficient, net photosynthetic rate, light-saturated net photosynthetic rate, light-saturation point, but higher light-compensation point. These differences in traits help explain why augmented plants grew faster than introduced plants. We found that *E. excelsum* can adapt to wide ranges of light intensity and water availability, including conditions encountered at the introduction site. Our findings suggest that some endemic and endangered plants with narrow distribution may adapt to different habitat conditions by rapidly altering their morphological, anatomical, and physiological traits.

Additional key words: assisted colonisation; morphological response; physiological response; reintroduction.

Introduction

Species around the globe are disappearing at an unprecedented rate because of several factors including narrow distribution ranges, reproduction obstacles, overexploitation, and habitat degradation (Maschinski and Haskins 2012, Mounce *et al.* 2017). These factors are generally exacerbated by climate change. An important method of preventing species extinction is conservation translocation (Hoegh-Guldberg *et al*. 2008, Seddon 2010, IUCN 2013). Conservation translocations can be facilitated by the *ex situ* collection of endangered species in botanical gardens through the provision of propagules or individual plants (Ren *et al*. 2012). There are three main types of conservation translocation: augmentation, reintroduction, and conservation introduction (Menges 2008, Liu *et al.* 2015). In augmentation, plants are added to an existing population in order to increase the population size or genetic diversity. In reintroduction, individuals of a species are planted into an area formerly inhabited by the species. In conservation introduction, plants are moved into new areas that are not part of the historic distribution of the species (IUCN 2013, Ren *et al*. 2014, Liu *et al*. 2015).

The monitoring of plant reintroductions is currently inadequate (Godefroid *et al.* 2011, Maschinski and Haskins 2012, Liu *et al*. 2015). In particular, plant functional traits and their responses to translocation sites, though important to explain reintroduction success or failure (Ren *et al*. 2010, Catoni and Gratani 2013), are seldom studied (Godefroid and Vanderborght 2011, Ren *et al*. 2014).

Received 15 November 2017, *accepted* 20 July 2018.

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Abbreviations: AQY – apparent quantum yield; Chl – chlorophyll; E – transpiration rate; EHZ – Ehuangzhang Nature Reserve; 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; In-Ds – Introduction-Dry season; In-Ws – Introduction-Wet season; IUCN – International Union for Conservation of Nature; LCP – light-compensation point; LSP – light-saturation point; NPQ – nonphotochemical quenching; P_{max} – light-saturated net photosynthetic rate; P_N – net photosynthetic rate; R_D – respiration rate; SCBG – South China Botanical Garden; WUE – instantaneous water-use efficiency; Φ_{PSII} – photochemical quenching coefficient.

Acknowledgments: The authors thank Dr. Nan Liu and Mr. Zhifang Lin for valuable suggestions and thank Mrs. Chunqing Long, Mrs. Rufang Deng, and Mrs. Xiaoying Hu for index measurements. This research was supported by the National Natural Science Foundation of China (31570422), Guangzhou Science and Technology Program (201710010137), and the Guangdong Science and Technology Program (2016A030303044). Thanks are also due to Prof. Bruce Jaffee for English editing and constructive comments.

Information on the physiological and morphological traits of rare and endangered plants could also be useful for both *in situ* conservation and *ex situ* conservation under climate change (Busch *et al*. 2011, Catoni and Gratani 2013).

Euryodendron excelsum H. T. Chang is an evergreen tree (Fig. 1) of the family Theaceae; the genus is monotypic. Worldwide, there are only about 100 individuals of this species, and these occur at ten isolated sites in Yangchun City, Guangdong Province, South China. Two populations in neighboring Guangxi Zhuang Autonomous Region have become extinct because of human destruction. *E. excelsum* is ranked as the second most endangered plant in China (Shen *et al*. 2007), listed on the first grade class protected plants in China (Shen *et al*. 2009), and classified as critically endangered by the International Union for Conservation of Nature (IUCN) (Barstow 2017).

Plants of *E. excelsum* are subject to habitat fragmentation, but showed a high level of genetic variations both within and between populations (Luo *et al*. 2005, Su *et al*. 2009). *E. excelsum* seeds are not dormant, with up to 70% germinate rates. The survival rate of cuttings is about 20% (Shen *et al*. 2016). *E. excelsum* grows slowly, but arbuscular mycorrhizal fungi can increase seedling survival and growth (Shen and Wang 2011). The ecophysiological traits of *E. excelsum* and its reintroduction possibilities have not been studied yet.

In this study, we conducted two translocation experiments. One experiment involved augmentation of an existing population, and the other included conservation introduction. The latter can be treated as an *ex situ* collection as well as it is located in a botanical garden. We compared the morphological, anatomical, and physiological traits of *E. excelsum* plants at the augmentation *vs*. introduction sites. We hypothesized that *E. excelsum* plants can adapt to different environments by changing their morphological and physiological traits.

Materials and methods

Study area: The study was conducted simultaneously at two sites. One site was at the Ehuangzhang Nature Reserve, Yangchun City, Guangdong Province, China (hereafter referred to as EHZ, the augmentation site) and the other was at the South China Botanical Garden (hereafter referred to as SCBG, the conservation introduction site), Guangdong Province, South China. SCBG had a fragment of secondary forest similar to that found at EHZ where *E. excelsum* originally grew. This natural habitat at SCBG has been subject to minimum human disturbance. Based on the climate change report, the climate at SCBG is predicted to become warmer and wetter within the next 20 years (Zhou *et al*. 2013). We hope to establish an *E. excelsum* population at SCBG as an *ex situ* conservation collection and a measure to address the climate change challenge to the species.

Fig. 1. A plant (*A*), flower (*B*), and fruit (*C*) of *Euryodendron excelsum*.

EHZ (21°54′36″N, 111°33′29″E) in Yangchun City is an original distribution area of *E. excelsum.* It has a lower subtropical monsoon climate, with a mean annual temperature of 21.7°C and a total annual rainfall of 2,003 mm (Yangchun Meteorological Station). The elevation is about 160 m. The soil is oxisolic with a high sand content in the upper layer and a pH ranging from 6.0 to 6.8 (Shen *et al*. 2009).

SCBG (23°35′30″N, 112°57′22″E) is about 230 km outside of the original distribution area of *E. excelsum.* It also has a lower subtropical monsoon climate, with a mean annual temperature of 20.8°C and a total annual rainfall of 1,612 mm (South China Botanical Garden Meteorological Station). The elevation is about 58 m. The soil is lateritic soil with a pH ranging from 4.5 to 6.0. It is slightly cooler and drier than EHZ.

Plant material: The *E. excelsum* plants used in this study were propagated from seeds collected from populations at EHZ. These seeds were germinated and seedlings were grown in a nursery. The soil used in the nursery were collected from areas nearby wild *E. excelsum* plants. In April 2012, 20 healthy, 3-year-old plants of roughly the same size were selected. Ten of these were transplanted to EHZ, *i.e.*, they were used to augment an existing population, and 10 were planted in the natural area at SCBG, *i.e.*, they were introduced into a site that was outside of *E. excelsum*'s indigenous distribution range. Hereafter, the transplants at EHZ are referred to as "augmentation plants" and those at SCBG as "introduction plants". The plants were transplanted with nursery soil at each site, and the plants were planted in 2.5 m × 2.5 m grid systems in such a way that each plant was 2.5 m apart from its closest neighbors at both sites. Because *E. excelsum* is sun-tolerant, we cleared all plants from both sites before planting to eliminate competition from other plants. We watered the plants several times after planting, and then left them to grow naturally. Neither planting site was fenced, fertilized, or mulched. We measured the height of each plant immediately after planting and measured the height and assessed the survival of each plant once every six months until June 2015.

Leaf morphological and anatomical traits: In April (wet season) and October (dry season) of 2015, we used a leaf area meter (*LI-3000, Li-Cor,* USA) to measure the leaf area of three fully expanded leaves and three young leaves from each of the five randomly selected plants at each site. These leaves were then oven-dried (65°C) to a constant mass. Specific leaf area was calculated as the ratio of leaf area to leaf dry mass (cm^2 g^{-1}). This sampling likely had a little effect on plant growth and survival because these plants had large numbers of leaves.

Transverse sections from three fully expanded leaves and three young leaves from each of the five plants at each site were used to assess leaf anatomical traits as in detail below. The sections were examined and photographed with a light microscope (*AX70, Olympus,* Tokyo, Japan). Leaf epidermal structure was observed with a scanning electron microscope (*JSM-6360LV, JEOL,* Japan), using 15 leaves of each type (three mature and young leaves from each of the five plants at each site).

Leaf pigment contents: We harvested three young leaves and three mature leaves from each of five randomly selected plants at each site to analyze leaf pigment contents. To determine chlorophyll (Chl) and carotenoid (Car) contents, leaf discs (0.6 cm in diameter) were immersed in 80% acetone and were kept in the dark at 4°C for 5 d. The light absorption of the extract was measured at 663, 645, and 440 nm with a UV-visible spectrophotometer (*UV-3802, Unico,* China), and the contents of Chl *a*, Chl *b,* and Car were then calculated (Wang *et al*. 2016).

To determine the anthocyanin content, the leaves were cut into small pieces, immersed in methanol and HCl (99:1, v/v), and kept at 4°C in the dark for 5 d. Absorption of the extract was measured at 530 and 650 nm with a UV-visible spectrophotometer, and the anthocyanin content was calculated (Reddy *et al*. 1995, Wang *et al*. 2016).

Chl fluorescence parameters: One mature leaf and one young leaf from each of five randomly selected plants from each site were randomly selected. The following Chl fluorescence parameters of PSII were measured with a portable fluorescence spectrometer (*PAM-2100, Heinz Walz,* Effeltrich, Germany): 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). These 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 08:30–11:00 h (Wang *et al.* 2016). 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 (PPFD of 2,700 μmol m^{-2} s^{-1}). 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 exposed to a second saturating pulse, and the steady-state fluorescence F_s was then recorded within 5 min. To measure F_0', the leaves were irradiated with 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 [$\Phi_{PSII} = (F_m' - F_s)/(F_m' - F_0')$], and nonphotochemical quenching [$NPQ = (F_m - F_m')/F_m'$] were calculated (Souza *et al*. 2004).

Photosynthetic light-response curve and photosynthetic parameters: A photosynthetic light-response curve was measured under constant conditions (CO_2 concentration of 400 μmol μmol^{-1}; 25°C) with a portable photosynthesis system (*LI 6400; LI-COR,* Lincoln, NE, USA) on a sunny day. The photosynthetic capacity was measured at a 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 (WUE =

P_N/E), net photosynthetic rate (P_N), transpiration rate (E), and instantaneous light-use efficiency (ILUE = P_N/PAR) were calculated by using the nonrectangular hyperbola model (*SigmaPlot 10.0*, *Systat Software*, San Jose, CA) of photosynthesis (Nijs *et al.* 1997, Wang *et al.* 2016).

Statistical analysis: When mean values were compared, a *t*-test (paired samples, two-tailed) in *SPSS* was used to assess the differences between young and mature leaves, augmentation and introduction, and dry and wet seasons. A $P \leq 0.05$ was considered statistically significant. Unless noted otherwise, means and standard deviations (SD) are presented.

Results

Plant survival rate and growth: After two years, survival rate of the transplanted plants was 100% at EHZ (the augmentation site) and 90% at SCBG (the introduction site). The initial height of all plants was 35.1 ± 2.1 cm. In June of 2015 (38 months after transplanting), the plants were taller at EHZ (85.2 ± 5.1 cm) than that at SCBG (56.5 ± 8.3 cm, mean ± SD). These results indicate that both survival rate and growth of augmentation plants were greater than that of the introduction plants.

Leaf morphological and anatomical traits: In all cases (dry *vs.* wet season, augmentation *vs.* introduction), leaf area was smaller, specific leaf area was greater, and leaf thickness was lesser in young leaves than hat of mature leaves (Table 1). Leaf area, specific leaf area, and leaf thickness of both young and mature leaves were lesser at the introduction site than that at the augmentation site in both dry and wet seasons (Table 1).

Although stomata density was greater for young than for mature leaves, the stomata of young leaves were smaller and were mostly closed (Fig. 2). The structure of the palisade tissue was developed in mature leaves but not in young leaves, and the ratio of palisade tissue/spongy tissue was lower in young leaves than that of mature leaves (Table 1).

Compared to the introduction plants, the augmentation plants had a greater specific leaf area, more developed palisade tissues, a greater palisade tissue/spongy tissue ratio, and a greater stomata density (Table 1).

Leaf pigment contents: For both introduction and augmentation plants in both wet and dry seasons, Chl and Car contents were lower and the anthocyanin content was higher in young than that in mature leaves (Table 2).

The Chl, Car, and anthocyanin contents were greater in the augmentation plants than that of introduction plants in both dry and wet seasons. The Chl content of the introduction plants did not significantly differ between dry and wet season, while the Chl content of augmentation plants was greater in the wet season than that in the dry season. The anthocyanin content was greater in the augmentation plants than that of introduction plants in the dry season (Table 2).

Table 1. Morphological and anatomical traits of *Euryodendron excelsum* leaves as affected by leaf age (young and mature), site (augmentation and introduction), and season (dry and wet). In-Ds – Introduction-Dry season; In-Ws – Introduction-Wet season; Au-Ds – Augmentation-Dry season; Au-Ws – Augmentation-Wet season. Values are means ± SD, $n = 15$. Within each row, different *uppercase* letters within the same season and same leaf age indicate significant differences between sites, different *lowercase* letters within the same site and same leaf age indicate significant differences between seasons, and asterisks within the same season and same site indicate significant differences between leaf ages at $P<0.05$.

Parameter	In-Ds		In-Ws		Au-Ds		Au-Ws	
	Young	Mature	Young	Mature	Young	Mature	Young	Mature
Leaf area [cm^2]	5.89 ± 1.28^{Ba}	$9.86 \pm 1.75B^{b*}$	5.68 ± 0.67^{Aa}	$11.01 \pm 1.64^{Ba*}$	6.32 ± 2.81^{Aa}	$20.15 \pm 4.01^{Aa*}$	5.69 ± 1.27^{Ab}	$19.76 \pm 5.57^{Aa*}$
Specific leaf area [cm^2 g^{-1}]	$88.36 \pm 8.66^{Bb*}$	73.82 ± 3.99^{Bb}	$155.94 \pm 10.20^{Aa*}$	88.02 ± 10.68^{Aa}	$108.81 \pm 29.56^{Aa*}$	107.00 ± 18.66^{Aa}	$158.05 \pm 10.12^{Ab*}$	99.27 ± 20.88^{Aa}
Blade thickness [µm]	126.98 ± 4.31^{Ba}	$187.91 \pm 3.73^{Ba*}$	119.47 ± 3.53^{Bb}	$204.20 \pm 5.84^{Aa*}$	131.89 ± 1.42^{Aa}	$195.58 \pm 3.73^{Ab*}$	136.37 ± 7.11^{Aa}	$177.15 \pm 6.17^{Bb*}$
Thickness of palisade tissue [µm]	35.55 ± 1.94^{Ba}	$60.99 \pm 10.17^{Aa*}$	35.47 ± 7.17^{Ba}	$63.45 \pm 2.87^{Aa*}$	41.74 ± 2.29^{Ab}	$66.54 \pm 4.96^{Aa*}$	47.00 ± 3.32^{Aa}	$65.55 \pm 8.75^{Aa*}$
Thickness of spongy tissue [µm]	58.73 ± 4.06^{Ab}	$98.70 \pm 14.63^{Aa*}$	64.05 ± 3.22^{Ba}	$107.56 \pm 6.09^{Aa*}$	59.66 ± 2.28^{Ab}	$94.48 \pm 5.54^{Ab*}$	73.42 ± 3.09^{Aa}	$81.18 \pm 7.31^{Bb*}$
Palisade/spongy ratio	0.61 ± 0.05^{Ba}	0.63 ± 0.16^{Ab}	0.55 ± 0.12^{Ba}	0.59 ± 0.06^{Bb}	0.70 ± 0.06^{Aa}	$0.71 \pm 0.07^{Aa*}$	0.64 ± 0.06^{Aa}	$0.82 \pm 0.17^{Aa*}$
Stomata density [number mm^2]	$495.29 \pm 51.41^{Bb*}$	421.64 ± 27.90^{Bb}	$724.88 \pm 36.53^{Ba*}$	552.84 ± 25.98^{Ba}	1,801.48 166.32^{Aa*}	$\pm$ 536.18 ± 56.43^{Ab}	$1,133.71 \pm 74.19^{Ab*}$	713.07 ± 110.39^{Aa}

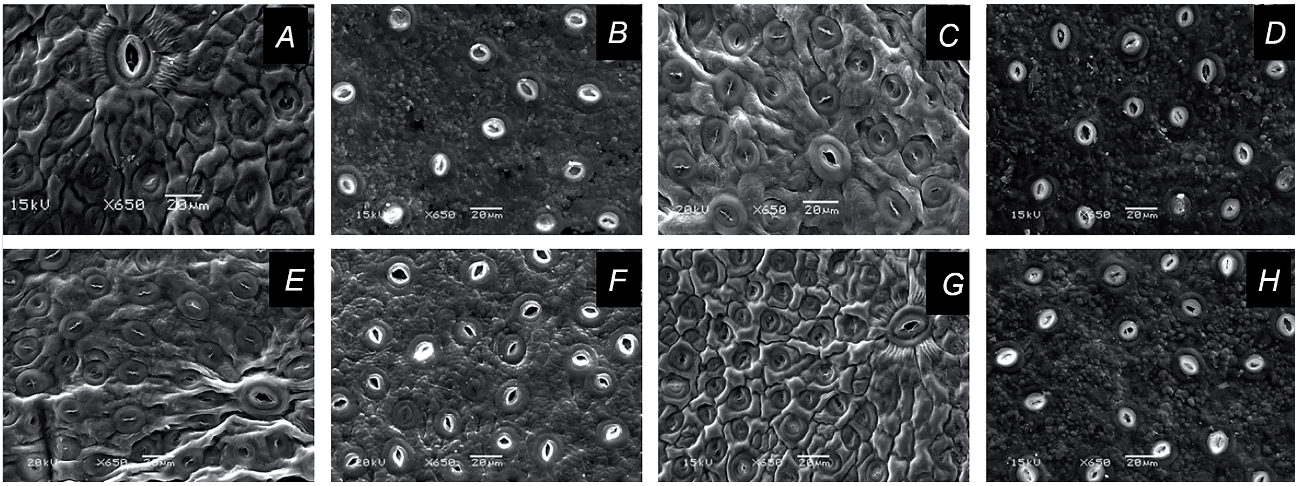

Fig. 2. SEM micrographs of stomata on leaves of augmentation and introduction *Euryodendron excelsum* plants. (*A*) young leaf, introduction, dry season; (*B*) mature leaf, introduction, dry season; (*C*) young leaf, introduction, wet season; (*D*) mature leaf, introduction, wet season; (*E*) young leaf, augmentation, wet season; (*F*) mature leaf, augmentation, wet season; (*G*) young leaf, augmentation, dry season; (*H*) mature leaf, augmentation, set season. Micrographs are at 650 × magnification. Bars = 20 µm.

Chl fluorescence parameters: F_v/F_m and Φ_{PSII} values were lower but NPQ values were higher for young leaves than for mature leaves. F_v/F_m and Φ_{PSII} values were lower but NPQ values were higher in the dry season than that in the wet season (Fig. 3).

F_v/F_m and Φ_{PSII} values of the PSII were significantly greater in the augmentation plants than that of introduction plants.

For introduction plants, the NPQ value was greater in the dry season than that in the wet season for young leaves, but the opposite was true for mature leaves.

Photosynthetic light-response curve and photosynthetic parameters: P_N increased rapidly as light intensity increased and then reached a steady state (Fig. 4). P_N was lower for young leaves than for mature leaves under different light intensities and reached saturation values at lower light intensities in young than that in mature leaves. Whether in the dry season or wet season, P_N of leaves of the same age was greater for augmentation plants than for introduction plants under the same light intensity (Fig. 4).

The average P_{max} and ILUE values of the young leaves of augmentation and introduction plants were similar in the dry and wet season and were lower than those of mature leaves (Table 3). Compared to the young leaves, mature leaves had lower LCP and higher LSP values.

P_{max} was lower in the young leaves than that of mature leaves of the augmentation plants in the wet season (Table 3). P_{max} was greater in the augmentation plants than that of for introduction plants. The mature leaves of augmentation plants had lower LCP and higher LSP values than the mature leaves of introduction plants.

The P_{max}, ILUE, AQY, and LSP values of the mature leaves of augmentation plants were lower in the dry season than that in the wet season, while the opposite was true for LCP and R_D values (Table 3). The P_{max}, ILUE, and AQY values of the mature leaves of introduction plants were greater in the dry season than in the wet season.

Like P_N, transpiration rate (*E*) increased with light intensity. For mature leaves of augmentation plants, *E* was higher in the wet season than in the dry season (Fig. 5*A*). The maximum WUE was higher in mature leaves than that of young leaves (Fig. 5*B*). For mature leaves, WUE values were greater in the dry season than in wet season. *E* values were greater for augmentation plants than that for introduction plants (Fig. 5*A*).

Discussion

The adaptive differences in functional traits of young *vs.* mature leaves: The changes in morphological and physiological traits between young and mature leaves of *E. excelsum* should help plants survive during augmentation and introduction. As leaves mature, the increase in specific leaf area, stomata density, and number of closed stomata, and the decrease in leaf area, leaf thickness, stomata size, and ratio of palisade tissue/spongy tissue should enable the plant to capture more light energy, adapt to drought, and increase their growth rate. These changes can also help reduce damage under strong light conditions.

The young leaves of *E. excelsum* are initially red. As the leaves mature, they gradually turn green, and their Chl and Car contents increase while their anthocyanin content decreases. Given that young leaves may experience significantly greater damage from herbivory and UV light than mature leaves, the red color of young leaves can be explained by two hypotheses. The photoprotection hypothesis suggests that red pigments (anthocyanins) protect against photoinhibition or photooxidation and thus allow a more efficient resorption of nutrients (Feild *et al.* 2001, Hoch 2001, Chen and Huang 2013). The animal–plant interaction hypothesis suggests that red leaves usually have better chemical defenses or poorer nutrition value than that of green leaves and that the red color signals these characteristics to herbivorous insects (Dominy 2002, Archetti 2009, Chen and Huang 2013).

Table 2. Pigment contents of *Euryodendron excelsum* leaves as affected by leaf age (young and mature), site (augmentation and introduction), and season (dry and wet). Values are means ± SD, $n = 3$. Within each row, different *uppercase* letters within the same season and same leaf age indicate significant differences between sites, different *lowercase* letters within the same site and same leaf age indicate significant differences for seasons, and asterisks within the same season and same site indicate significant differences between leaf ages at $P<0.05$. In-Ds – Introduction-Dry season; In-Ws – Introduction-Wet season; Au-Ds – Augmentation-Dry season; Au-Ws – Augmentation-Wet season; Chl *a* – chlorophyll *a;* Chl *b* – chlorophyll *b;* Chl – chlorophyll.

Parameter	In-Ds Young	In-Ds Mature	In-Ws Young	In-Ws Mature	Au-Ds Young	Au-Ds Mature	Au-Ws Young	Au-Ws Mature
Chl *a* [μg cm^{-2}]	4.524 ± 0.304^{Ba}	11.316 ± 0.421^{Ba*}	3.985 ± 0.908^{Aa}	11.194 ± 0.547^{Ba*}	12.469 ± 1.622^{Aa}	39.617 ± 4.569^{Aa*}	3.391 ± 0.699^{Ab}	22.290 ± 1.386^{Ab}*
Chl *b* [μg cm^{-2}]	1.702 ± 0.235^{Ba}	4.796 ± 0.215^{Ba*}	1.835 ± 0.381^{Aa}	4.737 ± 0.208^{Ba*}	5.459 ± 0.727^{Aa}	12.259 ± 1.383^{Aa}*	2.067 ± 0.705^{Ab}	10.769 ± 0.419^{Ab*}
Total Chl [μg cm^{-2}]	6.226 ± 0.531^{Ba}	16.112 ± 0.263^{Ba*}	5.820 ± 1.271Aa	15.931 ± 0.749^{Ba*}	17.928 ± 2.347^{Aa}	51.876 ± 5.947^{Aa*}	5.458 ± 1.392^{Ab}	33.059 ± 1.788^{Ab*}
Chl *a*/Chl *b*	2.685 ± 0.242^{Aa}	2.366 ± 0.187^{Ba}	2.169 ± 0.194^{Ab}	2.363 ± 0.031^{Aa}	2.285 ± 0.024^{Ba}	3.231 ± 0.035^{Aa*}	1.696 ± 0.211^{Bb}	2.069 ± 0.058^{Bb*}
Car [μg cm^{-2}]	3.075 ± 0.048^{Aa}	3.507 ± 0.252^{Bb*}	1.976 ± 0.315^{Ab}	4.248 ± 0.203^{Aa}*	3.413 ± 0.417^{Aa}	8.891 ± 1.088^{Aa*}	1.735 ± 0.157^{Ab}	2.687 ± 0.741^{Bb*}
Car/Chl	0.498 ± 0.053^{Aa*}	0.218 ± 0.018^{Ab}	0.343 ± 0.025^{Ab*}	0.268 ± 0.024^{Aa}	0.191 ± 0.004^{Bb}*	0.171 ± 0.003^{Ba}	0.328 ± 0.049^{Aa*}	0.081 ± 0.019^{Bb}
Anthocyanin [μmol g^{-1}(FW)]	0.942 ± 0.270^{Ba*}	0.686 ± 0.082^{Ba}	1.066 ± 0.039B^{a*}	0.543 ± 0.044^{Ab}	2.285 ± 0.542^{Ab*}	0.729 ± 0.064^{Aa}	2.752 ± 0.157^{Aa*}	0.404 ± 0.027^{Bb}

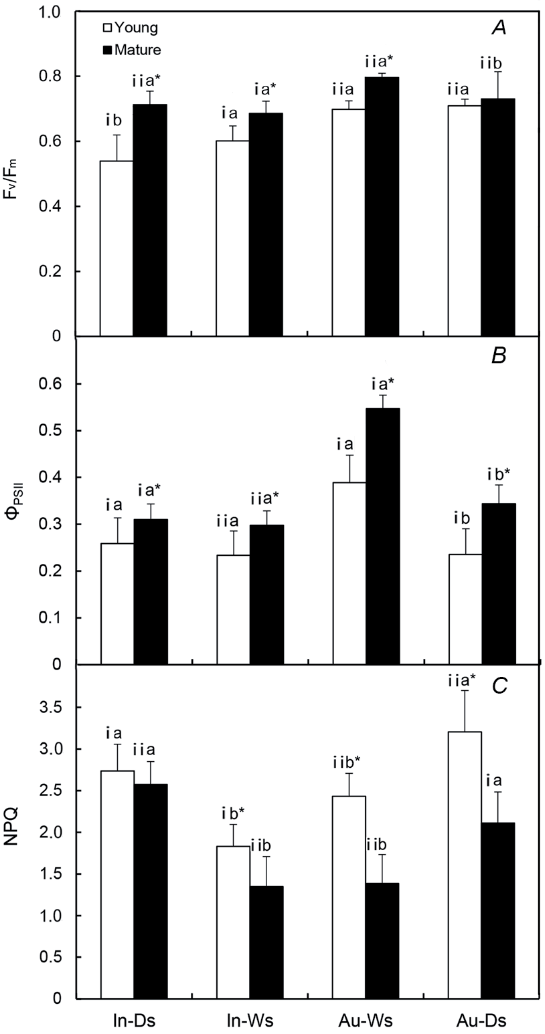

Fig. 3. Chlorophyll fluorescence characteristics of *Euryodendron excelsum* leaves as affected by leaf age (young and mature), site (augmentation and introduction), and season (dry and wet). In-Ds – Introduction-Dry season; In-Ws – Introduction-Wet season; Au-Ds – Augmentation-Dry season; Au-Ws – Augmentation-Wet season. Values are means ± SD, $n = 5$.

In addition, the high anthocyanin content and low Chl content of young leaves decrease the absorption of light energy and thereby prevent the generation of an excess of excitation energy (Manetas 2006). The higher Chl content of the mature leaves should increase their ability to absorb light energy. Although the anthocyanin content was low in mature leaves, the photosynthetic organs were well developed, and the high Car content could help prevent peroxidation injury.

The Chl fluorescence parameters of the young leaves showed that the PSII reaction center could effectively dissipate the surplus light energy by the nonphotochemical pathway; this would reduce the pressure on the PSII reaction center. In an arid environment, the enhanced PSII non-radiation dissipation capacity of *Begonia fimbristipula* leaves reduced the damage to the photosynthetic organs caused by excess light (Wang *et al.* 2016).

The young leaves had lower $P_{\max,}$ lower LSP, and

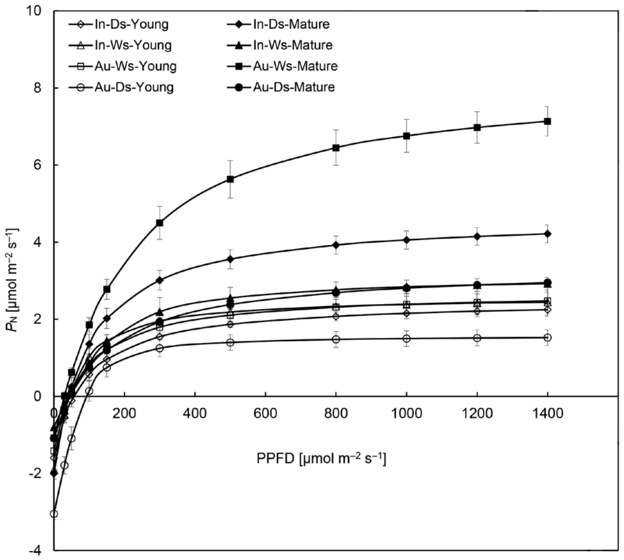

Fig. 4. Light-response curves of *Euryodendron excelsum* leaves as affected by leaf age (young and mature), site (augmentation and introduction), and season (dry and wet). In-Ds – Introduction-Dry season; In-Ws – Introduction-Wet season; Au-Ds – Augmentation-Dry season; Au-Ws – Augmentation-Wet season. Values are means ± SD, $n = 5$.

greater LCP values than that of the mature leaves. This could be related to the higher anthocyanin content in the young leaves. When plants are stressed by drought, high temperature, or strong light, they can protect the mesophyll by filtering and attenuating high-intensity blue-violet light in order to reduce photooxidation damage (Wang *et al*. 2016). The mature leaves had lower LCP and higher LSP values than the young leaves, which indicated that the mature leaves could use longer light-exposure times and a wider range of light wavelenghts than that of the young leaves, which would enable the mature leaves to accumulate more dry matter than the young leaves. In the dry season, the reduced transpiration rates for mature leaves were evidently a response to the arid environment.

Differences between functional traits in plants transplanted at augmentation *vs*. introduction sites: We found that the augmentation plants grew faster than introduction plants. Differences in the morphological and physiological traits between augmentation and introduction plants demonstrated the advantage of plants at its native site over the introduction site. They showed that the plants at the native habitat can capture more light, assimilate more CO_2, and grow faster than those at the introduction site. The relatively slow growth of the introduction plants in the non-native habitat was accompanied by a decrease in a leaf size, which might be a response to the slightly cooler environment.

The Chl content and Car contents were higher in the augmentation plants than that of introduction plants in on both dry and wet seasons and in both young and mature leaves. Therefore, the ability to utilize light energy was greater for augmentation plants, especially under drought stress. The anthocyanin content, also greater in

Table 3. Photosynthetic parameters of *Euryodendron excelsum* leaves as affected by leaf age (young and mature), site (augmentation and introduction), and season (dry and wet). In-Ds – Introduction-Dry season; In-Ws – Introduction-Wet season; Au-Ds – Augmentation-Dry season; Au-Ws – Augmentation-Wet season; P_{max} – maximum photosynthetic rate; R_D – dark respiration rate; AQY – apparent quantum yield; LCP – light -compensation point; LSP – light-saturation point; IIUE – instantaneous light-use efficiency. Values are mean ± SD, $n = 5$. Within each row, different capital letters within the same season and same leaf age indicate significant differences between sites, different lowercase letters within the same site and same leaf age indicate significant differences for seasons, and asterisks within the same season and same site indicate significant differences between leaf ages at $P<0.05$.

Parameter	In-DS		In-WS		Au-DS		Au-WS	
	Young	Mature	Young	Mature	Young	Mature	Young	Mature
P_{max} [μmol m⁻² s⁻¹]	4.095 ± 0.344^{Ba}	$6.658 \pm 0.085^{Aa*}$	4.511 ± 0.459^{Aa}	3.952 ± 0.287^{Bb}	4.637 ± 0.214^{Aa}	4.447 ± 0.163^{Bb}	4.135 ± 0.402^{Aa}	$9.308 \pm 0.216^{Aa*}$
R_D [μmol m⁻² s⁻¹]	1.596 ± 0.306^{Ba}	2.007 ± 0.140^{Aa}	$1.911 \pm 0.108^{Aa*}$	0.799 ± 0.111^{Bb}	$3.048 \pm 0.152^{Aa*}$	1.086 ± 0.104^{Ba}	1.417 ± 0.130^{Ab}	1.077 ± 0.191^{Aa}
AQY [mol mol⁻¹]	0.048 ± 0.019^{Ab}	0.068 ± 0.006^{Aa}	$0.062 \pm 0.011^{Aa*}$	0.022 ± 0.003^{Bb}	$0.046 \pm 0.011^{Aa*}$	0.032 ± 0.004^{Bb}	0.049 ± 0.009^{Ba}	0.040 ± 0.007^{Aa}
LCP [μmol m⁻² s⁻¹]	75.111 ± 6.686^{Aa}	57.017 ± 8.318^{Aa}	64.129 ± 5.478^{Ab}	46.268 ± 5.379^{Aa}	61.544 ± 6.434^{Aa}	57.253 ± 1.511^{Aa}	$61.544 \pm 6.434^{Aa*}$	33.028 ± 5.149^{Bb}
LSP [μmol m⁻² s⁻¹]	700	800	700	900	500	1,000	800	1,100
ILUE[μmol mmol⁻¹]	2.381 ± 0.190^{Aa}	$4.334 \pm 0.278^{Aa*}$	2.569 ± 0.247^{Aa}	3.175 ± 0.230^{Bb}	1.630 ± 0.221^{Ab}	$3.012 \pm 0.189^{Bb*}$	2.631 ± 0.282^{Aa}	$7.318 \pm 0.414^{Aa*}$

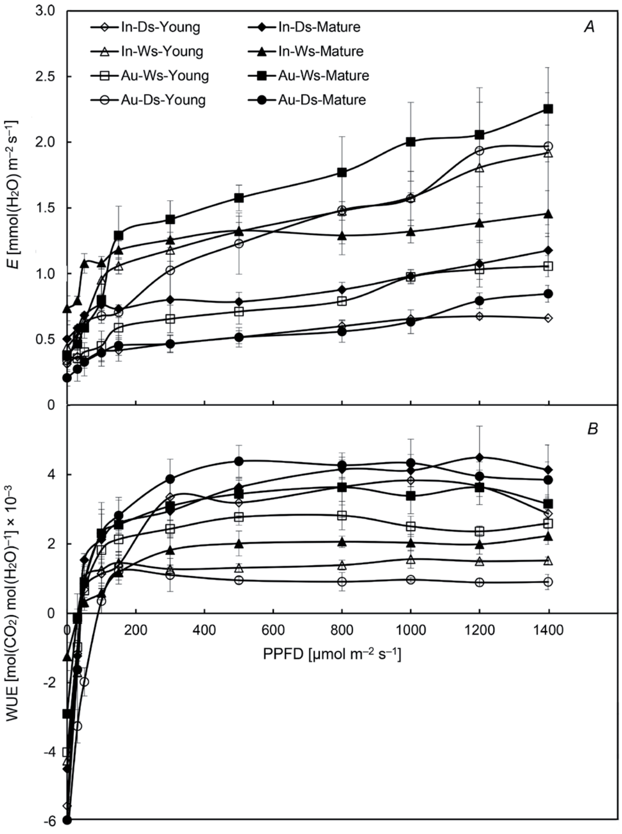

Fig. 5. Light-intensity responses of transpiration rate (*E*, *A*) and instantaneous water-use efficiency (WUE, *B*) of *Euryodendron excelsum* leaves as affected by leaf age (young and mature), site (augmentation and introduction), and season (dry and wet). In-Ds – Introduction-Dry season; In-Ws – Introduction-Wet season; Au-Ds – Augmentation-Dry season; Au-Ws – Augmentation-Wet season. Values are means ± SD, $n = 5$.

the augmentation than that of introduction plants, should reduce the damage to photosynthetic organs caused by excess light.

The difference in the photosynthesis parameters between the mature leaves of augmentation and introduction plants in the dry season indicated that water deficits experienced by the augmentation plants caused the decline in photosynthesis in the dry season. The introduction plants did not face a water deficit and did not experience a decline in photosynthesis in the dry season. These results indicate that *E. excelsum* can reduce its stomatal conductance and transpiration and can regulate its photosynthetic assimilation of carbon and its water-utilization strategy to cope with the drought environment during introduction.

Research on climate change has shown that, on average, global temperature would almost certainly increase in the future which could lead to reduced abundance and distribution or even extinction of many rare, endemic and endangered species (Maschinski and Haskins 2012, Liu *et al.* 2015, Mounce *et al.* 2017). Traditional conservation measures such as *in situ* protection, *ex situ* conservation, and reintroduction would continue to play an important role in rare plant conservation. Human-assisted plant movement, managed relocation or conservation introduction had been put forth as a strategy to conserve species threatened by the changing climate. The premise of conservation introduction is that threatened plants can be moved to locations where the future climate may be favorable for their persistence (Hoegh-Guldberg *et al.* 2008) and those threatened plants can adapt to a new habitat by changing functional traits (Li *et al.* 2015). However, some criticisms on conservation introduction argue that the relocated species may become invasive and have uncertain future in the new environment (Schlaepfer *et al.* 2009). Before we conducted conservation translocation, we had conducted species-specific habitat suitability assessments. Due to its slow growth, existing dispersal barrier, and low reproduction rate (Shen *et al.* 2007, 2009), *E. excelsum* maybe not become an invasive species in the near future. However, long-term experimentation and monitoring are clearly needed to provide the evidence for future debates.

Conclusion: Our results indicate that *E. excelsum* can be conserved *via* conservation and that augmentation may be more effective than introduction. The rapid changes in morphological and physiological traits in response to different habitat conditions also suggest that some endemic and rare species such as *E. excelsum* may have the ability to adapt to habitat changes. Conservation translocation can be used as a conservation tool to overcome plants' dispersal barriers under climate change.

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