



Adaptive photosynthetic strategies of the invasive plant *Sphagneticola trilobata* and its hybrid to a low-light environment

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Abstract

In stressful environments, invasive plants acclimate more efficiently than native plants and hybridization mainly contributes to this process. We examined changes in the morphological characteristics, photosynthetic characteristics, and antioxidant capacity of *Sphagneticola trilobata* and its hybrids in a low-light environment to explore their invasiveness, with *Sphagneticola calendulacea* serving as the control. The morphological plasticity of *S. trilobata* was not dominant, the maximal photochemical efficiency of PSII, actual quantum yield of PSII, and electron transport rate of PSII increased and nonphotochemical quenching decreased, while *S. calendulacea* and the hybrid produced opposite results. *S. trilobata* showed fewer spots stained for reactive oxygen species in tissues, with an increase in superoxide dismutase activity. Although *S. trilobata* is a heliophilous plant, we found that the shade tolerance of *S. trilobata* and the hybrid were stronger than that of *S. calendulacea*, which may be one important mechanism of invasion.

Keywords: growth strategy; hybridization; low light; photosynthesis; plant invasion.

Introduction

The rate of biological invasion has increased exponentially over time and is expected to continue to increase in the coming decades due to rapid globalization (Seebens *et al.* 2017, 2018, 2021). Invasive alien plants invade new areas through natural or artificial processes from their original distribution areas. These invasive species not only

reduce the local biodiversity and destroy the ecological community structure (Walker and Steffen 1997, Ju *et al.* 2016, Dong *et al.* 2017) but also cause huge economic losses to the invaded areas (Mack *et al.* 2010, Branco *et al.* 2015, Funk *et al.* 2016). Light intensity regulates plant morphogenesis, substance metabolism, and gene expression, which may affect the process of plant growth (Franklin and Whitelam 2005, Wada *et al.* 2010, Kim *et al.*

Highlights

- Shading promoted the plant height but decreased the stem length of *Sphagneticola trilobata*
- *S. calendulacea* has the strong photosynthetic capacity and antioxidant activity in the shade
- Shade tolerance of the hybrid is more efficient than that of *S. calendulacea*

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Abbreviations: ANOVA – analysis of variance; CAT – catalase; DAB – 3,3-diaminobenzidine; ETR – electron transport rate of PSII; F_v/F_m – maximal photochemical efficiency of PSII; GA – gibberellin; NBT – nitroblue tetrazolium; NPQ – nonphotochemical quenching; POD – peroxidase; SLA – specific leaf area; SMA – standardized major axis; SOD – superoxide dismutase; Φ_{PSII} – actual quantum yield of PSII.

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2011). In shaded urban green belts and under forests where little sunlight penetrates, light is one of the main limiting factors for shade-grown plants. In a low-light environment, plant photosynthetic performance may be reduced, thus affecting their growth and performance (Dong *et al.* 2019, Yang *et al.* 2020). Studies have shown that alien invasive plants better adapt to stressful environments (Siemann and Rogers 2001, Lee 2002). *Lantana camara*, which is native to tropical America, successfully invades by increasing the leaf area, leaf biomass, and leaf area index to adapt to weak light (Carrión-Tacuri *et al.* 2011). *Mikania micrantha* maintains high photosynthetic efficiency and synthesizes more growth regulators to promote the elongation of the main stem (Liang *et al.* 2022). Under low light, *Eupatorium adenophorum* Spreng. reduces the root biomass to supplement the supporting structure and reduces the leaf area index to weaken self-shading (Wang and Feng 2004). *Robinia pseudoacacia*, one of the most serious alien invasive plants in Italy, captures light as much as possible by reducing leaf thickness and increasing the specific leaf area (SLA), which is beneficial for adapting to low light in the understory (Granata *et al.* 2020). Therefore, invasive plants adapt to low light by adjusting their morphological indices and biomass allocation patterns to maximize light acquisition, improve photosynthetic efficiency, and promote their growth.

Sphagneticola trilobata is a perennial herb of the Asteraceae family that is native to South and Central America. It was introduced into China in the 1970s and escaped into the wild. It spreads and reproduces through stolons to form a patchy monodominant community, that seriously threatens the local ecosystem and species diversity (Wu *et al.* 2005). Therefore, it is listed as one of the '100 most harmful invasive alien species in the world' (Lowe *et al.* 2000). The main reasons for the successful invasion of *S. trilobata* are as follows: (1) it has a strong vegetative reproductive capacity (clonal growth) (Wu *et al.* 2005), (2) it secretes allelochemicals that inhibit the growth of surrounding plants and affect the structure of the soil microbial community and soil texture (Zhang *et al.* 2013, Sun *et al.* 2020a), and (3) it has a stronger CO₂-fixation capacity, wider effective photosynthetic radiation range, and higher light quantum-utilization efficiency than that of *S. calendulacea* (Song *et al.* 2010, Li *et al.* 2016). Studies have shown that *S. trilobata* has a higher photosynthetic nitrogen-utilization efficiency, stronger competitiveness, and higher plasticity at highly fluctuating water levels of 15 cm than *S. calendulacea*, and is more likely to invade wetlands (Javed *et al.* 2020). Compared to the native congener *S. calendulacea*, the stem of *S. trilobata* exhibits stronger tolerance and compensation at low temperatures, which are conducive to normal growth and asexual reproduction throughout the year (Cai *et al.* 2021). Under simulated extreme high-temperature conditions, the net photosynthetic rate of *S. trilobata* was significantly higher than that of *S. calendulacea*, indicating strong tolerance (Song *et al.* 2017). However, in a water-deficient and nitrogen-enriched environment, *S. trilobata* was less competitive than *S. calendulacea* (Azeem *et al.* 2022).

In 2013, a study identified a natural hybrid between *S. trilobata* and a native species of the same genus, *S. calendulacea*. The net photosynthetic rate, stomatal conductance, and transpiration rate of the hybrid were significantly higher than those of *S. calendulacea*, and its growth potential was similar to that of *S. trilobata* (Wu *et al.* 2013). Previous studies have shown that the competitive ability of the hybrid was comparable to that of *S. trilobata*, which was more sensitive to nitrogen deposition (Ni *et al.* 2014), and its high-temperature tolerance and drought resistance were between the two parents, which were stronger than those of the native species (Song *et al.* 2017, Zhang *et al.* 2020a). Hybridization promotes adaptive evolution mainly through evolutionary novelty, fixed heterosis, genetic variation, and dumping genetic load (Ellstrand and Schierenbeck 2000). Natural hybridization plays an important role in the process of biological invasion (Ellstrand and Schierenbeck 2000, Schierenbeck and Ellstrand 2009, Wu *et al.* 2013). For example, the hybrid of *Typha latifolia* and *Typha angustifolia* L. is more suitable to grow in environments with frequent disturbance and wetland ecosystems than their parents, and its litter plays a more important role in the invasion process than the hybrid (Farrer and Goldberg 2009, Olson *et al.* 2009, Larkin *et al.* 2012). The hybrid of the invasive plant *Xanthium strumarium* and the native plant *Xanthium sibiricum* as well as the hybrid of the invasive plant *Corymbia torelliana* have strong growth and reproductive abilities, which further strengthen their invasion (Dickinson *et al.* 2012, Xun *et al.* 2017). Therefore, monitoring and evaluating the invasiveness and adaptability of the hybrid of *S. calendulacea* and *S. trilobata* under adverse conditions is particularly important for the study of invasion ecology.

S. trilobata is a heliophile (Wu and Hu 2004), but many studies have shown that it exhibits a certain shade tolerance and the risk of spreading to shaded areas (Yi *et al.* 2014, Li *et al.* 2016, Zhang *et al.* 2022a). It has also been reported that hybridization is invasive through gene introgression (Ellstrand and Schierenbeck 2000, Xun *et al.* 2017). However, the performance ability of the hybrid in a low-light environment is not clear. Based on current relevant studies, we explored the shade tolerance mechanism of *S. trilobata* and whether the hybrid threatens the survival of *S. calendulacea* under low-light conditions to further promote the invasion of *S. trilobata*. We used *S. calendulacea* as the control plant, selected *S. trilobata*, and the hybrid as the research objects, and analyzed the morphological changes and physiological differences in the three *Sphagneticola* species through artificial shading treatment. We discussed the response strategy of *S. trilobata* and the hybrid to a low-light environment to further provide a theoretical basis for the prediction and control of the suitable habitat of *S. trilobata* and the hybrid.

Materials and methods

Materials and experimental design: Whole plants of *S. trilobata*, *S. calendulacea*, and their hybrid were collected from South China Botanical Garden, Chinese

Academy of Sciences, Guangzhou, China (23°10'N, 113°21'E). Plant materials were propagated through asexual propagation. After defoliation, the stem segments of two stem nodes were cut from the middle stem and then propagated into the water in an incubator [irradiance of 100–120 $\mu\text{mol}(\text{photon})\text{ m}^{-2}\text{ s}^{-1}$, culture temperature of 28/25°C day/night]. When rooting and growing 3–4 pairs of leaves (approximately 3 weeks), the healthy seedlings were transplanted to the flowerpot (12.2 \times 11.2 \times 9.5 cm), with one plant in each pot. The cultivation substrate was a mixture of local soil and potting soil (*Wing Fung Horticulture*, Guangzhou, China) with a volume of 1:1. When the length of the stem reached 20–30 cm, the plants were used for the experiments.

The experiment was conducted at the test site of the College of Life Sciences of South China Normal University from September to November 2021 (the average temperature in September and October in Guangzhou was 32 and 23°C, respectively, and the average relative humidity was 77%, and the average day length was 12 h). Two light environments were established: natural full light and low light (covered with a layer of black nylon net; the nylon net is used for 6-pin encryption). A photosynthesis light quantum meter (*TES-1339P*, Taiwan) was used to measure the number of photons received at a fixed horizontal position above the plant. The photosynthetic photon flux density (PPFD) of full light was 880 $\mu\text{mol m}^{-2}\text{ s}^{-1}$ and that of low light was 180 $\mu\text{mol m}^{-2}\text{ s}^{-1}$. In addition, the photosynthesis light quantum meter was used to measure the number of photons received at the position of the leaf in three *Sphagneticola* species. The ratio of the value and PPFD in the two light environments was reported as the relative irradiance above the leaf.

The three *Sphagneticola* species were exposed to the two light environments for 60 d, with 30 pots for each condition. Sufficient water was added daily and 10 g L⁻¹ fertilizer (250 ml) was applied twice weekly. The compound fertilizer (*Jiangsu Huachang Chemical Co. Ltd.*, China) contained 15% N, 15% P₂O₅, and 15% K₂O.

Determination of morphological and growth indices: On the first day of treatment, the main stem length, stem diameter, leaf length, and leaf width of the plants were measured, and the number of branches and leaf pairs was calculated. Then, the aforementioned indicators were measured every 15 d.

After 60 d of low-light treatment, the whole plant was harvested and washed with tap water. Afterward, the roots, stems, and leaves of each plant were separated, placed in envelopes, and dried in an oven at 75°C for 72 h. The dry masses of roots, stems, and leaves were weighed and recorded.

ImageJ software (*ImageJ*, National Institutes of Health, USA) was used to calculate the leaf area of fresh mature leaves by setting the unit length. SLA was measured by calculating the ratio of the leaf area to the dry mass of leaves. The measurement was replicated five times.

Chlorophyll (Chl) fluorescence parameters of plants were measured using a chlorophyll fluorescence imaging

system (*CF imager*, Technical Ltd., Colchester, UK). After 60 d of different light treatments, the third pair of leaves was cut off, and the fresh round leaves were fully dark-adapted for 30 min and then placed in the support *IMAG-MAX/L*. Five circular test target areas (AOIs, areas of interest) were selected for each plant, and the data listed below were measured. The relevant Chl fluorescence parameters include F_v/F_m , the maximal photochemical efficiency of PSII; Φ_{PSII} , the actual quantum yield of PSII; NPQ, nonphotochemical quenching; and ETR, the electron transport rate of PSII. These parameters were calculated using the methods reported by Gray *et al.* (1997) and Schreiber *et al.* (1995), and Genty *et al.* (1989).

Chemical localization and detection of H₂O₂ and superoxide anions (O₂⁻) in tissue: Reactive oxygen species (H₂O₂ and O₂⁻) were detected with *in situ* histochemical staining procedures using 3,3-diaminobenzidine (DAB) and nitroblue tetrazolium (NBT). After light treatment for 60 d, the third pair of leaves was quickly immersed in phosphate acid buffer solution (pH 7.0) containing 0.5 mg ml⁻¹ DAB or phosphate buffer (50 mM, pH 6.4) supplemented with 10 mM sodium azide and 0.1% NBT, vacuum pumped for 20 min until the leaves sank to the bottom without bubbles, and then placed in the dark at room temperature and incubated for 8–12 h. When brown spots appeared on the leaves, they were bleached with boiling 80% ethanol to remove Chl. Finally, the dyeing of leaves was observed and recorded with an *iPhone XR* camera (*Apple*, California, USA) (Zhang *et al.* 2011).

Determination of antioxidant enzyme activity: Leaves (0.1 g of the third or fourth pair of leaves) were homogenized on ice with a mortar and pestle in 50 mM phosphate buffer (1.5 ml) containing 0.1 mM EDTA, 0.1% *Triton X-100*, and 2% PVP. The grinding fluid was centrifuged at 12,000 \times g for 10 min at 4°C. The supernatant was collected as the enzyme extract for the determination of superoxide dismutase (SOD, EC 1.15.1.1), catalase (CAT, EC 1.11.1.6), and peroxidase (POD, EC 1.11.1.7) activities. Five replicates of each measurement were performed.

SOD activity was determined using the NBT-illumination method (Giannopolitis and Ries 1977). One unit of SOD activity was defined as the amount of enzyme that resulted in 50% suppression of the photochemical reduction of NBT. In this experiment, the reaction mixture contained 0.1 mL of the enzyme extract, 50 mM phosphate buffer (1.7 mL, pH 7.8), 130 mM methionine (0.3 mL), 0.75 mM NBT (0.3 mL), 0.1 mM EDTA-Na₂ (0.3 mL), and 0.02 mM riboflavin (0.3 mL) in a 3 mL volume. The enzyme solution in the positive and negative controls was replaced with phosphate buffer. The reaction was conducted under light (PPFD = 4,500 $\mu\text{mol m}^{-2}\text{ s}^{-1}$) for 15 min. However, the negative control was placed in the dark. Subsequently, the absorbance was determined immediately at 560 nm using a *UV-2450* spectrophotometer (*Shimadzu*, Tokyo, Japan).

POD activity was evaluated using the guaiacol method described by Chance and Maehly (1955). The change in optical density at 470 nm per min was 0.01 as an enzyme activity unit (U). The reaction mixture contained 0.1 mL

of the enzyme extract, 50 mM phosphate buffer (1.875 mL, pH 7.0), 30 mM H₂O₂ (1 mL), and 2-methoxyphenol (0.025 mL) in a quartz cuvette. The absorbance was immediately measured nine times (20 s each time) at 470 nm using a UV-2450 spectrophotometer (Shimadzu, Tokyo, Japan). The activity of POD was calculated per fresh mass.

CAT activity was determined using UV spectrophotometry as described by Chance and Maehly (1955) by measuring the decrease in absorbance at 240 nm for 1 min due to H₂O₂ consumption, and a reduction of A₂₄₀ by 0.01 within 1 min was regarded as an enzyme activity unit (U). The reaction mixture contained 0.1 mL of the enzyme extract and 30 mM H₂O₂ (2.9 mL). After 13 s of incubation, the absorbance was recorded nine times (20 s each time) at 240 nm using a UV-2450 spectrophotometer (Shimadzu, Tokyo, Japan). The activity of CAT was calculated per fresh mass.

Statistical analysis: All results are presented as the means \pm standard errors (SE). Significant differences were analyzed using IBM SPSS Statistics 19.0 software (IBM, NY, USA). Two-way analysis of variance (ANOVA) with plant species and light intensity as the grouping factors was used to compare all experimental parameters with Duncan's multiple comparison test. One-way ANOVA was performed to examine differences in traits among the three *Sphagneticola* species under full and low light. A standardized major axis (SMA) regression analysis was conducted to describe the relationship between each possible pairwise combination of traits. All variables were deemed normed following log transformation. On log-log axes, the SMA regression describes the best-fit scaling relationships between pairs of traits (Warton *et al.* 2006). SMA was performed using SMATR software (Falster *et al.* 2006). The figures were constructed using Origin 2018 (OriginLab, Northampton, MA, USA) and Adobe Photoshop CC 2014 (Adobe Systems Inc., USA). Significance levels for all statistical models were set to $P < 0.05$.

Results

Phenotypic characteristics: Under low light, the main stem length, leaf number, and branch number of the three *Sphagneticola* species increased with the growth process, but the increases were different. With the extension of treatment time, the main stem length of *S. calendulacea* and the hybrid treated with low light increased rapidly, which was significantly different from the full-light control group, while the growth of *S. trilobata* was slower (Fig. 1S, supplement).

On the 60th day, the main stem lengths of *S. calendulacea* and the hybrid were longer under low light than those under full light, increasing by 56 and 50%, respectively, while that of *S. trilobata* was shorter than that under full light, decreasing by 34% (Figs. 1A; 2S, supplement). A significant difference in the stem diameter of *S. trilobata* was not observed, but the stem diameters of both *S. calendulacea* and the hybrid increased significantly

under low light (Fig. 1B). The effect of the species on the stem diameter was significant (Table 1).

In the two light environments, the increasing rates of leaf pairs of the three *Sphagneticola* species were similar, and the difference in branch number increased over time. An obvious tendency for the increase in the branch number of the three *Sphagneticola* species was not detected in the first 15 d. Compared to the low-light treatment, the three *Sphagneticola* species grew more branches under full light after 15 d, while the branch number of the hybrid under low light increased rapidly after 45 d (Fig. 1S).

On the 60th day of low-light treatment, the branch number and leaf pairs of *S. trilobata* decreased significantly by 25 and 14%, respectively, compared to the full-light group (Fig. 1C,D). The ranges of variations in leaf length and leaf width of the hybrid were between those of the parents under low light. However, under low light, the range of increases in the leaf length of *S. trilobata* was smaller, at 20%, and the leaf width decreased significantly (Fig. 1E,F).

Plant height and specific leaf area (SLA): Both species and light intensity significantly affected plant height, the relative irradiance above leaf, and SLA (Table 1). In both light environments, the plant height of *S. trilobata* and the hybrid were significantly and positively correlated with the relative irradiance (Table 1S, supplement). Compared to full light, the vertical plant height of *S. trilobata* and the hybrid increased markedly under low light (Figs. 2A; 3S, supplement). Under low light, the relative irradiance of *S. trilobata* remained high, and did not change significantly, but the values of *S. calendulacea* and the hybrid remarkably improved (Fig. 2B). When the relative irradiance was the same, the plants of *S. trilobata* and the hybrid grown under low light were taller than that under full light (Fig. 4S, supplement). As a leaf shading index, SLA is generally greater when the light is weaker. Correspondingly, the SLA of the three *Sphagneticola* species increased significantly after low-light treatment, with the largest increase of 52% observed for *S. calendulacea* and the smallest increase of 12% observed for *S. trilobata* (Fig. 2C).

Biomass allocation: In different light environments, the distribution pattern of the biomass of each organ of *S. trilobata* was leaf > stem > root, while that of *S. calendulacea* and the hybrid was stem > leaf > root. The light intensity significantly affected the change in stem biomass (Table 1). Under low light, the leaf biomass of *S. trilobata* decreased by 24.8%. The root biomass of the hybrid decreased by 61%, and the root and leaf biomass of *S. calendulacea* decreased by 65 and 19%, respectively. After low-light treatment, the total biomass of *S. trilobata*, *S. calendulacea*, and their hybrid decreased by 20, 17, and 16%, respectively. However, under both light conditions, the total biomass of *S. trilobata* was higher than that of the other two species (Table 2).

Further analysis of the ratio of plant organs to total biomass showed that under low light, the root biomass ratio of *S. calendulacea* and the hybrid decreased by 56

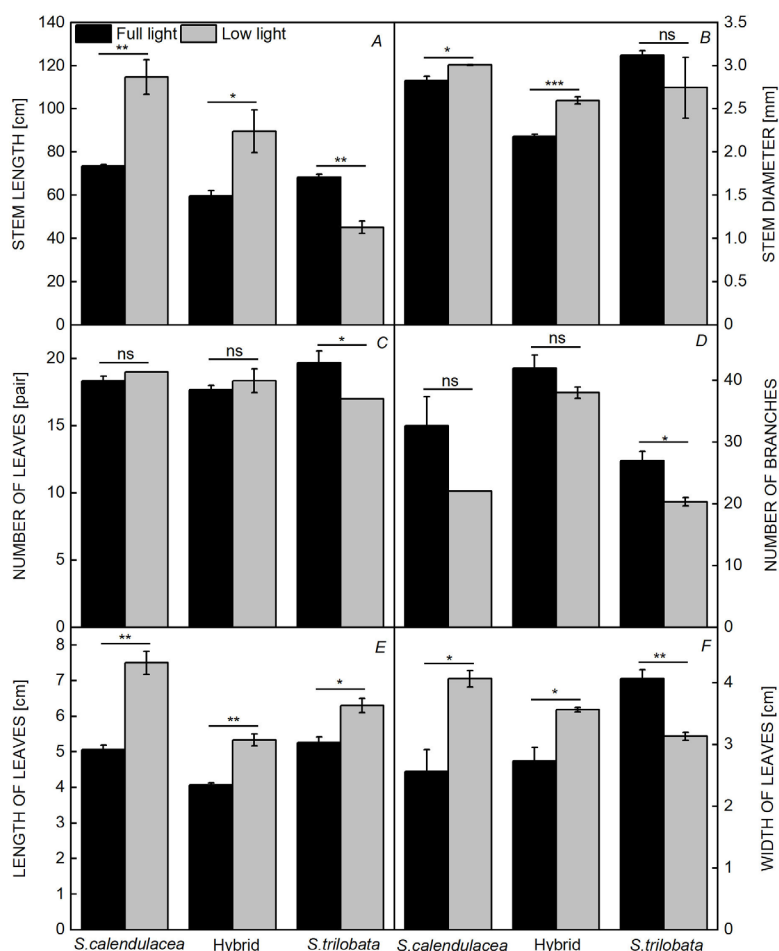


Fig. 1. Phenotypic characteristics of the three *Sphagneticola* species at 60 d of cultivation under low light. The main stem length (A), main stem diameter (B), leaf pairs (C), branch number (D), leaf length (E), and leaf width (F) of the three *Sphagneticola* species under full and low light. The data are shown as the means \pm SE of five biological replicates. Asterisks indicate different significant differences (* P <0.05, ** P <0.01, and *** P <0.001) according to one-way analysis of variance (ANOVA).

and 58%, the leaf biomass ratio increased by 19 and 26%, and the root/shoot decreased by 58 and 60%, respectively. The stem biomass ratio of the hybrid was reduced by 8%. Obvious differences in the aforementioned indices were not detected in *S. trilobata*. Under full light and low light, the leaf biomass ratio of *S. trilobata* was the largest among the three *Sphagneticola* species, the root biomass ratio of the hybrid was the largest, and the stem biomass ratio of *S. calendulacea* was the largest. The three *Sphagneticola* species showed different resource allocations and growth adaptations (Table 2).

Chlorophyll fluorescence parameters: Chl fluorescence parameters effectively reflect the internal photosystem performance of plants in stressful environments and have been used as indicators of plant stress tolerance (Chen *et al.* 2006). After low-light treatment, the F_v/F_m , Φ_{PSII} , and ETR of the same *Sphagneticola* species showed the same trends. These three indices increased noticeably in *S. trilobata* and remained at a high level under low light, while significant decreases were observed in the hybrid and *S. calendulacea*. *S. calendulacea* showed a greater decreasing trend (Fig. 3A,C,D). Under low light, the NPQ of *S. trilobata* was lower than that of the full-light control group, and the NPQ of *S. calendulacea* increased significantly, while their hybrid showed no remarkable

difference (Fig. 3B). The change in NPQ was strongly related to the species (Table 1).

O_2^- and H_2O_2 tissue localization: Under low light, the stained spots in the three *Sphagneticola* species were greater than those under full light. Under low and full light, the leaves of *S. calendulacea* contained a large number of indigo spots (NBT) and brown spots (DAB) that were deep colored, indicating that more O_2^- and H_2O_2 accumulated, respectively, in the leaves. After low-light treatment, the stained spots on the hybrid were slightly less intense than those of *S. calendulacea*, while the stained spots on *S. trilobata* were significantly less intense than those of the other two species, indicating lower accumulation of O_2^- and H_2O_2 (Fig. 4).

Antioxidant enzymes: CAT eliminates H_2O_2 and reduces the tissue damage caused by reactive oxygen species (Bashir *et al.* 2019). Comparing the interaction between light intensity and species, species was the main factor affecting the activity of antioxidant enzymes (Table 1). Upon low-light treatment, CAT activity in *S. calendulacea* increased by 11.8% and that of the hybrid decreased by 8.8%, but low-light stress did not affect CAT activity in *S. trilobata* (Fig. 5C). SOD is the key enzyme scavenging O_2^- (Bowler *et al.* 1992), and its activity in *S. trilobata* was

Table 1. Results from the two-way *ANOVA* of the effects of interactions between light intensity and three *Sphagneticola* species on all functional traits. The data are shown as the means \pm SE of five biological replicates. Asterisks indicate different significant differences (* P <0.05, ** P <0.01, and *** P <0.001) according to two-way analysis of variance (*ANOVA*). η^2 quantifies the effects of different factors.

Index/sources	Light intensity (L)			Species (S)			L \times S		
	<i>F</i>	<i>P</i>	η^2	<i>F</i>	<i>P</i>	η^2	<i>F</i>	<i>P</i>	η^2
Stem length	12.71	**	0.51	23.19	***	0.80	19.65	***	0.77
Stem diameter	0.38	ns	0.03	8.67	**	0.59	3.75	ns	0.39
Number of leaves	1.00	ns	0.08	0.75	ns	0.11	6.25	*	0.51
Number of branches	16.66	***	0.56	33.59	***	0.84	1.25	ns	0.16
Length of leaves	106.13	***	0.90	37.24	***	0.86	8.00	**	0.57
Width of leaves	9.19	**	0.43	2.91	ns	0.33	22.23	***	0.79
Plant height	44.96	***	0.79	59.32	***	0.91	6.58	*	0.52
Relative irradiance above leaf	22.22	***	0.65	17.98	***	0.75	1.60	ns	0.21
SLA	105.59	***	0.90	10.16	**	0.63	14.03	***	0.70
Root biomass	45.64	***	0.79	10.27	**	0.63	4.86	*	0.45
Stem biomass	6.05	*	0.34	0.79	ns	0.12	0.43	ns	0.07
Leaf biomass	10.88	**	0.48	71.66	***	0.92	7.56	**	0.56
Total biomass	40.27	***	0.77	21.67	***	0.78	0.89	ns	0.13
Root mass ratio	103.32	***	0.90	27.50	***	0.82	10.93	**	0.65
Stem mass ratio	3.60	ns	0.23	83.34	***	0.93	0.53	ns	0.08
Leaf mass ratio	39.68	***	0.77	162.96	***	0.96	0.56	ns	0.09
Root/shoot	105.34	***	0.90	28.53	***	0.83	11.85	***	0.66
F_v/F_m	46.25	***	0.79	22.35	***	0.79	80.71	***	0.93
NPQ	0.03	ns	0.00	59.44	***	0.91	25.95	***	0.81
Φ_{PSII}	53.34	***	0.82	158.52	***	0.96	101.75	***	0.94
ETR	53.34	***	0.82	158.52	***	0.96	101.75	***	0.94
SOD	3.43	ns	0.22	92.74	***	0.94	13.89	***	0.70
POD	1.60	ns	0.12	18.48	***	0.76	8.41	**	0.58
CAT	0.32	ns	0.03	32.59	***	0.85	12.51	***	0.68

markedly elevated by 12% under low light. In contrast, SOD activity in *S. calandulacea* was slightly lower than that in the control group (Fig. 5A). POD activity in *S. calandulacea* was also significantly reduced by 26.7% under low light, while *S. trilobata* and the hybrid showed no significant changes (Fig. 5B). SMA showed that the slopes of SOD and POD activities under low light were greater than those under full light, indicating that compared with full light, low light had a stronger promoting effect on SOD and POD activities than other factors in the same condition. In addition, the effect of the relative irradiance on increasing SOD and POD activities was stronger than that of plant height. The result for CAT activity differed from these results, showing that full light and plant height had greater promoting effect on CAT activity (Table 2S, supplement).

Discussion

S. trilobata optimizes resource allocation and reduces changes in morphological characteristics under low light

Most alien invasive plants have a stronger competitiveness than that of native plant species. In heterogeneous

environments, their adaptability is improved by changing their morphological and photosynthetic characteristics and self-regulatory mechanisms (Bassett *et al.* 2011, Cai *et al.* 2020, Sun *et al.* 2020b). Two-way *ANOVA* indicated that the morphological characteristics of the three *Sphagneticola* species exhibited significant differences in response to light intensity and species. Light intensity and species both exerted independent and interactive effects, and the mechanism underlying their effects was complex (Table 1). Givnish (1988) proposed that in a low-light environment, plants try to maintain the maximum ability to absorb light quanta and improve light energy-utilization efficiency; on the other hand, they maintain life activities by reducing respiration and energy consumption. After low-light treatment, *S. trilobata* adopted different growth strategies. Compared to full light, the main stem length, main stem diameter, leaf pairs, and leaf width of the hybrid and *S. calandulacea* increased significantly under low light, while the main stem length, leaf width, leaf number, and branch number of *S. trilobata* decreased noticeably (Fig. 1). Stem length was negatively correlated with plant height and relative irradiance, and the inhibitory effect of relative irradiance was stronger (Table 2S). In a low-light environment, *S. trilobata* grew to reach a higher position at the expense of main stem growth (Fig. 2A), consistent

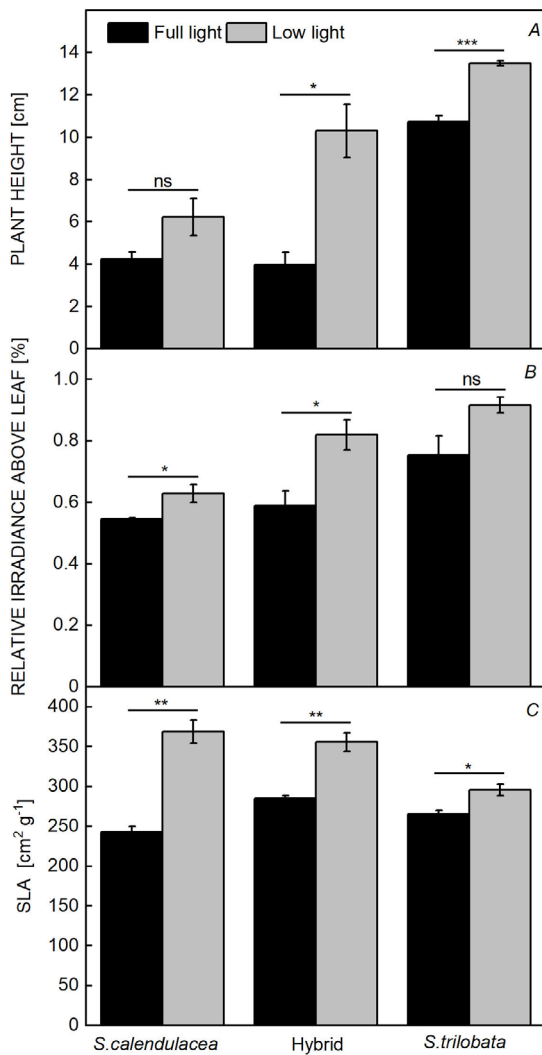


Fig. 2. Phenotype and relative irradiance of the three *Sphagneticola* species at 60 d of cultivation under low light. The plant height (A), relative irradiance above leaf (B), and specific leaf area (SLA) (C) of the three *Sphagneticola* species under full and low light. The data are shown as the means \pm SE of five biological replicates. Asterisks indicate different significant differences (* P <0.05, ** P <0.01, and *** P <0.001) according to one-way analysis of variance (ANOVA).

Table 2. The plant biomass of the three *Sphagneticola* species at 60 d of cultivation under full and low light. The data are shown as the means \pm SE of five biological replicates. Asterisks indicate different significant differences (* P <0.05, ** P <0.01, and *** P <0.001) according to two-way analysis of variance (ANOVA).

Index/ species and treatment	<i>S. calendulacea</i>				Hybrid				<i>S. trilobata</i>			
	Full light	Low light	<i>F</i>	<i>P</i>	Full light	Low light	<i>F</i>	<i>P</i>	Full light	Low light	<i>F</i>	<i>P</i>
Root biomass [g]	0.51 \pm 0.05	0.18 \pm 0.02	34.15	**	0.84 \pm 0.04	0.33 \pm 0.05	60.66	***	0.43 \pm 0.09	0.29 \pm 0.07	1.44	ns
Stem biomass [g]	6.60 \pm 0.31	5.78 \pm 0.03	6.83	ns	6.80 \pm 0.40	5.15 \pm 0.46	7.35	ns	5.90 \pm 0.44	5.15 \pm 1.02	0.45	ns
Leaf biomass [g]	4.02 \pm 0.06	3.25 \pm 0.09	49.90	**	3.86 \pm 0.13	4.20 \pm 0.31	1.05	ns	7.85 \pm 0.16	5.90 \pm 0.61	9.57	*
Total biomass [g]	11.13 \pm 0.23	9.20 \pm 0.06	67.97	***	11.49 \pm 0.26	9.68 \pm 0.19	32.36	**	14.18 \pm 0.58	11.34 \pm 0.76	8.79	*

with previous research on *Chrysanthemum grandiflorum* (Lei *et al.* 2015). Phytohormones are essential intrinsic regulators of plant morphological changes, among which gibberellin (GA) is one of the important factors promoting stem growth (Santner *et al.* 2009, Kurepin and Pharis 2014). Low light usually leads to the elongation of stems (Tan *et al.* 2018, Liang *et al.* 2022). In contrast, the stem length of *S. trilobata* decreased, possibly because low light reduced the content of GA in *S. trilobata* or its sensitivity to GA (Panda *et al.* 2022). Fewer leaf pairs and branch numbers reduce the consumption of organic matter and funnel energy to other processes to ensure sufficient resources for the adaptation to low-light stress (Jha *et al.* 2008, Chen *et al.* 2017). According to previous studies, an increase in SLA leads to the thinning and enlargement of leaves, which is conducive to light penetrating the leaf epidermis, improving the light capture ability of the leaves, accumulating more light assimilates, and enhancing competitiveness (Li and Bao 2005, Houter and Pons 2012, Liu *et al.* 2016). SLA was significantly negatively correlated with relative irradiance (Table 2S). The relative irradiance above the leaf of *S. trilobata* was significantly higher than that of the other two species, and the SLA was naturally lower but significantly higher than that obtained under full light (Fig. 2B,C). Plants adapt to different light conditions by changing their external shape and plasticity (Zhou *et al.* 2017, Coverdale and Agrawal 2021). The overall morphology of *S. trilobata* decreased, while that of the other two *Sphagneticola* species increased. The morphological characteristics of the hybrid are between those of the two parents, with a bias toward *S. calendulacea*. As a creeping plant, the vertical height of *S. trilobata* is higher than that of *S. calendulacea* under low light, and it intercepts more light when it grows to higher altitudes. However, *S. calendulacea* in the lower position is likely to be in a lower light-density environment due to the shade formed by *S. trilobata*, and thus the living environment of *S. calendulacea* is more difficult.

Generally, plants with large biomasses have relatively large coverage in ecosystems or natural habitats, occupying the advantage of living space (Zhang and Welker 1996). Invasive plants often maintain large biomass to obtain competitive advantages under stress (Liu *et al.* 2015, Zhang *et al.* 2020b). Under low light, the biomass of the three *Sphagneticola* species decreased, but the total

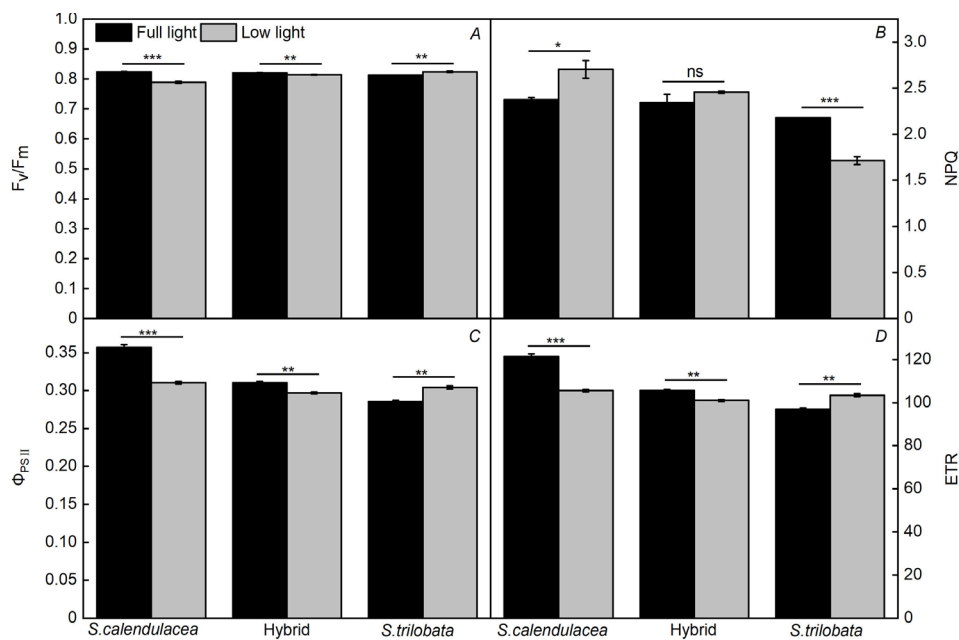


Fig. 3. Chlorophyll fluorescence parameters of the three *Sphagneticola* species at 60 d of cultivation under low light. The maximal photochemical efficiency of PSII (F_v/F_m) (A), nonphotochemical quenching (NPQ) (B), actual quantum yield of PSII (Φ_{PSII}) (C), and electron transport rate of PSII (ETR) (D) of the three *Sphagneticola* species under full and low light. The data are shown as the means \pm SE of five biological replicates. Asterisks indicate different significant differences (* $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$) according to one-way analysis of variance (ANOVA).

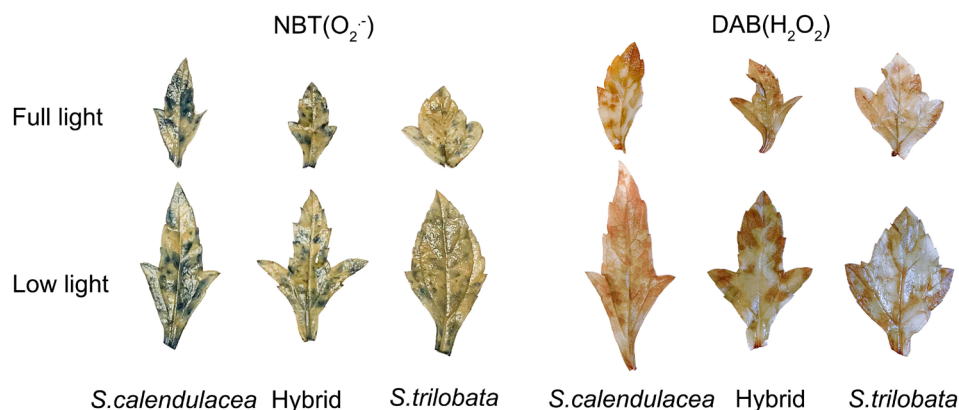


Fig. 4. Tissue localization of reactive oxygen species in the three *Sphagneticola* species at 60 d of cultivation under low light. O_2^- was detected using nitroblue tetrazolium (NBT) staining and H_2O_2 was detected using diaminobenzidine (DAB) staining in leaves of the three *Sphagneticola* species under full and low light.

biomass of *S. trilobata* was the largest. In terms of the ratio of each organ to the total biomass, the three species tended to increase the leaf biomass ratio. Among them, the largest leaf biomass ratio was observed for *S. trilobata*, and a significant difference was not observed under the two light conditions, consistent with the research of Chen *et al.* (2018). Nevertheless, the root biomass ratio of the hybrid was the largest, and the stem biomass ratio of *S. calendulacea* was the largest (Table 2). Seedlings of shade-tolerant tree species support growth by enlarging

the investment in leaves at the expense of roots, while non-shade-tolerant species do not employ this adaptation (Naumburg *et al.* 2001, Lusk 2004). Similarly, in terms of morphological structure, *S. trilobata* focuses more on the growth of leaves and reduces investment in underground organs, which is the adaptation strategy of *S. trilobata* to low light. This finding is also consistent with the hypothesis proposed by Poorter *et al.* (2012) that under stressful environments, plants allocate resources to the organs responsible for obtaining the most limited resources (Wang

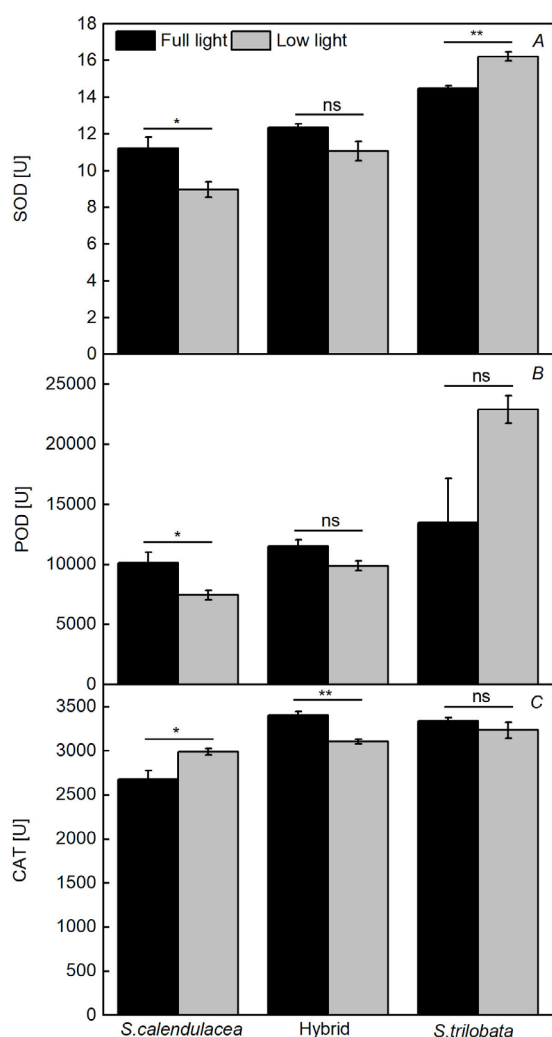


Fig. 5. The antioxidant enzyme activities in the three *Sphagneticola* species at 60 d of cultivation under low light. Superoxide dismutase (SOD) (A), peroxidase (POD) (B), and catalase (CAT) (C) of the three *Sphagneticola* species under full and low light. The data are shown as the means \pm SE of five biological replicates. Asterisks indicate different significant differences (* P <0.05 and ** P <0.01) according to one-way analysis of variance (ANOVA).

et al. 2016, Soda *et al.* 2017), which is related to changes in the distribution pattern of phytohormones (Hedden and Thomas 2012, Sugiura *et al.* 2016). *S. trilobata* may have evolved a phytohormone-dependent adaptation strategy by sensing and integrating environmental cues through hormone signaling in response to low light (Jiang *et al.* 2021).

***S. trilobata* has a strong photosynthetic capacity and active oxygen-scavenging capacity under low light**

Chl fluorescence is a probe of photosynthesis that accurately reflects the effect of shading on photosynthesis (Hallik *et al.* 2012). F_v/F_m is the efficiency of capturing

excitation energy in the photochemically open reaction centers (PSII). It is an important index used to measure the degree of photoinhibition, with a normal value of approximately 0.8, which decreases significantly under stress (Zhang 1999, Rascher *et al.* 2000, Griebeler *et al.* 2021). The F_v/F_m values of the three *Sphagneticola* species under full light were all greater than 0.8, indicating that the plant grew well under natural conditions. After low-light treatment, the F_v/F_m of *S. calendulacea* and the hybrid decreased significantly, indicating that the two plant species experienced low-light stress. The F_v/F_m of *S. calendulacea* was lesser than 0.8, indicating that it was seriously stressed; it might damage the photosynthetic structures, inhibit photosynthetic electron transfer, and lead to a decrease in photosynthetic capacity (Ojeda-Pérez *et al.* 2017). In contrast, the F_v/F_m of *S. trilobata* increased (Fig. 3A), suggesting that low light led to an increase in the openness of PSII reaction centers. The larger the NPQ, the greater the ability of plants to convert excess light energy into heat dissipation (Müller *et al.* 2001, Li *et al.* 2018). Under low light, the NPQ of *S. trilobata* decreased significantly (Fig. 3B), and the proportion of leaves used for heat dissipation decreased, suggesting that it tended to activate photochemical reactions and promoted the accumulation of carbon assimilation. This result is consistent with the research findings reported by Zhang *et al.* (2022b) for the shade-tolerant species *Panax notoginseng*. The F_v/F_m , Φ_{PSII} , and ETR of *S. trilobata* showed the same trends (Fig. 3A,C,D), consistent with the research results for *Fritillaria cirrhosa* adapted to shade (Li and Chen 2008).

Both biotic and abiotic stresses induce the production of a large number of reactive oxygen species. $O_2^{\cdot-}$ is not only an important reactive oxygen species in plants but also the main source of the formation of other reactive oxygen species (Bowler *et al.* 1992, Möller 2001). $O_2^{\cdot-}$ stimulates the activity of antioxidant enzymes (Wagner 1995). SOD catalyzes the conversion of $O_2^{\cdot-}$ to H_2O_2 with relatively low cytotoxicity, which may play a central role in the antioxidant protective mechanism of cells and improve stress resistance (Bowler *et al.* 1992, Zandalinas *et al.* 2017, Liu *et al.* 2018). In addition, POD and CAT are the main enzymes that scavenge H_2O_2 in cells (Bashir *et al.* 2019). Under low-light stress, *S. trilobata* accumulated $O_2^{\cdot-}$ and its SOD activity increased significantly, thereby catalyzing the production of H_2O_2 . However, POD and CAT activities did not change markedly, and H_2O_2 accumulation was not serious, showing that *S. trilobata* suffered less of low-light stress. *S. trilobata* was the species with advantages in terms of antioxidant capacity (Table 1). The CAT activity in the hybrid decreased significantly, the CAT activity in *S. calendulacea* apparently increased, and its SOD and POD activities decreased significantly (Fig. 4). The stained spots in the two species were serious (Fig. 5), indicating that a large amount of reactive oxygen species accumulated, the antioxidant system was severely damaged, and the capacity to scavenge reactive oxygen species was seriously weakened. A study of *Trollius chinensis* Bunge showed that exposure to shade for 40 d caused weak light stress and SOD activity decreased

continuously, shade cultivation for 80 d promoted growth and increased SOD activity, and shade cultivation for 120 d was not conducive to growth, and POD activity decreased (Lü *et al.* 2013). Studies of *Jasmine* suggested that shading promoted an increase in SOD and POD activities and a decrease in CAT activity (Deng *et al.* 2012). Moreover, after heavy shading (10% of full light), the SOD activity in *Bruguiera gymnorhiza* showed no significant change, CAT activity decreased, POD activity increased, and the plant could still maintain the normal operation of the antioxidant enzyme system (Zhu *et al.* 2022). Therefore, the changing trend of antioxidant enzymes caused by shading is closely related to the degree of shading and shading time, and significant differences have been observed between different species.

In summary, *S. trilobata* has a high Φ_{PSII} and the ETR (Fig. 3C,D) revealed a higher photosynthetic capacity under low light. According to the results for F_v/F_m , H_2O_2 and $O_2^{\cdot-}$ localization (Figs. 3A, 4), *S. trilobata* suffered less stress and increased the activity of antioxidant enzymes (Fig. 5), indicating that its ability to scavenge reactive oxygen species was enhanced and its antioxidant capacity became stronger. Compared to *S. calendulacea* and the hybrid, the morphological change of *S. trilobata* was reduced, showing weak morphological plasticity. Therefore, it invested more energy in physiological activities and showed stronger physiological plasticity, which is different from prior research showing that the adaptation of invasive species to light is based on morphological changes (Wang and Feng 2004, Wang *et al.* 2018). In contrast to the plasticity of morphological traits, the plasticity of physiological traits provides a faster, more reversible, and lower carbon consumption response for plants (Bradshaw 1965, Grime and Mackey 2002, Hou *et al.* 2015). Yamashita *et al.* (2002) postulated that plants with high physiological plasticity and moderate shade tolerance are most likely to become invasive species. Gruntman *et al.* (2017, 2020) also indicated that a physiological shade adaptation is more adaptive than investing in morphological traits because morphological traits require carbon allocation. Therefore, the greater plasticity of the physiological shade tolerance response is selected in the more mature population rather than in the invasive frontier population (Gruntman *et al.* 2020). Strong physiological plasticity plays an important role in the invasion of *S. trilobata* in a low-light environment.

Rhymer and Simberloff (1996) found that hybridization between alien and native plant species is one of the most important causes of habitat degradation of native species. As shown in the present study, the phenotypic plasticity of the hybrid was between the two parents, which was biased toward *S. calendulacea*, but its shade tolerance was better than that of *S. calendulacea*. This finding illustrated that the hybrid would further threaten the existence of *S. calendulacea* in a low-light environment, which is consistent with the study by Li *et al.* (2016). Wu *et al.* (2013) also discovered that the rapid expansion of *S. trilobata* in recent decades has substantially decreased the population of *S. calendulacea*. Therefore, *S. trilobata* can invade the low-light environment, which is inseparable

from its ability to not only reasonably allocate resources and improve its resistance but also crowd out local plants and become a dominant population by relying on the strong stress resistance of the hybrid offspring.

Conclusions: After low-light treatment, compared to *S. calendulacea*, *S. trilobata* showed a strong photosynthetic capacity, low oxidative stress, a certain shade tolerance, and further invasion of the living space and resources of *S. calendulacea*. The shade tolerance of the hybrid is between the two parents, which is better than that of *S. calendulacea* and plays an important role in the invasion process. Therefore, the existence of the hybrid should not be ignored while monitoring *S. calendulacea* and preventing it from invading shaded areas.

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