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2 Original Article

3 **Reversible color change in leaves enhances pollinator attraction and**
4 **reproductive success in *Saururus chinensis* (Saururaceae)**

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14 Running title: The role of reversible leaf colour change in *Saururus chinensis*

15 Abstract

- 16 ● *Background and Aims* Although there has been much experimental work on leaf
17 colour change associated with selection generated by abiotic environmental factors
18 and antagonists, the role of leaf colour change in pollinator attraction has been largely
19 ignored. We tested whether whitening of the apical leaves subtending the
20 inflorescences of *Saururus chinensis* during flowering enhances pollinator attraction,
21 and whether re-greening of the white leaves after flowering increases carbon
22 assimilation and promotes seed production.
- 23 ● *Methods* White leaves were removed or covered, and the effects of these
24 manipulations on pollinator visitation and subsequent reproductive success were
25 assessed. The net photosynthetic rates of leaves of different colour were measured and
26 their photosynthetic contributions to seed development were evaluated.
- 27 ● *Key Results* *Saururus chinensis* is able to self-pollinate autonomously, but depends
28 largely on flies for pollination. White leaves had different reflectance spectra than
29 green leaves, and white leaves attracted significantly more pollinators and led to
30 significantly higher fruit and seed set. Although leaf whitening resulted in a reduction
31 in photosynthetic capacity, it translated into only a small decrease in seed mass. White
32 leaves that turned back to green after flowering, had a similar photosynthetic capacity
33 as "normal" green leaves and increased seed production.
- 34 ● *Conclusions* The reversible leaf colour change in *S. chinensis* appears to be adaptive
35 because it enhances pollination success during flowering, with a small photosynthetic
36 cost, while re-greening of these leaves after flowering helps to meet the carbon
37 requirements for seed development.
- 38 *Key words:* carbon assimilation; leaf colour change; net photosynthetic rate; pollinator
39 attraction; seed production

40 INTRODUCTION

41 The organs of many higher plants exhibit remarkable colour changes during development;
42 these include both reproductive (i.e., flowers and fruits) and vegetative parts (i.e., leaves and
43 stems) (Lev-Yadun *et al.*, 2004). Such ontogenetic colour changes have been interpreted as
44 adaptations to several different selective forces (Haberlandt, 1914; Kursar and Coley 1992,
45 2003; Weiss, 1995; Feild *et al.*, 2001; Ida and Kudo, 2003; Herrera, 2005; Karageorgou and
46 Manetas, 2006; Karageorgou *et al.*, 2008; Pélabon *et al.*, 2015). Compared to the considerable
47 attention that has been paid to colour changes of reproductive organs, the adaptive
48 significance of colour change in leaves has been largely overlooked (Lev-Yadun *et al.*, 2002;
49 but see Kursar and Coley 1992, 2003).

50 In most cases, leaf colour change occurs in young, developing leaves or in old,
51 senescing leaves, mainly because of the accumulation of anthocyanins (together with other
52 pigments), the breakdown of chlorophyll (Lev-Yadun *et al.*, 2002), and/or the reversible
53 transformation of plastids (Ikeda 1979). The prevailing view among plant physiologists is that
54 leaf colour change is associated with resistance to photoinhibition because of the immature
55 photosynthetic machinery in young leaves and the risk of reactive oxygen production in
56 senescing leaves (Miranda *et al.*, 1981; Matile, 2000). An alternative, more ecologically
57 oriented view links leaf colour change with: a) reduced insect herbivory by signalling to
58 insects that the leaves are well defended or are a poor nutrient resource, such as red young
59 leaves or variegated adult leaves (Kursar and Coley, 1992, 2003; Numata *et al.*, 2004;
60 Lev-Yadun and Gould, 2007; Archetti *et al.*, 2009; Chen and Huang, 2013); b) increased leaf
61 temperature in cold regions (Taulavuori *et al.*, 2011); or c) enhanced seed dispersal by
62 providing visual guides to animals (Stiles, 1982). Thus far, no single general role of leaf
63 colour change for plant fitness has been accepted. In addition, it is worth noting that, in some
64 plant species, some leaves change colour during flowering, suggesting that they may take part
65 in plant reproduction, e.g., attracting pollinators by advertising flowers through high-contrast
66 colourful leaves, as showy extra-floral structures (e.g., bracts) helping to attract pollinators by
67 enhancing visual displays (Armbruster, 1997; Herrera, 1997; Borges, 2003; Armbruster *et al.*,
68 2005; Sun *et al.*, 2008; Song *et al.*, 2013). Furthermore, such visual signal in extrafloral
69 organs may be especially important in plant species lacking perianths or with inconspicuous

70 corollas (Keasar *et al.*, 2009). To date, however, very few studies have demonstrated the role
71 of colour changes in leaves in pollinator attraction and reproductive fitness.

72 Although plants may benefit reproductively from leaf colour change, such colour
73 changes usually involve trade-offs in terms of reduced photosynthesis and cost of pigment
74 synthesis. In the process of leaves changing from green to red, white or other colours, the
75 breakdown of chlorophyll and synthesis of anthocyanins and/or other pigments that absorb
76 visible light but have no direct role in photosynthesis may impose biosynthetic and
77 photosynthetic costs (Burger and Edwards, 1996; Karageorgou and Manetas, 2006;
78 Karageorgou *et al.*, 2008). For example, compared with green leaves, in *Coleus blumei*
79 quantum yield was reduced by 52% in red leaves because of the absorption of
80 photosynthetically active light by anthocyanin, resulting in a significant reduction in net
81 carbon gain (Burger and Edwards, 1996). Thus, colour change in leaves due to physiological
82 or other causes may negatively affect plant fitness in terms of fruit and seed production. In
83 this context, leaf colour change can be favoured by natural selection only when 1) the
84 photosynthetic cost can be paid-off by a fitness benefit associated with the colour change,
85 and/or 2) the post-pollination re-greening of leaves allows plants to minimize photosynthetic
86 losses and meet the increased carbon requirements of developing fruits and seeds.
87 Consequently, a complete understanding of the adaptive significance of various types of leaf
88 colour changes in plants requires the consideration of both their benefits and costs.

89 In *Saururus chinensis* (Saururaceae), two or three apical leaves near the inflorescences
90 show ontogenetic colour change from green before flowering, to white during flowering, and
91 then reversion back to green during fruit development (Fig. 1A-F). We investigated the
92 possible functions of this green-to-white-to-green colour change of apical leaves during
93 flowering and fruit development. We hypothesized that the white colour of the leaves during
94 flowering increases pollinator attraction by enhancing the visual display (flowers are
95 inconspicuous, being small and lacking a perianth). In addition, we hypothesized that the
96 re-greening of leaves after flowering could enhance photosynthetic capacity to the level of
97 normal green leaves and thereby increase the carbon supply for developing seeds. To
98 determine whether the colour change from green to white increased pollination success, we
99 compared pollinator visitation rates, and the subsequent fruit and seed set, in plants with and

100 without white leaves. In order to determine the possible costs of leaf whitening and the
101 function of leaf re-greening, we measured the net photosynthetic rates of leaves of different
102 colour and examined the effects of leaf removal at different developmental stages on seed
103 development.

104 **MATERIALS AND METHODS**

105 *Study species and sites*

106 *Saururus chinensis* (Lour.) Baill is a perennial medicinal herb in the Saururaceae; it has
107 alternate leaves, grows up to 1 m tall, and is found mainly in moist sites in southern China
108 from sea level to 1700 m a.s.l. (Chen *et al.*, 1982). Reproduction is exclusively by seeds. The
109 plant flowers between late May and late July. Each plant produces 2-3 lateral inflorescences
110 from the axils of terminal leaves each subtending an inflorescence; each inflorescence
111 comprises a single spike with 80-160 tiny flowers that mature in an acropetal succession
112 (Liang *et al.*, 1996). The flowers are 3.08 ± 0.06 mm ($n = 30$) wide, have no perianth, and
113 produce no nectar. At the beginning of anthesis, the apical 2-3 leaves subtending the
114 inflorescences change colour from green to white (Fig. 1 A, B, D, E). After *c.*15 days
115 (depending on the duration of anthesis), when all flowers have been pollinated, the white
116 leaves turn back to green (Fig. 1 C, F). The fruits mature between late August and
117 late-September (B. Song, pers. obs.).

118 This study was conducted at two sites: Jinfoshan Mountain (29° 12' 06" N, 107° 22'
119 41" E, 766 m a.s.l.), in Chongqing Municipality, Southwest China; and Kunming Botanical
120 Garden (25°08'42"N, 102°44'31"E, 1788 m a.s.l.), in Kunming City, Yunnan Province,
121 Southwest China.

122 *Pollinator observation and pollination experiments*

123 Pollinators visiting the flowers of *S. chinensis* were observed from 08:00 to 20:00 h for five
124 days at both sites in 2016. During one of the five nights of observation at both sites, no
125 visiting pollinators were observed. The visiting insects were collected and sent to the
126 Xishuangbanna Tropical Botanical Garden of the Chinese Academy of Sciences for
127 identification. In addition, the behaviour of visitors on flowers was observed. During the
128 observation period, the weather was clear and without strong winds.

129 To determine whether wind-pollination plays a role in *S. chinensis* reproduction, the

130 method of Yuan *et al.* (2008) was used. In the Jinfoshan Mountain population, five flowering
131 plants were selected and microscope slides covered with petroleum jelly were placed around
132 these plants at 0.5 m intervals, 30-50 cm above the ground, to a distance of 5 m away. The
133 slides were collected after two days and were checked under a light microscope for wind
134 dispersed *S. chinensis* pollen. To determine whether pollination of *S. chinensis* depends on
135 pollinators, two pollination treatments were applied to the Jinfoshan Mountain population: 1)
136 natural pollination: 50 inflorescences were randomly marked and these inflorescences were
137 not manipulated; 2) autonomous self-pollination: 50 inflorescences were selected randomly
138 and these inflorescences were bagged with nylon mesh bags throughout their anthesis to
139 exclude pollinating insects. When fruits were ripe, all infructescences were collected and
140 taken to the laboratory to determine fruit and seed set (seed number per fruit; hereinafter the
141 same).

142 ***Leaf morphology and anatomy***

143 Between May 25 and August 15, 2016, leaf development in *S. chinensis* was monitored by
144 examining 50 plants (25 for flowering plants and the rest for non-flowering plants) selected
145 randomly at both Kunming Botanical Garden and Jinfoshan Mountain. For the apical 2-3
146 leaves subtending the inflorescences that change colour during anthesis and post-anthesis on
147 each plant, three colour types were distinguished: 1) young green leaves: leaves that were
148 formed, but have not yet turned white (pre-anthesis); 2) white leaves: leaves have changed
149 from green to white (anthesis); 3) re-greened leaves: leaves have changed from white back to
150 green (post-anthesis). In addition, we defined leaves without colour changes as 4) normal
151 green leaves.

152 The four colour types of leaves described above were fixed on-site in formalin-acetic
153 acid-alcohol (FAA) then examined and photographed using a scanning electron microscope
154 (S-3000N, Hitachi High-technologies Corp., Japan) at Kunming Medical University. In
155 addition, the four types of leaves were also collected and were processed for frozen sectioning
156 with a Leica CM3050 cryostat (Leica Instruments, Germany) following the method of
157 Vogel-Mikus *et al.* (2008). The frozen section thickness was set at 20 μm and sections were
158 photographed using an Olympus microscope (Olympus Inc., Japan).

159 ***Colour measurement and pollinator visitation***

160 One green and one white leaf were randomly collected from each of 30 plants in the Kunming
161 Botanical Garden, and their reflectance spectra between 300 and 700 nm at 5 nm intervals
162 were measured relative to a magnesium sulphate (highly ultraviolet-reflecting white standard),
163 at an angle of 45°, using a spectroradiometer (USB Ocean Optics 2000+) equipped with a
164 Xenon Pulse X2 lamp light source, following Song *et al.* (2015). These leaves were kept fresh
165 and measured within 30 min.

166 To determine whether the presence of white leaves enhances visitation by pollinating
167 insects, 15 flowering plants at the Jinfoshan Mountain site were selected randomly each day
168 between June 15 and June 19, 2016, and separated into three treatment groups: 1) plants kept
169 intact; 2) plants with all white leaves removed; 3) plants with all white leaves covered by
170 recently detached green leaves and held in place with transparent tape. Inflorescences were
171 clipped as necessary in order to ensure that there were exactly two on each experimental plant.
172 We recorded pollinator visits following a schedule in which we observed each plant for 30
173 min, then moved to the next plant for another 30 min, etc., until all five plants in each group
174 had been monitored. Observations were carried out at 9:00, 12:00, 15:00 and 18:00 h by three
175 observers simultaneously. The plants used for this experiment were relatively isolated from
176 each other, ruling out the possibility of neighbouring plants adding to and confounding the
177 attraction effect. All censuses were carried out in warm and clear weather, and each plant was
178 considered to be a replicate.

179 To determine whether white leaves enhance fruit and seed set through attracting
180 pollinators, seventy-five plants about to flower were selected randomly from the Jinfoshan
181 Mountain population and the aforementioned three experimental treatments were applied
182 before young green leaves began to turn white. Inflorescences were clipped as necessary in
183 order to ensure that there were two on each plant, as described above. The selected plants
184 were randomly assigned to the three treatments. When fruits were ripe, all infructescences
185 were collected and taken to the laboratory to determine fruit and seed set. Each plant was
186 treated as a replicate.

187 ***Photosynthetic capacity, fecundity and progeny quality***

188 In order to investigate whether leaves of different colour types (described above) differ in
189 their photosynthetic capacity, we measured their net photosynthetic rates in the Kunming

190 Botanical Garden. For this, a portable photosynthesis measurement system with a
191 fluorescence chamber head (LI-6400-40, Li-Cor, Lincoln, NE, USA) was used (with CO₂
192 concentration at *c.*400 $\mu\text{mol mol}^{-1}$ and quantum flux at *c.*1200 $\mu\text{mol m}^{-2} \text{s}^{-1}$, representing
193 sunny conditions). Preliminary light response curves showed the selected light level to be
194 saturating but not inhibitory (data not shown). Measurements were made on two leaves of
195 each colour type per plant randomly selected on five different plants, and means were
196 calculated for each plant before analysis.

197 To measure the effects of leaf whitening and re-greening on reproductive success in
198 terms of seed development, plants about to flower were selected randomly from the Jinfoshan
199 Mountain population and used for the experimental treatments described below.
200 Inflorescences were clipped as necessary in order to ensure that there were two on each plant;
201 supplementary hand-pollination was performed before or after leaf treatment depending on
202 treatment type. The selected plants were exposed to the following five treatments: 1) natural
203 control: plants were left undisturbed after hand pollination; 2) young green leaves were
204 removed just before they turned white, thus preventing them contributing to photosynthesis
205 during flowering and fruiting; 3) young green leaves were trimmed by *c.*1 mm along their
206 edge just before they turned white as a control for leaf damage whilst mostly maintaining
207 their size; 4) white leaves were removed just before re-greening, thus preventing them
208 contributing to photosynthesis during fruiting; 5) half of each of the white leaves was
209 removed just before re-greening (the cut was made along the central vein, retaining petiole to
210 tip of half the leaf). When the fruits were ripe, all infructescences were collected and taken to
211 the laboratory to determine fruit set, seed set and mass of 10 randomly selected seeds from
212 each plant. Plants were treated as replicates, and each treatment included 25 plants.

213 It was difficult to measure directly the effect of leaf whitening and re-greening on
214 carbon balance, so the effects were estimated indirectly. Since the reduced net photosynthetic
215 rates of white leaves as compared with young green leaves was close to the net photosynthetic
216 rates of white leaves (see results), we used the decreased fecundity in plants from treatment 2)
217 as compared with treatment 4) to estimate the photosynthetic cost associated with the colour
218 change from green to white. Given that the net photosynthetic rate of white leaves was nearly
219 half that of the re-greened leaves (see results), we use the differences in fecundity from plants

220 between treatment 4) and treatment 5) to estimate the increased photosynthetic contribution of
221 the leaves after re-greening to carbon gain after the end of the white leaf phase.

222 *Data analysis*

223 Data obtained from spectral measurements were used to calculate the brightness of white and
224 green leaves. Four types of photoreceptors were involved in the colour vision of flies, R7p,
225 R7y, R8p and R8y (Troje, 1993). The brightness of object was calculated by simply summing
226 up the photon catch of these receptors. One-way ANOVA was used to test the effects of
227 pollination treatment on fruit and seed set, and the effects of leaf treatment on pollinator
228 visitation, fruit set, seed set and seed mass, respectively. Multiple comparisons of means were
229 performed using *Tukey's* test at the 0.05 significance level. A Mann-Whitney U-test was used
230 to compare the length and width of leaves between white and re-greened leaves, and the
231 brightness between white and green leaves. All analyses were performed using SPSS version
232 18.0. Measurements are reported as means \pm 1 SE.

233 **RESULTS**

234 *Pollinator observation and pollination experiments*

235 During our field observation, flowers of *S. chinensis* were visited by seven insect species (see
236 Table 1). Pollen-feeding syrphid flies (Syrphidae), including *Eristalis* sp., *Eupeodes luniger*,
237 and *Episyrphus balteatus*, and the tachinid fly *Leiophora* sp. (Tachinidae), were the major
238 visitors to the flowers at both sites. Of 998 and 1527 visits observed at Kunming Botanical
239 Garden and Jinfoshan Mountain, 96.1% and 98.8%, respectively, were by flies. The flies
240 tended to move from one flower to the next within an inflorescence before moving to another
241 inflorescence. Together, these observations suggest that flies are the most effective pollinators
242 of *S. chinensis*.

243 No pollen grains were captured on the microscope slides (14 cm²) coated with
244 petroleum jelly, suggesting that wind pollination is unlikely to play a role in the pollination of
245 *S. chinensis*. Flowers self-pollinated autonomously, but fruit set of bagged flowers was much
246 lower than in open-pollinated flowers (22.0 % \pm 1.50 vs. 67.0 % \pm 1.87, respectively; $F_{1,58} =$
247 368.16, $P < 0.001$; Fig. 2A). Similarly, seed number per fruit in autonomously self-pollinated
248 flowers was significantly lower than that in open-pollinated flowers (0.28 \pm 0.02 vs. 1.01 \pm
249 0.04, respectively; $F_{1,58} = 248.08$, $P < 0.001$; Fig. 2B). These results suggest that pollination

250 of *S. chinensis* depends mainly on visiting insects.

251 ***Leaf morphology and anatomy***

252 At both sites, leaves subtending the inflorescences always showed colour change from green
253 to white before the first flower is opened, and then reverted back to green after all flowers
254 had been pollinated; leaves on non-flowering plants showed no such colour change. Leaf
255 size increased significantly as colour changed from white to green ($Z = 2.167$, $n = 50$, $P < 0.05$;
256 $Z = 2.44$, $n = 50$, $P < 0.05$ for length and width, respectively, Mann-Whitney U-test; data from
257 the two sites were pooled since no significant difference was found between the two sites
258 (data not shown)). The leaves were 7.48 ± 0.12 cm long and 4.74 ± 0.06 cm wide when the
259 colour was white, and 7.83 ± 0.13 cm long and 4.94 ± 0.06 cm wide after re-greening.

260 The parenchyma cells of young green leaves contained many chloroplasts (Fig. 1 H, L).
261 When the leaves became white (during flowering), the palisade parenchyma cells contained
262 few chloroplasts (Fig. 1 I, M) because of the transformation of chloroplasts into leucoplasts.
263 However, when the white leaves turned back to green after flowering, the palisade
264 parenchyma cells were again full of chloroplasts, as in normal green leaves (Fig. 1 J, K, N, O),
265 because the leucoplasts had transformed into chloroplasts again.

266 ***Colour measurement and pollinator visitation***

267 The spectral measurements revealed that the reflectance of white leaves is significantly
268 different from that of green leaves in the visible range, but not in the ultraviolet range (Fig. 3).
269 Based on the sum of the excitation values of the four fly photoreceptors, the white leaves
270 were significantly brighter than green leaves ($Z = 6.06$, $n = 25$, $P < 0.001$, Mann-Whitney
271 U-test).

272 Fly visitation was significantly affected by leaf treatment ($F_{2, 72} = 7.20$, $P < 0.01$; Fig.
273 4). The number of fly visits to plants from which white leaves were removed (0.24 ± 0.10
274 visits per 30 min) and plants with white leaves covered by green leaves (0.32 ± 0.11 visits per
275 30 min) were not different from each other, but both were significantly lower than to intact
276 plants (0.96 ± 0.20 visits per 30 min). Similarly, fruit and seed set were also affected
277 significantly by leaf treatment (fruit set: $F_{2, 72} = 108.38$, $P < 0.001$; seed set: $F_{2, 72} = 111.39$, P
278 < 0.001 ; Fig. 5). Fruit set and seed number per fruit for plants with white leaves removed
279 (fruit set: $36.8 \% \pm 1.97$; seed number per fruit: 0.46 ± 0.02) and plants with white leaves

280 covered by green leaves (fruit set: $36.3 \% \pm 1.55$; seed number per fruit: 0.44 ± 0.02) were not
281 different from each other but both were significantly lower than intact plants (fruit set: 67.7%
282 ± 1.64 ; seed number per fruit: 0.97 ± 0.04). Fruit and seed set increased by *c.* 87% and 120%,
283 respectively, as a result of leaf colour change from green to white during flowering.

284 ***Photosynthetic capacity, fecundity and progeny quality***

285 The net photosynthetic rate differed significantly between leaves of different colours ($F_{3, 36} =$
286 201.58 , $P < 0.001$; Fig. 6). The net photosynthetic rate of white leaves was significantly lower
287 than in young green leaves (young green leaves: $7.23 \pm 0.09 \mu\text{mol m}^{-2} \text{s}^{-1}$; white leaves: $4.06 \pm$
288 $0.13 \mu\text{mol m}^{-2} \text{s}^{-1}$; reduced by *c.* 44%). However, when white leaves turned back to green,
289 again, these rates were significantly increased (*c.* 132%), and did not differ from the normal
290 green leaves (re-greened leaves: $9.40 \pm 0.30 \mu\text{mol m}^{-2} \text{s}^{-1}$; normal green leaves: 9.62 ± 0.28
291 $\mu\text{mol m}^{-2} \text{s}^{-1}$).

292 Leaf treatments (removing leaves at different developmental stages) had no significant
293 effects on fruit and seed set when flowers were hand pollinated (fruit set: $F_{4, 120} = 0.39$, $P =$
294 0.82 ; seed set: $F_{4, 120} = 0.29$, $P = 0.88$). On average, 81% of flowers produced fruits and each
295 flower produced 1.61 seeds. For seed mass, however, significant differences were found
296 between leaf treatments ($F_{4, 120} = 66.62$, $P < 0.001$; Fig. 7). Mean seed mass in plants with
297 young green leaves removed just before changing to white ($3.86 \pm 0.06 \text{ mg}$) was 0.25 mg less
298 than that from plants with white leaves removed just before re-greening ($4.11 \pm 0.08 \text{ mg}$).
299 This suggests that white leaves contribute to the photosynthetic budget, albeit at a lower rate
300 than would green leaves. This result also suggests that reduced photosynthetic capacity due to
301 leaf colour change from green to white eventually translated into an associated cost in terms
302 of decreased seed mass (*c.* 6%), given that the reduced net photosynthetic rates of white
303 leaves as compared with young green leaves was close to the net photosynthetic rate of white
304 leaves. Compared with the control plants ($5.08 \pm 0.04 \text{ mg}$), plants with white leaves removed
305 just before re-greening produced seeds that were 0.97 mg (*c.*20%) lighter. Seed mass in the
306 plants with half of each of the white leaves removed just before re-greening ($4.57 \pm 0.09 \text{ mg}$)
307 was greater by 0.46 mg (*c.*11%) than in plants with white leaves completely removed just
308 before re-greening, but was significantly lower than in the control plants. The mass of seeds
309 from the trimmed-control treatment were not different from the control treatment, indicating

310 that leaf damage alone did not affect carbon provisioning.

311 **DISCUSSION**

312 Here, we examined experimentally the possible functions of leaf colour change during
313 flowering and fruiting in *S. chinensis*. Unlike previous studies on leaf colour change, which
314 mainly focused on protection against abiotic factors such as ultraviolet radiation, low
315 temperature or oxygen toxicity, or against insect herbivory or fungal attacks (Miranda *et al.*,
316 1981; Matile, 2000; Lev-Yaun *et al.*, 2002; Numata *et al.*, 2004; Archetti *et al.*, 2009;
317 Taulavuori *et al.*, 2011), our study indicates that the colour change in leaves of *S. chinensis* is
318 functionally important because whitening of young green leaves enhances pollinator visitation
319 through an enhanced visual display during flowering. In addition, re-greening of white leaves
320 after anthesis appears to be advantageous because it promotes the development of ripening
321 seeds through increased photosynthetic capacity during fruiting. Compared with the
322 pollination benefit, the photosynthetic cost as a result of the temporary whitening of the
323 leaves during flowering, in terms of seed development, is modest. Consequently, our results
324 suggest that reversible leaf colour change in *S. chinensis* is an adaptive feature favoured by
325 natural selection.

326 ***Leaf colour change from green to white during flowering***

327 The mean pollinator visitation rate was 67% lower in plants with their white leaves removed
328 than in the controls, indicating that the whitening of leaves subtending the inflorescences in *S.*
329 *chinensis* is important for pollinator attraction. This is in agreement with previous reports on
330 the relationship between visual display by plants and pollinator attraction (Herrera, 1997;
331 Borges *et al.*, 2003; Armbruster *et al.*, 2005; Keasar *et al.*, 2009; Sun *et al.*, 2008; Song *et al.*,
332 2013, 2015). These findings can be explained as follows. First, white leaves of *S. chinensis*
333 contrast strongly with green leaves in all spectral ranges except the ultraviolet. This together
334 with the increased brightness should make the white leaves detectable to the pollinating
335 insects over long distances, since many fly species have been found to have the ability to
336 discriminate colours based on brightness (Vargas *et al.*, 1991; Cornelius *et al.*, 1999), as is the
337 case for “detection” of the bracts of *Davidia involucrata* (Nyssaceae) and *Mussaenda*
338 *frondosa* (Rubiaceae) (Borges, 2003; Sun *et al.*, 2008). Furthermore, several studies have
339 shown that this “detection effect” is more prominent for plants occurring at low densities

340 (Herrera, 1997; Borges, 2003; Keasar *et al.*, 2009). In the field, *S. chinensis* plants commonly
341 grow several meters apart (B. Song, pers. obs.), making it difficult for insects to find the
342 flowering plants. Second, a high synchrony between flower maturation and presentation of
343 white leaves suggested that the white colour may provide information on plant quality and
344 direct pollinating insects to rewarding flowers, similar to the role played by floral colour
345 change in some other plant species (Ida and Kudo, 2003; Sun *et al.*, 2005). What is important
346 is that increased pollinator visitation did result in greatly increased pollination success (fruit
347 and seed set were increased by 87% and 120%, respectively). Consequently, our results
348 suggest that leaf colour change from green to white during flowering greatly increased the
349 reproductive fitness of *S. chinensis* through enhancing pollinator visitation and subsequent
350 pollination success. However, we cannot rule out the possibility that extrafloral scents may
351 play a role in pollinator attraction. Further study should be conducted to test the relative role
352 of visual and olfactory signals provided by white leaves and floral scent in pollinator
353 attraction of *S. chinensis*. It is possible that leaf whitening might protect against various
354 abiotic factors (e.g., ultra violet irradiation, water shortage; Lev-Yadun *et al.*, 2002). However,
355 we largely rule out such a possibility, as leaves on non-flowering plants did not change their
356 colour during the growth season, albeit growing under similar environmental conditions as
357 plants with white leaves subtending the inflorescences, at least in the two study sites.

358 In previous studies on the interactions between plants and their pollinators,
359 enhancement of visual display mainly takes the form of larger flowers, aggregating flowers
360 into inflorescences, retaining of old flowers, or developing conspicuous secondary structures
361 associated with flowers, such as the showy bracts of many Araceae, Bromeliaceae,
362 Euphorbiaceae, Nyctaginaceae, and Rubiaceae (Heywood, 1978; Harder and Barrett, 1996;
363 Herrera, 1997; Blarer *et al.*, 2002; Suzuki and Ohashi, 2014; Gagliardi *et al.*, 2016). In
364 contrast to these findings, where visual displays are located in flowers themselves or in
365 secondary structures associated with flowers, our study demonstrates that pollinator attraction
366 has exerted a selective pressure on vegetative organs, namely leaves. Because of the sharp
367 reduction in chloroplasts in the palisade mesophyll, the colour change from green to white to
368 attract pollinators undoubtedly comes at a photosynthetic cost, with photosynthetic rate
369 decreased by at least 44% compared to the young green leaves. However, from a reproductive

370 viewpoint, the effect of temporary decreased photosynthesis on reproductive fitness (e.g., fruit
371 set, seed set and seed mass) as a result of the presence of the white leaves requires direct
372 validation. The subtle decrease in seed mass (c.6%) in plants with young green leaves
373 removed just before they turned white as compared to plants with white leaves removed just
374 before re-greening, in combination with the absence of obvious variations in fruit and seed set,
375 suggested that the cost of reduced photosynthesis was modest compared with the enhanced
376 pollination benefit from the presence of the white leaves. A weakness of this study was that
377 we examined only indirectly the photosynthetic cost of changing colour in terms of reduction
378 in seed production. However, the reduced value in net photosynthetic rate due to the colour
379 change from green to white was lower than that of white leaves. Thus, the real photosynthetic
380 cost of white leaves in terms of reduction in seed mass should not be more than the
381 contribution of white leaves that we estimated (6%). In addition, our results also suggest that
382 in the measurement of reproductive effort, just assessing the reproductive parts may
383 underestimate the reproductive cost if the investment in associated vegetative parts is not
384 taken into account (Bazzaz and Carlson, 1979; Obeso, 2002). It is also possible that the
385 presence of white leaves during flowering might increase floral herbivory, and thus reduce
386 plant reproductive fitness, as traits that are attractive to pollinators may also attract florivores
387 (Armbruster, 1997; Perez-Barrales *et al.*, 2013). However, we did not find any evidence of
388 floral predation (B. Song *et al.*, unpublished), indicating that leaf whitening in *S. chinensis*
389 was unlikely to result in a florivory cost, at least in both sites studied.

390 ***Leaf colour change from white to green during fruiting***

391 Leaf re-greening after flowering was associated with a significant increase in photosynthetic
392 capacity, to a level comparable with normal green leaves, suggesting that the re-greened
393 leaves may help to meet the carbon requirements of the developing seeds in *S. chinensis*. As
394 has been mentioned previously, however, this proposition also needs to be verified directly,
395 because even if the assimilates produced by re-greened leaves increased, this was not
396 necessarily translated into a significant enhancement of fruit or seed development: assimilates
397 could have remained in the leaves or been transferred to storage organs other than nearby
398 seeds as a result of architectural constraints (Watson and Casper, 1984; Nakano *et al.*, 1997).
399 In the treatment where we removed half of each white leaf just before re-greening, there was

400 no significant effect on fruit and seed set, but seed mass was significantly greater (c.11%)
401 than in plants with white leaves removed completely just before re-greening. We largely
402 eliminated the possibility that the variation in seed mass resulted from the damage inflicted
403 because there was no difference in seed mass between the trimmed-control treatment and the
404 control treatment. These results indicate that the re-greened leaves of *S. chinensis* contributed
405 substantial assimilates to the development of seeds, similar to the way that re-greening in
406 persistent reproductive organs, such as bracts and sepals, contributes to reproductive output in
407 other species. For example, the involucre bracts in *Dalechampia scandens* permit carbon
408 assimilation that contributes to the carbon demand of developing seeds by changing colour
409 from white to green after flowering (Pélabon *et al.*, 2015). In *S. chinensis*, white leaves
410 accounted for up to 38 % of the total number of leaves on an individual plant. Thus, it is not
411 surprising that re-greening of white leaves substantially contributes to the carbon needs of
412 developing seeds in *S. chinensis*, in contrast to the limited overall contribution of bract
413 photosynthesis for the seed development in *Dalechampia scandens* (Pélabon *et al.*, 2015).
414 Thus, re-greening of the white leaves after flowering increased the local production of
415 photosynthates and decreased the earlier costs imposed by leaf colour change from green to
416 white to enhance pollination success during flowering.

417 In conclusion, the colour change from green to white of the apical 2-3 leaves in *S.*
418 *chinensis* during flowering enhances the visual display and increases pollination success by
419 increasing pollinator attraction, with only limited photosynthetic cost in terms of seed
420 production. Furthermore, these leaves present a similar photosynthetic capacity as normal
421 green leaves after turning back to full green during fruiting, and they help to meet the carbon
422 requirements of the developing seeds, thereby also compensating for the photosynthetic cost
423 resulting from leaf whitening during flowering. Thus, our results suggest that the reversible
424 leaf colour change in *S. chinensis* is an adaptive strategy resulting from selection for enhanced
425 pollination success during flowering combined with providing carbon supply for seed
426 development. Although leaf colour change occurs in many plants, this study is the first to
427 document experimentally that selection from pollinators is likely to play an important role in
428 leaf colour change and thereby it contributes to our understanding of how the interaction
429 between plant and pollinator can allow leaves to evolve a new function in flowering plants.

430

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552 **93**:523-536.

553 **Table 1** Insect visitors observed on the flowers of *Saururus chinensis* at Kunming
 554 Botanical Garden and Jinfoshan Mountain.

Taxon	Kunming Botanical Garden		Jinfoshan Mountain	
	Total number of visits	Percentage of total visits	Total number of visits	Percentage of total visits
Diptera				
<i>Eristalis</i> sp.	898	90.0	1362	89.2
<i>Eupeodes luniger</i>	42	4.2	97	6.4
<i>Leiophora</i> sp.	19	1.9	18	1.2
<i>Episyrphus balteatus</i>			31	2.0
Hymenoptera				
<i>Anthophora</i> sp.	22	2.2	19	1.2
Coleoptera				
<i>Oenopia</i> sp.	8	0.8		
Lepidoptera				
<i>Spindasis syama</i>	9	0.9		

555 **LEGENDS TO FIGURES**

556 **Fig. 1** Leaves of *Saururus chinensis* at different developmental stages. A plant at pre-anthesis
557 stage (A); a plant at anthesis stage (B); a plant at fruit developmental stage (C); a green leaf at
558 pre-anthesis stage and its cross section (D, H, L); a white leaf at anthesis stage and its cross
559 section (E, I, M); a re-greened leaf and its cross section (F, J, N); a normal green leaf and its
560 cross section (G, K, O). Bars: (H-K) 100 μm ; (L-O) 10 μm .

561

562 **Fig. 2** Fruit set (A) and seed number per fruit (B) of *Saururus chinensis* flowers that were
563 open-pollinated and autonomously self-pollinated (bagged). Different letters denote
564 significant differences at $P < 0.05$.

565

566 **Fig. 3** Spectral reflectance of white leaves (solid line) and green leaves (dotted line) of
567 *Saururus chinensis*.

568

569 **Fig. 4** Number of fly visits per census (30 min) to plants subjected to three experimental
570 treatments. Different letters denote significant differences at $P < 0.05$. *IN*: Intact; *-WL*: all
571 white leaves on a plant were removed; *+GL*: all white leaves on a plant were covered by
572 green leaves.

573

574 **Fig. 5** Fruit set (A) and seed set (B) of *Saururus chinensis* flowers subjected to three
575 experimental treatments. Different letters denote significant differences at $P < 0.05$. *IN*: Intact;
576 *-WL*: leaves were removed just before whitening; *+GL*: leaves were covered by green leaves
577 just before whitening.

578

579 **Fig. 6** Net photosynthetic rates of leaves of different colour. Different letters denote
580 significant differences at $P < 0.05$. *YL-BW*: young green leaves before whitening; *WH*: white
581 leaves; *GL-AR*: green leaves after re-greening; *NG*: normal green leaves.

582

583 **Fig. 7** Seed mass of *Saururus chinensis* flowers when the 2-3 apical leaves that will change
584 colour were subjected to different experimental treatments. Flowers used for the experimental

585 treatment were hand-pollinated. Different letters denote significant differences at $P < 0.05$.
586 *NA*: Natural; *LRBW*: leaves were removed just before whitening; *LRBWC*: leaves were
587 trimmed *c.*1 mm from their edge just before whitening; *LRBR*: white leaves were removed
588 just before re-greening; *HLRBR*: half of each of the white leaves was removed just before
589 re-greening.
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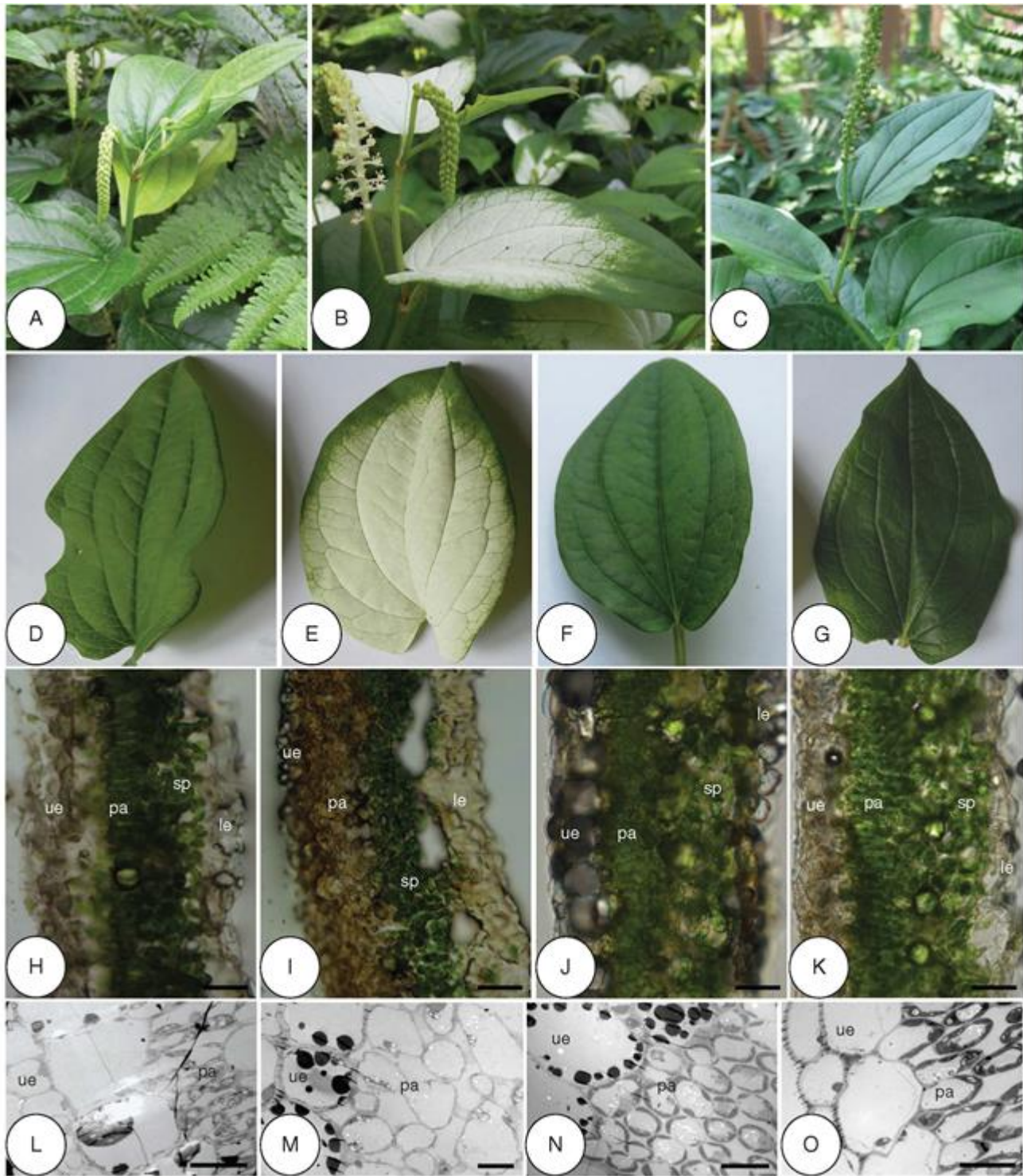


FIG. 1. Leaves of *Saururus chinensis* at different developmental stages. (A) A plant at pre-anthesis stage. (B) A plant at anthesis stage. (C) A plant at fruit development stage. (D, H, L) A green leaf at pre-anthesis stage and its cross-section. (E, I, M) A white leaf at anthesis stage and its cross-section. (F, J, N) A re-greening leaf and its cross-section. (G, K, O) A normal green leaf and its cross-section. Scale bars: (H–K) = 100 μ m; (L–O) = 10 μ m. le = lower epidermis, pa = palisade parenchyma, sp = spongy parenchyma, ue = upper epidermis.

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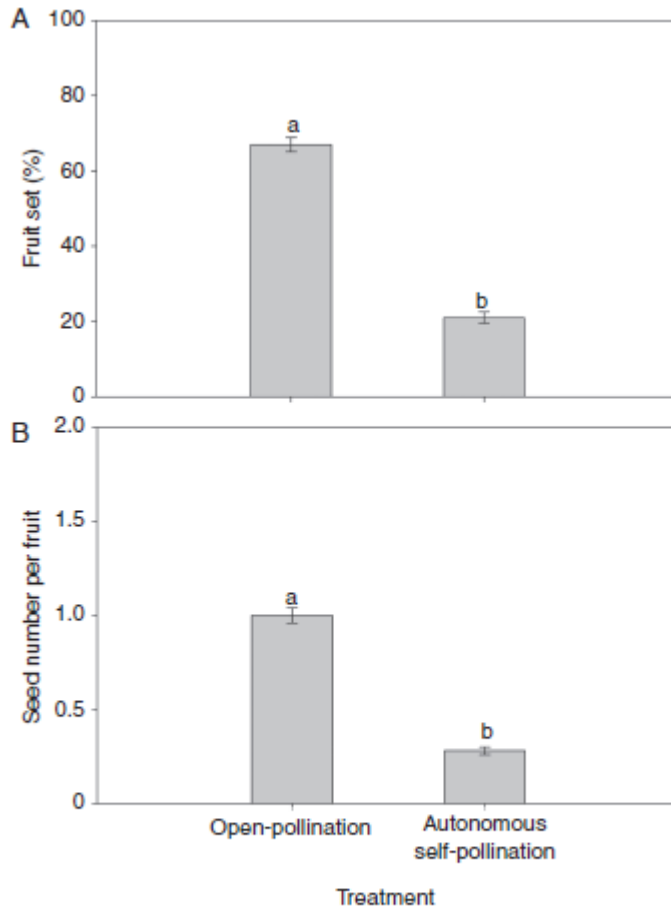


FIG. 2. Fruit set (A) and seed number per fruit (B) of *Saururus chinensis* flowers that were open-pollinated and autonomously self-pollinated (bagged). Different letters denote significant differences at $P < 0.05$.

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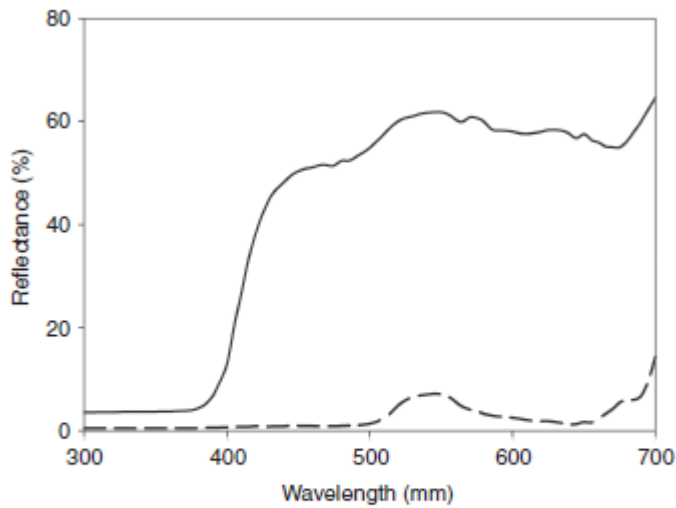


FIG. 3. Spectral reflectance of white leaves (solid line) and green leaves (dashed line) of *Saururus chinensis*.

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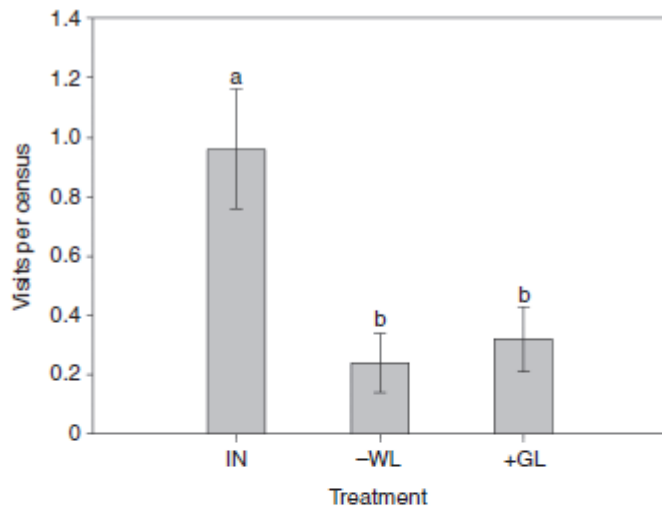


FIG. 4. Number of fly visits per census (30 min) to plants subjected to three experimental treatments. Different letters denote significant differences at $P < 0.05$. IN, intact; -WL, all white leaves on a plant were removed; +GL, all white leaves on a plant were covered by green leaves.

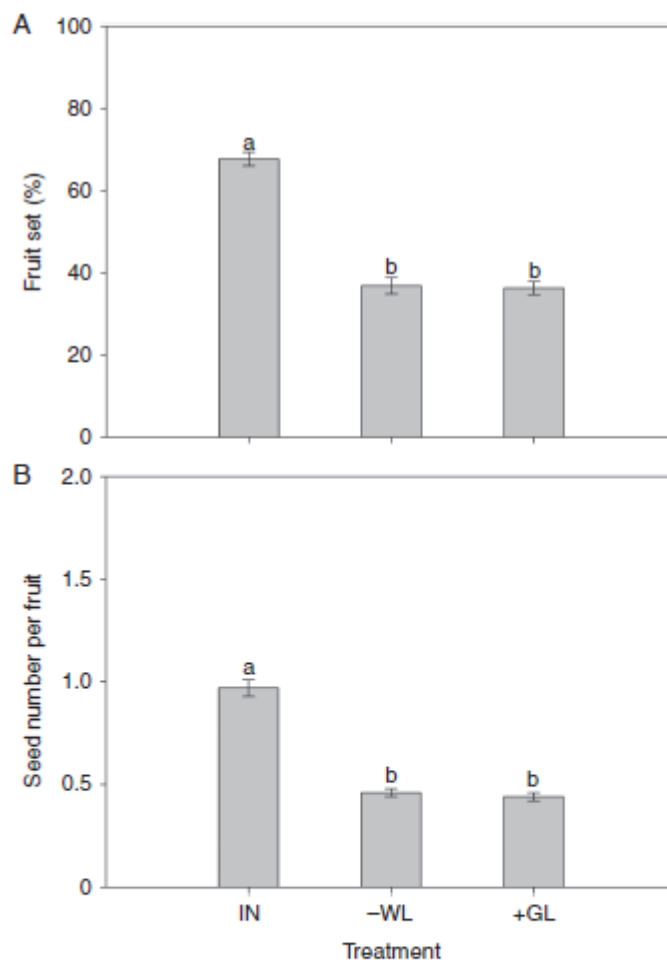


FIG. 5. Fruit set (A) and seed set (B) of *Saururus chinensis* flowers subjected to three experimental treatments. Different letters denote significant differences at $P < 0.05$. IN, intact; -WL, leaves were removed just before whitening; +GL, leaves were covered by green leaves just before whitening.

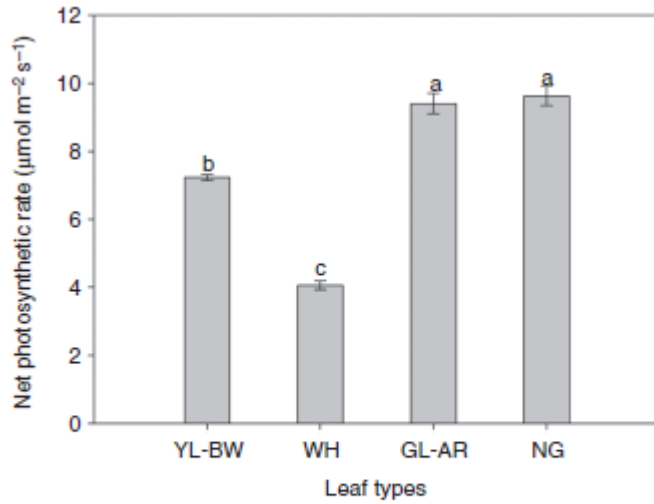


FIG. 6. Net photosynthetic rates of leaves of different colour. Different letters denote significant differences at $P < 0.05$. YL-BW, young green leaves before whitening; WH, white leaves; GL-AR, green leaves after re-greening; NG, normal green leaves.

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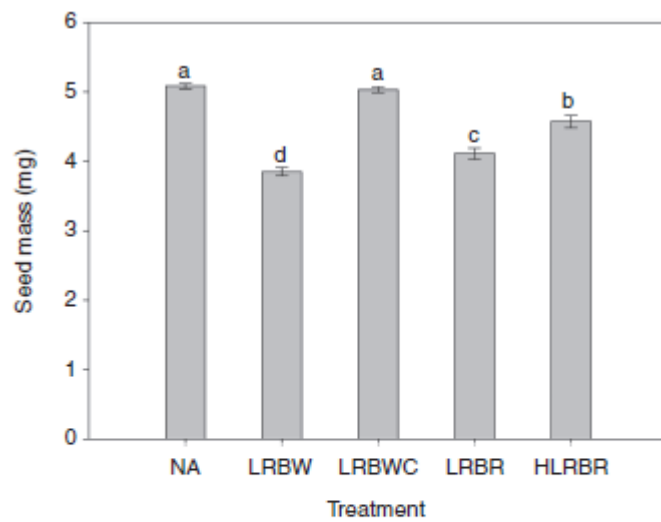


FIG. 7. Seed mass of *Saururus chinensis* flowers when the two or three apical leaves that will change colour were subjected to different experimental treatments. Flowers used for the experimental treatment were hand-pollinated. Different letters denote significant differences at $P < 0.05$. NA, natural; LRBW, leaves were removed just before whitening; LRBWC, leaves were trimmed ~1 mm from their edge just before whitening; LRBR, white leaves were removed just before re-greening; HLRBR, half of each of the white leaves was removed just before re-greening.

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