

1 **Cloning capacity helps tropical seeds counter animal predation**

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26 **Abstract**

27 Seed predators have the potential to act as agents of natural selection that
28 influence seed traits. Accordingly, plants deploy a variety of mechanisms (e.g.
29 resistance and tolerance strategy) to lessen the impact of predation on seed crop or on
30 an individual seed. In this study, we found a novel mechanism (i.e. cloning strategy)

31 in a tropical plant species in countering animal predation. We found both rodent
32 damaged and human artificially damaged seed fragments of a large-seeded tree
33 *Garcinia xanthochymus* in the Xishuangbanna tropical forest of China could develop
34 into seedlings in both field and laboratory conditions. *G. xanthochymus* seed has no
35 endosperm in seeds, and its seed tissue own strong capacity of differentiation and
36 cloning. Seed damage would negatively affect seedling growth and germination, but
37 the seed germination rate was remarkably high. Our study suggests that, as a novel
38 strategy countering animal predation, seed cloning would play a significant role in
39 stabilizing the mutualism between plant and animals.

40

41 **Key words:** cloning strategy, mutualism, rodent, seed dispersal, seedling
42 establishment, seed predation

43

44 **Introduction**

45 Seed dispersal is an essential stage in plant regeneration (Jansen *et al.*, 2014;
46 Garzon-Lopez *et al.*, 2015). Many plants rely heavily on animals for seed dispersal
47 (Vander Wall, 2001; Lichti *et al.*, 2017). Most of these plants attract animals for seed
48 dispersal by producing large amounts of nutrient-rich seeds (Wang *et al.*, 2016;
49 Corlett *et al.*, 2017). While animals feed on seeds in order to obtain their required
50 energy, seeds of the plant are dispersed away from their parent trees (Hollander
51 & Vander Wall, 2004; Hirsch *et al.*, 2012). Dispersed seeds are often scatter-hoarded
52 in soil that will contribute to seedling establishment if they escape from predation by
53 rodents (Briggs *et al.*, 2009). However, predation on plant seeds by animals is often
54 very high (Hulme & Hunt, 1999; Blendinger & Diaz-Velez, 2010), which may
55 destabilize the mutualism between plants and animals. Therefore, seed predation by
56 animals should be an important selection pressure on the evolution of seed traits
57 (Janzen, 1971). Accordingly, plants may have evolved a variety of mechanisms to
58 counter predation, so as to minimize the negative impacts of animal predation on seed
59 survival, and then to stabilize the mutualism between plants and rodents.

60 Plants are known to adopt several mechanisms for countering the seed predation

61 by animals, including mechanisms of resistance (Grubb *et al.*, 1998; Zhang & Zhang,
62 2008; Chen *et al.*, 2012) and tolerance (Dalling & Harms, 1999; Vallejo-Marín *et al.*,
63 2006; Xiao *et al.*, 2007; Perea *et al.*, 2011). Resistance mechanisms involve physical
64 traits (e.g., thick, spiny fruits, hard seed coats) or chemical traits (toxic chemical
65 compounds) that reduce consumption of plant seeds and/or negatively affect
66 predator's performance (Strauss & Agrawal, 1999; Guimaraes *et al.*, 2003; Shimada *et al.*,
67 2015; Zhang *et al.*, 2016). However, excessively high defensive traits of seeds
68 may reduce dispersal effectiveness by animals (Vander Wall, 2010; Zhang *et al.*,
69 2016). Thus, plants developed tolerant seed traits to increase seed survival under
70 extensive animal predation (Xiao *et al.*, 2007; Loayza *et al.*, 2014). Tolerance
71 mechanisms mean that the seed is able to germinate and establish an intact seedling
72 after being partially eaten or damaged by animals (Dalling & Harms, 1999;
73 Vallejo-Marín *et al.*, 2006). Seeds with strong tolerance to animal predation are often
74 large and contain rich nutrient in the endosperm or cotyledons, which make it possible
75 for partially damaged seeds to successfully germinate and establish into seedlings
76 (Mendoza & Dirzo, 2009; Perea *et al.*, 2018).

77 The embryo in a seed is essential for seed germination and seedling
78 establishment, but it is easily destroyed by animals. In nut-bearing trees, particularly
79 in oak (genus *Quercus*) acorns, embryo damage is common and has been, in general,
80 considered equivalent to seed death (Steele *et al.*, 2001; Branco *et al.*, 2002; Xiao *et al.*,
81 2009; Perea *et al.*, 2011; Yang *et al.*, 2012), resulting in a transition from
82 mutualism to predation. However, a few studies have documented that survival and
83 germination of embryo-damaged acorns can occur if the vulnerable embryo is
84 partially retained, but with significantly lower germination rates as compared to intact
85 acorns (McEuen & Steele, 2005; Yi & Yang 2010; Xiao & Zhang 2010; Bartlow *et al.*,
86 2018). Seed with proportionally larger embryos (radicle plus plumule) provide greater
87 tolerance to seed damage by rodents, allowing successful germination (Perea *et al.*,
88 2018). A few studies reported that some plant species had seed fragments that were
89 able to establish into seedlings after cutting by rodents (Cao *et al.*, 2011) or by people
90 (Joshi *et al.*, 2006; Teixeira & Barbedo, 2012), suggesting seeds may possess

91 regeneration capacity in order to counter for animal predation.

92 Cloning is a common phenomenon in plants for asexual reproduction. In many
93 plant species, tissues of leaves, roots or stems can easily develop into seedlings
94 (Thorpe, 2007). However, the cloning capacity of seeds and its link to animal
95 predation has been rarely quantified. In this study, we found a tropical plant species
96 *Garcinia xanthochymus* that developed a novel mechanism of seed cloning (meaning
97 that any part of the seed tissue can develop into seedlings) for countering seed
98 predation by animals. *G. xanthochymus* is an evergreen tree in Xishuangbanna
99 tropical forests in Yunnan, China. Seeds of *G. xanthochymus* are large (average
100 weight 4.35 g), with a thin testa (< 1mm) and low tannin (1.56%), and also rich in
101 nutrients (up to 28.26% fat content). Our previous field study showed that rodents
102 prefer to eat and hoard seeds of *G. xanthochymus* (Wang *et al.*, 2019). Seed fragments
103 are often left on the ground after rodent predation (Wang *et al.*, 2019). The rate of
104 seedling recruitment was over 20% under rodent predation in field conditions, two
105 times larger than that of the sympatric tree *Scleropyrum wallichianum* (Wang *et al.*,
106 2019), suggesting *G. xanthochymus* had a high level mutualistic relationship with
107 rodents. Our artificial cutting and enclosure tests indicated that *G. xanthochymus*
108 possessed the cloning capacity to account for rodent predation. We quantified seed
109 consumption and hoarding behavior of rodents on *G. xanthochymus* seeds in
110 enclosures and tested the germination success of seed fragments in the field by
111 simulating partial seed predation. We also conducted in vitro tissue culture
112 experiments to examine the cloning capacity of *G. xanthochymus* seeds.

113

114 **Materials and methods**

115 **Study site and species**

116 We conducted this study in the Menglun Nature Reserve, Xishuangbanna,
117 Yunnan, China. This region is dominated by a typical tropical monsoon climate with
118 distinct rainy (May to October) and dry seasons (November to April). The average
119 annual temperature of this area is 21.8 °C. The annual precipitation varies from 1200
120 to 1700 mm, of which approximately 85% occurs during the rainy season. The dry

121 season is characterized by heavy radiation fog, which supplements the deficiency of
122 rainfall in this season (Zhang & Cao, 1995).

123 *G. xanthochymus* is an evergreen tree that is widely distributed in the
124 Xishuangbanna tropical forests in southwest China. Its flowering period is from
125 March to May, ripe fruit is available from September through November. Fruit crop
126 per individual tree is highly variable, ranging from 31 to 357 fruits (average $256 \pm$
127 143 , mean \pm SD, $n = 8$). Ripe fruits are globose or ovoid, fleshy, yellow berries that
128 generally contain 1–3 arillate seeds. The fruit mass is 118.04 ± 26.15 g, fruit length is
129 6.21 ± 0.64 cm, fruit diameter is 6.01 ± 0.56 cm ($n = 60$). Seeds are oblong or ovoid,
130 seed mass is 4.35 ± 0.9 g, seed length is 3.01 ± 0.75 cm, seed diameter is 1.67 ± 0.25
131 cm ($n = 60$).

132 Fruits of the genus *Garcinia* possess a sugary water-rich pulp which attract
133 frugivorous vertebrates, such as elephants, deer and primates (Stevenson *et al.*, 2000;
134 Kitamura *et al.*, 2002). Due to extensive human disturbances in the tropical forest in
135 the Xishuangbanna region during past decades, populations of large vertebrates have
136 significantly declined, or even suffered local extinction. By using infrared cameras,
137 we found there were very few large vertebrates visiting the fruiting trees; piles of *G.*
138 *xanthochymus* fruit were found rotten under parent trees. Many small rodents (e.g.
139 *Maxomys surifer* and *Niviventer confucianus*) were frequently found to eat and
140 remove the *G. xanthochymus* seeds (Wang *et al.*, 2019). These scatter-hoarding
141 rodents play a significant role in seed dispersal and seedling recruitment of *G.*
142 *xanthochymus*. Germination was seen from the seed fragments in field, suggesting
143 their seeds might possess cloning capacities (personal observation).

144 **Seed collection**

145 Seeds were obtained from mature *G. xanthochymus* fruit in October 2010 from
146 eight trees. All fruit was subsequently taken to the laboratory, where we manually
147 removed seeds from the fruit, without damaging the seed coat. We used only intact
148 seeds for our experiments.

149 **Seed predation and hoarding by rodents**

150 We studied seed predation and hoarding behavior of two dominant rodent

151 species, red spiny rats *M. surifer* and Chinese white-bellied rats *N. confucianus* on
152 seeds of *G. xanthochymus* by using four 10 × 10 m semi-natural enclosures (for
153 details of the enclosure see: Wang *et al.*, 2018). To prevent rodents escaping from the
154 enclosures, and to prevent other animals from entering it, the walls of the enclosures
155 were built using concrete and poured 0.5 m below the ground surface level. The top of
156 the enclosures were covered with a plastic sheet to prevent rain from entering during
157 the experiments. One underground nest was provided for the rodents in the corner of
158 each enclosure. We captured rodents in the field using live traps (L × W × H = 30 ×
159 14 × 14 cm) made of steel wire mesh. Each trap was baited with fresh peanuts and
160 checked before sunrise and sunset. The species name, body weight and reproductive
161 status of each captured animal were recorded before bringing them back to laboratory.
162 Pregnant females or juveniles were immediately released in the field in situ. All adult
163 animals were kept individually in a cage (L × W × H = 40 × 25 × 30 cm) and
164 provided with adequate food, water and nest material. A photoperiod cycle of 12:12 h
165 (light:dark) was maintained. During the experiments, one animal was placed in the
166 enclosure and observed for two consecutive days. The animal was provided with
167 laboratory food on the first day in order to ease adaptation to the new environment.
168 On the second day, twenty intact seeds of *G. xanthochymus* were placed in the center
169 of enclosure. Seeds were marked by attaching a small coded plastic tag to each seed
170 through a thin steel thread (for details see: Wang *et al.*, 2018). Seeds were classified
171 as eaten seeds, scatter-hoarded seeds or larder-hoarded seeds by following Wang *et al.*
172 (2018). Seed fragments of *G. xanthochymus* after being partially eaten by rodents in
173 this experiment were collected and used for the following field germination
174 experiments. Ten healthy adult *N. confucianus* (4 females and 6 males, average body
175 weight 85.4 ± 9.7 g) and sixteen *M. surifer* (9 females and 7 males, average body
176 weight 117.9 ± 10.9 g) were used in this experiment.

177 **Germination of rodent and human artificially damaged seed fragments**

178 In October 2010, we set up a small enclosure for germination experiments in the
179 gully rain forest in the Xishuangbanna Tropical Botanical Garden of the Chinese
180 Academy of Sciences. The enclosure (L × W × H = 3 × 1.2 × 0.6 m) was surrounded

181 and covered by wire mesh (mesh size = 1 × 1 cm) and extended 20 cm into the soil to
182 prevent from entry of vertebrates, especially rodents. The enclosure was divided into
183 small 10 × 10 cm grids by using nylon ropes, one grid square was used for one seed.
184 To study the consequences of partial seed predation on the germination and seedling
185 survival of *G. xanthochymus* seeds, three artificial cutting treatment groups (the left
186 seeds after artificial cutting were defined as the human damaged seed fragments) were
187 conducted: (1) one end of the intact seed was removed, the left seed part was retained;
188 (2) both ends of the intact seed were removed and the middle part was left retained; (3)
189 the middle part of the seed was removed, and the left seed was kept (Fig. 1). Each
190 treatment consisted of 60 seed fragments. To see the size effect of the seed fragment
191 on germination rate and growth, each treatment group was divided into three
192 sub-groups, with the proportion of removed part being controlled into three categories:
193 <25%, 25%–50%, and >50%. The sample size of each subgroup was 20 seeds. Each
194 seed was individually weighed to calculate the mass removed after artificial cutting.
195 In addition to the artificial cutting treatment, a control group consisting of 20 intact
196 seeds and a rodent predation group consisting partially eaten seeds by rodents (here
197 defined as the rodent damaged seed fragments) that were collected in the enclosure
198 experiments were also tested. In November 2010, all tested seeds were sown
199 individually in the small grids of the enclosure. A coded plastic tag (2.5 × 3.6 cm) was
200 attached to the nylon ropes of the small grids to distinguish the seeds of each
201 treatment group. In order to simulate the scatter-hoarding behavior of rodents, we
202 buried the tested seeds in the shallow surface and covered them with a layer of fallen
203 leaves. The dormancy period for *G. xanthochymus* seeds is more than four months.
204 From the end of March 2011, seeds were monitored for germination and sprouting,
205 and after seedling establishment, leaf production and growth in height were measured
206 once a week. We recorded the time of germination and the percentage of germination
207 for intact seeds, human damaged seed fragments and rodent damaged seed fragments.
208 A seed or its fragment was defined as germinated when a bud grew out of the soil and
209 was clearly visible. We also quantified seedling establishment rates when germinated
210 seeds produced a main stem with its first leaf.

211 **Laboratory germination experiments**

212 We removed the epidermis of *G. xanthochymus* seeds and planted them in
213 nutritious soil to observe their germination and seedling establishment. The culture
214 room temperature was maintained at 30 °C and the photoperiod cycle was 12:12 h
215 (light:dark).

216 **Laboratory tissue culture experiments**

217 We studied cloning capacity of human damaged seed fragments of *G.*
218 *xanthochymus* through tissue culture in laboratory. Firstly, we removed the testa of
219 intact seeds, then soaked them in 70% alcohol for 30 s. Secondly, using sterile
220 distilled water, we rinsed the seed twice, then transferred it to 0.1% mercuric chloride
221 solution for 15 min. Thirdly, we rinsed seeds with sterile distilled water five times,
222 then blotted the moisture with sterile filter paper. After cutting seeds into two halves
223 longitudinally, we put them in 1/2 MS (Murashige and Skoog medium) medium for
224 culturing. During the cultivation process, we found that two seedlings could be
225 formed on the top of the longitudinally sliced seed; thus, we cross-cut the seed and cut
226 both ends longitudinally into eight small pieces (Fig. 1), and placed the pieces on
227 medium supplemented with different cytokinin (BA, 6-Benzylaminopurine) and auxin
228 (NAA, 1-Naphthylacetic acid) with matching ratios (Table 1). We evaluated the
229 effects of different mediums on plant regeneration and counting the number of
230 regenerated plants. Eight seeds were used as replicates for each medium. The medium
231 in this experiment included the following four types: (1) 1/2 MS; (2) 1/2 MS + 0.5
232 mg L⁻¹ 6-BA + 0.25 mg L⁻¹ NAA; (3) 1/2 MS + 0.5 mg L⁻¹ 6-BA; and (4) 1/2 MS +
233 0.25 mg L⁻¹ NAA (Table 1). We added 15 g L⁻¹ sucrose and 7 g L⁻¹ agar into the
234 medium, adjusted PH value to 5.8, then sterilized them for 20 minutes at 121 °C. A
235 photoperiod cycle of 12:12 h (light:dark) and temperature 25 ± 2 °C was maintained
236 in the culture room. Test materials were subcultured every two weeks. After
237 subculturing for about two months, complete plants were formed.

238 **Statistical analysis**

239 The difference between seed germination rate (binomial distribution) or seedling
240 height between treatments (control, cut one end, cut middle part, and cut both ends)

241 was compared by Generalized Linear Model (GLM). We also used GLM to analyze
242 the effects of seed mass lost (%) or seed mass retained (g) after artificial seed cutting,
243 considering treatments as a factor variable, on seed germination rate (binomial
244 distribution) and seedling height, respectively. All data was analyzed using R
245 software (R Core Team, 2017).

246

247 **Results**

248 **Seed predation and hoarding by rodents**

249 Sixteen red spiny rats (*M. surifer*) harvested 15.9 ± 1.5 (mean \pm SE) seeds of *G.*
250 *xanthochymus* within 24 h; ate 2.3 ± 0.5 seeds, and scatter-hoarded 13.6 ± 1.4 seeds.
251 Ten Chinese white-bellied rats (*N. confucianus*) harvested 7.3 ± 1.0 seeds of *G.*
252 *xanthochymus* within 24 h, ate 2.4 ± 0.3 seeds and scatter-hoarded 1.6 ± 0.5 seeds. A
253 total of 37 seeds were eaten by the red spiny rats, of which 27 were partially eaten
254 (73%). The white-bellied rats consumed a total of 24 seeds, of which 15 were partially
255 eaten (62.5%).

256 **Germination of rodent damaged seed fragments in field conditions**

257 The intact *G. xanthochymus* seeds germinated easily under natural environmental
258 conditions, with a germination rate of 95% (19/20), and all germinated seeds or 100%
259 (19/19) established into healthy seedlings. In contrast, the germination rate and
260 seedling establishment rate of rodent damaged seed fragments (collected from the
261 enclosures after rodent predation) was 38.09% (16/42) and 93.75% (15/16),
262 respectively. There was significant difference of germination rate between the control
263 seeds and rodent damaged seed fragments ($P < 0.05$).

264 **Germination of human artificially damaged seed fragments in field conditions**

265 Artificially cutting seeds into fragments had a significant effect on the seed
266 germination rate of *G. xanthochymus* (Wald test, $\chi^2 = 14.349$, $df = 3$, $P = 0.002$).
267 Cutting both ends of intact seeds significantly reduced the germination success of *G.*
268 *xanthochymus* seeds as compared with the control group ($P = 0.018$); there was no
269 significant difference of germination rate between the other treatments and the control
270 group (all $P > 0.05$) (Fig. 1a).

271 The seed mass after artificial cutting had a significant positive effect on the
272 germination rate ($z = 4.501$, $df = 199$, $P < 0.001$) (Fig. 2a). While the percentage of
273 seed mass loss by artificial cutting had a significant negative effect on seed
274 germination rate ($z = -4.842$, $df = 199$, $P < 0.001$) (Fig. 2b).

275 Artificial cutting treatment had a significant effect on *G. xanthochymus* seedling
276 growth (as measured by plant height) ($F = 3.122$, $df = 3$, $P = 0.028$). Cutting both
277 ends of intact *G. xanthochymus* seeds had lower seedling height than the control
278 group ($P < 0.05$); however, there was no significant difference of seedling height
279 between the other treatments and the control group (all $P > 0.05$) (Fig. 1b).

280 The weight of seed mass after artificial cutting had a significant positive effect
281 on seedling height. With an increase in seed weight, seedling height increased
282 significantly ($F = 41.26$, $df = 1$, $P < 0.001$) (Fig. 2c). There was a significant
283 interaction between artificial cutting treatments and seed mass after artificial cutting
284 on seedling height ($F = 3.443$, $df = 3$, $P = 0.018$) (Fig. 3a).

285 Seed mass loss also negatively affected seedling height ($F = 57.69$, $df = 1$, $P <$
286 0.0018) (Fig. 2d). There was a significant interaction between artificial cutting
287 treatments and the percentage of seed mass loss on seedling height ($F = 4.6528$, $df = 2$,
288 $P = 0.011$) (Fig. 3b).

289 **Germination of human artificially damaged seed fragments in cultured** 290 **conditions**

291 In laboratory conditions, when intact seeds were buried in soil and germinated,
292 they became reddish-brown, with 2 to 4 pairs of sterile leaves growing up from the
293 seeds (Fig. 4a). Seeds did not segregate from seedlings after germination (Fig. 4b).
294 Both the young stem and the main root bud sprouted linearly from one end of the
295 cotyledon at the same time, and then the weaker roots emitted at the other end (Fig.
296 4b). The whole plant could be formed after the germinating seeds were transplanted
297 into nutrient-rich soil (Fig. 4c).

298 Intact seeds were cultured on 1/2 MS medium after longitudinal cutting. Buds
299 were observed in the second week (Fig. 5a), and sub-cultured at about two months,
300 they could form intact seedlings (Fig. 5b). The survival rate of these seedlings when

301 transplanted in nutrient-rich soils exceeded 90% (n = 10) (Fig. 5c).

302 After one *G. xanthochymus* seed was cross-cut into eight small pieces (see Fig.
303 6a), the pieces were cultured in a 1/2 MS medium supplemented with cytokinin (BA)
304 and auxin (NAA) in matching ratios (Table 1). After one week, the top part of the
305 seeds cultured in these media were the first to grow small buds (Fig. 6b, c). Then after
306 a subculture, small buds appeared one after another in the middle part of the seed (Fig.
307 6d, e) and at the base (the end with the umbilicus) (Fig. 6f, g). Seed fragments
308 cross-cut from one single seed could establish one to five whole seedlings. The small
309 buds grew faster and produced more seedlings on 1/2 MS medium supplemented with
310 BA and NAA. The other three treatments had little difference in the number of plants
311 produced (Table 1).

312 During the cultivation process, we found that both the seed epidermis and the
313 inside could germinate small buds and develop into normal plants. Many bud spots
314 could sprout in the seed epidermis (Fig. 7a, b, c), but only a few buds could develop
315 into normal plants (Fig. 7b). There are fewer small buds that germinate inside the seed
316 and all will form a complete plant (Fig. 7d, e, f).

317

318 **Discussion**

319 Our study indicates that seeds of *G. xanthochymus* have developed cloning
320 capacity to counter animal predation. Both rodent damaged and human damaged seed
321 fragments of *G. xanthochymus* were able to germinate and established into normal
322 seedlings, even with up to a 75% loss of their seed mass. The unique seed properties
323 of *G. xanthochymus*, such as having no endosperm and with the embryonic axis
324 extending along the entire seed, might contribute to the cloning capacity. We found
325 that all seed tissues contained embryo cells. Our study revealed this novel mechanism
326 of cloning capacity in these plants in order to counter animal predation, which likely
327 helps to stabilize the mutualism between plants and animals.

328 The partial consumption of seeds by animals usually reduces seed vigor and
329 germination success, and it is unfavorable to subsequent growth and development of
330 seedlings (Janzen, 1976; Branco *et al.*, 2002; Jansen *et al.*, 2006; Xiao *et al.*, 2009;

331 Perea *et al.*, 2011). Seeds of many plant species are highly tolerant to animal's partial
332 consumption (Cao *et al.*, 2011; Teixeira & Barbedo, 2012). The germination success
333 and growth performance of damaged seeds largely depend on the structural
334 characteristics of the seeds and the degree of damage to the seeds (Yi & Yang 2010;
335 Teixeira & Barbedo 2012; Zhang *et al.*, 2014). Some plant seeds are not able to
336 germinate under more than 10% weight loss (Vallejo-Marín *et al.*, 2006), while others
337 are able to germinate and successfully develop into normal seedlings with up to 60%
338 of their reserves removed, provided that the vulnerable embryonic parts are not
339 damaged (Giertych & Suszka, 2011; Yi *et al.*, 2015). In our study, we found that intact
340 *G. xanthochymus* seeds germinate easily under natural environmental conditions, and
341 all the germinated seeds established into healthy seedlings. Although artificial cutting
342 (simulating partial consumption by rodents) reduced germination rate and growth of
343 seedlings, the germination rate (83.33%) were remarkably high. Many seeds with up
344 to 75% of their reserves removed successfully germinated and established into healthy
345 seedlings. This finding implied that the cloning capacity of *G. xanthochymus* seed
346 may be an adaptation to animal predation.

347 The tolerance strategy is widely used by plants to counter animal predation. It
348 emphasizes that the embryo of a seed retains the capacity of establishing a seedling
349 even if it is partially damaged by predators (Dalling & Harms, 1999; Vallejo-Marín *et*
350 *al.*, 2006). In contrast to the tolerance strategy, our study indicated that any parts of
351 the *G. xanthochymus* seed could become a seedling, suggesting that seeds of *G.*
352 *xanthochymus* evolved a cloning capacity to counter animal predation. In this study,
353 the cloning strategy was defined here in order to show a different strategy from the
354 tolerance strategy. The cloning strategy emphasizes that a single seed is able to
355 produce more seedlings under heavy predation by animals.

356 The cloning capacity of *G. xanthochymus* seeds may be closely related to its
357 unique structural characteristics. Its seed has no endosperm, with the embryonic axis
358 extending along the entire seed. Partial consumption by rodents (or artificially cutting)
359 does not destroy the embryo because all tissues contain embryo cells. This
360 observation was further confirmed in laboratorial tissue culture experiments. Seeds of

361 *G. xanthochymus* were placed in a medium after being cut into many parts and both
362 the skin and the interior of the seeds could sprout to produce intact seedlings. In our
363 field germination experiments, we found only a few seeds failed to germinate, and
364 some seedlings occasionally died after germination, probably due to infection of
365 saprophytic microorganisms or pathogens or a deficiency in nutrition. It is notable
366 that seed germination rate (38.09%) from the seed fragments of *G. xanthochymus*
367 collected from the enclosures was much lower than that of human artificially cut seed
368 fragments (83.33%); this is probably because the rodent damaged seed fragments
369 were temporarily stored in the incubator for a few days before the tests, which may
370 have reduced vitality.

371 For simulated rodent damaged seed fragments, the greater the seed mass retained,
372 the higher the germination probability was, which is consistent with several previous
373 studies (Bonfil, 1998; Strauss & Agrawal, 1999; Mendoza & Dirzo, 2009). In addition,
374 we also found seed mass retained after human artificially cutting was significantly and
375 positively correlated with the height of seedlings after germination, while seed mass
376 loss was significantly negatively correlated with the height of seedlings after
377 germination. These results indicated that the nutrients stored in the seeds are not only
378 used for germination, but also used to support the subsequent growth and
379 development of the seedlings (Kennedy *et al.*, 2004; Yi *et al.*, 2015). Seed loss caused
380 by human artificial cutting had an adverse effect on the growth of the seedlings
381 (Mendoza & Dirzo, 2009). The large seed fragments with relatively large reserves
382 grew faster and the seedlings were taller. Many studies have demonstrated that the
383 time when the remaining part of the seed that was attached to the seedling was
384 removed significantly affects survival and growth of seedlings (Garcia-Cebrian *et al.*,
385 2003).

386 Animals not only consume seeds, but also disperse and cache seeds. Thus, tree
387 species and animals form a mutualistic relationship (Wang *et al.*, 2014). However,
388 over predation of seeds damages mutualism between seeds and rodents, by shifting
389 the mutualism to a predation interaction (Lichti *et al.*, 2017). Anti-predation or
390 tolerance strategies are known to alleviate seed predation by animals, which helps

391 stabilize the mutualism between plants and animals. In this study, we found that in
392 both enclosure and field conditions, rodents preferred to eat and hoard *G.*
393 *xanthochymus* seeds from the mother tree. Many *G. xanthochymus* seeds were seen to
394 be partially consumed by small rodents, which resulted in secondary dispersal of the
395 *G. xanthochymus* seeds (Wang *et al.*, 2019). As compared to intact seeds, these
396 partially eaten seeds were mostly discarded and were rarely eaten or hoarded again by
397 rodents. Because rodent damaged seed fragments of *G. xanthochymus* had cloning
398 capacities, these partially damaged seed fragments were able to grow into seedlings.
399 Indeed, the seedling recruitment rate of tagged seeds released in field was over 20%
400 under rodent predation in field conditions, far higher than most plant species studied in
401 this region (Cao *et al.*, 2011; Wang *et al.*, 2019). Therefore, the cloning capacity of
402 seeds plays an important role in stabilizing mutualism between plants and animals,
403 and needs further investigation.

404

405 **Data Accessibility Statement**

406 We archived our data in Dryad and would share these data after the article is
407 accepted.

408

409 **Competing Interests**

410 We declare that no part of this study has been published elsewhere or being sent
411 to other places for publications. There is no conflict of interests in this study.

412

413 **Author Contributions**

414 Wang ZY and Zhang ZB conceived and designed the experiments. Wang ZY,
415 Niu YD performed the experiments. Wang ZY and Yan C analyzed the data. Wang
416 ZY and Zhang ZB wrote the manuscript; Cao L and Chong K provided editorial
417 advice.

418

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426 **References**

- 427 Bartlow AW, Agosta SJ, Curtis R, Yi XF, Steele MA. 2018. Acorn size and tolerance
428 to seed predators: the multiple roles of acorns as food for seed predators, fruit for
429 dispersal and fuel for growth. *Integrative Zoology* **13**:251–266.
- 430 Blendinger PG, Diaz-Velez MC. 2010. Experimental field test of spatial variation in
431 rodent predation of nuts relative to distance and seed density. *Oecologia*
432 **163**:415–423.
- 433 Bonfil C. 1998. The effects of seed size, cotyledon reserves, and herbivory on
434 seedling survival and growth in *Quercus rugosa* and *Q. laurina* (Fagaceae).
435 *American Journal of Botany* **85**:79–87.
- 436 Branco M, Branco C, Merouani H, Almeida MH. 2002. Germination success, survival
437 and seedling vigour of *Quercus suber* acorns in relation to insect damage. *Forest*
438 *Ecology and Management* **166**:159–164.
- 439 Briggs JS, Vander Wall SB, Jenkins SH. 2009. Forest rodents provide directed
440 dispersal of Jeffrey pine seeds. *Ecology* **90**:675–687.
- 441 Cao L, Xiao ZS, Wang ZY, Guo C, Chen J, Zhang ZB. 2011. High regeneration
442 capacity helps tropical seeds to counter rodent predation. *Oecologia* **166**: 997–
443 1007.
- 444 Chen X, Cannon CH, Conklin-Brittan NL. 2012. Evidence for a trade-off strategy in
445 Stone Oak (*Lithocarpus*) seeds between physical and chemical defense highlights
446 fiber as an important antifeedant. *PLOS One* **7**: e32890.
- 447 Corlett RT. 2017. Frugivory and seed dispersal by vertebrates in tropical and
448 subtropical Asia: An update. *Global Ecology and Conservation* **11**:1–22.
- 449 Dalling JW, Harms KE. 1999. Damage tolerance and cotyledonary resource use in the

450 tropical tree *Gustavia superba*. *Oikos* **85**:257–264.

451 Garcia-Cebrian F, Estesó-Martínez J, Gil-Pelegrin E. 2003. Influence of cotyledon
452 removal on early seedling growth in *Quercus robur* L. *Annals of Forest Science*
453 **60**:69–73.

454 Garzon-Lopez CX, Ballesteros-Mejía L, Ordoñez A, Bohlman SA, Olf H, Jansen PA.
455 2015. Indirect interactions among tropical tree species through shared rodent
456 seed predators: a novel mechanism of tree species coexistence. *Ecology Letters*
457 **18**:752–760.

458 Grubb PJ, Metcalfe DJ, Grubb EAA, Jones GD. 1998. Nitrogen-richness and
459 protection of seeds in Australian tropical rainforest: a test of plant defence theory.
460 *Oikos* **82**:467–482.

461 Guimaraes PR, Jose J, Galetti M, Trigo JR. 2003. Quinolizidine alkaloids in *Ormosia*
462 *arborea* seeds inhibit predation but not hoarding by agoutis (*Dasyprocta*
463 *leporina*). *Journal of Chemical Ecology* **29**:1065–1072.

464 Hirsch BT, Kays R, Pereira VE, Jansen PA. 2012. Directed seed dispersal towards
465 areas with low conspecific tree density by a scatter-hoarding rodent. *Ecology*
466 *Letters* **15**:1423–1429.

467 Hollander JL, Vander Wall SB. 2004. Effectiveness of six species of rodents as
468 dispersers of singleleaf pinon pine (*Pinus monophylla*). *Oecologia* **138**:57–65.

469 Hou XG, Yi XF, Yang YQ, Liu WJ. 2010. Acorn germination and seedling survival of
470 *Q. variabilis*: effects of cotyledon excision. *Annals of Forest Science* **67**:711–
471 717.

472 Hulme PE, Hunt MK. 1999. Rodent post-dispersal seed predation in deciduous
473 woodland: predator response to absolute and relative abundance of prey. *Journal*
474 *of Animal Ecology* **68**:417–428.

475 Jansen PA, Bongers F, Prins HHT. 2006. Tropical rodents change rapidly germinating
476 seeds into long-term food supplies. *Oikos* **113**:449–458.

477 Jansen PA, Visser MD, Joseph Wright S, Rutten G, Muller-Landau HC. 2014.
478 Negative density dependence of seed dispersal and seedling recruitment in a
479 neotropical palm. *Ecology Letters* **17**:1111–1120.

480 Janzen DH. 1971. Seed Predation by Animals. *Annual Review of Ecology and*
481 *Systematics* **2**:465–492.

482 Janzen DH. 1976. Reduction of *Mucuna-Andreana* (Leguminosae) seedling fitness by
483 artificial seed damage. *Ecology* **57**:826–828.

484 Joshi G, Kumar ANA, Gowda B, Srinivasa YB, 2006. Production of
485 super-numbererary plants in *Garcinia gummi-gutta*: evolutionary implication of
486 mammalian frugivory. *Current Science* **91**:372–377.

487 Kennedy PG, Hausmann NJ, Wenk EH, Dawson TE. 2004. The importance of seed
488 reserves for seedling performance: an integrated approach using morphological,
489 physiological, and stable isotope techniques. *Oecologia* **141**:547–554.

490 Kitamura S, Yumoto T, Poonswad P, Chuailua P, Plongmai K, Maruhashi T, Noma N.
491 2002. Interactions between fleshy fruits and frugivores in a tropical seasonal
492 forest in Thailand. *Oecologia* **133**:559–572.

493 Lichti NI, Steele MA, Swihart RK. 2017. Seed fate and decision-making processes in
494 scatter-hoarding rodents. *Biological Reviews* **92**:474–504.

495 Loayza AP, Carvajal DE, Garcia-Guzman P, Gutierrez JR, Squeo FA. 2014. Seed
496 predation by rodents results in directed dispersal of viable seed fragments of an
497 endangered desert shrub. *Ecosphere* **5**:1–9.

498 McEuen AB, Steele MA. 2005. Atypical acorns appear to allow seed escape after
499 apical notching by squirrels. *American Midland Naturalist* **154**:450–458.

500 Mendoza E, Dirzo R. 2009. Seed Tolerance to Predation: Evidence from the Toxic
501 Seeds of the Buckeye Tree (*Aesculus Californica*; Sapindaceae). *American*
502 *Journal of Botany* **96**:1255–1261.

503 Perea R, Fernandes GW, Dirzo R. 2018. Embryo size as a tolerance trait against seed
504 predation: Contribution of embryo-damaged seeds to plant regeneration.
505 *Perspectives in Plant Ecology Evolution and Systematics* **31**:7–16.

506 Perea R, San Miguel A, Gil L. 2011. Leftovers in seed dispersal: ecological
507 implications of partial seed consumption for oak regeneration. *Journal of*
508 *Ecology* **99**:194–201.

509 Shimada T, Takahashi A, Shibata M, Yagihashi T. 2015. Effects of within-plant

510 variability in seed weight and tannin content on foraging behaviour of seed
511 consumers. *Functional Ecology* **29**:1513–1521.

512 Steele MA, Turner G, Smallwood PD, Wolff JO, Radillo J. 2001. Cache management
513 by small mammals: Experimental evidence for the significance of acorn-embryo
514 excision. *Journal of Mammalogy* **82**:35–42.

515 Stevenson PR, Quinones MJ, Ahumada JA. 2000. Influence of fruit availability on
516 ecological overlap among four neotropical primates at Tinigua National Park,
517 Colombia. *Biotropica* **32**:533–544.

518 Strauss SY, Agrawal AA. 1999. The ecology and evolution of plant tolerance to
519 herbivory. *Trends in Ecology & Evolution* **14**:179–185.

520 Teixeira CC, Barbedo CJ. 2012. The development of seedlings from fragments of
521 monoembryonic seeds as an important survival strategy for *Eugenia* (Myrtaceae)
522 tree species. *Trees-Structure and Function* **26**:1069–1077.

523 Thorpe TA. 2007. History of plant tissue culture. *Molecular Biotechnology* **37**:169–
524 180.

525 Vallejo-Marin M, Dominguez CA, Dirzo R. 2006. Simulated seed predation reveals a
526 variety of germination responses of neotropical rain forest species. *American*
527 *Journal of Botany* **93**:369–376.

528 Vander Wall SB. 2001. The evolutionary ecology of nut dispersal. *Botanical Review*
529 **67**:74–117.

530 Vander Wall SB. 2010. How plants manipulate the scatter-hoarding behaviour of
531 seed-dispersing animals. *Philosophical Transactions of the Royal Society*
532 *B-Biological Sciences* **365**:989–997.

533 Wang ZY, Cao L, Zhang ZB. 2014. Seed traits and taxonomic relationships determine
534 the occurrence of mutualisms versus seed predation in a tropical forest rodent
535 and seed dispersal system. *Integrative Zoology* **9**:309–319.

536 Wang ZY, Wang B, Yi XF, Yan C, Cao L, Zhang ZB. 2018. Scatter-hoarding rodents
537 are better pilferers than larder-hoarders. *Animal Behaviour* **141**:151–159.

538 Wang ZY, Wang B, Yi XF, Yan C, Zhang ZB, Cao L. 2019. Re-caching behaviour of
539 rodents improves seed dispersal effectiveness: Evidence from seedling

540 establishment. *Forest Ecology and Management* **444**:207–213.

541 Wang ZY, Zhang YH, Zhang DY, Li J, Yi XF. 2016. Nutritional and defensive
542 properties of Fagaceae nuts dispersed by animals: a multiple species study.
543 *European Journal of Forest Research* **135**:911–917.

544 Xiao ZS, Gao X, Jiang MM, Zhang ZB. 2009. Behavioral adaptation of Pallas's
545 squirrels to germination schedule and tannins in acorns. *Behavioral Ecology*
546 **20**:1050–1055.

547 Xiao ZS, Gao X, Steele MA, Zhang ZB. 2010. Frequency-dependent selection by tree
548 squirrels: adaptive escape of nondormant white oaks. *Behavioral Ecology*
549 **21**:169–175.

550 Xiao ZS, Harris MK, Zhang ZB. 2007. Acorn defenses to herbivory from insects:
551 Implications for the joint evolution of resistance, tolerance and escape. *Forest*
552 *Ecology and Management* **238**:302–308.

553 Xiao ZS, Zhang ZB, Wang YS. 2005. The effects of seed abundance on seed predation
554 and dispersal by rodents in *Castanopsis fargesii* (Fagaceae). *Plant Ecology*
555 **177**:249–257.

556 Yang YQ, Yi XF, Yu F. 2012. Repeated radicle pruning of *Quercus mongolica* acorns
557 as a cache management tactic of Siberian chipmunks. *Acta Ethologica* **15**:9–14.

558 Yi X F, Wang ZY, Liu CQ, Liu GQ, Zhang MM. 2015. Acorn cotyledons are larger
559 than their seedlings' need: evidence from artificial cutting experiments. *Scientific*
560 *Reports* **5**.

561 Yi XF, Yang YQ. 2010. Large acorns benefit seedling recruitment by satiating weevil
562 larvae in *Quercus aliena*. *Plant Ecology* **209**:291–300.

563 Zhang HM, Zhang ZB. 2008. Endocarp thickness affects seed removal speed by small
564 rodents in a warm-temperate broad-leafed deciduous forest, China. *Acta*
565 *Oecologica-International Journal of Ecology* **34**:285–293.

566 Zhang JH, Cao M. 1995. Tropical forest vegetation of Xishuangbanna, SW China and
567 its secondary changes, with special reference to some problems in local nature
568 conservation. *Biological Conservation* **73**:229–238.

569 Zhang MM, Dong Z, Yi XF, Bartlow AW. 2014. Acorns containing deeper plumule

570 survive better: how white oaks counter embryo excision by rodents. *Ecology and*
571 *Evolution* **4**:59–66.

572 Zhang ZB, Wang ZY, Chang G, Yi XF, Lu JQ, Xiao ZS *et al.* 2016. Trade-off between
573 seed defensive traits and impacts on interaction patterns between seeds and
574 rodents in forest ecosystems. *Plant Ecology* **217**:253–265.

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600 **Table 1** The number of cloning seedlings of *G. xanthochymus* seed after multiple cuts
601 and culturing in different mediums (BA is 6-benzylaminoadenine one kind of
602 cytokinin; NAA is 1-naphthylacetic acid one kind of auxin)

Culture mediums	Seeds (n)	Number of cloning seedlings (n)
1/2 MS	8	16
1/2 MS+0.5 mg L ⁻¹ 6-BA	8	18
1/2 MS+0.25 mg L ⁻¹ NAA	8	17
1/2 MS+0.5 mg L ⁻¹ 6-BA+0.25 mg L ⁻¹ NAA	8	26

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623 **Figure legends**

624 **Fig. 1** The germination rates (a) and the average seedling height (b) of seeds of *G.*
625 *xanthochymus* under the different treatments. * $p < 0.05$.

626

627 **Fig. 2** Relationship between germination rate and seed mass retained (a) or percentage
628 of seed mass loss (b) after human artificially cutting seeds of *G. xanthochymus*; and
629 relationship between seedling height and seed mass retained (c) or percentage of seed
630 mass loss (d) after human artificially cutting seeds of *G. xanthochymus*

631

632 **Fig. 3** Relationship between seedling height and seed mass retained after human
633 artificially cutting (a); relationship between seedling height and seed mass loss after
634 human artificially cutting (b).

635

636 **Fig. 4** Germination characteristics of intact *G. xanthochymus* seed (a); seedlings of *G.*
637 *xanthochymus* after planted in the nutrient soil (b, c).

638

639 **Fig. 5** Tissue culture of the *G. xanthochymus* seed in 1/2 MS medium after
640 longitudinal cutting: bud (a) and seedling (b); tissue culture seedlings after
641 transplanting to nutrient soil (c).

642

643 **Fig. 6** Seed of *G. xanthochymus* used for tissue culture after multiple cutting (a), and
644 germination characteristics of various parts of *G. xanthochymus* seed after multiple
645 cuts: germination from the top of seed (b, c); germination from the middle part of seed
646 (d, e); germination from the base of seed (f, g).

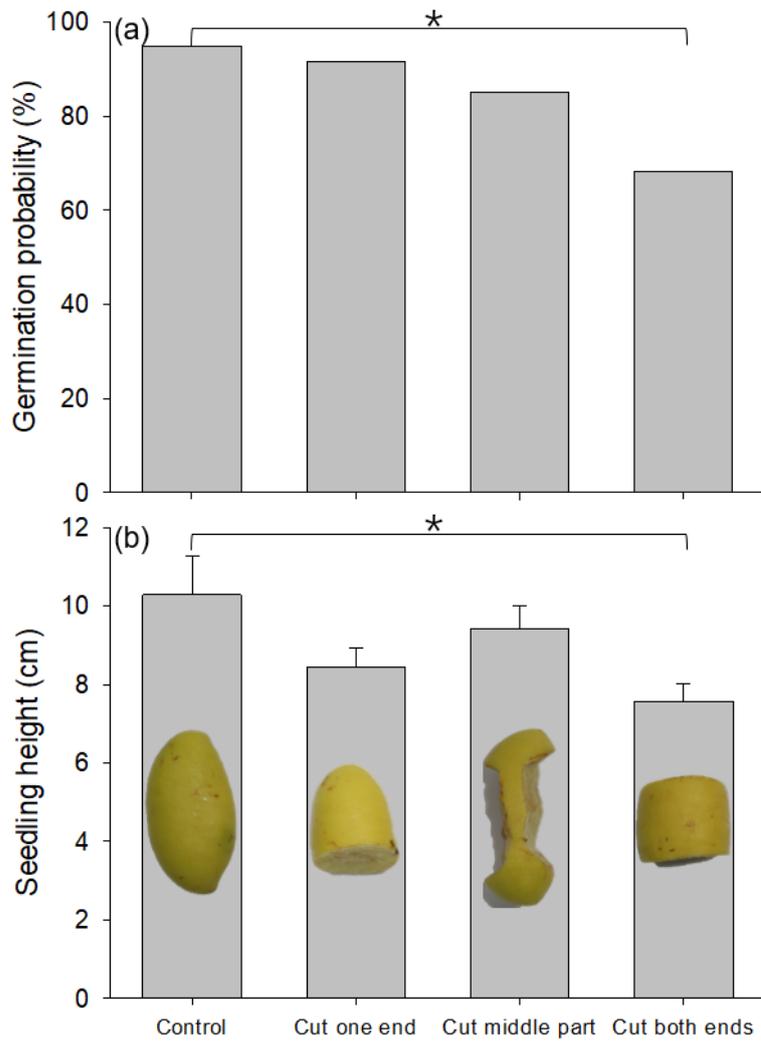
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648 **Fig. 7** Germination characteristics of various parts of *G. xanthochymus* seed after
649 multiple cuts: buds germinated from the skin of seeds (a, b, c); buds germinated from
650 the internal part of seeds (d, e, f).

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653 **Fig. 1**



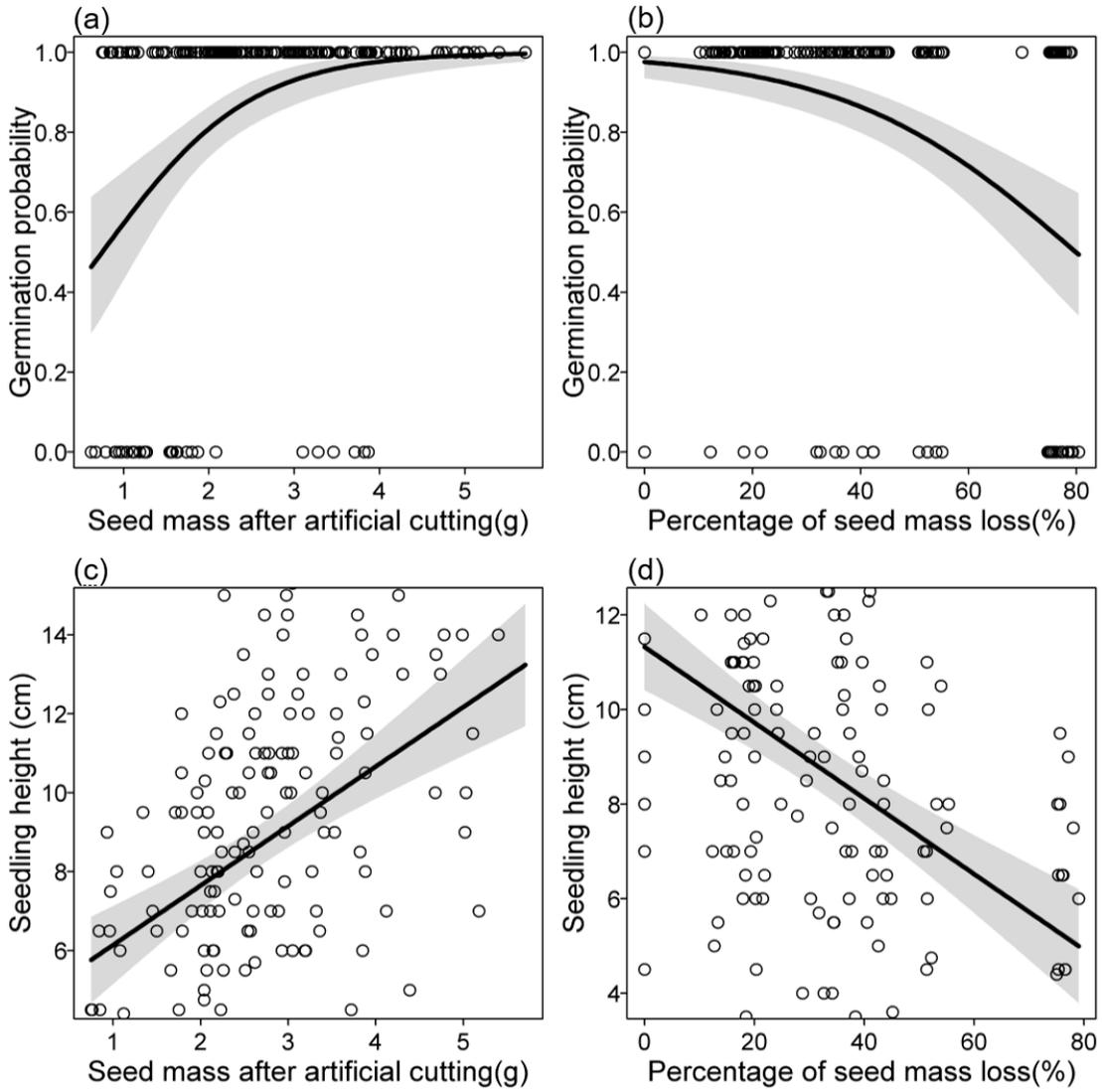
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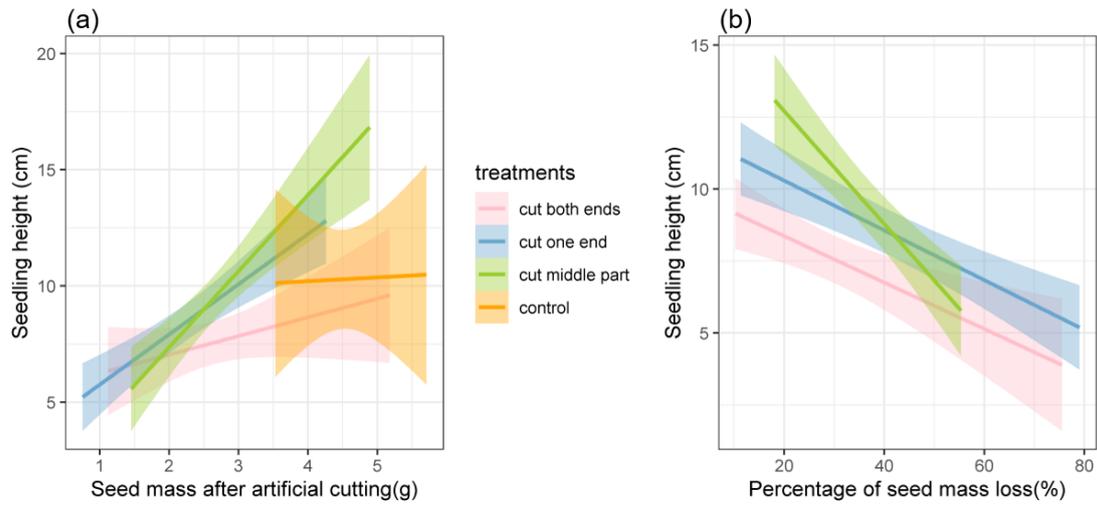
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662 **Fig. 3**



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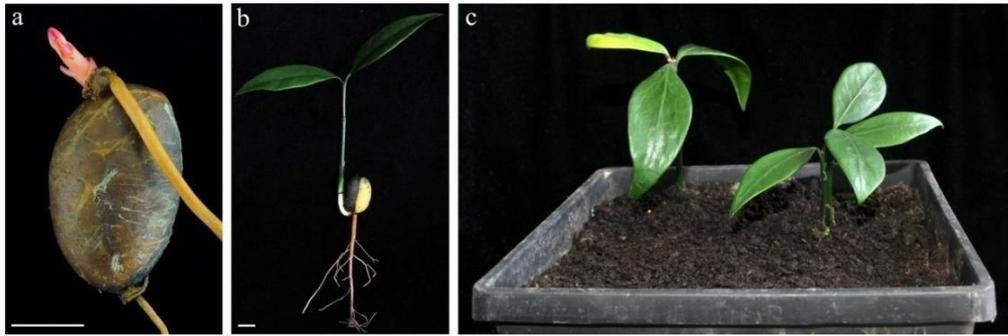
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684 **Fig. 4**



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709 **Fig. 5**



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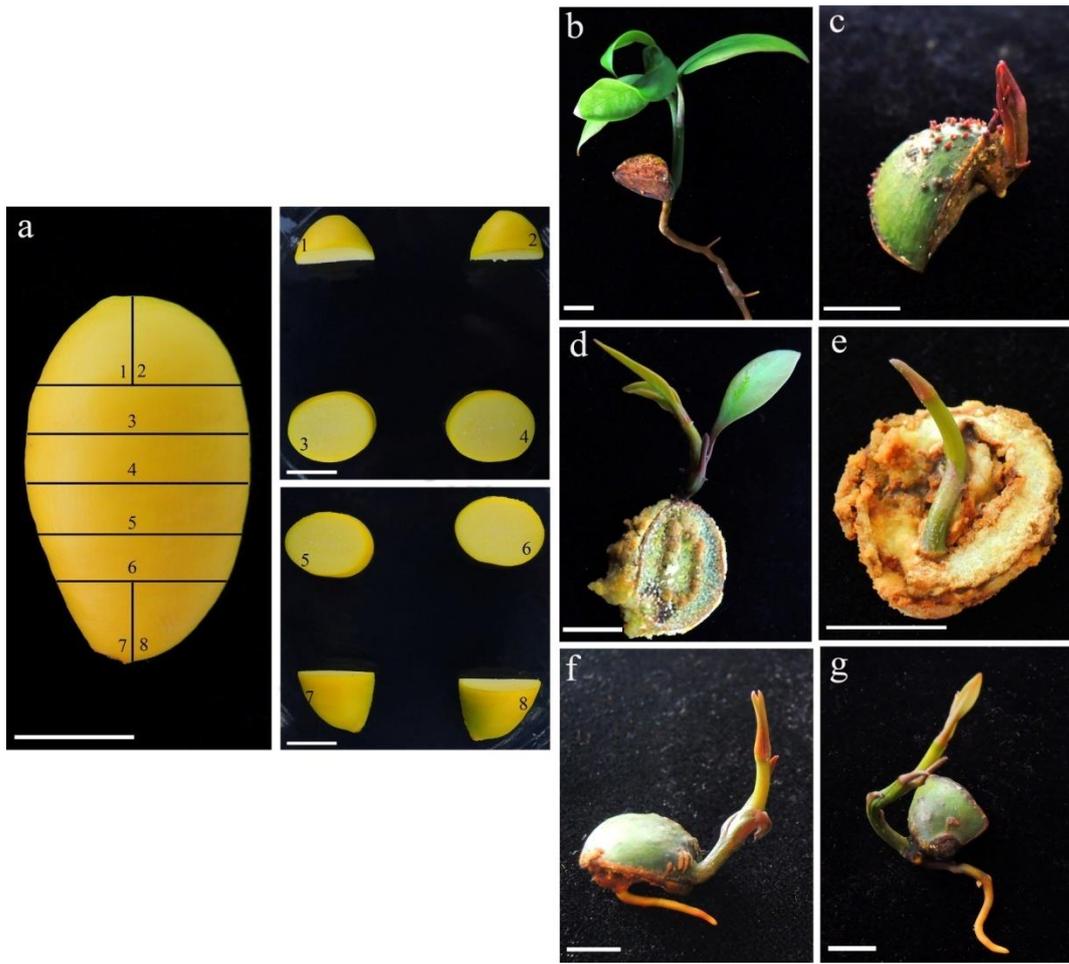
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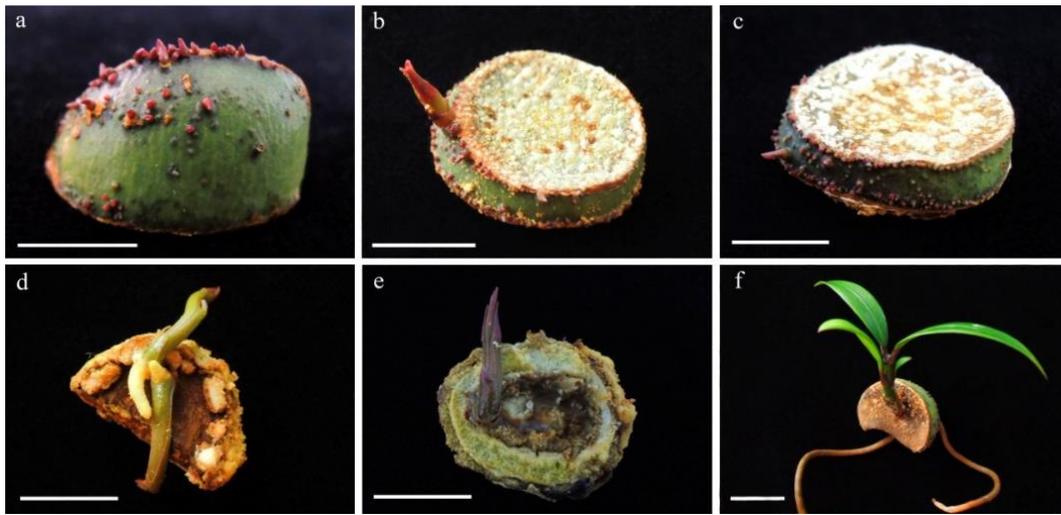
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745 **Fig. 7**



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