

# 1 Cloning capacity helps tropical seeds counter animal predation

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3 Zhenyu Wang <sup>a, b</sup>, Lin Cao <sup>c</sup>, Chuan Yan <sup>a</sup>, Yuda Niu <sup>d</sup>, Kang Chong <sup>d</sup>, Zhibin Zhang <sup>a\*</sup>

4 <sup>a</sup> State Key Laboratory of Integrated Management of Pest Insects and Rodents in  
5 Agriculture, Institute of Zoology, Chinese Academy of Sciences, Beijing 100101,  
6 China

7 <sup>b</sup> Key Laboratory of Poyang Lake Wetland and Watershed Research (Ministry of  
8 Education), College of Life Sciences, Jiangxi Normal University, Nanchang 330022,  
9 China

10 <sup>c</sup> Institute of Ecology and Geobotany, College of Ecology and Environmental Science,  
11 Yunnan University, Kunming 650091, China

12 <sup>d</sup> Institute of Botany, Chinese Academy of Sciences, Beijing 100093, China

13  
14 \*Corresponding author: Zhibin Zhang, [zhangzb@ioz.ac.cn](mailto:zhangzb@ioz.ac.cn)

15 Address: 1 Beichen West Road, Chaoyang District, Beijing 100101, P.R.China

16  
17 E-mail address:

18 Zhibin Zhang, [zhangzb@ioz.ac.cn](mailto:zhangzb@ioz.ac.cn)

19 Zhenyu Wang, [zhenyuwang1983@163.com](mailto:zhenyuwang1983@163.com)

20 Lin Cao, [lincao@ynu.edu.cn](mailto:lincao@ynu.edu.cn)

21 Chuan Yan, [yanchuan@lzu.edu.cn](mailto:yanchuan@lzu.edu.cn)

22 Yuda Niu, [niuyuda@ibcas.ac.cn](mailto:niuyuda@ibcas.ac.cn)

23 Kang Chong, [chongk@ibcas.ac.cn](mailto:chongk@ibcas.ac.cn)

## 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)

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

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

## Introduction

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

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

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

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

regeneration capacity in order to counter for animal predation.

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

## Materials and methods

### Study site and species

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

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

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

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

#### **Seed collection**

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

#### **Seed predation and hoarding by rodents**

We studied seed predation and hoarding behavior of two dominant rodent

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

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

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

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

## Laboratory germination experiments

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

## Laboratory tissue culture experiments

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

## Statistical analysis

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



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

## Results

### Seed predation and hoarding by rodents

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

### Germination of rodent damaged seed fragments in field conditions

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

### Germination of human artificially damaged seed fragments in field conditions

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

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

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

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

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

#### **Germination of human artificially damaged seed fragments in cultured conditions**

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

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

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

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

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

## Discussion

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

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

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

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

The cloning capacity of *G. xanthochymus* seeds may be closely related to its unique structural characteristics. Its seed has no endosperm, with the embryonic axis extending along the entire seed. Partial consumption by rodents (or artificially cutting) does not destroy the embryo because all tissues contain embryo cells. This 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.

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**Table 1** The number of cloning seedlings of *G. xanthochymus* seed after multiple cuts and culturing in different mediums (BA is 6-benzylaminoadenine one kind of 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 <sup>-1</sup> 6-BA	8	18
1/2 MS+0.25 mg L <sup>-1</sup> NAA	8	17
1/2 MS+0.5 mg L <sup>-1</sup> 6-BA+0.25 mg L <sup>-1</sup> NAA	8	26

**Figure legends**

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

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

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

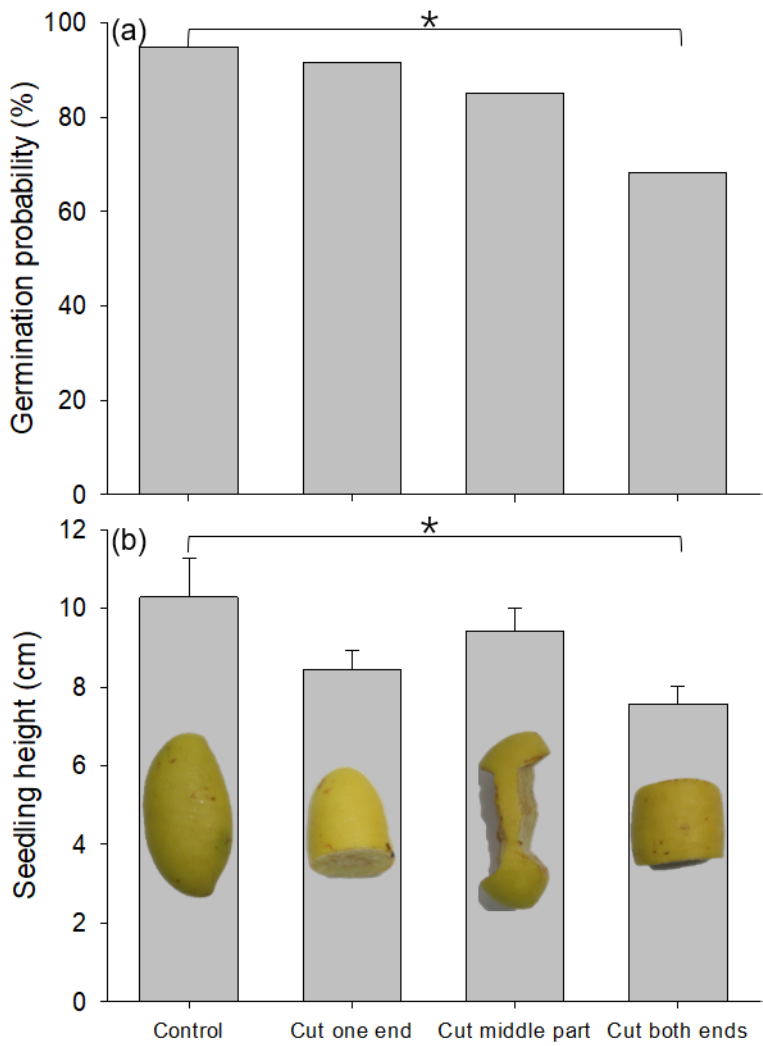
**Fig. 4** Germination characteristics of intact *G. xanthochymus* seed (a); seedlings of *G. xanthochymus* after planted in the nutrient soil (b, c).

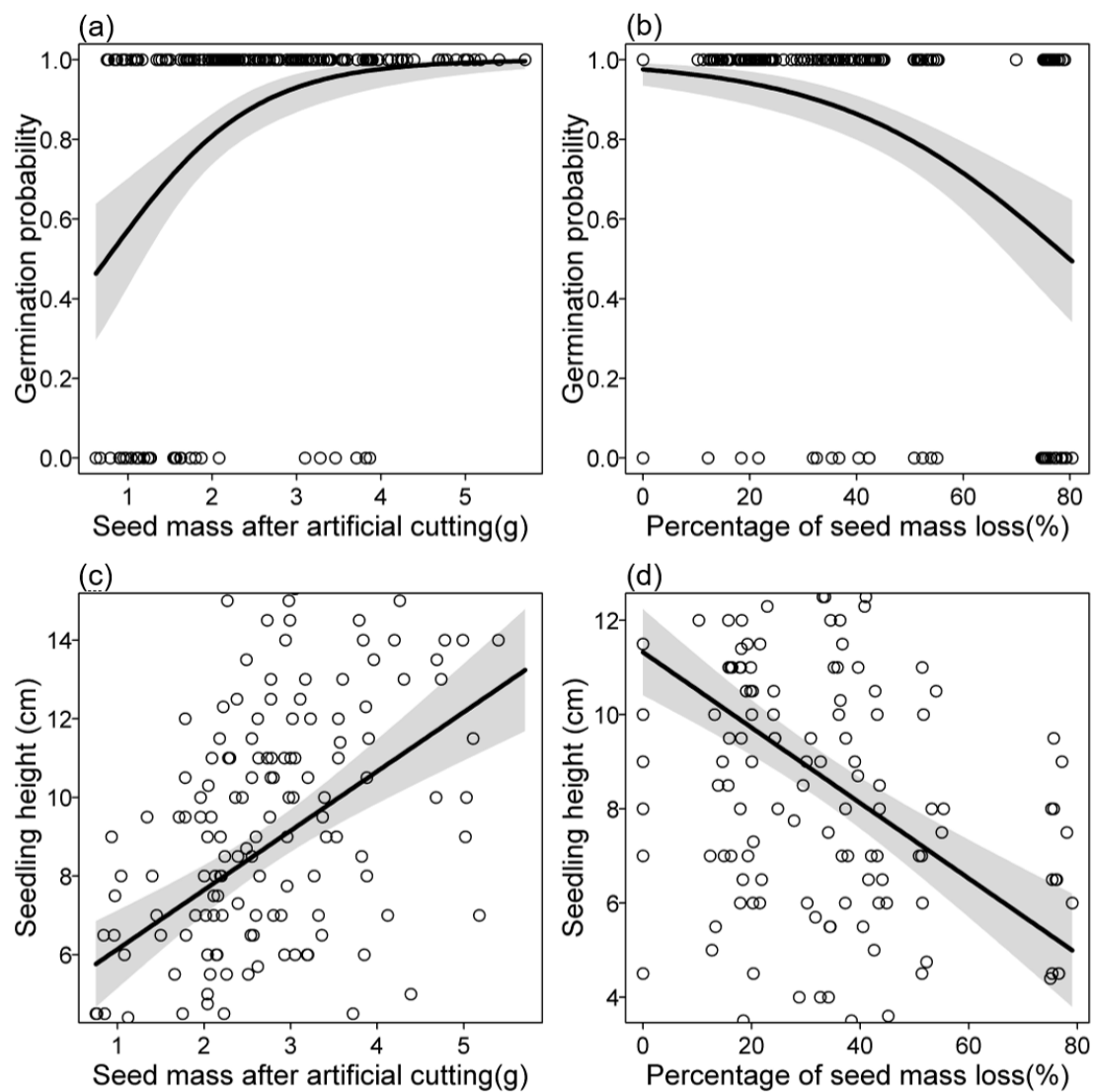
**Fig. 5** Tissue culture of the *G. xanthochymus* seed in 1/2 MS medium after longitudinal cutting: bud (a) and seedling (b); tissue culture seedlings after transplanting to nutrient soil (c).

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

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

**Fig. 1**



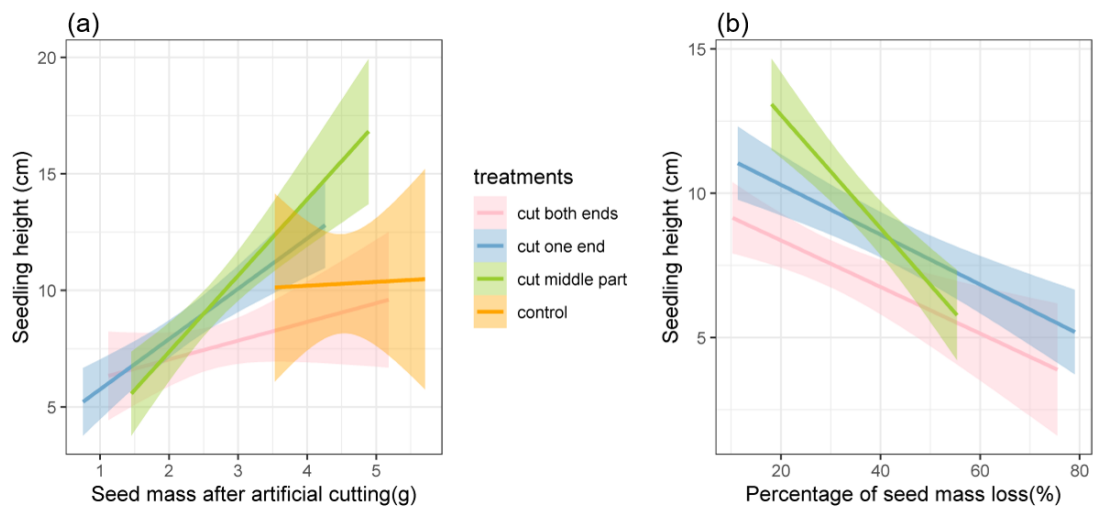


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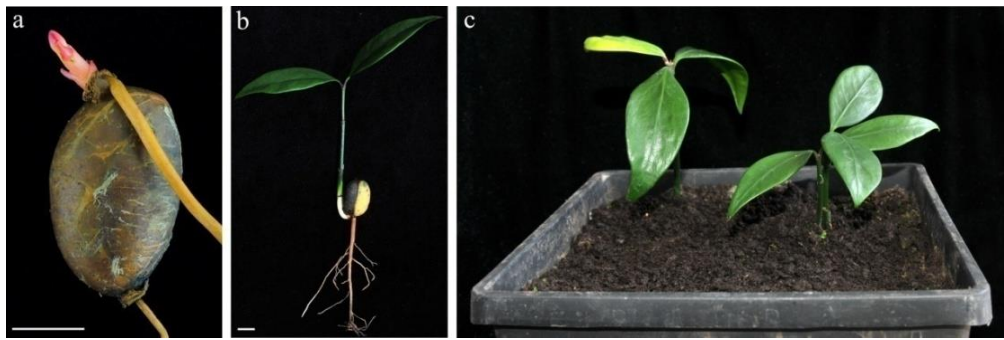
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**Fig. 3**

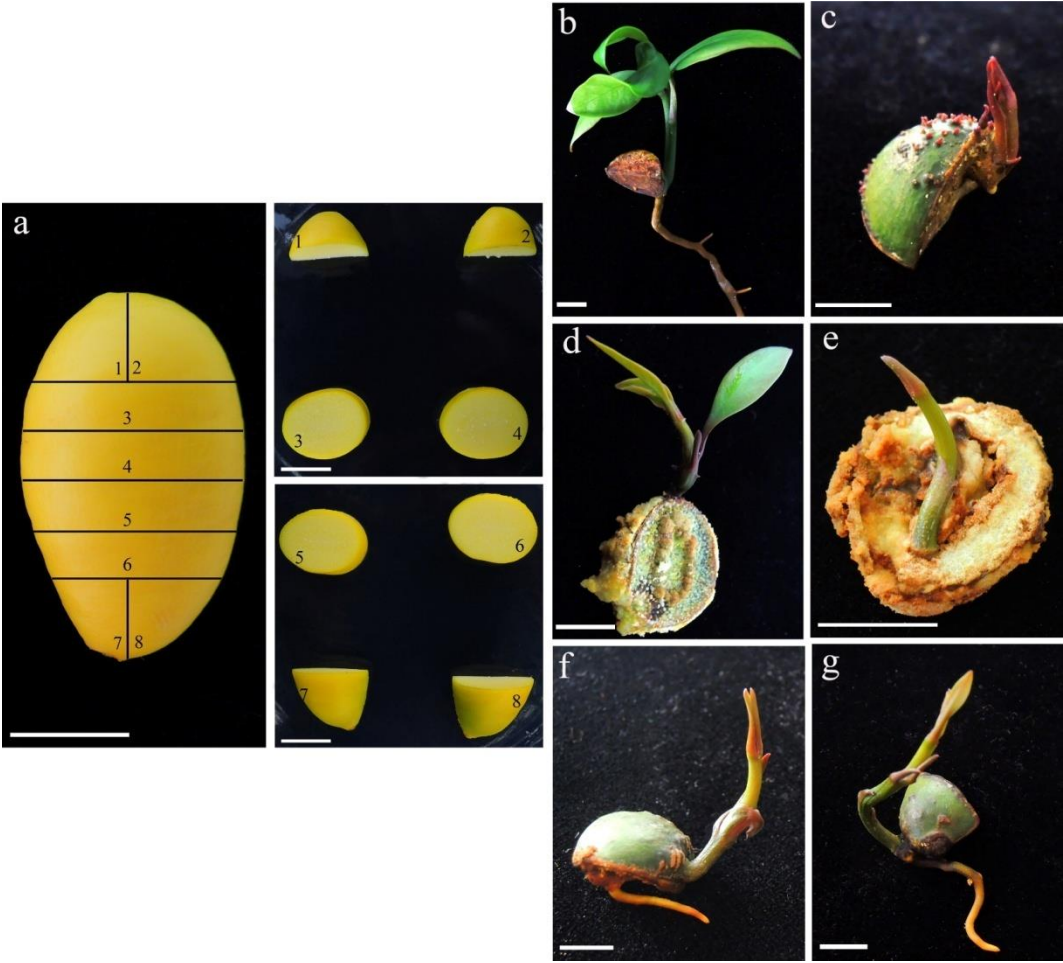


**Fig. 4**



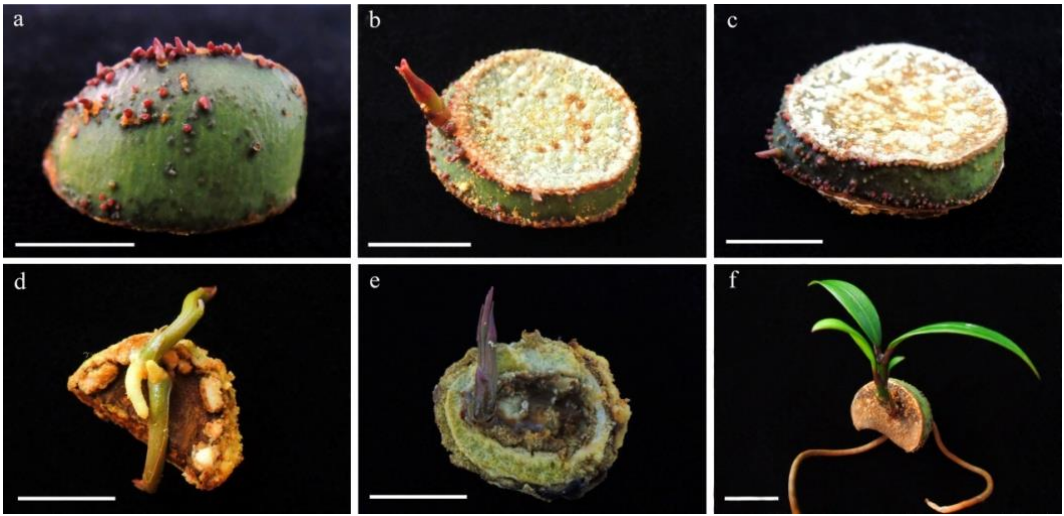
**Fig. 5**





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**Fig. 7**



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