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Thidiazuron increases fruit number in the biofuel plant *Jatropha curcas* by promoting pistil development

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ABSTRACT

Jatropha curcas L. is a potential biofuel plant because the composition of its seed oil is suitable for biodiesel and bio-jet fuel production and it is able to grow in unproductive subtropical or subdesert soils. Many studies have been performed to improve the seed yield of *J. curcas* to meet the needs of the biodiesel industry. As female flower number is an important factor affecting seed yield, an increase in the number of female flowers through the modification of sex expression is critical to the improvement of *J. curcas* for use as a biofuel. In this study, thidiazuron (TDZ), a synthetic compound with cytokinin (CK) activity, was exogenously applied to inflorescence meristems in four developmental stages to study its effect on sex expression in *J. curcas*. The results revealed that TDZ treatments of 75 μM and 225 μM promoted pistil development, which significantly increased the number of female flowers along with the development of inflorescence meristems. Number of female flowers reached a peak (40.0 female flowers per inflorescence) at 225 μM TDZ on stages III and IV inflorescence meristems. TDZ also reversed stamen abortion in stages II, III, and IV female flowers and induced bisexual flowers, which largely depends on the development stage of the inflorescence meristems. Furthermore, TDZ treatment increased the branch orders of the dichasia on the inflorescence, as observed by scanning electron microscopy. However, the total number of flowers was significantly decreased, as a result of the abortion of flower buds caused by TDZ. The number of mature fruits, which determines seed yield, was significantly increased by TDZ treatment, although this treatment resulted in a greater number of premature fruits. This study found that treatment with TDZ improved the fruit number of *J. curcas* by promoting pistil development. TDZ may play dual roles in the determination of flower sex, i.e., promoting pistil development and reversing stamen abortion in female flowers, which could shed light on the mechanism of sex determination in *J. curcas* and/or other non-model plants.

1. Introduction

In the context of increased demand for biofuels, the potential of the non-food species *Jatropha curcas* L. (hereafter referred to as *Jatropha*) has been recognized because the quality of its seed oil is suitable for biodiesel production and it is able to grow in unproductive subtropical or subdesert soils (Edrisi et al., 2015; Sujatha et al., 2013). Inflorescences of *Jatropha* are composed of five to nine dichasia on the upper part of the inflorescence rachis and one or two secondary inflorescences at the base of the inflorescence rachis (which also include 1–3 dichasia) (Supporting information Fig. S1a) (Fresnedo-Ramírez, 2013). There are four orders of branches on each dichasium, and the sex of the flower is dependent on its location on the four orders of the branches. The terminal flower produced at the joint of the first order of dichotomous branching is usually female (designated as the 1st flower, Supporting information Fig. S1b); flowers produced at the joint of the second dichotomous branching (the 2nd flowers, Supporting information Fig. S1b) may be female or male, which varies from plant to plant and population to population in response to time, climate, and nutrition; all flowers produced at the joint of the third and fourth dichotomous branching are male (the 3rd and 4th flowers, respectively; Supporting information Fig. S1b) (Wu et al., 2011). Therefore, female flowers are very limited in *Jatropha*, and increasing the number of female flowers through the modification of sex expression seems critical for the improvement of seed yield (Nietsche et al., 2015).

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Plants have evolved various mechanisms to promote cross-pollination, including the production of unisexual flowers on the same or different plants. Approximately 10% of angiosperms produce unisexual flowers (Yampolsky, 1922), and two broad categories of unisexual flowers have historically been recognized. In one type (type I), flowers become unisexual through the abortion of male or female reproductive organs, and in the second type (type II), flowers are unisexual from inception, as sex differentiation occurs before the initiation of stamens and carpels (Mitchell and Diggle, 2005). Most *Jatropha* plants produce unisexual flowers in inflorescences (Carels, 2009; Fresnedo-Ramírez, 2013), and Wu et al. (2011) reported that female and male flowers are formed via different mechanisms in *Jatropha*. The female flower is a type I unisexual flower that exhibits bisexual organs upon initiation, in which there is a rudiment of the nonfunctional male organ, whereas the male flower is a type II unisexual flower that bears no vestigial sexual organ during development. The existence of these two modes is also supported by the two whorls with five minute staminodes each at the base of the ovary, which develop into functional stamens, causing the *Jatropha* flower to sometimes become bisexual (Nair and Abraham, 1962).

Unisexual flower formation has been reported to be regulated by various phytohormones (Gerashchenkov and Rozhnova, 2013; Zhang et al., 2014), among which cytokinin (CK) has been shown to have a feminizing effect on a number of plant species. For example, the exogenous application of CK converted male flowers to hermaphroditic flowers in *Vitis vinifera* (Negi and Olmo, 1966) and induced female flowers in *Momordica charantia* (Ghosh and Basu, 1982), *Luffa acutangula* (Bose and Nitsch, 1970) and *Luffa cylindrica* (Takahashi et al., 1980). We have previously reported that 6-benzyladenine (BA, a synthetic compound with CK activity) treatment significantly increased the number of female flowers per inflorescence and induced bisexual flowers (Pan and Xu, 2011), which may have resulted from the differential expression of a large number of genes, such as those related to phytohormone biosynthesis and signaling and the regulation of the cell cycle (Chen et al., 2014; Pan et al., 2014). However, BA treatment produced too many flowers (both male and female), most of which were not well developed, and did not contribute to final seed yield. Thidiazuron (TDZ), a diphenylurea derivative, has been reported to have a high degree of intrinsic CK-like activity, much higher than that of BA (Thomas and Katterman, 1986), and it functions as an inhibitor of CK oxidase activity mainly through a non-competitive mechanism that is different from that of BA (Kieber and Schaller, 2014). Here, we report the effects of TDZ on sex expression in *Jatropha* through the exogenous application of various concentrations of TDZ onto inflorescence meristems (IMs) at four developmental stages. Our results indicate that TDZ may play dual roles in flower sex determination, i.e., promoting pistil development and reversing stamen abortion in female flowers, resulting in an increase in the number of fertile flowers (female and bisexual flowers) and, consequently, fruits. This study may shed light on the mechanism of sex determination in *Jatropha* and/or other non-model plants.

2. Materials and methods

2.1. Plant materials and growth conditions

Jatropha cuttings from a local population were grown at the beginning of March 2011 in a field at the Xishuangbanna Tropical Botanical Garden (XTBG; 21°54′N, 101°46′E; 580 m in altitude) of the Chinese Academy of Sciences located in Mengla County, Yunnan Province, southwestern China. The average rainfall, temperature and relative humidity in April 2012 and 2013 when the experiments were conducted were 41.3 mm, 23.9 °C and 75%, and 142.9 mm, 23.7 °C and 80%, respectively (data from Xishuangbanna Station for Tropical Rain Forest Ecosystem Studies). Plants were monocultured at a density of 1.5 m × 3 m under normal fertilization.

2.2. Thidiazuron (TDZ) application

A stock solution (454.0 mM) of thidiazuron (TDZ, Bio Basic Inc., Toronto, Ontario, Canada) was prepared by dissolving 0.1 g of TDZ with 1 mM NaOH and bringing the final volume to 1 ml with distilled water. Tween-20 (Polysorbate-20, Shanghai Sangon Biological Engineering Technology & Services Co., Ltd., China) was added at a final concentration of 0.05% (V/V) as a wetting agent to all of the TDZ working solutions. Inflorescence meristems (IMs) in four developmental stages (Supporting information Fig. S2) were used for TDZ application, as the stages may respond differently to TDZ treatment: stage I—the 1st flower appeared the primordial stamen (Supporting information Fig. S3a); stage II—the 2nd flower appeared the primordial stamen (Supporting information Fig. S3b); stage III—the 3rd flower appeared the primordial stamen (Supporting information Fig. S3c); stage IV—the 4th flower appeared the primordial stamen (Supporting information Fig. S3d). Three milliliters of each of the various concentrations of TDZ working solutions (0, 25, 75 and 225 μM) that contained equal volumes of 1 mM NaOH and 0.05% (V/V) Tween-20 were sprayed on each IM and its surrounding leaves using a hand sprayer. Spraying was conducted once on a sunny day. Thirty-five IMs were used for each treatment. The results of this study were confirmed by two replicated experiments at the end of April 2012 and 2013.

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The total number of flowers and number of each sex type per inflorescence and the number of fruits per infructescence were counted. A female flower was defined as a flower with only a pistil, and a male flower was defined as a flower with only stamens. Bisexual flowers were defined as flowers with both pistils and visible stamens, and an aborted flower bud was defined as a flower bud observed in the early inflorescence development stage after TDZ treatment that was unable to bloom and eventually withered. The total flower number is the sum of the male, female, and bisexual flowers. A mature fruit contained mature seeds, whereas an immature fruit contained small seeds that were unable to mature.

2.3. Scanning electron microscopy (SEM)

Stages I and IV IMs were collected 6 days after TDZ treatment and fixed overnight in FAA (50% ethanol, 5% acetic acid, and 3.7% formaldehyde) and dehydrated through a graded ethanol series to 100%. Materials were critical point dried using liquid CO_2, mounted on aluminum stubs with double-sided tape, gold-coated with an Edwards S150B sputter coater, and then examined through a scanning electron microscope (EVO LS10, Germany, at 10 kV).

2.4. Characterization of seeds

After being air-dried for 2 months, seeds from control and TDZ-treated plants were analyzed to determine their weight and oil content. Seed oil contents were determined with a minispec mq-one Seed Analyzer (Bruker Optik GmbH, Germany) with *Jatropha* seed oil used as a reference.

2.5. Statistical analysis

Data were analyzed using Statistical Product and Service Solutions software (SPSS Inc., Chicago, IL, USA, version 16.0). Differences

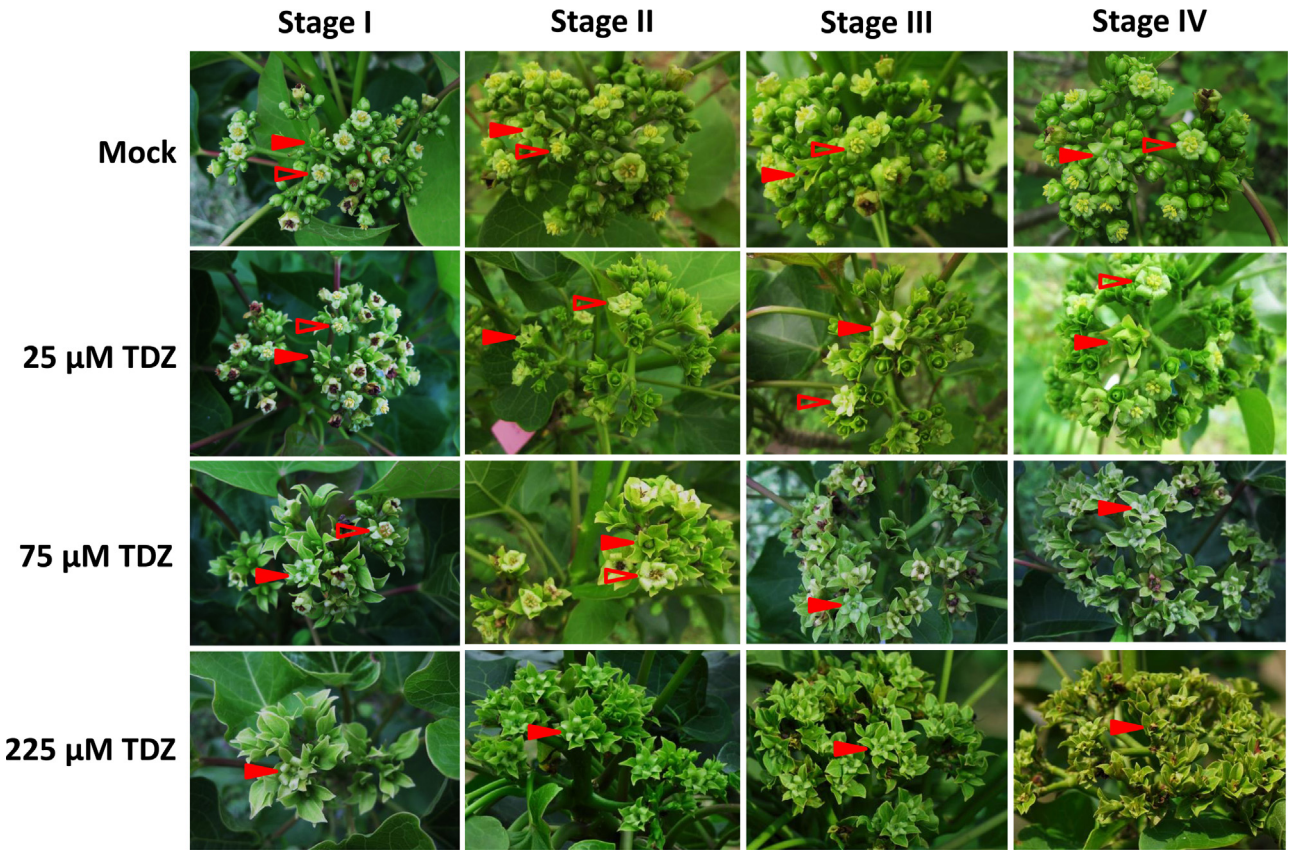

Fig. 1. Effects of TDZ treatment on flower number and sex expression in *Jatropha*.
Female and male flowers are indicated by solid triangles and hollow triangles, respectively.

among means were determined by one-way ANOVA with Tukey's or Tamhane's post hoc tests. Graphics were drawn with SigmaPlot (version 12.5; Systat Software, Inc., Point Richmond, CA).

3. Results

3.1. Reducing the total number of flowers through the induction of flower bud abortion

To study the effect of TDZ on flower sex expression in *Jatropha*, inflorescence meristems (IMs) of four developmental stages (Supporting information Fig. S2) were treated with various concentrations of TDZ. Treatments of meristems at all four stages with various concentrations of TDZ (Supporting information Fig. S2) reduced the total number of flowers per inflorescence (Figs. 1–3a–c), as a result of the abortion of flower buds (Supporting information Table S1, Figs. S4 and S5).

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Each dichasium on each inflorescence usually had four orders of branches in the non-treated IMs (Supporting information Fig. S1b). After the TDZ treatments, fewer orders of inflorescence branches were found when the flowers were in bloom (Fig. 4a–c). However, based on observations under scanning electron microscopy (SEM), TDZ treatments promoted the initiation of more orders of branches on the dichasium by six days after treatment (Fig. 4d–g, Supporting information Fig. S1c). However, the flower buds on the last orders of branches were aborted and eventually withered (Supporting information Fig. S4). Therefore, the newly induced branches were not well developed and thus were not visible, and/or the orders of the branches were reduced when the inflorescences matured (Fig. 4a–c). The number of aborted flower buds increased along with the development of IMs and increased concentrations of TDZ (Supporting information Table S1). Through dissection, most of the aborted flower buds were found to contain pistils (Supporting information Fig. S5). Moreover, the 1st, 2nd and/or 3rd flowers on some of the dichasia of the TDZ-treated IMs were found to be aborted during inflorescence development (Supporting information Fig. S4). Because of these aborted flower buds, the number of total flowers was significantly decreased (Figs. 1–3a–c). A significant reduction in the number of male flowers was also found after exposure to TDZ, and the effect was greater with increasing concentrations of TDZ (Figs. 2 and 3a–c).

3.2. Increasing the number of female flowers

TDZ treatment significantly increased the number of female flowers per inflorescence (Figs. 1–3a–c), which was generally proportionate to the concentration of TDZ within a specific development stage (Fig. 2). Although significantly more female flowers were produced on the IMs in all four stages with higher concentrations of TDZ, different responses to TDZ were found among the different IM developmental stages (Fig. 3a–c). The numbers of female flowers increased along with the development of the IMs and reached their peaks at stages III and IV (225 μM TDZ) (Figs. 2 c and d and 3 c). However, at the lowest TDZ concentration (25 μM), the number of female flowers on the treated IMs shows no significant difference compared to the control in most stages (I–III), except for stage IV, in which a significant decrease in female flower number was found (Fig. 3a).

Because the 3rd and 4th flowers on a dichasium are exclusively male and the stamens in the stage IV IMs emerged before TDZ treat-

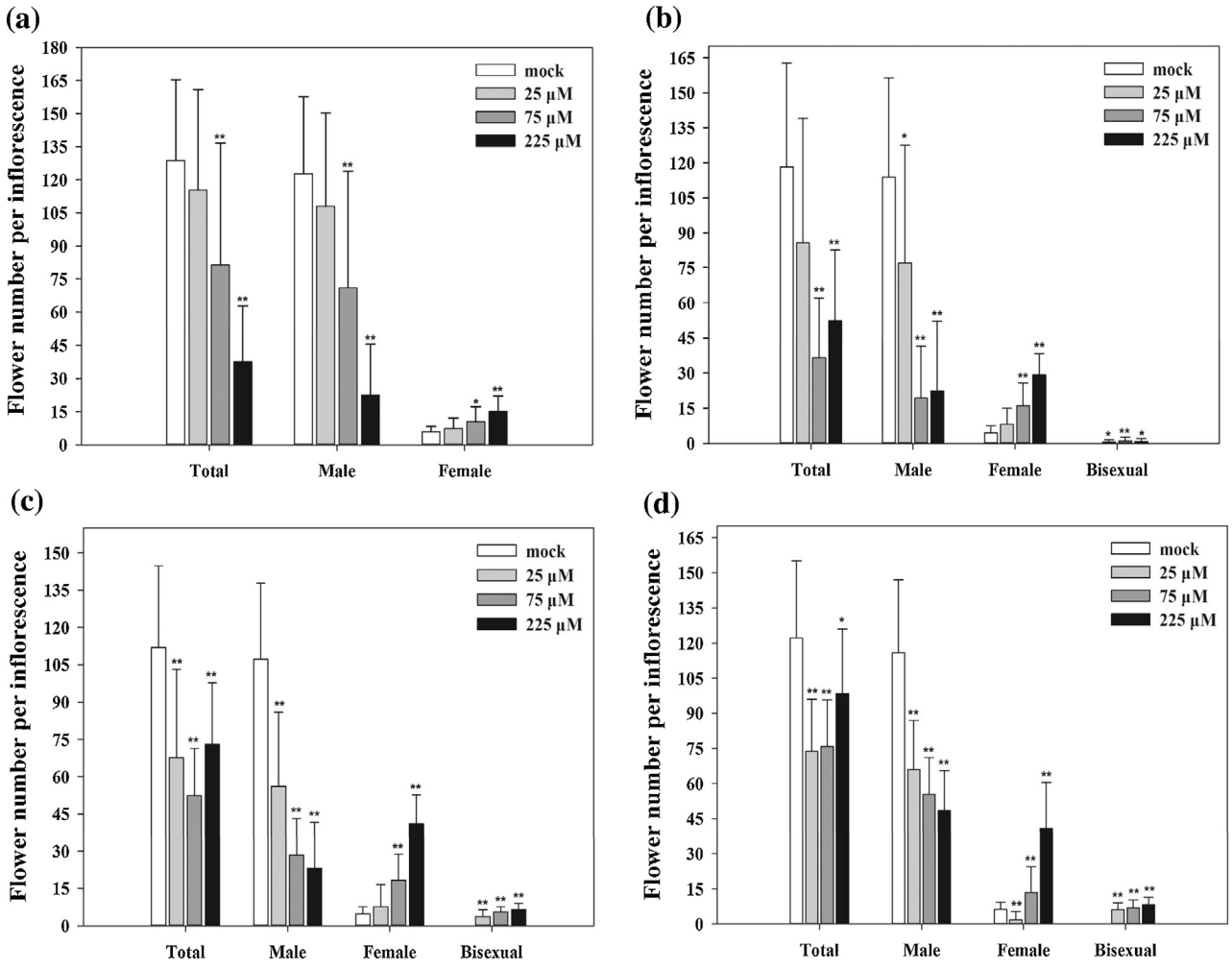

Fig. 2. Effects of treatment with various concentrations of TDZ on flower sex.
(a) Stage I; (b) stage II; (c) stage III; (d) stage IV. Values are means ± standard deviations (n = 30 inflorescences). *Statistically significant at the 5% level. **Statistically significant at the 1% level.

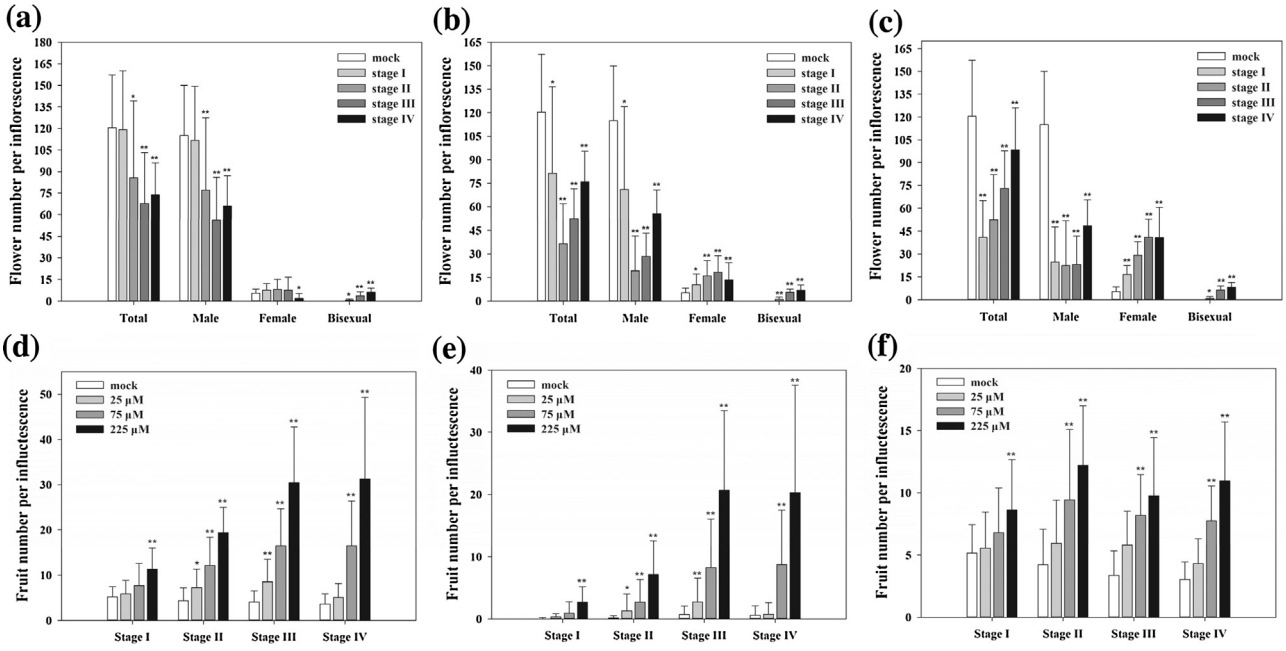

Fig. 3. Effects of TDZ treatment on flower sex and fruit number in different IM developmental stages.
(a) 25 μM TDZ; (b) 75 μM TDZ; (c) 225 μM TDZ; (d) total number of fruits; (e) number of premature fruits; (f) number of mature fruits. Values are means ± standard deviations (n = 30 inflorescences or infructescences). *Statistically significant at the 5% level. **Statistically significant at the 1% level.

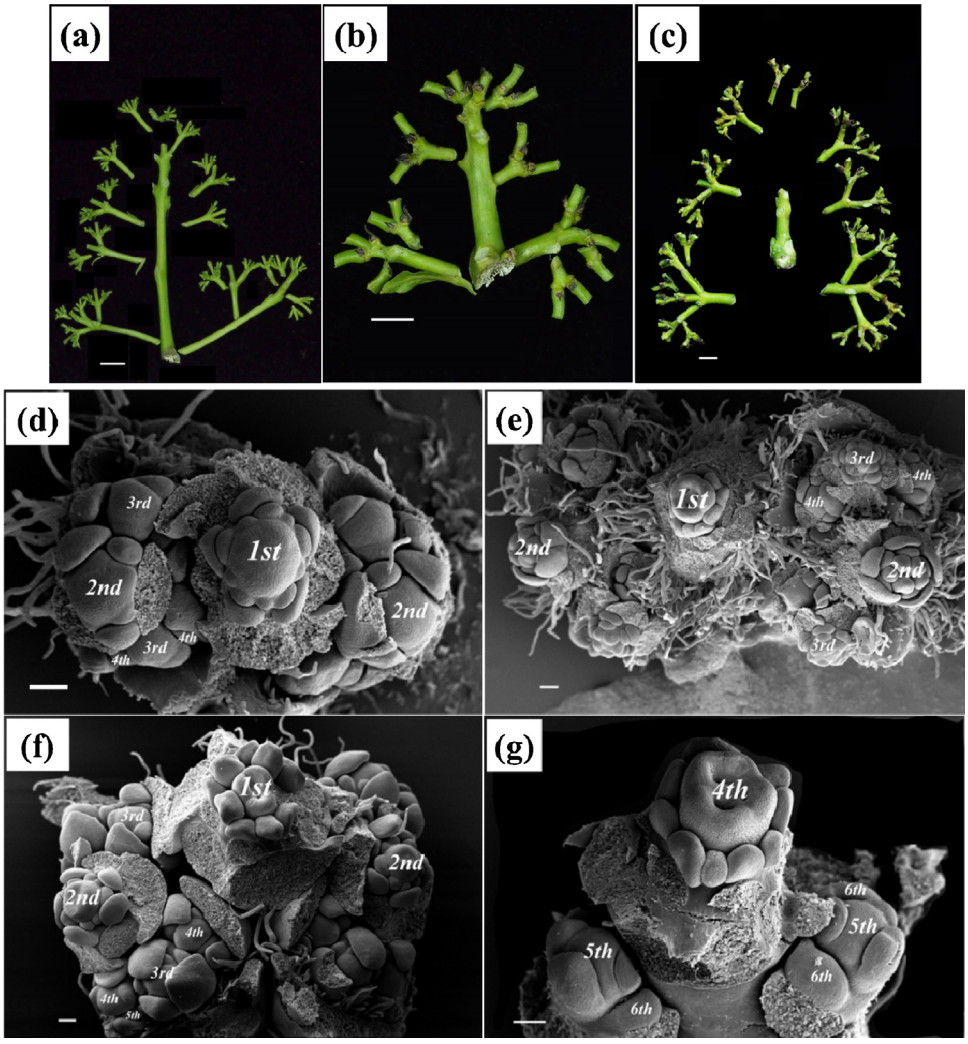

Fig. 4. Inflorescence branching with and without TDZ treatment in *Jatropha*.
(a)–(c) Branching of inflorescences in the control (a), and inflorescences with TDZ treatment at stage I (b) and stage IV (c). To clearly show the pattern of inflorescence branching, all flowers were removed. Bars represent 1 cm. (d) and (e) SEM observations of branching in control IMs (stages I and IV). (f) and (g) SEM observations of branching in IMs 6 days after TDZ treatment at stages I and IV. The primordia of the flowers at different stages were labeled with 1st to 6th. Bars in (d)–(g) represent 100 μm.

ment (Supporting information Fig. S3), these two types of flowers were dissected to observe reproductive organogenesis by SEM. Six days after TDZ treatment, the ovaries were initiated on the fourth whorl of the 3rd and 4th flowers (Fig. 5); thus, TDZ treatment increased the number of female flowers through the promotion of pistil development.

3.3. Inducing the formation of bisexual flowers

Female *Jatropha* flowers (Fig. 6a) bear vestigial stamens (Fig. 6b), which developed into bisexual flowers (Fig. 6c) after treatment with TDZ. The IM development stage significantly affected the number of bisexual flowers. In stage I, bisexual flowers were not induced, whereas the numbers in stages II–IV increased along with the development of the IMs (Fig. 3a–c), which also increased with the concentration of TDZ (Fig. 2b–d). Stage IV IMs responded to the lowest concentration (25 μM) of TDZ with an increased number of bisexual flowers, whereas the number of female flowers significantly decreased (Figs. 2 d and 3 a), indicating that at least some of the TDZ-induced bisexual flowers may be derived from female flowers by reversing the abortion of stamens. Moreover, this effect was more dependent on the developmental stages of the IMs than on the concentration of TDZ (Fig. 7).

As both female and bisexual flowers contribute to fruit number, the sum of the number of female and bisexual flowers was investigated in different treatments. At each developmental stage, the sum increased along with increasing concentrations of TDZ and reached its peak at 225 μM in stages III and IV (Table 1).

Table 1
The total number of female and bisexual flowers per inflorescence in *Jatropha* after TDZ treatment. Values are mean ± standard deviation (n = 30 inflorescences).

		TDZ concentration (μM)		
IM stages	Mock	25	75	225
Stage I	5.9 ± 2.4	7.4 ± 4.7	10.4 ± 6.8[a]	15.1 ± 7.0[b]
Stage II	4.4 ± 3.0	8.7 ± 6.8[a]	17.2 ± 10.0[b]	30.0 ± 9.5[b]
Stage III	4.7 ± 3.0	11.5 ± 8.1[b]	23.9 ± 11.6[b]	47.5 ± 13.1[b]
Stage IV	6.2 ± 3.1	7.8 ± 4.3	20.3 ± 12.2[b]	48.9 ± 21.5[b]

[a] Significantly different from the control at the 5% level.
[b] Significantly different from the control at the 1% level.

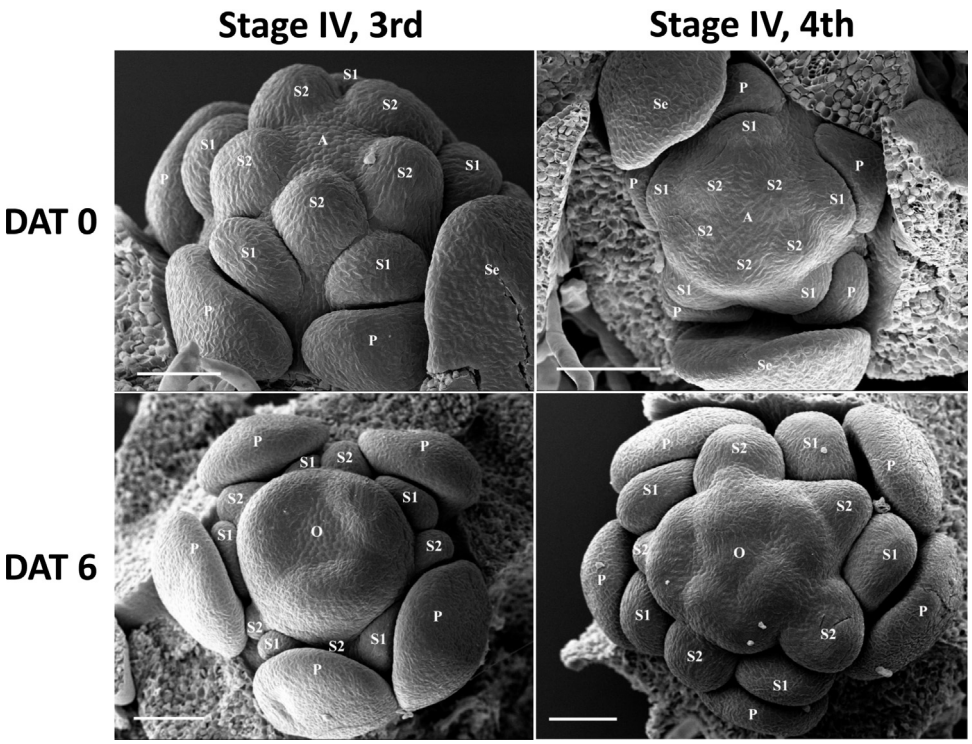

Fig. 5. TDZ treatment promoted pistil development in the 3rd and 4th flowers in dichasia from IMs in stage IV.
(A) Apical meristem (S1) the first whorl of the stamen primordium (S2) the second whorl of the stamen primordium; (P) petal primordium (Se) sepal primordium. Bars = 100 μm.

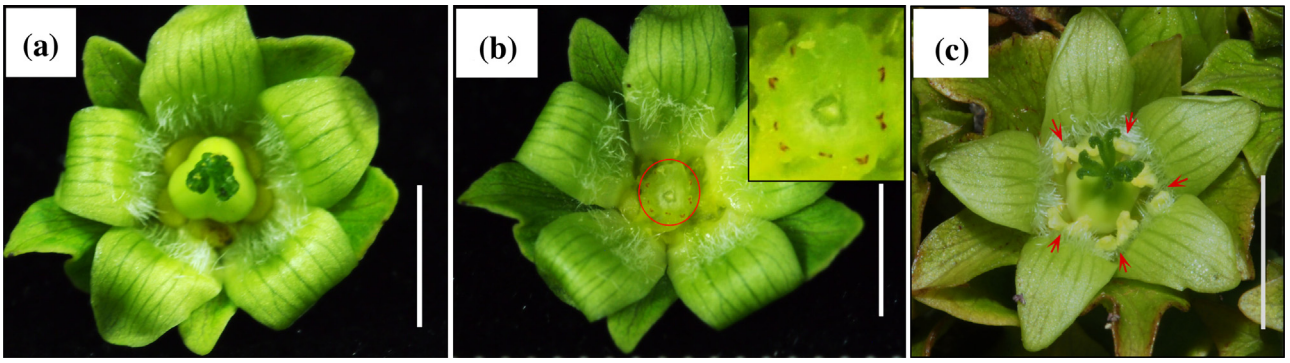

Fig. 6. TDZ treatment reversed the abortion of stamens in female flowers.
(a) Normal female flower; (b) the vestigial stamens (inside the red circle) in the female flower (pistil was removed); (c) bisexual flower induced by TDZ. The reversed stamens are indicated by red arrows. Bars = 5 mm. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3.4. Regulation of fruiting and seed development in Jatropha by TDZ treatment

More female flowers and newly induced bisexual flowers from IMs treated with TDZ produced many more fruits than the control IMs (Figs. 8 and 3d), but many of these fruits were not well developed (Fig. 3e). The number of premature fruits increased with developmental stage and the concentration of TDZ, reaching a peak at 225 μM in stages III and IV (Fig. 3e). Nevertheless, the numbers of mature fruits from TDZ-treated IMs were still significantly higher than those from the control IMs (Fig. 3f). The effect of TDZ treatment on the total number of fruits at different developmental stages (Fig. 3d) was similar to that on the number of female flowers, i.e., the highest number of female flowers was found at stages III and IV (Figs. 2 and 3a–c). However, the highest number of mature fruits per infructescence was found in the 225 μM TDZ treatment at stage II and was a result of relatively fewer premature fruits compared to stages III and IV (Fig. 3e).

The weight of the seeds from the 225 μM TDZ treatment at stage I was reduced, whereas seed weight from the other treatments was not significantly affected (Supporting information Table S2). The oil content of seeds from various TDZ treatments was not significantly different from the control (Supporting information Table S2). These results indicate that the application of TDZ has great potential to improve seed yield in *Jatropha*.

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4. Discussion

4.1. Promoting pistil development

TDZ treatment promoted pistil development in *Jatropha* flowers (Fig. 5), resulting in an increase in the number of female flowers (Figs. 2 and 3a–c). Under normal growth conditions, the meristems of the 3rd and 4th flowers in stages III and IV only contain sta-

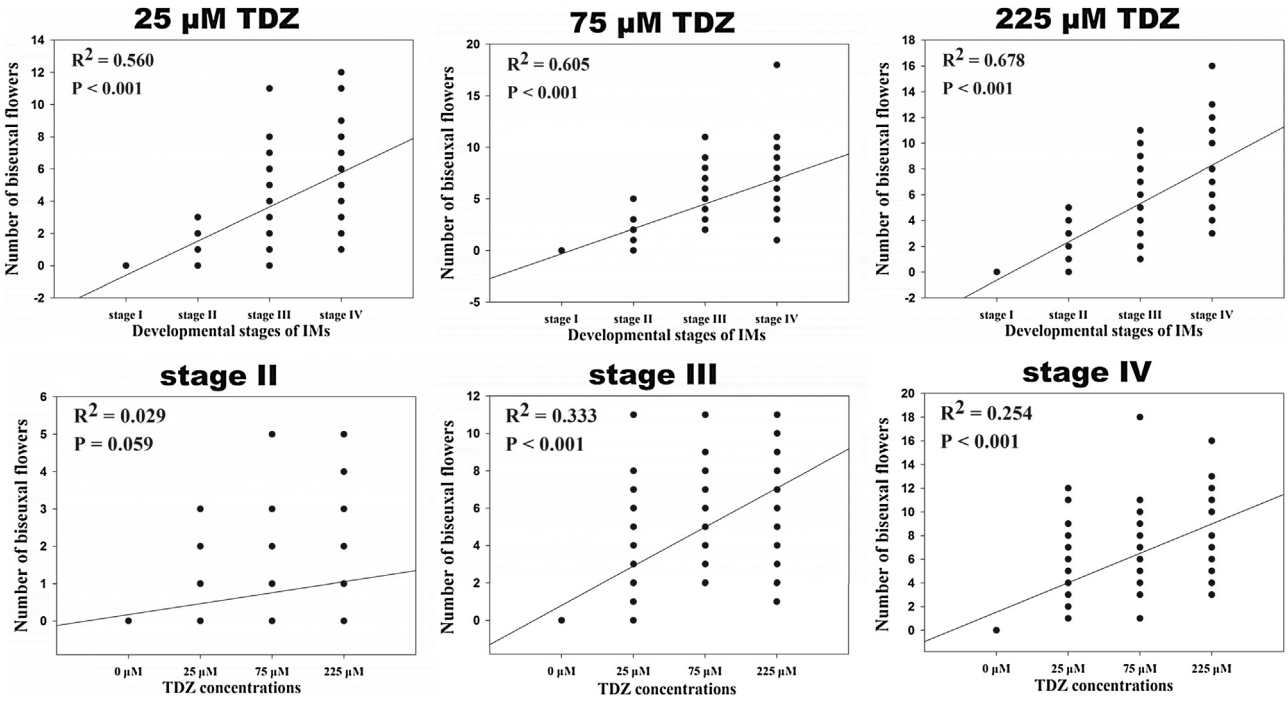

Fig. 7. Linear regression of the development of inflorescence meristems and the concentration of TDZ versus the number of bisexual flowers per inflorescence in *Jatropha*.

mens, which develop into male flowers, but no pistils (Supporting information Fig. S3c and d). However, pistils emerged in the 3rd and 4th flowers six days after the application of TDZ (Fig. 5). The development of female flowers in many other flowering plants is also regulated by species-specific hormones. In *Mercurialis annua*, which also belongs to the Euphorbiaceae family, high levels of endogenous CK appear to be correlated with the development of floral primordia into carpels (Dauphinguerin et al., 1980). CK treatment promotes floral feminization in *Plukenetia volubilis*, a promising oilseed crop, which also belongs to the Euphorbiaceae family (Fu et al., 2014). In cucumber, ethylene acts on both the development of pistil primordia and the arrest of stamen primordia, which results in the induction of femaleness (reviewed by Yamasaki et al., 2005). The availability of GAs plays an essential role in the expression of feminizing *An1* (*Anther earl*) and *D* (*Dwarf*) genes in maize flowers (Dellaporta and Calderon-Urrea, 1994). In another study, the expression of the CK-synthesizing enzyme *isopentenyl transferase* (*IPT*) gene under the control of the *Arabidopsis* senescence-inducible promoter *SAG12* (a senescence-associated gene) was found to reverse the aborted pistil of the lower floret in a female maize inflorescence (Young et al., 2004). As both CK and gibberellin play important roles in the feminization of maize, it is possible that IPT might function by increasing the level of gibberellin (Dellaporta and Calderon-Urrea, 1994). Gibberellin treatments have been reported to increase the number of female flowers (Makwana et al., 2010) and bisexual flowers in andromonoecious *Jatropha* (Miftahudin et al., 2014), but in our previous study, an exogenous gibberellin treatment was not found to promote pistil development in *Jatropha* (Pi et al., 2013). Therefore, how TDZ promotes pistil development in *Jatropha* flowers remains to be determined.

4.2. Reversing the abortion of stamens in female flowers

Our results indicate that TDZ treatments reversed the abortion of stamens in some female flowers in stages II–IV, and ultimately resulted in the formation of bisexual flowers (Fig. 6c), whereas there were only vestigial stamens in the control female flowers (Fig. 6b). The number of bisexual flowers increased along with the development of the treated IMs (Figs. 2 and 3a–c), which may have resulted from the short time window during which TDZ is able to reverse stamen abortion during a critical period in stamen development. During this critical period, the endogenous CK content in control female flowers may be insufficient for normal stamen development, resulting in abortion. We performed an additional TDZ treatment after 10 days of the first treatment and found that the number of bisexual flowers significantly increased in all developmental stages (data not shown). The hormonal regulation of stamen development has been extensively studied (Gerashchenkov and Rozhnova, 2013; Khryanin, 2002). In *M. annua*, the exogenous application of CK to different male fertile lines resulted in a range of sterile conditions, i.e., from inhibition at the tetrad stage to empty anthers (Louis and Durand, 1978). Gibberellin has been widely implicated in stamen development (see the review by Sawhney and Shukla, 1994). The *gid1a gid1b gid1c* (the genes encoding gibberellin receptors) triple mutant exhibited the failed filament elongation and arrested anther development at floral stage 9 in *Arabidopsis* (Griffiths et al., 2006). The specific expression of *gai*, a gene involved in gibberellin signal transduction, in the anthers and pollens of tobacco (*Nicotiana tabacum*) and *Arabidopsis* resulted in the abortion of these respective tissues, which was reversible by the exogenous application of kinetin (Huang et al., 2003). Our previous studies reported that benzyladenine and gibberellin treatments can induce bisexual flowers in *Jatropha* (Pan and Xu, 2011; Pi et al., 2013), suggesting that male flower development in *Jatropha* may be co-regulated by crosstalk among CK and gibberellin and/or other unidentified signals or components.

To improve *Jatropha* seed production, various plant growth regulators (PGRs) were used to increase female flower production, including gibberellin (Makwana et al., 2010; Pi et al., 2013), CK (Pan and Xu, 2011), paclobutrazol (Song et al., 2013; Xu et al., 2013) and auxin (Miftahudin et al., 2014). Compared to other CKs, TDZ is relatively stable in plants (Mok and Mok, 1985), and has much higher activity (Thomas and Katterman, 1986). Hence only one application of TDZ in a flowering season would lead to a significant increase in fruit number of *Jatropha*. For a large commercial plantation, how-

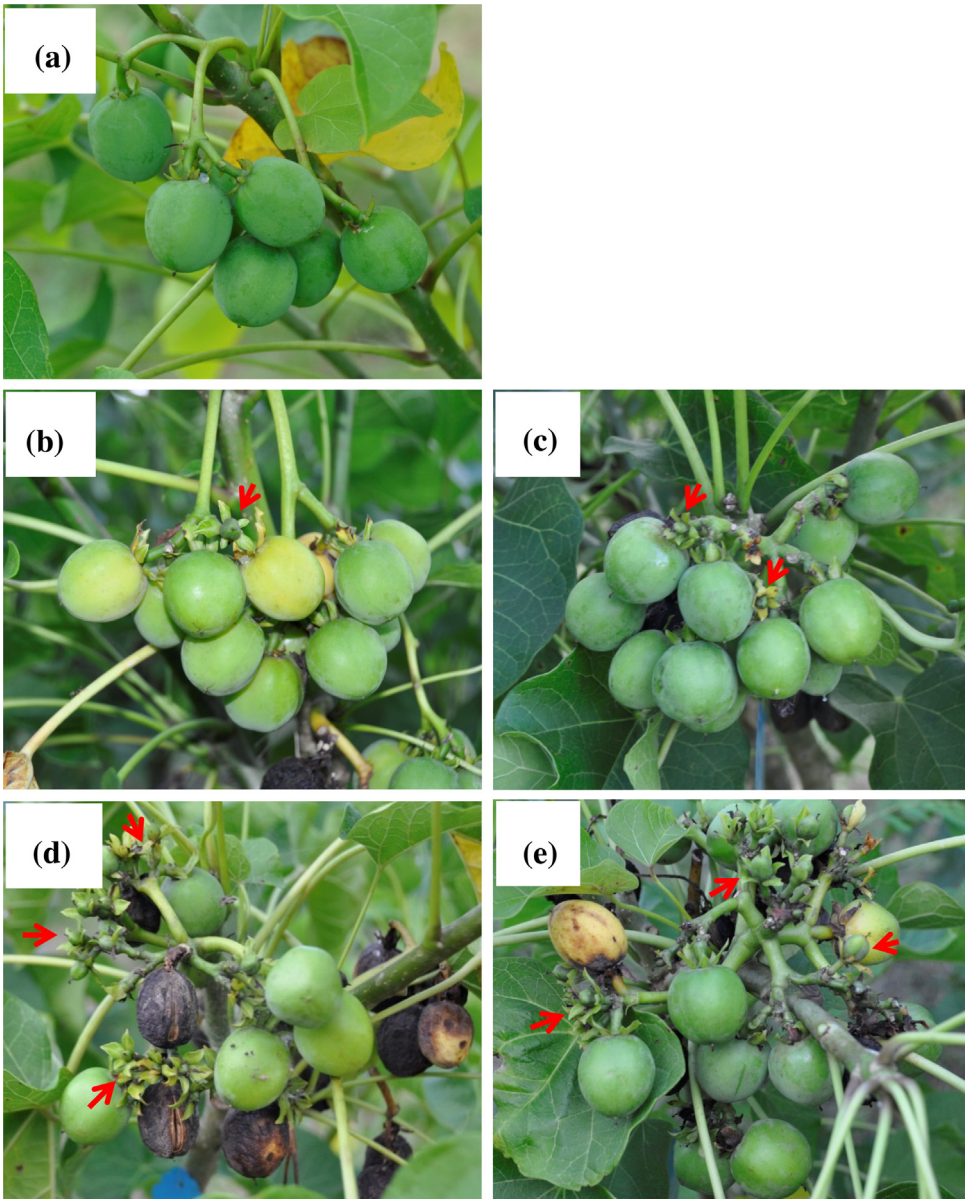

Fig. 8. Effects of the 225 μM TDZ treatment on fruiting in *Jatropha*.
(a) Mock; (b) stage I; (c) stage II; (d) stage III; (e) stage IV. The premature fruits are indicated by red arrows in (b)–(e). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

ever, it is not economically viable to improve *Jatropha* seed yield by exogenous application of PGRs.

Comparing the results of this study to our previous report on the effects of benzyladenine treatment on *Jatropha* flower development (Chen et al., 2014; Pan et al., 2014; Pan and Xu, 2011), we found that both treatments can induce bisexual flowers and increase female flower number. However, TDZ produced more aborted flowers, resulting in a reduction in the total number of flowers (Figs. 1–3a–c). Multiple factors affect the abortion of flowers, such as light intensity and sucrose supply (Aloni et al., 1997; Van Tuyl et al., 1985), source and sink strength (Marcelis et al., 2004), heat stress (Guilioni et al., 1997), and hormones (Nagel et al., 2001).

5. Conclusions

TDZ treatment increased the number of female flowers by promoting pistil development and induced bisexual flowers by reversing stamen abortion during *Jatropha* flower development, which indicates that the development of both pistils and stamens in *Jatropha* requires CK. As both female and bisexual flowers influence seed yield, TDZ treatment can significantly increase the number of fruits, and thus final seed yield. In addition, these results shed light on the mechanism of sex determination in *Jatropha* and other monoecious plants.

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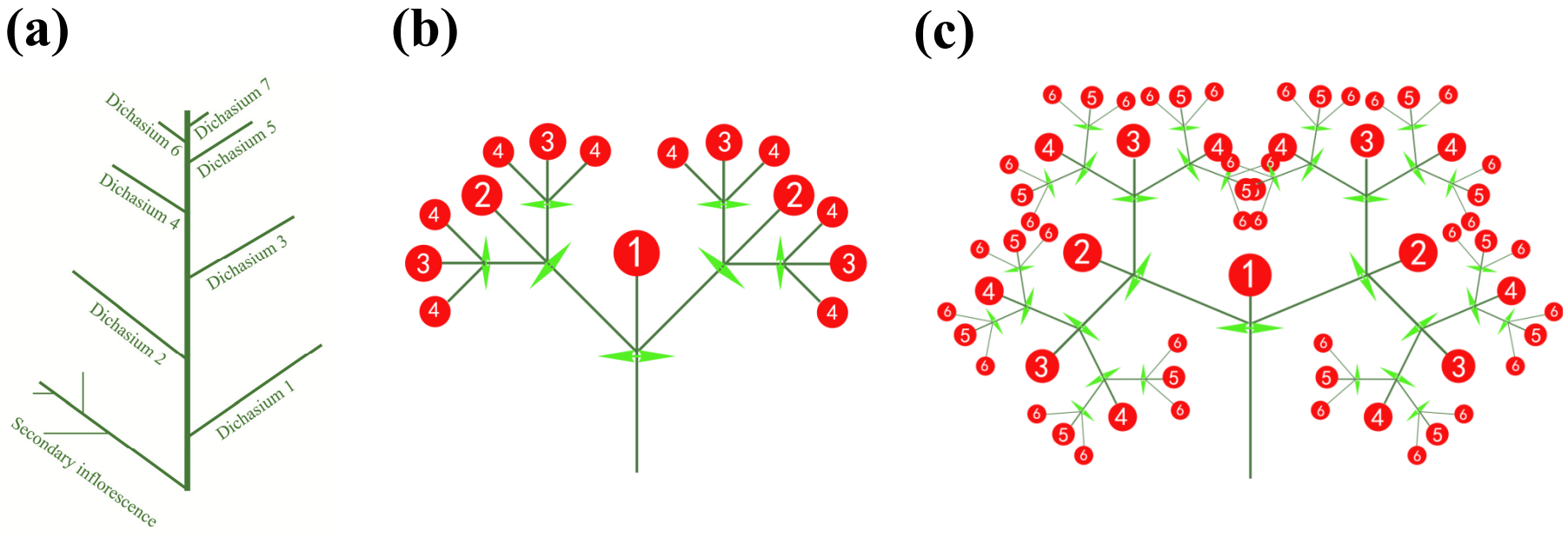

Figure S1 Diagram of *Jatropha* inflorescence. (a) Inflorescence branching; (b) diagram of a dichasium on an inflorescence; (c) diagram of a dichasium after TDZ treatment based on scanning electron microscopy (SEM).

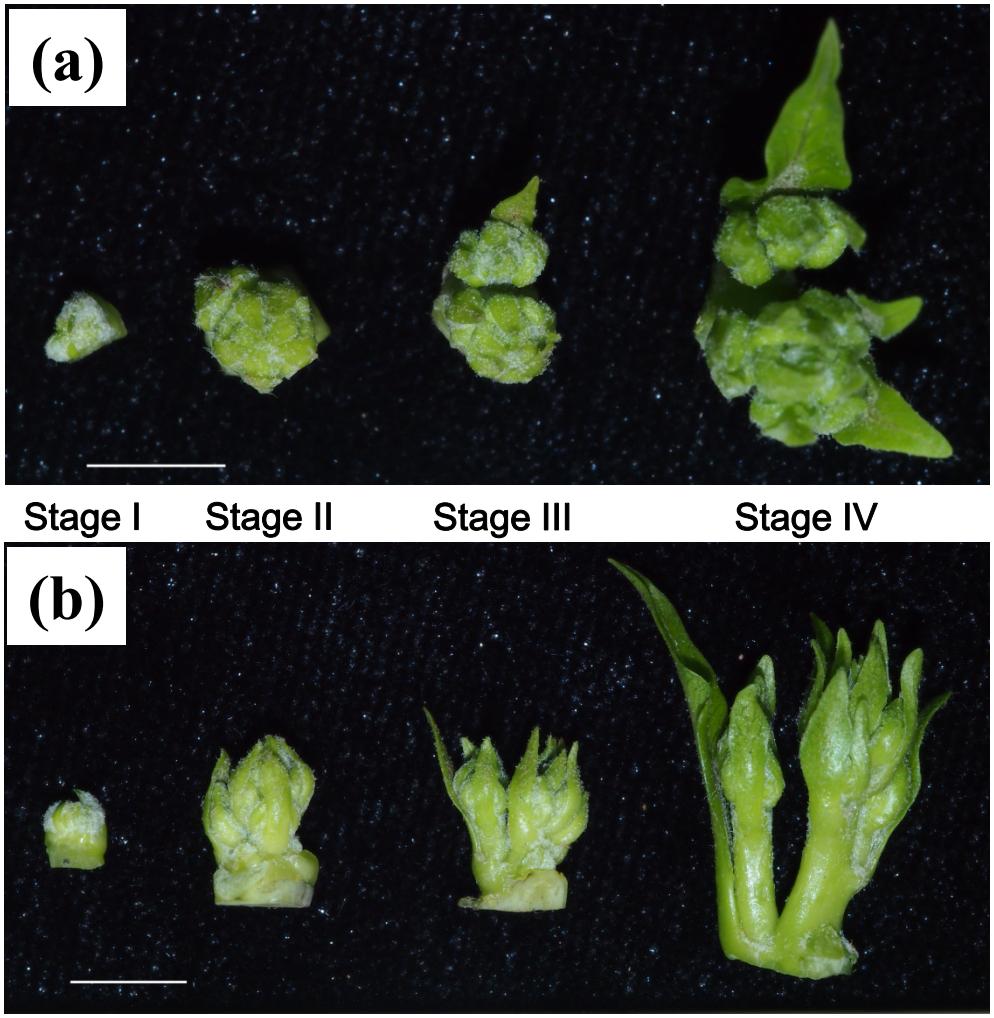

Figure S2 Stage I to stage IV inflorescence meristems. (a) Apical view of inflorescence meristems; (b) lateral view of inflorescence meristems. Bars = 5 mm.

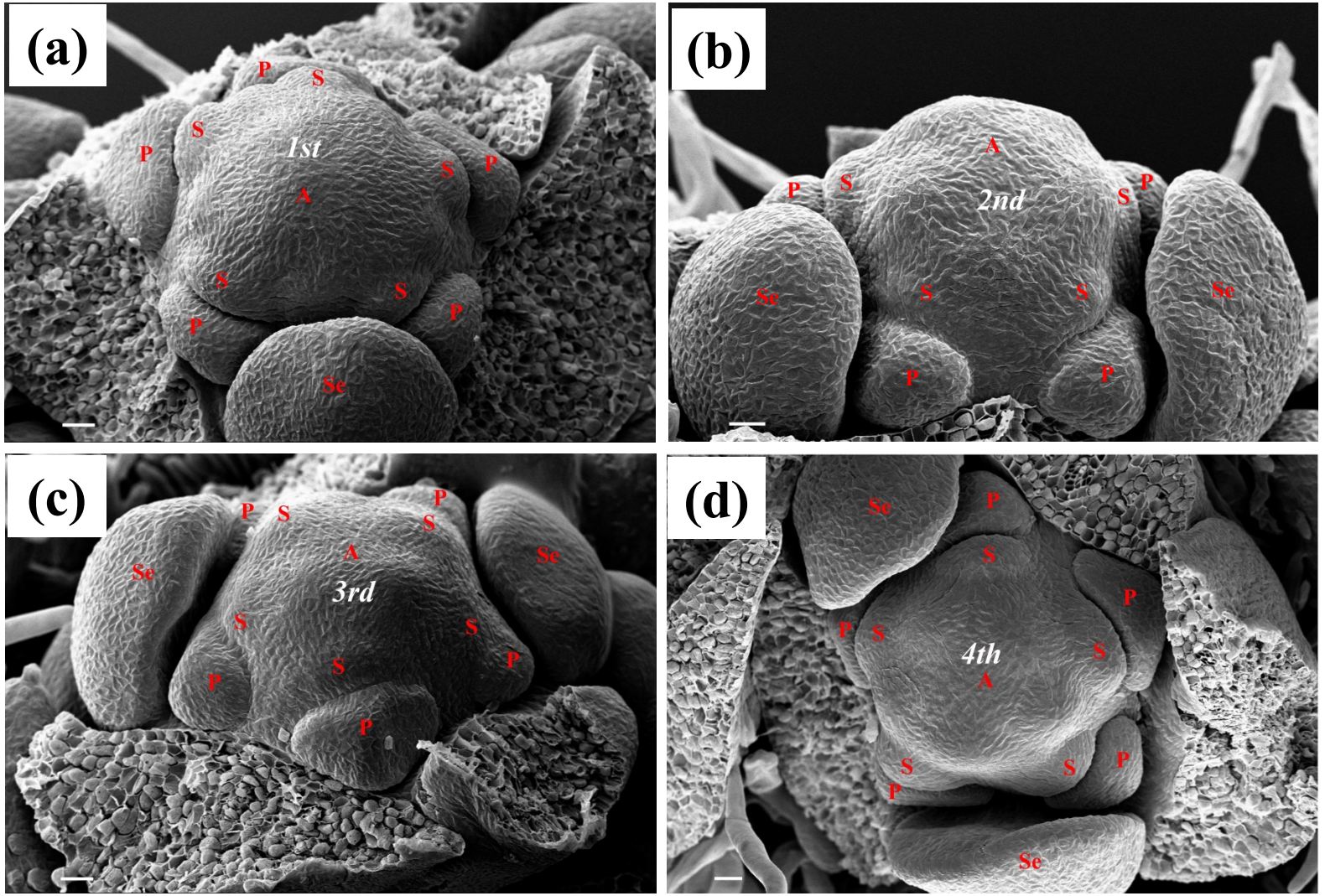

Figure S4 SEM observations of stage I - IV *Jatropha* flowers. (a) The 1st flower on a stage I IM dichasium; (b) the 2nd flower on a stage II IM dichasium; (c) the 3rd flower on a stage III IM dichasium; (d) the 4th flower on a stage IV IM dichasium. A: apical meristem, S: stamen primordium, P: petal primordium, Se: sepal primordium. Bars = 20 μm.

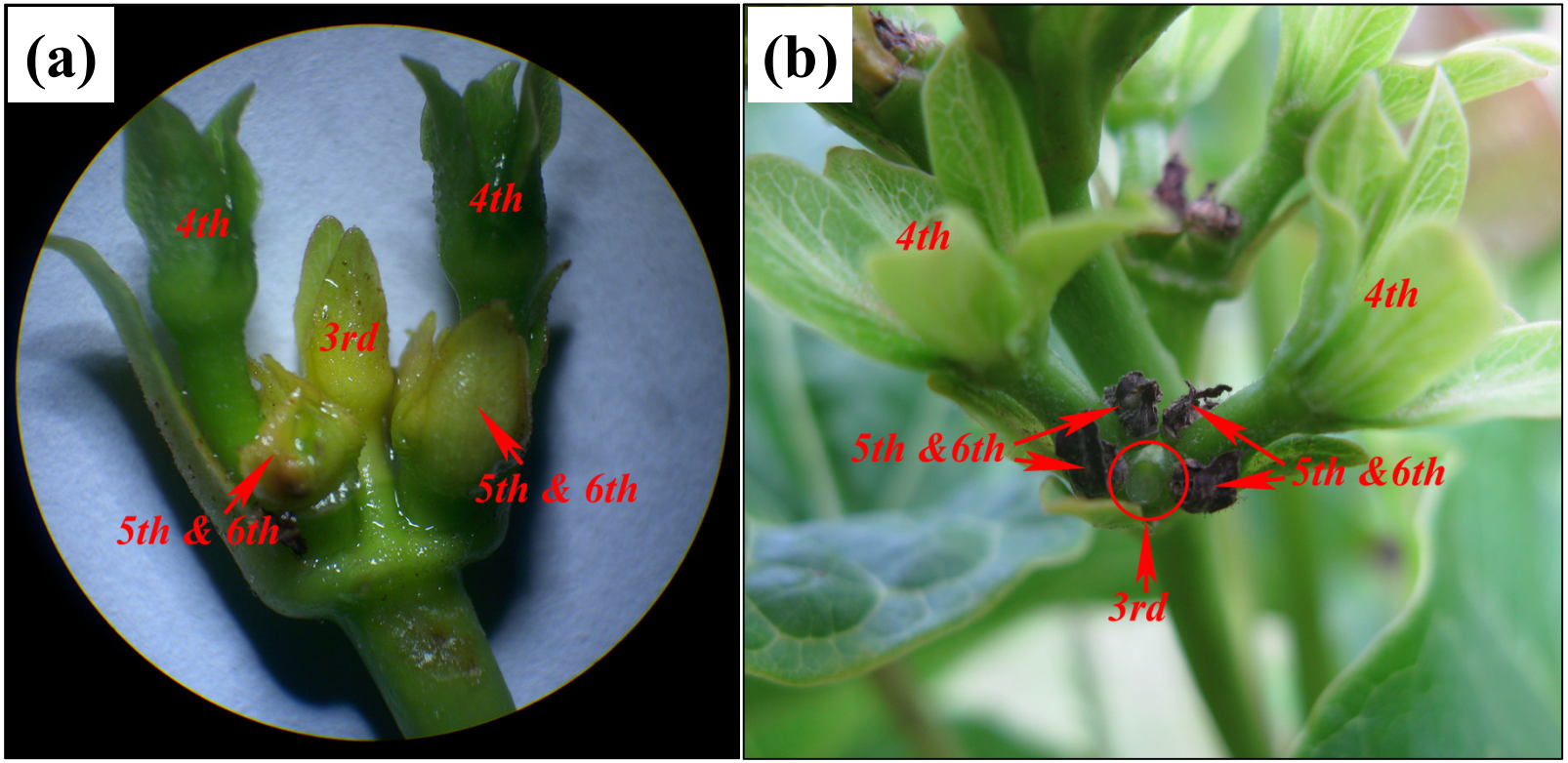

Figure S4 Abortion of flower buds from stage IV IMs treated with TDZ. (a) The 3rd, 5th and 6th flowers were yellowing after 20 days of TDZ treatment. (b) The 3rd, 5th and 6th flowers withered after 40 days of TDZ treatment.

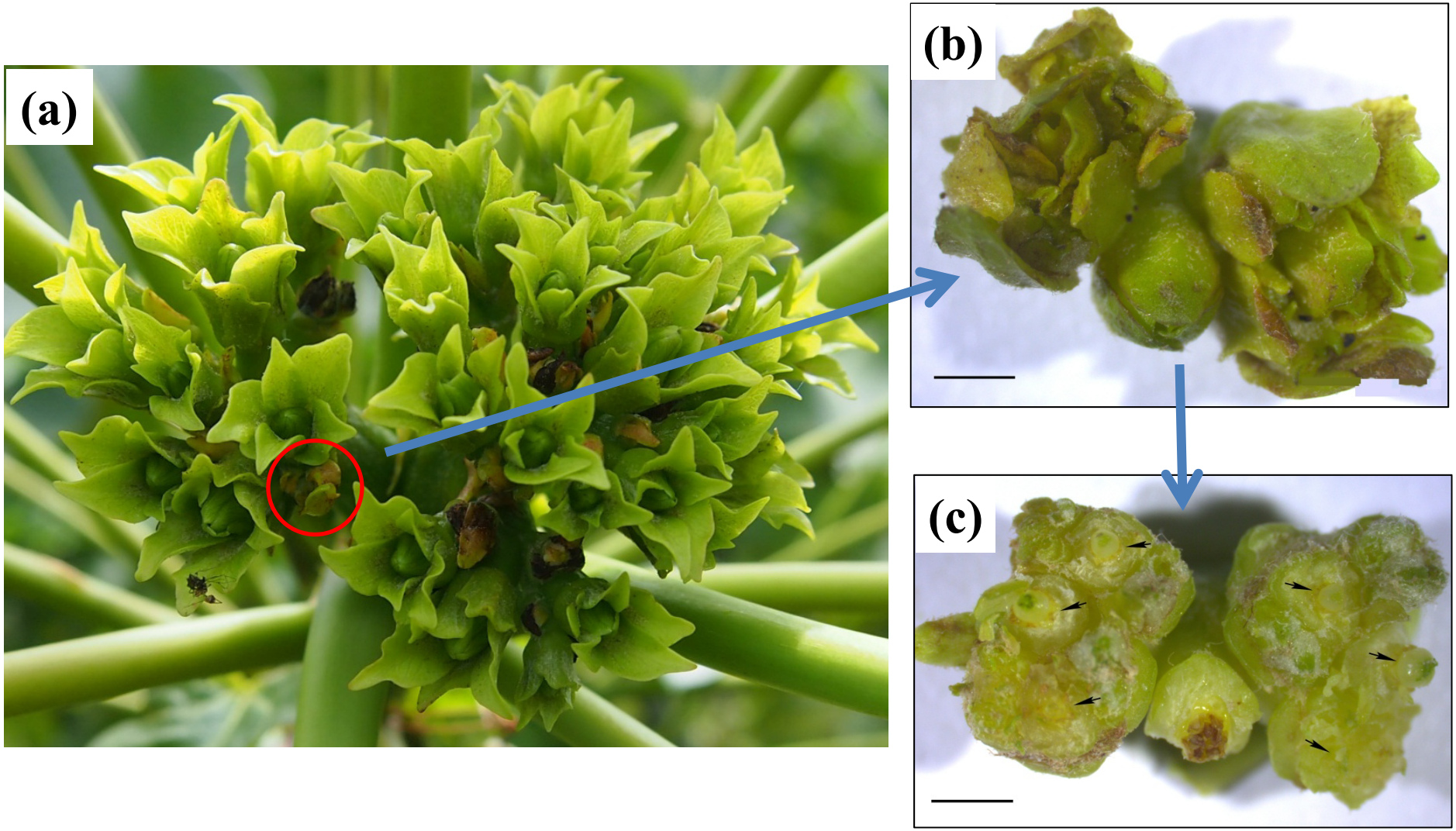

Figure S5 Aborted flower buds were found to contain pistils after dissection. (a) TDZ-treated inflorescence before blooming; (b) aborted flower buds on (a); (c) Pistils in aborted flower buds are indicated by black arrows (sepals and petals were removed). Bars = 5 mm.

Table S1 The number of aborted flower buds per inflorescence after TDZ treatments in *Jatropha*.

	TDZ Concentration (μM)			
IM stages	mock	25	75	225
stage I	0	4.23 ± 5.94*	7.57 ± 6.96*	13.38 ± 6.90*
stage II	0	18.25 ± 8.34*	18.61 ± 9.15*	30.13 ± 13.00*
stage III	0	34.91 ± 9.19*	33.62 ± 8.26*	49.52 ± 11.98*
stage IV	0	38.31 ± 13.72*	39.44 ± 9.77*	61.12 ± 18.46*

Values are mean ± standard deviation (n = 30 inflorescences). * Statistically different from the control at 1% level.

Table S2 Single-seed weight (g) and seed oil content (%) of *Jatropha* by TDZ treatment.

	mock		25 μM TDZ		75μM TDZ		225μM TDZ	
IM stages	Seed weight	Oil content	Seed weight	Oil content	Seed weight	Oil content	Seed weight	Oil content
stage I	0.69 ± 0.07	38.20 ± 4.06	0.63 ± 0.08	36.92 ± 5.89	0.66 ± 0.07	38.66 ± 3.30	0.59 ± 0.07*	36.07 ± 3.05
stage II	0.67 ± 0.06	37.83 ± 2.39	0.64 ± 0.08	38.16 ± 2.32	0.64 ± 0.06	38.36 ± 2.60	0.62 ± 0.06	37.50 ± 3.69
stage III	0.63 ± 0.07	38.26 ± 2.30	0.63 ± 0.07	38.33 ± 2.63	0.64 ± 0.07	37.75 ± 3.25	0.62 ± 0.07	37.35 ± 2.51
stage IV	0.67 ± 0.08	36.54 ± 2.88	0.65 ± 0.07	37.85 ± 3.55	0.63 ± 0.07	37.41 ± 2.46	0.66 ± 0.05	37.98 ± 2.95

A total of 900 seeds per treatment were measured. Values are mean ± standard deviation. * Significantly different from the control at the 1% level.