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Adaptation analysis of insect-resistant transgenic line after introducing *mcry1F* gene in maize

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Abstract: The ability to adapt, survive, and compete with weeds of transgenic plants is the necessary evaluation content to release transgenic lines in target regions. We compared weediness and agronomic traits of transgenic maize lines G1F-8 and G1F-19 carrying the *mcry1F* gene with their near-isogenic maize inbred line Zheng 58 in the wasteland and cultivated field under natural conditions for two consecutive years. The results showed that there was no significant difference identified in the species, quantity, and relative coverage ratio (RCR) of weeds between fields with G1F-8, G1F-19, and Zheng 58, regardless of the sowing pattern in the wasteland. Compared with the vigour of weeds, none of G1F-8, G1F-19, and Zheng 58 showed survival advantages, and all showed weak growth potential with no final grain yield. Meanwhile, no volunteer seedlings were found upon investigation in the following year. The simulated seed overwintering experiment in the wasteland further showed that the three kinds of maize could not germinate in the second year. In cultivated land, G1F-8 and G1F-19 had the same growth stages, plant height, and RCR as Zheng 58 throughout two years. In conclusion, the transgenic lines G1F-8 and G1F-19 exhibited no adaptability risk in Gongzhuling, Jilin, China.

Keywords: *Zea mays* L.; transgenic technology; competition; ecological suitability; wilderness

To meet the growing global demand for food, fibre, and fuel, innovative transgenic technology has become widely used worldwide (Lee et al. 2017, ISAAA 2018). Among the most important grain crops globally, maize was also one of the first crops to be developed as a transgenic cultivar to achieve resistance to insects, herbicides, and adverse environments through transgenic technology. In 2019, the total planting area of transgenic maize was 60.90 million hectares worldwide (ISAAA 2021). With the global development and promotion of transgenic maize, its environmental safety has also attracted much attention. The existing literature mainly focuses on functional efficiency (Wang et al. 2016, Fu et al. 2021), ecological fitness (Huang et al. 2016, 2017, Fu et al. 2018), the impact on non-target organisms, and biodiversity (Yang et al. 2014, 2015, 2018), and the evolution of resistance to target pests (Zhang et al. 2011, Wan et al. 2012, Berg et al. 2013, Santos-Amaya et al. 2016, Xv et al. 2018). The evaluation indexes of ecological adaptability of genetically modified crops mainly include the differences in main agronomic

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traits and weediness risk between transgenic crops and non-transgenic controls, such as plant height, relative coverage ratio (RCR), seed overwintering ability, and competition with other weeds (Yang et al. 2014, Lu et al. 2014).

In China, insect-resistant transgenic maize lines G1F-8 and G1F-19 carrying *mcry1F* genes (*Bacillus thuringiensis*), which China Agricultural University developed, have entered the environmental release stage and showed excellent potential for industrialisation. However, no studies have examined the adaptation of transgenic maize G1F-8 and G1F-19 in their targeted popularisation areas, where G1F-8 and G1F-19 are expected to be expanded plangting. Field trials were conducted for two years in the Gongzhuling city of Jilin Province, a primary maize production zone in China, to systematically evaluate the ecological suitability of transgenic insect-resistant maize lines G1F-8 and G1F-19 to support their future promotion in the spring corn region of China.

MATERIAL AND METHODS

Plant materials. Transgenic maize inbred lines G1F-8 and G1F-19, and their near-isogenic line Zheng 58 were selected as study materials. Zheng 58 is an elite inbred line in China. G1F-8 and G1F-19 were developed by China Agricultural University and feature insect resistance resulting from the foreign *mcry1F* gene that is stably inherited. Currently, G1F-8 and G1F-19 are in the environmental release stage in China.

Field experiment and design. The field experiment was conducted at the National Centre for Transgenic Plants Research & Commercialisation in Gongzhuling (43°30'N, 124°49'E), Jilin province, China, which is located one of the three maize golden belts worldwide, covers 40 hectares and is specially used to assess the environmental safety of genetically modified maize. The survival adaptability and competitiveness with weeds of G1F-8 and G1F-19 were assessed in both wasteland and cultivated land in 2017 and 2018. The near-isogenic line Zheng 58 was used as a control. The Ministry of Agriculture and Rural Affairs, China, approved the experiment.

Artificial abandoned land was used as wasteland; every plot (6 m^2, 2 m × 3 m) was separated by 1 m spacing. The experiment was designed with randomised blocks of four replicates, with 150 seeds per plot for each time. Sowing was conducted on April 25th and repeated on May 25th and June 25th at the same plot, 2017, respectively, aiming to simulate different seed-dropping periods in natural conditions. Each maize was planted with two designs: broadcast sowing, in which seeds were spread on the soil surface, and deep sowing, in which seeds were sown at 5 cm soil depth with a precision dibbler. After sowing, no additional management was carried out. These two patterns were used to simulate the competitive environment in which seeds fall into the wasteland. In 2018, maize seeds were not sown in the original wasteland used in 2017, and an adjacent wasteland was used to perform the same experiment as a biological replicate.

Each plot was designed to be 25 m^2 (5 m × 5 m) in cultivated land. A randomised block design with three replicates was adopted for comparing G1F-8, G1F-19, and Zheng 58. Seeds were sown on May 5th of both years at a rate of 320 kernels per plot with 1 kernel per hole. The quantity of seeds and field practices followed the local tillage management method, with other local maize fields, according to the actual situation of the field management, such as weeding, and insect control, to ensure the normal growth of maize.

Methods. In 2017 and 2018, the number of maize plants per plot in the wasteland was counted at 60, 90, 120, and 150 days after sowing. In addition, weed occurrence was investigated at 1, 30, 60, 90, 120, and 150 days after sowing in both broadcast- and deep-sown plots. Using the diagonal-line sampling method, we selected five areas of 0.25 m^2 per plot to investigate the weed number and species. The relative coverage ratio (RCR) of weeds was investigated based on the agricultural industry standards of the People's Republic of China (NY/T 720.2, 2003).

The maize planted in the wasteland was not harvested in 2017 and 2018, and no seed was planted in the following year. Then, the volunteer seedlings were investigated in the following May and June.

Thirty seeds of each maize line were individually placed into small nylon mesh bags (0.425 mm sieve) and sealed tightly. These bags were buried at 3 cm and 20 cm depths in the wasteland (snowy and rainy conditions, but no irrigation) in December 2018 using a completely randomised design with four replicates. In April 2019, all bags were retrieved and left in darkness at 25 °C. The germinated seeds in each small bag were counted after two weeks, which was conducted according to the national standard (GB/T 3543.4, 1995).

In the cultivated experimental land, we recorded the maize growth stage, plant height, and RCR. The investigated growth stages included seedling emergence

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(VE), tasseling (VT), silking (R1), and physiological maturity stages (R6). Plant height was evaluated at seedling (seven days after seedling, V3), middle whorl (small horn stage, V6), end whorl (big horn stage, V11), tasseling (VT), and silking stages (R1). When measuring maize plant height and RCR in each plot, five sampling sites were randomly selected, and ten plants per site were measured.

Data analysis. Analysis of variance was conducted using Excel (Microsoft, Redmond, USA). The measured traits were compared using the least significant difference test ($P < 0.05$).

RESULTS

Investigation of surviving maize plants in the wasteland. In the wasteland, the transgenic G1F-8 and G1F-19 and the control Zheng 58 were sown three times. The seedlings of the latter two sowings could not survive the third leaf stage due to the vigorous growth of weeds; only the first set of seedlings grew past this stage, so the first set of seedlings was investigated and analysed only. Broadcast sowing and deep sowing showed no significant differences in surviving plants among the three maize treatments on the same day after sowing (DAS) (Figure 1). In the wasteland, maize growth was strongly inhibited by weeds. Finally, all maize plants in the wasteland failed to bear seeds for the next generation due to the effect of weeds.

The investigation of weeds species, quantity and RCR in the wasteland. In 2017 and 2018, the RCR of weeds in G1F-8, G1F-19, and Zheng 58 plots remained similar from 30 to 150 DAS regardless of the sowing pattern. At 90 DAS, the weed RCRs reached 100.00% (Figure 2).

Weed species were similar across plots. The dominant weeds were barnyard grass (*Echinochloa crusgalli* [Linn.] Beauv.) and lamb's quarters (*Chenopodium album* Linn.), which represented 64.48% and 18.60%, respectively, of the RCR of total weeds at 30 DAS. The horseweed herb (*Conyza canadensis* [Linn.] Cronq.) became the dominant weed at 90 DAS in 2017, and in 2018, it became dominant at 120 DAS. Other species, including amaranth (*Acalypha australis* Linn.), pie-marker (*Abutilon theophrasti* Medicus), *Calystegia hederacea* Wall., alfalfa (*Medicago falcata* Linn.), and dayflower (*Commelina communis* Linn.) were present at lower densities (Figure 3).

Investigation of volunteer seedlings and seed germination rate after overwintering among G1F-8, G1F-19, and Zheng 58. All surviving G1F-8, G1F-19, and Zheng 58 plants in the wasteland were infertile throughout the two years. Thus, no volunteer seedlings were found upon investigation in May and June 2018 and 2019.

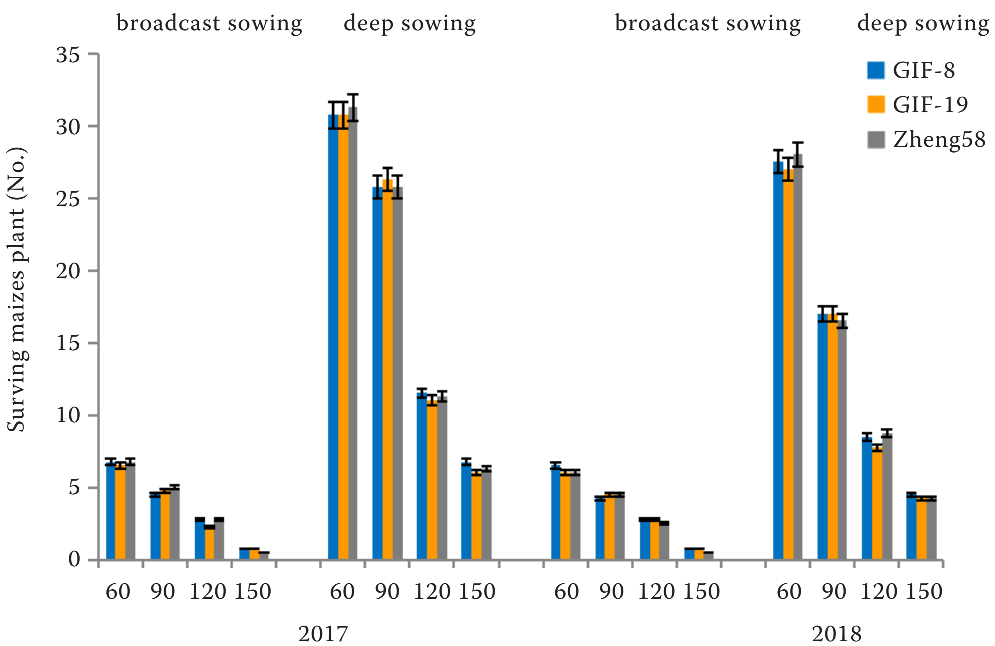

Figure 1. The surviving maize plants are at different investigated stages in the wasteland. Note no different letters indicate that there is no significant difference ($P < 0.05$)

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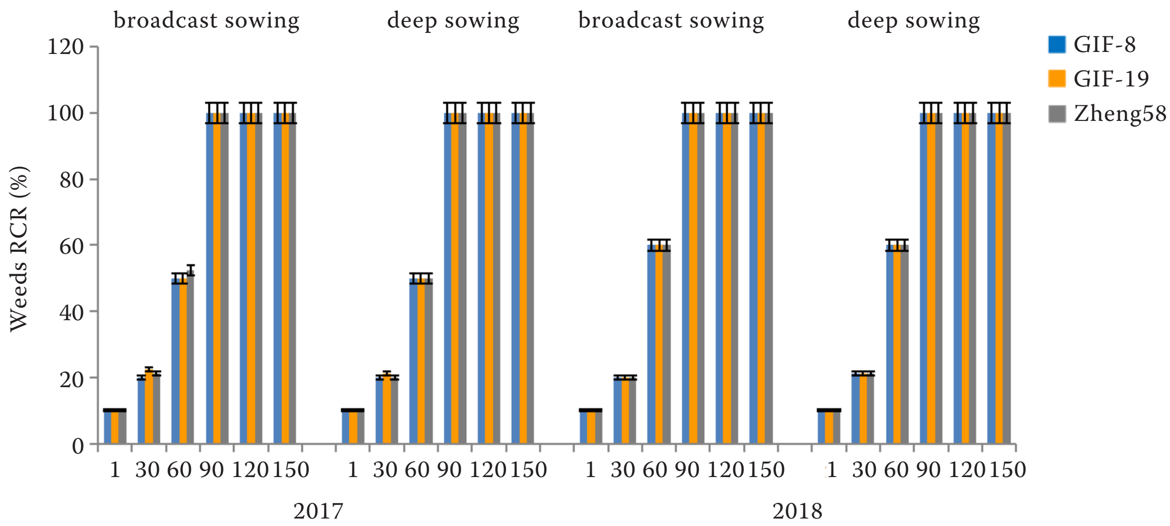

Figure 2. The relative coverage ratio (RCR) of weeds from 1 to 150 days after sowing (DAS) in the wasteland. Note no different letters indicate that there is no significant difference ($P < 0.05$)

After overwintering in the wasteland under simulated natural conditions, the seed germination experiment showed that none of the three different lines germinated.

Performance of agronomic traits of three different lines in cultivated land. In cultivated land, G1F-8, G1F-19, and Zheng 58 reached each growth stage at approximately the same date, including seedling emergence, tasseling, silking, and physiological maturity stages.

In both years, all three lines consistently maintained similar heights at seedling, middle whorl, end whorl, tasseling, and silking stages. At the silking stage, G1F-8, G1F-19, and Zheng 58 reached 211.33, 211.20, and 212.00 cm in 2017 and 211.67, 211.53, and 211.20 cm in 2018, respectively (Figure 4). In cultivated land, the plant heights of G1F-8 and G1F-19 were similar to that of Zheng 58 throughout the growth period in both years, which showed no significant difference.

There were no significant variations between the RCRs of G1F-8, G1F-19, and Zheng 58 plants at the five maize growth stages in 2017 and 2018 (Figure 5).

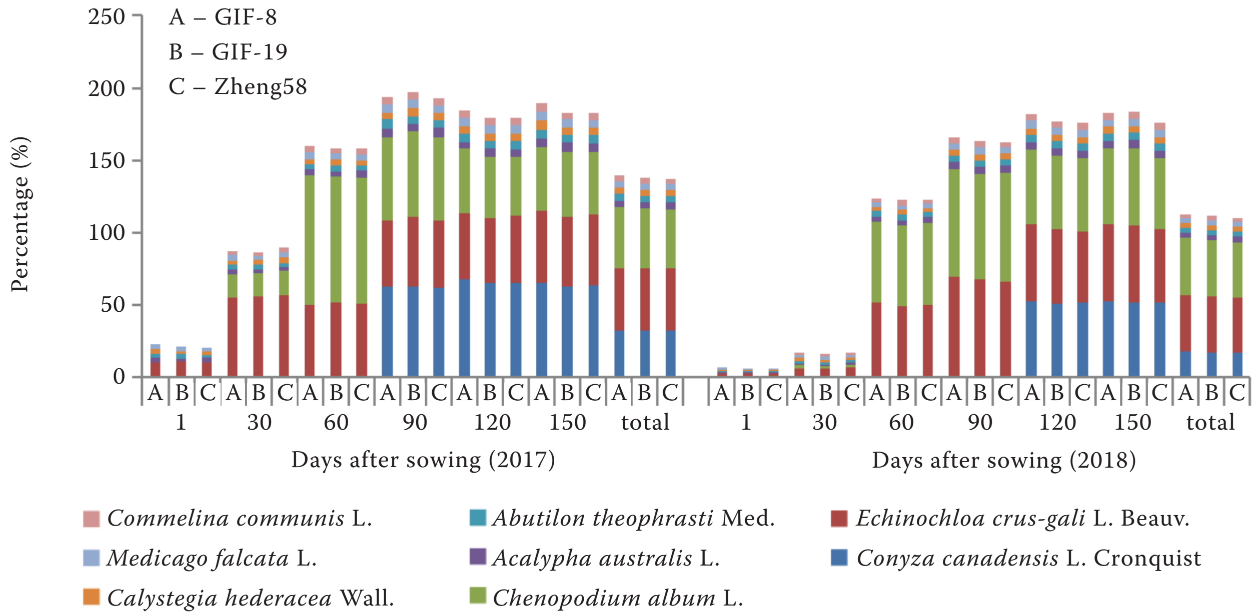

Figure 3. The percentages of weeds in G1F-8, G1F-19, and Zheng 58 plots. This diagram illustrates the weeds with a percentage > 1%

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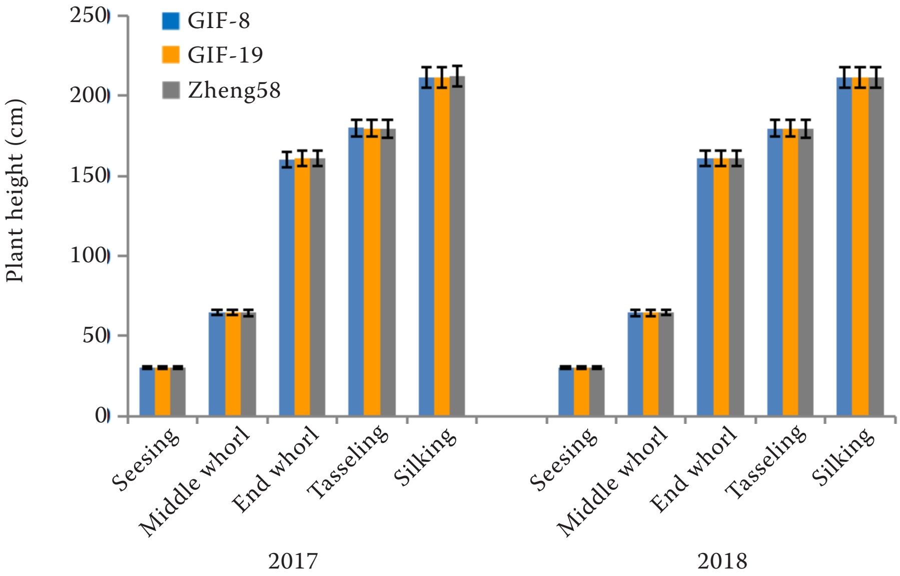

Figure 4. Plant heights of the three maize lines in the field. Data of the same maize line from the seedling to the silking stage were pooled for analysis. Note no different letters indicate that there is no significant difference ($P < 0.05$)

DISCUSSION

When evaluating the weediness of genetically modified crops, the whole growth period and the important indicators of weediness at each stage should be fully considered. For example, Raybould et al. (2012) suggest that if the agronomic characteristics of genetically modified crops and their near-isogenic partners are nearly identical, they will show similar weediness even when growing in the wild, which indicates that no ecological risks will happen when cultivating genetically modified crops. Fu et al. (2018) evaluated the fitness of *cry1Ab/c* transgenic rice in both farmland and saline-alkaline soils, assessed the expression pattern of exogenous *cry1Ab/c* protein, and examined the vegetative and reproductive fitness of rice; they determined that the ecological risk of *cry1Ab/c* transgenic rice is not expected to be higher than that of its parental rice cultivar if the former escapes from natural saline-alkaline soil. Lu et al. (2014) estimated the life-cycle fitness of transgenes introgressed to wild rice relatives, the result showed that transgenic hybrid progeny derived from crop-wild/weed crosses showed increased fitness under the presence of insects, but no significant differences in fitness were detected between transgenic and non-transgenic populations under low the presence of insects. Liu et al. (2015) investigated the relative plant growth and reproduction of transgenic insect-resistant *Brassica napus* and

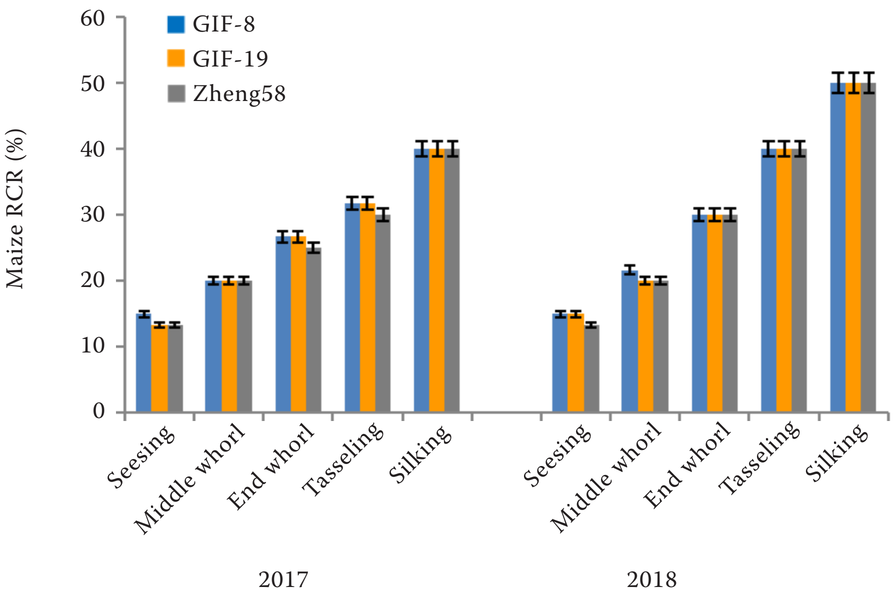

Figure 5. The relative coverage ratios (RCRs) of the three maize lines from the seedling stage to the silking stage after sowing in the field. Note no different letters indicate that there is no significant difference ($P < 0.05$)

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its non-transgenic control. The conclusion is trBC2 plants performed better than ntrBC2 plants under the pressure of target pests (Liu et al. 2015). Huang et al. (2016) compared stacked transgenic rice T1c-19 (*cry1C*/bar* genes) with two non-transgenic rice cultivars under field conditions and without selection pressures. The results demonstrated that exogenous insect and herbicide resistance genes did not confer a competitive advantage to T1c-19. We conducted a similar study with Huang and concluded that there was no significant difference in ecological adaptability between transgenic maize and its near-isogenic maize inbred line under natural conditions without the presence of insects. Meanwhile, during evolution, the germinating capacity of plants is closely related to weediness and is thus an important indicator for evaluating the weediness of transgenic crops (Herman et al. 2011, Sammons et al. 2014, Marral et al. 2020, Raybould et al. 2012). Our study's simulated seed overwintering experiment in the wasteland showed that the three kinds of maize could not germinate in the second year. The results of our research are basically consistent with the above studies.

The experimental methods were not sufficiently comprehensive among previous studies that evaluated the weediness of genetically modified crops. Huang et al. (2016) solely conducted field trials in a cultivated environment, and Fu et al. (2018) experimented with greenhouses. Moreover, the conclusions of Huang et al. (2016) and Fu et al. (2018) were based on the data from only one year of experiments. However, most studies showed that it is necessary to include seeds dropped in the wasteland when evaluating the weediness of genetically modified crops. In the wasteland, surviving maize plants, weed species, quantity, RCR, and volunteer maize seedlings are closely related to weediness. Therefore, field trials in both the cultivated field and wasteland were conducted for two years to evaluate the effects of the environments in our experiment. There was no significant difference in the quantity of surviving maize plants between G1F-8, G1F-19, and Zheng 58 in the wasteland, suggesting that the insertion of the exogenous gene failed to change the survival ability of maize. Moreover, we investigated the dominant weed species and quantities in the wasteland and observed that the weed community remained unchanged in the plots of G1F-8, G1F-19, and Zheng 58, which demonstrated that none of the three maize types could compete with weeds. Furthermore, we assessed volunteer seedlings; no ones were found upon investigation in the following year. The experiment of seed germination after overwintering in the wasteland. The results indicated that overwintering in the wasteland, seed viability similarly decreased to zero among the three maize lines.

In cultivated land, the maize growth stage, plant height, and RCR are important indices for adaptability (Jiang et al. 2015, Zhang et al. 2017). In the present study, we monitored four stages of G1F-8, G1F-19, and Zheng 58 for two years and concluded that the growth stages of three types of maize were identical, suggesting that the insertion of the exogenous gene did not alter the maize growth stages. Plant height is another crucial factor that determines the competitiveness of a crop community (Raybould et al. 2011, Finger et al. 2011, Garcia-Alonso et al. 2014). Taller plants can acquire more sunshine during their growth, and photosynthetic products will increase accordingly, thus providing them with an improved competitive ability compared to shorter plants. In our experiment, we analysed the plant heights and RCRs of G1F-8, G1F-19, and Zheng 58 throughout two years and found no significant differences between the three maize lines.

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