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Tracking and Monitoring Leaf Development, Coupling Law and Regulation Techniques during Flowering Period of Hybrid Foxtail Millet (*Setaria italica* (L.) P. Beauv.) Parental Lines

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Abstract

The determining factor of *Setaria italica* (L.) P. Beauv. is the coupling of its flowering stage and outcrossing rate which leads to low and unstable seed yields in self-pollinated foxtail millet hybrids and thereby limits their large-scale application. In this study, Datong 27, Datong 29 and gu 83 were screened and identified through meticulous observations of their pollination habitats. High exposure rate, degree of exposure and plump of stigma are good factors to accept foreign pollen. Datong 27 and Datong 29 have some additional characteristics, such as long filaments and exposed and full anthers that contain a large amount of pollen. We transformed into a series of stigma-exposed and plump sterile lines that easily accepted exotic pollen. New restorer lines with anthers that were full of powder and exhibited quick recovery, which improved the parental lines' heterosexual characteristics. By tracking and monitoring the leaf development of the new sterile and restorer lines, a coupling law of leaf development was determined and a series of flowering control measures were formulated. These factors ensured that the parental lines encounter one another during the flowering stage. By utilizing fertilizer and water, the vitality of the female stigma, amount of powder scattered and powder loosening time were prolonged, which increased hybrid

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seed yields from 1500 to 3000 kg/hm^2. These findings were helpful in resolving the technical problems of seed production that restricted the propagation of foxtail millet hybrids and supporting future large-scale applications.

Keywords

Foxtail Millet, Hybrids, Leaf Age Coupling Law, Flowering Regulation, Seed Production Technology

1. Introduction

In recent years, the utilization of foxtail millet heterosis has progressed in China. The yield per unit of foxtail millet hybrids producing over 11,250 kg/hm^2 [1] [2]. As a self-pollinating crop, foxtail millet has a small amount of pollen, short propagation distance, low outcrossing rate, and sensitive photo-temperature response. During the seeding process, changes in the environment can lead to florescence in hybrid parents that do not meet [3]. As a result, florescences that do not meet and low outcrossing rates lead to low hybrid seed yields and instability, thereby limiting the large-scale application of foxtail millet hybrids.

Because foxtail millet is mainly distributed developing countries, such as China and India, research on foxtail millet lags behind that of other cereal crops. Coupled with self-pollination, foxtail millet heterosis has been difficult to study. Research on foxtail millet in Japan and the United States has mainly focused on its conservation, utilization of resources and gene mining [4]. In France and Canada, breeding of herbicide-resistant varieties has been conducted [5] [6] [7]. In Australia, foxtail millet varieties have been developed for foraging grass [8]. Meanwhile, India and similar countries still rely on conventional breeding of foxtail millet [9]. Currently, there were no reports on the use of millet heterosis.

China is the only country in the world that systematically studies the utilization of millet heterosis [3]. Since 1980s, the researchers have attempted to increase the seed yields of foxtail millet hybrids by various methods, such as the selection and application of photo-thermophilic male sterile lines [10]. In the cold-growing hilly area of a seed production site [11]. Using the cultivation method, the ratio of female and male were reduced and the planting line spacing of the sterile lines was shortened, in order to decrease the distance between sterile and restorer lines [12], and ensured that the parental lines encounter one another during the flowering stage by sowing within a certain time interval [13]. However, these effects on seed production were limited. Due to foxtail millet's sensitivity to light and temperature, the flowering stage will not be synchronized and production is thereby reduced. Currently, a high-crossing rate of a sterile or restorer line has not been achieved and the development of leaf age in sterile and restorer lines has not been identified, and a series of control measures have not been formulated to ensure that the parental lines encounter one another during the flowering stage to improve seed yields.

In other crops, research on high-yield and highly-efficiently seed production techniques has been reported. For example, in cross-pollinating crops and regular pollen crops, such as maize and sorghum, the flowers possess a large amount of pollen and the transmission distance is long. Based on the heading period of the male and female parental lines, when sowing is conducted within a certain time interval, the parental lines encounter one another during the flowering stage. When the male parental line has more tassel branches and full anthers, pollination is improved [14] [15] and seed yields are increased. Moreover, these crops are not sensitive to light and temperature.

Rice and foxtail millet are self-pollinating crops, and the promotion speed of rice hybrids is limited by seed yields. For this reason, rice researchers have conducted some studies on the exposure rates and degrees of rice stigma. For example, some researchers believed that rice stigma exposure rate was a quality trait controlled by major genes [16]. Some studies had suggested that the stigma exposure rate was a quantitative trait controlled by multiple genes, but the dominant effect was larger [17] [18]. Other researchers suggested that this trait was controlled by multiple genes and that the broad heritability was as high as 90% - 96.86% [19] [20]. However, these studies had mainly focused on the inheritance of the stigma exposure rate and related gene mining. A few reports were available on the creation of rice sterile lines with high stigma exposure rates and no restorer lines with high outcrossing rates. Moreover, the thousand-grain weight of rice was generally 18 - 34 g [21], and the flowers were relatively large, while the foxtail millet weight is only 2.2 - 3.3 g, and the flowers were small, ~1/9 that of rice [22]. Most of the results of rice do not apply in foxtail millet.

The relationship between leaf age development and ear differentiation had been investigated in different crops [23]. In recent years, the influence of the relationship between leaf and ear development by nutrients has been studied [24]. However, such studies rarely used seed production technology, and no studies have reported on the coupling law of parental leaf age development based on the encounter of hybrid parental lines.

In terms of flowering regulation, some studies on different seedling sizes and hormone regulation have been conducted in hybrid parental lines [25] [26], but were lacking in regulatory measures of specific flowering types.

Based on foxtail millet's sensitivity to light and temperature, self-pollination, and low-crossing rate, this study investigated the floral characteristics of foxtail millet, identifying and screening some resources with floral openings at a high level, high stigma exertion rates, high degrees of exertion, plump stigma fullness, long filaments and exposed anthers. Additionally, through crossing, backcrossing and other methods, these characteristics were separately introduced in sterile and restoring lines. Sterile lines with strong pollination and restorative lines with strong loose powder abilities were created to improve outcrossing rates. By tracking and monitoring the developmental dynamics of foxtail millet hybrid parental leaves, it was possible to detect and promote coupling during the flowering stage of hybrid parental lines, as well as determine key leaf age nodes that regulate the parental lines based on the relationship between leaf and ear differentiation. Moreover, by studying agronomic regulation, fertilizer and water regulation, physiological and chemical control measures, a coupling law of leaf development for the parental lines was formulated, and a series of targeted control measures were developed to ensure foxtail millet hybrid parental line encounters in the flowering stage, enhanced outcrossing rates and seed yields. Thus, the findings of this study resolve the problem of restricted extension of foxtail millet hybrids that had low and unstable seed yields.

2. Materials and Methods

2.1. Identification and Screening of Foxtail Millet Parental Lines

The experiment was carried out in Shijiazhuang, China, from 2007 to 2008. Three thousand varieties of foxtail millet were collected from various ecological areas throughout China. Randomly planted in identification nursery of germplasm resource, and used to identify foxtail millet resources.

At the flowering stage, a magnifying glass was used for preliminary observation and screening. Three plants (three replication) with high stigma exertion rates were screened, and the ears were transferred to the laboratory for observation under stereo microscope (SZ61, Olympus, Tokyo, Japan), one grain was selected from the middle, upper and middle-lower parts of the ear every plant. The stigma exertion rate, exposure degree, and pinnate stigma development were further observed from 6:30 to 7:30 am every day. During the observation of the stigma exposure rate, the flowering number and stigma exposure were counted every day, and the flowering florets were removed. The remaining florets on the grain were placed in a beaker of water and observed after the next day of flowering. Flowering takes 3 - 4 days and one spikelet was observed. Through the above observations, foxtail millet resources with obvious stigma exertion, high exertion rates and plump stigma were screened, photographed, and labeled as R11, R12, R13 and so on.

Moreover, a magnifying glass was used to screen the anthers from the selected test materials, and the anthers of these test materials were completely exposed. Then, the ears were transferred to the laboratory for observation under a stereo microscope. Materials with full anthers and loose powder were selected, photographed separately, and labeled as R21, R22, R23 and so on.

2.2. Emergence of Foxtail Millet Sterile Lines with High Outcrossing Rates

The sterile line, 1066A, was used as the female parental line to prepare a series of hybrids with male parental lines selected from the foxtail millet breeding materials, R11, R12, R13, etc., that exhibited obvious stigma exertion, high exertion rates, and plump stigma.

The first generation of hybrids (a series of hybrid combinations) was prepared from the aforementioned parental lines, and sowing was conducted in a selective nursery. After maturity, 3 - 4 plants with good seed and full individuals were selected, harvested and threshed for seeding in the next year.

Single plants harvested from the previous generation were interlaced and planted separately with the male parental lines, R11, R12, R13, etc. The stigma developmental characteristics of isolated sterile plants were observed using a magnifying glass. Single plants with high exogenous rates were selected. These infertile plants were backcrossed with the male parent. After maturity, sterile plants were mixed and harvested. The backcrossed first-generation seeds were subsequently obtained and labeled as B11, B12, B13 and so on.

After continuous observation of the stigma traits of the sterile offspring plants and continuous directional selection, multiple stable sterile ear rows with the target traits were obtained. Then, after two consecutive years, observation of the stigma traits met the target trait requirements; the ear row was a sterile line created with obvious stigma exposed, a high exposure rate and plump stigma.

2.3. Creation of Foxtail Millet Restoration Lines with Powder-Removing Ability

First, the female materials, R21, R22, R23, etc., which had full anthers and loose powder contents, were crossed with the male materials K359 and K492 with herbicide-resistance and strong restorability. A series of hybrid combinations were carried out.

Then, the first generation of hybrids (a series of hybrid combinations) sowing was conducted in a selective nursery. Herbicides were sprayed at the 4-leaf stage to kill pseudo-hybrids that were not resistant. Then, 3 - 4 plants with good seed and full plants were harvested and threshed for planting in the next year.

From the first generation, after selecting single plants with full anthers and loose powder contents, backcrossing was conducted with K359 and K492 four times to establish the next generation with nuclear replacement. Then, the backcrossed progeny was oriented and selected. After continuous selection for 4 - 5 generations, stable hybrid progeny was selected. After selecting a single plant, the remaining parts of the ear line were mixed and harvested. After two consecutive years of observation of the anther traits, the rows of spikes that meet the requirements of the target traits, anti-sethoxydim recovery lines with full and exposed anthers and a large amount of powder were obtained.

2.4. Detection of Leaf Age Dynamics in Foxtail Millet Hybrid Parental Lines

The leaf age dynamics experiment was conducted at the Luannan Research and Experiment Station of Tangshan Normal University from 2016 to 2017. The newly selected sterile lines, DZ759A, KM58A and KM249A, and the restorer lines, HK902 and HK950, were used as test materials. First, the restorer and sterile lines were sowed at the same time. Afterward, the sterile line was planted once every day a total of 20 times. During the seeding stage, three representative plants was selected for each sowing period. Each piece of the unfolded leaves was marked with a marker pen. Leaves were subsequently observed, the dates of development for each period were recorded. The sowing rules regarding the sterile and restorer lines that meet during flowering stage were also explored. By tracking and monitoring leaf age development, a dynamic database of leaf age development of the foxtail millet hybrids was established. Based on this, a coupling model of leaf age development of the male and female parental lines was also established.

2.5. Regulation Technology for the Foxtail Millet Hybrid Parental Lines

The regulation technology experiment was conducted at the Luannan Millet Research and Experiment Station of Tangshan Normal University from 2016 to 2017. Plot regulation and the seed field control experiments were conducted simultaneously. Flowering regulation of the foxtail millet hybrid parental lines was investigated from three aspects: agronomy, fertilizer and water, and chemical regulation.

The plot experiment was randomly arranged in 14 rows with a row spacing of 0.4 m, row length of 3.5 m, and three replicates. In order to avoid the influence of different control measures, the sterile and restorer lines were planted in a 2:1 ratio in every unit (2 sterile lines on both sides and 1 restoring line). The female parent was sowed at 5 days, 2 days before the male parental line, on the same day and at 2 days, 5 days and 10 days after the male parental line with a total of six levels. The seed field control experiment was not repeated and was conducted under the same conditions as the plot test with six levels.

Based on the co-extension relationship between leaf and ear differentiation, four regulatory nodes were established: the maternal 4-leaf stage, which consisted of seedlings that did not rely on seed vegetative growth that exhibited self-sufficient fertility; 8-leaf stage, the earliest stage of panicle differentiation; 12-leaf stage, the beginning of the booting stage; 16-leaf stage, the late booting stage [27].

At each regulatory node, based on the leaf development coupling law of the sterile and restorer lines, agronomic regulation was adopted as follows: development of fast seedlings, delayed thinning time, controlled growth; slow development, accelerated thinning time, big seedling and strong seedling leaves, and growth promotion. Fertilizer and water regulation were as follows: 0.5% - 1.0% ammonium bicarbonate water or urea irrigation roots and 0.5% - 1.0% potassium dihydrogen phosphate irrigation roots. Hormone regulation was as follows: late parental leaves were sprayed with 2×10^{-5} gibberellin and 2×10^{-5} naphthalene acetic acid. The regulation effects of various control measures were investigated to provide technical support and ensure that the parental lines encounter one another during the flowering stage and increased seed production.

3. Results

3.1. Identification and Screening of Foxtail Millet Resources with Some Object Characters

After two consecutive years of field magnifying inspection and further screening using a microscope in the laboratory, four parental lines with obvious exertion, high exertion rates, and plump stigma were obtained from 300 total breeding materials; they are: 206083, Datong 29, Datong 27 and 1066A (**Figure 1**). The

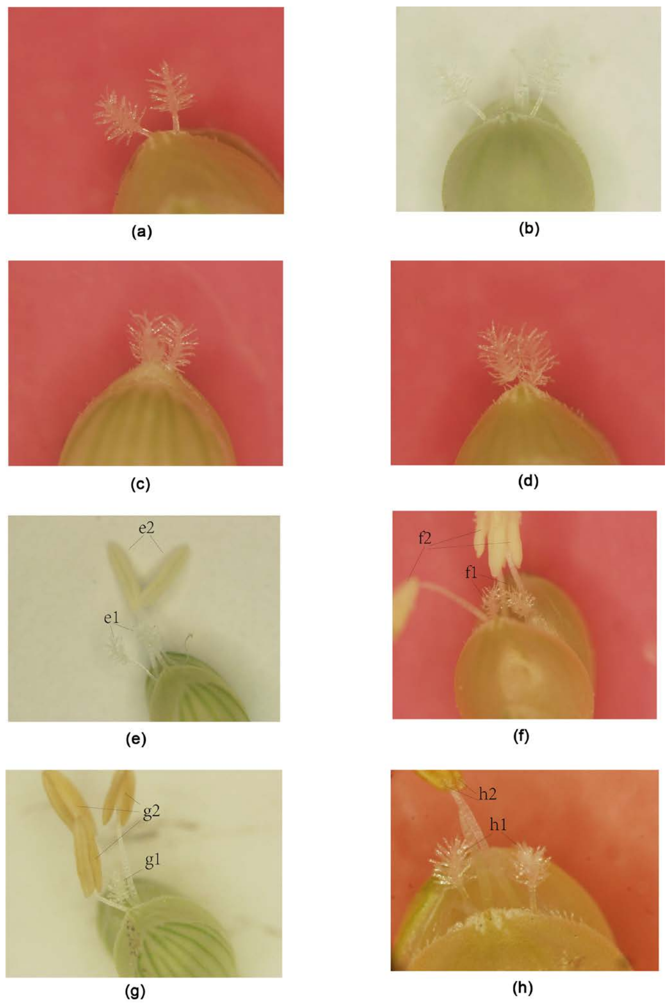

Figure 1. The organs development of foxtail millet. (a). The stigma development of 206083 in 2007. (b). The stigma development of 206083 in 2008. (c). The stigma development of 1066A in 2007. (d). The stigma development of 1066A in 2008. (e). The development of Datong 27 in 2007, e1. stigma, e2. anther. (f). The development of Datong 27 in 2008, f1. stigma, f2. anther. (g). The development of Datong 29 in 2007, g1. stigma, g2. anther. (h). The development of Datong 29 in 2008, h1. stigma, h2. anther.

stigma of 206083 were completely exposed and plump with an exposure rate of 85.69%, Datong 27 stigma were completely exposed and plump with an exposure rate of 77.57%, Datong 29 stigma were completely exposed and moderately plump with an exposure rate of 68.83%, and 1066A stigma were 3/4 exposed and plump with an exposure rate of 78.23% (Table 1, Table S1-1 and Table S1-2). Collectively, these results indicate that the breeding materials have better stigma development and strong abilities to accept pollen.

Two parental lines with long filaments and anthers that were completely exposed and full were screened; they are Datong 29 and Datong 27 (Figure 1). These lines were used to transfer the recovery line with strong loose powder abilities (Table 2, Table S2-1 and Table S2-2). These findings indicated that Datong 27, Datong 29 stigma and anther development were better and had a high outcrossing rate.

3.2. Emergence of Foxtail Millet Sterile Lines with Obvious Stigma Exertion, High Exertion Rates, Plump Stigma and Easy Exotic Pollination Acceptance

Using 1066A as the female parental line and 206083, Datong 27 and Datong 29 as the male parental lines, a series of hybrid combinations were prepared. Then, 206083, Datong 27 and Datong 29 were used as recurrent parents, and generations were subsequently used as experimental materials. After interval generation backcrossing four times and orientation selection, the stigma traits of the offspring were observed. In 2012, the target traits were bred, DZ759A (after the hybrid combination 1066A × 206083 was combined, backcrossed 4 times with

Table 1. The stigma development of 206083, 1066A, Datong 27 and Datong 29.

Material	Stigma exsertion rate (%)			Degree of exposure (%)			Fullness			Overview
	2007	2008	Mean Value	2007	2008	Mean Value	2007	2008	Mean Value	
206083	86.07	85.31	85.69	100	100	100	high	high	high	good
1066A	77.56	78.90	78.23	75	83	79	high	high	high	good
Datong 27	77.46	77.67	77.57	100	100	100	medium	medium	medium	good
Datong 29	68.64	69.01	68.83	100	100	100	medium	medium	medium	good

Table 2. The anther development of Datong 27 and Datong 29.

Material	Anther exposure rate (%)			Degree of exposure (%)			Fullness			Overview
	2007	2008	Mean Value	2007	2008	Mean Value	2007	2008	Mean Value	
Datong 27	76.88	76.96	76.92	100	100	100	high	high	high	good
Datong 29	79.73	78.26	79.00	100	100	100	high	high	high	good

206083), KM58A (after the hybrid combination 1066A × Datong 27 was combined, backcrossed 4 times with Datong 27) and sterile lines including KM249A (after combining 1066A × Datong 29, using Datong 29 to backcross 4 times). Then, from 2013 to 2014, the stigma traits of these sterile lines were further observed for two consecutive years and "DZ759A, KM58A, KM249A" were determined to be the new types of sterile lines with the created stigma and other target traits (Figure 2; Table 3, Table S3-1 and Table S3-2).

3.3. Emergence of Foxtail Millet Restoration Lines with Exposed and Full Anthers and Strong Powder-Removing Ability

Using Datong 27 and Datong 29 as the female parental lines, while K359 and K492 as the male parental lines, a series of hybrid combinations were prepared. Then, K359 and K492 were used as recurrent parents, backcrossing was conducted

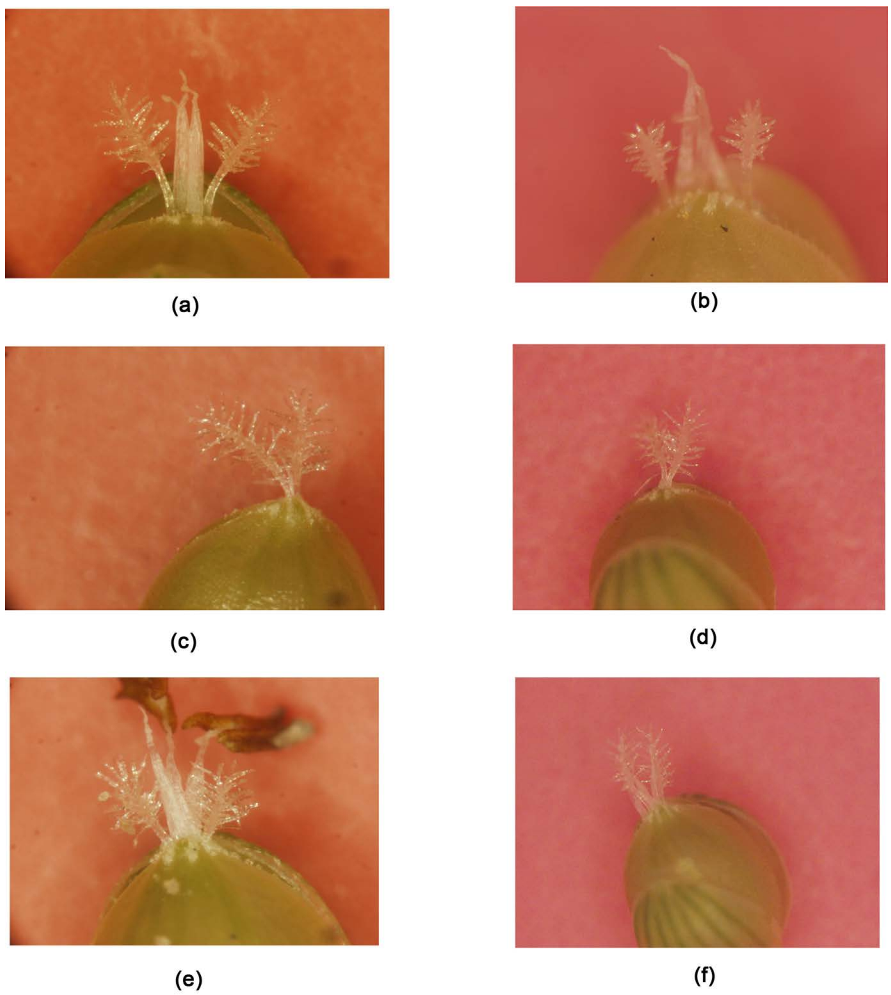

Figure 2. The stigma development of foxtail millet. (a). The stigma development of DZ759A in 2013. (b). The stigma development of DZ759A in 2014. (c). The stigma development of KM249A in 2013. (d). The stigma development of KM249A in 2014. (e). The stigma development of KM58A-1 in 2013. (f). The stigma development of KM58A-1 in 2014.

Table 3. The stigma development of DZ759A, KM58A and KM249A.

Material	Stigma exsertion rate (%)			Degree of exposure (%)			Fullness			Overview
	2013	2014	Mean Value	2013	2014	Mean Value	2013	2014	Mean Value	
DZ759A	82.92	82.82	82.87	100	100	100	high	high	high	good
KM58A	79.63	78.89	79.26	100	100	100	high	high	high	good
KM249A	74.26	74.94	74.60	100	100	100	high	high	high	good

four times. After orientation selection for anther traits, in 2012, the outstanding target traits were bred HK902 (after combining Datong 27 × K359, backcrossed 4 times with K359), HK950 (after combining Datong 29 × K492, backcrossed 4 times with K492), etc. Then, from 2013 to 2014, the anther traits of these restorer lines were observed for two consecutive years, while "HK902, HK950" were determined to be the new restorer lines with target traits, such as anthers, that met the requirements (Figure 3; Table 4, Table S4-1 and Table S4-2).

3.4. Detection and Coupling Law of Leaf Age Dynamics in Foxtail Millet Hybrid Parental Lines

From 2016 to 2017, the tracking and monitoring results of the leaf developmental dynamics of the sterile lines, DZ759A, KM58A and KM249A, while the restoration lines, HK902 and HK950, revealed that in Luannan and similar ecological areas, when DZ759 and HK902 were the parental lines of the hybrid seeds, 2 - 4 days after the male parental lines advanced sowing the female parental lines, the parental lines encountered one another during the flowering stage; 3 days is the most conducive time for encounters during the flowering stage. When the DZ759 and HK950 group were the parent lines of the hybrid seeds, the male parental lines encountered the female parental lines 3 - 5 days in advance; 4 days is the most conducive time for encounters during the flowering stage. When the KM58A, KM249A and HK902 groups were prepared, the male parental lines encountered the female parental lines 9 - 15 days in advance. When the KM58A, KM249A and HK950 groups were prepared, the male parental lines encountered the female parental lines 10 - 18 days in advance. Additionally, due to the sterile line's sensitive light-temperature response, the leaves of the sterile lines, DZ759A, KM58A and KM249A, exhibited a decreasing trend as the sowing date was delayed, but this trend was not regular. The temperature was high enough during the sowing period and the leaves were not reduced. After further analysis of the developmental dynamics of leaf age at suitable sowing dates, the relationship between the parent control nodes was determined (Figure 4 and Figure S1; Table 5, Table S5 and Table S6).

3.5. Regulation Techniques for Foxtail Millet Hybrid Parental Lines

From 2016 to 2017, the research on the regulation of the flowering period of

Table 4. The anther development of HK902 and HK950.

Material	Anther exposure rate (%)			Degree of exposure (%)			Fullness			Overview
	2013	2014	Mean Value	2013	2014	Mean Value	2013	2014	Mean Value	
HK902	77.13	77.19	77.16	100	100	100	high	high	high	good
HK950	78.13	77.86	78.00	100	100	100	high	high	high	good

Table 5. The relationship of leaves on control nodes between the sterile lines (DZ759A, KM58A and KM249A) and the restorer lines (HK902 and HK950).

Restorer	Sterile line											
	DZ759A (The number of expanded leaves)				KM58A (The number of expanded leaves)				KM249A (The number of expanded leaves)			
	4	8	12	16	4	8	12	16	4	8	12	16
HK902 (The number of expanded leaves)	5 - 6	8 - 10	13 - 14	17 - 18	7 - 9	11 - 12	16 - 17	19 - 20	7 - 8	11 - 12	16 - 17	19 - 20
HK950 (The number of expanded leaves)	5 - 6	9 - 10	13 - 14	17 - 18	8 - 9	12 - 13	16 - 17	20 - 21	8 - 9	12 - 13	16 - 17	20 - 21

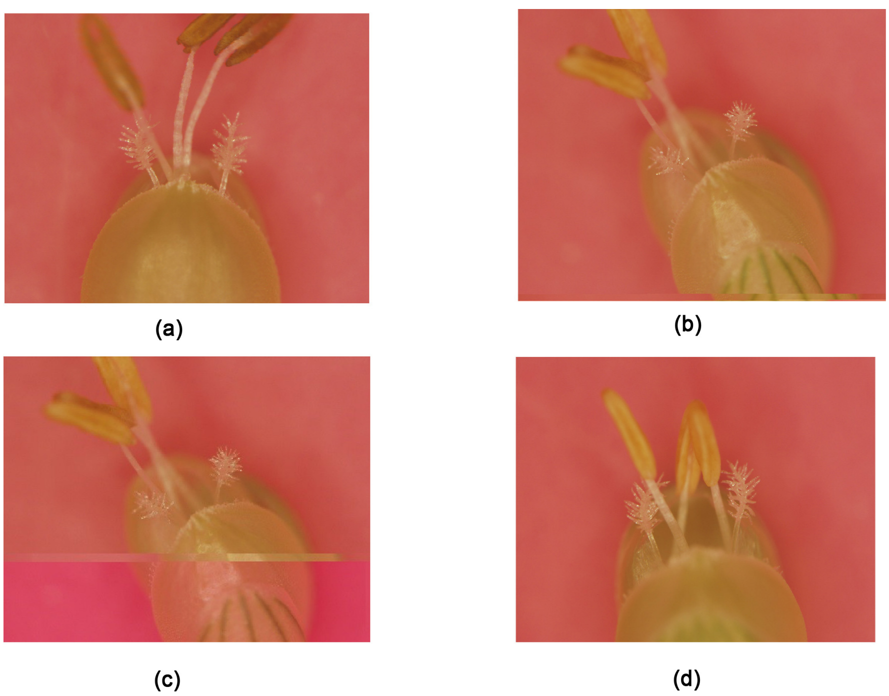

Figure 3. The anther development of foxtail millet. (a). The stigma development of HK902 in 2013. (b). The stigma development of HK902 in 2014. (c). The stigma development of HK950 in 2013. (d). The stigma development of HK950 in 2014.

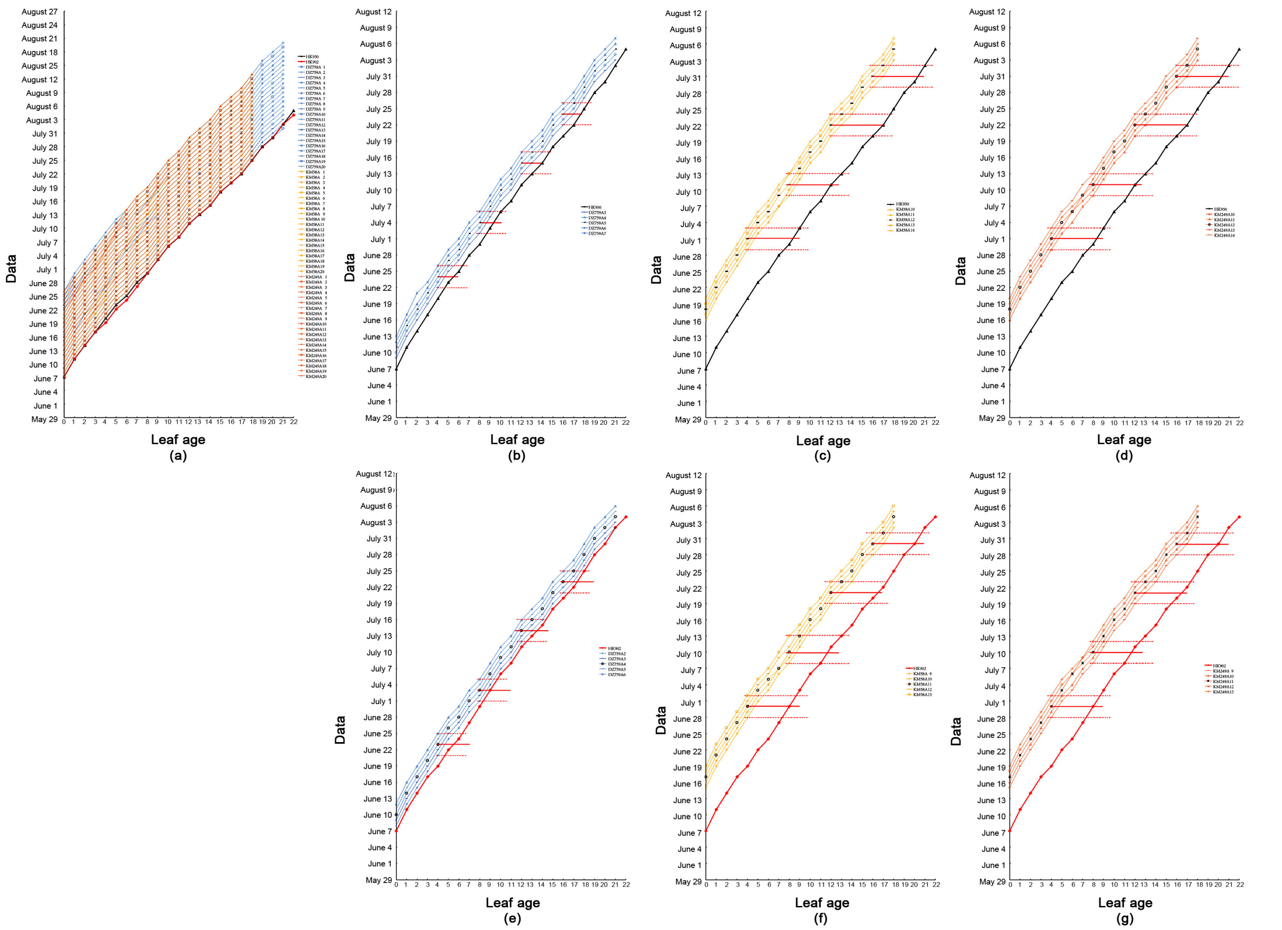

Figure 4. The developmental dynamics of leaf age and the corresponding maps of different control nodes of the sterile lines (DZ759A, KM58A and KM249A) and restorer lines (HK902 and HK950) during 2016 (The graph's horizontal axis shows the leaf age of the tested material, and the vertical axis shows the sowing date and the leaf development date). (a). Developmental dynamics of leaf age of the sterile lines (DZ759A, KM58A and KM249A) and restorer lines (HK902 and HK950) during 2016. (b). The control nodes of the restorer line (HK950) and sterile lines (DZ759A). (c). The control nodes of the restorer line (HK950) and sterile lines (KM58A). (d). The control nodes of the restorer line (HK950) and sterile lines (KM249A). (e). The control nodes of the restorer line (HK902) and sterile lines (DZ759A). (f). The control nodes of the restorer line (HK902) and sterile lines (KM58A). (g). The control nodes of the restorer line (HK902) and sterile lines (KM249A).

sterile lines and restorer lines achieved the following results:

The flowering regulation results revealed that during the seedling 4-leaf stage, there was uncoordinated growth in the female and male parental lines. Thus, it is necessary to adopt agronomic measures to regulate and control the growth of parental seedlings. For fast-developing parental lines, seedlings underwent delayed thinning and leaves of small seedlings were used to control growth. For slow-developing parental lines, seedlings were thinned in advance and leaves of big and strong seedlings were used to promote growth.

At the 8-leaf stage, uncoordinated parental growth was observed, but the development of parental lines was not noticeably different (1 - 2 leaves). Plants were regulated by fertilizer and water treatments, and early-developing parental lines rooted in 0.5% - 1.0% urea solutions, an appropriate extension of vegetative growth. For late-developing parental lines, 0.5% - 1.0% potassium dihydrogen phosphate was used to accelerate reproductive growth. If the development of the parental lines was considerably different (up to four leaves), it was necessary to conduct artificial root-cutting. When the roots were broken, 1/3 of the root system was cut obliquely at 5 cm along the base of the foxtail millet stalk and were uncoordinated at 45˚. The next day, the leaves appeared mildly wilted and regulation was achieved.

At the 12-leaf stage, uncoordinated flowering was observed and hormone regulation was used. When the difference was not large, the upper leaves of the late-developing parental lines, were sprayed with 2×10^{-5} gibberellin solution; and the difference between the parental lines was greater than 10 days (3 - 4 leaves). Plants were sprayed once every 24 hours and continuously sprayed three times. When the difference between the parental lines was within 10 days, the number of applications was reduced and the medication time was prolonged. Afterwards, foliar spray fertilizer consisted of 0.5% potassium dihydrogen phosphate and 1% urea to ensure the normal development of new leaves and tassels.

At the 16-leaf stage, the parental flowering period was uncoordinated. The 2×10^{-5} naphthalene acetic acid solution was used to spray late-developing parental leaves. The difference of the parental lines was about 10 days in the flowering stage, as flowering can anastomosis after hormone regulation. However, more than 15 days must be adjusted by agronomic measures for plants to conduct artificial root-cutting.

4. Discussion

Previous studies demonstrated that stigma and anther traits are qualitative traits or quantitative traits with high heritability, and their characteristics are not greatly affected by the environment [16] [17] [18] [19] [20]. In this study, two annual trials were established. Based on the results, the new sterile lines and restorer lines that were bred indeed met the requirements of the breeding goals, indicating that the experimental results of this study are reliable.

The stigma traits of the sterile lines determine the ability of the sterile lines to accept foreign pollen, and the anther characteristics of the restorer lines determine the pollen characteristics of the restorer lines. The stigma traits of the sterile lines and the anther characteristics of the restorer lines can be inherited; previous studies found part of a QTL that controls stigma and anther traits [28] [29] [30] [31]. Only by creating a sterile line with full stigma, obvious exposure, and high exposure rate, can the sterile line's ability to accept foreign pollen be improved. Additionally, it is necessary to create a restorer line with full anthers and full exposure to protect the glume in order to improve the heterogeneity of the sterile line. The seed setting rate, in turn, increases the seed yield. Thus, this project began with the transformation of the floral characteristics of the sterile and restorer lines of millet. Through the creation of a sterile line that is easy to accept foreign pollen and a restorer line with strong powder dispersing ability, the outcrossing seed setting rate of millet was improved, and the stigma traits and stigma of the sterile line were further improved. The heritability of the anther traits of the restorer line was complementary to a previous conclusion that the stigma and anther traits are qualitative traits or quantitative traits with higher heritability, and thus mutually verify each other.

Many researchers have attempted to increase the seed yields of foxtail millet hybrids by various methods, such as the selection and application of photo-thermophilic male sterile lines [10]. In the cold-growing hilly area of a seed production site [11] and some cultivation method such as sowing within a certain time interval etc. [12] [13]. However, the photo-thermophilic male sterile line exhibits infertility in cold environments, such as the long-term cold zone of Northern China. Comparatively, fertility in the short-day, high-temperature zone of Southern China was greatly improved [10]. Light and temperature can maintain the sterile line and improve it, but the ability of the sterile line to receive pollen is minimal. Meanwhile, production in the shallow hills area and reducing the planting ratio of the female to male, which extended the time for receiving pollen and decreased the distance that the male's loose powder needed to travel. However, these effects on seed production were limited. Due to foxtail millet's sensitivity to light and temperature, even if sowing was conducted within a certain time interval, based on the growth and development regulation of the hybrids' parents, climatic conditions during the growth period still play an important role. Thus, the flowering stage will not be synchronized and production is thereby reduced. Moreover, even if these methods ensured that the parental lines encountered one another during the flowering stage, outcrossing rates have not been improved, and foxtail millet hybrid seed production was difficult to increase, and foxtail millet hybrid seed production was difficult to increase, which was generally less than 1500 kg/hm^2 [2]. A few reports were available on the creation of sterile lines with high stigma exposure rates and no restorer lines with high outcrossing rates in foxtail and other crops. In this study, Datong 27, Datong 29 and gu 83 were screened and identified through meticulous observations of their pollination habitats. The three resources possess object characteristics. We transformed into a series of stigma-exposed and plump sterile lines that easily accepted exotic pollen (DZ759A, KM58A, KM249A); and new restorer lines with anthers that were full of powder and exhibited quick recovery (HK950, HK902), which improved the parental lines' heterosexual characteristics, and improved outcrossing rate of the sterile line, and laid a foundation for increasing seed yield of foxtail millet hybrid.

Based on the above findings, this study adopted the tracking and detection of leaf age development dynamics to detect and confirm the coupling law of leaf age development in the meeting during the flowering stage of foxtail millet hybrid parental lines, and based on the co-extension relationship between leaf development and ear differentiation, four regulatory nodes that ensured that the parental lines encountered one another were summarized through a series of regulation and control measures. These technologies belonged to previous studies on the coextensive relationship between leaf development and panicle differentiation [23] [24] and further research and application in hybrid seed production technology, which can effectively ensure the self-pollination of millet, which is sensitive to light and temperature. In the process of crop hybrid seed production, the parents meet during the flowering stage, thereby increasing seed yield. Applied in practice, good results were achieved. On August 26 and 27, 2016, the Hebei Provincial Department of Science and Technology organized experts to conduct an on-site inspection of 1.67 hm^2 farmland in Xindian Village, Luojiatun Town, Qianxi County, China, and adopted random sampling methods. Results revealed that the average yield of seed yield was 3019.8 kg/hm^2, thus overcoming the bottleneck that previously restricted the cultivation of foxtail millet hybrids and laying a foundation for the large-scale extension of foxtail millet hybrids.

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Author Contributions

ZL and SL contributed to the study conception and design. ZL, QL, SY and XZ performed the experiments. DL, YC and SL analyzed the data. ZL and GM wrote the manuscript. All authors read and approved the final manuscript.

Conflicts of Interest

The authors declare that they have no conflict of interest.

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Supplementary Materials

Table S1-1. The statistical results of stigma exposure rate of 206083, 1066A, Datong 27 and Datong 29 in 2007.

Material	Repeat	Part	The number of exposed small flowers in each column	Total number of small flowers in each part	Total number of exposed small flowers on the stigma	Total number of small flowers	Single column head exposure rate (%)	Stigma exsertion rate (%)
206083	First strain	Above	62	69	179	201	89.05	86.07
		Middle	69	75				
		Below	48	57				
	Second strain	Above	58	71	182	209	87.08	
		Middle	73	76				
		Below	51	62				
	Third strain	Above	52	68	167	203	82.27	
		Middle	66	74				
		Below	49	61				
1066A	First strain	Above	53	67	162	208	77.88	77.56
		Middle	61	74				
		Below	48	67				
	Second strain	Above	49	63	159	197	80.71	
		Middle	57	68				
		Below	53	66				
	Third strain	Above	43	61	143	193	74.09	
		Middle	54	68				
		Below	46	64				
Datong 27	First strain	Above	48	59	147	190	77.36	77.46
		Middle	55	70				
		Below	44	61				
	Second strain	Above	45	56	148	184	80.43	
		Middle	56	67				
		Below	47	61				
	Third strain	Above	42	51	135	181	74.59	
		Middle	51	66				
		Below	42	64				
Datong 29	First strain	Above	37	63	127	189	67.20	68.64
		Middle	52	67				
		Below	38	59				
	Second strain	Above	38	61	136	195	69.74	
		Middle	57	69				
		Below	41	65				
	Third strain	Above	39	63	129	187	68.98	
		Middle	54	66				
		Below	36	58				

Table S1-2. The statistical results of stigma exposure rate of 206083, 1066A, Datong 27 and Datong 29 in 2008.

Material	Repeat	Part	The number of exposed small flowers in each column	Total number of small flowers in each part	Total number of exposed small flowers on the stigma	Total number of small flowers	Single column head exposure rate (%)	Stigma exsertion rate (%)
206083	First strain	Above	63	68	184	211	87.20	85.31
		Middle	67	76				
		Below	54	67				
	Second strain	Above	57	72	181	215	84.19	
		Middle	63	74				
		Below	61	69				
	Third strain	Above	54	70	175	207	84.54	
		Middle	62	71				
		Below	59	66				
1066A	First strain	Above	54	69	161	203	79.31	78.90
		Middle	53	71				
		Below	54	63				
	Second strain	Above	52	67	162	209	77.51	
		Middle	55	73				
		Below	55	69				
	Third strain	Above	44	68	151	189	79.89	
		Middle	51	64				
		Below	56	57				
Datong 27	First strain	Above	51	70	164	215	76.28	77.67
		Middle	57	74				
		Below	56	71				
	Second strain	Above	53	72	174	219	79.45	
		Middle	62	74				
		Below	59	73				
	Third strain	Above	46	62	153	198	77.27	
		Middle	58	71				
		Below	49	65				
Datong 29	First strain	Above	46	64	135	201	67.16	69.01
		Middle	47	70				
		Below	44	67				
	Second strain	Above	52	70	146	212	68.87	
		Middle	50	73				
		Below	44	69				
	Third strain	Above	47	64	147	207	71.01	
		Middle	57	75				
		Below	43	68				

Table S2-1. The statistical results of anther exposure rate of Datong 27 and Datong 29 in 2007.

Material	Repeat	Part	Number of exposed small flowers in various parts of the valley	Total number of small flowers in each part	Anthers exposed total number of small flowers	Total number of small flowers	Single anther exposure rate (%)	Anther exposure rate (%)
Datong 27	First strain	Above	45	59	148	190	78.07	76.88
		Middle	53	70				
		Below	50	61				
	Second strain	Above	45	56	143	184	77.83	
		Middle	52	67				
		Below	46	61				
	Third strain	Above	40	51	135	181	74.73	
		Middle	52	66				
		Below	43	64				
Datong 29	First strain	Above	49	63	152	189	80.43	79.73
		Middle	61	67				
		Below	42	59				
	Second strain	Above	42	61	150	195	76.89	
		Middle	62	69				
		Below	46	65				
	Third strain	Above	45	63	153	187	81.87	
		Middle	59	66				
		Below	49	58				

Table S2-2. The statistical results of anther exposure rate of Datong 27 and Datong 29 in 2008.

Material	Repeat	Part	Number of exposed small flowers in various parts of the valley	Total number of small flowers in each part	Anthers exposed total number of small flowers	Total number of small flowers	Single anther exposure rate (%)	Anther exposure rate (%)
Datong 27	First strain	Above	54	71	162	213	76.06	76.96
		Middle	57	76				
		Below	51	66				
	Second strain	Above	51	61	165	207	79.71	
		Middle	60	76				
		Below	54	70				
	Third strain	Above	41	57	151	201	75.12	
		Middle	61	75				
		Below	49	69				
Datong 29	First strain	Above	48	60	162	204	79.41	78.26
		Middle	64	79				
		Below	50	65				
	Second strain	Above	46	68	163	209	77.99	
		Middle	67	72				
		Below	50	69				
	Third strain	Above	45	67	154	199	77.39	
		Middle	57	71				
		Below	52	61				

Table S3-1. The statistical results of stigma exposure rate of DZ759A, KM58A and KM249A in 2013.

Material	Repeat	Part	The number of exposed small flowers in each column	Total number of small flowers in each part	Total number of exposed small flowers on the stigma	Total number of small flowers	Single column head exposure rate (%)	Stigma exsertion rate (%)
DZ759A	First strain	Above	39	51	133	163	81.60	82.92
		Middle	53	61				
		Below	41	52				
	Second strain	Above	42	51	141	169	83.43	
		Middle	54	64				
		Below	45	54				
	Third strain	Above	45	54	144	172	83.72	
		Middle	55	66				
		Below	44	52				
KM58A	First strain	Above	43	52	136	166	81.93	79.63
		Middle	51	61				
		Below	42	53				
	Second strain	Above	40	52	138	173	79.77	
		Middle	53	67				
		Below	45	54				
	Third strain	Above	39	53	132	171	77.19	
		Middle	51	65				
		Below	42	53				
KM249A	First strain	Above	40	58	137	189	72.49	74.26
		Middle	56	72				
		Below	41	59				
	Second strain	Above	40	55	127	171	74.27	
		Middle	45	63				
		Below	42	53				
	Third strain	Above	41	50	121	159	76.10	
		Middle	43	61				
		Below	37	48				

Table S3-2. The statistical results of stigma exposure rate of DZ759A, KM58A and KM249A in 2014.

Material	Repeat	Part	The number of exposed small flowers in each column	Total number of small flowers in each part	Total number of exposed small flowers on the stigma	Total number of small flowers	Single column head exposure rate (%)	Stigma exsertion rate (%)
DZ759A	First strain	Above	40	47	131	159	82.39	82.82
		Middle	52	63				
		Below	39	49				
	Second strain	Above	46	53	147	174	84.48	
		Middle	58	66				
		Below	43	55				
	Third strain	Above	35	48	133	163	81.60	
		Middle	48	64				
		Below	40	51				
KM58A	First strain	Above	44	54	141	177	79.66	78.89
		Middle	53	68				
		Below	44	55				
	Second strain	Above	40	48	134	169	79.29	
		Middle	51	68				
		Below	43	53				
	Third strain	Above	38	61	136	175	77.71	
		Middle	55	70				
		Below	43	54				
KM249A	First strain	Above	43	56	136	183	74.32	74.94
		Middle	51	69				
		Below	42	58				
	Second strain	Above	42	60	137	187	73.26	
		Middle	54	71				
		Below	41	56				
	Third strain	Above	38	48	129	167	77.25	
		Middle	48	61				
		Below	43	58				

Table S4-1. The statistical results of anther exposure rate of HK902 and HK950 in 2013.

Material	Repeat	Part	Number of exposed small flowers in various parts of the valley	Total number of small flowers in each part	Anthers exposed total number of small flowers	Total number of small flowers	Single anther exposure rate (%)	Anther exposure rate (%)
HK902	First strain	Above	46	60	147	190	77.37	77.13
		Middle	51	68				
		Below	50	62				
	Second strain	Above	49	62	153	192	79.69	
		Middle	56	69				
		Below	48	61				
	Third strain	Above	47	62	139	187	74.33	
		Middle	49	66				
		Below	43	59				
HK950	First strain	Above	49	61	146	187	78.07	78.13
		Middle	55	65				
		Below	42	61				
	Second strain	Above	50	63	149	189	78.84	
		Middle	54	66				
		Below	45	60				
	Third strain	Above	46	61	141	182	77.47	
		Middle	53	63				
		Below	42	58				

Table S4-2. The statistical results of anther exposure rate of HK902 and HK950 in 2014.

Material	Repeat	Part	Number of exposed small flowers in various parts of the valley	Total number of small flowers in each part	Anthers exposed total number of small flowers	Total number of small flowers	Single anther exposure rate (%)	Anther exposure rate (%)
HK902	First strain	Above	48	67	164	209	78.47	77.19
		Middle	63	77				
		Below	53	65				
	Second strain	Above	48	59	152	198	76.77	
		Middle	57	77				
		Below	47	62				
	Third strain	Above	45	62	158	207	76.33	
		Middle	54	76				
		Below	49	69				
HK950	First strain	Above	43	58	159	203	78.33	77.86
		Middle	69	81				
		Below	47	64				
	Second strain	Above	41	57	148	192	77.08	
		Middle	63	74				
		Below	44	61				
	Third strain	Above	44	57	154	197	78.17	
		Middle	63	77				
		Below	47	63				

Table S5. The schedule of leaf development dynamics of the sterile lines (DZ759A, KM58A and KM249A) and restorer lines (HK902 and HK950) in the foxtail millet research experimental station of Luanan of Tangshan normal university during 2016.

Variety	Sowing date	1 leaf	2 leaf	3 leaf	4 leaf	5 leaf	6 leaf	7 leaf	8 leaf	9 leaf	10 leaf	11 leaf	12 leaf	13 leaf	14 leaf	15 leaf	16 leaf	17 leaf	18 leaf	19 leaf	20 leaf	21 leaf	22 leaf
HK950	6/7	6/11	6/14	6/17	6/20	6/23	6/25	6/28	6/30	7/3	7/6	7/8	7/11	7/13	7/15	7/18	7/20	7/22	7/25	7/28	7/30	8/2	8/5
HK902	6/7	6/11	6/14	6/17	6/19	6/22	6/24	6/27	6/30	7/3	7/6	7/8	7/11	7/13	7/15	7/18	7/20	7/22	7/25	7/28	7/30	8/2	8/4
DZ759A1	6/7	6/11	6/14	6/17	6/20	6/23	6/25	6/28	6/30	7/3	7/6	7/8	7/11	7/13	7/15	7/18	7/20	7/22	7/25	7/28	7/30	8/1	
DZ759A2	6/8	6/12	6/15	6/18	6/21	6/24	6/26	6/29	7/1	7/4	7/7	7/9	7/12	7/14	7/16	7/19	7/21	7/23	7/26	7/29	7/31	8/2	
DZ759A3	6/9	6/13	6/16	6/19	6/22	6/25	6/27	6/30	7/2	7/5	7/8	7/10	7/13	7/15	7/17	7/20	7/22	7/24	7/27	7/30	8/1	8/3	
DZ759A4	6/10	6/14	6/17	6/20	6/23	6/26	6/28	7/1	7/3	7/6	7/9	7/11	7/14	7/16	7/18	7/21	7/23	7/25	7/28	7/31	8/2	8/4	
DZ759A5	6/11	6/15	6/18	6/21	6/24	6/27	6/29	7/2	7/4	7/7	7/10	7/12	7/15	7/17	7/19	7/22	7/24	7/26	7/29	8/1	8/3	8/5	
DZ759A6	6/12	6/16	6/19	6/22	6/25	6/28	6/30	7/3	7/5	7/8	7/11	7/13	7/16	7/18	7/20	7/23	7/25	7/27	7/30	8/2	8/4	8/6	
DZ759A7	6/13	6/17	6/21	6/23	6/26	6/29	7/1	7/4	7/6	7/9	7/12	7/14	7/17	7/19	7/21	7/24	7/26	7/28	7/31	8/3	8/5	8/7	
DZ759A8	6/14	6/18	6/21	6/24	6/27	6/30	7/2	7/5	7/7	7/10	7/13	7/15	7/18	7/20	7/22	7/25	7/27	7/29	8/1	8/4	8/6	8/8	
DZ759A9	6/15	6/19	6/22	6/25	6/27	7/1	7/3	7/6	7/8	7/11	7/14	7/16	7/19	7/21	7/23	7/26	7/28	7/30	8/2	8/5	8/7	8/9	
DZ759A10	6/16	6/20	6/23	6/26	6/29	7/2	7/5	7/7	7/9	7/12	7/15	7/17	7/20	7/22	7/24	7/27	7/29	7/31	8/3	8/6	8/8	8/10	
DZ759A11	6/17	6/21	6/24	6/27	6/30	7/3	7/5	7/8	7/11	7/13	7/16	7/18	7/21	7/23	7/25	7/28	7/30	8/1	8/4	8/7	8/9	8/11	
DZ759A12	6/18	6/22	6/25	6/28	7/1	7/4	7/6	7/9	7/11	7/14	7/17	7/19	7/22	7/24	7/26	7/29	7/31	8/2	8/5	8/8	8/10	8/12	
DZ759A13	6/19	6/23	6/26	6/29	7/2	7/5	7/7	7/10	7/12	7/15	7/18	7/20	7/23	7/25	7/27	7/30	8/1	8/3	8/6	8/9	8/11	8/13	
DZ759A14	6/20	6/24	6/27	6/30	7/3	7/6	7/8	7/11	7/13	7/16	7/19	7/21	7/24	7/26	7/28	7/31	8/2	8/4	8/7	8/10	8/12	8/14	
DZ759A15	6/21	6/25	6/29	7/1	7/4	7/7	7/9	7/12	7/14	7/17	7/20	7/22	7/25	7/27	7/29	8/1	8/3	8/5	8/8	8/11	8/13	8/15	
DZ759A16	6/22	6/26	6/30	7/2	7/5	7/8	7/10	7/13	7/16	7/18	7/21	7/23	7/26	7/28	7/30	8/2	8/4	8/6	8/9	8/12	8/14	8/16	
DZ759A17	6/23	6/27	6/30	7/3	7/6	7/9	7/11	7/14	7/16	7/19	7/22	7/24	7/27	7/29	7/31	8/3	8/5	8/7	8/10	8/13	8/15	8/17	
DZ759A18	6/24	6/28	7/1	7/4	7/7	7/10	7/12	7/15	7/17	7/20	7/23	7/25	7/28	7/30	8/1	8/4	8/6	8/8	8/11	8/14	8/16	8/18	
DZ759A19	6/25	6/29	7/2	7/5	7/8	7/11	7/13	7/16	7/18	7/21	7/24	7/26	7/29	7/31	8/2	8/5	8/7	8/9	8/12	8/15	8/17	8/19	
DZ759A20	6/26	6/30	7/3	7/6	7/9	7/12	7/14	7/17	7/19	7/22	7/25	7/27	7/30	8/1	8/3	8/6	8/8	8/10	8/13	8/16	8/18	8/20	
KM58A1	6/7	6/11	6/14	6/17	6/20	6/23	6/25	6/28	6/30	7/3	7/6	7/8	7/11	7/13	7/15	7/18	7/20	7/22	7/25				
KM58A2	6/8	6/12	6/15	6/18	6/21	6/24	6/26	6/29	7/1	7/4	7/7	7/9	7/12	7/14	7/16	7/19	7/21	7/23	7/26				
KM58A3	6/9	6/13	6/16	6/19	6/22	6/25	6/27	6/30	7/2	7/5	7/8	7/10	7/13	7/15	7/17	7/20	7/22	7/24	7/27				
KM58A4	6/10	6/14	6/17	6/20	6/23	6/26	6/28	7/1	7/3	7/6	7/9	7/11	7/14	7/16	7/18	7/21	7/23	7/25	7/28				
KM58A5	6/11	6/15	6/18	6/21	6/24	6/27	6/29	7/2	7/4	7/7	7/10	7/12	7/15	7/17	7/19	7/22	7/24	7/26	7/29				
KM58A6	6/12	6/16	6/19	6/22	6/25	6/28	6/30	7/3	7/5	7/8	7/11	7/13	7/16	7/18	7/20	7/23	7/25	7/27	7/30				
KM58A7	6/13	6/17	6/20	6/23	6/26	6/29	7/1	7/4	7/6	7/9	7/12	7/14	7/17	7/19	7/21	7/24	7/26	7/28	7/31				
KM58A8	6/14	6/18	6/21	6/23	6/27	6/30	7/2	7/5	7/7	7/10	7/13	7/15	7/18	7/20	7/22	7/25	7/27	7/29	8/1				

Continued

KM58A9	6/15	6/19	6/22	6/25	6/28	7/1	7/3	7/6	7/8	7/11	7/14	7/16	7/19	7/21	7/23	7/26	7/28	7/30	8/2
KM58A10	6/16	6/20	6/23	6/26	6/29	7/2	7/4	7/7	7/9	7/12	7/15	7/17	7/20	7/22	7/24	7/27	7/29	7/31	8/3
KM58A11	6/17	6/21	6/24	6/27	6/30	7/3	7/5	7/7	7/10	7/13	7/16	7/18	7/21	7/23	7/25	7/28	7/30	8/1	8/4
KM58A12	6/18	6/22	6/25	6/28	7/1	7/4	7/6	7/9	7/11	7/14	7/17	7/19	7/22	7/24	7/26	7/29	7/31	8/2	8/5
KM58A13	6/19	6/23	6/26	6/29	7/2	7/5	7/7	7/10	7/13	7/15	7/18	7/20	7/23	7/25	7/27	7/30	8/1	8/3	8/6
KM58A14	6/20	6/24	6/27	6/30	7/3	7/6	7/8	7/11	7/13	7/16	7/19	7/21	7/24	7/26	7/28	7/31	8/2	8/4	8/7
KM58A15	6/21	6/25	6/28	7/1	7/4	7/7	7/9	7/12	7/14	7/17	7/20	7/22	7/25	7/27	7/29	8/1	8/3	8/5	8/8
KM58A16	6/22	6/25	6/28	7/1	7/5	7/8	7/10	7/13	7/15	7/18	7/21	7/23	7/26	7/28	7/30	8/2	8/4	8/6	8/9
KM58A17	6/23	6/26	6/29	7/2	7/5	7/9	7/11	7/14	7/16	7/19	7/22	7/24	7/27	7/29	7/31	8/3	8/5	8/7	8/10
KM58A18	6/24	6/27	6/30	7/3	7/6	7/9	7/12	7/15	7/17	7/20	7/23	7/25	7/28	7/30	8/1	8/4	8/6	8/8	8/11
KM58A19	6/25	6/28	7/1	7/4	7/7	7/10	7/13	7/16	7/18	7/21	7/24	7/26	7/29	7/31	8/2	8/5	8/7	8/9	8/12
KM58A20	6/26	6/29	7/2	7/5	7/8	7/11	7/14	7/17	7/19	7/22	7/25	7/27	7/30	8/1	8/3	8/6	8/8	8/10	8/13
KM249A1	6/7	6/11	6/14	6/17	6/20	6/23	6/25	6/28	6/30	7/3	7/6	7/8	7/11	7/13	7/15	7/18	7/20	7/22	7/25
KM249A2	6/8	6/12	6/15	6/18	6/21	6/24	6/26	6/29	7/1	7/4	7/7	7/9	7/12	7/14	7/16	7/19	7/21	7/23	7/26
KM249A3	6/9	6/13	6/16	6/19	6/22	6/25	6/27	6/30	7/2	7/5	7/8	7/10	7/13	7/15	7/17	7/20	7/22	7/24	7/27
KM249A4	6/10	6/14	6/17	6/20	6/23	6/26	6/28	7/1	7/3	7/6	7/9	7/11	7/14	7/16	7/18	7/21	7/23	7/25	7/28
KM249A5	6/11	6/15	6/18	6/22	6/24	6/27	6/29	7/2	7/4	7/7	7/10	7/12	7/15	7/17	7/19	7/22	7/24	7/26	7/29
KM249A6	6/12	6/16	6/19	6/22	6/25	6/28	6/30	7/3	7/5	7/8	7/11	7/13	7/16	7/18	7/20	7/23	7/25	7/27	7/30
KM249A7	6/13	6/17	6/20	6/23	6/26	6/29	7/1	7/4	7/6	7/9	7/12	7/14	7/17	7/19	7/21	7/24	7/26	7/28	7/31
KM249A8	6/14	6/18	6/21	6/24	6/27	6/29	7/2	7/5	7/7	7/10	7/13	7/15	7/18	7/20	7/22	7/25	7/27	7/29	8/1
KM249A9	6/15	6/19	6/22	6/25	6/28	7/1	7/3	7/6	7/8	7/11	7/14	7/16	7/19	7/21	7/23	7/26	7/28	7/30	8/2
KM249A10	6/16	6/20	6/23	6/26	6/29	7/2	7/4	7/7	7/9	7/12	7/15	7/17	7/20	7/22	7/24	7/27	7/29	7/31	8/3
KM249A11	6/17	6/21	6/24	6/27	6/30	7/3	7/6	7/8	7/10	7/13	7/16	7/18	7/21	7/23	7/25	7/28	7/30	8/1	8/4
KM249A12	6/18	6/22	6/25	6/28	7/1	7/4	7/6	7/9	7/11	7/14	7/17	7/19	7/22	7/24	7/26	7/29	7/31	8/2	8/5
KM249A13	6/19	6/23	6/26	6/29	7/2	7/5	7/7	7/9	7/12	7/15	7/18	7/20	7/23	7/25	7/27	7/30	8/1	8/3	8/6
KM249A14	6/20	6/24	6/27	6/30	7/3	7/6	7/8	7/11	7/13	7/16	7/19	7/21	7/24	7/26	7/28	7/31	8/2	8/4	8/7
KM249A15	6/21	6/25	6/29	7/1	7/4	7/7	7/9	7/12	7/14	7/17	7/20	7/22	7/25	7/27	7/29	8/1	8/3	8/5	8/8
KM249A16	6/22	6/25	6/28	7/1	7/5	7/8	7/10	7/13	7/15	7/19	7/21	7/23	7/26	7/28	7/30	8/2	8/4	8/6	8/9
KM249A17	6/23	6/26	6/29	7/2	7/5	7/9	7/11	7/14	7/16	7/19	7/22	7/24	7/27	7/29	7/31	8/3	8/5	8/7	8/10
KM249A18	6/24	6/27	6/30	7/3	7/6	7/9	7/12	7/15	7/17	7/20	7/23	7/25	7/28	7/30	8/1	8/4	8/6	8/8	8/11
KM249A19	6/25	6/28	7/1	7/4	7/7	7/10	7/13	7/16	7/18	7/21	7/24	7/26	7/29	7/31	8/2	8/5	8/7	8/9	8/12
KM249A20	6/26	6/29	7/2	7/5	7/8	7/11	7/14	7/17	7/19	7/22	7/25	7/27	7/30	8/1	8/3	8/6	8/8	8/10	8/13

Table S6. The schedule of leaf development dynamics of the sterile lines (DZ759A, KM58A and KM249A) and restorer lines (HK902 and HK950) in the foxtail millet research experimental station of luanan of tangshan normal university during 2017.

Variety	Sowing date	1 leaf	2 leaf	3 leaf	4 leaf	5 leaf	6 leaf	7 leaf	8 leaf	9 leaf	10 leaf	11 leaf	12 leaf	13 leaf	14 leaf	15 leaf	16 leaf	17 leaf	18 leaf	19 leaf	20 leaf	21 leaf	22 leaf
HK950	6/13	6/17	6/20	6/22	6/24	6/27	6/29	7/2	7/4	7/7	7/10	7/13	7/15	7/18	7/20	7/22	7/25	7/28	7/30	8/1	8/4	8/7	8/9
HK902	6/13	6/17	6/20	6/22	6/24	6/27	6/29	7/1	7/3	7/5	7/8	7/11	7/14	7/17	7/19	7/21	7/23	7/26	7/28	7/30	8/2	8/5	8/8
DZ759A1	6/13	6/17	6/20	6/23	6/25	6/27	6/30	7/2	7/5	7/8	7/10	7/12	7/15	7/18	7/20	7/22	7/24	7/27	7/29	8/1	8/3	8/5	
DZ759A2	6/14	6/18	6/21	6/24	6/26	6/29	7/1	7/4	7/6	7/8	7/11	7/13	7/16	7/19	7/21	7/23	7/25	7/28	7/30	8/2	8/4	8/6	
DZ759A3	6/15	6/19	6/22	6/25	6/27	6/29	7/2	7/5	7/8	7/10	7/12	7/14	7/17	7/20	7/22	7/24	7/26	7/28	7/31	8/3	8/5	8/7	
DZ759A4	6/16	6/20	6/23	6/26	6/28	6/30	7/3	7/6	7/9	7/12	7/14	7/16	7/18	7/21	7/23	7/25	7/27	7/29	8/1	8/3	8/6	8/8	
DZ759A5	6/17	6/21	6/24	6/27	6/30	7/2	7/4	7/7	7/10	7/12	7/15	7/17	7/19	7/21	7/24	7/26	7/28	7/30	8/2	8/4	8/7	8/9	
DZ759A6	6/18	6/22	6/25	6/28	7/1	7/3	7/5	7/7	7/10	7/13	7/15	7/18	7/20	7/22	7/24	7/27	7/29	8/1	8/3	8/5	8/7	8/10	
DZ759A7	6/19	6/23	6/26	6/29	7/2	7/5	7/7	7/9	7/11	7/14	7/16	7/19	7/21	7/23	7/25	7/27	7/30	8/2	8/4	8/6	8/9	8/11	
DZ759A8	6/20	6/24	6/27	6/30	7/2	7/5	7/8	7/10	7/12	7/14	7/17	7/20	7/22	7/24	7/26	7/28	7/31	8/3	8/5	8/7	8/10	8/12	
DZ759A9	6/21	6/25	6/28	6/30	7/3	7/6	7/8	7/11	7/13	7/15	7/18	7/21	7/23	7/25	7/27	7/29	8/1	8/4	8/7	8/9	8/11	8/13	
DZ759A10	6/22	6/26	6/29	7/1	7/3	7/6	7/9	7/12	7/14	7/16	7/19	7/22	7/24	7/26	7/28	7/30	8/2	8/5	8/8	8/10	8/12	8/14	
DZ759A11	6/23	6/27	6/29	7/1	7/4	7/7	7/10	7/13	7/15	7/17	7/20	7/23	7/25	7/28	7/31	8/3	8/5	8/8	8/10	8/12	8/14		
DZ759A12	6/24	6/28	6/30	7/2	7/5	7/8	7/11	7/14	7/16	7/18	7/20	7/23	7/26	7/29	8/1	8/4	8/6	8/9	8/11	8/13	8/15		
DZ759A13	6/25	6/28	7/1	7/3	7/6	7/9	7/12	7/15	7/18	7/20	7/22	7/24	7/27	7/30	8/2	8/5	8/7	8/10	8/12	8/14	8/16		
DZ759A14	6/26	6/29	7/2	7/4	7/7	7/10	7/13	7/16	7/18	7/21	7/23	7/25	7/28	7/31	8/3	8/6	8/8	8/11	8/13	8/15	8/17		
DZ759A15	6/27	6/30	7/3	7/5	7/8	7/11	7/13	7/16	7/19	7/22	7/24	7/26	7/29	8/1	8/4	8/7	8/9	8/11	8/14	8/16	8/18		
DZ759A16	6/28	7/1	7/4	7/6	7/9	7/11	7/14	7/17	7/20	7/22	7/24	7/27	7/30	8/2	8/5	8/8	8/10	8/12	8/15	8/17	8/19		
DZ759A17	6/29	7/2	7/5	7/8	7/10	7/12	7/15	7/18	7/21	7/23	7/25	7/28	7/31	8/3	8/5	8/8	8/11	8/13	8/16	8/18	8/20		
DZ759A18	6/30	7/3	7/6	7/9	7/11	7/14	7/16	7/18	7/21	7/23	7/26	7/29	8/1	8/4	8/7	8/9	8/12	8/14	8/17	8/19	8/21		
DZ759A19	7/1	7/4	7/7	7/9	7/12	7/15	7/17	7/19	7/21	7/24	7/27	7/30	8/1	8/4	8/7	8/10	8/13	8/16	8/18	8/20	8/22		
DZ759A20	7/2	7/5	7/8	7/10	7/13	7/16	7/18	7/20	7/22	7/25	7/28	7/31	8/2	8/5	8/8	8/11	8/14	8/17	8/19	8/21	8/23		
KM58A1	6/13	6/17	6/20	6/23	6/25	6/28	6/30	7/2	7/5	7/8	7/10	7/13	7/15	7/18	7/20	7/22	7/24	7/27	7/29				
KM58A2	6/14	6/18	6/21	6/24	6/27	6/29	7/1	7/4	7/6	7/8	7/10	7/13	7/16	7/19	7/21	7/23	7/25	7/28	7/30				
KM58A3	6/15	6/19	6/22	6/24	6/27	6/29	7/2	7/5	7/8	7/10	7/12	7/14	7/17	7/19	7/22	7/24	7/26	7/28	7/31				
KM58A4	6/16	6/20	6/23	6/25	6/28	6/30	7/3	7/6	7/9	7/11	7/14	7/16	7/18	7/21	7/23	7/25	7/27	7/29	8/1				
KM58A5	6/17	6/21	6/24	6/27	6/30	7/2	7/5	7/7	7/10	7/12	7/15	7/17	7/19	7/21	7/24	7/26	7/28	7/30	8/2				
KM58A6	6/18	6/22	6/25	6/28	7/1	7/3	7/5	7/7	7/10	7/13	7/16	7/18	7/20	7/22	7/24	7/27	7/29	8/1	8/3				
KM58A7	6/19	6/23	6/26	6/29	7/2	7/5	7/7	7/9	7/11	7/14	7/17	7/19	7/21	7/23	7/25	7/27	7/30	8/2	8/4				
KM58A8	6/20	6/24	6/27	6/30	7/2	7/5	7/8	7/10	7/12	7/15	7/17	7/20	7/22	7/24	7/26	7/28	7/31	8/3	8/5				

Continued

KM58A9	6/21	6/25	6/28	6/30	7/3	7/6	7/9	7/11	7/13	7/15	7/18	7/21	7/23	7/25	7/27	7/29	8/1	8/4	8/7
KM58A10	6/22	6/26	6/29	7/1	7/3	7/6	7/9	7/11	7/14	7/16	7/19	7/22	7/24	7/26	7/28	7/30	8/2	8/5	8/8
KM58A11	6/23	6/27	6/29	7/1	7/4	7/7	7/10	7/12	7/15	7/17	7/20	7/23	7/25	7/28	7/31	8/3	8/5	8/8	
KM58A12	6/24	6/28	6/30	7/2	7/5	7/8	7/11	7/14	7/16	7/18	7/20	7/23	7/26	7/29	8/1	8/4	8/6	8/9	
KM58A13	6/25	6/28	7/1	7/3	7/6	7/9	7/12	7/15	7/18	7/20	7/22	7/24	7/27	7/30	8/2	8/5	8/7	8/10	
KM58A14	6/26	6/29	7/2	7/4	7/7	7/10	7/13	7/16	7/19	7/21	7/23	7/25	7/28	7/31	8/3	8/6	8/8	8/11	
KM58A15	6/27	6/30	7/3	7/6	7/8	7/10	7/13	7/16	7/19	7/22	7/24	7/26	7/29	8/1	8/4	8/7	8/9	8/11	
KM58A16	6/28	7/1	7/4	7/6	7/9	7/12	7/14	7/17	7/20	7/22	7/24	7/27	7/30	8/2	8/5	8/8	8/10	8/12	
KM58A17	6/29	7/2	7/5	7/8	7/10	7/13	7/15	7/18	7/21	7/23	7/25	7/28	7/31	8/3	8/5	8/8	8/11	8/13	
KM58A18	6/30	7/3	7/6	7/9	7/11	7/14	7/16	7/18	7/21	7/24	7/26	7/29	8/1	8/4	8/7	8/9	8/12	8/14	
KM58A19	7/1	7/4	7/7	7/9	7/12	7/15	7/17	7/19	7/21	7/24	7/27	7/30	8/1	8/4	8/7	8/10	8/13	8/16	
KM58A20	7/2	7/5	7/8	7/10	7/13	7/16	7/18	7/20	7/22	7/25	7/28	7/31	8/2	8/5	8/8	8/11	8/14	8/17	
KM249A1	6/13	6/17	6/20	6/23	6/25	6/27	6/30	7/2	7/5	7/7	7/9	7/12	7/15	7/18	7/20	7/22	7/24	7/27	7/29
KM249A2	6/14	6/18	6/21	6/24	6/26	6/29	7/1	7/4	7/6	7/8	7/11	7/13	7/16	7/19	7/21	7/23	7/25	7/28	7/30
KM249A3	6/15	6/19	6/22	6/25	6/27	6/29	7/2	7/5	7/8	7/10	7/12	7/15	7/17	7/20	7/22	7/24	7/26	7/28	7/31
KM249A4	6/16	6/20	6/23	6/26	6/28	6/30	7/3	7/6	7/9	7/12	7/14	7/16	7/18	7/20	7/23	7/25	7/27	7/29	8/1
KM249A5	6/17	6/21	6/24	6/27	6/30	7/2	7/4	7/7	7/10	7/13	7/15	7/17	7/19	7/21	7/24	7/26	7/28	7/30	8/2
KM249A6	6/18	6/22	6/25	6/28	7/1	7/3	7/5	7/7	7/10	7/13	7/15	7/18	7/20	7/22	7/24	7/27	7/29	8/1	8/3
KM249A7	6/19	6/23	6/26	6/29	7/2	7/5	7/7	7/9	7/11	7/14	7/16	7/19	7/21	7/23	7/25	7/27	7/30	8/2	8/4
KM249A8	6/20	6/24	6/27	6/30	7/2	7/5	7/8	7/10	7/12	7/14	7/17	7/19	7/22	7/24	7/26	7/28	7/31	8/3	8/5
KM249A9	6/21	6/25	6/28	6/30	7/3	7/6	7/8	7/11	7/13	7/15	7/18	7/20	7/23	7/25	7/27	7/29	8/1	8/4	8/7
KM249A10	6/22	6/26	6/29	7/1	7/3	7/6	7/9	7/12	7/14	7/16	7/19	7/22	7/24	7/26	7/28	7/30	8/2	8/5	8/8
KM249A11	6/23	6/27	6/29	7/1	7/4	7/7	7/10	7/13	7/15	7/17	7/20	7/23	7/25	7/28	7/31	8/3	8/5	8/7	8/9
KM249A12	6/24	6/28	6/30	7/2	7/5	7/8	7/11	7/14	7/16	7/18	7/20	7/23	7/26	7/29	8/1	8/4	8/6	8/9	
KM249A13	6/25	6/28	7/1	7/4	7/6	7/9	7/12	7/15	7/18	7/20	7/22	7/24	7/27	7/30	8/2	8/5	8/7	8/10	
KM249A14	6/26	6/29	7/2	7/5	7/7	7/10	7/13	7/16	7/18	7/21	7/23	7/25	7/28	7/31	8/3	8/6	8/8	8/11	
KM249A15	6/27	6/30	7/3	7/6	7/8	7/11	7/13	7/16	7/19	7/22	7/24	7/26	7/29	8/1	8/4	8/7	8/9	8/11	
KM249A16	6/28	7/1	7/4	7/7	7/9	7/11	7/14	7/17	7/20	7/22	7/24	7/27	7/30	8/2	8/5	8/8	8/10	8/12	
KM249A17	6/29	7/2	7/5	7/7	7/10	7/12	7/15	7/18	7/21	7/23	7/25	7/28	7/31	8/3	8/5	8/8	8/11	8/13	
KM249A18	6/30	7/3	7/6	7/8	7/11	7/14	7/16	7/18	7/21	7/23	7/26	7/29	8/1	8/4	8/7	8/9	8/12	8/14	
KM249A19	7/1	7/4	7/7	7/10	7/12	7/15	7/17	7/19	7/21	7/24	7/27	7/30	8/1	8/4	8/7	8/10	8/13	8/16	
KM249A20	7/2	7/5	7/8	7/11	7/14	7/16	7/18	7/20	7/22	7/25	7/28	7/31	8/2	8/5	8/8	8/11	8/14	8/17	

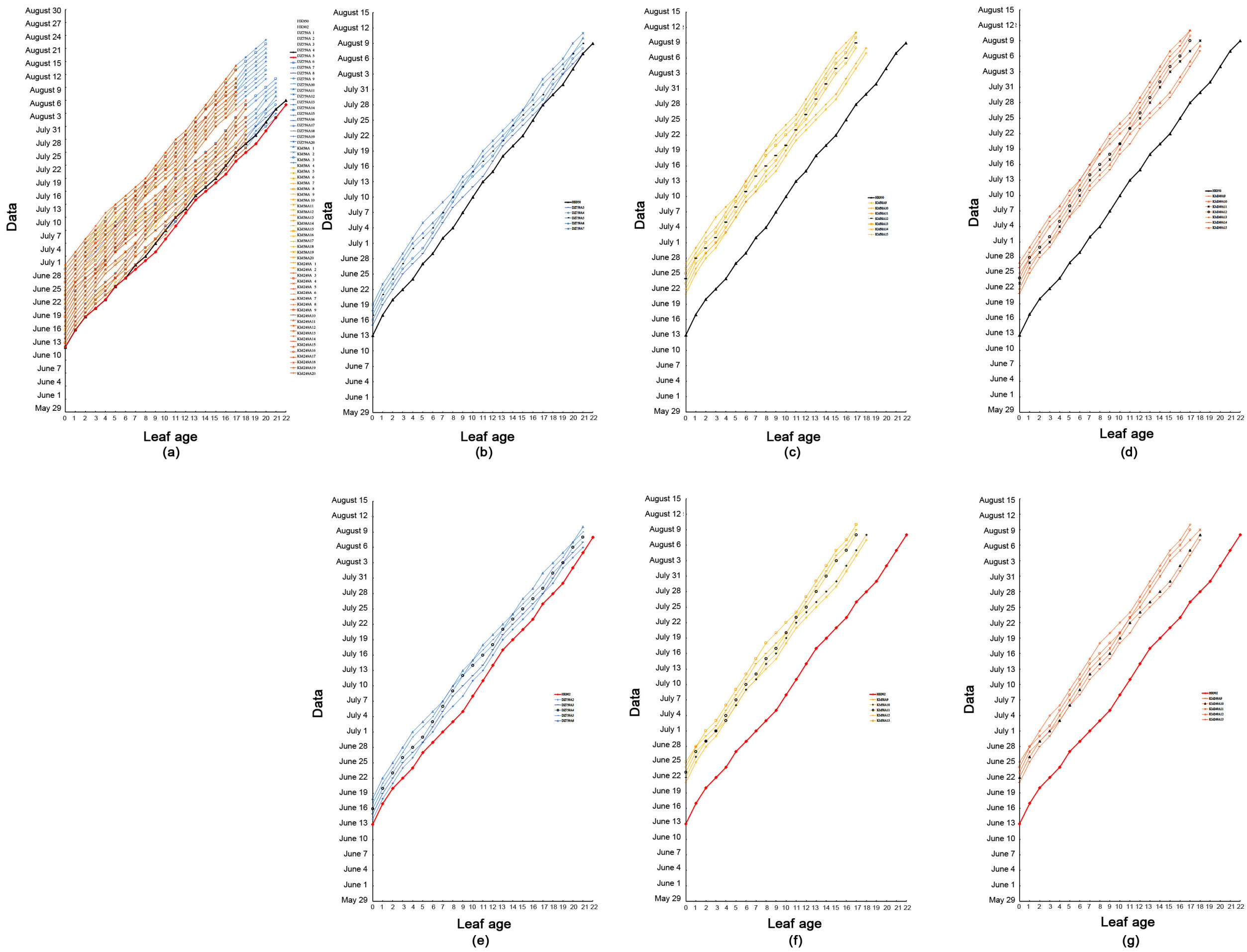

Figure S1. The developmental dynamics of leaf age and the corresponding maps of different control nodes of the sterile lines (DZ759A, KM58A and KM249A) and restorer lines (HK902 and HK950) during 2017 (The graph's horizontal axis shows the leaf age of the tested material, and the vertical axis shows the sowing date and the leaf development date). (a). Developmental dynamics of leaf age of the sterile lines (DZ759A, KM58A and KM249A) and restorer lines (HK902 and HK950) during 2017. (b). The control nodes of the restorer line (HK950) and sterile lines (DZ759A). (c). The control nodes of the restorer line (HK950) and sterile lines (KM58A). (d). The control nodes of the restorer line (HK950) and sterile lines (KM249A). (e). The control nodes of the restorer line (HK902) and sterile lines (DZ759A). (f). The control nodes of the restorer line (HK902) and sterile lines (KM58A). (g). The control nodes of the restorer line (HK902) and sterile lines (KM249A).