# Chapter 6
# Foxtail Millet Breeding in China

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**Abstract** Although there is a long history of foxtail millet cultivation in China, modern foxtail millet breeding was only initiated in China in the 1950s and 1960s, with significant progress being made since the 1980s. Most of the research on foxtail millet breeding has been conducted in China, where it is an important regional cereal. The main research activities from the 1950s to 1970s were comparisons among landraces and individual selection, followed by cross-based pedigree selection in the 1970s. These comparisons and cross-based pedigree selections contributed greatly to foxtail millet improvement in China, including the development of the super cultivars 'Yugu 1' and 'Zhaogu 1' in the 1980s. Radiation and chemical-induced mutations have also been used in foxtail millet breeding to create novel types, such as dwarf lines. Although different types of male sterile lines have been developed over the past 50 years in China, only partial genetic male sterile lines (PAGMS) have been used successfully in hybrid seed production, allowing the use of heterosis to become a reality in recent years. The foxtail millet eco-regions, breeding phases, breeding methodology, and main cultivars grown at different times since the 1950s in China are reviewed in this chapter. With the rapid advances in foxtail millet genomic sciences, mining and elucidation of quantitative trait loci related to important traits will accelerate foxtail millet breeding in the near future.

**Keywords** Foxtail millet • Pedigree selection • Cultivar development • Heterosis • Setaria

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## 6.1 Foxtail Millet Breeding in China

### *6.1.1 Eco-Regions and Ecotypes of Foxtail Millet in China*

Foxtail millet is an ancient crop in China, where it has probably been cultivated for 10,000 years. Under long-term selective pressures in different geographical, climatic, and anthropogenic regions, foxtail millet has evolved into diverse ecotypes for field production in different regions. Each ecotype includes many varieties with common genetic characteristics that allow them to adapt to certain ecological conditions, including differences in photoperiod. The ecological classification of foxtail millet is pivotal to understanding the characteristics of different groups of varieties and to establish and exchange germplasm collections. Studies on the ecological responses of foxtail millet varieties have been concentrated in the main foxtail millet production areas of northern China. Several different methods for classifying eco-regions have been reported, including classification according to the natural climatic conditions of the different foxtail millet-producing areas, and the heading dates of various groups of foxtail millet varieties (Wang and Guo 1997; Diao et al. 2011). The ‘four ecological regions’ theory was developed based on these classifications, and this has been largely adopted by Chinese foxtail millet breeders. The four ecological regions of foxtail millet are the Northeast Plain, North China Plain, Inner Mongolia Plateau, and the Northwest Plateau.

#### 6.1.1.1 The Northeast Plain Eco-Region

This ecological zone includes Heilongjiang, Jilin, and the northern part of Liaoning Province in northeast China, which are the northernmost regions of China (latitude ≥40°). In this eco-region, the altitude ranges from about 50 to 200 m, the frost-free period is short, and it is mainly the spring-sowing region for foxtail millet cultivation, except for a few zones located in the south of Liaoning. The annual rainfall in this region is about 400–700 mm, and rainfall is concentrated during summer during the growing season of foxtail millet. The accessions in this region have medium–tall plant height (average height, 141.8 cm), medium panicle length (22.6 cm), an average 1000-grain weight of 2.77 g, and an average growing period of about 110–130 days. Recent simple sequence repeat (SSR) and single nucleotide polymorphism (SNP) data have shown that accessions from Heilongjiang Province are greatly diverged from those in Jilin and Liaoning provinces. The varieties from Heilongjiang were shown to be more light- and temperature-sensitive, and consequently, were classified as the early spring-sowing region type. The varieties from Jilin and Liaoning provinces were shown to be genetically much closer to the summer-sowing types cultivated on the North China Plain (Wang et al. 2012; Jia et al. 2013).

#### 6.1.1.2 The North China Plain Eco-Region

This eco-region covers the area from the south of the Great Wall to the north of the Huaihe River, to the west of the Taihang Mountains and most regions in Hengshan distributed in Henan, Hebei, Shandong, Anhui, Jiangsu, Beijing, Tianjin, and other provinces or cities (latitude ≤40°). In this eco-region, the average altitude is about 100 m, and the frost-free period is about 6–8 months. The annual rainfall is about 500–700 mm, with rainfall concentrated during the foxtail millet growing period in summer. This area is appropriate for both spring- and summer-sowing types of foxtail millet. The average growing period of spring-sowing varieties is about 110–120 days and that of summer-sowing varieties is about 80–90 days. The accessions in this region have a medium plant height (average, 127.6 cm), medium to small panicles, and relatively low average 1000-grain weight (2.74 g) compared with those of other ecotypes. Foxtail millet was a spring-sowing crop in this region before the 1960s, but became a summer-sowing crop after the harvesting of wheat from the 1970s onward.

#### 6.1.1.3 Inner Mongolia Plateau Eco-Region

This eco-region includes Hohhot and Chifeng in Inner Mongolia, Fuxin and Chaoyang in Liaoning, Chengde and Zhangjiakou in Hebei, Yanbei in Shanxi, and the Yulin region in Shannxi Province. This ecological zone is located at high latitudes, with an average altitude of 1000 m. The annual rainfall in this region is about 400 mm, and the frost-free period is about 5 months. This area is appropriate for spring-sowing varieties, with an average growing period of 110–120 days. The foxtail millet varieties from this region have an average plant height of 135 cm, large panicles (22.9 cm long), and large grains (average 1000-grain weight, 3.52 g).

#### 6.1.1.4 Northwest Plateau Eco-Region

This eco-region includes Gansu, Shannxi, Xinjiang, and the southern part of Shanxi Province. In this eco-region, the average altitude is about 1000–1500 m, the annual precipitation is about 400–500 mm, and the frost-free period is about 150–180 days. This area is mainly appropriate for spring-sowing varieties with a growing period of about 110–130 days. Some locations in this region are also appropriate for summer-sowing types with a growing period of 85–95 days. The foxtail millet varieties in this region have a medium plant height (average, 135.5 cm), medium-long panicles (average, 29.3 cm), and medium-large grains (average 1000-grain weight, 3.44 g). Recent morphological and molecular studies have identified that varieties from Gansu Province are much more light- and temperature-sensitive than those from other provinces in this region and are genetically similar to varieties from Heilongjiang Province; consequently, they should be classified as the early spring-sowing region type (Wang et al. 2012; Jia et al. 2013).

### *6.1.2 A Brief Introduction to Modern Foxtail Millet Breeding in China*

Modern foxtail millet breeding started in the 1950s and 1960s, and significant progress in foxtail millet cultivar improvement has been made since 1980s. Several books or chapters in Chinese have summarized the progress of foxtail millet breeding, including “Foxtail Millet Breeding” (1997) edited by Li Yinmei, “Principles of Foxtail Millet Genetics and Breeding” by Li (1997), “Genetic Improvement of Foxtail Millet” by Diao et al. (2011), and “Foxtail Millet Production and Research System in China” by Diao (2011). The development of foxtail millet breeding programs in China can be divided into three phases: the direct reselection phase, the pedigree cross-breeding phase, and the multiple-methods-integrated breeding phase.

#### 6.1.2.1 Direct Reselection Phase

Modern improvement of foxtail millet varieties began in the 1920s at several institutes such as the former Jinling University in Nanjing, the Yanjing Crop Improvement Station in Beijing, and the North China Agricultural Research Institute in Beijing. Researchers at these institutes started to evaluate and compare genotypes, leading to the release of some outstanding cultivars such as ‘Yanjing 811,’ ‘Kaifeng 48,’ and ‘Huanong 4.’ During the 1950s–1960s, direct selection from populations with natural variation was the main method to improve foxtail millet. The collected landraces were also extensively compared during the 1950s, and superior varieties were selected. The average grain yield of foxtail millet was about 915 kg/ha during the 1920s to the 1950s in China.

#### 6.1.2.2 Pedigree Cross-Breeding Phase

The period from the 1960s to the 1970s can be described as the pyramiding cross-breeding phase. The Xinxiang Agricultural Research Institute in Henan Province initiated cross-breeding and released the cultivar ‘Xinnongdong 2’ in 1959. In this method, hybrids are produced by crossing different varieties and super individuals or lines are screened from their offspring. After this period, cross-breeding became popular throughout the country and it is still the main method of foxtail millet breeding today. Since the 1980s, cross-breeding has continued to make substantial contributions to foxtail millet improvement in China. Many leading cultivars developed during this period include ‘Yugu 1’ and ‘Zhaogu 1,’ which were developed in the 1980s by the Anyang Agricultural Research Institute in Henan Province and the Chifeng Agricultural Research Institute in Inner Mongolia, respectively. Thereafter, these two varieties became landmark cultivars contributing to national foxtail millet production in China after the 1980s. Many newly developed cultivars are derivatives of these two landmark cultivars. The grain yield of foxtail millet in China reached 1485–1852.5 kg/ha during this period.

#### 6.1.2.3 Integrated Breeding Phase

Since the 1960s, mutations caused by radiation and chemical treatments have been widely used for foxtail millet breeding. In the 1980s, somaclonal variations arising in tissue culture were also exploited for foxtail millet breeding. In the integrated breeding phase, artificial mutations were integrated with hybridization-based pedigree selection to improve foxtail millet. Mutation breeding began in 1963 at the Zhangjiakou Baxia Agricultural Research Institute in Hebei province. The cultivar 'Zhangnong 1' was developed using $^{60}Co\gamma$-ray mutagenesis. Beginning in the 1970s, mutation breeding was widely used for foxtail millet breeding in China, and this method was responsible for approximately 30 % of all cultivars developed over the following 20 years (Yi 1997). Somatic variations induced by tissue culture were also exploited to improve foxtail millet (Diao et al. 2002), resulting in several cultivars, including 'Ai 88,' that were released in China. Sterility of the $F_1$ progeny resulting from interspecies hybridization has also been relieved through the use of tissue culture (Luo et al. 1993; Zhou et al. 1988). During this period, the average grain yield of foxtail millet in China increased to 2250–3000 kg/ha$^2$.

#### 6.1.2.4 Release of the Super Cultivars 'Yugu 1' and 'Zhaogu 1' in China

In the 1980s, the grain yields of summer-sown foxtail millet varieties in northern China were extremely low because of lodging and leaf diseases. The cultivar 'Yugu 1' was developed by the Anyang Institute of Agricultural Science in Henan province in the early 1980s by pedigree selection from the offspring of a cross between the land races Riben 60 Days and Tulong. In 1983, 'Yugu 1' was authorized and released by the Crop Assessment Committee of Henan Province. This variety exhibited excellent agronomic traits, including lodging resistance, a high production rate of effective tillers (over 95 %), and high grain yield. 'Yugu 1' represented a significant breakthrough in combining grain yield and taste quality. During a 2-year field trial in 1985 and 1986 in Xinjiang Hami City, 'Yugu 1' was identified as a first-order drought-tolerant variety. This variety also showed resistance to valley blast, brown stripe disease, brown spot, and smut disease. 'Yugu 1' also showed wide adaptability to environmental conditions, and it has been cultivated in 24 provinces of China since its release. Ever since the 1980s, 'Yugu 1' has been used frequently as a parent in hybrid-based pedigree selection breeding. Currently, nearly 80 % of the cultivars released in the summer-sowing regions of China are direct or indirect derivatives of 'Yugu 1.' Today, 'Yugu 1' is not only widely cultivated in China, but it is also used extensively in breeding and basic research. For example, it was used to generate the foxtail millet trisomic series (Wang et al. 1994) and the foxtail millet reference genome (Bennetzen et al. 2012).

Around the same time in the 1980s, 'Zhaogu 1' was released by the Chifeng Institute of Agricultural Sciences in eastern Inner Mongolia. This variety showed outstanding lodging resistance, thus solving the lodging problem for spring-sowing foxtail millet varieties. Compared with conventional foxtail millet varieties, 'Zhaogu 1' was shown to be more resistant to white hair diseases. Consequently, this variety led to a new generation of high- and stable-yielding and adaptable varieties in spring-sowing foxtail millet breeding.

#### 6.1.2.5 Breeding for Taste and Nutritional Quality

As well as higher grain yields, superior taste and nutritional properties are breeding targets in Chinese foxtail millet breeding programs. In the long history of foxtail millet cultivation, many varieties have been selected for their superior taste, including the four well-known Chinese varieties 'Qinzhouhuang,' 'Jinmi,' 'Longshanmi,' and 'Taohuami.' Although several researchers have tried to establish breeding methods to improve taste and nutritional quality over the past 30 years (Gao and Wang 1997), little is known about the heredity of these characters, which hampers effective breeding.

Starting from the national "Seventh-Five-Year Plan" in the 1980s, taste and nutritional quality were established as breeding targets and several high-quality and special-use cultivars were released. For instance, 'Jingu 21' with superior commercial quality was released by the Institute of Economical Crops, Agricultural Academy of Shanxi Province, and 'Xiaoxiangmi,' 'Jiyou 2,' and 'Jite 4' were released by the Institute of Millet Crops, Hebei Academy of Agricultural and Forestry Sciences. Since 1988, the Millet Section of the Crop Science Society of China continued a national assessment of foxtail millet grain quality, focusing on commercial use and superior taste, and more than 80 varieties were designated as good-quality foxtail millet cultivars. In the past 60 years, approximately 770 varieties have been registered or released in China, but only a small subset are still cultivated. Table 6.1 lists the leading cultivars grown in China from the 1950s to the present day.

### *6.1.3 Foxtail Millet Breeding in Countries Other than China*

The main foxtail millet breeding programs in countries other than China are in India and France. In India, breeding of foxtail millet cultivars began in the 1930s, and the cultivars released from the 1970s to the 2000s included the 'Co' series (Coinbatore), the 'HK' series (Bangalore), the 'K' series (Bangalore), 'Arjuna,' 'SIA326,' 'Chitra' (Andhra Pradesh), the 'SIA' series (Bihar), 'K221' (Karnataka), 'Rs118' (Karnataka), and the 'SIC' series (Maharashtra). Most of the foxtail millet breeding programs in Korea and Japan were conducted in the early 1900s, and almost no breeding has been conducted in those countries since the 1970s. In France, foxtail millet was improved for bird-feed production, and herbicide-resistant green foxtail was used to breed the herbicide-resistant foxtail millet varieties that were introduced into China in the late 1990s (Wang et al. 2001) (also see Chap. 15). Work related to foxtail millet breeding has also been conducted in America, Russia, and some other countries.

**Table 6.1** Leading cultivars of foxtail millet from the 1950s to the present

| Cultivar | Cross | Year of release | Areas of adaptation |
|---|---|---|---|
| Xinnong 724 | Mihuanggu reselection | 1955 | Henan, Hebei, Shandong |
| Moligu | Jiansuijinmiaohuang reselection | 1958 | Hebei, Beijing, Shanxi |
| Hualian 1 | Huaidehualiangu reselection | 1959 | Jilin |
| Angu 18 | Daqingmiao reselection | 1965 | Heilongjiang |
| Lugu 2 | 60 days Huancang reselection | 1971 | Shandong |
| Jingu 1 | Baimujizui reselection | 1973 | Shanxi |
| Zhaogu 1 | Shuangguayin reselection | 1977 | Inner Mongolia, Liaoning |
| Baisha 971 | Baishagu reselection | 1978 | Jilin |
| Jigu 6 | Japan 60 days × Xinnong 724 | 1982 | Hebei, Henan, Shandong |
| Yugu 1 | Japan 60 days × Tulong | 1983 | Henan, Hebei, Shandong |
| Longgu 25 | Harbin 5 × Longgu 23 | 1986 | Heilongjiang |
| Yugu 2 | An 30 × Xiaoliugen | 1989 | Henan, Hebei, Shandong |
| Jingu 21 | Jinfen 52 mutation | 1991 | Shanxi, Shanxi, Inner Mongolia |
| Lugu 10 | Yugu 1 × 5019-5 | 1995 | Shandong, Hebei |
| Jigu 14 | Lusuigu mutation | 1996 | Hebei, Henan, Shandong |
| Gufeng 2 | 95307 × 8337 | 2002 | Hebei, Henan, Shandong |
| Jigu19 | Ai88 × Qingfenggu | 2004 | Hebei, Henan, Shandong |
| Yugu 18 | Yugu1 × Bao282 | 2012 | Henan, Hebei, Shandong, Liaoning |
| | | | Xinjiang, Beijing |

From Cheng and Dong (2010), with some modifications

## *6.1.4 Conventional Foxtail Millet Breeding Technologies*

### 6.1.4.1 System Selection

Natural mutations occur frequently in cultivated foxtail millet, and variations can also arise from hybrids derived from outcrossing. Therefore, it is possible to develop new cultivars from direct selection from the farm field. At the early breeding stage in the 1950s, direct selection was popular in China, and even today it is a simple and effective method for breeders.

There are two options for direct selection: single plant selection and pooled selection. For single plant selection, healthy panicles from well-developed robust plants with characters matching breeding targets are selected, numbered, and preserved. Each panicle becomes a line in the next generation, and different lines are compared against the original varieties. Then, conventional pedigree selection over the following years results in lines with superior characters. 'Gonggu 6,' 'Changnong 1,' 'Hengyan 130,' 'Lugu2,' and other cultivars were developed using this method. These cultivars showed 5–30 % yield increases compared with those of the original varieties. The second method, pooled selection, is when varieties cultivated for a long time are classified into types based on their variations. For pooled selection, plants with similar characters are harvested together as a pool. Then, in the following year, comparisons are made among the pools, the original variety, and the check variety in field trials, and superior varieties are selected. The foxtail millet cultivars 'Huanong 4,' 'Baisha 971,' 'Changwei 69,' 'Lugu 4,' and others were developed by the pooled method. Sometimes the pooled method can be used in combination with the single plant selection method, depending on the degree of variation in the basic varieties.

#### 6.1.4.2 Cross-Based Pedigree Selection

Cross-based pedigree selection can combine superior characters from different varieties, and it is the most popular method for foxtail millet improvement. The choice of the parents and the selection of the best individuals and lines from the offspring are the key steps for successful pedigree selection.

##### Hybrid Parent Selection and Arrangement

Only those varieties with many good traits and few unfavorable ones can be used as parents. The two parents in a given cross should have complementary characters, especially the main agriculturally important characters. The female parent, which is usually a local cultivated variety, cannot have serious defects. Varieties with weak photoperiod-sensitivity are usually more adaptable and are good options as parents. The parents can have common advantages, but they should not have common weaknesses. The breeding targets can be very different for different regions and purposes, so parent selection and arrangement can differ widely depending on the local breeding targets.

##### Method of Hybridization

Because the spikelets of foxtail millet are very small and the flowering time differs among spikelets on the same panicle, it is difficult to hybridize different foxtail millet varieties. Chinese breeders have created various methods of hybridization, whose labor inputs and effectiveness differ widely (Cheng and Liu 1997). The first is artificial emasculation of the foxtail millet spikelet with scissors and tweezers before flowering, followed by pollination with male parent pollen. This method is labor intensive and time consuming, and it is rarely used today. The second, frequently used, method, is hot water emasculation. In this method, the female panicle is soaked in water at 47 °C for 8–10 min and then bagged with male panicles for pollination. In the third method, the female panicle or plants are sealed in plastic bags in the field for 2–3 days, depending on the environmental conditions. The high temperature inside the bag kills the pollen, allowing for subsequent pollination with the desired pollen. Various chemicals to kill pollen were also tested for foxtail millet emasculation. Although some were effective, they are rarely used in breeding because of their limited availability.

All of these foxtail millet emasculation methods have a common problem; that is, the kill rate of the pollen in the female panicle is not 100 %, and so genuine hybrids must be identified in the next generation. During breeding, the male parent is often a variety with a dominant character, for example, purple vs. green seedling sheath, green vs. yellow leaf, or orange vs. white anther. In the $F_1$ generation, individuals with the dominant characters are usually genuine hybrids that can be used for subsequent breeding activities. Some quantitative characters can also be used for hybrid identification. For example, early heading and tillering are usually dominant traits, and the $F_1$ hybrids could have heading dates similar to that of the early heading parent or between those of the two parents.

### Pedigree Selection of Hybrid Offspring

Segregation in the second-generation ($F_2$) is complex, especially for those hybrids whose parents are very different phenotypically and genetically, or whose parents are from different ecotype origins. The population size of the $F_2$ generation depends on the degree of difference between the parents, and a larger population size is required for hybrids of vastly different parents and/or those with characters that have a low frequency of recombination, to increase the chances of selecting targeted individuals. Breeding experience has verified that the selection of heading date, maturity date, disease and insect resistance, plant height, grain color, kernel color, and stem strength (lodging resistance) in the $F_2$ generation is effective, because those characters are easy to select for in early generations. Relatively larger numbers of individuals with different combinations of characters should be selected from hybrids with superior parents.

In the $F_3$ generation, most agronomically important characters are still segregating, and individuals with new combinations of different traits are also emerging. In some lines, the degree of segregation in the $F_3$ generation seems to be smaller than that in the $F_2$ generation. For this generation, the selection focus should be on characters with complex genetic backgrounds such as yield and nutritional characters. Individual plant selection should be carried out to obtain lines with superior characters that are still segregating. Those lines that do not match the breeding target should be rejected. The $F_2$ and $F_3$ generations are the key generations for pedigree selection, and it is important to establish appropriate selection criteria for individual plants and to keep a sufficient but not an excessive number of lines. It is important to select individual plants according to the established objectives.

From the fourth to the seventh generations, more and more lines become genetically stable with the increasing number of generations, and the degree of segregation becomes smaller. The selection focus is to identify superior lines that match the breeding targets and to select lines that are still segregating. Comprehensive comparisons among lines should focus on the breeding targets as criteria, and only a small number of lines matching the breeding targets with superior characters and stable inheritance should be selected for the final test. Most other lines will be abandoned at this point. If some important traits are still segregating in a superior line, individual plant selection is still necessary.

The final test of the selected lines is to compare grain yield and quality among different locations and different years. Only those lines with superior characteristics and wide adaptability can be registered as new cultivars and released to farmers.

Breeding experience has verified that the grain yield of foxtail millet is positively correlated with grain weight of the main panicle and flag leaf area, and the straw yield is positively correlated with plant height and growth duration. The taste and nutritional qualities are usually tested at higher generations. Protein and fat contents are positively related to seed size and negatively related to panicle length. Those corelationships can be used to guide the selection of individuals and lines.

Foxtail millet is sensitive to photoperiod, temperature, and other environmental factors, and so it is important to grow hybrid offspring in different environments to select for wide adaptability. Lines with few morphological changes in different environments usually have wider adaptability. For successful breeding, the nursery field should be a little more fertile than the targeted extension field.

#### 6.1.4.3 Breeding Using Radiation and Chemical-Induced Mutations

The roles of radiation and chemical-induced mutations in the breeding of foxtail millet have been summarized by Yi (1997). Because dry seeds are easy to store, transport, and treat, they are usually the materials used for radiation breeding. However, wet seeds, pollen, and even entire plants can be subjected to radiation to induce mutations. There are external and internal radiation treatments for seeds. The external radiation treatment is usually $^{60}Co$ γ rays irradiated onto dry seeds with a 30,000–40,000 roentgen dosage, as dosages higher than this are lethal to seeds. If the seed moisture content is high, the treatment dosage is lower. For internal irradiation, seeds of foxtail millet are generally soaked in solutions of chemicals containing $^{32}P$ or $^{35}S$. When these elements are absorbed, they emit radiation and cause DNA mutations in the treated plants. Other chemicals have also been used to induce DNA mutations in foxtail millet, including ethyl methanesulfonate (EMS) (Li et al. 2015) and other chemicals (Yi 1997).

The treated seeds and the plants obtained from them are the $M_1$ generation, and usually there is no selection in this generation. However, if there is clear segregation, some individual plants can be selected. The $M_1$ seeds and plants must be cultivated carefully in optimum conditions. After harvesting, 15–20 seeds are collected from each of the $M_1$ individual plants, so as to form a mixed pool designated as $M_2$. The number of seeds collected from each $M_1$ plant varies depending on the number of $M_1$ plants and the size of the $M_2$ pool that have been specified in the experimental design. Because of genetic segregation in the $M_2$ generation, this generation must be observed carefully throughout the whole growing period, in order to select individuals with characters matching the breeding objective. A large number of plants should be selected for those pools with fine characters and wide segregation, and pools with no segregation and/or with bad agronomic phenotypes should be discarded. The selected $M_2$ individuals are grown as lines in the $M_3$ generation. In this generation, the selection should focus on line identification, and individual plants should be selected from the best-performing lines. Because most lines become genetically stable in the $M_4$

generation, the main task for this generation is to comprehensively assess the lines. Lines with good characters matching the breeding targets are selected based on yield and other characters for the final regional adaptation trials.

Most radiation-induced mutations are recessive mutations and can only be selected in the homozygous form after the $M_3$ generation. However, many researchers have found dominant mutations in the $M_1$ generation, and these can be selected in the early generations. So far, more than 40 foxtail millet cultivars have been developed by radiation breeding (Yi 1997) and EMS-induced mutation has played an important role in functional genomics research (Li et al. 2015, 2016).

#### 6.1.4.4 Polyploid Breeding

The ploidy levels of *Setaria* species vary, but domesticated foxtail millet is a true diploid. Because tetraploid species usually have larger seeds, scientists have been very interested in the breeding of tetraploid foxtail millet. In the 1960s and 1970s, polyploid breeding programs developed some tetraploid foxtail millet lines such as 'Wulijin,' 'Jiaqihuang,' and 'Chaoyanggu.' Also, a few foxtail millet landraces with large seeds were identified as natural tetraploids.

Compared with diploid lines, tetraploid foxtail millet lines have shorter, thicker, wider leaf blades, and the blade surface is wrinkled. They also have larger spikelets, pollen grains, and seeds, later heading dates, and longer growth duration. The tetraploid plants are shorter than diploid ones and have smaller panicles. The seed setting rate of the tetraploid foxtail millet is low, leading to low grain yield (Ahanchede et al. 2004). The low grain yield is the main reason why no tetraploid foxtail millet cultivars have been released so far.

However, there are other purposes for tetraploid foxtail millet besides grain production. Tetraploid foxtail millet can be used for distant hybridizations with other tetraploid *Setaria* species such as *Setaria faberii* (Chen et al. 1997; Wu and Bai 2000) and *Setaria yunnanensis* (Zhou et al. 1988). Tetraploid foxtail millet has also been used to construct the foxtail millet trisomic system and in other genetic research (Wang et al. 1994).

There are several different ways to develop tetraploid foxtail millet. The most popular method is to treat seeds with an aqueous solution of colchicine (0.02–0.05 % w/v) for 3–5 days. Other methods include variable-temperature processing of panicles and treatment of young seedlings or tissue-cultured calli.

#### 6.1.4.5 Distant Hybridization

Distant hybridization between different species and/or between different genera can transfer genes from wild to domesticated cultivars. Because foxtail millet is a relatively regional crop, there are only a few research groups that specifically focus on it. Therefore, few distant hybridization breeding and related studies have been conducted for foxtail millet. The earliest report of distant hybridization in foxtail millet was in 1942 by Li et al., who described the hybridization of foxtail millet with green foxtail and *S. faberii*. Based on this study, they identified that green foxtail is the ancestor of foxtail millet. In the 1960s to 1990s, there were many attempts to hybridize foxtail millet with green foxtail, *S. faberii*, *Setaria glauca*, *S. yunnanensis*, *Setaria verticillata,* and other species, with the goal to develop cytoplasmic male sterile (CMS) lines. Although many distant hybridization attempts were made, real hybrids were only obtained from crosses between foxtail millet and green foxtail, *S. faberii*, *S. verticillata,* and *S. yunnanensis* (Zhu et al. 1987; Chen et al. 1997; Zhou et al. 1988; Wu and Bai 2000; Zhi et al. 2007). Since 2000, distant hybridizations were successfully used to transfer herbicide resistance genes from wild green foxtail into domesticated foxtail millet (Chap. 15). The wild germplasm of *Setaria* is certainly a rich gene pool for the improvement of foxtail millet. Therefore, it is reasonable to expect that distant hybridizations will be a powerful tool to improve foxtail millet in the future.

Because of the close relationship between foxtail millet and green foxtail, crosses between these two types cannot be regarded as distant hybridization. They produce a fertile hybrid, which makes it possible to construct recombinant inbred lines for domestication-related studies and other developmental research, such as the characterization of the foxtail millet root system (Zhang et al. 2014).

## 6.2 Heterosis Utilization in Foxtail Millet

### *6.2.1 Brief History of Research on Foxtail Millet Heterosis Utilization in China*

Heterosis, where hybrids show better growth and grain yield than their parents, is a common phenomenon in crops. Because foxtail millet is a self-fertilizing species with a small spikelet, hybrid seeds can only be produced with the development and use of male sterile lines. The successful use of cytoplasmic male sterile lines in the breeding of rice and other crops prompted foxtail millet breeders to try to develop CMS lines during the 1960s, when the foxtail millet heterosis-utilization breeding program started. The first male sterile foxtail millet was reported by Takahashi (1942) from Japan. In China, the first male sterile line ‘Yanxing’ was reported in 1967 by Zhu Guanqin and was developed at the Yan’an Agricultural Research Institute of Shannxi Province (Zhu et al. 1991). Since then, many male sterile materials have been identified in farm fields, but none has been successfully developed into a CMS line.

Researchers attempted to develop CMS lines via distant hybridizations of foxtail millet with other *Setaria* species in the 1970s and 1980s. Only one CMS line was produced, and it was never used in hybrid seed production (Zhu et al. 1991). In the early 1970s, the first partial genetic male sterile (PAGMS) line, ‘Suanxi 28,’ was developed by Cui Wensheng at the Baxia Institute of Agricultural Science, Hebei province. This led to the successful utilization of heterosis in foxtail millet with a two-line system (Cui et al. 1979). In this system, the male sterile line retains approximately 3 % male fertile spikelets, which can reproduce the male sterile line, and hybrid seeds are produced via hybridization of the PAGMS line with restoration lines. A foxtail millet hybrid cultivar produced using this system, 'Suanxi 28' × 'Zhangnong 10,' was released in the late 1970s. Even today, the two-line system is the only one used for foxtail millet hybrid seed production. In the 1980s, a genetic dominant male sterile line was developed via hybridization between the landraces Aodaliyagu and Tulufan, and a hybrid seed production system was suggested with this dominant male sterile line (Hu et al. 1986, 1993).

In the 1990s, many foxtail millet breeders in China focused on developing photo-thermo sensitive genetic male sterile (PTGMS) lines, and although several PTGMS lines were developed during this period (Zhao et al. 1994, 1996; Wang et al. 2003), none of them has been used successfully for hybrid seed production. In the first decade of this century, the development of herbicide-resistant restoration lines made the PAGMS two-line system a great success in foxtail millet heterosis utilization, and several new hybrid cultivars were released, including 'Zhangzagu 5,' 'Zhangzagu 8,' and 'Changza 2.' Compared with conventional varieties, hybrid foxtail millet varieties show greatly increased grain yield in suitable cultivation regions, with reported grain yields as high as 7500 kg/ha.

### *6.2.2 Heterosis Performance in Foxtail Millet*

Many studies have shown that hybrid vigor, or heterosis, of foxtail millet is evident in many characteristics including seedling viability, growth potential, biotic and abiotic stress resistance, and grain yield. However, the specific advantages differ widely among different hybrids.

Compared with their parent lines, hybrids showed faster seed germination and 1–2 days' earlier seedling emergence from soil (Cui et al. 1979), indicating hybrid growth vigor over conventional lines. The growth-related characterization of six foxtail millet hybrids, including '38A' × 'Hui 329,' showed that the dry weight of hybrid seedlings was 15 % greater than that of their parents. Also, the hybrid plants were taller, produced more secondary roots, and had a greater stem diameter, compared with their parents and the control. Li et al. (1963) reported that the heading date of hybrid foxtail millet is usually earlier than those of their parents. Cui et al. (1987) observed that the foxtail millet hybrids 'Suanxi 28' × 'Zhangnong 10' and 'Huangxi 4' × '1007' were more drought resistant than conventional varieties. A drought in 1984 resulted in reduced heading or no heading in conventional varieties, but did not affect heading in these three foxtail millet hybrids.

Many studies have indicated that heterosis is more evident for grain yield per plant than for other yield-related characters, with grain yield being 20–30 % higher in hybrids than in their parents (Li et al. 1963; Du 1984). The main reason for this increase is increased number of spikelets per panicle. However, not all hybrids show heterosis in grain yield. In one study, 57 % of 533 hybrids showed heterosis over their parents, but only 4 % of those hybrids produced a higher grain yield than that of the local control cultivar (Cui et al. 1987). In addition to grain yield, some hybrids show heterosis in protein content, vitamin content, and other characteristics (Ji 1990).

The performance of foxtail millet heterosis is closely related to the geographical origin of their parents and/or genotype differences. Among 251 hybrids produced from the male sterile lines 'Suanxi 28' and 'Huangxi 4,' hybrid vigor was stronger in the offspring of distantly related parents than in the offspring of two local parents. Hybrids produced with cultivars as parents showed 71.4 % heterosis, while hybrids produced with landraces as parents showed only 30.0 % heterosis, indicating that the newly developed cultivars are advantageous in hybrid foxtail millet production (Cui et al. 1987).

### *6.2.3 Development of CMS Lines of Foxtail Millet*

In the early days of development of male sterile foxtail millet lines in the 1960s and 1970s, spontaneous male sterile mutations identified in farm fields were crossed with other genotypes to develop CMS lines, but no true CMS line was developed. Cui Wensheng from the Baxia Agricultural Institute and other breeders conducted many crosses with landraces from distant geographical origins, and although these crosses created several male sterile foxtail millet lines, no CMS line was developed (Cui and Gui 1984). As the wild ancestor of domesticated foxtail millet, green foxtail has been used as the maternal parent in crosses with domesticated foxtail millet to develop CMS lines. More than 30 crosses with green foxtail were conducted in 1987 by Zhu et al. and several hundred similar crosses were conducted the 1970s by different breeders in China. Although a few male sterile lines were created (Zhu et al. 1987; Zhi et al. 2007), no true CMS line was developed. In the late 1980s and early 1990s, the research group lead by Zhu Guanqin at the Shanxi Academy of Agricultural Sciences conducted many distant crosses between *S. faberii*, *S. verticillata,* and *S. yunnanensis* as the female parents and foxtail millet as the male parent. Hybrid plants were successfully obtained from *S. faberii* and *S. verticillata* as parents, but no $F_1$ individual was obtained from the cross using *S. yunnanensis* as the female parent, even when hybrid embryos were rescued by tissue culture (Zhou et al. 1988). Because of the tetraploid nature of *S. faberii*, *S. verticillata*, and *S. yunnanensis*, tetraploid foxtail millet lines created by artificial chromosome duplication were used for crosses, and then back-crossed several times with diploid foxtail millet to restore the diploid character. Among the two successful distant crosses with *S. faberii* and *S. verticillata* as the female parents, a single hybrid offspring of *S. verticillata* was developed into a CMS line. It was designated as the Ve CMS line of foxtail millet, because its cytoplasm comes from *S. verticillata* (Zhu et al. 1991). This is the only CMS line reported in foxtail millet breeding so far, and for unknown reasons this line has not been used to produce hybrid seeds.

### 6.2.4 Identification of the Ch Dominant Male Sterile Line in Foxtail Millet

Hu et al. (1986) reported the first genetic dominant male sterile line 'Ch78182' identified among the offspring of a cross between the Australian landrace Aodaliyagu, (meaning Australia) and the Chinese landrace Tulufan from Xinjiang. 'Ch78182' was designated as the Ch dominant male sterile line, because it was developed in Chifeng Agricultural Institute of Inner Mongolia. The individual male sterile plant was first identified in 1976, and an inheritance study confirmed its genetically dominant nature (Hu et al. 1993). Screening for restoration lines identified that '181–5' could fully restore the male fertility of 'Ch78182.' An inheritance study showed that lines '181–5' carry the epistasis gene *GG*, which suppresses the expression or function of the dominant male sterile gene *ChCh*. These two genes, *GG* and *ChCh*, were found to be linked in the foxtail millet genome. The male sterile individuals could be genotyped as ChChgg and Chchgg, and any genotype with --G- was male fertile (Hu et al. 1993). The main reason for the male sterility of 'Ch 78182' line was shown to be the elimination of anther dehiscence. That study showed that pollen development is nearly normal inside the anther, and fully developed mature pollen grains accumulate inside the anthers (Diao et al. 1991). Later, Hu et al. (1993) identified that 'Ch 78182' could self-pollinate to some degree, leading to 6–10 % seed setting in Hainan Province of Southern China, implying that environmental factors affect anther dehiscence. Plants from those self-pollinated seeds are thoroughly male sterile in northern China, where foxtail millet is cultivated for grain production. According to all those characters of the dominant male sterile line 'Ch 78182' and its putative restoration lines '181–5,' a hybrid seed production system was suggested, as shown in Fig. 6.1 (Hu et al. 1993). However, because of the complexity of this system, it has not been used for the commercial production of foxtail millet hybrid seeds.

### 6.2.5 Development of Photo- or Thermo-Sensitive Genic Male Sterile Lines

Photo- or thermo-sensitive genic male sterility (PTGMS) was successfully used in rice hybrid seed production in China from the 1990s, and this captured the interest of many foxtail millet breeders. The first foxtail millet PTGMS line '292' was developed in 1989 from pedigree selection of a cross between two landraces, Cai 5 and Ce35-1, at the Baxia Institute of Agricultural Sciences (Zhao et al. 1994). The '292' line was made male fertile to some degree by a short photoperiod treatment (13 h or shorter), and made male sterile by a longer photoperiod treatment (14 h or longer). This suggested that male sterile lines could be produced in short-day conditions (winter) in Hainan by self-pollination of '292,' and hybrid seeds could be produced in long-day conditions in summer in northern China. Based on line '292,' many crosses were made

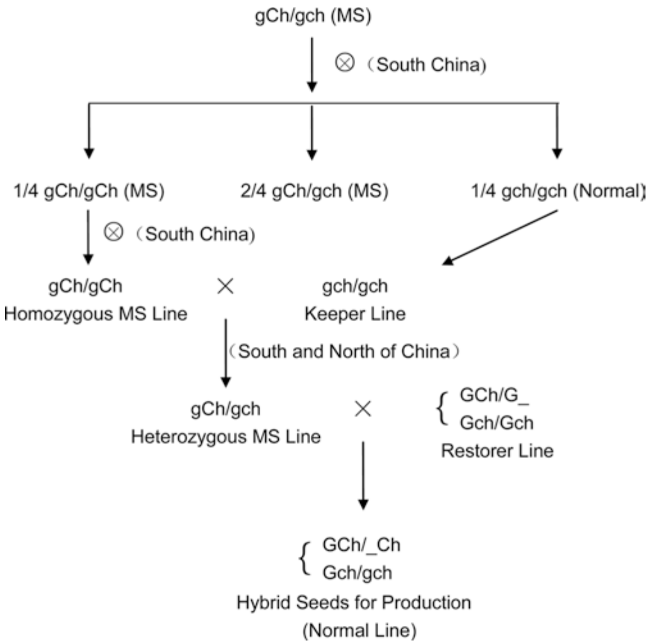


**Fig. 6.1** Chart of hybrid seed production using Ch dominant male line, modified from Hu et al. (1993). *Chch* represents dominant male sterile gene and *Gg* represents epistasis gene of *Ch*. Chchgg genotype can self-pollinate in southern China (Hainan or Zhanjiang) with a 6–10 % seed setting rate, thereby producing ChChgg and chchgg lines. ChChgg genotype can maintain itself in southern China by self-pollination, and is crossed with chchgg genotype to enlarge male sterile seed pool. Chchgg is crossed with GG restoration line to produce hybrid seeds for commercial foxtail millet production

with different landraces and cultivars, and different PTGMS lines were developed, including '821' which was much more stable than '292.' An inheritance study on '821' identified that the PTGMS male sterile gene was a single recessive one (Zhao et al. 1996). Wang et al. (2003) developed the PTGMS lines 'SMPA 1,' 'SMPA 2,' 'SMPA 3,' and 'SMPA 4,' all derivatives of '292.' The grain yields of the hybrids produced from those lines were more than 15 % higher than that of the local control cultivar. Another PTGMS foxtail millet line, 'Guang A1,' was developed by Cui et al. (1991). This line was derived from pedigree selection of the cross between the landraces Aodaliyagu and Zhongweizhuyeqing. 'Guang A4' was developed from the offspring of a cross between 'GuangA1' and the landrace Jiugenqi by Zhao and Cui (1994). Characterization of photosensitivity showed that a short photoperiod treatment (10–12 h) resulted in 45 % sterile pollen in 'Guang A4' with 33.2–38.2 % seed setting by self-pollination. A longer photoperiod treatment (15 h) resulted in 99 % sterile pollen with a 0.1 % seed setting rate (Zhao and Cui 1994). Although there have been several reports of PTGMS in foxtail millet, no hybrid cultivar developed using this technology has been released so far. This is probably because the stability of the male sterile character of PTGMS differs in unstable environmental conditions and from year to year. This should be studied in detail in the future.

### *6.2.6 Successful Heterosis Utilization in Foxtail Millet with PAGMS Lines*

Cui Wenshen from the Baxia Institute of Agricultural Sciences identified an individual male sterile foxtail millet plant from the landrace Hongmiaosuanpibai in 1969. Consecutive selections developed this male sterile plant into a partial genic male sterile line (PAGMS) line, which was named 'Suanxi 28.' A characterization and inheritance study demonstrated that 97 % of its spikelets were male sterile and 3 % were male fertile. With its 3 % male fertile spikelets, 'Suanxi 28' could achieve approximately 5 % seed setting by self-pollination, and the progeny of those self-pollinated seeds retained the 97 % male sterile character. An inheritance study of the male sterile character demonstrated that it is controlled by a single recessive gene, and all normal landraces or cultivars can restore male sterile to male fertile ones (Cui et al. 1979). This is a distinct type of male sterility, which was named Gaoduxiongxingbuyu in Chinese, meaning highly male sterile, but the term "partial genic male sterile line" is more appropriate in English (Diao 2014). Similar to 'Suanxi 28,' other PAGMS lines were also developed from different source of male sterile origin, such as 'Huangxi 1,' 'Huangxi 4,' and 'Huangmi 1.' The male sterile genes of 'Suanxi 28' and 'Huangxi 1' were transferred into different foxtail millet genotypes and several PAGMS lines were developed. Other PAGMS lines similar to 'Suanxi 28' were developed at different institutes in China in the late 1980s and 1990s, such as '1066 A,' '350 A,' 'Jinfen A,' and 'Gao 146A' (Wang et al. 1993; Li et al. 2011; Yang et al. 2007).

The advantage of this system is that it is easy to maintain male sterile seeds with one line and easy to screen restoration lines from common cultivars. The disadvantage of this system is that false hybrid seeds must be eliminated from the hybrid seed pool. In the late 1970s, false hybrid plants were removed by hand at the seedling stage in field trials, and were identified based on color, as false male sterile hybrid seedlings were yellow instead of green. The labor-intensive nature of this system restricted the use of these hybrid cultivars, even though several cultivars showed substantially increased grain yields, such as 'Suanxi 28' × 'Zhangnong 10' and 'Huangxi 4' × '1007' (Cui et al. 1979). In the first decade of this century, an herbicide resistance gene was transferred into foxtail millet from wild green foxtail, and herbicide-resistant cultivars were released (see Chap. 15). Hybrid seeds produced using herbicide-resistant foxtail millet cultivars as the restoration line retain the herbicide-resistant character, and so false male sterile plants can be easily killed by spraying with herbicide. This has created a new opportunity for the successful utilization of PAGMS foxtail millet lines (Wang et al. 1996).

The development of PAGMS lines and herbicide-resistant restoration lines has made it possible to utilize foxtail millet heterosis. Consequently, more than ten hybrid cultivars produced using this system have been released during the past 10 years in China. Most of these cultivars were developed at the Zhangjiakou Institute of Agricultural Sciences in Hebei Province. According to data from the National Test for Regional Adaptability (TRA) of foxtail millet cultivars at the National Extension Center for Agricultural Technology from 2000 to 2002, the grain yield of hybrid foxtail millet ‘Zhangzagu 1’ was 5494.5 kg/ha, 19.78 % greater than that of the control variety ‘Datong 14.’ In the TRA trial in 2004, Zhangzagu 3 had a grain yield of 4455 kg/ha, 19.13 % greater than that of the control variety. In the TRA trial in Shanxi Province, the grain yield of the foxtail millet hybrid ‘Changzagu 2’ was 17 % greater than that of the control.

Today, foxtail millet is an important crop in arid and semi-arid regions, and it is potentially important for a much drier and warmer climate in the future. As a self-pollinated diploid species with a small genome, foxtail millet is fast becoming a new model plant for functional genomics and its wild progenitor, green foxtail, is becoming a model plant for research on $C_4$ photosynthesis and abiotic response. Progress in research on the germplasm, conventional breeding, functional genomics, and molecular breeding of foxtail millet will certainly improve research on heterosis in this crop.

Several breeding groups in China are focusing on the utilization of foxtail millet heterosis with PAGMS lines and have developed new super hybrid cultivars. However, few researchers are attempting to create new types of male sterile lines. Since 2007, three foxtail millet hybrid varieties have been released in Shanxi Province, and at least five new varieties are being evaluated in field trials in the National Test for Regional Adaptation of foxtail millet cultivars. Research efforts have improved foxtail millet heterosis utility, and analyses of population structure and heterotic groups by combining ability tests and molecular methods are underway. Therefore, we forecast a promising future for the utilization of foxtail millet heterosis.

## 6.3 Perspectives for Foxtail Millet Breeding

As a cereal with a long history of cultivation as a staple crop in arid and semiarid regions, foxtail millet is now recognized as a crop suitable for cultivation in the drier and warmer conditions predicted in the future. As such, it is receiving more attention as a sustainable agricultural crop (Li and Brutnell 2011; Lata et al. 2013; Diao et al. 2014). In the past 60 years in China, four great achievements have been made in foxtail millet breeding. First, a comprehensive germplasm collection of *Setaria* was established, comprising 27,760 accessions from all of the ecological regions of foxtail millet cultivation in China. These accessions are housed at the Chinese Gene Bank. Second, the grain yield and food quality of foxtail millet were greatly improved in the 1980s through the development of the super cultivars ‘Yugu 1’ and ‘Zhaogu 1,’ with most of the recently released cultivars being derivatives of these two genotypes. Third, the introduction of the gene conferring resistance to the herbicide sethoxydim from green foxtail into foxtail millet made its cultivation system more suitable for modern agriculture. Finally, the development of hybrid cultivars of foxtail millet through the PAGMS two-line system created a new method of heterosis utilization for this crop, distinct from the well-known CMS and PTGMS systems. All of those achievements have not only enriched the theory of plant breeding but have also established a firm foundation for the development of *Setaria* as a model species.

Compared with rice, wheat, maize, and other major cereals, conventional and hybrid cultivars of foxtail millet still have a low grain yield potential. Increasing the yield potential is one of the main goals of foxtail millet breeding. In northern China, foxtail millet is mainly consumed as a gruel for breakfast and dinner, mainly because the kernels of foxtail millet are more suitable for gruel than for preparation as a main-meal grain like rice. If the quality of foxtail millet kernels can be improved to a point where it can be prepared as a main food, then this will promote foxtail millet as a future main crop. Foxtail millet is a well-known drought-tolerant crop. This has been learned mainly from experience, and there is no detailed scientific data on its drought tolerance or water use efficiency. Consequently, it has been difficult to establish breeding programs to improve its drought tolerance and water use efficiency. Other characteristics that are important in breeding include resistance to diseases such as leaf rust and blast disease, and pests such as nematodes and other insects.

The recent advances in genome sequencing and functional genomics research on *Setaria* will lead to substantial changes in the breeding methods for foxtail millet in the near future. The elucidation of characters related to photosynthesis and grain yield and the molecular interaction networks between them will accelerate improvements in the yield potential of foxtail millet. It is possible that foxtail millet will become a major crop in dry land agriculture, with a grain yield potential similar to those of maize and sorghum. Using a combination of association and linkage mapping populations, the Foxtail Millet Research Team at the Chinese Academy of Agricultural Sciences (CAAS) is now elucidating coding genes and dominant alleles of foxtail millet related to taste and commercial and nutritional quality, with the aim to improve foxtail millet to the point where it can become a widely used crop. Breeders at the Institute of Millet Crops, Hebei Academy of Agricultural Sciences, and at the Institute of Crop Sciences, CAAS, are currently developing markers for resistance to leaf rust and blast disease. Several research groups in India, China, and America are currently deciphering genes, alleles, and molecular networks related to drought resistance of foxtail millet (Wang et al. 2016; Qie et al. 2014; Qi et al. 2013). The results of those studies, as well as recent progress in research on the drought resistance of other crop species, will be useful for the molecular breeding of highly drought-tolerant cultivars of foxtail millet and other crops in the near future.

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