

Chapter 2

Population Genetics and Genome-Wide Association Mapping of Chinese Populations of Foxtail Millet and Green Foxtail

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Abstract Green foxtail (*Setaria viridis*) and foxtail millet (*Setaria italica*) are new biological and genomic models for investigation of the biology of C₄ photosynthesis and grass evolution. Green foxtail is the ancestor of foxtail millet, an ancient cereal of great importance in arid and semi-arid regions of the world, especially in China and India. To date, China has been recognized as the center of origin and improvement of foxtail millet, and over 80% of the world's *Setaria* accessions are conserved in the National Gene Bank of China. Assessment of germplasm samples collected in China can help to reveal the domestication history and potential for improvement of cultivated foxtail millet. Recently, the molecular diversity, genetic structure, eco-geographical distribution and selection history of foxtail millet cultivars has been revealed through large scale germplasm characterization, genomic analysis, and genome-wide association mapping of QTLs controlling agronomic traits. These achievements have laid the foundation for further exploration of functional genes controlling vital characters in *Setaria* and will be powerful tools for improved marker-assisted breeding of foxtail millet cultivars. In this chapter, recent studies on the Chinese *Setaria* gene pool will be discussed, as well as their potential for benefiting future genetic investigations in *Setaria*.

Keywords *Setaria* • Germplasm • Diversity • Structure • Domestication • Breeding • Genome-wide association studies (GWAS) • Quantitative trait loci (QTL)

2.1 Introduction

According to recent archeological evidence, foxtail millet (*Setaria italica*) has been domesticated and grown for harvesting grains as food in North China for over 11,500 years (Lu et al. 2009; Yang et al. 2012). As one of the oldest cereals cultivated

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widely in China, large numbers of foxtail millet variants have emerged since domestication. To date, over 27,500 accessions of foxtail millet collected from China have been conserved in the National Gene Bank of China, operated by the Chinese Academy of Agricultural Sciences (CAAS). China is also the center of foxtail millet crop production and contributes over 80 % of the global grain yield of this ancient cereal. For a long time, China has been the main region where most foxtail millet improvement has been conducted, despite the fact that basic research in *Setaria* has been carried out recently in a number of countries across the world (Doust et al. 2009; Li and Brutnell 2011; Diao et al. 2014).

Evaluation and identification of genetic diversity and population structure of germplasm accessions is pivotal for resource management and hybrid breeding of foxtail millet. In addition, association mapping of Quantitative-Trait-Loci (QTLs) controlling agronomically important traits through Genome-Wide-Association-Studies (GWAS) can provide more opportunities for breeders to characterize genetic backgrounds and to pyramid favorable traits by molecular marker approaches.

This chapter describes the diversity and structure of Chinese *Setaria* accessions, including landraces, improved cultivars, and wild relatives, together with an overview of molecular and genetic analyses to identify genomic regions contributing to important morphological traits in foxtail millet. Finally, conclusions and research perspectives will be presented, especially in the context of the rapid development of a new generation of high-throughput genome re-sequencing technologies.

2.2 Diversity and Structure of Chinese *Setaria* Accessions

The first-generation haplotype map of genomic variants of foxtail millet was constructed through genome re-sequencing of 916 accessions of foxtail millet lines (Jia et al. 2013a). Sequence diversity (π) of cultivated *Setaria* accessions was estimated to be ~ 0.0010 , which is comparable to the diversity of *indica* (~ 0.0016) and *japonica* (~ 0.0006) subspecies of rice (*Oryza sativa*). The population structure of Chinese *Setaria* accessions (including both landraces and improved elite cultivars) is highly consistent with the geographic distribution of eco-regions in China. Two divergent subgroups, Part 1 and Part 2, which include accessions sourced from north China and south China respectively, are inferred by phylogenetic analysis (Jia et al. 2013a). Linkage disequilibrium (LD) decay rate in foxtail millet is ~ 100 Kb on average, similar to cultivated rice (Huang et al. 2010) and sorghum (*Sorghum bicolor*) (Morris et al. 2013), which are also self-pollinated organisms.

2.2.1 Landraces

A landrace is a kind of locally adapted variety domesticated from wild species of animals or plants, by long-term human or environmental selection and population isolation. Landraces usually display a high level of diversity in morphological

phenotypes, physiological characters, agronomic traits, and even genomic sequences. In China, prehistoric remains chart the long history of foxtail millet domestication. In northern China, the domestication of foxtail millet was initiated 11,500 years ago and was the predominant food crop until 4,000 years ago (Lu et al. 2005). The area under cultivation continued to increase up until the 1950s. There exist ancient agricultural documents that list foxtail millet as an important cereal food crop, and several officials in the Qing and Ming dynasties in China were designated as “officers of foxtail millet management” (Cao 1986).

The diversity of foxtail millet landraces is the result of conscious selection by farmers for specific phenotypes for harvesting and sowing, including (1) Higher yield potential; (2) Better biotic and abiotic stress tolerance, good adaptability, and lodging resistance; (3) Suitability for local farming environmental conditions and for intercropping; and (4) Good quality of kernel/grain ratio (ratio of milled grain to harvested grain), and better nutrition and flavor (Li and Wu 1996).

According to a morphological analysis of accessions conserved in the Chinese national gene bank reported by Li et al. (1995), foxtail millet landraces sourced from different eco-regions of China are very diverse. Landraces differed in all measured morphological traits (Table 2.1). Overall, grain dry weight and number of tillers were more variable (exhibited a higher Coefficient of Variation, CV) than other traits. For landraces collected from other countries, grain dry weight and number of tillers also exhibited a higher level of phenotypic variation. In terms of yield-related characters, Chinese landraces produced a higher level of grain yield in field production, but crude protein contents of Chinese landraces were lower than traditional cultivars sampled from other countries. Reddy et al. (2006) reported on the morphological diversity of foxtail millet germplasm collections conserved at the International Crops Research Institute for Semi-Arid Tropics (ICRISAT) in India and showed that there was more variation among landraces sampled from China than among landraces sampled from any other country. Overall, however, variation among landraces outside China was greater than that within China (Table 2.1), which may be due to fewer number of samples collected and conserved in other countries (Li et al. 1995).

The genetic diversity of foxtail millet landraces was analyzed by Jusuf and Pernes (1985) using ten isozymic polymorphic loci. Their results suggested that China has the highest level of molecular diversity for the species. RAPD markers (Li et al. 1998; Schontz and Rether 1999), AFLP markers (Le Thierry d’Ennequin et al. 2000), and ISSR markers (Li et al. 2012) have also been utilized for foxtail millet landrace diversity analysis. Jia et al. (2009) characterized genetic diversity of 40 foxtail millet landraces sourced from China using SSR markers. Van et al. (2008) detected the LD extent in Waxy gene sequences from a set of a worldwide collections of foxtail millet, and Wang et al. (2010) analyzed extent of LD in nine selected genomic fragments from 50 landrace accessions, with both studies revealing low levels of LD in foxtail millet. A report of population structure analysis by transposon display (TD) classified foxtail millet landraces into eight clusters that are linked closely to geography and suggest a monophyletic origin of foxtail millet domestication (Hirano et al. 2011). In recent studies reported by Wang et al. (2012), a total of 250 representative foxtail millet landraces collected from China were sampled for population genetic analysis using microsatellite markers. For each SSR locus, an average of 20.9 alleles

Table 2.1 Mean values of quantitative traits of foxtail millet landraces sourced from China and other countries

Province/ country	Till	Hgt (cm)	CulD (cm)	Node#	PanL (cm)	DaysM (day)	DWP (g)	DW1000 (g)	Prot (%)
Anhui	1.5	154.1	0.50	16.0	20.0	107.0	11.5	2.68	13.00
Beijing	1.4	139.0	0.67	15.0	24.0	105.3	10.8	2.68	14.32
Fujian	1.0	170.9	0.60	18.2	27.9	124.0	14.5	1.10	11.45
Guangdong	2.4	123.7	0.66	16.7	24.8	129.0	5.2	1.57	16.04
Guangxi	1.1	140.3	0.70	23.4	21.7	112.8	3.8	2.14	13.20
Guizhou	1.8	147.3	0.67	16.0	23.3	119.7	7.9	2.25	16.22
Hebei	2.5	131.8	0.70	17.4	21.0	111.8	11.9	2.63	14.00
Henan	2.2	131.4	0.66	13.8	20.9	116.4	11.1	2.52	15.12
Hunan	2.2	129.4	0.70	16.9	23.5	127.0	10.4	1.63	15.10
Jiangsu	1.7	113.4	0.70	14.4	18.4	110.0	10.1	2.60	14.30
Shaanxi	1.3	138.8	0.75	14.4	24.9	123.3	9.1	2.71	15.42
Shandong	2.0	130.4	0.67	14.7	21.5	113.8	12.0	2.70	13.94
Shanxi	1.2	132.4	0.73	13.9	25.0	117.1	10.1	2.97	15.19
Xinjiang	2.4	113.8	0.47	12.3	27.0	95.4	5.3	2.86	17.36
Yunnan	1.4	122.8	0.42	12.8	18.7	98.7	5.3	1.57	15.04
Zhejiang	1.4	121.1	0.70	12.3	21.2	94.5	9.6	2.34	14.96
Average	1.7	133.8	0.64	15.5	22.7	112.9	9.3	2.31	14.67
CV%	29.22	11.10	15.00	17.75	12.19	9.47	32.30	24.00	9.56
Afghanistan	6.4	74.4	0.27	9.1	10.1	90.7	2.6	2.43	15.33
Albania	1.1	132.9	0.50	11.5	26.1	108.0	5.0	2.50	15.06
Australia	2.9	96.1	0.37	8.3	14.9	97.7	5.9	2.60	16.48
Bulgaria	2.6	121.3	0.40	11.1	16.6	105.0	5.0	2.02	19.58
Denmark	1.6	102.4	0.30	8.8	16.1	85.0	2.7	2.40	14.22
Finland	1.1	70.0	0.30	8.4	17.5	90.0	1.3	2.04	17.37
France	1.0	121.0	0.42	11.9	26.0	96.2	3.8	2.45	15.94
Hungary	2.1	110.1	0.48	10.1	13.6	94.3	3.6	2.55	17.79
India	4.4	137.0	0.53	16.7	17.1	120.0	6.5	2.29	16.54
Iran	4.3	83.6	0.30	10.8	12.5	90.0	2.4	2.40	15.78
Italy	1.0	132.0	0.50	12.5	24.5	111.0	8.0	2.88	17.27
Japan	1.4	136.3	0.68	15.3	23.6	114.4	11.3	2.23	14.66
Kenya	4.2	146.7	0.54	16.5	17.9	115.6	8.1	2.23	16.44
Korea	1.4	137.2	0.60	13.4	24.5	105.1	9.2	2.32	14.58
Lebanon	16.8	70.8	0.26	11.0	7.6	99.8	2.5	2.04	16.18
Nepal	1.2	120.8	0.35	13.0	19.6	112.0	2.6	2.18	16.89
Netherlands	1.7	117.4	0.60	12.3	19.8	93.9	6.1	2.62	17.56
Poland	3.5	73.6	0.30	8.2	9.1	90.0	2.7	2.39	15.63
Romania	2.8	104.1	0.50	9.6	18.4	93.0	6.0	2.55	15.53
Russia	2.3	94.3	0.38	9.8	19.8	89.2	4.9	2.41	16.23
South Africa	5.4	153.1	0.50	16.5	17.4	117.0	7.8	2.40	18.81
USA	4.3	128.5	0.52	15.0	15.9	114.0	6.1	2.45	15.78

(continued)

Table 2.1 (continued)

Province/ country	Till	Hgt (cm)	CulD (cm)	Node#	PanL (cm)	DaysM (day)	DWP (g)	DW1000 (g)	Prot (%)
Average	3.3	111.9	0.4	11.8	17.6	101.4	5.2	2.4	16.3
CV%	101.39	23.06	27.72	23.55	29.56	10.83	50.08	8.69	8.17

Modified from Li et al. (1995)

Abbreviations: *Till* number of tillers including main culm, *Hgt* plant height to panicle base, *CulD* diameter of main culm base at maturity, *Node#* visible node number of main culm, *PanL* length of main panicle, *DaysM* days from emergence to maturity, *DWP* grain dry weight per plant including tillers, *DW1000* 1000-grain dry weight, *Prot* crude protein content, *CV%* coefficient of variation (= standard deviation divided by the mean) $\times 100\%$

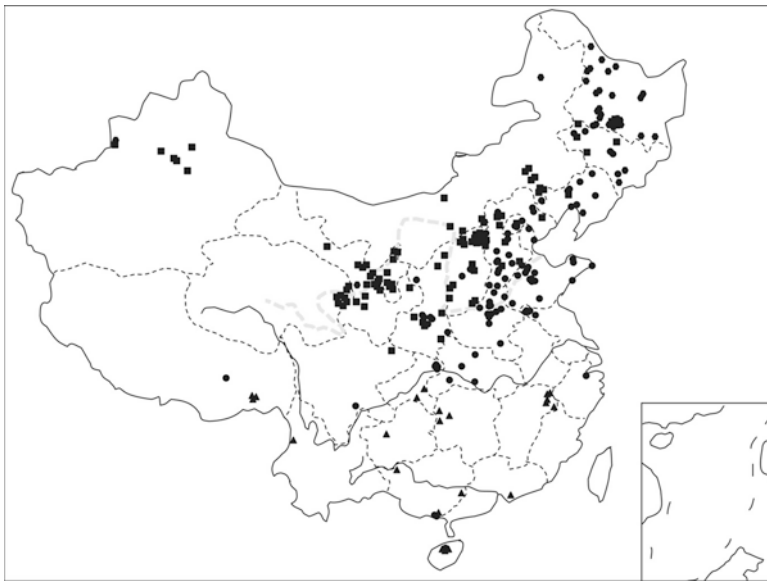


Fig. 2.1 Geographical distributions of subpopulations of Chinese foxtail millet landraces. *Black triangle*—SCR Group (all provinces south of Qinling Mountain); *black circle*—SSSR Group (Henan, Hebei, Shandong, Beijing, Tianjin, and connected parts of Jiangsu, Liaoning, and Jilin provinces); *black square*—SR Group (Shanxi, Shannxi, Inner Mongolia, Gansu, Ningxia, Xianjiang, and western part of Jilin and Liaoning provinces); *black hexagon*—ESR Group (northeast Heilongjiang province). The Yellow River valley is marked a *grey broken line* (modified from Wang et al. 2012)

were identified, which suggest highly diversified genomic variants within and among Chinese ecotypes. Four subpopulations (Fig. 2.1) were inferred, corresponding to the early-spring sowing region (northeast Heilongjiang province, where foxtail millet is sowed in late April or early May annually), spring sowing region (Shanxi, Shannxi, Inner Mongolia, Gansu, Ningxia, Xianjiang, and western part of Jilin and Liaoning provinces, where foxtail millet is sowed in May), summer and spring sowing region (Henan, Hebei, Shandong, Beijing, Tianjin, and connected parts of Jiangsu, Liaoning

and Jilin provinces, where foxtail millet can be sowed from late April to early July), and southern china region (all provinces south of Qinling Mountain, where foxtail millet can be sowed even in late summer and early autumn). The highest level of molecular diversity was detected in accessions grouped into subpopulations sourced from eco-regions around the Yellow River valley in north China.

2.2.2 *Elite Varieties*

China is considered as the center of foxtail millet production and most crop improvement efforts. Since the 1950s, over 550 foxtail millet elite varieties developed by more than 40 breeding programs were released and registered with local and national authorities in China. According to studies by Jia et al. (2013a), phenotypic changes involved in modern breeding of foxtail millet include yield-related traits such as grain yield per plant, grain yield per main stem, panicle weight per main stem, panicle weight per plant, 1000-grain weight, and number of total panicles per plant. Using microsatellite markers, Jia et al. (2015) sampled 348 accessions and detected an average of 17.9 allele number per locus in Chinese elite varieties.

Two clear subgroups, designated as spring-sowing and summer-sowing types, were identified by Jia et al. (2015). These correspond to the eco-regions of the two main foxtail millet production areas in China. In addition, analyses of subgroups associated with breeding histories, breeding preference, and planting configuration indicated that the genetic background of most elite varieties released in recent years was from summer-sowing ecotypes, as a result of the response of breeders to the recent increase of the multi-cropping index (number of crops that can be sown and harvested in a year) in China (Xin et al. 2009).

The spring-sowing cluster is comprised of varieties developed prior to the 1980s, of which most are not currently used in grain production. The summer-sowing cluster is composed of elite varieties that were mostly developed after the 1980s. Elite varieties in the spring-sowing cluster possess higher levels of genetic diversity compared to those in the summer-sowing cluster, and varieties from the same breeding programs and similar eco-environmental conditions tend to be more closely related. Chinese foxtail millet elite varieties released by breeding programs conducted in Shanxi and Jilin provinces were dissimilar from most other lines, suggesting that unique germplasm was incorporated into these breeding programs (Jia et al. 2015).

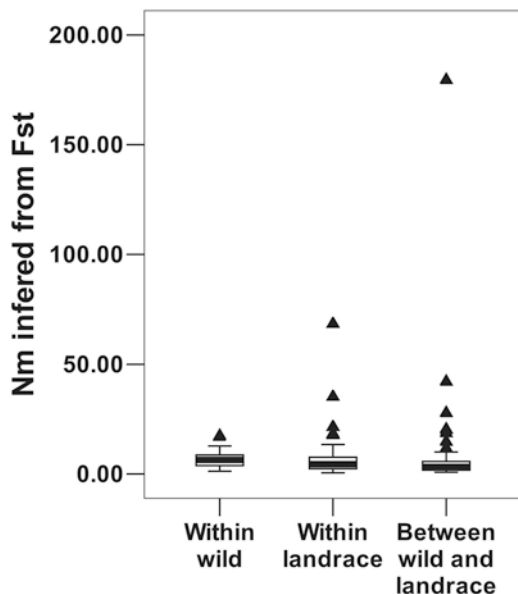
2.2.3 *Wild Relatives*

Green foxtail (*Setaria viridis*), the ancestor and wild relative of cultivated foxtail millet, is distributed worldwide. To date, little morphological research on the diversity of Chinese green foxtail populations has been published. Investigations of genetic diversity of Chinese green foxtail accessions using transposon display (Hirano et al. 2011), intersimple sequence repeat (Li et al. 2012), and amplified

fragment length polymorphism markers (Le Thierry d'Ennequin et al. 2000) have found very high levels of molecular diversity. Green foxtail is also becoming a model for functional genomics studies, focusing on abiotic stress tolerance (Qie et al. 2014), crop domestication (Doust et al. 2014), and grass evolution (Doust et al. 2009). The well-studied green foxtail accession A10 that has been used as model for morphological development and C_4 photosynthesis research was collected in Canada, but is no doubt originally from China (Brutnell et al. 2010; Li and Brutnell 2011; Bennetzen et al. 2012; Caemmerer et al. 2012).

Genetic characterization of DNA sequence polymorphisms from green foxtail germplasm is pivotal for analyzing domestication, evolution, and potential for breeding in this wild grass species (Huang et al. 2014). In the analysis reported by Jia et al. (2013b), the microsatellite diversity of Chinese green foxtail accessions is 50 % higher than cultivated foxtail millet, with an average of 33.5 alleles per locus. Subclusters in the south and north are inferred according to eco-geographical regions designated in China, and higher molecular diversity was found in the northern subcluster. Phylogenetic studies of both Chinese foxtail millet and green foxtail samples also suggest that North China may be the first domestication center, or at least one of the oldest locations for cultivation of this ancient cereal crop. Genetic introgression analysis illustrates lower levels of gene flow within green foxtail subclusters coupling with higher frequency of introgressions between cultivated and wild Chinese *Setaria* accessions (Fig. 2.2). Similar results were also detected by Wang et al. (2010) through re-sequencing of nine genomic regions in both wild and cultivated *Setaria* samples. Decay of LD was found to be faster in *S. viridis* than *S. italica* (Wang et al. 2012), which is consistent with higher rates of cross pollination

Fig. 2.2 Gene flow identified by N_m inferred from classical F test within and between green foxtail and foxtail millet landraces (modified from Jia et al. 2013b)



within wild *Setaria* accessions. Using high density SNPs markers, Huang et al. (2014) also detected a decline in LD decay distance (<45 Kb) compared with that of cultivated *Setaria* accessions (~100 Kb) (Jia et al. 2013a).

Using simulation analysis (Wang et al. 2010), a low level of gene flow from cultivated landraces to wild relatives was detected, but the reverse process was even smaller. Gene flow between *S. italica* and *S. viridis* was also identified through microsatellite analysis (Jia et al. 2013b) and introgressions of herbicide-resistant genes detected in the field (Shi et al. 2008). These observations illustrate that under long-term intensive human selection, alleles transferred from wild green foxtail to cultivated foxtail millet were mostly eliminated, but genes introgressed into the wild relatives might be retained at a higher level, as evidenced by many weedy types (labeled as “giant foxtail”) that are morphologically similar to cultivated foxtail millet in and around foxtail millet fields (Li et al. 1942, 1945).

2.3 Selection Bottlenecks

Domestication and improvement of ancient cereal crops always leads to a dramatic decline in morphological diversity and genetic polymorphisms. Characteristics like loss of shattering, less tillering, plant height variation, and grain yield improvement have been widely identified in crop species such as rice, wheat, maize, barley, etc. For *Setaria*, morphological changes from the wild green foxtail to cultivated landraces include larger panicles, higher grain yield, less tillering/branching, longer period of flowering, loss of seed shattering and dormancy, and variation in biotic/abiotic stress tolerance, etc. (Doust et al. 2004, 2005, 2014; Brutnell et al. 2010; Mauro-Herrera et al. 2013; Qie et al. 2014; Mauro-Herrera and Doust 2016).

Through re-sequencing of nine genomic regions of wild and cultivated *Setaria*, Wang et al. (2010) reported a 55% loss of nucleotide diversity from wild relatives to cultivated *Setaria* accessions. To date, only one candidate gene (*Si037789m*) has been identified for the control of seed shattering in *Setaria*, based on comparative genetic analysis (Lin et al. 2012) and an 855-bp insertion or deletion in coding regions confirmed between *Setaria italica* and *Setaria viridis* (Jia et al. 2013a). Furthermore, several candidate loci involved in controlling branching (Doust et al. 2004; Mauro-Herrera and Doust 2016), panicle formation (Doust et al. 2005), and flowering time (Mauro-Herrera et al. 2013; Doust et al. 2014) have been identified in *Setaria* by QTL mapping analysis (see Chap. 12).

Marked changes have also been observed between Chinese traditional landraces and modern cultivars, especially in yield and morphological traits such as panicle weight, tiller number, hull color, and leaf angle. A total of 36 genomic regions corresponding to improvement of elite *Setaria* varieties have been characterized, but no genes have yet been identified (Jia et al. 2013a).

Genetic bottlenecks between wild relatives, landraces, and elite varieties have also been detected by microsatellite variation (Wang et al. 2012; Jia et al. 2013b, 2015). Studies using the same set of SSR markers identified an average of 33.5

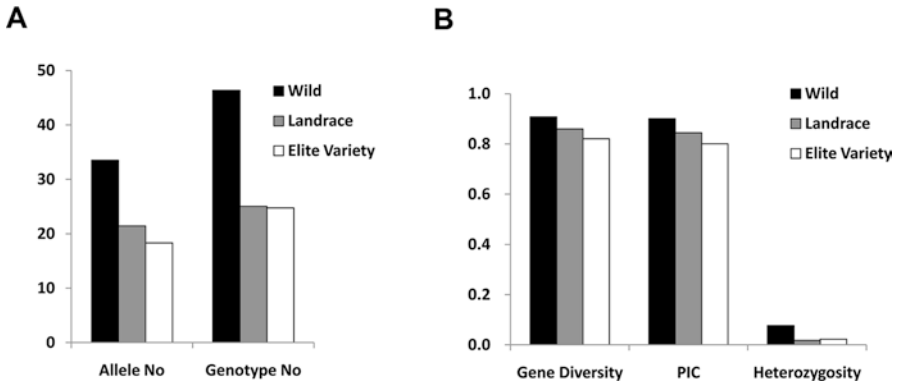


Fig. 2.3 Selective bottlenecks detected in *Setaria*. Allele and genotype no. (a) and gene diversity, PIC values, and heterozygosity (b) per locus detected in three panels of *Setaria* gene pools (modified from Jia et al. 2015)

alleles per locus in Chinese green foxtail, 20.9 alleles per locus in Chinese foxtail millet landraces, and 17.8 alleles per locus in Chinese elite foxtail millet varieties. This result is consistent with a loss of genetic diversity between landrace and elite gene pools as a result of two levels of genetic bottlenecks during the development of modern foxtail millet elite varieties in China. Selection for the domestication of green foxtail appears to be stronger than that for the improvement of cultivated foxtail millet (Fig. 2.3). Genomic regions contributing to the domestication process of *Setaria* have also received attention in recent years (Doust et al. 2014). For elite varieties, selective sweep regions of foxtail millet improvement are ~ 200 Kb in average, and selective pressures are modest (the largest π/π_m value is 6), comparable to what has been observed in maize (*Zea mays*) (Jia et al. 2013a).

2.4 QTLs Revealed by GWAS

Quantitative trait loci (QTLs) are genomic regions containing or linked to genes controlling or involved in a quantitative trait. Mapping of QTLs is an important first step for functional gene isolation, metabolic pathway construction, and marker-assisted selection (MAS), as well as pyramiding of target phenotypes in breeding programs. In foxtail millet, recombinant segregating population-based linkage analysis (Doust et al. 2004, 2005, 2014; Mauro-Herrera et al. 2013; Qie et al. 2014; Mauro-Herrera and Doust 2016) and natural accession-based association analysis (Jia et al. 2013a, 2015; Gupta et al. 2014) have been used to localize QTLs into specific genomic regions that are involved with phenotypes. Over 900 QTLs (Table 2.2) controlling 47 agronomical important characters have been identified under diverse environmental conditions by GWAS, using over a million SNPs and microsatellite markers (Jia et al. 2013a, 2015; Gupta et al. 2014).

Table 2.2 Selected results of GWAS for QTL detection in *Setaria*

Trait	Chr.	Marker name/physical position	Location of trials	Reference
Anther color	6	34,378,428	Chaoyang, Beijing, Changzhi and Anyang of China	Jia et al. (2013a)
Branch number per main stem	2	2,465,005	Chaoyang, China	Jia et al. (2013a)
	5	10,414,336	Beijing, China	Jia et al. (2013a)
	9	44,633,850	Anyang, China	Jia et al. (2013a)
	1	27,146,578	Changzhi, China	Jia et al. (2013a)
	5	31,851,805	Sanya, China	Jia et al. (2013a)
Tiller number	8	35,517,380	Chaoyang, China	Jia et al. (2013a)
	2	2,364,917	Beijing, China	Jia et al. (2013a)
	2	2,374,147	Anyang, China	Jia et al. (2013a)
	9	56,850,515	Changzhi, China	Jia et al. (2013a)
1000-grain weight	2	2,411,360	Changzhi, China	Jia et al. (2013a)
	1	37,243,809	Anyang, China	Jia et al. (2013a)
	1	b260/37,750,853-37,751,008	New Delhi, India	Gupta et al. (2014)
	5	P75/-	New Delhi, India	Gupta et al. (2014)
	5	b129/7,959,308-7,959,430	New Delhi, India	Gupta et al. (2014)
Bristle length	1	37,748,213	Beijing, Anyang, Changzhi of China	Jia et al. (2013a)
	1	37,751,602	Sanya, China	Jia et al. (2013a)
Heading date	2	49,164,136	Beijing and Anyang of China	Jia et al. (2013a)
	1	37,215,226	Anyang, China	Jia et al. (2013a)
	8	28,507,351	Changzhi, China	Jia et al. (2013a)
	2	2,711,177	Sanya, China	Jia et al. (2013a)
Leaf length	9	P44/-	Beijing, Changzhi and Anyang of China	Jia et al. (2015)
	2	49,119,122	Beijing, China	Jia et al. (2013a)
	2	49,117,404	Anyang, China	Jia et al. (2013a)

(continued)

Table 2.2 (continued)

Trait	Chr.	Marker name/physical position	Location of trials	Reference	
Panicle length	2	22,845,341	Beijing, China	Jia et al. (2013a)	
	1	37,343,439	Anyang, China	Jia et al. (2013a)	
	4	b109/1,290,206~1,290,107	Beijing, Changzhi and Anyang of China	Jia et al. (2015)	
	1	b182/32,831,786~32,831,986	Beijing, Changzhi and Anyang of China	Jia et al. (2015)	
	9	b217/—	Beijing, Changzhi and Anyang of China	Jia et al. (2015)	
	4	b236/37,687,774~37,687,917	Beijing, Changzhi and Anyang of China	Jia et al. (2015)	
	4	b247/—	Beijing, Changzhi and Anyang of China	Jia et al. (2015)	
	4	b266/40,054,016~40,053,821	Beijing, Changzhi and Anyang of China	Jia et al. (2015)	
	9	b269/—	Beijing, Changzhi and Anyang of China	Jia et al. (2015)	
	1	P8/37,804,404~37,804,237	Beijing, Changzhi and Anyang of China	Jia et al. (2015)	
Bristle color	5	b129/7,959,308~7,959,430	Beijing, Changzhi and Anyang of China	Jia et al. (2015)	
	4	7,400,384	New Delhi, India	Gupta et al. (2014)	
	4	7,390,482	Chaoyang, China	Jia et al. (2013a)	
	4	7,390,404	Beijing, China	Jia et al. (2013a)	
	4	7,215,041	Anyang, China	Jia et al. (2013a)	
	4	7,284,766	Changzhi, China	Jia et al. (2013a)	
	4	7,284,766	Sanya, China	Jia et al. (2013a)	
	7	26,883,452	Chaoyang, China	Jia et al. (2013a)	
	7	26,908,254	Beijing, China	Jia et al. (2013a)	
	4	7,322,156	Beijing, China	Jia et al. (2013a)	
Leaf sheath color	7	26,859,601	Anyang, China	Jia et al. (2013a)	
	4	7,349,812	Anyang, China	Jia et al. (2013a)	
	7	26,910,276	Anyang, China	Jia et al. (2013a)	
	4	7,390,404	Changzhi, China	Jia et al. (2013a)	
	7	26,908,030	Changzhi, China	Jia et al. (2013a)	
	7	26,908,030	Sanya, China	Jia et al. (2013a)	
	4	7,322,156	Sanya, China	Jia et al. (2013a)	

(continued)

Table 2.2 (continued)

Trait	Chr.	Marker name/physical position	Location of trials	Reference	
Hull color	1	5,480,917	Chaoyang, China	Jia et al. (2013a)	
	9	54,490,294	Chaoyang, China	Jia et al. (2013a)	
	1	5,467,847	Anyang, China	Jia et al. (2013a)	
	9	54,533,849	Anyang, China	Jia et al. (2013a)	
	1	5,476,983	Changzhi, China	Jia et al. (2013a)	
	9	54,490,656	Changzhi, China	Jia et al. (2013a)	
	Height of main stem (plant height)	5	MPGA31/27,433,840~27,433,863	Beijing, Changzhi and Anyang of China	Jia et al. (2015)
		9	X4/98,538~98,850	Beijing, Changzhi and Anyang of China	Jia et al. (2015)
		4	P2/1,221,537~1,221,682	Beijing, Changzhi and Anyang of China	Jia et al. (2015)
Pedicule length	3	b225/11,756,544~11,756,666	New Delhi, India	Gupta et al. (2014)	
	5	b129/7,959,308~7,959,430	New Delhi, India	Gupta et al. (2014)	
	5	P75/—	New Delhi, India	Gupta et al. (2014)	
	7	b123/—	Beijing, Changzhi and Anyang of China	Jia et al. (2015)	
	3	P98/15,515,665~15,515,807	Beijing, Changzhi and Anyang of China	Jia et al. (2015)	
Stem diameters	7	P29/—	Beijing, Changzhi and Anyang of China	Jia et al. (2015)	
	2	49,164,136	Beijing, China	Jia et al. (2013a)	
Early blooming date	1	37,210,883	Anyang, China	Jia et al. (2013a)	
	6	SIGMS9645/—	New Delhi, India	Gupta et al. (2014)	
	5	b129/7,959,308~7,959,430	New Delhi, India	Gupta et al. (2014)	
Flag leaf width	7	P59/—	New Delhi, India	Gupta et al. (2014)	
	8	b185/—	New Delhi, India	Gupta et al. (2014)	
Grain yield	5	P75/—	New Delhi, India	Gupta et al. (2014)	
	5	b129/7,959,308~7,959,430	New Delhi, India	Gupta et al. (2014)	
	5	b129/7,959,308~7,959,430	New Delhi, India	Gupta et al. (2014)	
Grain shape	3	P61/—	New Delhi, India	Gupta et al. (2014)	
	3	b225/11,756,544~11,756,666	New Delhi, India	Gupta et al. (2014)	

Physical positions of QTLs loci were acquired according to reference genome sequence of Yugu1 (*Setaria italica* version 2.1) from website: <http://phytozome.jgi.doe.gov/pz/portal.html>

Results of QTL mapping suggest that several coloration-related traits are controlled by Mendelian genes, but that other quantitative traits including yield components, disease resistance, growth time, and morphological development are controlled by a number of genomic regions with diverse minor effects. Association mapping analysis also suggests that most marker and phenotype associations were environment-specific and only acted on a single trait (Jia et al. 2015). Venn plot analysis of QTLs identified under different environmental conditions (Jia et al. 2013a) implied that a majority of morphological characters were closely related to locally adapted responses in *Setaria* to various climatic and ecological environments, so that *Setaria* can potentially be used as the complementary model to *Arabidopsis* for future ecological genomics studies.

2.5 Perspectives

Domestication and breeding processes have profoundly influenced the genetic diversity and population structure present in *Setaria*. Understanding the genetic basis of domestication and phenotypic variation in *Setaria* can help us efficiently utilize these genetic resources for variety improvement and relevant biological studies including C_4 photosynthesis, stress tolerance, plant architecture, and panicle development.

Morphological and genetic diversity detected in *Setaria* germplasm suggests that the sequence diversity (π) of cultivated *Setaria* accessions is approximately ~ 0.0010 , on a similar scale to that found in rice. A high level of diversity and the ability of *Setaria* to adapt to extreme local environments will make *Setaria* an attractive model for agronomic studies of crop species like maize (*Zea mays*), wheat (*Triticum aestivum*), barley (*Hordeum vulgare*), sorghum (*Sorghum bicolor*), switchgrass (*Panicum virgatum*), and oat (*Avena sativa*).

Genome variations in *Setaria* can be characterized by genotyping each individual accession. Recently, many kinds of molecular markers have been developed in *Setaria* (Jia et al. 2009; Yadav et al. 2015; B et al. 2013; Zhang et al. 2014; Pandey et al. 2013; Muthamilarasan et al. 2014; Gupta et al. 2012; Wang et al. 1998; Gale and Devos 1998). New generations of genotyping technologies (Sequencing-based genotyping, genotyping by sequencing, RNA-seq-based genotyping, Exon-sequencing-based genotyping, etc.) have also been developed in recent years (Huang and Han 2014), including a low coverage whole-genome re-sequencing method for analyzing the origin and domestication of rice (Huang et al. 2012) and construction of haplotype maps in rice (Huang et al. 2010) and *Setaria* (Jia et al. 2013a). We believe that these genomic approaches will continue to reveal variability in the genetic architecture of valuable traits in *Setaria* in the future. Likewise, with continued significant decreases in sequencing costs, we can predict that more and more such sequencing data sets will be available to facilitate GWAS and domestication studies in *Setaria*.

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