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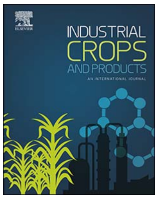

SSR markers associated with fiber yield traits in ramie (*Boehmeria nivea* L. Gaudich)

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ABSTRACT

Marker-assisted selection (MAS) can effectively promote fiber yield improvement for ramie. However, so far, the lack of molecular markers associated with fiber yield traits impede the application of MAS in ramie breeding. The objectives of this study were to identify simple sequence repeat (SSR) loci associated with fiber yield traits in ramie and to infer population structure and evolutionary genetic relationships. Genetic diversity, population structure, and association mapping analyses were conducted using 107 ramie accessions and 95 pairs of SSR markers. The results indicate that the average number of alleles per locus was 2.64 and that Shannon diversity index values ranged from 0.3797 to 1.4298. The 107 accessions were divided into two subpopulations with extensive genetic variation, indicating that the natural population was representative and could be used for further association analysis of quantitative traits. Association analysis detected a total of 16 stable molecular markers related to yield traits in ramie, including six for plant height, two for ramet number, five for stem diameter, three for skin thickness, and three for fiber percentage. Of these, the SSR marker RAM290 was associated with stem diameter in three environments and can explain 8.32%–23.42% of the phenotypic variation, indicating its potential utility in molecular breeding. These findings will be useful in the genetic improvement of ramie yield traits through marker-assisted selection.

1. Introduction

Ramie (*Boehmeria nivea* L. Gaudich) or "China grass," which originated in China, is a perennial root herb that is cultivated as an important natural textile crop (Luan et al., 2015a,b). At present, ramie is China's second largest fiber crop (Xiong, 2008). However, although there have been marked improvements in ramie fiber production, improvement in fiber yield is still an important goal for ramie breeding. Crop yield traits, often being quantitative characters, represent complex genetic mechanisms in plant growth and development, including environmental interactions (Lu et al., 2016). Thus, yield traits are typically controlled by many genes. Ramie fiber yield component traits include plant height, stem diameter, skin thickness, fiber percentage, and ramet number (Liu et al., 2012). Currently, there is a consensus that the set traits are quantitative and controlled by many genes with small phenotypic effects (Xiong et al., 1998). Thus, the genetic improvement of these traits by traditional breeding technology is limited.

In modern breeding, a plant genetic diversity platform combined with advanced genome technology is needed to discover new genes or alleles for targeted traits (Jin and Bao, 2009). Using molecular markers linked to the quantitative trait of interest, marker-assisted selection can enhance the genetic improvement process (Wang et al., 2007). In recent years, exploring quantitative trait loci (QTLs) (and even nucleotides, QTNs) through association analysis has been one of the most active research topics in plant quantitative genetics (Zhao et al., 2014; Giraud et al., 2014; Awais et al., 2012). Association analysis, also known as linkage disequilibrium (LD) mapping or association mapping, is based on LD and uses polymorphism analyses of genes and loci of interest to identify marker loci closely related to phenotypic variation (Myles et al., 2009). Association analysis offers many advantages compared to traditional linkage analysis, including the use of natural populations as experimental material, detection of multiple alleles on the same locus, and targeting of single genes or nucleotides (Flint-Garcia et al., 2005). At present, only a few molecular markers for traits related to ramie fiber production have been reported through linkage analysis (Liu et al., 2014), which has limited the use of molecular markers in ramie fiber yield improvement.

In this study, 107 core ramie accessions from four countries, China, Brazil, India, and Indonesia, were used as a natural population panel, and were genotyped at 95 simple sequence repeat (SSR) molecular

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Table 1
Ramie germplasms.

code	name	variety types	Geographical origin	code	name	variety types	Geographical origin
1	Xijieba	landraces	Hunan	515	Nanchengbopizhuma	landraces	Jiangxi
2	Chishanxijieba	landraces	Hunan	525	Xiaoyelugan	landraces	Jiangxi
6	Yuanjiangdayebai	landraces	Hunan	527	Yedouzi	landraces	Jiangxi
8	Hanshougangma	landraces	Hunan	534	Xiaogubai	landraces	Jiangxi
10	Yizhangyuanma	landraces	Hunan	548	Gaoanma	landraces	Jiangxi
12	Zuodouma1	landraces	Hunan	560	Yangsujigubai	landraces	Guangxi
14	Hongjiaoma	landraces	Hunan	573	Heipima	landraces	Guangxi
15	Niutima	landraces	Hunan	579	Gebuqingma	landraces	Guangxi
33	Gegengma	landraces	Hunan	590	Hexianjiama	landraces	Guangxi
40	Xinningqingma	landraces	Hunan	607	Tongmuqingma	landraces	Guangxi
55	Xiangzhu1hao	varieties	Hunan	684	Qingyezhuma	landraces	Hainan
59	Qingmazhu	landraces	Hainan	757	Yangxinxiyelv	landraces	Hubei
60	Huangmazhu	landraces	Hainan	775	Wuchangshanpozhuma1hao	landraces	Hubei
61	Heipidou	landraces	Guangxi	797	Xianningdayelv	landraces	Hunan
71	Qianzhu1hao	varieties	Guizhou	822	Honggujing	landraces	Hubei
79	Chuanzhu2hao	varieties	Sichuan	847	Shanyangzhuma	landraces	Shanxi
80	Rongchangzhuma	landraces	Chongqing	860	Qingpidama	landraces	Sichuan
81	Xunyanglvbaima	landraces	Shanxi	913	Nanchongzhuma	landraces	Sichuan
82	Hanzhongzhuma	landraces	Shanxi	957	Xiaoqinggan	landraces	Sichuan
83	Sima	landraces	Shanxi	966	Pingchangjiama	landraces	Sichuan
86	Yinduzhuma	landraces	India	991	Hejiangqingma	landraces	Sichuan
102	Kuguaqing	landraces	Hunan	1025	79 – 20	varieties	
111	Leiyanghuangkema	landraces	Hunan	1065	Wuchuanbaima	landraces	Guizhou
119	Changshaqingyema	landraces	Hunan	1180	Shengbaxianma	landraces	Hubei
120	Youqima	landraces	Hunan	1210	Huangjindou	landraces	Hubei
124	Xiyebai	landraces	Hunan	1214	Enshiqingma2hao	landraces	Hubei
126	Huangjiuma	landraces	Hunan	1225	Xiningxianma	landraces	Chongqing
185	Jiangkouqingpizhuma	landraces	Guizhou	1226	Manyuanchuan1hao	landraces	Hubei
188	Ziyangdayepao	landraces	Shanxi	1258	S-1–6	varieties	Hunan
192	Yichuntongpiqing	landraces	Jiangxi	1276	Suiningqingma	landraces	Hunan
195	Tiantaitiema	landraces	Zhejiang	1345	Xinpuqingma	landraces	Guizhou
208	Quxianzhuma	landraces	Zhejiang	1352	Simaohongzhuma	landraces	Yunnan
248	Guangpima	landraces	Jiangxi	1360	Anlongzhuma2hao	landraces	Sichuan
267	Lidazhuma	landraces	Yunnan	1363	Guangdonghuangpidou2hao	landraces	Guizhou
275	Menglazhuma	landraces	Yunnan	1372	Limuqingma	landraces	Guizhou
296	Ribenzhuma5hao	landraces	Brazil	1377	Dayujiandaobai	landraces	Hubei
337	Rongjiangbbaima1hao	landraces	Guizhou	1406	Yinni1hao	landraces	Indonesia
340	Jinpingqingma	landraces	Guizhou	1407	Yinni2hao	landraces	Indonesia
414	Huangpinghuangganma	landraces	Guizhou	1408	Yinni3hao	landraces	Indonesia
429	Zunyichuangenma	landraces	Guizhou	1421	Ximatuma	landraces	Guangxi
440	Xinminqingma	landraces	Guizhou	1431	Dingyezhuma	landraces	Guangxi
449	Qingpigan	landraces	Jiangxi	1439	Tianpaishanyema	landraces	Guangxi
450	Fenyihuangzhuangdou	landraces	Jiangxi	1441	Fulisima	landraces	Sichuan
454	Lvzhubai	landraces	Jiangxi	1443	Xieliqingma	landraces	Sichuan
456	Baiyema	landraces	Jiangxi	1454	Gaotiqingma	landraces	Sichuan
461	Dayehongzhameng	landraces	Jiangxi	1455	Sichuangaotibaima	landraces	Sichuan
466	Lichuanhoupizhuma	landraces	Jiangxi	1464	Miaobazhuma	landraces	Sichuan
468	Zixima	landraces	Jiangxi	1474	Shanqingbaima	landraces	Chongqing
471	Nanchenghoupizhuma	landraces	Jiangxi	1479	Shuiqingqingma	landraces	Chongqing
484	Tianbaoma	landraces	Jiangxi	1484	Yihancongma	landraces	Sichuan
485	Yihuangjiama	landraces	Jiangxi	ZHS	Zhongsuzhu1hao	varieties	Hunan
489	Yushanma	landraces	Jiangxi	ZZ-1	Zhongzhu1hao	varieties	Hunan
502	Huangqingdou	landraces	Jiangxi	ZZ-2	Zhongzhu2hao	varieties	Hunan
509	Ningduyema	landraces	Jiangxi				

marker loci. In addition, plant height, stem diameter, ramet number in three environments, and skin thickness and fiber percentage in two environments were evaluated in the 107 core collection accessions. The objectives of our study were (1) to analyze the genetic population structure of this core collection of ramie and (2) to detect marker loci associated with fiber production traits.

2. Materials and methods

2.1. Plant materials

One hundred and seven ramie accessions from a core germplasm collection (Table 1) were used to construct a mapping panel. Of these 107 accessions, 99 are landraces and eight are domesticated cultivars (cultivated varieties). The accessions were originally collected from four countries, namely, China (94 landrace accessions), Brazil (1), India (1), and Indonesia (3). In China, the landraces were collected from 11 provinces, notably Jiangxi, Hunan, Sichuan, and Guizhou.

2.2. Field experiments and phenotypic data collection

The 107 core accessions were planted in the Wangcheng Experimental Station of the Institute of Bast Fiber Crops, Chinese Academy of Agricultural Sciences, Changsha, Hunan Province. The experiment was conducted using a randomized block design with two replicates. Each plot had single rows of 3 m long × 1 m apart. Five individuals were planted in each plot, and standard cultural practices were performed following the methods described by Liu et al. (2012) for three successive seasons in 2015: from March to June (environment 1), June to August (environment 2), and August to October