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Full Length Article

SSR-Based Diversity of Domesticated and Local Cotton (*Gossypium* Spp.) Populations Collected in Indonesia

Jakty Kusuma[1*], Muhammad Zahir Ahsan[2], Wawan Setiawan[3], Khalid Abdullah[4] and Muhammad Tahir[1]
[1]*Politeknik Negeri Lampung, Indonesia*
[2]*Cotton Research Station, Sahiwal, Pakistan*
[3]*University of Lampung, Indonesia*
[4]*Pakistan Central Cotton Committee, Pakistan*
[*]For correspondance: kusuma@polinela.ac.id

Abstract

This research was aimed to understand the genetic diversity of agronomic traits among cotton germplasms based on agronomic and molecular markers. Parental selection of potentially high yielding and oilseed content was revealed by Simple Sequence Repeat (SSR). SSR markers that are linked to high yield and high oil seed content were used against 22 accessions of cotton germplasm as prospective parental line (s) to produce cotton varieties that has a dual purpose character. To determine the cotton diversity, PCR and clustering analysis was performed with six primers that are flanked with agronomic traits. Dendrogram exhibited consistency between genetic and phenotypic trees indicating that broad genetic diversity appears to exist among tested accessions. This report provides information on elaborating relationship on three different species of cotton, from phenotypical and molecular perspectives. © 2018 Friends Science Publishers

Keywords: SSR; Cotton; Germplasm; Dendrogram analysis

Introduction

Cotton is a fiber crop that has a major role to meet the needs of human clothes, which is grown by more than 70 countries. In Indonesia, production of cotton is 1,871 tons and has a productivity of 288 kg ha^{-1} (Ministry of Agriculture, 2014), as a comparison productivity of cotton in China, Australia and Pakistan as much as 1.380, 2,151 and 1.689 kg ha^{-1}, respectively (Johnson *et al.*, 2014). These results can only fulfill the national fiber stock as much as 0.5% (Balai, 2014), and the rest >99% were imported from other cotton producing countries. This situation is more aggravated by decreasing of cotton acreage planting from 10.194 ha in 2010 to 8.738 ha in 2013. The fall off in cotton acreage crops caused by farmers replace it with other commodities with more economically advantageous.

As next to its fiber producing capability, cotton seed is the second major product (Hamilton *et al.*, 2004; Ashokkumar and Ravikesan, 2008). Cotton seed can be used as a substitute for palm oil or another crop-based ethanol to produce a renewable energy source. Cotton seed oil can be widely accepted by the processed food industry since its stable and its production costs are low (Dowd *et al.*, 2010). Nabi *et al.* (2009) reported that cottonseed oil can reduce carbon footprint, this suggests that cotton seed oil can compete with other sources of bioethanol. It is indispensable to suppress the dependence on cotton imports and development of renewable source of energy is inevitable. By integrating these two main uses of cotton, farmers would have a benefit and provide added value in the agribusiness cotton. Dual purpose crop can contribute of added value in the agribusiness of selected crop. The development of dual purpose crop has been recently reported by Bell *et al.* (2015) and Kingwell and Squibb (2015).

Genetic diversity can provide convenience for cotton breeders to select parental lines for carrying out the selection. The selection process by using molecular markers proved that producing the desired varieties are faster than the conventional way. Selection of the appropriate type of molecular markers can detect genetic diversity effectively, taking into account the level of polymorphism and high stability (Tu *et al.*, 2014). Molecular markers also offer efficient tools for dissecting QTL (*Quantitative Trait Loci*) affecting traits with complex genetic inheritance, and facilitate *Marker Assisted Selection* (MAS) (Park *et al.*, 2005).

Recent publications related to genetic diversity of cotton had been done by Saeed *et al.* (2014), Tyagi *et al.* (2014), Moiana *et al.* (2015), using SSR markers, which reported there were high level of polymorphism. Development of SSR markers and genetic maps have been reported to mark the traits which are flanked to yield and oilseed-related content traits (Nguyen *et al.*, 2004;

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He *et al.*, 2005; Lin *et al.*, 2005; Shen *et al.*, 2007; Zeng *et al.*, 2009; Zhang *et al.*, 2013; Shi *et al.*, 2015). Those marker development reports could serve as powerful selection tools for enhancing selection efficiency and curtailing time and resources involved in traditional selection methods (Xu and Crouch, 2008).

Related topics on cotton genetic diversity in Indonesia remains infrequent, however some genotypes which are available in Indonesian Sweetener and Fiber Crop Institute (Balittas) reported has a broad genetic diversity (Nuraida, 2012). Sulistyowati (2011) reviewed that the direction of the development of cotton varieties were conducted by the Balittas is divided into several sections, drought tolerant and cotton with high photosynthetic efficiency. The development of cotton with *dual-purpose* characters in Indonesia alone has been yet conducted. Based on these reasons above, we aimed to develop cotton that has high productivity and utilization of cottonseed as a source of ethanol approached by molecular markers. The objectives are to determine the genetic diversity of agronomic characters in 22 accessions of cotton germplasm and selecting parental line (s) of cotton fiber has the character of high productivity and has a high oil yield, which will be used as a source of donor genes for breeding of dual purpose cotton.

This research is expected to contribute to the development of dual-purpose cotton varieties, which could subsidize economically for agribusiness cotton. The important information about using the polymorphic molecular markers in this research is expected to help in switching from traditional breeding to marker assisted breeding of cotton.

Materials and Methods

Planting Materials

22 accessions derived from cotton-growing areas of Indonesia and introduced cultivars from Pakistan selected for genotype screening and evaluation of yield and oil seed-related traits. All accessions were introducted from different sources germplasm lines and parental lines with potential outstanding yield. Those accessions were kindly supplied by Indonesian Sweetener and Fiber Crop Institute and Pakistan Central Cotton Committee.

Genomic DNA Extraction

During vegetative stage, fresh young leaves from each accessions are sampled for DNA extraction following described protocols (Wu *et al.*, 2009). Isolated DNA of cotton leaf tissue previously ground with a Qiagen Mixer Mill MM 300 and using DNeasy Plant mini kit following the modified manufacture's protocol. An addition of sodium metabisulfite to the lysis buffer at concentration of 10 μM as descibed by Horne *et al.* (2004).

PCR Amplification and Electrophoresis

SSR primers that are associated with yield and oilseed-related traits were selected carefully and obtained from three different sources (Table 1): CIR, BNL, and NAU primers whose sequences are available at cottongen.org.

PCR will be performed under optimum condition. Amplification reaction with 30 μL total volume which contain 1x PCR buffer, 0.32 m*M* dNTPs, 0.8 m*M forward* and *reverse primer*, 1 Unit *Go Taq Green Polymerase* (Promega) and 20 ng DNA template (genomic). Amplification fragment using *thermocycler* with following profile: three minutes of one cycle 93°C denaturation; one minutes of 30 cycles at 94°C denaturation; 30 sec of annealing at 55–57°C; two min of elongation at 72°C; five minutes of final elongation at 72°C. Amplification products then separated in an 8% non-denaturing polyacrylamide gel at a constant voltage of 15 W for approximately 4 h at room temperature. After electrophoresis, all of the DNA fragments will be visualized by silver staining.

Genetic Diversity Analysis

The bands of DNA fragments scored as present (1) or absent (0) as described by Guang and Du (2006). Binary scores are applied to determine the genetic diversity of all cotton accessions to calculate PIC, heterozigosity and allele frequency. Clustering analysis will be performed using clustering software NTSYS ver. 2.1to calculate the genetic similarity and constructing dendrogram by unweight pair group method of arithmetic averages (UPGMA).

Phenotypic Data Analysis

The observed traits divided into two main criteria based on their agronomic characters yield and oilseed-related content which follow: (1) boll weight (g/boll), boll per plant, lint yield; (2) seed cotton yield (g), seed oil percent (%), seed weight (1000/g), seed per boll. The analysis of morphological diversity of 8 characters measured in 22 genotypes on each replication will be analyzed for estimation of standard error using variance analysis. The data on 7 agronomic traits from 22 genotypes are subjected to multivariate cluster analysis using cluster analysis software.

Result

SSR Allelic Diversity

Based on the SSRs analysis on 22 cotton accessions, we generated one to three alleles on each locus. From 6 SSR markers amplified 20 allele, with average 3,3 alleles per microsatellite locus (Table 2).

In particular, NAU 0816 and BNL 3259 amplified two alleles, and BNL 3345 with CIR0005 generate 4 alleles (Table 2). Highest alleles number were shown by BNL3871.

Table 1: List of primer sequences used

No	Primer	Chr	Type	Trait Name	References
1	CIR0005	10	SSR	Boll size	Nguyen *et al.*, 2004
2	BNL3445	18	SSR	Boll weight	Zeng *et al.*, 2009
3	NAU0816	24	SSR	Boll per plant	Zhang *et al.*, 2013; Shen *et al.*, 2007
4	BNL1231	25	SSR	Lint yield	Lin *et al.*, 2005; He *et al.*, 2005
5	BNL3871	7	SSR	Seed cotton yield	Lin *et al.*, 2005; He *et al.*, 2005
6	BNL3259	3	SSR	Seed per boll	Lin *et al.*, 2005; He *et al.*, 2005

Table 2: Genetic Diversity's Index Estimated From 6 SSR Markers

Locus	A	Major Allele	Product size (bp)	HE	HO	PIC
CIR0005	4	0,63	160 bp - 200 bp	0,477	0,421	0,357087499
BNL3445	4	0,41	160 bp - 240 bp	0,665	0,5	0,571300821
NAU0816	2	1	112 bp - 128 bp	0	0	0
BNL1231	3	0,58	146 bp-164 bp	0,5	0,722	0,367959105
BNL3871	5	0,44	190 bp - 216 bp	0,668	0,529	0,575715988
BNL3259	2	1	122 bp - 130 bp	0	0	0
Mean	3,33					0,312

Allelic frequencies for all populations by locus registered various number, with BNL 3445 resulting moderate frequency (0,41). The highest allele frequency showed by BNL 1231 with value of 0,58.

Polymorphism Information Content (PIC) value were calculated to determine the informativeness of each locus (Table 2), with a mean 0,312. Results showed that PIC value ranged from 0,357 to 0,575, with lowest PIC are shown by marker CIR 0005 and BNL 1231 (0,375 and 0,367 respectively). On the other hand, highest PIC value are shown by marker BNL 3445 and BNL 3981 (0,571 and 0,575 respectively).

Genetic Structure, Distance and Diversity

The Principal Coordinate Analysis (PCoA) and UPGMA dendrogram clustering were performed to estimate genetic structure and diversity among 22 cotton accessions (Fig. 1). Binary and allelic SSR data were used for PcoA. All introduced germplasm are considered as an outgroup. PCoA based on the first two components (factors) in all cotton accessions using combined molecular data when the eigenvalues for the first and second axis were 47.37% and 41.23%, respectively. The pattern explained of all *G. arboreum* are separated from all accessions and attributed the highest eigenvalues.

Significant genetic diversity did occur to exist among tested accessions. Similarity distance is ranging from 0,40 to 0,90. Clustering analysis based on UPGMA method (Fig. 2) showed that the population divided into two main groups. Group I in tree graph contains two accessions from diploid cotton *G. arboreum* species (Marvi and FDH 834), showed constant similarity coefficient about 90%. The result showed consistency as observed in PCoA analysis, which confirming their status as different species. In group II, the accessions were clustered into two different

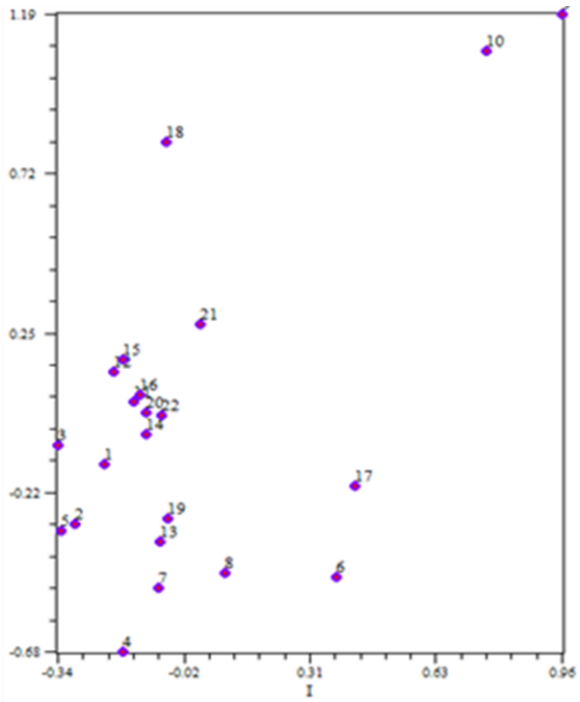

Fig. 1: Principal Coordinate Analysis of 22 accessions based on 6 SSR loci

sub groups and all accessions derived from tetraploid cotton *G. hirsutum* and *G. barbadense*. Sub group IIA consists of CRIS 667, Kanesia 19, CRIS 342, Kanesia 15, Kanesia 11, CRIS 669 and CRIS 670 accessions.

It is interesting to note that Indonesian cotton were considered has similarities with Pakistan cotton. Identical things were also showed on sub group IIB, where consists of CRIS 664, CRIS 665, CRIS 666, Kanesia 18, SA 2476, SA 2465, CRIS 668, Kanesia 12, Kanesia 14, Kanesia 13, Kanesia 16, Kanesia 19 and Kanesia 20 accessions. All *G. barbadense* accessions were belong to same group with *G. hirsutum* since they share the same AD genome group. Different information shown by diploid cotton *G. arboreum* at group I, that caused separation from other accessions.

Furthermore, *G. barbadense* species are included in the same subgroup with *G. hirsutum* because molecular markers were flanked with agronomic and other quantitative traits, where there is a slight significant differences between *G. barbadense* and *G. hirsutum* regarding to agronomic traits.

Agronomic Evaluation

To explore agronomic differentiation and variation, we performed variance analysis (Table 3) and dendrogram clustering analysis (Fig. 3).

All tested accessions were performing moderate variation based on their genotypic and phenotypic performance.

Table 3: Genotypic and Phenotypic Variance of Yield and Oil-related Traits of 22 Cotton Accessions

Traits	V_G	V_P	Results	Range
Boll Weight (g)	0,640	0,839	Narrow	1,74 – 5,82
Boll per Plant	44,234	55,586	Broad	23,2 – 52,10
Yield (kgha^{-1})	251872,076	412675,097	Broad	707,13 – 2849, 24
Seed Oil Content (%)	11,057	12,275	Broad	14,45 – 28,48
Seed Index (%)	0,583	0,774	Narrow	5,69 – 8,68
Seed per Boll	23,642	44,032	Broad	23,5 - 43
Seed Yield (kgha^{-1})	90673,947	128563,035	Broad	424,08 – 1709,55

V_G = Genetic variance; V_P = Phenotypic variance

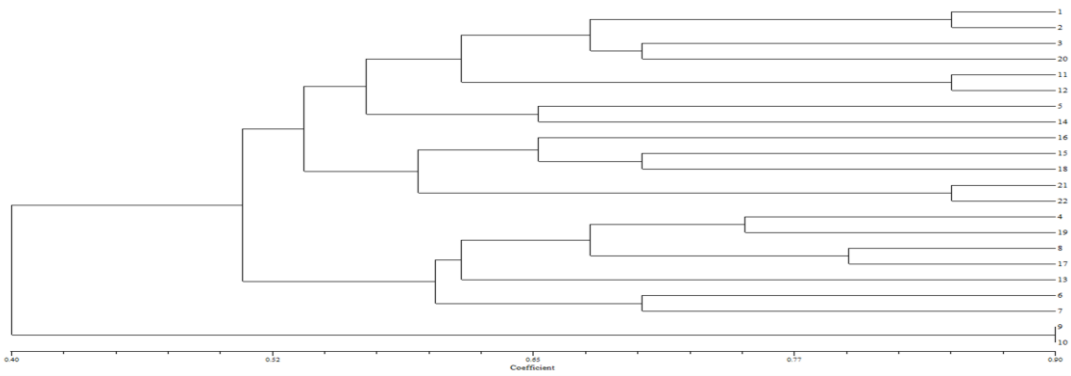

Fig. 2: Distribution of 22 cotton accessions based on UPGMA clustering.1 = CRIS 664; 2 = CRIS 665; 3 = CRIS 666; 4 = CRIS 667; 5 = CRIS 668; 6 = CRIS 669; 7 = CRIS 670; 8 = CRIS 342; 9 = Marvi; 10 = FDH 834; 11 = SA 2467; 12 = SA 2465; 13 = Kanesia 11; 14 = Kanesia 12; 15 = Kanesia 13; 16 = Kanesia 14; 17 = Kanesia 15; 18 = Kanesia 16; 19 = Kanesia 17; 20 = Kanesia 18; 21 = Kanesia 19; 22 = Kanesia 20

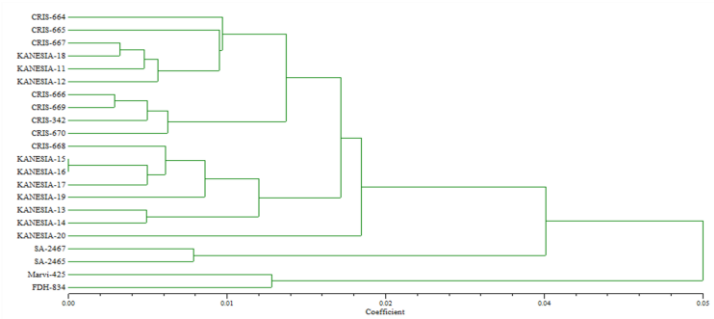

Fig. 3: Dendrogram cluster of 22 cotton accession based on agronomic traits

Clustering groups on dendrogram analysis were shown a consistency compare to genetic diversity as revealed by SSR markers. Cotton accessions were divided into 2 large group, separates accessions based on species. Group I consist 2 accessions from *G. arboreum* species, Marvi and FDH 834. Group II branched onto sub group IIA which consist all accessions from *G. barbadense* species. All *G. hirsutum* species were numbered on sub group IIB. This result demonstrated that phenotypical performance can also differentiate cotton species. According to agronomic performance by variance analysis, only boll weight and seed index resulted a narrow diversity, showing that these traits were controlled by low numer of gene and does not involve an environmental effect. Similar report (Ahsan *et al.*, 2015) resulting narrow genetic variability of boll weight and seed index traits.

Based on examination from all accessions, CRIS 664 appears to establish greater performance than any other accessions. Both yield and seed oil content are surpassing all accessions. Phenotypic appearance of CRIS 664 may be referenced as dual purpose donor parent. Broad genetic diversity as we showed on dendrogram and scatterplot, provide benefit for cotton breeder to enhance yield quality and oilseed quantity.

Discussion

This result is higher if compare to Bertini *et al*. (2006) who used 31 primer pairs to analyze diversity of 53 *Gossypium hirsutum*. Gutierrez *et al*. (2002) also reported an average 2 alleles per locus derived from 60 primer pairs. In this report, several locus produced three to fivee alleles which could be possible for tetraploid cotton, including *Gossypium hirsutum* and *Gossypium barbadense* species were used in this experiment. Liu *et al*. (2000) used 56 polymorphic primer pairs to amplify 62 cotton loci and produce a total of 325 alleles with average of 5 alleles per locus.

Lacape *et al*. (2007) explained that variation number of alleles on each marker depends on the marker itself and accession to be genotyped. Lower alleles (3.0) per locus in upland cotton were also reported by Tyagi *et al*. (2014).

The PIC values are indicating informative potential was moderated. Moiana *et al*. (2015) reported PIC range from 0,090 to 0,580 with an average number 0,361, which is close similar with this report. Based on this result, the most informative loci are BNL 3445 and BNL 3981, and moderate informativeness are shown by CIR 0005 and BNL 1231. The two BNL loci mentioned above can be used to examine genetic profile for cotton, to improve traits such as boll weight and seed yield in particular. Informativeness of those four loci could lead to marker assisted selection on yield and oil-related traits in next investigation.

Despite they share the same subgroup, there is considerable similarity coefficient with species *G. hirsutum*. Zhang *et al*. (2005) reported that using a low number of SSR markers does not provide whole genome information, however it can be used as a preliminary breeding to detect desirable traits on certain accessions.

Pakistan cotton breeding source approximated derived from USA on early 20th century (Iqbal *et al*., 2001; Bertini *et al*., 2006). Similar information were reported by Indonesian Research and Development Center of Estate Crops (2005), that most of the Indonesian cotton were introducted from USA during the period of 1975 until 2000. It is believed they share the same breeding source, and causing that Indonesian and Pakistan cotton are fused into a same sub group. There could be a possibility several traits of these accessions were co-segregated (Morton, 2005), which is the result of domestication process from several generations).

From the results of the present study, it can be determined that direct selection can be done for most of the yield attributing traits since it exhibited high genetic variability and high range of variation. A high phenotypic variance and genotypic variance for the characters studied indicated that environment influences on the expression of these traits were minor.

Conclusion

All cotton accessions are divided based on their sub genome, and perform moderate to high genetic diversity among species. These putative fiber traits-associated and oilseed SSR markers identified in the present study provides useful information for further investigations. We found the *G. barbadense* share similarity with *G. hirsutum*, this appears to occur since these two species were in a same sub genome. Four from six SSR markers resulted an average to moderately high polymorphism and can be used as selection marker related to yield and oil content of cotton. We propose that Kanesia as a local-adaptive accessions, and can be used as ovule source. CRIS 664 as a donor parent, and possibility of *G. barbadense* and *G. arboreum* as breeding source to enhace oilseed content in cotton plants.

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