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ТУРЛИ ГЕОГРАФИК ҲУДУДЛАРГА ТЕГИШЛИ GOSSYPIUM HIRSUTUM ВА G. BARBADENSE ҒЎЗА ЛИНИЯЛАРИДАГИ ГЕНЕТИК ХИЛМА-ХИЛЛИКНИ ҚИЁСИЙ БАҲОЛАШ

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Аннотация. *Селекционерлар, одатда, генотипларни ядровий геном билан тартибга солинувчи морфологик хусусиятларига кўра танлашади. Ваҳоланки, цитоплазматик геном, хусусан, митохондрия ва хлоропласт геномлари ҳам биологик функцияларда муҳим роль ўйнайди. Цитоплазматик ва ядровий геномнинг генетик ирсийланиш усули ва хусусиятлари ген оқими, эволюцияси, популяция тарихини ўрганиш учун қўшимча қимматли маълумотлар беради. Генетик тадқиқотлар учун цитоплазматик ДНК маркерларини ишлаб чиқиш, шунингдек, G. hirsutum ва G. barbadense линиялари мажмуасидаги цитоплазматик ва ядровий геном ўзгаришларини аниқлаш учун ДНК маркерларини қўллаш мақсад қилиб олинди. Тола белгиларига алоқадор бўлган 61 та ядровий SSR ва 49 та цитоплазматик геномга хос бўлган индел ва SSR праймер жуфтлари 20 та G. hirsutum ва 74 та G. barbadense линиялари учун ишлатилди. Цитоплазматик индел маркерларнинг цитоплазматик SSR маркерларга нисбатан полиморфик эканлиги кузатилди. Умумий натижалар сараланган 94 та G. hirsutum ва G. barbadense линияларининг иккита катта гуруҳга бўлинишини кўрсатди. Бу эса биринчи марта ғўза цитоплазматик геномини ўрганиш учун генетик восита ҳамда G. hirsutum ва G. barbadense линияларининг цитоплазматик ва ядровий геномларида генетик хилма-хилликни таққослайдиган ҳисоботни тақдим этди. Тадқиқот натижалари селекционерларга ғўза навларини яхшилаш учун генетик хилма-хилликнинг таъсирини максимал даражада оширишга қаратилган навдорлик стратегиясини ишлаб чиқишда ёрдам беради.*

Таянч тушунчалар: *хилма-хиллик, Gossypium, цитоплазма, ядровий, SSR, дендограмма, шажара дарахти, G. hirsutum, G. barbadense.*

СРАВНИТЕЛЬНАЯ ОЦЕНКА ГЕНЕТИЧЕСКОГО РАЗНООБРАЗИЯ ЦИТОПЛАЗМАТИЧЕСКОГО И ЯДЕРНОГО ГЕНОМА В УЛУЧШЕННЫХ ЛИНИЯХ ХЛОПЧАТНИКА GOSSYPIUM HIRSUTUM И G. BARBADENSE ИЗ РАЗНООБРАЗНОГО ГЕОГРАФИЧЕСКОГО ПОЛОЖЕНИЯ

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Аннотация. *Селекционеры обычно выбирают генотипы на основе морфологических признаков, в первую очередь регулируемых ядерным геномом. Однако цитоплазматический геном, включая митохондрии и хлоропластные геномы, также играет важную роль при выполнении многих биологических функций в селекции хлопчатника. Характеристики и способ генетического наследования цитоплазмы и ядерного генома дают дополнительную ценную информацию для изучения потока генов, эволюции и истории популяции. Авторы стремились разработать цитоплазматические ДНК-маркеры в качестве инструмента генетических исследований и использовать ДНК-маркеры в качестве инструмента для выявления генетических изменений в цитоплазматическом и ядерном геноме в наборе линий G. hirsutum и G. barbadense. В исследовании использованы 61 пара праймеров SSR, связанная с важными специфичными для волокна признаками ядерного генома, и 49 пар праймеров Indel и SSR, специфичных для цитоплазматического генома, для скрининга 20 линий G. hirsutum и 74 G. barbadense из различных географических мест. Согласно наблюдениям, среди линий цитоплазматические маркеры Indel более полиморфны по сравнению с цитоплазматическими маркерами SSR. Общие результаты показали, что отобранные 94 линии в целом можно разделить на две широкие группы G. hirsutum и G. barbadense. Данное исследование впервые предоставило инструмент для генетического изучения цитоплазматического генома хлопчатника и отчет о сравнении генетического разнообразия в цитоплазматическом и ядерном геномах линий G. barbadense и G. hirsutum. Результаты исследования помогут селекционерам разработать селекционную стратегию, максимально увеличивающую эффект генетического разнообразия для улучшения линии хлопчатника.*

Ключевые слова: *разнообразие, Gossypium, цитоплазма, ядерный, SSR, дендрограмма, филогенетическое дерево.*

COMPARATIVE ASSESSMENT OF GENETIC DIVERSITY IN CYTOPLASMIC AND NUCLEAR GENOME IN IMPROVED GOSSYPIUM HIRSUTUM AND G. BARBADENSE COTTON LINES FROM DIVERSE GEOGRAPHICAL LOCATION

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Annotation. *Breeders normally select genotypes based on morphological characters, primarily regulated by nuclear genome. However, cytoplasmic genome, including mitochondria and chloroplast genomes, also play an important role to perform a number of biological functions in cotton breeding. The characteristics and the mode of genetic inheritance of cytoplasm and nuclear genome provide complementary valuable information to study gene flow, evolution, and population history. The study is aimed to develop cytoplasmic DNA markers as a tool for genetic research and to use the DNA markers as a tool to detect genetic variation in the cytoplasmic and nuclear genome in a set of G. hirsutum and G. barbadense lines. The study involved 61 SSR primer pairs associated with important fiber specific traits of the nuclear genome and 49 indel and SSR primers specific to the cytoplasmic genome to screen 20 G. hirsutum and 74 G. barbadense lines from diverse geographical locations. It showed that cytoplasmic indel markers are more polymorphic compared to the cytoplasmic SSR markers among the lines. The overall results showed that the selected 94 lines could widely be divided into two broad groups of G. hirsutum and G. barbadense. The research for the first time provided a genetic tool to study cotton cytoplasmic genome and a report comparing the genetic diversity in the cytoplasmic and nuclear genomes of G. barbadense and G. hirsutum lines. The following research will help breeders to develop a breeding strategy maximizing the effects of genetic diversity to improve cotton lines.*

Key words: *diversity, Gossypium, cytoplasm, nuclear, SSR, dendrogram, phylogenetic tree, G. hirsutum, G. barbadense.*

Introduction

Cotton is the leading textile fiber and the second most important oilseed crop in the world generating over 26 Mt of fibre in 2014/2015 on more than 33 million hectares [1]. Upland cotton (Gossypium hirsutum L., AD1, 2n = 52) is the most widely grown cotton species worldwide due to its superior yield potential, whereas G. barbadense L. (AD2, 2n = 52) is the only other cultivated tetraploid species, grown in some areas because of the price advantage due to superior fiber quality[2] (Figure 1). The genetic improvement of any crop species depends on the extent of genetic variation for desirable alleles and the accurate characterization of the variability among germplasm accessions. Breeders normally select genotypes based on morphological characters, primarily regulated by nuclear genome. However, cytoplasmic genome, including mitochondria and chloroplast genomes, plays also an important role to perform many biological functions. The narrow genetic base of cultivated cotton germplasm is the primary impediment in its genetic improvement. The characteristics and genetic inheritance mode of cytoplasm and nuclear genome provide complementary valuable information to study gene flow, evolution, and population history in any cotton breeding program. Hence, the limited information is available on the genetic variation especially on cytoplasmic genome of the improved cotton lines of G. hirsutum and G. barbadense. The aimof the research is: 1) to develop DNA markers specific to the cotton cytoplasmic genome as a tool for genetic studies and 2) to make use of DNA markers to detect genetic diversity in the cytoplasmic and nuclear genome respectively in a set of 94 improved G. hirsutum and G. barbadense lines.

Materials and Methods

The study involved 61 SSR primer pairs associated with important fiber specific traits of the nuclear genome and 49 primers specific to the cytoplasmic genome to screen 20 G. hirsutum and 74 G. barbadense improved lines from diverse geographical locations. The overall methods of standard technique of PCR and gel methods using an ABI 3130XL with a 96-capillary system for molecular analysis have been used. The nuclear SSR primer pairs were selected based on the previous studies considering their presence covering almost the whole genome and association with important fiber traits. The molecular marker data have been assessed as a dominant marker to avoid ambiguous scoring for allelic relationship without pedigree data of the cotton lines in the present experiment (Figure 2). The marker data were analyzed to evaluate the genetic similarity between cultivars based on the simple matching coefficient (SI) and constructed phylogenetic trees using JMP Genomics software (SASTM). 27 CPSSR and 22 indel specific primer pairs were detected from the cotton chloroplast genome using NCBI database using a cost-effective data mining strategy.

DNA extraction

Approximately two mg of fresh leaf samples of individual line for DNA extraction using a QIAGEN DNeasy Plant Maxi kit (QIAGEN Inc., CA) and/or with a QIAGEN DN easy Plant Mini kit following the manufacturer's protocol have been used. DNA solutions were diluted to a working concentration of 10 ng µl and stored at 40C until PCR amplification.

PCR method with universal labeled primer for cpSSR

PCR reactions were performed in 10 µl volumes containing 10 ng of cotton template DNA, 19 GeneAmp PCR Gold buffer from Applied Biosystems, (109, 150 mM Tris–HCl, PH 8.0, 500 mM KCl), 1 mM MgCl2, 0.2 mM dNTPs,0.5 uM of forward and reverse primer mixtures, 0.35 ul of AmpliTaq Gold, (Applied Biosystems). Dye-labeled chromosome specific forward and unlabeled reverse primers were used in all PCR reactions. We used a modified PCR protocol to be cost effective by using a universal Fluorescent labeled HPLC Purified T13 primer. In order to use a universal primer, a 19 bp long sequence of CAGTTTTCCCAGTCACGAC were added to the 50 left end of each forward primer, and a 4 bp short sequence of GTTT to the 50 left end of the respective reverse primer (CAGTTTTCCCAGTCACGAC) [3]. The modified forward and reverse SSR primers were dissolved in water respectively to make a 100 uM stock solution. Afterwards, the forward and reverse SSR primers were combined to make a diluted 5 uM working solution. PCR reaction was carried out in 10-ul reactions containing

2.5 ul of DNA, 10x Gold Taq Buffer, 20 mM MgCI2, 10 mM dNTPS, 0.3 of each 3 primers, 1 unit of Taq polymerase and 4.4 milliQ water. The PCR amplification profile consisted of an initial denaturation of DNA at 95 0C for 3 min, followed by 95 0C for 1 m, 60 0C for 1 m; GOTO 2:1 time, 95 0C for 30 s, 60 0C for 30 s, 68 0C for 30 s; GOTO 5; 26 times and a final extension of 4 m at 68 0C.

Gel electrophoresis

The PCR products were diluted 1:20 before loading into capillaries and run in a denaturing capillary electrophoresis in an ABI 3130Xl with a 96-capillary system using POP-7 polymer (Applied Biosystems, USA) following the overall methods[4]. The size of amplified products was detected using GeneMapper 3.7 (Applied Biosystems, USA) as well as confirmed by visual corrected for appropriate sizes. The nuclear SSR product sizes were also confirmed based on the available SSR amplicon product sizes where available in the panel of CMD web page [5].

Result and discussion

Results from the hybrids (F1) of the reciprocal crosses between G. hirsutm (TM-1) and G. barbadense (Pima 3-79) confirmed the association of these markers specific only to the cotton cytoplasmic genome. also It was observed that cytoplasmic indel markers are more polymorphic compared to the cytoplasmic SSR markers among the lines. The overall results from the dendrogram revealed that the selected 94 lines could be divided widely into two broad groups of G. hirsutum and G. barbadense. However, the dendrogram results also showed that two accessions of G. hirsutum and two accessions of G. barbadense clustered with different species group respectively suggesting the interspecific introgression of gene pool in the development of these lines. The overall results suggested that the genetic diversity in the group of G. hirsutum is narrower in both cytoplasmic and nuclear genomes compared to the group of G. barbadense. The average coefficient of similarity based on IBD value (identical alleles) in the cytoplasmic genome is 0.48 (S.E. ± 0.005). However, the nuclear genome average IBD value is 0.44 (S.E. ± 0.005), which suggests the genetic diversity in the cytoplasmic genome to be more conserved compared to the nuclear genome among the lines. The average IBD value is 0.42 (S.E. ± 0.005) combining both of the cytoplasmic and nuclear markers among the lines. Results from the dendrogram pattern showed that the accessions in some cases from the similar breeding sources or geographic locations clustered together suggest the use of similar in-house gene pool in the breeding program. Genetic variation for desirable alleles for fiber traits and the accurate characterization of the variability in the targeted lines of interest are the foundation for any successful breeding program. The study provided for the first time a genetic tool to study cotton cytoplasmic genome and a report comparing the genetic diversity in the cytoplasmic and nuclear genomes of improved G. barbadense and G. hirsutum lines. The research will help breeders to develop a breeding strategy maximizing the effects of genetic diversity to improve cotton lines.

Conclusion

Twenty-sevenSSR and 22 indel specific cytoplasmic primer pairs were developed using a cost-effective data mining strategy as a genetic tool to study cotton cytoplasmic genome. Results from the hybrids (F1) of the reciprocal crosses between TM-1) and Pima 3-79 confirmed the association of these markers to be specific only to the cotton cytoplasmic genome (Table 1); The overall results from the dendrogram revealed that the selected 94 lines could broadly be divided into two distinct groups of G. hirsutum and G. barbadense; Results suggested that the genetic diversity in G. hirsutum lines is narrower in both cytoplasmic and nuclear genome compared to G. barbadense lines. The average coefficient of similarity based on IBD value (identical alleles) in the cytoplasmic genome is 0.48 (S.E. ± 0.005). However, the nuclear genome average IBD value is 0.44 (S.E. ± 0.005) suggesting the genetic diversity in the cytoplasmic genome is little more conserved compared to the nuclear genome among the lines. Results from the dendogram pattern showed that Acala Ultima (G. hirsutum) at all time clustered with G. barbadense accessions and Bahamas-1 (G. barbadense) grouped with the G. hirsutum accessions suggesting the possibility of interspecific introgression in these accessions. Results from the dendrogram showed that the accessions in some cases from

the similar breeding sources or geographic locations clustered together suggest the use of similar in house gene pool in the development of these accessions; The research provided for the first time a genetic tool to study cotton cytoplasmic genome and a report comparing the genetic diversity in the cytoplasmic and nuclear genomes of improved G. barbadense and G. hirsutum accessions (Figure 3; 4).

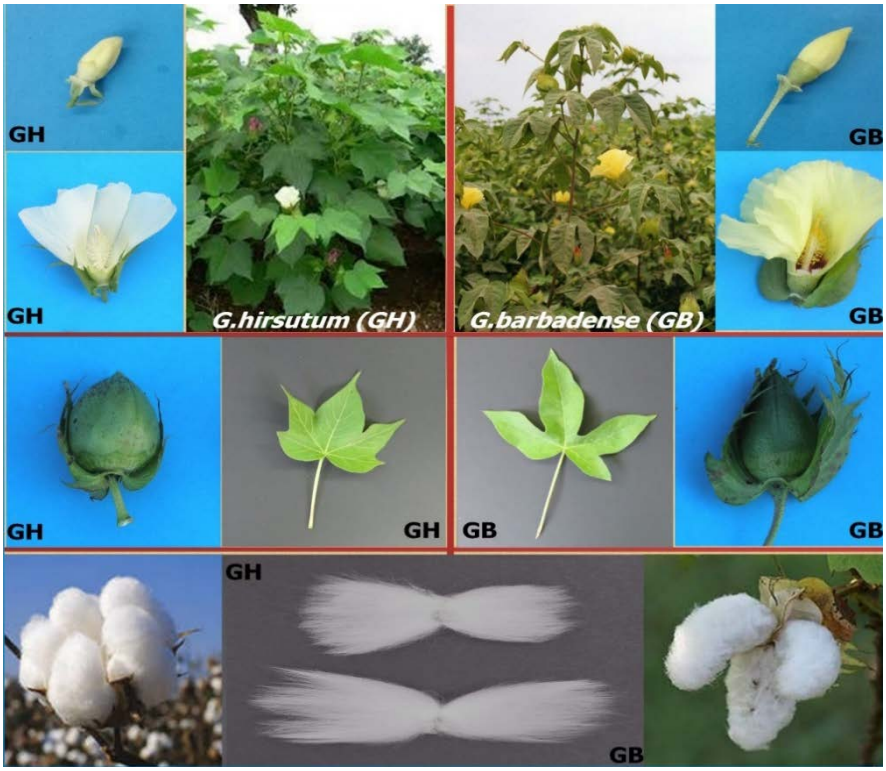
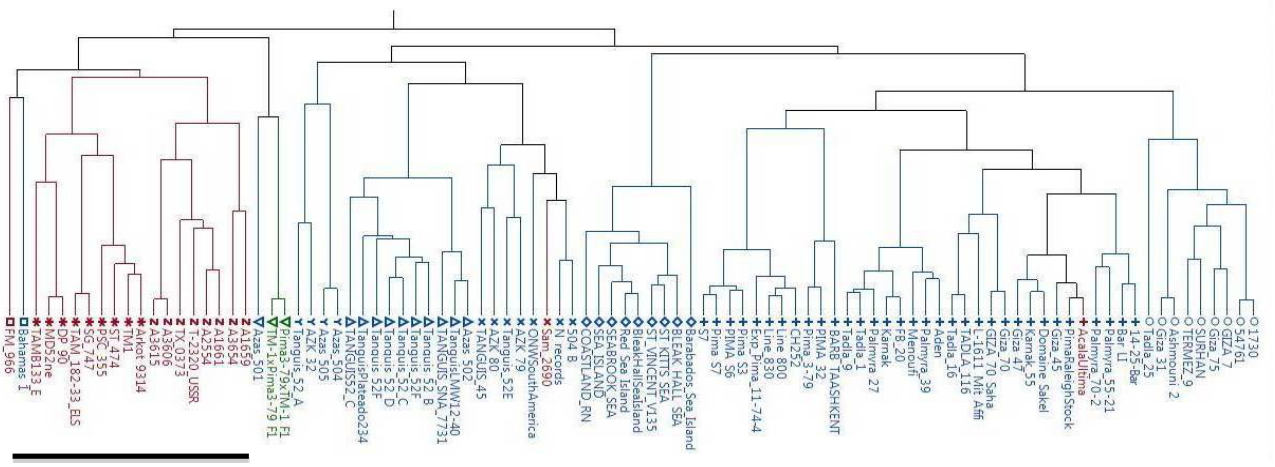


Figure 1. Morphological differences between G.hirsutum and G.barbadense

Figure 2. Electrophorogram gel picture of ABI 3130XL Capillary electrophores shows the polymorphic nuclear SSR markers between G. hirsutum and G. barbadense



G. hirsutum *G. barbadense*

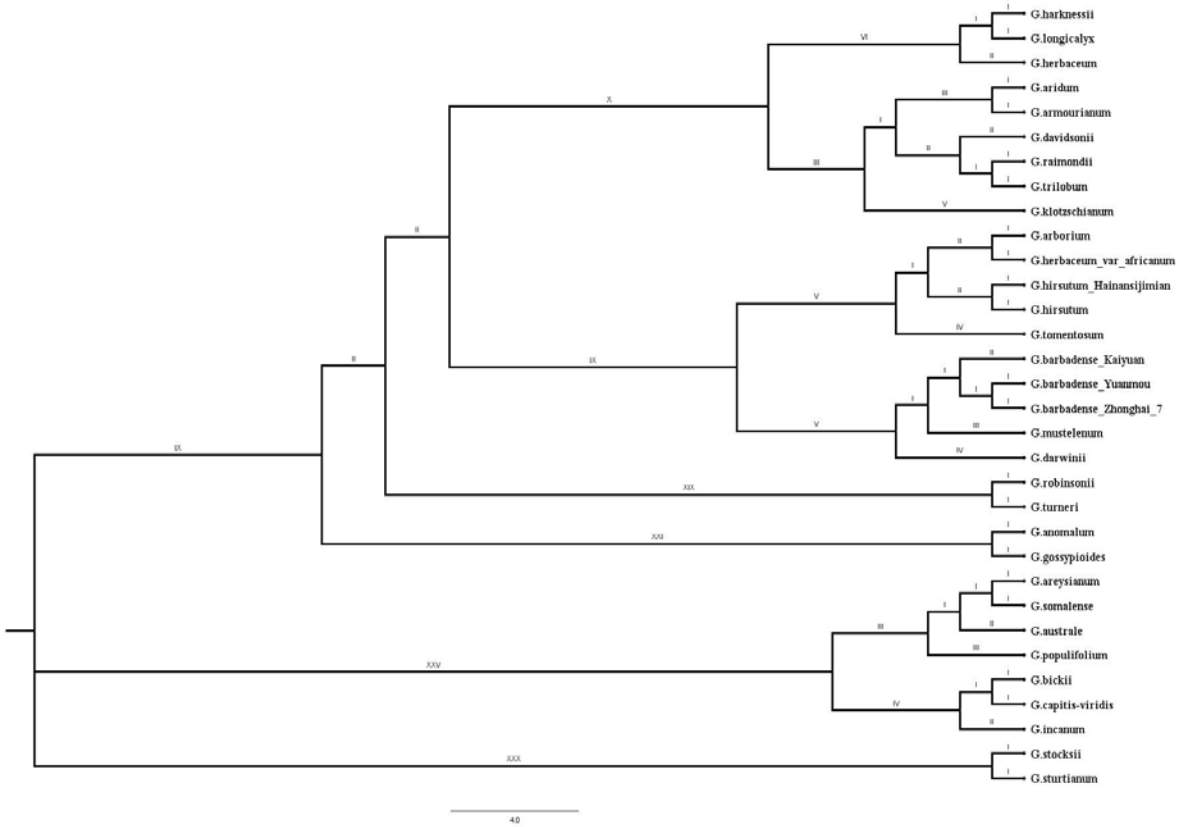


Figure 4. Cytoplasmic genome sequences from different Gossypium species

Table 1

Used chloroplast specific SSR and indel primer pairs. Genotyped by parental crossing

Entry name	Total SSRs	CP SSRs	Indel SSRs	Polymorphic		Monomorphyc		NA	
				CP SSRs	Indel SSRs	CP SSRs	Indel SSRs	CP SSRs	Indel SSRs
TM1	56	32	24	13	14	18	8	1	2
(TM-1xPima 3-79)	56	32	24	13	14	18	8	1	2
Pima_3-79	56	32	24	13	14	18	8	1	2
(Pima3-79xTM-1)	56	32	24	13	14	18	8	1	2
Acala Ultima	56	32	24	13	14	18	8	1	2

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