

## **ACHIEVEMENTS AND PERSPECTIVES OF COTTON “OMICS” IN UZBEKISTAN**

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### **ABSTRACT**

To timely address current and projected issues in cotton farming, and for boosting its cotton production, Uzbekistan has paid a particular attention to develop “omics” science based cotton programs, established modern research facilities, prepared new generation of biotechnologists and molecular breeders, and enhanced global collaborations with advanced centres worldwide. Scientists have concentrated and significantly advanced on molecular marker technologies, genetic mapping of important loci conditioning complex agronomic traits, cloning and characterization of important cotton genes and sequence signatures, application of marker-assisted selection (MAS) and genetic engineering technologies that resulted in development of Uzbekistan's own 'biotech' and MAS-derived novel cotton cultivars, which are being commercialized in near future. Here the key achievements, current developments, and future perspectives were revisited.

### **INTRODUCTION**

Cotton research is one of the priorities of Uzbekistan that concentrated on improvement of cotton fibre quality and lint yield, productivity, maturity, resistance to various diseases and abiotic stresses. Uzbekistan roughly produces about 5% of world fibre production and exports 10% of world fibre that puts Uzbekistan in the rank of sixth largest cotton producer and the second largest cotton exporter in the world (Abdurakhmonov, 2013). Cotton farming occupies around 30% of all lands available for cultivation in the country, which produces 0.85-1.0 million metric tons of fibre valued at ~US\$0.9 to 1.2 billion (Abdurakhmonov, 2007; Campbell *et al.*, 2010).

Uzbekistan cotton production is affected by reduction in area engaged for growing the cotton, policy environment (e.g., food security), the bio-security issues threatening cotton, and environmental stress factors (Abdullaev *et al.*, 2007) such as shortages in irrigation and water deficiencies as well as soil salinization issues (Cotton Fact Sheet, ICAC, 2011; Abdurakhmonov, 2013). These are gradually influencing the fibre quality and productivity of cotton. Uzbekistan's cotton lint fibre yield was 753 kg/ha in 2010/11 (Cotton Fact Sheet, ICAC, 2011) and estimated at 804 kg/ha in 2012/13 or 812 kg/ha in 2013/14 (Cotton World Statistics, ICAC, 2009; Abdurakhmonov, 2013), which is close to the world average (Cotton World Statistics, ICAC, 2009).

To address issues standing in cotton production, during past century cotton farming, Uzbekistan, being the northernmost cotton growing region, has built a sustainable agricultural system for cotton production, gained sufficient expertise, collected the richest cotton germplasm resources (Abdurakhmonov, 2007; Campbell *et al.*, 2010; Abdullaev *et al.*, 2013), bred highly adapted, early maturing cotton cultivars suitable to be grown in the northern latitudes and arid zones (Abdukurimov *et al.*, 2003; Ibragimov *et al.*, 2008; Abdullaev *et al.*, 2013), and promoted cotton science through its multiple research institutions, centres and associations devoted to cotton growing, research, fibre preparation and its export.

Further, to address current and projected issues in cotton production, along with contemporary cotton breeding programs and institutions in the country, Uzbekistan paid a particular attention to develop its own “omics” science based scientific programs. A stepping stone into this development was Uzbekistan President's personal attention and government's significant investment during the past 22-years of independence of the country.

This resulted in development of effective research environment, building a well-equipped modern laboratories for genomics and biotechnology research, and training of qualified scientists in this direction (Abdurakhmonov *et al.*, 2012a; Abdurakhmonov, 2013). Researchers developed strong international collaborations with leading cotton genomics and biotechnology laboratories worldwide to shape up the world science level cotton research program in Uzbekistan (Abdurakhmonov *et al.*, 2012a).

These collaborations accelerated the cotton genomics and biotechnology of Uzbekistan, enhanced the development of genetically engineered cotton varieties, promoted technology transfer, and resulted in jointly patenting of newly developed products (AAAS-ASU joint report, 2012). Achievements and perspectives of Uzbekistan's “omics” science programs related to cotton was highlighted by Abdurakhmonov (2013) in detail. Here, the most important achievements on this direction were briefly revisited and some ongoing efforts and the future perspectives of scientific development were discussed.

## **MOLECULAR MARKER DEVELOPMENT AND GENETIC MAPPING**

A large number of cotton microsatellites and candidate gene-specific markers were developed (Reddy *et al.*, 2001; Abdurakhmonov, 2001; Abdurakhmonov *et al.*, 2007a). Several agronomically important quantitative trait loci (QTLs), controlling the fibre length (Abdurakhmonov, 2001), lint yield (Abdurakhmonov *et al.*, 2007b) and natural leaf defoliation (Abdurakhmonov *et al.*, 2005) traits were mapped. To study photoperiodic flowering in cotton, a collection of photoperiod-converted radiomutants (32P) including their wild-type parental lines was investigated using SSR markers (Abdurakhmonov *et al.*, 2007c).

Further using bi-parental mapping populations developed via crossing the photoperiod-converted, day-neutral flowering radiomutants to the original photoperiodic wild parents, QTL regions responsible for photoperiodic conversions

and causing the day-neutral flowering after the mutation were mapped. These QTL regions were localized on chromosome 5 of cotton (Kushanov *et al.*, 2010; Kushanov *et al.*, unpublished; Abdurakhmonov, 2013).

To address emerging threats of Fusarium wilt disease in Uzbekistan (Marupov *et al.*, 2012), a race/genotype distribution of FOV fungi in Uzbekistan was studied (Egamberdiev *et al.*, 2013) and most frequently occurring FOV races and their pathogenicity (Egamberdiev *et al.*, unpublished) were identified. Several QTL loci contributing to FOV resistance in cotton were mapped using SSR markers (Abdullaev *et al.*, 2010; Abdullaev *et al.*, 2014, unpublished; Abdurakhmonov, 2013).

Molecular tagging of major QTLs conditioning salt, drought and heat tolerance in cotton is in progress to apply modern molecular breeding tools in the development of abiotic-stress tolerant cotton cultivars. These ongoing efforts will address current needs of cotton farming in the event of projected climate change and water deficiency (Sutton *et al.*, 2013; World Bank report, 2013) as well as land salinization increase in the region.

According to reports, the chromosome substitution (CS-B) lines are effective way in enhancing fibre quality (Saha *et al.*, 2012) because one of the chromosome or chromosome arm of CS-Bs was replaced with Pima 3-79 line (*G. barbadense* L.), which have superior fibre quality. The direct comparative analysis of CS-B lines showed that CS-B25 had significantly lower micronaire than TM-1 and seven CS-B lines (2, 6, 16, 18, 5Lo, 22Lo, 22sh) had greater lint percentage than 3-79 or TM-1 (Saha *et al.*, 2004). In addition, CS-B lines showed positive additive effect associated with fibre traits when these lines were crossed to five US cultivars (Jenkins *et al.*, 2006, 2007).

To conduct fine QTL-mapping of fibre quality genes and further release of improved germplasm resources bearing Pima fibre quality loci in Upland cotton background, Uzbekistan cotton cultivar specific chromosome substituted recombinant inbred line (UzCS-RIL) mapping populations have been developed (Makamov *et al.*, unpublished). For this purpose, within the frame of USDA/ARS-Uzbekistan cooperation programs, a set of CS-B lines developed by USDA/ARS scientists (Stelly *et al.*, 2005; Saha *et al.*, 2012) was obtained, and sexual crosses between several improved Uzbek cultivars and CS-B lines for chromosomes 16, 22, and 25 were performed. At present fifth generation of UzCS-RIL mapping populations have been developed and they are being phenotypically evaluated in multi-environmental conditions for fibre and major agronomic traits to conduct QTL-mapping using set of chromosome specific DNA markers (Makamov *et al.*, unpublished).

## **ASSOCIATION MAPPING**

To apply modern genetic mapping methodology, (Abdurakhmonov and Abdugarimov, 2008) for the first time, Uzbek cotton germplasm resources including a global set of ~1000 *Gossypium hirsutum* L. (so called Upland cotton - one of the widely grown allotetraploid cotton species) accessions from 37 cotton growing countries and 8 breeding ecotypes as well as wild landrace stocks, were characterized at molecular

level (Abdurakhmonov *et al.*, 2004; Abdurakhmonov *et al.*, 2006; Abdurakhmonov *et al.*, 2008a; Abdurakhmonov *et al.*, 2009; Abdurakhmonov *et al.*, 2012b) and the extent of linkage disequilibrium (LD) for the cotton genome were identified. The feasibility of LD-mapping in tagging of useful genes from germplasm resources was shown (Abdurakhmonov *et al.*, 2008a; Abdurakhmonov *et al.*, 2009).

Further, the important fibre quality (fibre length and strength, micronaire, uniformity, reflectance, elongation and ect.) traits were measured in two distinct environments of Uzbekistan and Mexico. This study allowed researchers to design an “association mapping” (AM) study to find biologically meaningful marker-trait associations for important fibre quality traits that accounts for population confounding effects (Abdurakhmonov and Abdurakhmonov, 2008). Several SSR markers associated with main fibre quality traits along with donor accessions were identified and selected for MAS programs (Abdurakhmonov *et al.*, 2008a; Abdurakhmonov *et al.*, 2009).

To enhance power (Stich and Melchinger, 2010) of AM in cotton (Abdurakhmonov *et al.*, 2012b; Kumptala *et al.*, 2012), based on above-mentioned association mapping studies on a global set of *G. hirsutum* germplasm resources, 17 most diverse *G. hirsutum* accessions were selected and used in genetic crosses with Uzbek cultivar Namangan-77, a genetic standard for *G. hirsutum*. This effort led to create a nested association mapping (NAM) populations for cotton. A panel of 3400 F<sub>2:3</sub> generation NAM population individuals was developed and is being phenotyped for important agronomic and fibre traits in multiple environmental conditions of Uzbekistan. These NAM population individuals are also being genotyped with a large number of SSR markers to conduct fine association mapping studies (Abdurakhmonov *et al.*, 2014, unpublished) on targeted traits of interest.

In addition, 286 *G. barbadense* (so called long staple fibre Pima cotton) accessions were selected from Uzbek cotton germplasm that represent wide geographic origin covering 17 countries worldwide. These Pima cotton accessions were genotyped over 290 polymorphic SSR markers that helped to estimate molecular diversity, phylogeny, population structure, linkage disequilibrium level. These Pima cotton accessions were phenotypically evaluated for major fibre quality traits in the Uzbekistan and USA growing environments. Further, using similar approach reported for a global set of *G. hirsutum* (Abdurakhmonov *et al.*, 2008a; 2009), a mixed linear model based association mapping of fibre quality traits in *G. barbadense* AM panel was conducted that identified several key SSR markers useful for MAS programs to mobilize superior quality fibre QTLs into commercial cultivars (Abdullaev *et al.*, 2014 unpublished).

## **MARKER-ASSISTED SELECTION**

The first MAS effort was performed in Uzbekistan using molecular markers associated with important fibre traits from AM studies in a global set of cotton germplasm accessions (Abdurakhmonov *et al.*, 2008a; Abdurakhmonov *et al.*, 2009). Fibre trait-associated DNA markers were used as a tool to manipulate the transfer of QTL loci during a genetic hybridization. For genetic crosses, 37 donor cotton genotypes were selected that bear important QTLs for major fibre traits. These donor

genotypes were crossed with 9 commercial cultivars of Uzbekistan (as recipients) in various combinations with the objective of improving one or more of fibre characteristics of these recipients. The hybrids from each crossing combination were tested using DNA-markers at the seedling stage, and hybrids bearing DNA-marker bands from donor plants were selected for further backcross breeding (**Fig.1**; Abdurakhmonov, 2011; Abdurakhmonov *et al.*, 2011ab; Abdurakhmonov *et al.*, 2012b).

As a result, novel haplotypes of quantitative trait loci (QTLs), underutilized in current cotton breeding, were successfully mobilized from donor genotypes to several commercialized recipient cultivars using traditional backcrossing with the aid of trait-associated molecular markers. Testing the major fibre quality traits using a high volume instrumentation (HVI) in MAS hybrids revealed that mobilization of the specific marker bands from donors has positively improved the trait of interest in recipient genotypes. Currently, fourth generation MAS hybrids (F<sub>1</sub>BC<sub>4</sub>), carrying new markers and having a better quality of fibre compared to the recipient parents, are being grown and tested (**Fig.2**). Using these effective molecular markers as a breeding tool, researchers aim to pyramid major fibre quality traits into single genotype of several commercial Upland cotton cultivars of Uzbekistan (**Fig.3**; Abdurakhmonov, 2011; Abdurakhmonov *et al.*, 2011ab). Thus, genetic mapping and MAS efforts are advancing in Uzbekistan that will help to address many problems associated with improving and boosting yield and quality.

## COTTON GENE CHARACTERIZATIONS

To develop cotton biotechnology and produce Uzbekistan's own biotech cottons, several genes representing the cotton phytochrome gene family involved in cotton photomorphogenesis and flowering (Abdurakhmonov, 2001; Abdurakhmonov *et al.*, 2010), and genes with important role in plant defence and disease resistance (Buriiev *et al.*, 2010; 2011) were cloned and sequenced.

A large number of small RNAs from developing ovules (Abdurakhmonov *et al.*, 2008b), root tips of developing cotton seedlings (Devor *et al.*, 2009), salt stressed cotton tissues (Shapulatov *et al.*, 2014 unpublished), and root-knot (RKN)/Fusarium wilt (FOV) infected cotton tissues (Shapulatov *et al.*, 2014 unpublished) were cloned and annotated. Genes characterized in these studies are being widely used in genetic engineering programs in Uzbekistan (Abdurakhmonov, 2013).

## COTTON PROTEOMICS AND METABOLOMICS

Uzbek researchers are actively working on cotton proteomics research area, and recently have collaboratively characterized major root proteins in several cotton species. Root proteins have not been examined extensively for cultivated and wild *Gossypium* species. Unique cotton root proteins from *G. hirsutum*, *G. barbadense*, *G. arboreum*, and *G. longicalyx* were identified in 2-D gels, which were then characterized by Q-TOF tandem mass-spectral sequencing. The subsequent *in silico* bioinformatics annotation of Q-TOF sequenced fragments from selected major spots

revealed proteins that were associated with primary and secondary metabolism, defence and stress conditions, and growth and developmental functions. This study should be useful as a preliminary inventory of root proteome in different cotton species (Mavlonov *et al.*, 2009).

In addition, several anti-fungal (from latex of Ficus plant) and insecticidal (from melon seed) plant proteins were characterized (Mavlonov *et al.*, 2008) and patented (Abdulkarimov *et al.*, 2006) that are being applied to create transgenic cotton cultivars resistant against wilt diseases and insect pest (boll worms). Combination of cotton genomics efforts and proteomics advancements will greatly ensure the development cutting-edge technologies responding cotton industry needs of Uzbekistan.

## GENETIC ENGINEERING AND BIOTECHNOLOGY

Further, with the specific aim of developing Uzbekistan's own tissue culture derived 'biotech' cottons as well as to study the biological function of these *de novo* characterized cotton genes mentioned above, an efficient high-throughput cotton tissue culture and transformation system was established (Abdurakhmonov *et al.*, 2013). RNAi (RNA interference) approach was used for genes characterized in the centre using available RNAi vector systems (Wesley *et al.*, 2001; Helliwell *et al.*, 2002) as well as newly developed synthetic RNAi constructs that are highly specific to a gene or small RNA/microRNA signature of interest to reduce (knock-out) its function (Abdulkarimov *et al.*, 2011) .

For instance, RNAi of cotton phytochrome gene family and its signal transduction system was conducted to create genetically engineered cottons for Uzbekistan in collaboration with Texas A&M University and USDA-ARS laboratories. There was a particular interest in phytochrome gene family of cotton because of its multiple gene effects in plant development (Abdurakhmonov, 2013; Abdudarkhmonov *et al.*, 2013). Several studies in model plants have shown that phytochromes play important role in conditioning of yield potential and productivity (Smith, 1994; Robson and Smith, 1997; Robson *et al.*, 1996; Thiele *et al.*, 1999; Rao *et al.*, 2011), plant flowering and architecture (Fankhauser and Chory, 1997), and cotton fibre quality (Kasperbauer *et al.*, 2000; Abdurakhmonov, 2001). Studies in model plants also have demonstrated possible association of phytochrome genes in salt tolerance (Datta *et al.*, 2007; 2008), in regulation of nitrate reductase (Jonassen *et al.*, 2008; Lillo, 2008), in cold/freezing and drought tolerance (Kim *et al.*, 2002; Franklin and Whitelam, 2007; Beck *et al.*, 2007), in fungal disease resistance (Xie *et al.*, 2011).

As a result of cotton phytochrome RNAi study (**Fig.4**), the first generation biotech cotton varieties in Uzbekistan with improved fibre length (38-40 mm fibre length; **Fig.5** and **Fig.6**) and Micronaire (3.9-4.2), early maturity (for 5-10 days) and higher seed cotton yield (~10-18% higher) as well as improved root system (two fold) were developed (Abdurakhmonov *et al.*, 2012c; Abdurakhmonov *et al.*, 2013). This technology demonstrated a great potential to develop superior cultivars in a globally important crop species in a short-time without any adverse effect on yield (Abdurakhmonov *et al.*, 2012c; Abdurakhmonov *et al.*, 2013) and creates longer and stronger new Upland cotton fibre.

The resistance of phytochrome RNAi varieties to various biotic and abiotic stresses, their photosynthetic and nitrate assimilation characteristics, transcriptome and metabolome contents, and cotton seed oil content and quality are being investigated in order to better understand the biochemical modification and genetic mechanism of simultaneous trait improvements behind cotton *PHYA1* gene RNAi. Additionally, these new phytochrome gene RNAi based varieties are being tested in multi-environmental large field trails in Uzbekistan for the purpose of future commercialization to timely address and boost cotton production in Uzbekistan (Abdurakhmonov, 2013; Abdurakhmonov *et al.*, 2013).

Similarly, using RNAi approach, biotech cottons for drought, salt, and wilt (FOV) resistance traits are being developed using characterized genes and sequence signatures in Uzbekistan laboratory. In perspectives, researchers concentrate to work on new genetic engineering technologies and cotton genome editing (Miller *et al.*, 2011) to develop native biotech cotton cultivars for Uzbekistan farmers, which is in progress (Abdurakhmonov, 2013).

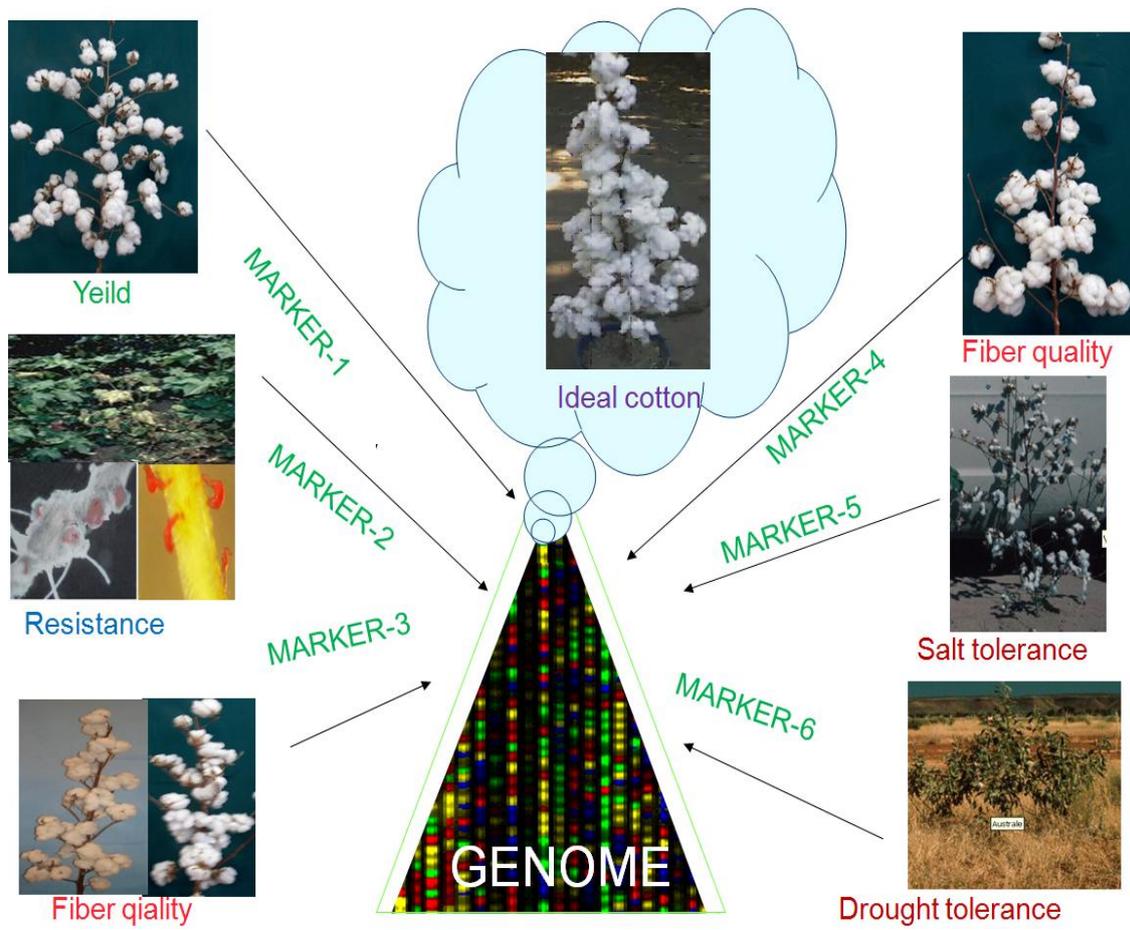
## **CONCLUSIONS**

Thus, past decade efforts, in a support of Uzbekistan government and global international collaborations, resulted in significant advancement in cotton genomics and biotechnology in Uzbekistan. As a key outcome of these efforts, the establishment of modern “omics” science oriented well-equipped laboratories, preparation of new generation specialists with expertise and clear vision, and development of novel 'biotech' and MAS cotton cultivars can be exemplified. This will ensure the quality of scientific investigations and help to timely address and safeguard the cotton production in the country.

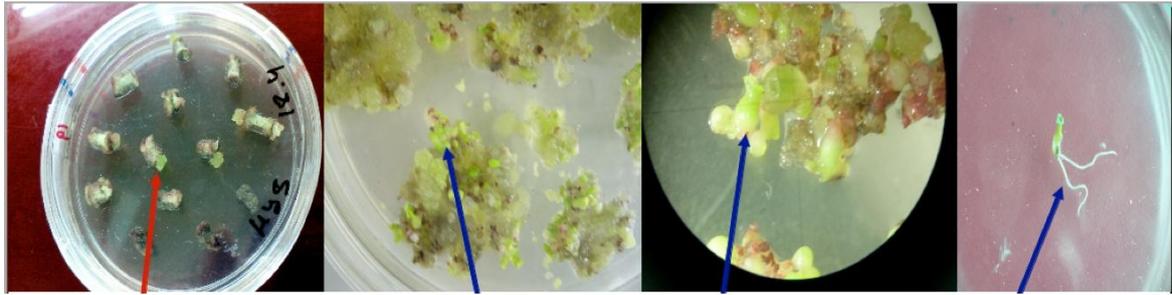
## **ACKNOWLEDGEMENTS**

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**Fig.3.** Schematic representation of gene pyramiding using molecular markers.



**Transformation      Callus formation and Somatic embryogenesis**

**Embryo plantlets      Transferring into pots and fields**



**Fig.4.** Cotton somatic embryogenesis process with PHYA1 RNAi interference (Source: Abdurakhmonov *et al.*, 2013).



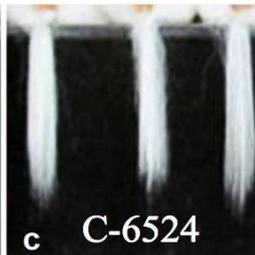
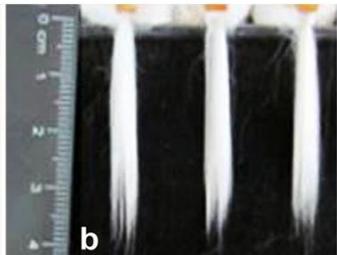
RNAi plants

Controls

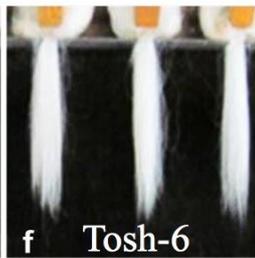
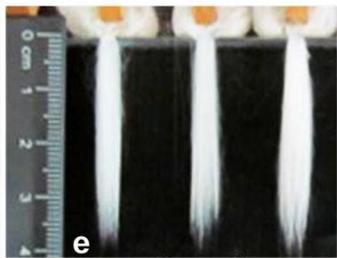
**Fig.5.** *PHYA1* RNAi versus traditional Upland cotton fibre (Source: Abdurakhmonov *et al.*, 2013).

Novel RNAi varieties

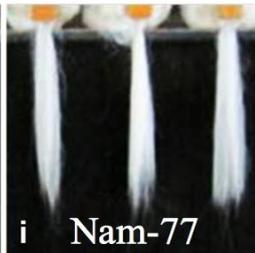
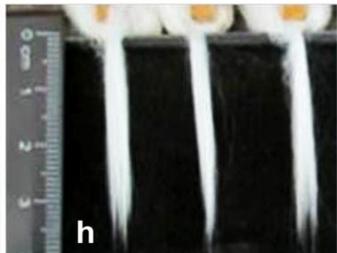
RNAi variety  
(C-6524)



RNAi variety  
(Toshkent-6)



RNAi variety  
(Namangan-77)



**Fig. 6.** Transferring *PHYA1* RNAi effects into traditional Uzbek Upland cottons: novel RNAi varieties (a-g) with fibre length of RNAi versions (b-h) versus original version (c-i; Source: Abdurakhmonov *et al.*, 2013).

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