# PYRAMIDING OF NEW QTL LOCI INTO A SINGLE GENOTYPE IN COTTON

**M.M.Darmanov,**
**F.N.Kushanov,**
**A.Kh.Makamov,**
**O.S.Turaev,**
**B.K.Rakhmanov,**
**A.T.Adilova,**
**Z.T.Buriev,**
**I.Y.Abdurakhmonov**
**Center of Genomics and Bioinformatics, Academy of Sciences of Uzbekistan**
**Tashkent, Uzbekistan**

## Abstract

The goal of this research is to pyramid QTLs, which control cotton fiber quality traits such as fiber length, strength and elongation using marker-assisted selection technique. In this study, the commercial Uzbek cotton cultivar Andijan-35 was used as a recipient parent, L-141 and Saenr Pena85 lines as donor genotypes. The recipient parent, Andijan-35 is an elite cultivar that has high yield, but poor fiber quality. Andijan-35 was crossed with L-141 (possessing marker loci associated with fiber length and strength) and Saenr Pena85 141 (having marker loci associated with fiber elongation) donor lines. Resulting two different $F_1$ hybrids were crossed with each other in order to combine two different QTLs of fiber length, strength and elongation. Subsequently, complex $F_1$ hybrids obtained have been backcrossed with the recipient genotype for the next four generations, which were resulted in $BC_4F_2$ hybrids with improved fiber quality properties. Results of fiber quality analysis of $BC_4F_2$ hybrids revealed that QTLs, which were stacked into a new cotton lines, had a significant impact on improving the fiber quality traits of $BC_4F_2$ hybrids. Molecular breeding experiments confirmed DNA markers used in this study as an effective regulator tool for combining targeted gene loci in single genotype.

## Introduction

New technologies and approaches associated with the achievements of genetics and biotechnology are actively applied to develop new agricultural crops in modern plant breeding programs. One of them is the "gene pyramiding", which allows combining several economically valuable properties into one plant genotype using trait-associated DNA markers. Nowadays, this technology is considered as the main acceptable strategy for developing new varieties of crops [1, 2].

Application of gene/loci pyramiding for developing cotton varieties, which effectively assemble the genes/loci with several parameters for fiber quality, for example, micronaire, strength, length and elongation that can significantly improve the capacity of cotton production in terms of the textile industry. It is known that the thinner, stronger and longer cotton fiber is considered more valuable and preferable for textile industry, since the best quality fabrics will be woven from it [3].

The purpose of this research was to develop new cotton lines having high fiber quality by pyramiding more two QTL loci associated different fiber quality parameters (length, strength and elongation) into one genotype.

## Materials and Methods

### Plant materials

Two cotton lines have been chosen as initial material for hybridization as donors. This is the L-141 line, which has a marker locus in the genome responsible for the fiber length and strength, and the second donor is Saenr Pena 85 with identified QTL locus determining the fiber elongation. As a recipient genotype, the commercial variety Andijan-35 was selected [4]. In addition, as a control genotype elite cultivar Namangan-77 was used. At the first stage of the study, the hybrids were obtained in two crossing combinations: (Andijan-35 x L-141) and (Andijan-35 x Saenr Pena85). At the next stage, these hybrids were crossed with each other to obtain the first generation of a complex hybrid [(Andijan-35 x L-141) x (Andijan-35 x Saenr Pena85)]. Finally, the obtained complex hybrids were backcrossed with the recipient Andijon-35 in order to eliminate unnecessary genome parts except fiber quality loci of donor genotypes from the complex hybrids and consolidate this hybrid with remaining features of the variety Andijan-35. As a result, $BC_4F_2$ genotypes were obtained.

**Isolation of genomic DNA**

The genomic DNAs were isolated by CTAB method [7] from each backcross generations and parental lines.

**DNA Markers and PCR analysis**

Prior to the subsequent crosses, the PCR screening of genomic DNAs of the first backcross ($BC_1$) generation were carried out using DNA markers such as BNL1604 marker associated with the fiber length and fiber strength, and BNL3650 associated with fiber elongation [5, 6]. Microsatellite genotyping was performed according to the method of Reddy et al., 2001 [8]. Positive hybrids, which are bearing targeted gene alleles of our interest were subjected for further backcrossing.

**Analysis of the fiber quality parameters**

The fiber quality characteristics, such as fiber length (UHM), strength (STR), micronaire (MIC), elongation (ELO), uniformity (UI) of samples were tested using High Volume Instrumentation (HVI) technique at the special fiber quality testing center "SIFAT", Tashkent, Uzbekistan. Initial analysis of phenotypic and genotypic data was performed using Microsoft® Office EXCEL 2013 program.

**Statistical analysis**

Statistical analysis of the data was carried out using the statistical programs SOFA STATS and NCSS 2003 with the ANOVA (Analysis of Variance) method. Multiple comparisons of samples were conducted according to the Kruskel-Wallis criterion. Differences were considered reliable at a value of $p<0.05$. The Bonferroni test (multiple t-test with alpha correction) and criterion multiple comparisons the LSD Fisher were used to evaluate for the reliability differences within groups.

## RESULTS AND DISCUSSIONS

Based on the results of molecular genetic analysis of $BC_4F_2$ generation, individual plants possess homozygous QTL loci similar to both donor lines (L-141 and Saenr Pena85) were identified using labeled DNA markers BNL1604 and BNL3545 in ABI3130 *xl* capillary electrophoresis (figure 1). These homozygous genotypes were selected for future studies of the technological parameters of fiber quality and for evaluation the effect of introgressed QTL alleles on their quality.

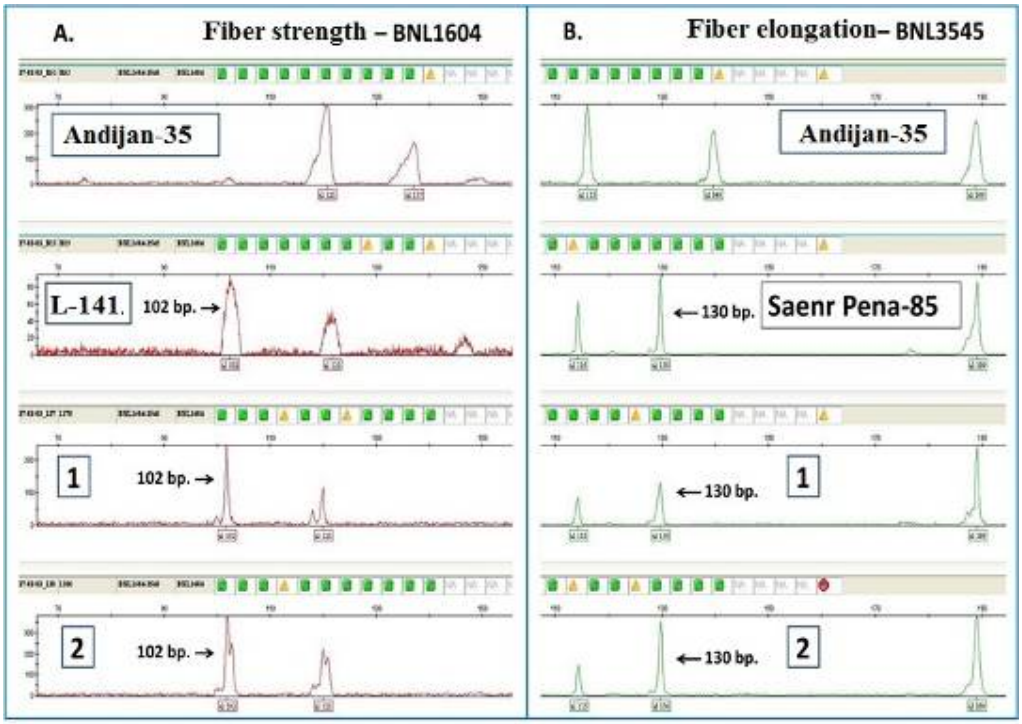


**Figure 1.** Electropherogram results by ABI3130 xl capillary electrophoresis showing homozygous state of $BC_4F_2$ generation plants that have the same allele of both donor genotypes for (1) BNL-1604 associated with fiber strength and length (A) and (2) BNL-3545 associated with fiber elongation (B).

The results of fiber quality analysis of the homozygous plants in $BC_4F_2$ generation showed the mean fiber length – 1.18 inches, strength – 35.2 g/tex and fiber elongation – 10.8%. These parameters are significantly lower in the recipient genotype Andijon-35, in which fiber length is 1.13 inches, strength – 32.5 g/tex and fiber elongation – 9.0 %. L-141 line is one of the donor genotypes of $BC_4F_2$ generation that has a mean fiber length and strength of 1.24 inches and 40.1 g/tex and fiber elongation 9.2%. The second donor, Saenr pena85 has a mean fiber length - 1.09, fiber strength - 30.1 g/tex and fiber elongation – 9.7 % (figure 2).
Thus, the fiber quality of the obtained homozygous plants in $BC_4F_2$ generation is significantly higher than the recipient genotype Andijon-35, where fiber length increased by 4.3 %, strength by 7.7 %, and elongation by 16.6 %. Comparing to the variety Namangan-77, the advantages of homozygous genotypes of $BC_4F_2$ generation are also noticeable than the control genotype.

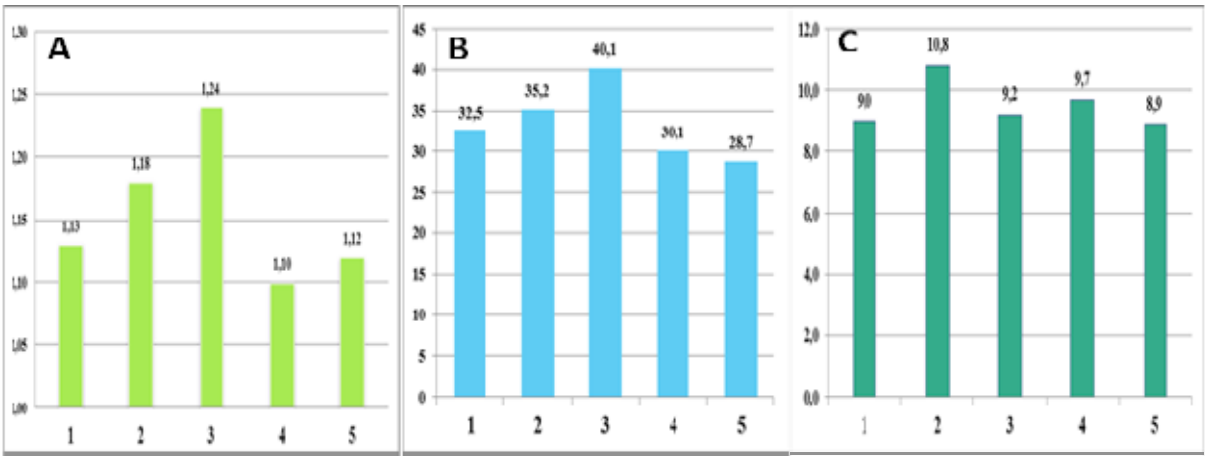


**Figure 2.** Comparison of fiber quality parameters in studied cotton genotypes: A- fiber length; B- fiber strength; C- fiber elongation. 1-5 studied genotypes: 1- Andijan-35, 2- $BC_4F_2$ hybrids, 3 and 4 donors L-141, and Saenr Pena85, 5- standard variety Namangan-77.

One-way analysis of variances (Analysis of Variance, ANOVA) was used to evaluate the influential degree of introgressed QTL alleles on fiber quality. The results displayed that analysis of intergroup dispersions in the studied cotton genotypes significantly differ ($p<0.001$) among in all fiber parameters. Moreover, according to the data of statistical analysis, there are significantly differences in all parameters of fiber quality between hybrids of $BC_4F_2$ generation and control samples of Andijan-35 and Namangan-77, which indicates a significant influence of introduced QTL-loci on the regulation of fiber quality characteristics in cotton genotypes $BC_4F_2$ (Tab1).

**Table 1**. Multiple comparison of fiber quality parameters for the studied genotypes by the Kruskel-Wallis criterion (Kruskal-Wallis Multiple-Comparison Z-Value Test).

| Fiber elongation, **%** | | | | | |
|---|---|---|---|---|---|
| **Samples** | **Andijan-35** | **$BC_4F_2$ hybrids** | **L-141** | **Namangan-77** | **Saenr pena85** |
| **Andijan-35** | 0.0000 | 4.0530** | 0.1182 | 1.3119 | 0.3591 |
| **$BC_4F_2$ hybrids** | 4.0530** | 0.0000 | 3.4468 | 5.3861** | 2.0800 |
| **L-141** | 0.1182 | 3.4468 | 0.0000 | 1.2488 | 0.2659 |
| **Namangan-77** | 1.3119 | 5.3861** | 1.2488 | 0.0000 | 1.1058 |
| **Saenr pena85** | 0.3591 | 2.0800 | 0.2659 | 1.1058 | 0.0000 |
| Fiber length, inches | | | | | |
| | **Andijan-35** | **$BC_4F_2$ hybrids** | **L-141** | **Namangan-77** | **Saenr pena85** |
| **Andijan-35** | 0.0000 | 1.7291* | 5.0761 | 0.9611 | 0.5817 |
| **$BC_4F_2$ hybrids** | 1.7291* | 0.0000 | 3.3350 | 2.6751** | 0.4675 |
| **L-141** | 5.0761 | 3.3350 | 0.0000 | 6.0077 | 2.6586 |

| | | | | | |
|---|---|---|---|---|---|
| **Namangan-77** | 0.9611 | 2.6751** | 6.0077 | 0.0000 | 1.1316 |
| **Saenr pena85** | 0.5817 | 0.4675 | 2.6586 | 1.1316 | 0.0000 |
| Fiber strength g / tex | | | | | |
| | **Andijan-35** | **$BC_4F_2$ hybrids** | **L-141** | **Namangan-77** | **Saenr pena85** |
| **Andijan-35** | 0.0000 | 0.3897 | 3.3483 | 4.6296 | 1.8394 |
| **$BC_4F_2$ hybrids** | 0.3897 | 0.0000 | 2.8593 | 4.7504** | 2.0360 |
| **L-141** | 3.3483 | 2.8593 | 0.0000 | 7.3989 | 3.8626 |
| **Namangan-77** | 4.6296 | 4.7504** | 7.3989 | 0.0000 | 0.7678 |
| **Saenr pena85** | 1.8394 | 2.0360 | 3.8626 | 0.7678 | 0.0000 |

There is a significant difference in medians with alpha = 0.05
* - at a value of z> 1.6954 - was used the common test,
** - at a value of z> 2.6121 - was used the Bonferroni test.

Thus, mobilization of new QTL regions from donor lines to the recipient genome (Andijan-35) through "gene pyramiding" has significantly improved the parameters of the fiber length, strength and elongation in obtained $BC_4F_2$ hybrids, without affecting or altering other fiber quality parameters. Furthermore, the results of statistical analysis confirm the existence of a reliable association of DNA markers BNL1604 and BNL3545 with the examination of fiber quality traits.
As a conclusion, several homozygous genotypes were identified based on DNA markers. Hence, individual samples having high fiber quality were selected in order to evaluate agronomical and morphological performances in a breeding nursery. Gene pyramiding once again shows an effective approach for improvement of different economical important traits in cotton.

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