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A Study to Check the Nitrogen Used Efficiency (NUE) by Utilizing Large Assortment of Strategies for yield improvement of Cotton

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Cotton is an important crop for the textile industry and cottonseed as a source to extract cooking oil and feed animals is widely acknowledged. In methodology, the measurement of plant architecture and physiological traits and analysis of components of phenotypic data has been done by using statistical tools, extraction tools, protein structure analysis, LD analysis, association mapping, qRT-PCR, and VIGS assay. The results of this study improved the understanding of Nitrogen Used Efficiency (NUE) which is largely responsible to develop cultivars with the enhancement of protein level, yield potential, and quality of the cotton. The identified results also enhance our knowledge to understand the molecular mechanism governing the efficiency of nitrogen in cotton and identified the potential targets for genetic advancement. This study helps to identify the plant architecture related to height, the number of sympodial branches and root-related traits, the identification of candidate genes involved in the NUE regulation and their role for improvement in plant architectural components, and understanding the genetic mechanism of plant architecture under low and high N environment.

Keywords: Nitrogen Used Efficacy, Physiological traits, Yield potential, Genetic advancement, Plant architectural components

INTRODUCTION

Cotton is one of the most widely cultivated and essential fiber products globally. The genus *Gossypium* of cotton consists of 46 diploid and 6 allotetraploid species (Chen et al., 2017) but cultivated species are *G. arboreum*, *G. barbadense*, *G. herbaceum,* and *G. hirsutum*(Jonathan and Richard, 2003). Among these species, *G. hirsutum* (Upland cotton or American cotton) is most extensively cultivated, Contributing 95% of total cotton production globally due to its wide adaptability across countries (Pasricha et al., 2007). Cotton as an important commodity for the textile industry and cottonseed as a source to extract cooking oil and to feed animals is widely acknowledged.

The increasing global population is increasing food demand and this can be fulfilled by going after affordable protein sources. This can be done by increasing cotton production up to the mark. ((Bellaloui, Turley, &Stetina, 2021; Laule, Thurston, Alford, &Bloomsmith, 1996).

According to the USDA report 2018, global cotton consumption is expected to reach 125.4 million bales in 2018/2019. The leading spinners of raw cotton are China, India, and Pakistan; together these countries are expected to account for 62 percent. Projected global cotton production for the year 2018/2019 is 121.2 million bales which are one percent decline as compared to

2017/2018. China, India, and United States are projected to produce 62% of cotton (2018/2019) globally as compared to 2017/2018 production which was 63%. Other reports by USDA are also showing a decline in cotton production and an increase

in consumption (Cotton, world markets and trade, November 2018). These all are demanding a need to improve the yield of cotton and this can be done by extensive studies of traits that are important in yield

contribution in upland cotton.

Nitrogen is a key and enslaved element in seedling development and fertility (Jarwar et al., 2019) and has crucial input in the horticultural production, therefore it is the primary integral of numerous macromolecules, metabolites pathways, and signaling of chemicals required for the productivity and development of the plant(Malcolm John Hawkesford, 2012). The fertilization of nitrogen has raised food yield dramatically, alleviating the pressures of the growth of the world population(Gojon, 2017). The availability of suboptimal nitrogen is a severe restraint in crop production and can result in crop losses of up to 50%(Jones, Clode, Kilburn, Stockdale, & Murphy, 2013). As a result, substantial volumes of nitrogenous fertilizer are used to boost growth and production(Sarasketa, González-Moro, González-Murua, & Marino, 2014), and are used to predict the triple in the future (Good, Shrawat, &Muench, 2004). Nonetheless, the enormous utilization of chemical Nitrogen fertilizers diminishes N-use efficiency (NUE) and produces major environmental damage, such as the contamination of groundwater and acidification of soil (Liang, Jiao, Jaroniec, &Qiao, 2012). Furthermore, the extensive utilization of Nitrogen fertilizers raises costs management dramatically. As a result, there is an urgent commitment to find and produce output genotypes with more advanced NUEs regulate to eliminate the valuable fundamentals of crop manufacturing (Den Herder, Van Isterdael, Beeckman, & De Smet, 2010).

NUE is a complicated attribute with two primary innards: N-uptake efficiency (NUpE) and N-utilization efficiency (NUtE), which are controlled by phenology, environmental reactions, biochemistry, and architecture(Malcolm J Hawkesford& Griffiths, 2019). The N supply influences the functions of NUpE and NUtE in defining the long-term NUE (Garnett, Plett, Heuer, & Okamoto, 2015). Crops have acquired a diversity of methods to boost the consumption of Nitrogen (Bascuñán-Godoy et al., 2018). Competent genotypes have special physiological processes that allow them to obtain sufficient N quantities (intake efficiency) or use their N uptake higher efficiently (utilization efficiency). NUpE is linked to the advancement of roots and architecture(Xu, Fan, & Miller, 2012). Because root is a primary organ for the uptake of Nitrogen and its morphological properties, such as length and area of the root, enhance the ability of a plant to acquire Nitrogen(Zhou et al., 2017). The expanded appropriation of biomass to roots for the formation of a branching and heavy root system ends in an enhanced NUpE and reduces the environmental impact of Nitrogen fertilizer (Brackin et al., 2015). The uptake of nitrogen is also affected by the rate of root consumption, which is influenced by energy fund and assessment from the assimilation of the root of nitrogen (Zhou et al., 2017). Low nitrate levels have been shown to induce root elongation. Under low conditions, (Luo, Wang, Dooner, & Clarke, 2015) discovered that root lengths by 54% and surface area rose by 49%, subsequently, whereas (Bahrman et al., 2005) found a drop in root length but an increase in the ratio of root/shoot. (Lynch, 2013) shown that the root length and frequency are critical for N abduction. Clarifying the alterations in the architecture of the cotton root system under different nitrate concentrations provides knowledge on root N-uptake potentiality. Anyhow, the expansion of NUtE is a far more challenging task (Xiao et al., 2017). The technology of genetic engineering has been utilized to develop plant practices linked to NUtE, such as carbon/N storage, regulation of N metabolism, signaling, remobilization, and translocation(Hu et al., 2018). To isolate the important variables of N utilization in cotton, detailed experiments to discover the underlying features of NUtE concealed by diverse nitrate absorption are necessary.

Cotton (*Gossypium* L.) is important economically and socially around the world because it is one of the top ten agricultural wealth generators (Sacramento et al., 2013). Nonetheless, the biggest issue for cotton growers is the high production cost, particularly Nitrogen fertilization(Wen et al., 2020). To lower the cost of crop production, cotton genotypes with high NUEs must be identified and developed right away. Many crops have been studied for N-efficient genotypes along with rice crop (Cheng, Farooq, & Johansen, 2011)maize crop(Gallais&Hirel, 2004), rapeseed(Bouchet et al., 2016), tomato plant(Abenavoli et al., 2016), barley(Shah et al., 2017), and wheat(Iqbal et al., 2020). Anyhow cotton cultivators with nitrogen efficacy are still to be found and produced due to our poor understanding of the processes and features that contribute to NUE (Zhang & Cue, 2018). Recent developments in the techniques of genotyping and high-throughput sequencing provided modern sequencing opportunities, such as Specific-locus Amplified Fragment Sequencing (SLAF-Seq),Genotyping by sequencing, and Restriction Site-Associated DNA sequencing (RAD-Seq) which have enabled to obtain markers of upland cotton in a large quantity at lower cost (Sun et al., 2018)

The number of QTLs for different parameters such as the height of the plant, fruiting branch length, fruiting branch angle, position of the first fruiting branch, number of fruiting branches, and length of stem node, have been identified using linkage mapping(Sun et al., 2018). Wide genome studies have been applied to *G. hirsutum* to discover genes, based on biparental linkage analysis. So far, many SNPs and SSR markers related to plant architectural elements have been identified (Li, Duan, Fang, Gong, & Jiang, 2020). The cottonSNP63K Array for analysis of diversity in *G. hirsutum* has revealed genome-wide variations. Thousands of SNPs have been identified to discriminate against cultivated cotton globally. These molecular polymorphisms are correlated to the variations in phenotypic traits(Naaldijk et al., 2017)

Wide genome association studies based on linkage disequilibrium have detected naturally occurring allelic variations and candidates. The objectives of this study are to understand the elements responsible for NUE would be valuable for improving crop production. Therefore, the identification of genetic variations and genes controlling

NUE is necessary. Among these components, genetic variations in Plant height, number of sympodial branches, and number of bolls bearing seeds in response to N application in the cotton plant have not been studied extensively and remain poorly understood.

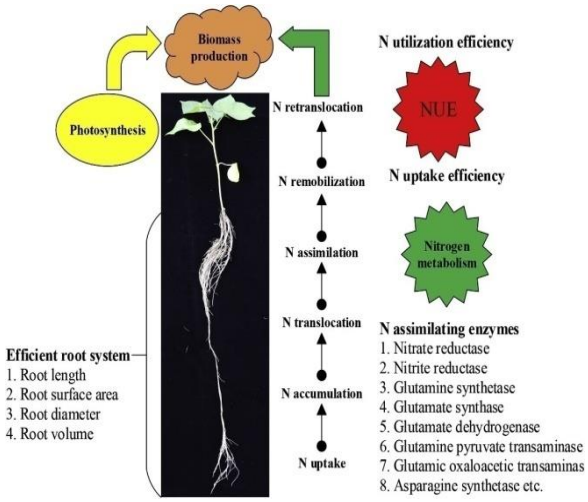

Figure 01:The summary of the physiological process of NUE in cotton (Iqbal et al. 2020)

MATERIALS AND METHODS

Plant Material

Diverse germplasm of upland cotton was collected from different germplasm resources in Pakistan. These collected accessions are categorized according to cotton-growing regions. Cotton genotypes were chosen from the preliminary experiment for screened genotypes occupying biomass and NUE in the pot analysis, then select N-efficient and three N-inefficient. Active seeds of a particular genotype originated in a germinator for one week in a concoction of sand and vermiculate. After the full openings of two cotyledons, the seedling with constant height was selected and emigrated into 7 L jars in the development room (16/8 h light/dark cycle, 28 °C temperature, 60 percent relative humidity). During the first week, seedlings provided a 1/2-strength Hoagland solution by following transplanting, outlawed by a full-strength Hoagland solution. Seedlings were subjected to varied nitrate doses of 0, 0.5, 1, 2, 5, and 10 mM as Ca(NO3)2. Furthermore, an absolute absorption of 0–4 mM L1 CaCl2 was supplemental to the low N treatment to adjust calcium concentration between analyses. Plants exhibited evident N treatment symptoms after four weeks, and eight plants from each group were taken for additional investigation.

Field trial and phenotyping

All the cotton germplasm was grown at the experimental field agricultural department in Faisalabad, Pakistan with appropriate agronomic practices and irrigation systems. Field experiments are designed according to randomized complete block design (RCBD) with three replications.

Measurement of plant architecture and physiological traits under low and high N environment

Plant architectural traits were measured at the boll opening stage i-e plant height and number of sympodial branches, physiological and biochemical traits, and root growth-related traits.

Analysis of components of phenotypic data using statistical tools

The variation in phenotypic data was analyzed using the procedure of the Mixed Linear Model of software SAS 9.2.

Sampling and DNA extraction

A sampling of young leaves was done to extract DNA following an optimized protocol. (CTAB method

Gene search

For the identification of candidate genes, physical positions of SNP loci associated with significant trait was used.

RNA isolation

Total RNA was extracted from young leaves by Plant RNA Purification Kit.

Reverse transcription-polymerase chain reaction (qRT-PCR) for gene profiling

For qRT-PCR, cDNA was synthesized by reverse transcription procedure and the expression level of candidate genes was determined. The $2^{-\Delta\Delta CT}$ method was used to estimate relative gene expression levels.

Virus-induced gene silencing (VIGS) assay

A virus-induced gene silencing assay was conducted to study and confirm the functions of the identified genes. qRT-PCR was performed to check and confirm the effects and expression levels of silenced genes.

RESULTSAND DISCUSSION

Genotypic variations in the morphology and physiology of cotton plant under low and high N application

All the result is based on the findings of data from other crops and model plants. The experiment observed the symptoms of N-deficiency were definite in genotypes N-inefficient in comparison with genotypes N- efficient. Plants that matured in less-nitrate concentration were short in height with enormous systems of roots in comparison with those developed in high-nitrate and moderate absorption.

To appraise these changes, several morphological traits are analyzed. Under the low-nitrate (0.5 mM) and zero

(0 mM) absorptions, the length (cm) of the shoot, amount to dry matter, and single leaf markedly reduced; anyhow, the decrease was more prominent in N-inefficient genotypes in comparison with N-efficient genotypes. The morphology of roots under diverse nitrate absorptions among contrasting N-efficient genotypes of cotton was compared to that is grown in hydroponic cultures. Enhancement in nitrate absorptions in the medium enormously hindered the development of roots as determined by little surface fields, smaller root height, or quantity of root in comparison to those plants which grow in low and moderate-nitrate concentrations. (Figure 03)

Genotypic variations in the traits of NUE

Different concentrations of nitrate approximately afflicted accretion or N concentrations in the plant of cotton. Commonly, low-nitrate concentrations or under no, compelling depletion in accretions or under no were noticed.

Identification of candidate QTL/s for NUE

This study showed the momentous concentration of nitrate, genotype, and interaction between the concentration of (nitrate concentration × genotype) nitrate and genotype holdings. The concentrations of nitrate influenced every trait identified with an absolute variation of 13%–99%.

Variations in morphophysiological quantities encompassed by cotton genotypes

The cotton plant showed behavioral diversity in return to several levels of equipped N. As roots of the plant perform an important part in adjusting Nitrogen accessibility changes and disturbs the plant to change the root system. In Arabidopsis and exceptionally matched variation in the root, morphology happened extensions in response to several levels of Nitrogen accessibility. Furthermore, photosynthesis is very hypersensitive to the diversity in Nitrogen availability because in chloroplast leaves 57% of nitrogen is placed and used for the blending of associated enzymes and photosynthetic entrails. The photosynthetic capability is emphatically correlated with the leaf Nitrogen content, and a positive correlation was acclaimed between the activity of photosynthesis and N concentration.

Variations in N consumption are firmly related to NUE

The NUE expansion is essential to achieve high productivity with a comparatively low nitrogen supply. Therefore, to understand the traits and processes that contribute to NUE, the best approach is to analyze the genotypic feedback under different N conditions. Shoot dry weight was used to identify genotypes with excellent NUE values, which are already known for the tomato cultivars were described. In addition, large genotype-dependent differences have been claimed in the basic enzymes that regulate N metabolism: conversion to glutamine and glutamate, amino acids, and other N compounds are synthesized. N-assimilating enzymes play a central role in the complex matrix of plant N metabolism. Nitrate is taken up by the roots and transported to the shoots. Nitrate reductase (NR) reduces nitrate to nitrite. Nitrite is then reduced to ammonium by nitrite reductase (NiR). Ammonium is then incorporated into glutamate through the action of glutamine synthetase (GS)/glutamate synthase (GOGAT). Glutamate dehydrogenase (GDH) provides 2 OG to the TCA cycle during carbon starvation. The amino acids are equipped in such a way that they sink into the utilization of proteins synthesized in the source as an N source.

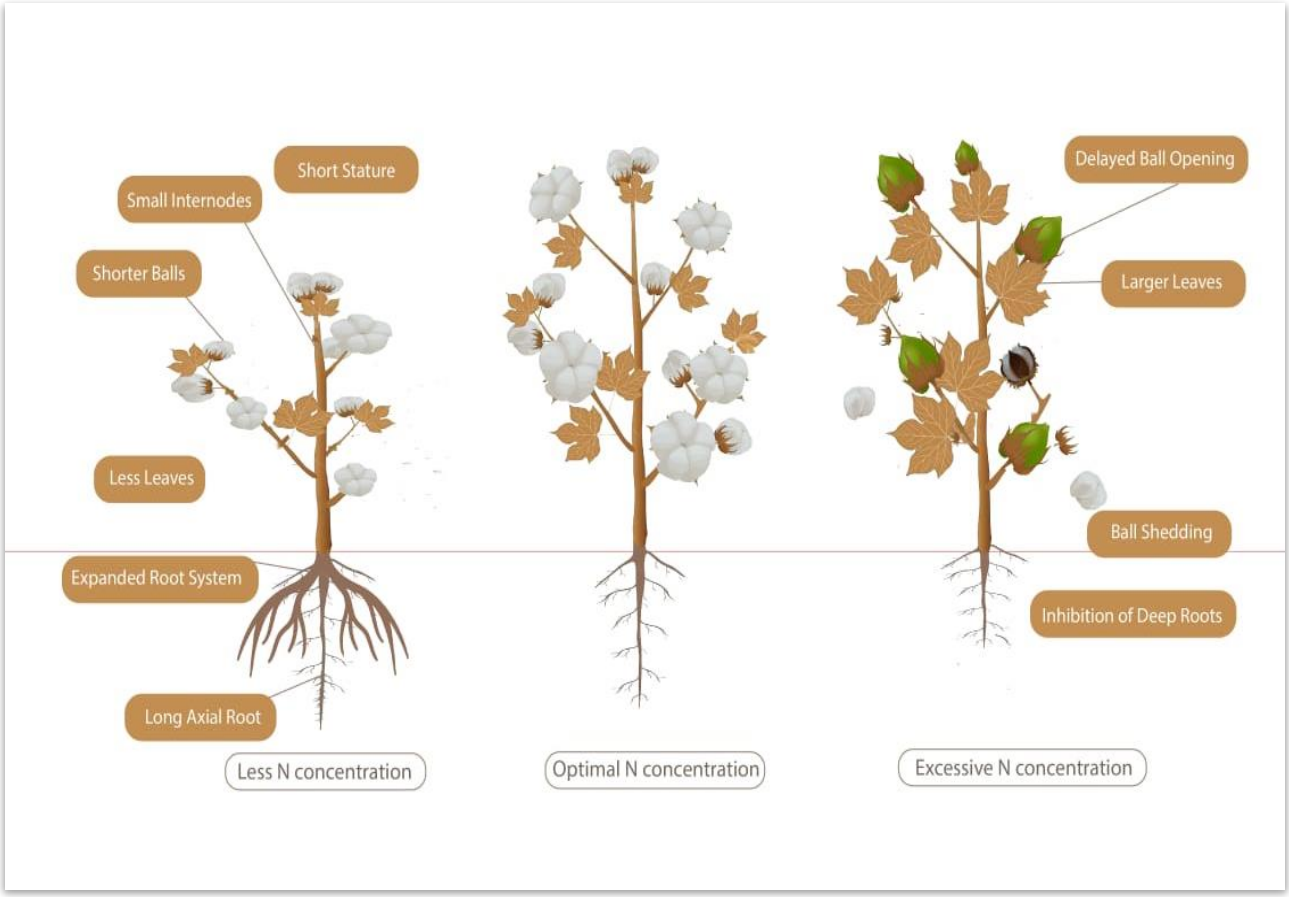

Figure 02: Plants that matured in less-nitrate concentration were short in height with enormous systems of roots in comparison with those developed in high-nitrate and moderate absorption. To appraise these changes, several morphological traits are analyzed. Under the low-nitrate (0.5 mM) and zero (0 mM) absorptions, the length (cm) of the shoot, amount to dry matter, and single leaf markedly reduced; anyhow, the decrease was more prominent in N-inefficient genotypes in comparison with N-efficient genotypes. The morphology of roots under diverse nitrate absorptions among contrasting N-efficient genotypes of cotton was compared to that is grown in hydroponic cultures. Enhancement in nitrate absorptions in the medium enormously hindered the development of roots as determined by little surface fields, smaller root height, or quantity of root in comparison to those plants which grow in low and moderate-nitrate concentrations.

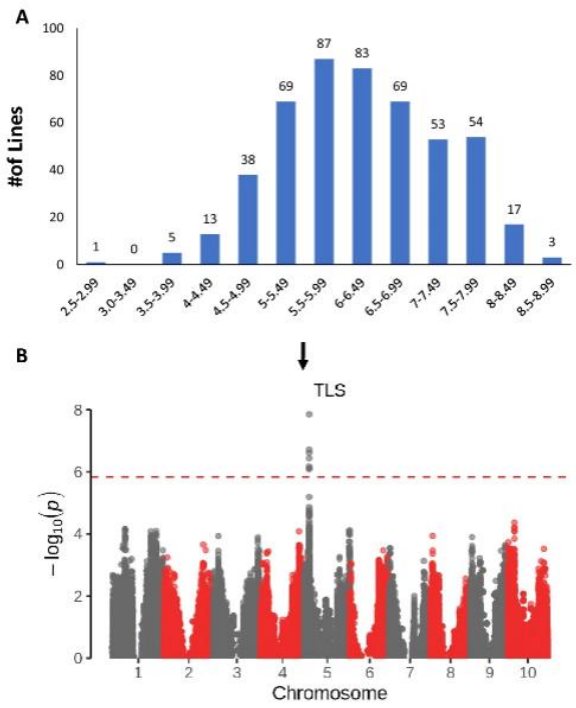

Fig 03. Significant SNPs are indicated by arrow using Mixed Linear Model of software SAS 9.2.

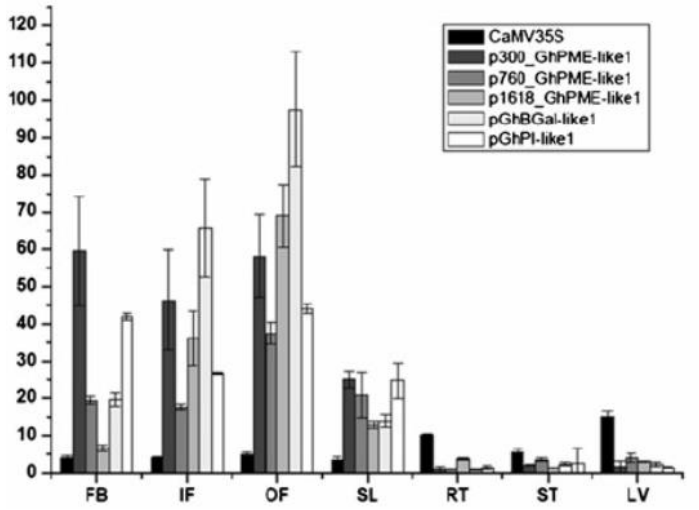

Fig. 04. The $2^{-\Delta\Delta CT}$ method used to estimate relative gene expression levels,as measured by qtPCR.FB;Flower Buds,IF; Inflorescense, OF; Open Flowers,SL; Siliques, RT; Roots, ST; Stems and LV; Leaves.

CONCLUSION

This study concluded that understanding the elements responsible for NUE, is valuable for improving crop production, and therefore, identification of genetic variations and genes controlling NUE is necessary. Among these components, genetic variations in Plant height, number of sympodial branches, and number of bolls bearing seeds in response to N application in the cotton plant have not been studied extensively and remain poorly understood. This study was conducted for architectural components of upland cotton including Plant height, number of sympodial.

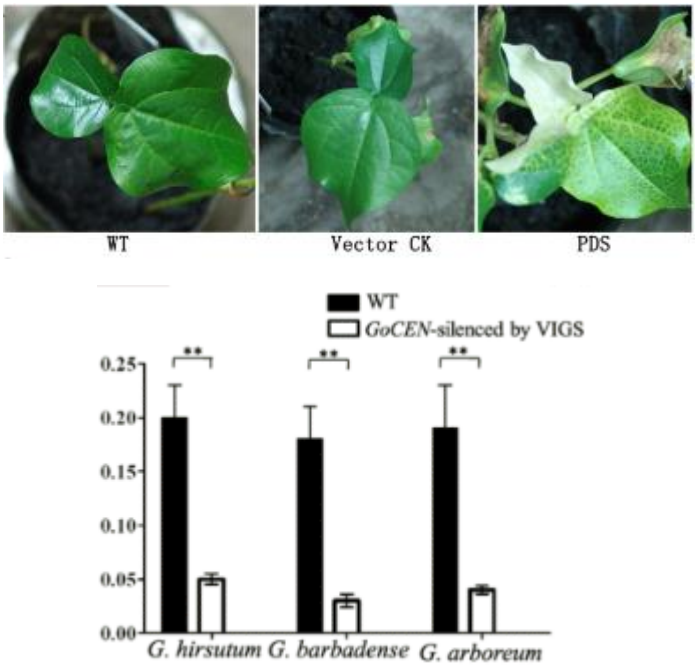

Fig 05. On x axis, relative expression is shown and on y axis, different genes are shown. qRT-PCR was performed to check and confirm the effects and expression levels of silenced genes.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENT

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AUTHOR CONTRIBUTIONS

Muhammad Usman and Muhammad Arslan collected the Diversegermplasm of upland cotton from different germplasm and arrange their categories. Field trial and phenotyping were done by Hafiz Khawar.DNA extraction was done by Muhammad Hussnain Babar. KanzaBatool,RehmatKabir and Israr Ahmad have done the Statistical analysis such as qRT-PCR and VIGS Assay was performed by Muhammad Hasnain.Funding was done by Muhammad Amir Muawiya and Mohammad Aslam and, gene search was performed by Aqsa Qurban.

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