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Modern High-Biotechnologies for Improvement of Superior Fibre, Productive and Early Maturing Upland Cotton Cultivars

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Worldwide agricultural production is significantly suffering from day-to-day worsening environmental situations because of land degradation, decreased level of crop genetic diversity, increased biosecurity threats and global climate change. This has decreased soil water availability, increased heat stress of plants, and altered the development cycles of crops. Soil salinity and drought stress accounts for large reductions in the yield of a wide variety of crops worldwide. The area of salt-affected land is

very large, and rapidly increasing due to increased irrigation, farming practices in arid zones, and global warming. There is a noticeable downward trend in cotton production during the past decades that is due to genetic (narrow genetic diversity), environmental (drought, heat, salinity, etc.) and a number of policy factors (food security, low fibre price, etc.). In particular, the decrease in area allocated to cotton sowing is attributed to issues concerning food security and problems with irrigation and soil salinisation.

The main bio-security issue threatening agricultural production, including cotton and wheat, is related to this issue of problematic shortages in irrigation and water deficiencies that will remain a priority danger for Central Asian region and beyond¹. Water deficiency and high temperature, combined with saline conditions, are seriously affecting crop production and yield, and affect the economy on a year-to-year basis. This problem may be significantly aggravated due to global warming².

The utilisation of available freshwater resources for irrigation of cotton plantations causes many ecological problems in the region, including soil salinity and human population health issues, mainly associated with decreasing the level of Aral Sea basin, which has become a global problem for the past several decades³. In that note, most irrigated land is subject to natural salinisation and cropland suffering from secondary salinisation in Central Asia, including Uzbekistan.

Being one of the important cash crops, cotton (*Gossypium* spp.) is the unique, most important natural fibre crop in the world with a significant economic impact. The worldwide cotton industry is estimated at \$500 billion/yr with an annual utilisation of 115-million bales or 27-million metric tons (MT) of cotton fibre valued for \$27 - 29 billion. Additionally, cotton provides a significant food (cottonseed oil) and feed (cottonseed meal, cottonseed hulls and whole cottonseed) source for humans and livestock. Compared to other many field crops including tropics such as soybeans, corn, maize, rice and wheat (with a net return of \$312, \$234, \$138, \$126 and \$116 per hectare respectively), the average net return from cotton is significantly higher and valued for US\$415 per hectare⁴.

Genome of allopolyploid cotton (*Gossypium* ssp.) is poorly studied and cotton lags behind many crop genomics and genetics as well as marker-assisted selection (MAS) due to existence of low molecular polymorphisms among cultivar germplasm caused by a 'genetic bottleneck' during cotton domestication. There are two tetraploid species of cultivated cotton grown, *Gossypium hirsutum* (so called Upland cotton) and *Gossypium barbadense* [so called Extra Long Staple (ELS) or Pima/Sea Island cotton]. Upland cotton cultivars are grown over 90% the world cotton area because of their productivity and early maturity and moderately good fibre properties. ELS cotton cultivars, however, is grown in only 5% worldwide. Although ELS cultivars produce very fine fibre qualities, their productivity, and other key agronomic properties are poorer than Upland cottons. Improvements of fibre quality of Upland cottons like what is in ELS varieties without affecting early-flowering, early-maturity and productivity is a very difficult task using conventional breeding methods, because it has been observed that normally negative correlations exist between major fibre quality and yield or maturity traits. It is important and imperative to develop Upland cotton cultivars with increased yield, early maturity while producing longer and stronger fibres to be competitive in the global market and over synthetic fibres. However, traditional breeding successes have been minimal over 100 years of worldwide breeding efforts that suffered from linkage drag and distorted segregation in interspecific hybrid progenies from Upland and Pima sexual crosses. This prompted the cotton community to develop an "innovative new generation crop technology" to address this largely eluded and fundamentally longstanding challenge in worldwide cotton improvement programs⁵.

This issue was even more challenging due to a global climate change coupled with accelerated rise in atmospheric carbon dioxide (CO₂), shifted vegetation period and crop cycle, and increased biological and

environmental threats, that is expected to be negatively impacting world agricultural farming including cotton and many other crops. Similar challenges of negative correlation between productivity/maturity and crop quality as well as a narrow genetic diversity do exist in many crop species of world agriculture, where a solution would provide an opportunity to benefit more from marginal agricultural production of high quality in shorter vegetative periods that helps to save land resources and environment. Here it should be noted that based on 11,275 approved field trials for genetically engineered (GE) crops that covered more than 20 years of research and 13 years of commercialization, helped to have no boost of intrinsic (or potential) yield of agricultural crop with marginal 3-4% operational yield gains. This underlies the necessity for novel biotechnology tools to solve these outstanding problems of yield increase, in particular intrinsic yield improvement in all crops⁵.

We took innovative approaches to timely overcome above-mentioned obstacles in agriculture in an example of cotton research. We added new results to the world literature on genetic mapping of complex agriculturally important complex traits such as early leaf defoliation, lint percentage, photoperiodic flowering, wilt disease resistance and major fiber quality traits. Further, our team analysed a global set of 1000 accessions of worldwide Upland cotton germplasm from Uzbekistan collection, and for the first time estimated "yet unknown" linkage disequilibrium (size of recombination blocks; LD) in complex poorly characterized cotton genome. A genome-wide averages of LD extended up to genetic distance of 25 cM at $r^2 > 0.1$ and ~5-6 cM at $r^2 > 0.2$ in variety germplasm. Genome wide LD at $r^2 > 0.2$ was reduced on average to ~1-2 cM in the landrace stock germplasm and 6-8 cM in photoperiodic variety germplasm, providing evidence of the potential for association mapping of agronomically important traits in cotton. Results suggest that linkage, selective sweeps, inbreeding and genetic drift as the potential LD-generating factors in cotton⁵.

Efforts resulted in association mapping of major fibre quality traits in two globally diverse environments of Uzbekistan and Mexico using mixed liner model (MLM), considering both kinship (K) and population structure (Q) to minimise spurious associations. LD-based association mapping was found to be effective in cotton. Efforts provided first insight into understanding environment-specific functions of genes controlling fiber development that increases the effectiveness of cotton marker-assisted breeding programs in similar latitudes. This study demonstrated that successful application of genetic association analysis using large numbers of populations accelerates the discovery rate of gene and quantitative trait loci (QTL) alleles, tagging



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useful genes and biologically meaningful markers useful for marker-assisted selection (MAS) of cotton⁵.

Most importantly, these pioneering investigations, for the first time, resulted in designing and completing a successful MAS programs in Uzbekistan. We succeeded in creating modern MAS breeding platform for improving complex cotton fibre quality traits of any Upland variety in a short-term using DNA markers and donor lines as well as prepared new generation molecular breeders. MAS concept and platform developed through examining a global set of worldwide Upland cotton germplasm resources provided opportunity to utilise “yet untapped” QTLs from cotton germplasm that widened the genetic background of commercial cotton cultivars conventionally suffered by a narrow genetic diversity⁵. This should increase adaptability of commercial cultivars to the harsh environmental abiotic and biotic stresses in the era of global climate change and warming as well as global biosecurity threats of crops in the era of global technological advance. To address crop biosecurity more specifically, efforts are in progress in our laboratory to breed, for instance, highly Fusarium wilt disease (FOV) resistant MAS cotton varieties keeping superior fibre quality, maturity, and productivity using molecular breeding.

In particular, the first generation novel and high impact MAS cotton cultivars, named as “Ravnaq” series (translates as “Advance”), bear novel and “yet-unused in Uzbek cotton breeding” fibre quality QTLs and have improved cotton fibre quality from coarse types of 4-5 or from code of 35-36 (typically marketed worldwide) to the finest type of 2-3 or code of 38-39 fibre. These improvements add higher premium price for each pound of fibre that should bring significant economic income and help to benefit more, therefore, if necessary, may allow cut the acreages engaged to cotton saving the land resources. Modern MAS breeding platform significantly cuts breeding period from traditionally required 10-12 years of tedious traditional breeding with minimal success to 3-4 years for improving cotton fibre qualities of any Upland variety with keeping early maturity and high yield characteristics - helping to save resources and accelerated solving of any key issues in breeding. This demonstrated the power of using MAS DNA marker panel and novel QTL-bearing donor genotypes in cotton breeding. This helps to decrease the cost and resources needed for breeding that accelerate cotton-breeding efforts worldwide.

Currently “Ravnaq” MAS cultivars are being tested by State Variety Testing Committee of Uzbekistan across different cotton growing soil-climatic zones of the country with the aim of its commercialization. MAS cultivars has passed 2-years

of large field evaluations in 2013 and 2014 seasons and have already proved their genetic and agronomic superiority to conventional Upland cultivars. It is expected that after completion 3rd year of field evaluations in 2015/16 season the first generation MAS cotton cultivars will be considered for a large-scale cotton farming.

Secondly, to develop cotton biotechnology and produce Uzbekistan’s own biotech cottons, we put specific effort to de novo characterize important cotton gene families and sequence signatures involved in cotton photomorphogenesis and flowering, fibre and seed development, root development, and in plant defense and disease resistance. We have established an efficient high-through put cotton tissue culture and transformation system in order to study newly cloned gene functions. For instance, because of multiple gene effects in plant development, yield potential and productivity, plant flowering and architecture, salt tolerance, cold/freezing and drought tolerance in model plant *Arabidopsis* and fungal disease resistance in rice, for the first time, we characterised cotton phytochrome gene family. Our team genetically associated cotton phytochrome and its signal transduction factors to cotton fibre quality, yield potential, and maturity traits in his genetic mapping and targeted RNA interference studies^{5, 6}.

PhytochromeRNAi study provided the first molecular evidence of importance of the phytochrome gene family in cotton fibre development and demonstrated the role of phytochrome-specific RNAi, simultaneously improving several important agronomic (e.g., early maturity, high yield) and fiber quality traits (length, strength, fineness, elasticity, and uniformity) in somatically single-cell regenerated RNAi Coker 312 cotton plants. Previous studies in model plant *Arabidopsis* have shown physiological consequences associated with modulation of expression of phytochromes and cross-regulatory effects as the manifestations of a compensatory regulatory network of phytochromes. Our results sharply contrast with findings from *Arabidopsis* in which loss-of-function *phyA* mutations showed no increase in *PHYB* expression. *PHYA1*RNAi cotton lines with 70% decreased level of *PHYA1* expression showed increased transcript levels for *PHYA2*, *PHYB*, *PHYC* and *PHYE*. These observations indicate that the phytochrome regulatory network of cotton may have a fundamentally different dynamic architecture than that of *Arabidopsis*^{5, 6}.

Our effort proved that RNAi of cotton *PHYA1* gene generated agronomically useful phytochrome-associated RNAi phenotypes in somatically regenerated RNAi Coker-312. Efforts improved fibre quality (38-40 mm fibre length versus 29-32 mm), micronaire (3.9-4.2 vs. 4.9-6), early maturity



Figure 1. General field and cotton bush view of novel RNAi cultivars series "Porloq" grown and field trialed in 2014 season in Uzbekistan.

(for 5-10 days early) and higher seed cotton yield (~10-18% higher) with developed root system (two times longer) and better adaptation to salt/drought and heat conditions. A concept of this technology demonstrates a great potential to develop superior cultivars in a globally important crop species in a short time without any adverse effect on yield and other desirable agronomic traits^{5, 6}.

Using this state-of-art PHYA1 RNAi concept and results, we developed the first generation novel generation GE cotton variety series "Porloq-1", "Porloq-2", "Porloq-3" and "Porloq-4" (translates to "Great future"; Figure 1). These RNAi cultivars have successfully passed three years (2012-2014) of extended field trials across 13 different soil-climatic regions in Uzbekistan⁶⁻⁸. Results of field trials demonstrated superiority of RNAi cultivars to any traditional Uzbekistan varieties both in terms of fiber quality, adaptation to harsh environmental conditions across Uzbekistan, early maturity and significant increase in seed cotton yield or production of average lint fibre. It is noteworthy to mention this here that these first generation RNAi cultivars developed from application of state-of-art RNAi technology concept, to the best of current knowledge, are the world's first biotech cotton with improved fibre quality and other agriculturally important characteristics. This is also the first example of successfully field trialed RNAi cotton cultivars worldwide.

The state-of-art novel RNAi cultivar series "Porloq" demonstrated a huge impact⁶⁻⁸ and superiority of these GE cultivars to any traditional Uzbekistan cotton varieties in terms of fibre quality (38-41 codes vs. 35-36 of ordinary). Further, the superiority is evidenced by adaptation to harsh

environmental conditions (salt and drought/heat stress) across Uzbekistan, early maturity for 5-10 days, and at least 10-18% increase in seed cotton yield or production of average lint fibre over 1000 kg per hectare versus current estimate of ~800 kg/ha in Uzbekistan. RNAi cotton varieties yield more cottonseed with 25-30% increase in 1000 seed mass providing an opportunity to increase food and feed products.

Production of "yet not existed in world fibre market" novel Upland cotton fibre with 38-41 code (versus possibility of production of fibre with only maximum 35-37 code from existing ordinary Upland cottons) would have a premium price and increased in income per acre in Uzbekistan and worldwide⁸. Increased yield and fibre quality improvement of RNAi cotton varieties should allow for expanded cotton production on marginal land and create a new cotton fibre/cloth market. This would provide opportunity of planting more other food crops ensuring food security of people and sustainability of the environment suffering from cotton production and application of agricultural chemicals. A patent⁹ on the new RNAi technology was filed in Uzbekistan (IAP: 20120069) and USA (USPTO: 13/445696) and internationally (PCT application; National phase applications in India, China, Egypt and Russia) the technology is ready for licensing to other cotton growing countries.

Differing from existing transgenic cottons and technologies, the phytochrome-specific and RNAi based cotton cultivars, bearing only cotton genes, are "ecologically safer and could potentially have a multi-billion-dollar impact on the global cotton industry and help cotton farmers fend off increasing