GMOs' status in the world and in Uzbekistan

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GMO

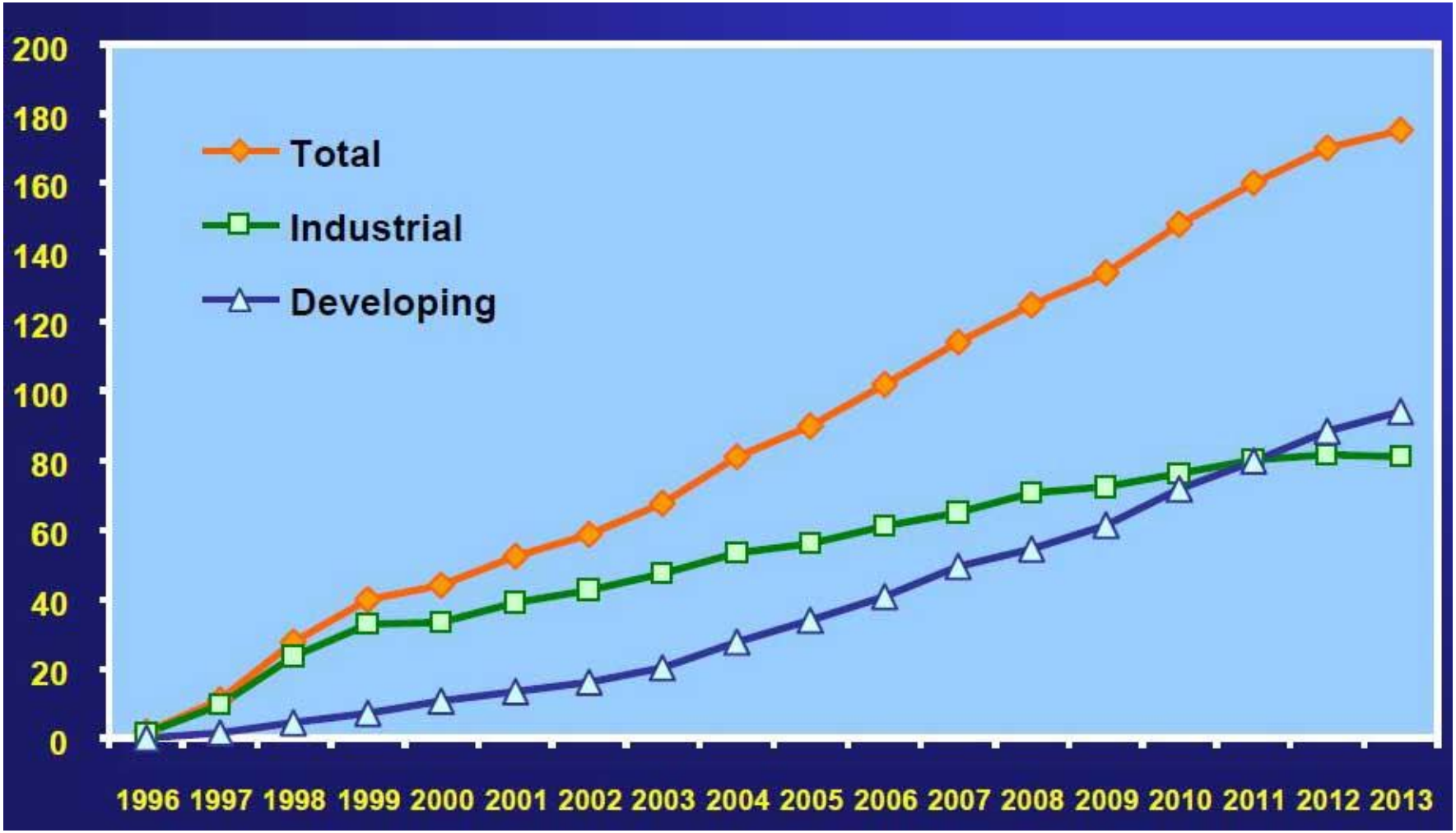
(Genetically Modified Organism) —

is an organism whose genetic material has been altered using genetic engineering techniques

Purpose of Genetic Engineering on Plants

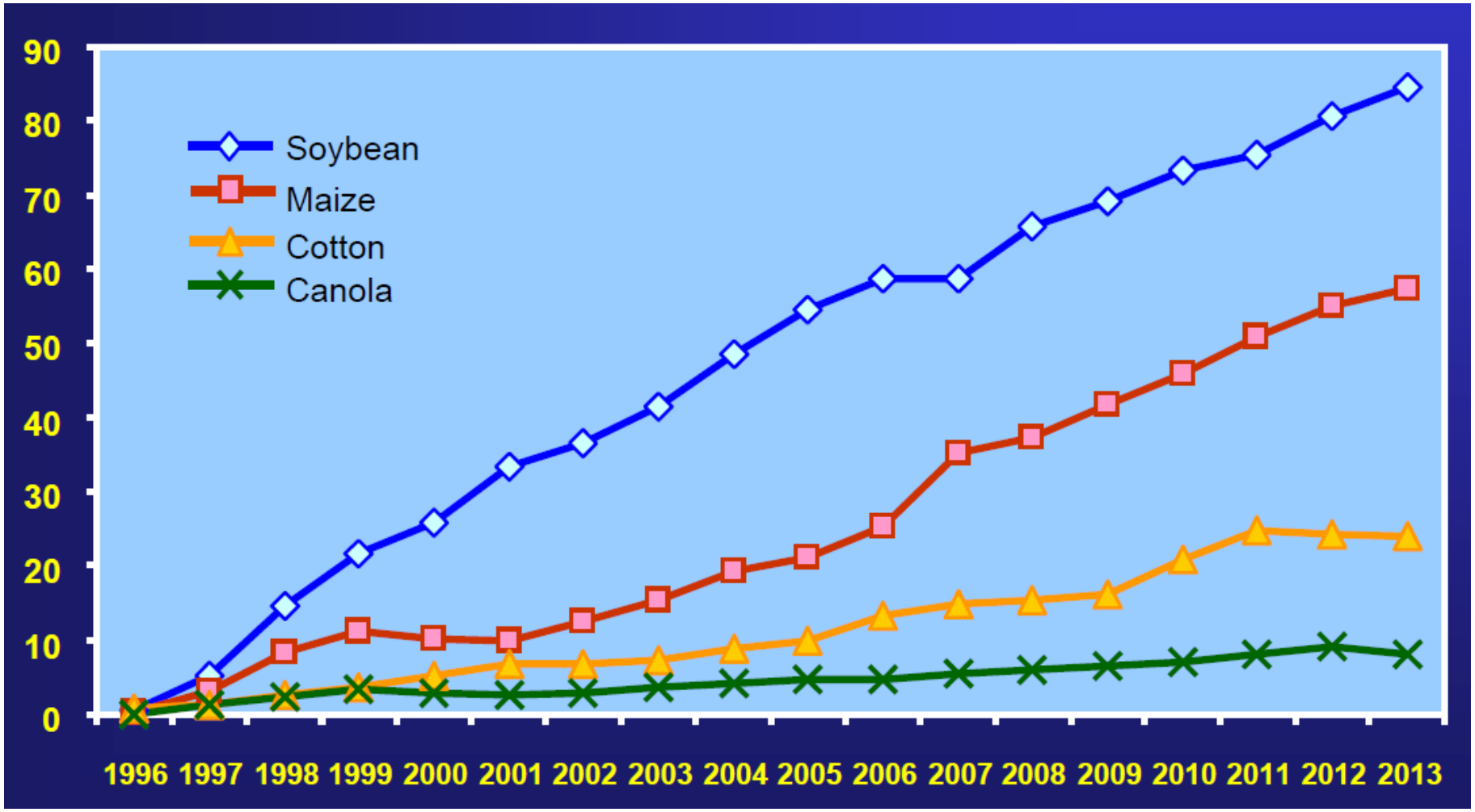
- Resistance to diseases and pathogens
- Resistance to novel herbicides
- Protection against abiotic stress
- Improved nutritional value in different food products
- Increased amount of vitamins in products (golden rice – provitamin A)
- Improved aroma, taste and structure of agricultural products
- Improved yield of agricultural crops
- Improved fiber quality (cotton)

Global Area of Biotech Crops, 1996 to 2013.
175,2 million hectares in 2013



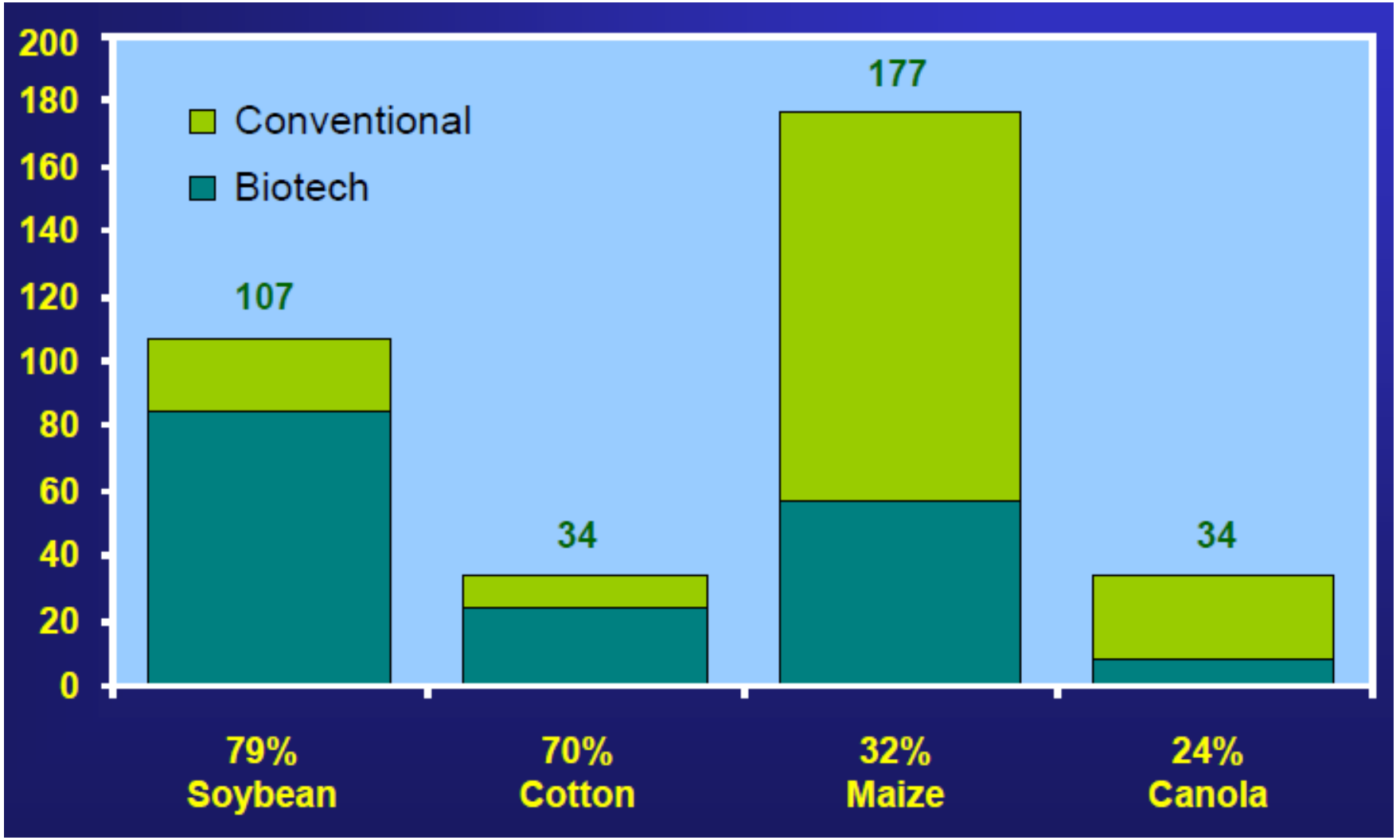
Source: Clive James, 2013. http://www.isaaa.org/

Global Area of Biotech Crops, 1996 to 2013.
By crop (million hectares)



Source: Clive James, 2013. http://www.isaaa.org/

Global Adoption Rates (%) for Principal Biotech Crops (million hectares), 2013



Source: Clive James, 2013. http://www.isaaa.org/

"Transgenics" or "Biotech cotton"

Insect resistant (Bt-cotton) notably affecting the larvae of moths *Helicoverpa* ssp.

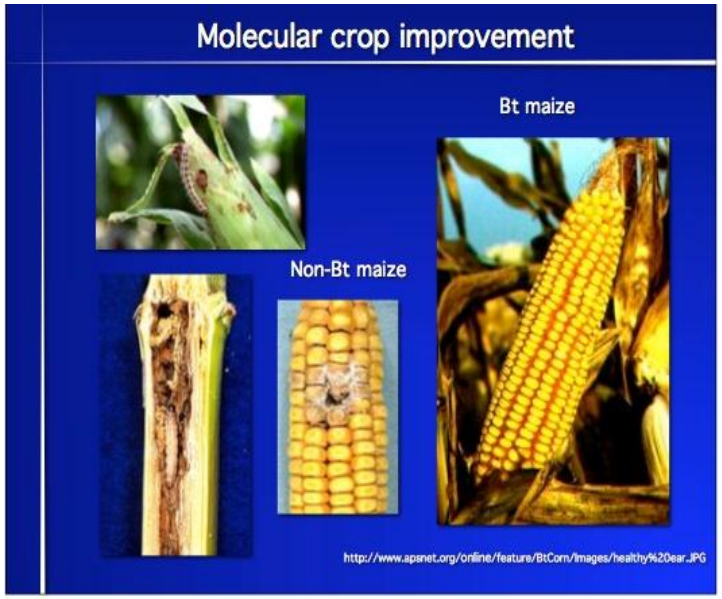
Herbicide tolerant (HT-cotton) providing tolerance to the herbicide glyphosate or BXN gene providing tolerance to the herbicide bromoxynil

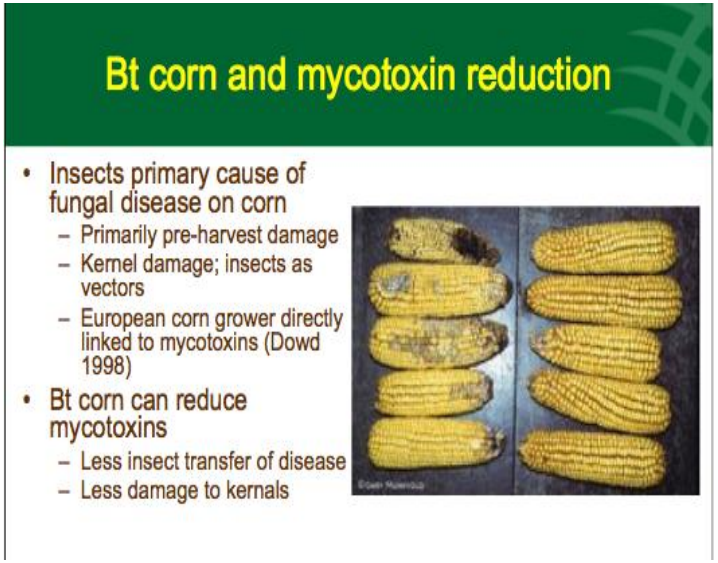
1.7 million hectares in 1996
170 million hectares in 2012

Currently grown by 28 countries and on more than 66% of world cotton area (ICAC report, 2012/13).

Advantages

- Reduced use of pesticides
- Increased yield
- Less use of herbicides
- Quality improvement
- Herbicide tolerance
- Insect resistance
- Virus resistance





The European Commission in a report following a **15 year study** (1985-2000) involving **400 public research** institutions, to the cost of **70 million Euros** stated "… genetically modified plants and products derived from them present **NO RISK to human health or the environment**……these crops and products are **EVEN SAFER** than plants and products generated through conventional processes" (European Commission, 2001).

In a subsequent report covering the next decade the EU commission affirmed this outcome and reiterated: "The main conclusion to be drawn from the efforts of more than **130 research projects**, covering a period of more than **25 years of research**, and involving more than **500 independent research groups,** is that biotechnology, and in particular GMOs, are **NOT** per se more **RISKY** than e.g. conventional plant breeding technologies" (European Commission, 2010).

EC Research, 2001; http://europa.eu.int/comm/research/quality-of-life/gmo; http://www.gmo-safety.eu/pdf/biosafenet/Economidis.pdf. European Commission, 2010; http://ec.europa.eu/research/biosociety/pdf/a_decade_of_eu_funded_gmo_research.pdf.

CURRENT STATE OF WORLD BIOTECH

- **20** years of research
- **13** years of commercialization
- **11275** approved field trials:
  - **3630** field trails for Bt – crops
  - **4626** field trials for HT – crops
  - **139** field trials for bacterial resistance
  - **713** field trials for fungal resistance
  - **51** field trials for nematode resistance
  - **884** field trials for virus resistance
  - **583** field trials for abiotic stresses
  - **652** field trials for yield traits

3-4 % operational yield increase

No increase of intrinsic yield of any crops

Gurian-Sherman, D. 2009. Failure to yield: performance of genetically engineered crops. Union of Concerned Scientists (available at http://www.ucsusa.org/, verified on September 16, 2013).

Genes with More Complex Effects

Photosynthetic genes

Transcription factors

Light perception genes

Genes from cell cycle machinery

Signal transduction factors

Plant hormones

Small RNA and microRNA genes

SIDE EFFECTS????!!!!

APETALA2

FASCIATED EAR2

PHYTOCHROME B

BRASSINOSTEROID

SUCROSE SYNTHASE

AUXIN

MiR156, 319, 396

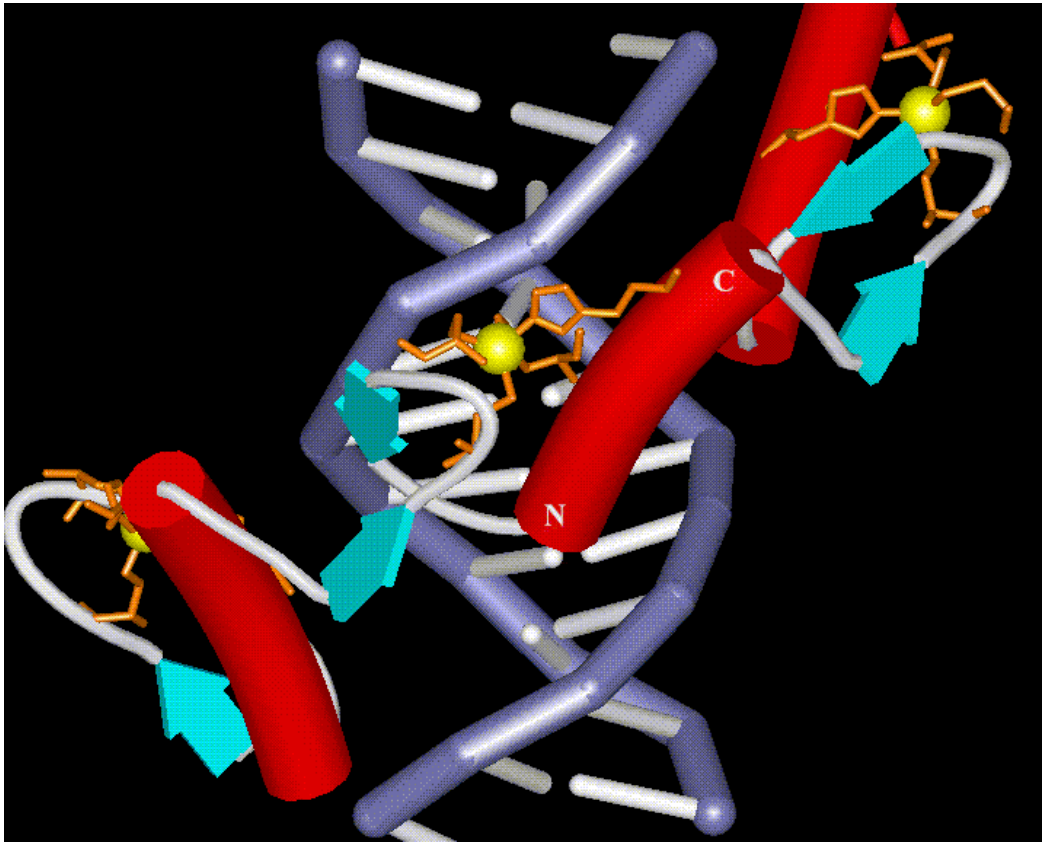
OsSPL14

AtNHX1

CASEIN KINASE

Novel Transgenomics Tools

- RNAi interference
- Anti-sense oligonucleotides
- Artificial microRNAs
- Zinc fingers
- Transcription activator like endonuclaeases (TALENS)



GMO Research in Uzbekistan

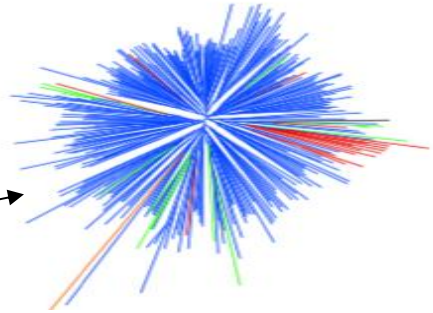
Importance Cotton for Uzbekistan

- **One of the main economic resources**
- **Annually brings an average of ~$0.9 to 1.2 billion economic income**
- **Represented 22% of all Uzbek exports from 2001-2003**
- **Accounts for roughly 11% of the Uzbekistan's GDP in 2009**
- **NO “Biotech Cotton” is cultivated**
- **Lint fibre yield was 753 kg/ha in 2010/11 and estimated at 804 kg/ha in 2012/13 or 812 kg/ha in 2013/14**

Source: http://www.state.gov/r/pa/ei/bgn/2924.htm; Abdurakhmonov 2013, ICAC RECORDER

NEED FOR OMICS VARIETIES

- **Narrowness of genetic diversity of currently grown Uzbek cultivars**



- **Salinity problem**
- **Water deficiency problem**

Current water deficit may further be intensified by over 500%, spreading from 2 km cube in 2005 up to 13 km cube in 2050 (World Bank country note, 2010).

- **Global warming**
- **Crop Biosecurity (e.g., FOV)**
- **Food security**

"Additionally, average annual rainfall may drop by about 10 mm in the highlands and increase by 40 to 50 mm in the desert areas of the country. If no adaptation measures are taken beyond changing planting dates in response to climate change, and taking reduced water availability into account, nearly all crop yields could fall 20 to 50 percent by 2050" (World Bank report, 2013).

Development of Genomics Sciences in Uzbekistan

Visit of the president of the Uzbekistan, I. Karimov to our laboratory:

- **Organization of Research Institute of Genetics**
- **Special assignment to develop cotton genomics research in Uzbekistan**
- **Prioritization of preparation and education highly qualified scientists and**
- **Granting a special ‘hard currency” government funding for investigations, training and research facility modernization**
- **Prioritization and encouragement of building wide range of international collaboration**
- **Prioritization of continual ”matching funds” to international funds received**
- **Prioritization of links between scientific efforts and agricultural production**

Center of Genomics and Bioinformatics

A dedicated research organization for “omics” science

Achievements

- Uzbek Cotton germplasm characterization at molecular level
- DNA barcoding of commercial Uzbek cultivars
- Estimation linkage disequilibrium level
- Genetic (QTL and association) mapping of important agronomic traits
- Successful marker-assisted selection and gene pyramiding programs
- Characterization of novel genes, gene families and small RNAs
- Efficient tissue culture and transformation
- **“OWN” GE cottons targeting multiple effect genes using RNAi**

Uzbek “biotech cotton”

The main objective of world cotton breeding

Low-yield superior fiber quality of Pima cotton (*G. barbadense*), 8% grown in the world

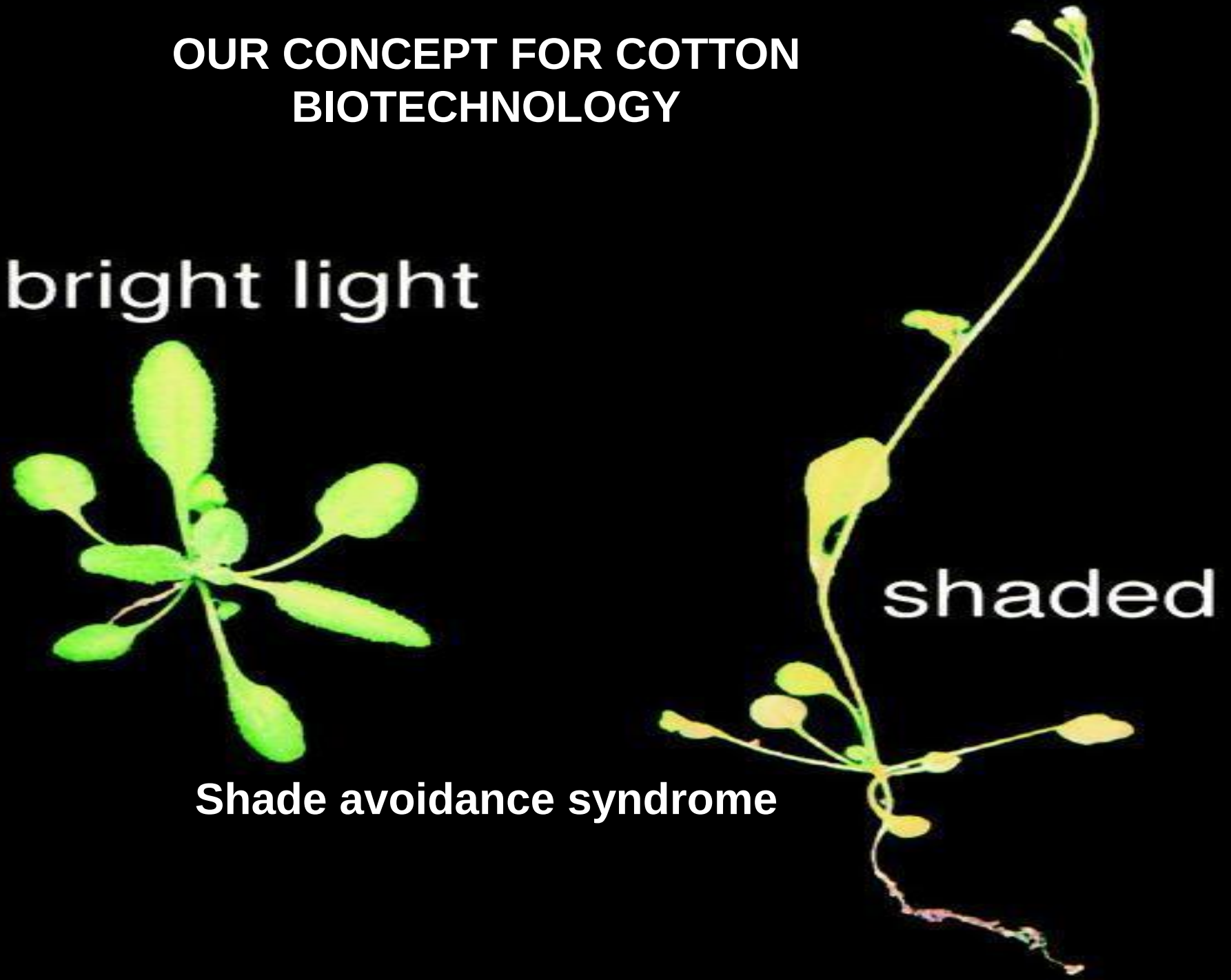
Mobilization of fiber quality genes

High yield middle fiber (*G. hirsutum*) Upland cotton, 90% grown in the world

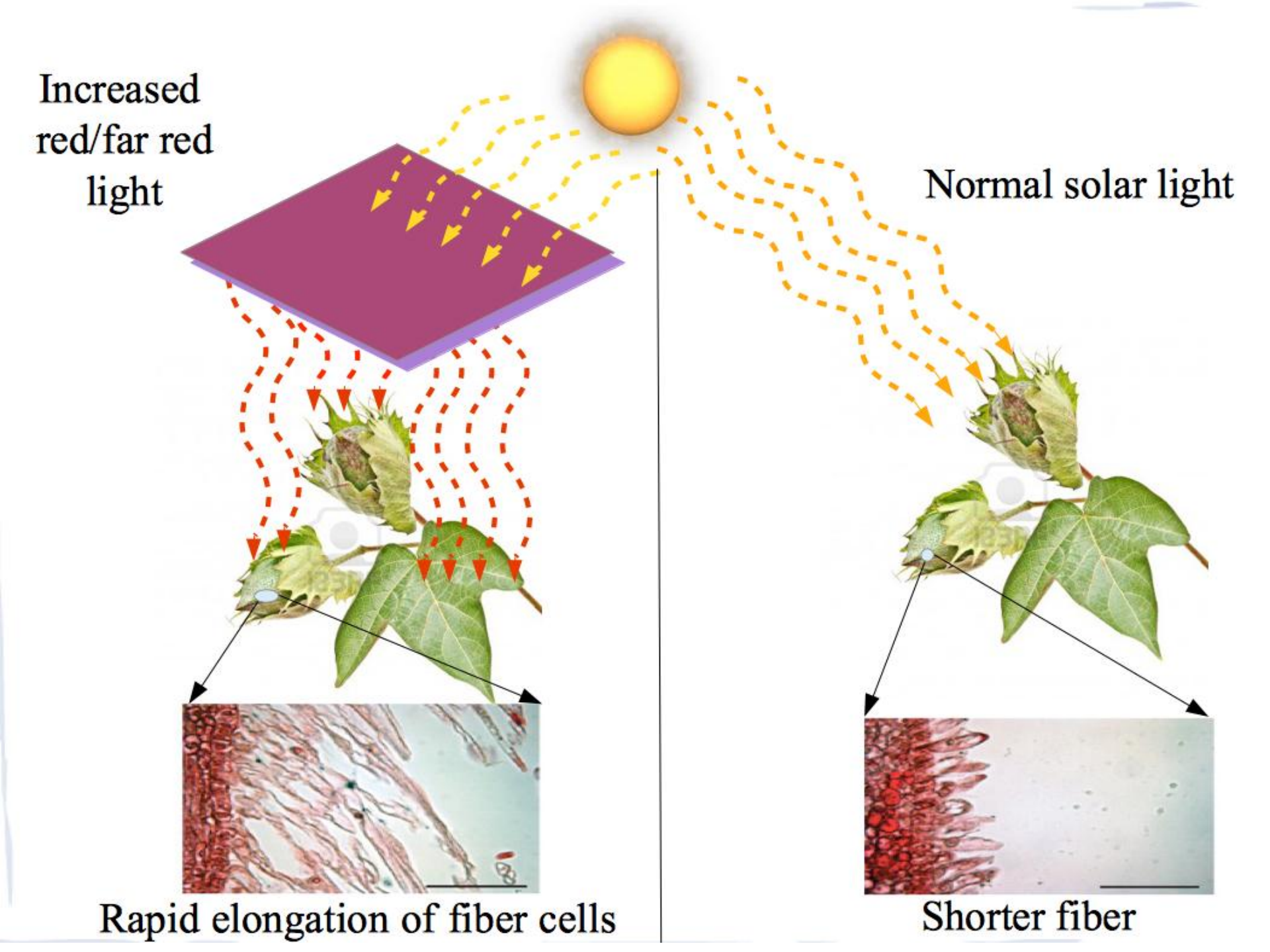
Long-standing conventional problem

A kilogram of superior quality long fiber with good microniare (3.8-4.9) brings 8 to 10 cents more income

OUR CONCEPT FOR COTTON BIOTECHNOLOGY

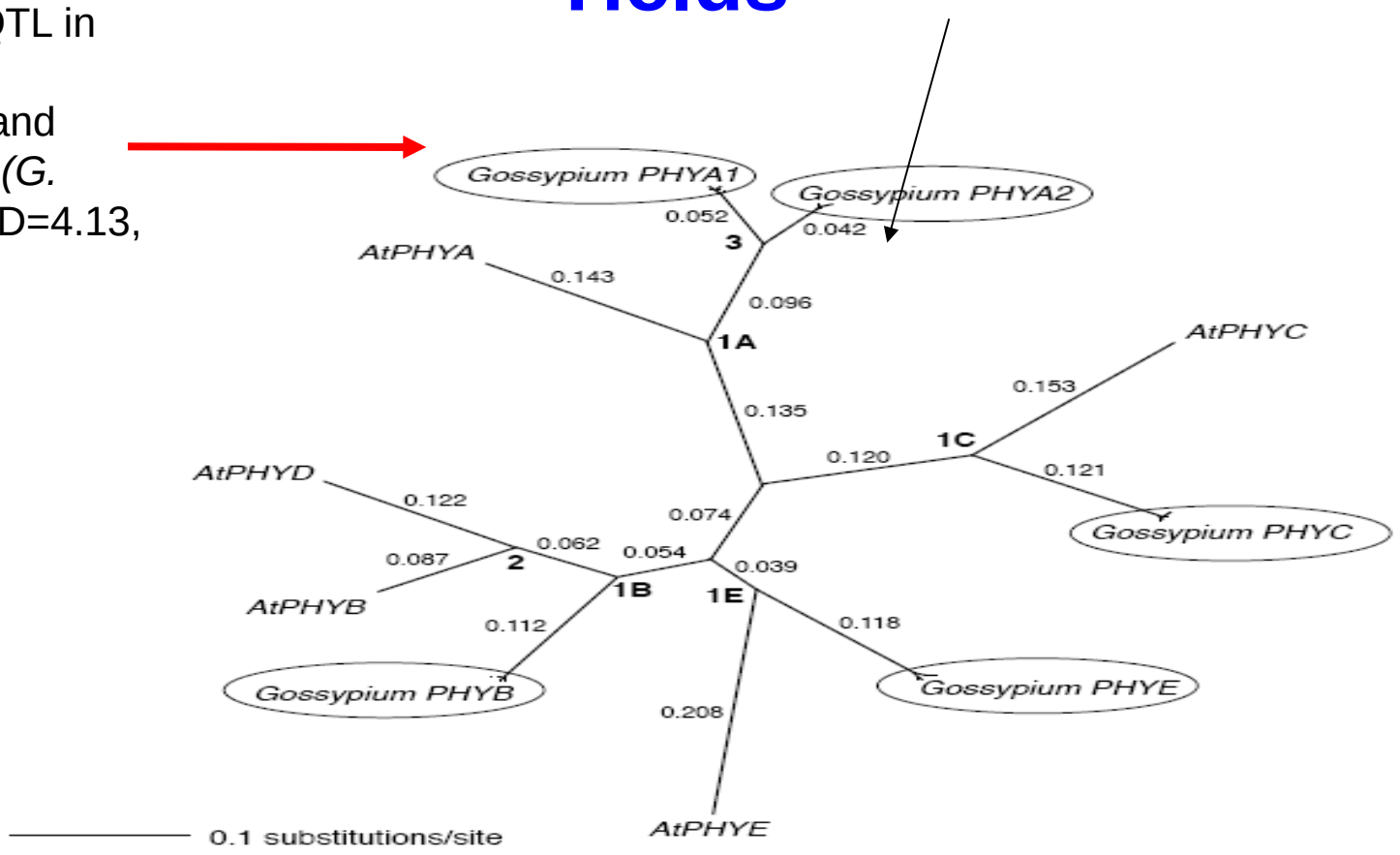


Shade avoidance syndrome

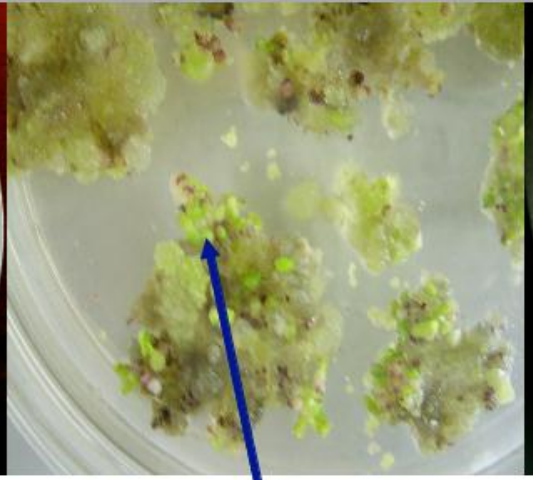

Increased red/far red light
Normal solar light
Rapid elongation of fiber cells
Shorter fiber

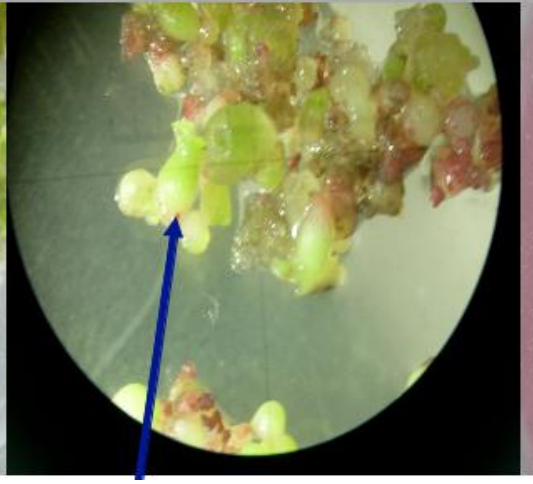
Cotton *PHYA1* to Improve Fiber Quality, Flowering, Maturity and Yields

Fiber length QTL in Pima S-7 *(G. barbadense)* and Tamcot SP37 *(G. hirsutum)* (LOD=4.13, P<0.0001)



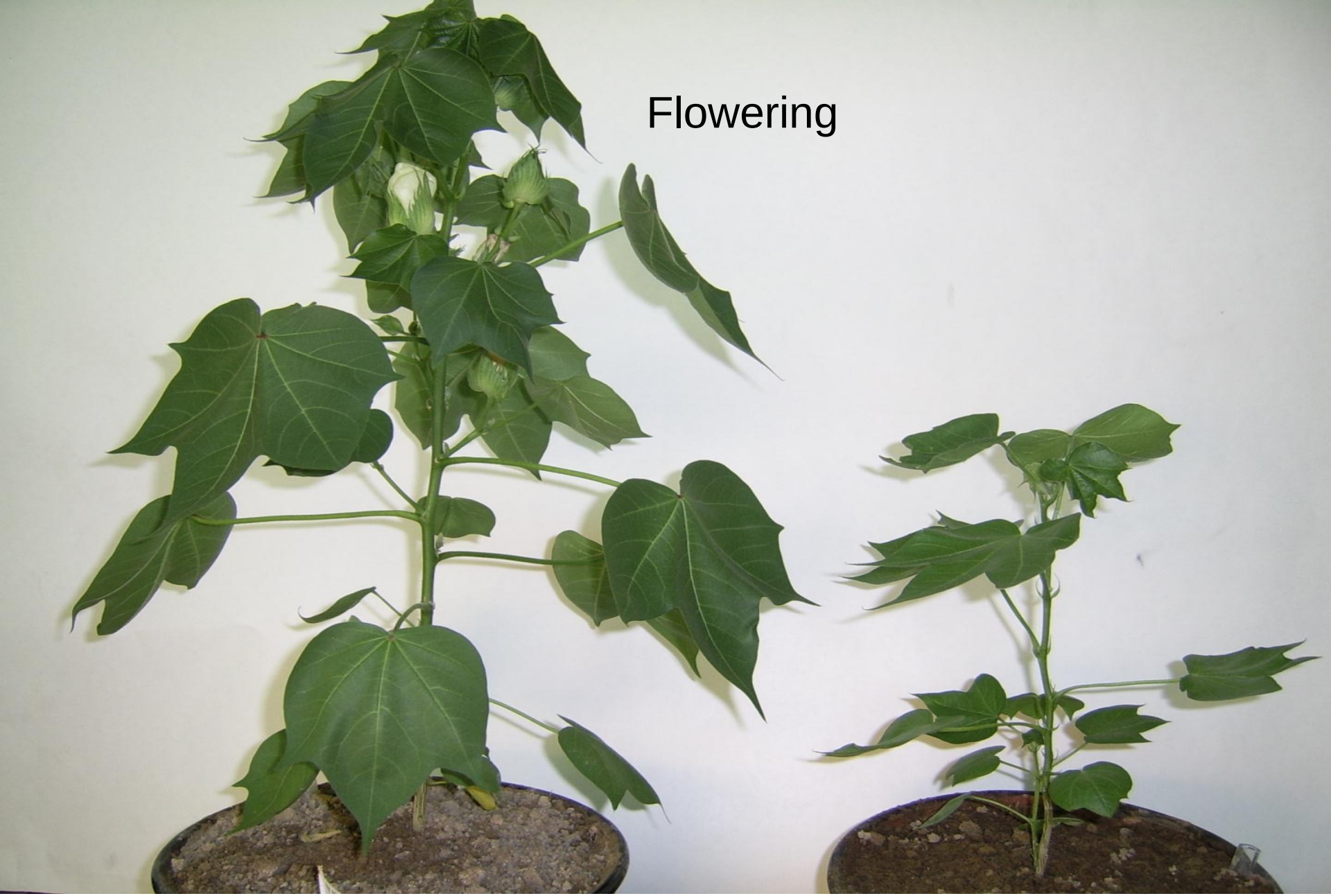
Source: Abdurakhmonov et al. 2010. BMC Plant Biology





Transformation **Callus formation and Somatic embryogenesis**

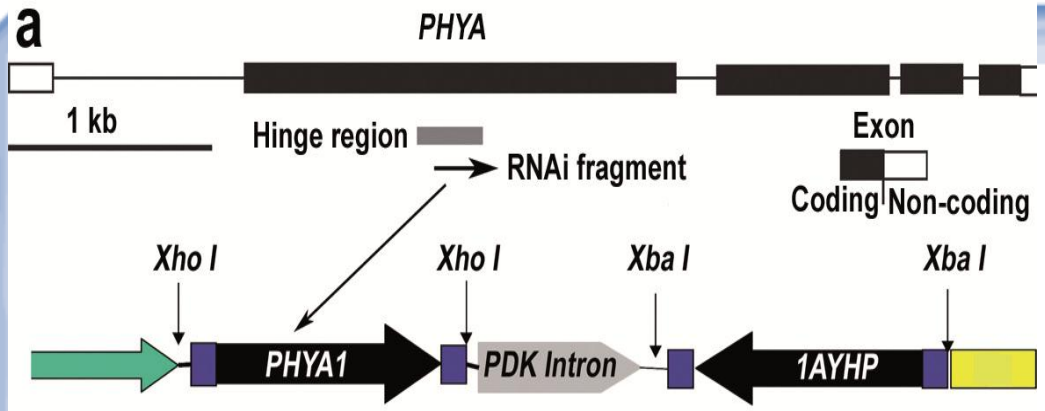
Embryo plantlets **Transferring into pots and fields**

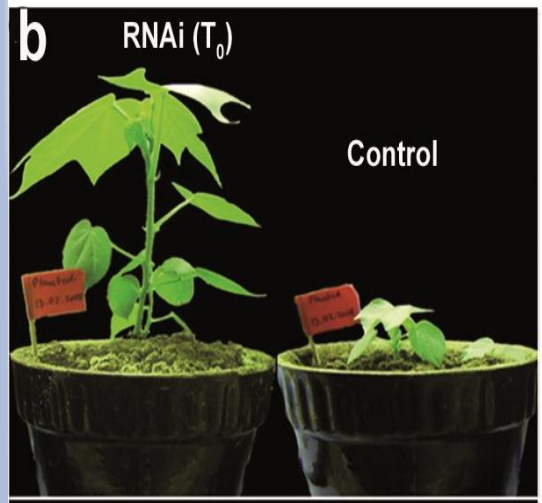


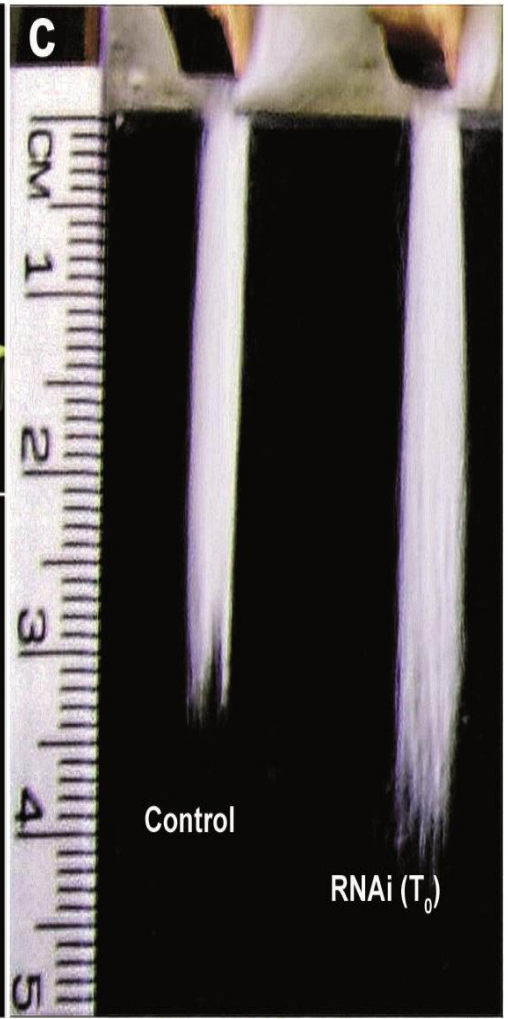
RNAi T0 plants

Control

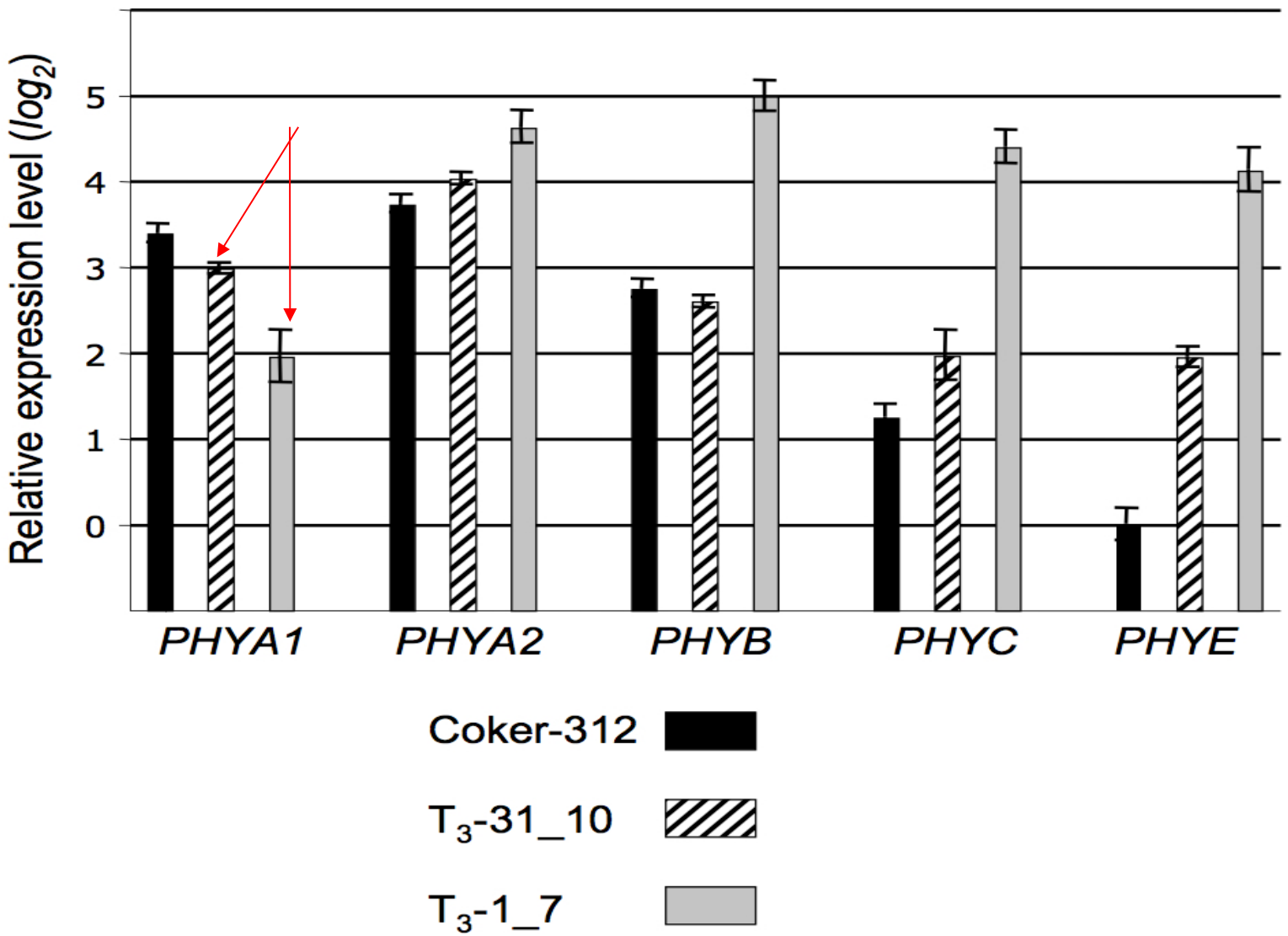
The same day planted

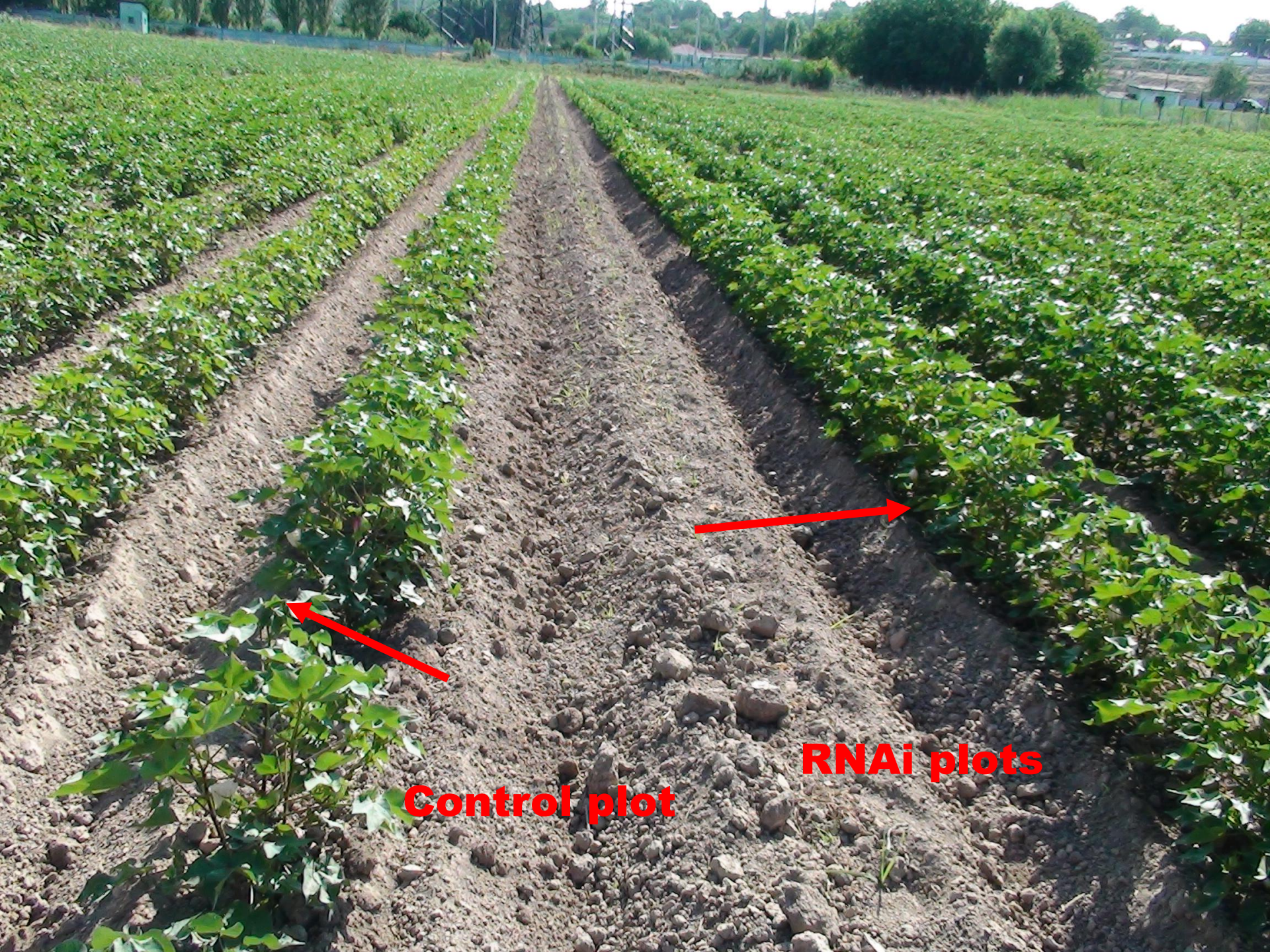

a
PHYA
1 kb
Hinge region
RNAi fragment
Exon
Coding
Non-coding
Xho I
Xho I
Xba I
Xba I
PHYA1
PDK Intron
1AYHP


b
RNAi (T0)
Control


c
CM
1
2
3
4
5
Control
RNAi (T0)

GENE KNOCKOUT LEVELS




RNAi plots
Control plot

Early Boll Maturity and Plant Senescence

RNAi Coker-312

wild-type Coker-312

Cotton Fiber Length in T_{1-3} Generation RNAi

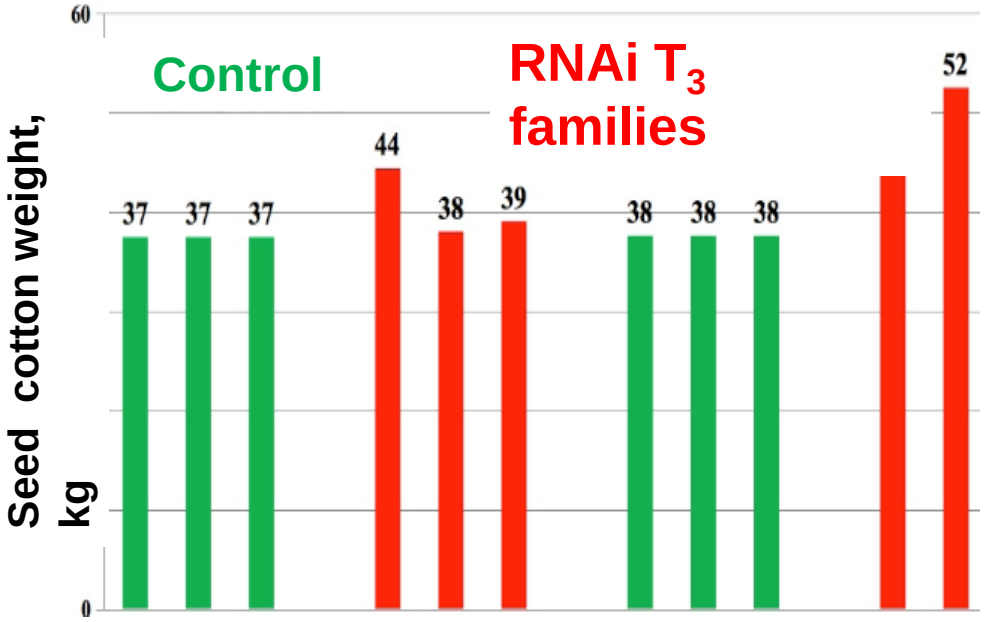
RNAi plants

Controls

Cotton Fiber Characteristics

Traits	T-1 family (RNAi)	T-31 family (RNAi)	Coker-312 (wild-type)	Null segregants (control)
UHM (SE)	1.29 (0.003)^{acd}	1.28 (0.002)^{acd}	1.23 (0.004)^{b}	1.22 (0.006)^{b}
MIC (SE)	4.99 (0.07)^{acde}	5.21 (0.04)^{acd}	5.43 (0.08)^{b}	5.40 (0.13)^{b}
STR (SE)	31.20 (0.28)^{ac}	31.25 (0.13)^{ac}	29.71 (0.37)^{b}	31.01 (0.31)^{b}
ELO (SE)^{s}	9.82 (0.18)^{acd}	10.50 (0.09)^{acde}	9.58 (0.21)^{b}	10.34 (0.38)^{b}
UI (SE)	88.42 (0.46)^{a}	88.06 (0.08)^{a}	87.25 (0.25)^{b}	87.13 (0.39)^{b}
RD (SE)	76.50 (0.26)	77.11 (0.16)	75.70 (0.28)	77.41 (0.99)
+B (SE)^{s}	8.97 (0.11)^{d}	9.47 (0.08)^{de}	9.51(0.22)	8.60 (0.18)

Improved Cotton Yield Potential with *PHYA1* RNAi



Seed cotton yield in 0.01 hectare land plot (700 plants)

18-20% higher yield

Transferability of RNAi Effects

[RNAi Coker-312 x local cultivar] x local cultivar

AN-Boyovut-2

Porloq-1

AN-Boyovut-2

Porloq-1

Novel RNAi varieties

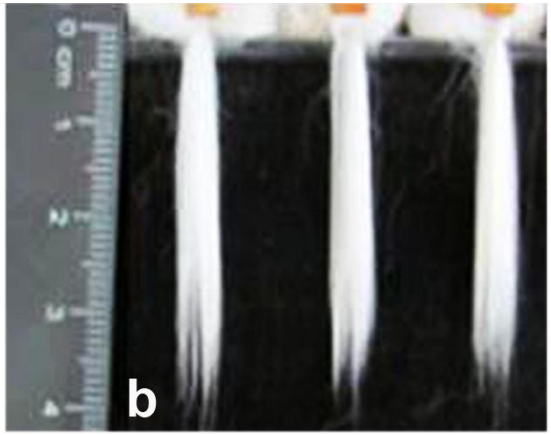
PHYA1 RNAi

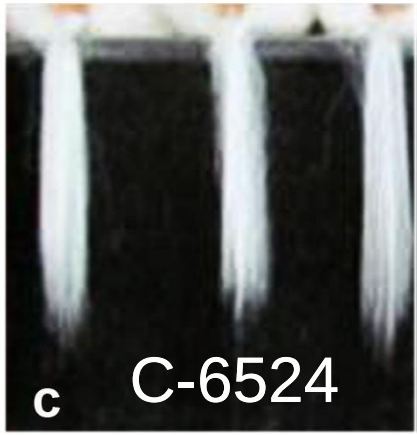
Controls

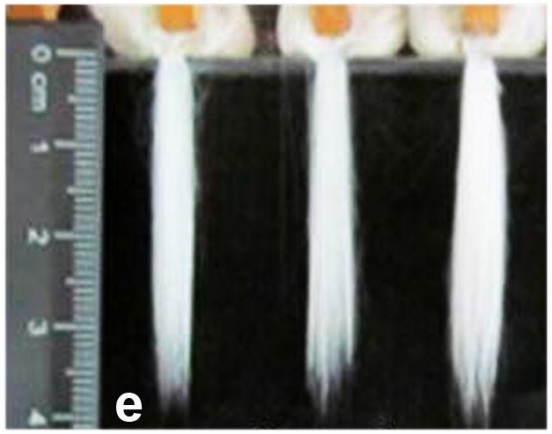
PORLOQ-2
(C-6524)

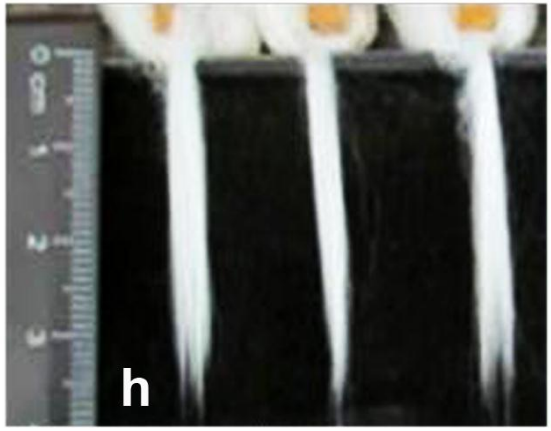
PORLOQ-3
(Toshkent-6)

PORLOQ-4
(Namangan-77)









Textile Quality of Yarn From RNAi Fiber

Name	CVm %	Thin-40%/km	Thin-50%/km	Thick +35% /km	Thick +50% /km	Neps +140% /km	Neps +200% /km	H	Tenacity R/km	Exportability
RNAi	10.54	2.5	0	64	2.5	79.5	13	5.84	21.47	15.5
Control	11.03	9.5	0	102	5.5	209	25.5	6.60	17.0	35

DNA typing for counterfeiting

We made high quality cottor yarn and fabric from NOVEL RNAi fiber

YARN DNA TYPING PROVED YARN MADE FROM TOTALY UPLAND FIBER

FIBERTYPING textile genotyping

applieddnasciences the ultimate reality check

Cotton Authentication Report- Starting Materials

Sample Ref. No.: JR1182 **Lab Notebook Ref. No.:** APDN2013-002 p60-62 **Date Tested:** July 12-16, 2013

Job Ref. No.: N/A **Client:** Cotton Inc. **Manufactured By:** N/A **Country:** Uzbekistan

Color: White **Style:** Shirt **Additional Comments:** special treatment comparable to Pima cotton

Item Tested ☒ *(only **one** item (front/back) per report)*

☐ Raw Cotton		☐ Yarn		☐ Yarn (cone)		☐ Greige Fabric		☒ Dyed Fabric	
									
Sample ID	Sample Result	Sample ID	Sample Result	Sample ID	Sample Result	Sample ID	Sample Result	Sample ID	Sample Result
								N/A	Upland*

* The sample identity has been confirmed by more five consistent results.

ELS Product Conformance Criterion

Test	Result	Criterion
FiberTyping™	ELS	Samples tested contain DNA from *Gossypium barbadense* or ELS cotton.
	Upland	Samples tested contain DNA from *Gossypium hirsutum* or upland cotton.
	Blend	Samples tested contain a blend of DNA from *Gossypium hirsutum & Gossypium barbadense*
	Inconclusive	Samples tested DID NOT contain DNA, cotton content could not be determined.

FiberTyping™ Test Result:

The material indicated above was evaluated and ☐ ELS ☒ Upland ☐ Contains a Blend or ☐ was Inconclusive with regards to the ELS content indicated on the product's label.

In no event shall APDN be liable for any loss, claim, damage or liability of any kind or nature, that may arise from the reporting of these results. Client agrees to hold harmless APDN and APDN's officers, directors, agents and employees from any liability, loss or damage they might suffer as a result of claims, demands, costs, or judgments against them arising out of the use of the Authentication Reports by client.

Tested By: _____ **Date:** 071613

Reviewed by: _____ **Date:** 07162013

Form No. 090-154.07

KEY Message and achievement

!

NOVEL superior quality Upland cotton fiber exploiting an internal potential of Upland cotton genome (without using any genes or components from from Pima cotton) and without adverse effects on key agronomic traits including maturity and productivity;

Novel fiber "*could potentially have a multi-billion-dollar impact on the global cotton industry and help cotton farmers fend off increasing competition from synthetic fibers*"

Protection of the technology

AGENCY
ON INTELLECTUAL PROPERTY
OF THE REPUBLIC OF
UZBEKISTAN

O'ZBEKISTON RESPUBLIKASI
INTELLEKTUAL MULK
AGENTLIGI

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20 MAR 2012 № 01/595

СПРАВКА

Агентство по интеллектуальной собственности Республики Узбекистан настоящим удостоверяет, что приложенные материалы являются точным воспроизведением первоначальных материалов заявки на выдачу патента на изобретение IAP 2012 0069, поданной 28 февраля 2012 года.

Название изобретения: РНК-интерференция *PHYA1* хлопчатника улучшающая качество волокна, удлинение корня, цветение, созревание и урожайность у Gossypium hirsutum L.

Заявители: Центр геномных технологий института генетики и экспериментальной биологии растений Академии наук Республики Узбекистан, UZ

ЗЕ ЮНАЙТЕД СТЭЙТС ОФ АМЕРИКА, ЭЗ РЕПРЕЗЕНТИД БАЙ ЗЕ СЕКРЕТЭРИ ОФ АГРИКАЛЧЕ, US

ЗЕ ТЕКСЭС Аэнд М ЮНИВЕРСИТИ СИСТЕМ, US

Уполномоченный заверить копию заявки на объекты промышленной собственности

Первый заместитель генерального директора
З.Б.Гиясов

A patent application for this work has been filed in the Uzbekistan (IAP 20120069), USA (USPTO:13/445696), and internationally (PCT/US13/27801).

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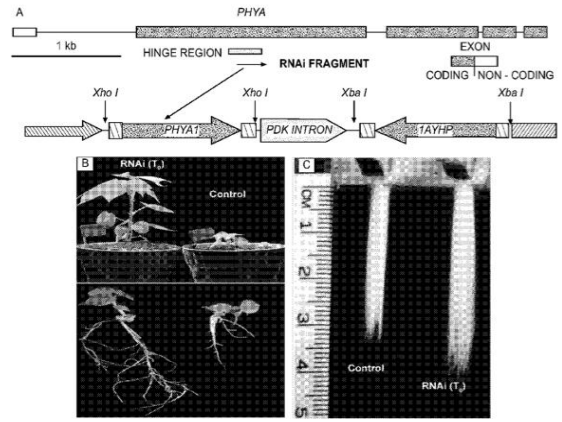
[Continued on next page]

(54) Title: COTTON PHYA 1 RNAI IMPROVES FIBER QUALITY, ROOT ELONGATION, FLOWERING, MATURITY AND YIELD POTENTIAL IN GOSSYPIUM HIRSUTUM L.



(57) Abstract: Improvement of fiber quality of Upland cultivars (*Gossypium hirsutum*), while maintaining early maturity and productivity, is a fundamental problem in conventional cotton breeding. Phytochromes play a fundamental role in plant development, flowering and cotton fiber length. Targeted RNAi of PHYA 1 genes in cotton suppressed expression of PHYA 1 and/or PHYB, resulting in over-expression of the remaining PHYA2/B/C/E genes. This altered expression induced a number of phytochrome- associated phenotypes, including increased root length and mass, increased anthocyanin-pigment, vigorous shoot development and vegetative growth, early flowering, early boll maturity, increased fiber length and increased seed cotton yield compared to control plants. These RNAi phenotypes were stably inherited and expressed through four generations (T_{0-3}) and were transferable from RNAi Coker-312 plants to Upland cultivars via conventional hybridization. These effects in Upland cotton breeding can offer a new paradigm in cotton breeding resulting in the development of productive, early-maturing Upland cultivars with increased fiber length and fiber strength.

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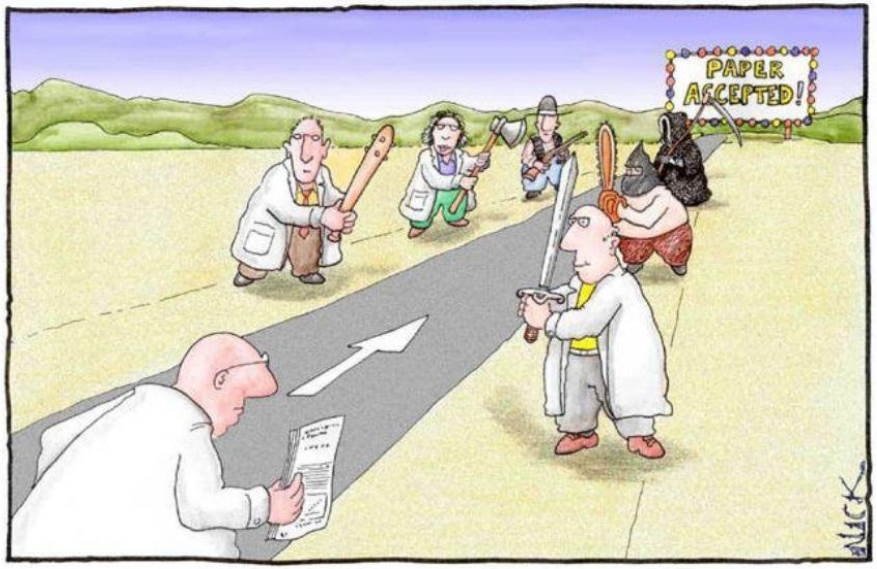
Phytochrome RNAi enhances major fibre quality and agronomic traits of the cotton *Gossypium hirsutum* L

Ibrokhim Y. Abdurakhmonov[1,†], Zabardast T. Buriev[1,†], Sukumar Saha[2], Johnie N. Jenkins[2], Abdusattor Abdukarimov[1,†] & Alan E. Pepper[3]

Simultaneous improvement of fibre quality, early-flowering, early-maturity and productivity in Upland cotton (*G. hirsutum*) is a challenging task for conventional breeding. The influence of red/far-red light ratio on the fibre length prompted us to examine the phenotypic effects of RNA interference (RNAi) of the cotton *PHYA1* gene. Here we show a suppression of up to ~70% for the *PHYA1* transcript, and compensatory overexpression of up to ~20-fold in the remaining phytochromes in somatically regenerated *PHYA1* RNAi cotton plants. Two independent transformants of three generations exhibited vigorous root and vegetative growth, early-flowering, significantly improved upper half mean fibre length and an improvement in other major fibre characteristics. Small decreases in lint traits were observed but seed cotton yield was increased an average 10–17% compared with controls. RNAi-associated phenotypes were heritable and transferable via sexual hybridization. These results should aid in the development of early-maturing and productive Upland cultivars with superior fibre quality.

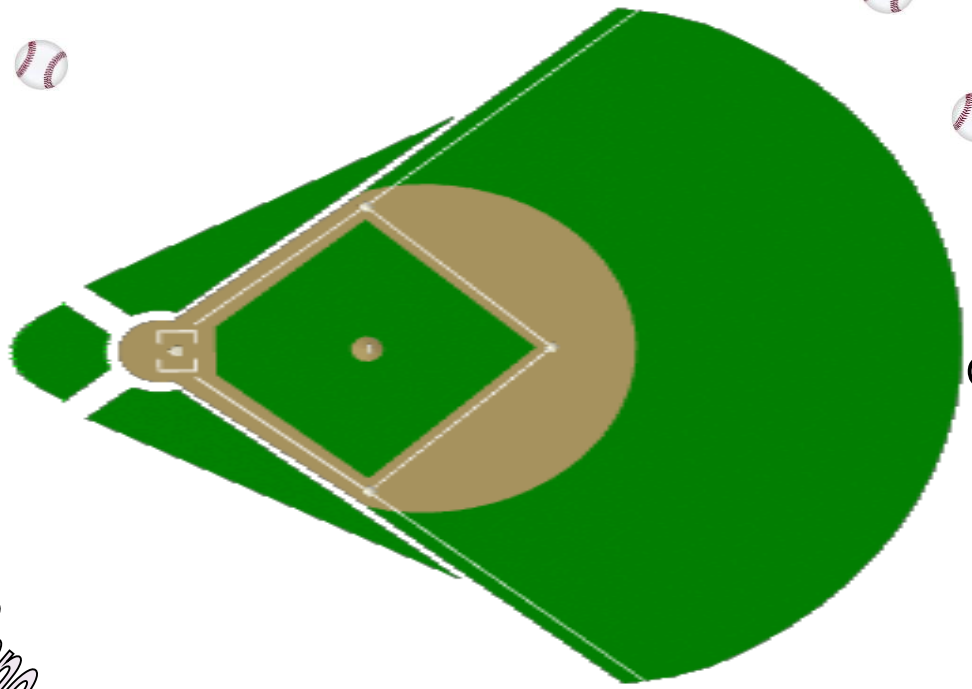
The paper describing entire technology has been published in one of the leading Nature family journals!

Getting published



HOME RUN!

- Proof of Concept – Technology - Product – Patent - Publication



Commercialization
- Licensing

Physiology - Gen.

Exciting mission!

Uzbekistan CGB

Future perspectives

- **Commercialization of these new generation RNAi varieties in Uzbekistan and worldwide through interested companies;**

- **Bringing novel Upland fiber into world market:**
  - *Identification of the price of the RNAi fiber*
  - *Make the larger volume of RNAi fiber yarn*
  - *Get involvement of local and world textile companies for the use of Novel Upland RNAi fiber*

- **Application of the same approach to other crops;**

- **Transcriptome and metabolome profiling of RNAi genotypes;**

Convert the RNAi to the new generation genome editing and transgenomics tools (amiR constructs, Zinc fingers, TALEN).

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- **SIFAT fiber testing center**
- **UzTEX Company**