Phytochrome RNA interference enhances major fiber quality and agronomic traits of cotton (*Gossypium hirsutum* L.)

Prof. Ibrokhim.Y. Abdurakhmonov

Center of Genomics and bioinformatics, Uzbekistan

The main objective of world cotton breeding

Superior fiber quality of Pima cotton (*G. barbadense*), 8% grown in the world

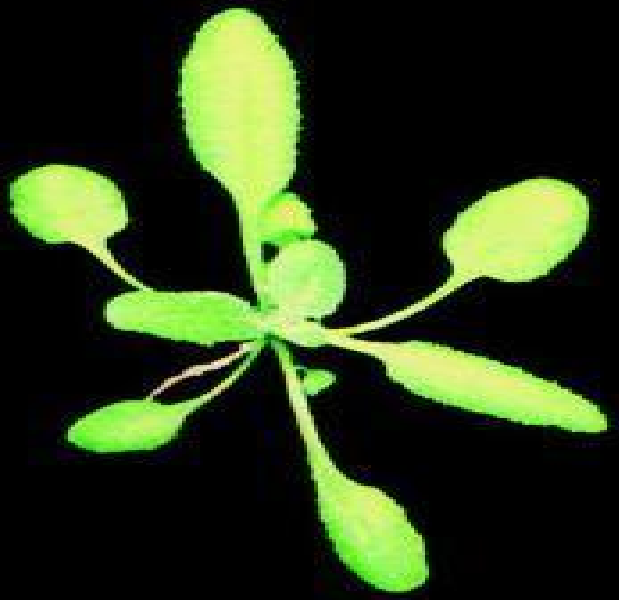
Mobilization of fiber quality genes

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

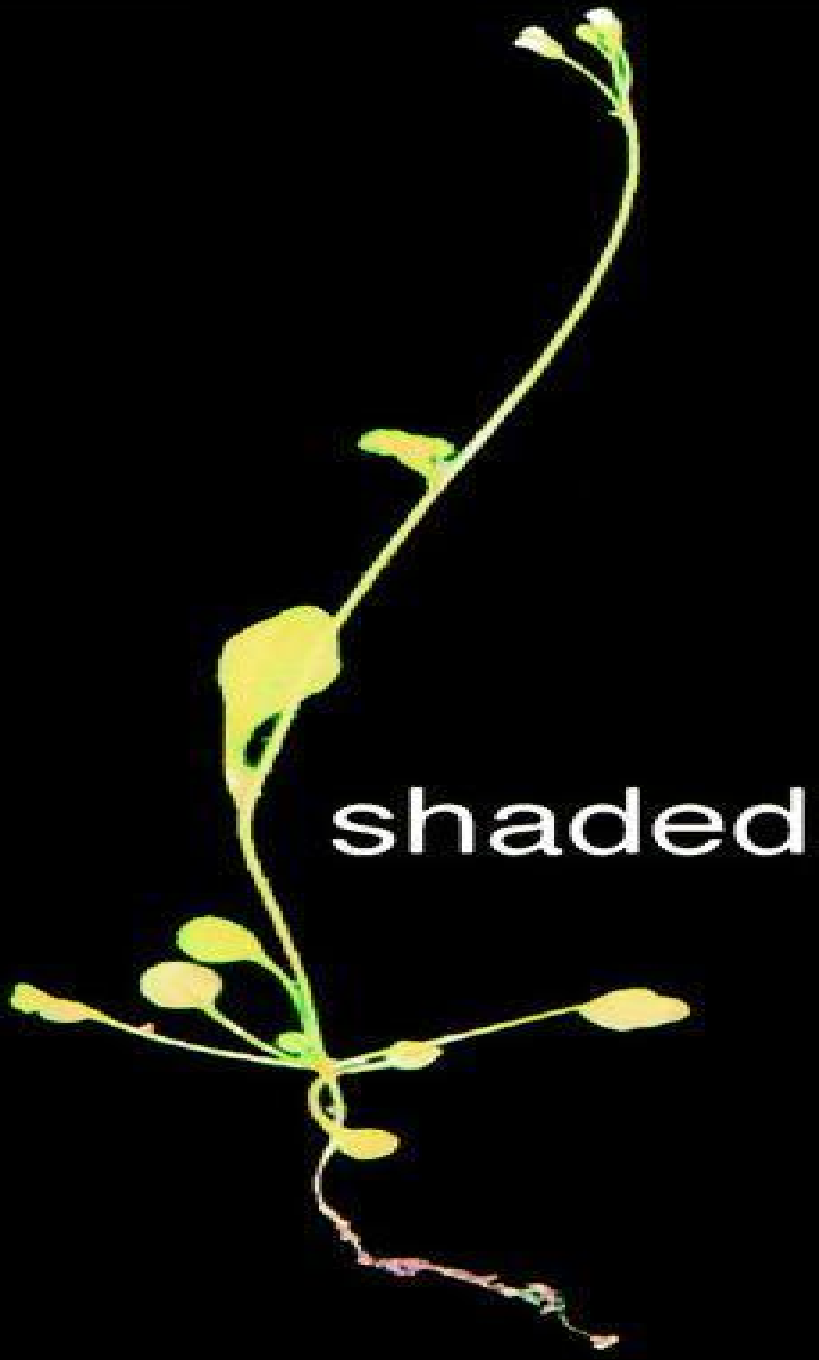
Long-standing conventional problem

A kilogram of superior quality long fiber with good microniare (3.8-4.9) brings 8 to10 cents more income

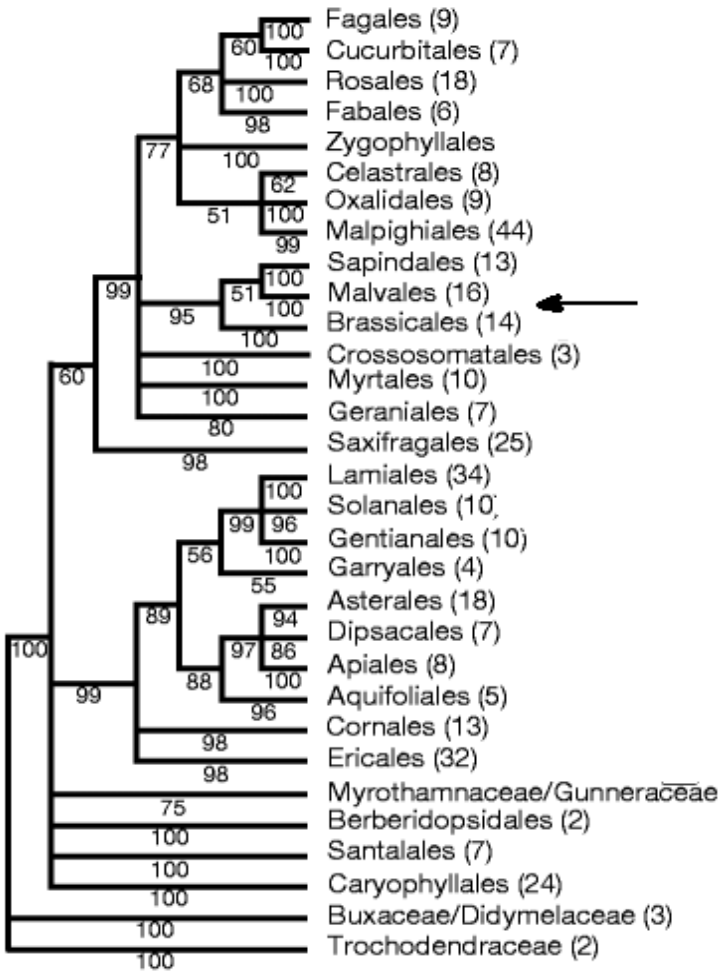
bright light



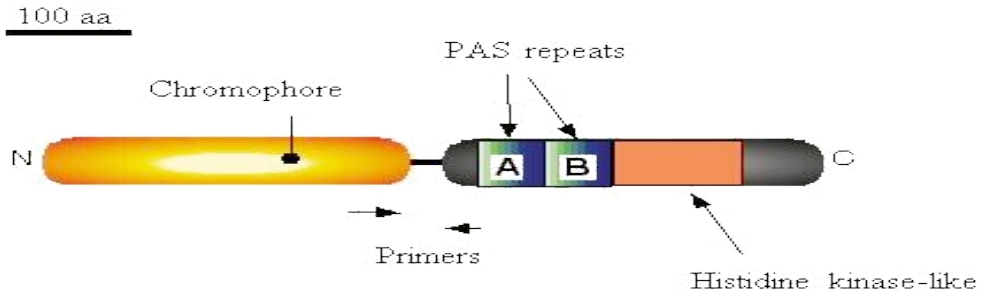
shaded



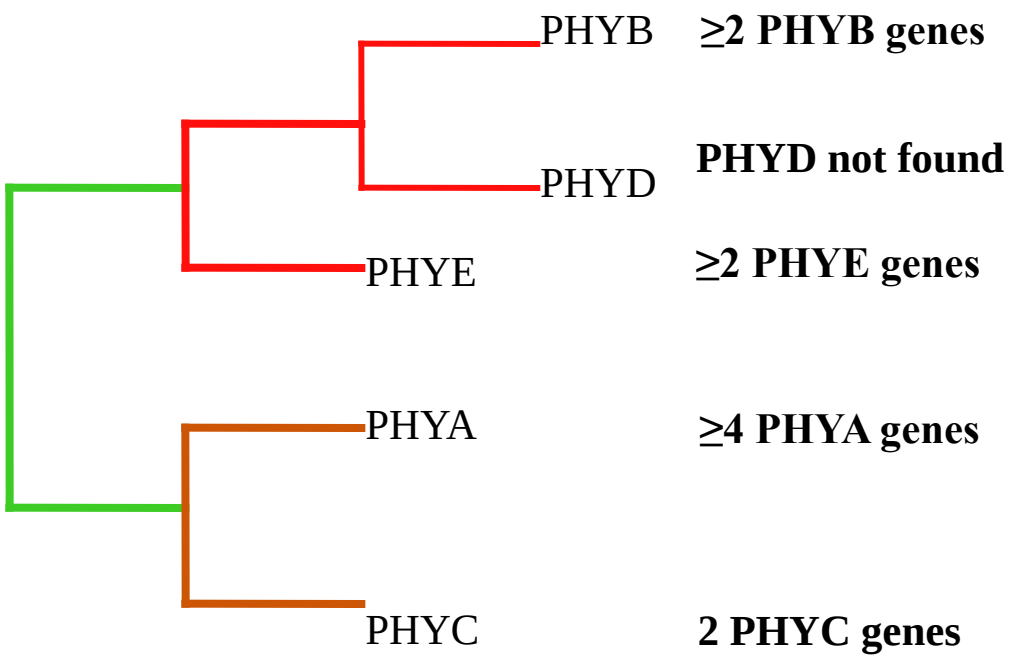
Cotton phytochrome gene family characterization



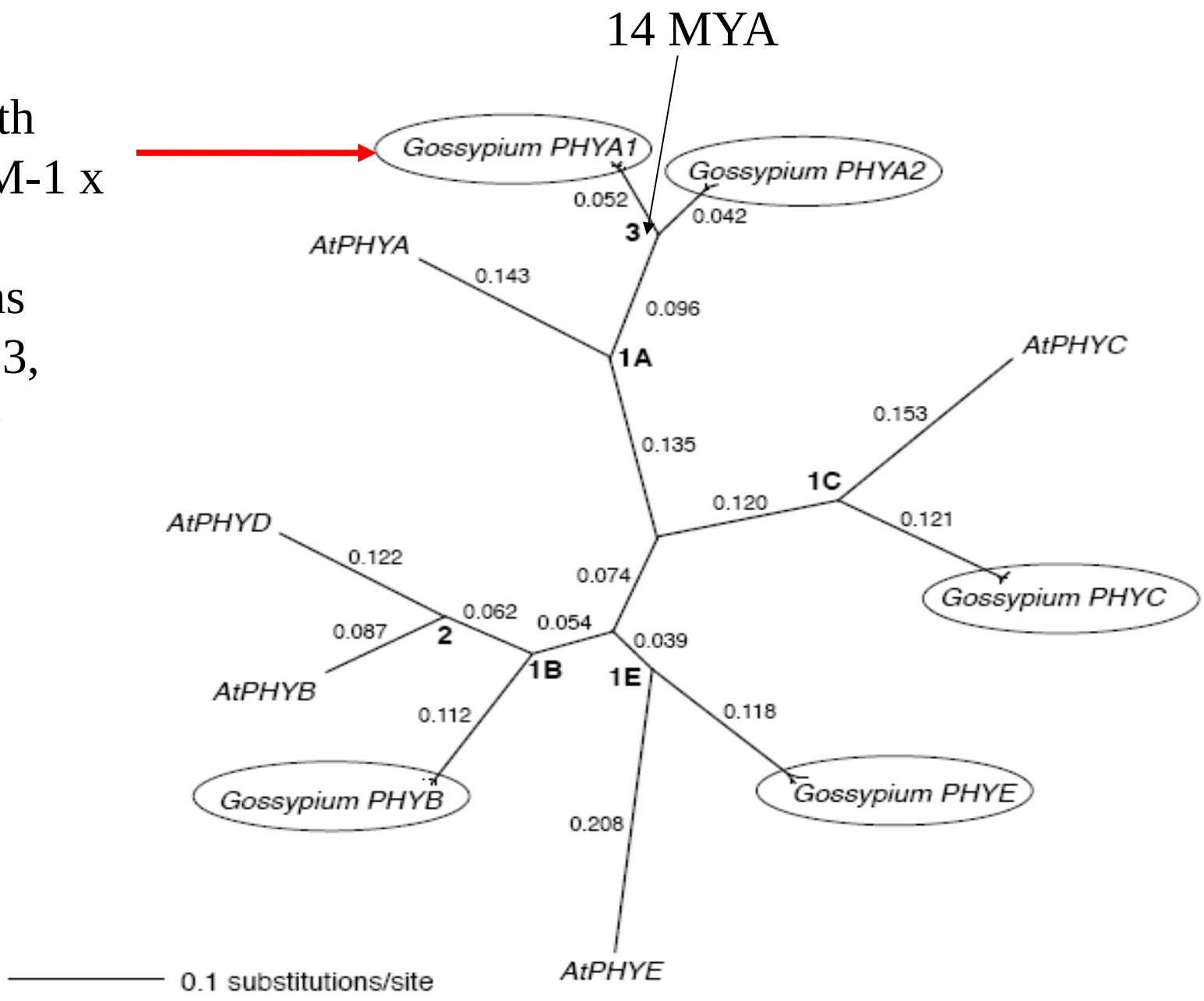
Soltis et al. 1999. Nature **402**: 402-404.



Arabidopsis **Tetraploid cotton**

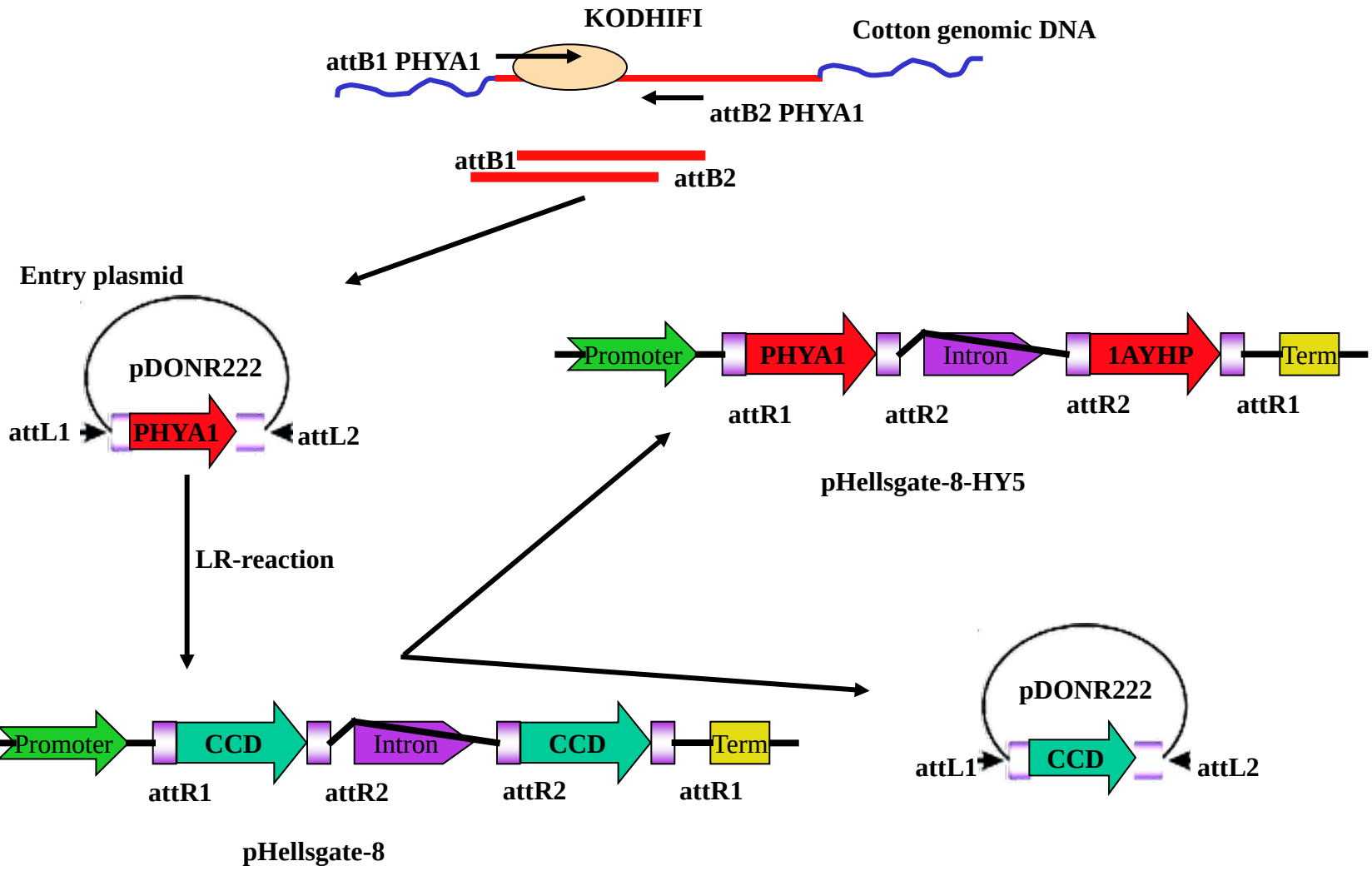


Fiber length QTL in TM-1 x Pima 3-79 populations (LOD=4.13, P<0.0001)

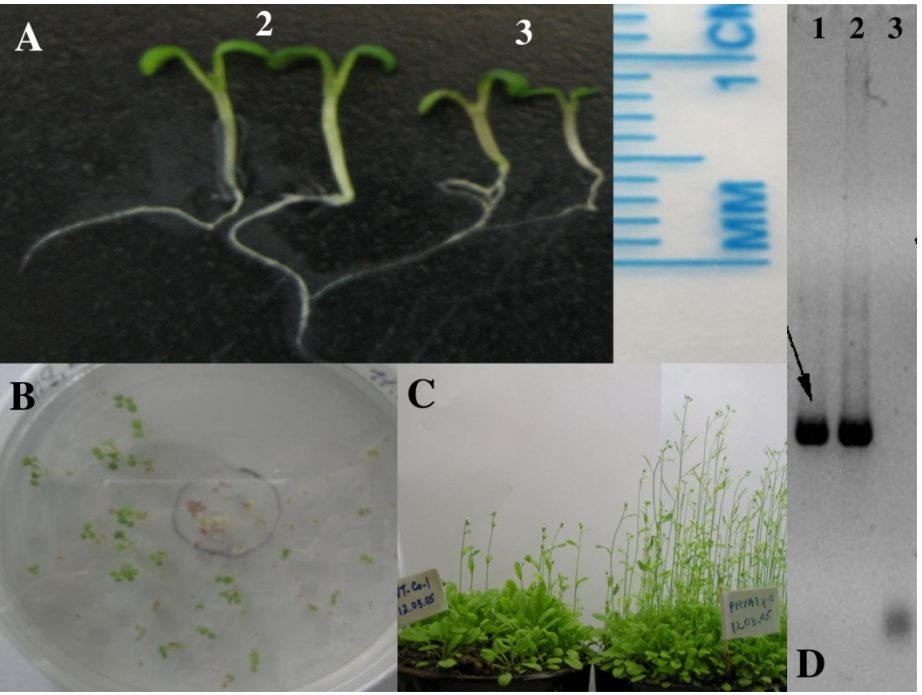


Source: Abdurakhmonov et al. 2010. BMC Plant Biology

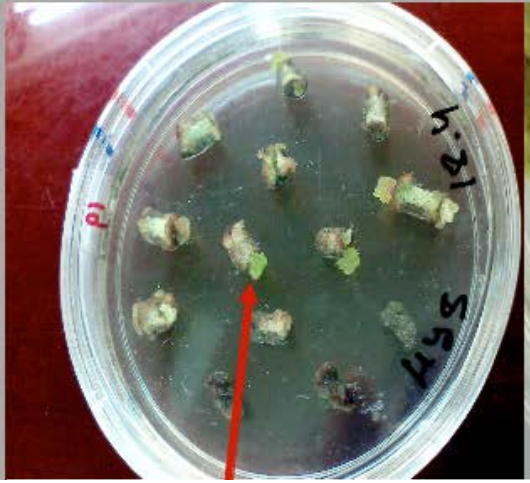
RNAi vector designing for *PHYA1*

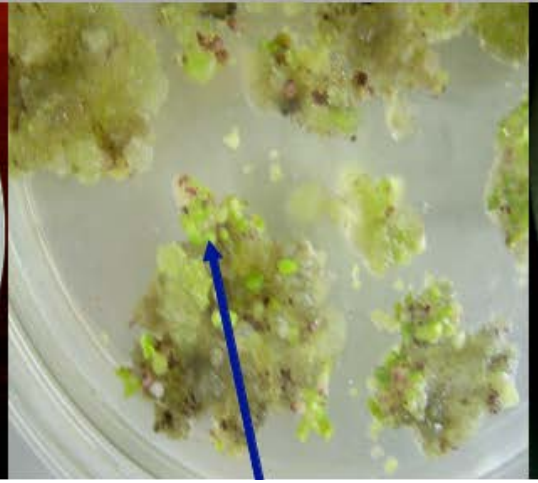


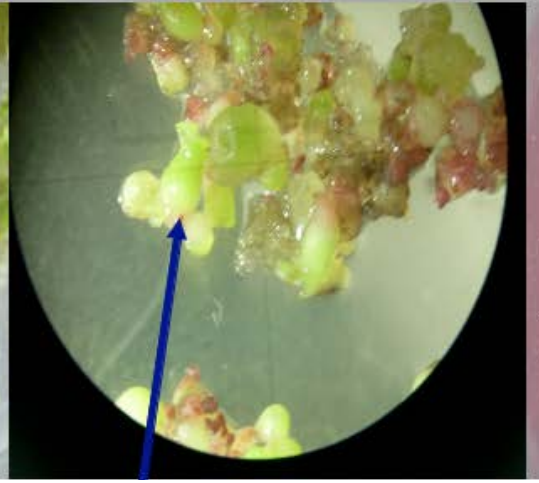
Helliwell et al. 2002. Funct. Plant. Biol. 29:1217-1225



Arabidopsis transformed with cotton *PHYA1* hairpin construct

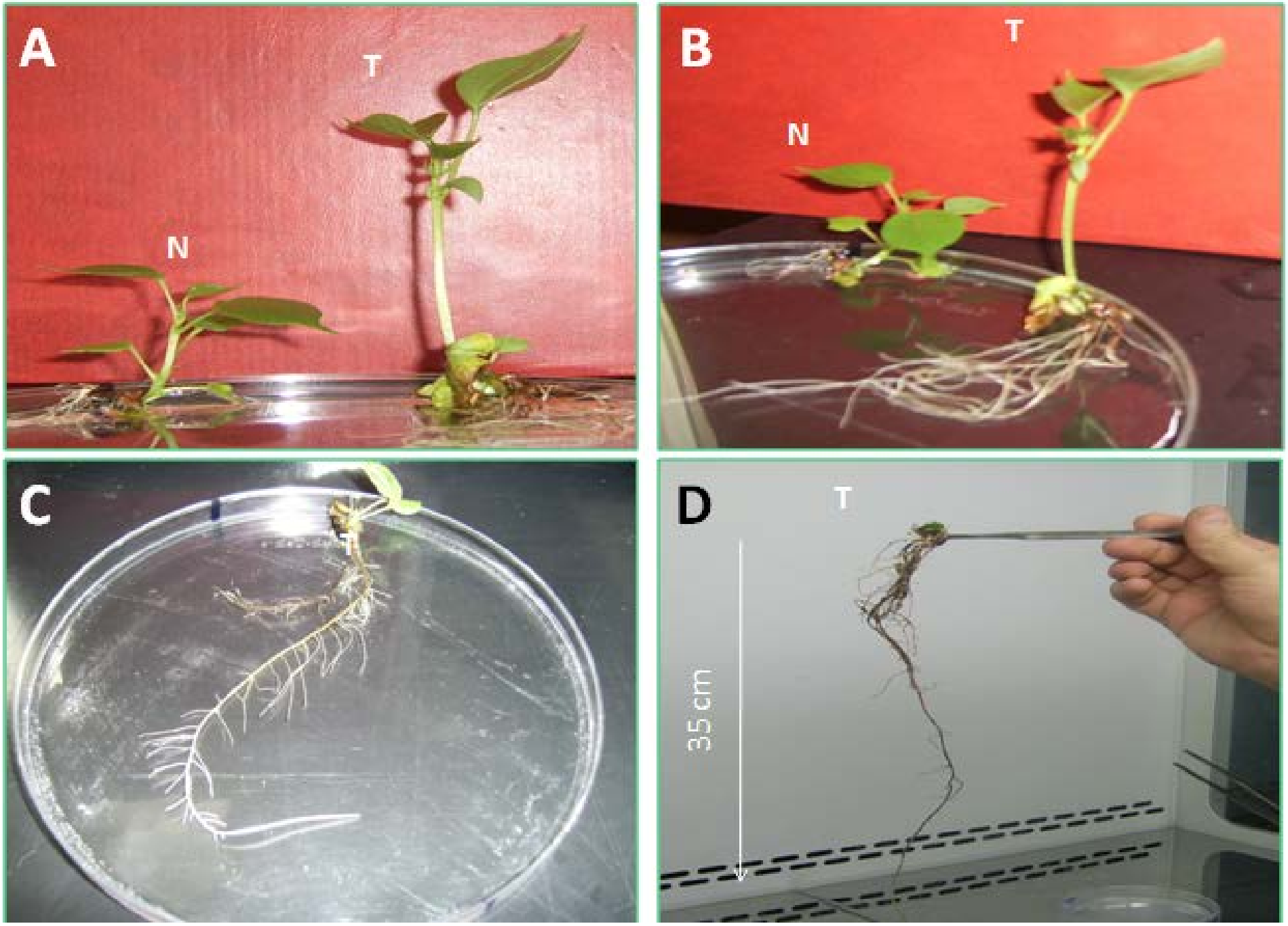


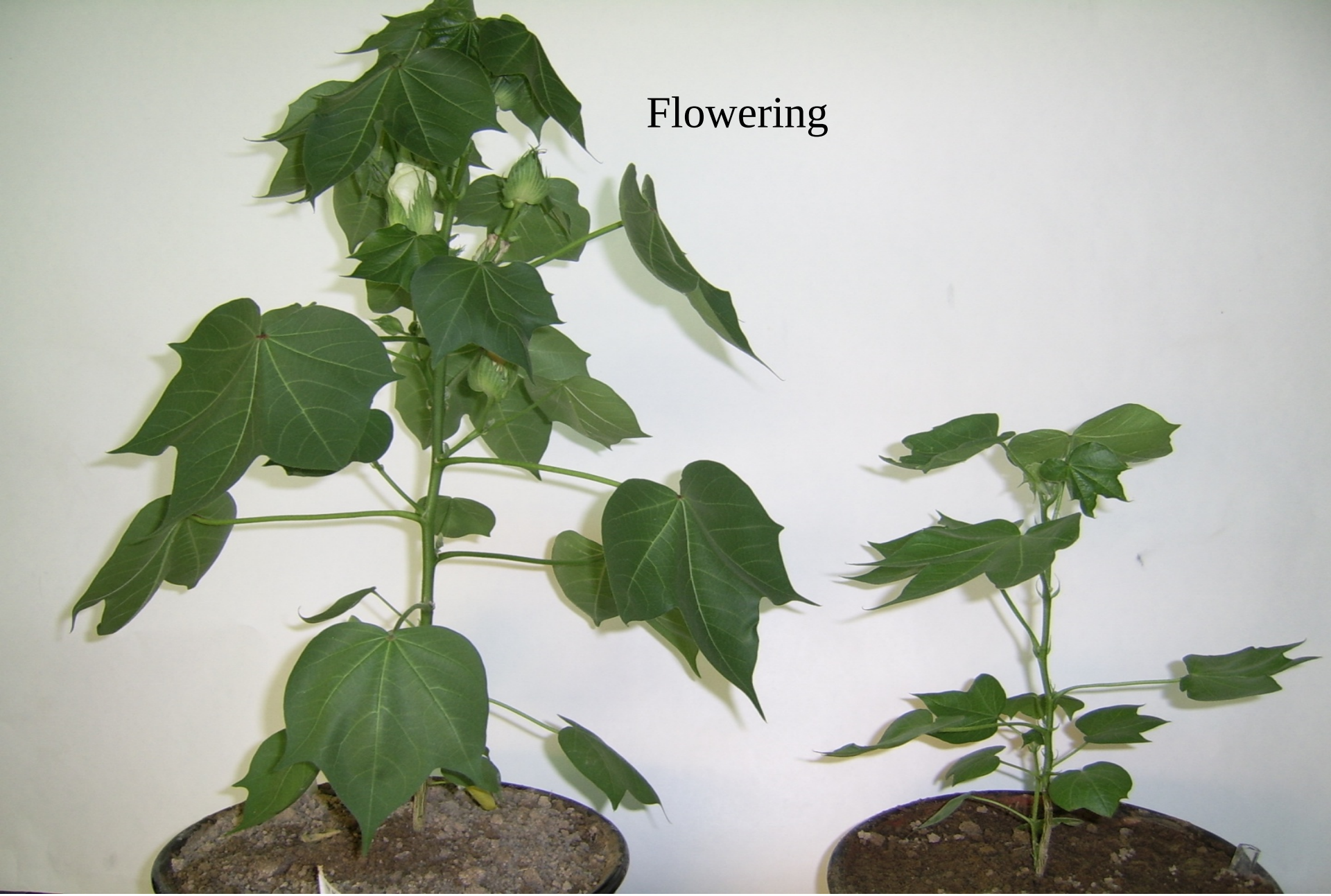




Transformation **Callus formation and Somatic embryogenesis**

Embryo plantlets **Transferring into pots and fields**


A
T
N
B
T
N
C
T
D
T
35 cm



RNAi T0 plants

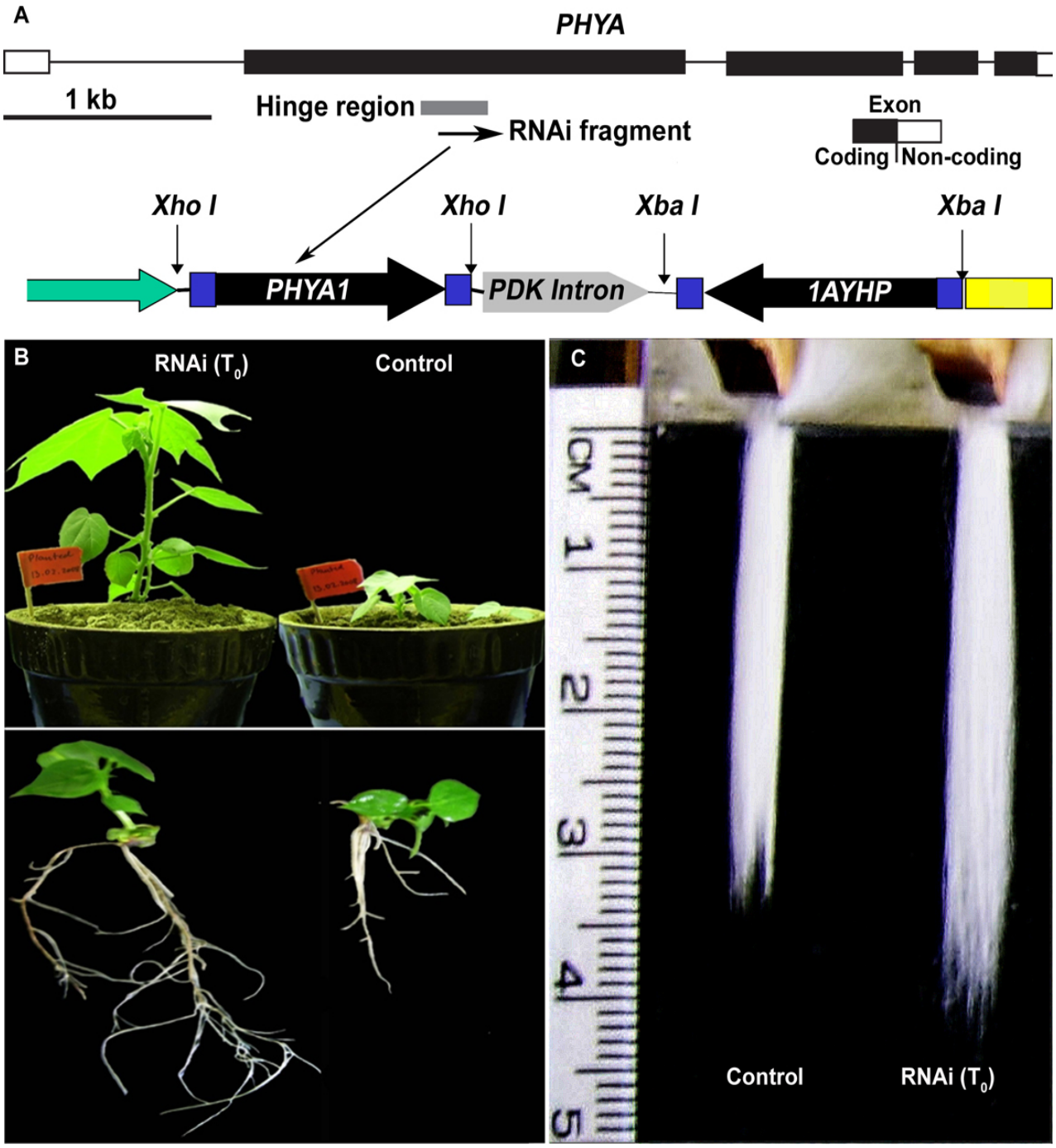
Control

The same day planted

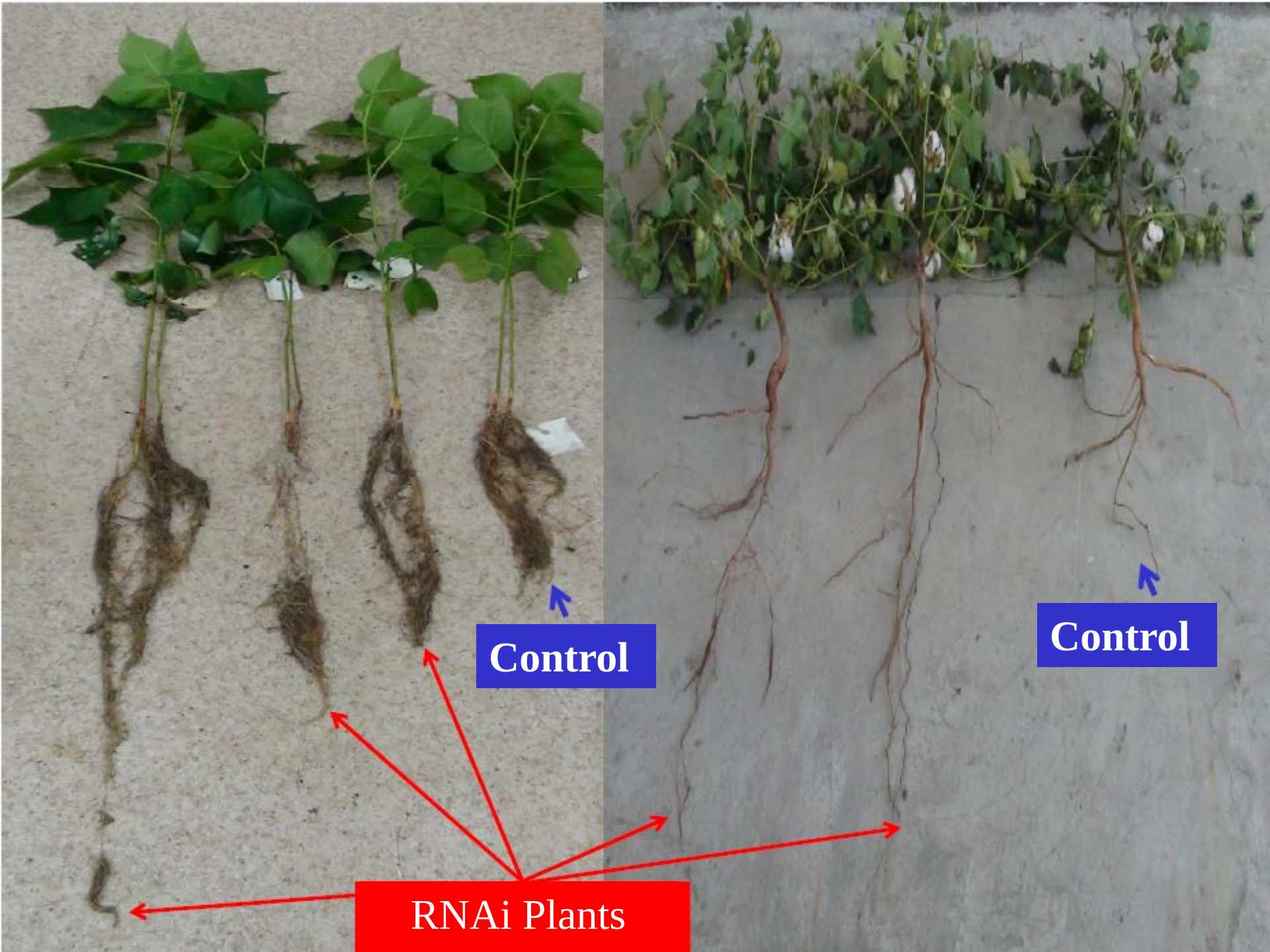
Hypocotyl length $T_{1:5}$

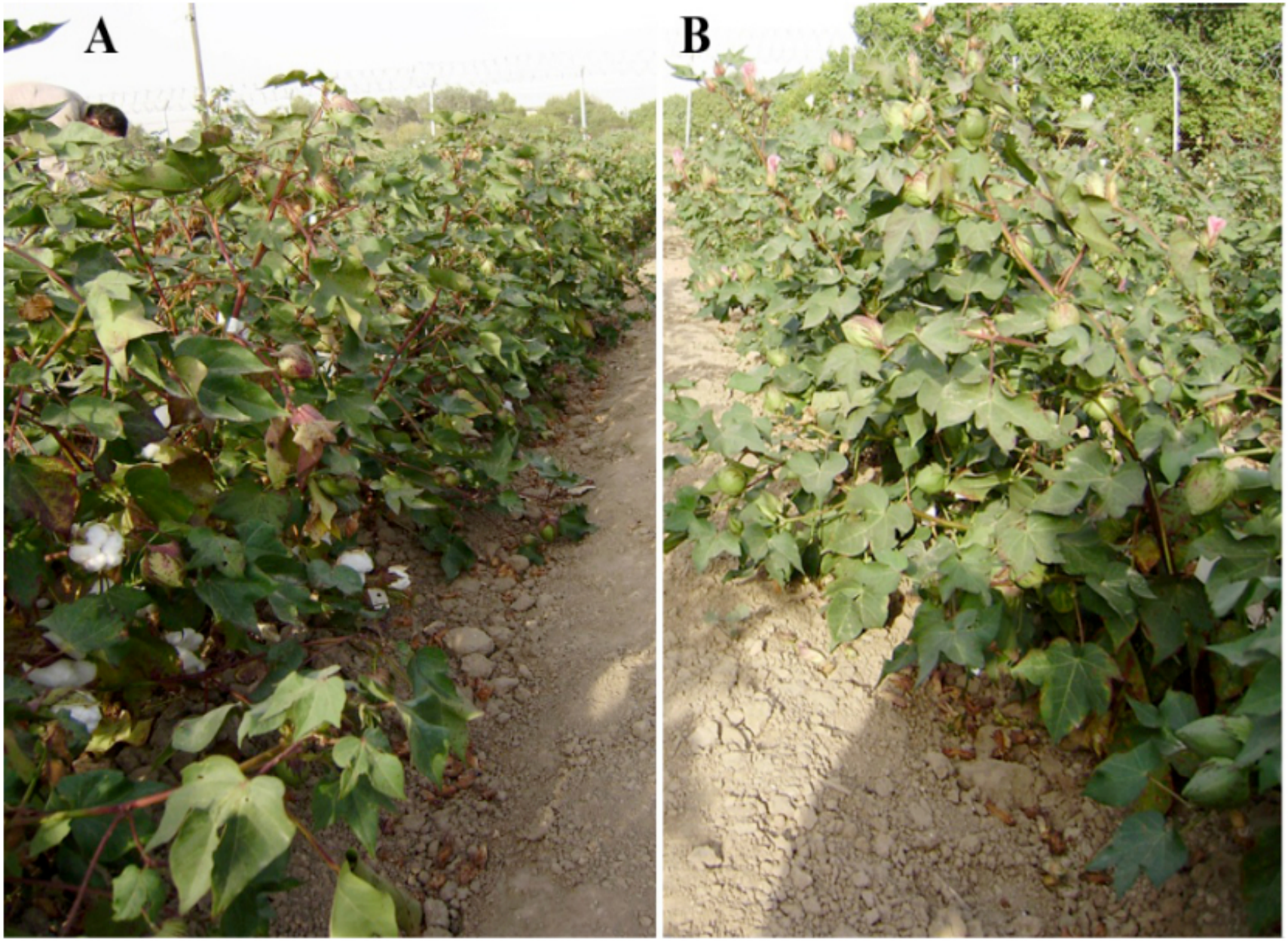
Coker-321

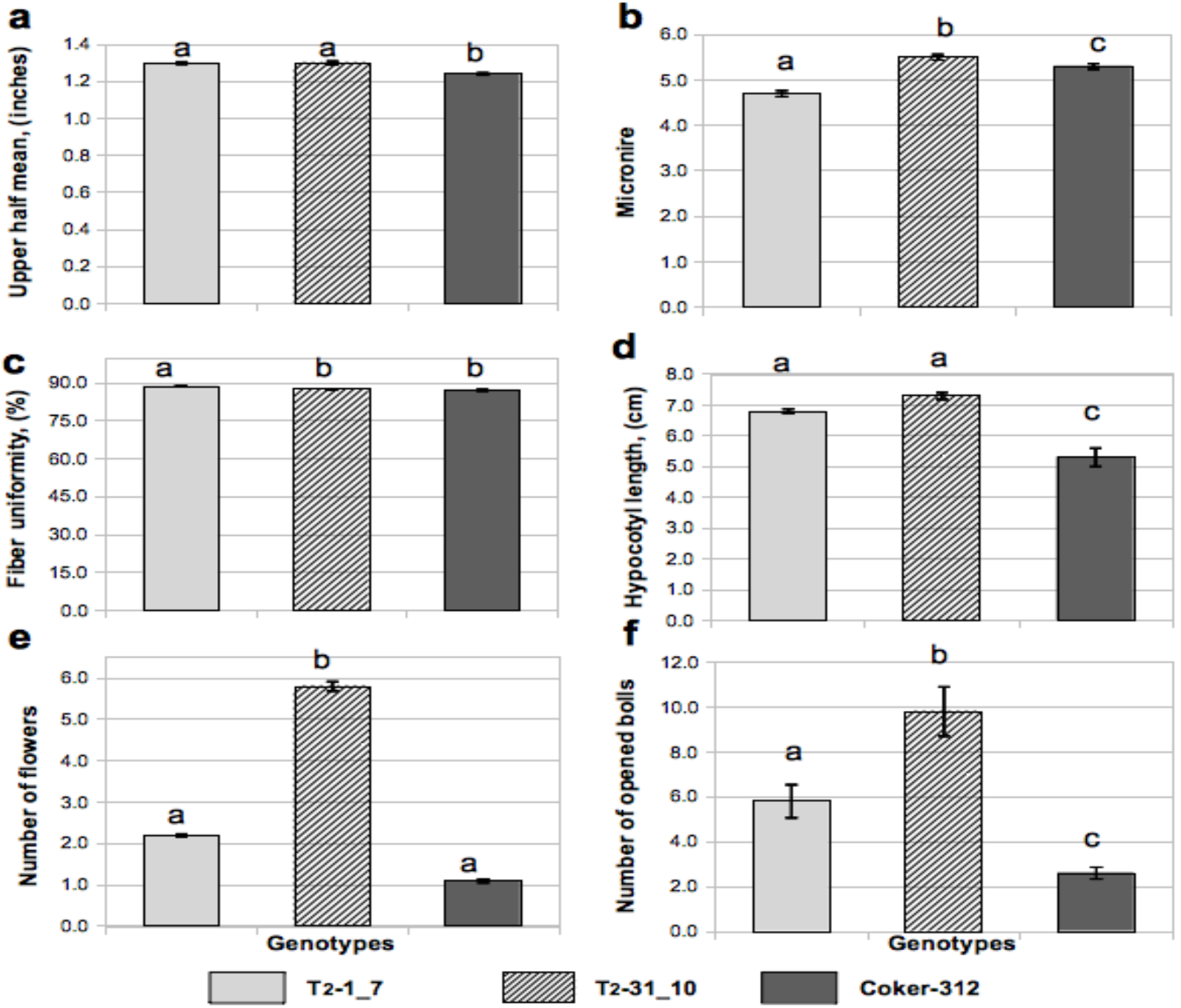
RNAi


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)


RNAi plots
Control plot


Control
Control
RNAi Plants

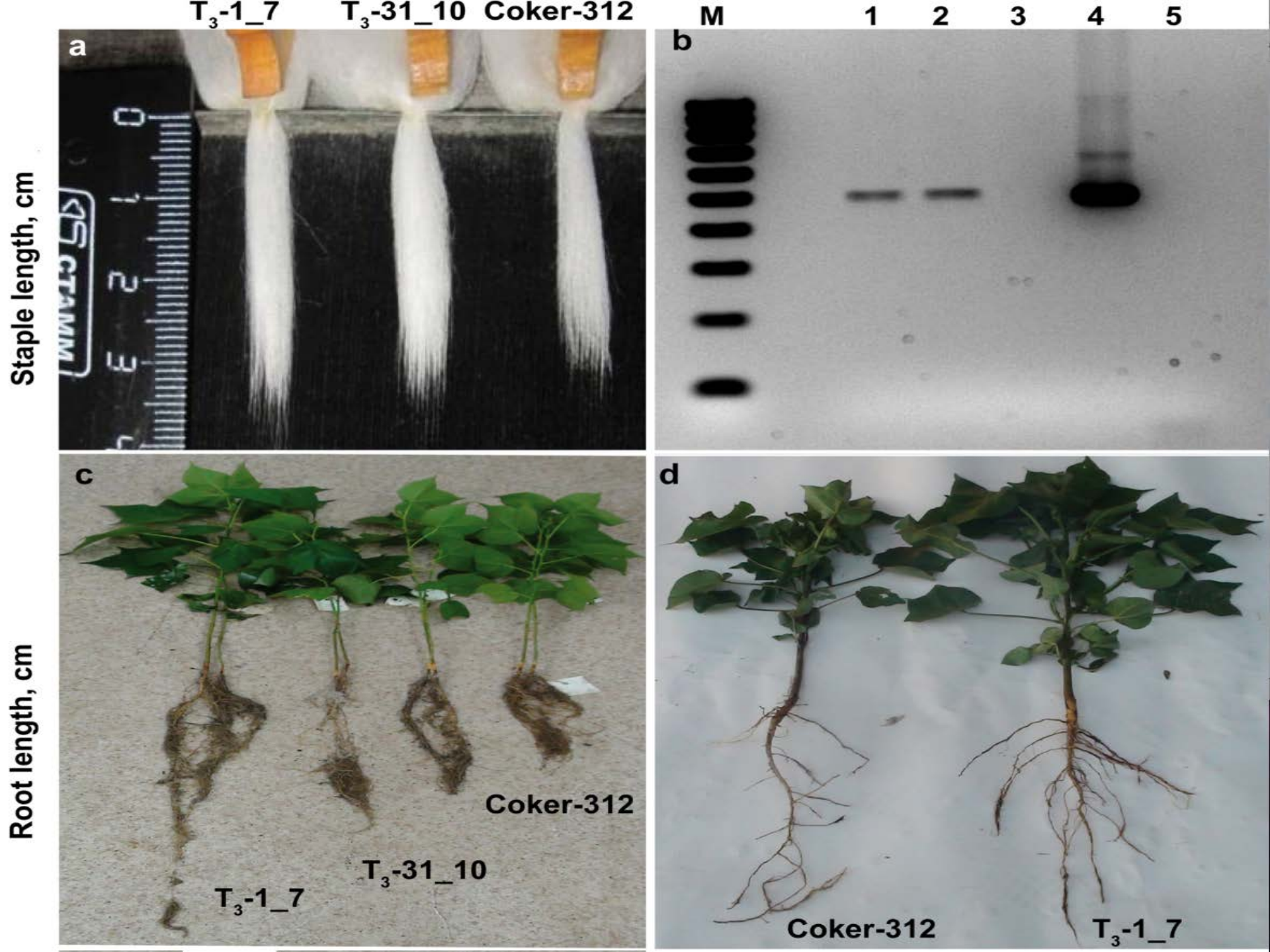

A
B


a
Upper half mean, (inches)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
a
a
b
b
Micronire
6.0
5.0
4.0
3.0
2.0
1.0
0.0
a
b
c
c
Fiber uniformity, (%)
90.0
75.0
60.0
45.0
30.0
15.0
0.0
a
b
b
d
Hypocotyl length, (cm)
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
a
a
c
e
Number of flowers
6.0
5.0
4.0
3.0
2.0
1.0
0.0
a
b
a
Genotypes
f
Number of opened bolls
12.0
10.0
8.0
6.0
4.0
2.0
0.0
a
b
c
Genotypes
T2-1_7
T2-31_10
Coker-312

Cotton fiber length in $T_{1;3}$ generation RNAi – field grown

RNAi plants

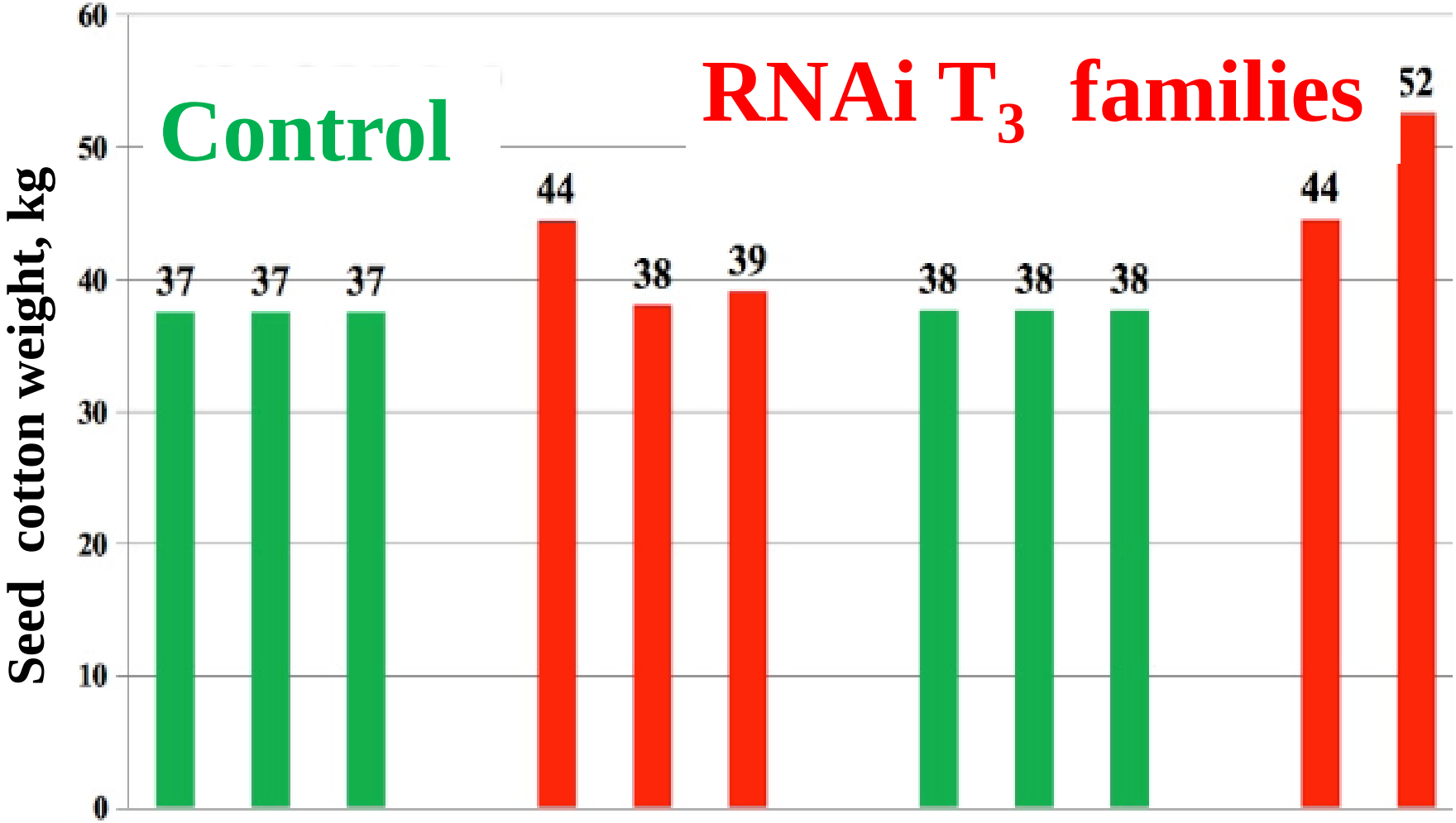
Controls


T3-1_7
T3-31_10
Coker-312
M
1
2
3
4
5
a
b
c
d
Staple length, cm
Root length, cm
Coker-312
T3-31_10
T3-1_7
Coker-312
T3-1_7

Cotton fiber characteristics

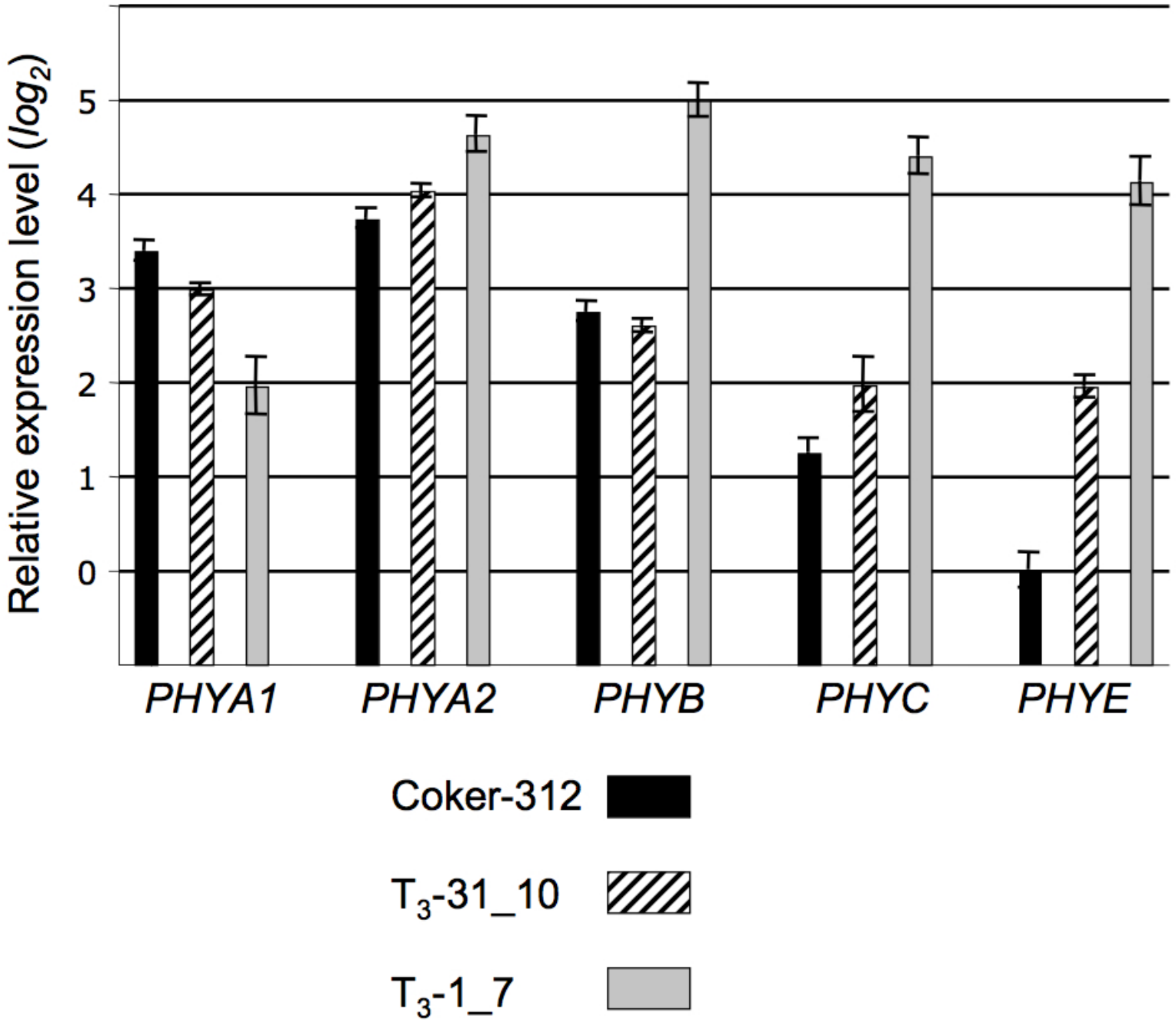
Traits	Coker-312 (control)	T_3_1-7	T_3_31-10
UHM (SE)	1.22 (0.005)	1.3 (0.004)***	1.3 (0.01)***
MIC (SE)	5.6 (0.12)	5 (0.13)**,a	5.4 (0.08)**a
STR (SE)	28.8 (0.31)	30.9 (0.62)**,a	29.7 (0.23)*,a
UI (SE)	87.5 (0.34)	89.2 (0.60)*	88.1 (0.30)*
ELO (SE)	9 (0.17)	10 (0.15)***	10.1 (0.18)***
Weight of 100 seeds, g (SE)	12.9 (0.2)	12.4 (0.3)	12.4 (0.2)
Lint% (SE)	38.7 (0.4)	37.3 (0.4)*	37.1 (0.4)*
Seed weight% (SE)	61.3 (0.4)	62.7 (0.4)*	62.9 (0.4)*
Lint index (SE)	8.1 (0.1)	7.4 (0.2)***	7.3 (0.1)***

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



Estimated numbers of *nptII* in third generation (T_3) RNAi cotton lines

Samples	Average Ct values*	SD	SE	CV	**X_0/R_0	Estimated copy number	cv***
*nptII*_T_3-1_7	22.01	0.75	0.24	3.4	3.08	3	0.037
*nptII*_T_3-31_10	21.07	0.29	0.09	1.4	1.67	2	0.025
*nptII*_Bt-cotton	24.45	0.27	0.11	1.1	1.07	1	0.035
*GhUBC1*_ T_3-1_7	23.27	0.46	0.19	2			
*GhUBC1*_ T_3-31_10	21.42	0.46	0.16	2.1			
*GhUBC1*_Bt-cotton	24.15	0.79	0.35	3.3			


Relative expression level (log_2)
5
4
3
2
1
0
PHYA1
PHYA2
PHYB
PHYC
PHYE
Coker-312
T_3-31_10
T_3-1_7

TRANSFERABILITY TO UPLAND CULTIVARS

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

Variety-1

RNAi

Variety-2

RNAi

Boll size and form

Variety-1

RNAi

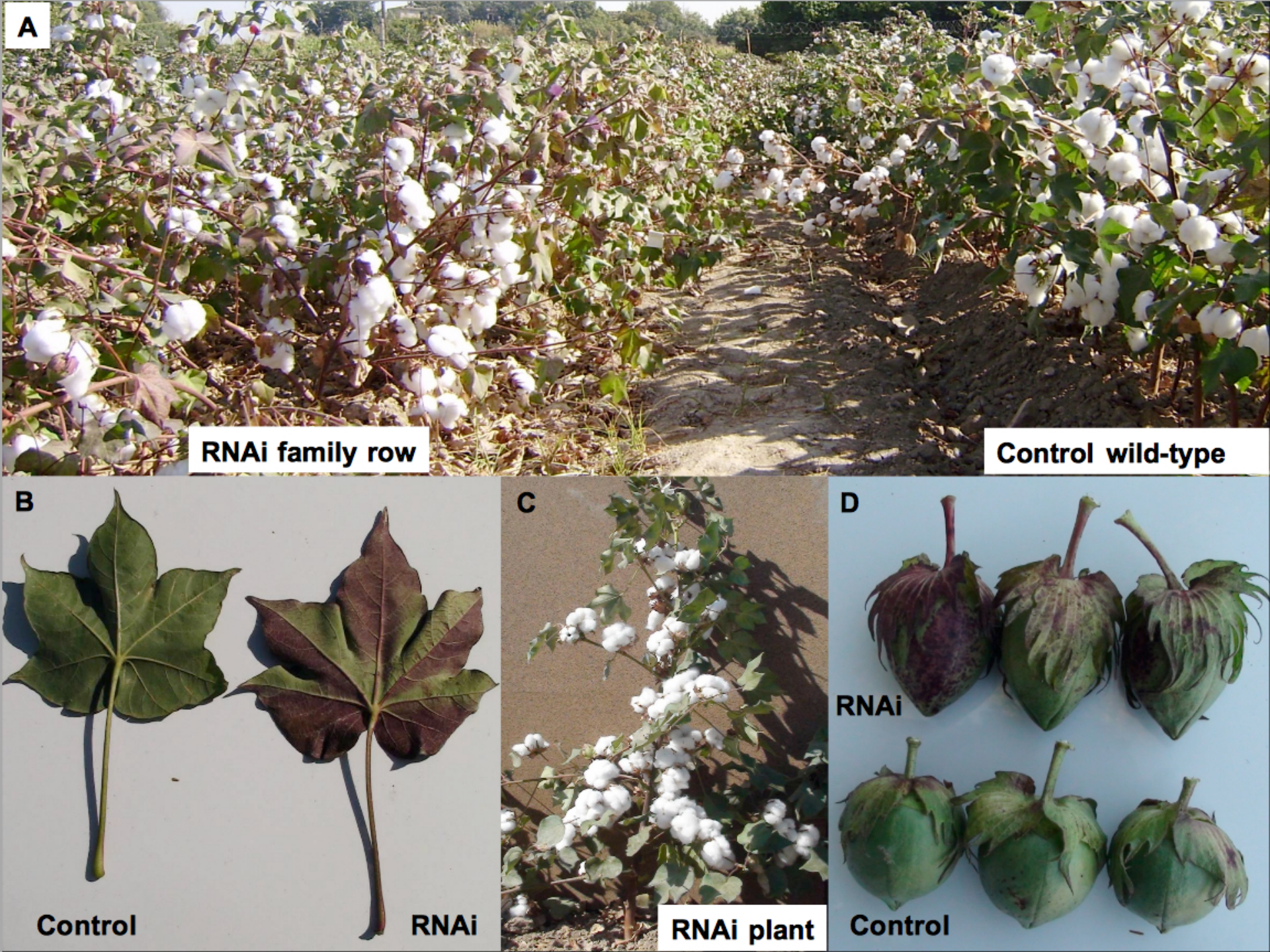
Control plots

RNAi variety plot

Major fiber characteristics and agronomic quality of RNAi cultivars (4-generation, 2011)

[RNAi Coker312 x local cultivar] x local cultivar

Cultivar name	UHM, inch	STR, g/tex	MIC	UI, %	Earliness, days	Yield, MT/ha
ANB-2 RNAi	1.10 1.27	30.28 37.90	4.5 4.2	84.5 86.0	120 110	4.0 5.8
C6524 RNAi	1.15 1.28	33.59 42.80	4,2 4.0	83.5 87.3	125 120	4.0 5.4
Tosh-6 RNAi	1.10 1.28	27.50 33.80	4.7 4.2	81.3 84.9	120 105	3.5 4.5


A
RNAi family row
Control wild-type
B
Control
RNAi
C
RNAi plant
D
RNAi
Control

AGENCY
ON INTELLECTUAL PROPERTY
OF THE REPUBLIC OF
UZBEKISTAN

O'ZBEKISTON RESPUBLIKASI
INTELLEKTUAL MULK
AGENTLIGI

100000, Tashkent, Mustakillik avenue, 59
Tel.: (99871) 232-00-13, Fax: (99871) 233-45-56
E-mail: info@ima.uz web-site: www.ima.uz

100000, Toshkent shahri, Mustaqillik shox ko'chasi, 59 uy
Tel.: (99871) 232-00-13, Faks: (99871) 233-45-56
E-mail: info@ima.uz, web-site: www.ima.uz

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

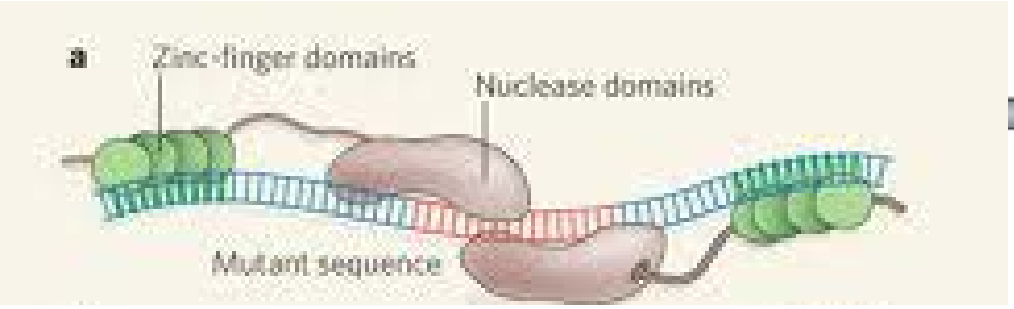
A patent application for this work has been filed in the Uzbekistan (IAP 20120069) and USA (USPTO:13/445696).

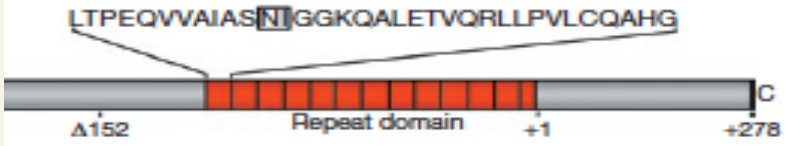
Future perspectives

- Commercialization of these new generation RNAi varieties;
- 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).





RVD:	NI	HD	NN	NG
Base:	A	C	G/A	T

Acknowledgments

Contributors:

- Dr. Zabardast Buriev
- Dr. Abdusattor Abdukarimov

- Dr. Alan Pepper
- Dr. Sukumar Saha
- Dr. Jonnie Jenkins

Funding:

- USDA-FSU program
- Cabinet of Ministries of Uzbekistan
- Academy of Sciences of Uzbekistan
- Ministry of Agriculture and Water Resources of Uzbekistan
- "UzCottonindustry" association,
- Association of "Oil-Fat& Food Industry"
- Holding company «Uzvinprom Holding»

We thank P. Waterhouse and C. Helliwell, CSIRO, Australia, for providing pHellsgate vector systems for our study.