



Characterization of yield potential for RH-662: A new high yielding, stress and salinity tolerant cotton variety

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Abstract

RH-662 is a high yielding, CLCuV, salinity and heat tolerant cotton (Bt) variety developed by Cotton Research Institute, Khanpur (RYK), Pakistan. It is a local cross developed through hybridization linking two different cotton genotypes i.e., 319/08 and FH-113. F₂ to F₆ generations of this cross were advanced by modified bulk pedigree method during 2006-13. The variety was tested for the multilocation trials under different agro-climatic conditions during 2014-2015 and under national uniform trial during two consecutive years, 2016-17 and 2017-18. The variety was finally approved for cultivation in 2018 by Punjab Seed Council (PCS). The plant type of RH-662 is medium compact with 130-160 cm height along with yellow pollen. It has semi erect sympodial and monopodial branches and medium leaf size. This variety has solitary boll shape with 3.7-3.9 gm boll weight having 40-50 bolls per plant. The flower of variety emerges from 45-55 days and matures within 140-150 days. This variety has good boll opening with excellent fiber characteristics and 39.9 GOT. Its seed cotton yield potential is 6400 kg per hectare and average yield is 4260 kilo gram per hectare under different agro-ecological zones. This variety is highly tolerant to marginal and saline soils and also proved resistant to CLCuV and drought conditions. The release of this variety may be helpful to meet the needs of farmers, picking labor and all other stockholders including cotton industry.

Keywords: *Gossypium hirsutum*, Cotton variety RH-662, excellent boll size, salinity tolerant

Introduction

Gossypium hirsutum L. is an important fiber crop that playing vital role in the progress of the textile industries. Demand of extra long and strong fiber with good shining color is a permanent desire of the industry to raise the economy of a country. To fulfill of the export standards with good quality and quantity of the cotton, increase in the cotton yield per acre, boll size and weight, length and strength of the fiber, lint color, drought resistance, and temperature tolerance is very important [1, 2]. Due to the increase in the human population and fast growth of the economic, irrigation water scarcity and salinity are going to become a serious fundamental and chronic problem for the betterment and sustainable progress of the agriculture in arid and semi arid areas of the world [3, 4]. Similarly, in Pakistan farmers use ground water in order to meet the deficiency of the canal water especially in south Punjab [1]. Use of the water unfit for agriculture adding high amount of salts that causing salinity in our lands day by day [5, 6]. Furthermore, intensive metrological changes in the environment in tropical and subtropical regions stressing the cotton cultivars and reinforcing the water scarcity issue. The present cultivars collection must be furnished against marginal and saline soils with high potential of the yield [4, 7]. Last few years an increase in the attack of cotton insect pests and diseases causing a huge yield loss in Pakistan. Increase in the attack of white fly in the month of the July to September effect the cotton foliage and deposition of the sooty mold.

While, in the month of August to November heavy attack of the pink bollworm is a serious threat to our mature crop. A partial attack of the spotted and American bollworm was also observed in Punjab. Almost all the cotton cultivars growing in the field are being affected with CLCuV disease. Therefore, pest resistance in the cotton cultivars is mandatory in future [1, 8, 9].

Thus breeding process for the purpose of resistance development is an incessant process for the improvement of the new cotton cultivars through various means like straight breeding techniques and molecular methods that help in controlling these factors [4, 10-13]. Major objectives for a cotton variety development are resistant to pests and diseases, long staple length, early maturity, fiber strength, fineness of fiber, high yield and improved lint to seed ratio. Consequently, release of the new CLCuV, drought and salinity resistant variety with high yield and good quality characters would enhance the sustainable cotton production in the country [7, 14, 15]. RH-662 is newly developed Bt-cotton variety possessing superior characteristics of high yield potential and improved fiber quality traits by Cotton Research Institute CRI, Khanpur. RH- 662 is highly heat and drought tolerant to withstand and yield out in harsh, hot and dry climatic conditions in the Punjab. RH-662 was developed through one-way hybridization and gene pyramiding technique of elite parental genotypes accompanied by pedigree selection method through for incorporation of excellent combinations of fiber quality

traits and CLCuV tolerance with higher yield potential right from F₁ population. The superior plant combinations selected in F₂-F₅ generation were entirely based on phenotypic plant characteristics, field look, progeny yield potential, plant shape, number of bolls/plant, average boll weight (g) and fiber quality traits over standard varieties that were screened in field conditions. RH-662 (Bt.) is proficiently embraced with the harmony of desirable economic yield and fiber quality traits for all the cotton stakeholders from farmers-ginners-textile industry. RH-662 is high yielding newly developed Bt. cotton variety topped in yield in Punjab in NCVT-2017-18 with big boll size and good fiber quality traits. It is highly tolerant to heat, CLCuV and salinity. Furthermore, possesses high levels of Bt. insect resistance against bollworms (American & Spotted) and better tolerance to plant sucking insect species. It is highly input responsive can be cultivate in all areas of the Punjab and perform well even under low fertile soil and stress conditions.

Materials and Methods

Two parents with desirable characters i.e., 319/08 (Female) and FH-113 (Male) were crossed in 2006-07 season for the development of a new cotton variety (RH-662) at Cotton Research Institute Khanpur (RYK) Pakistan. Resultant F₁ was grown in greenhouse in 9 x 12" clay pots for generation advancement as (F₂). During 2008-09 F₂ generation was grown in the field and superior plant

selected. F₃ was grown in Field and superior plants were selected in 2009-10. During crop season of 2010-11 F₄ single plant progeny rows were studied in the field and superior single progeny rows were selected. While in 2011-12 F₅ generation was Normal sown in field. Several superior progeny rows in F₅ generation were studied in 2012-13 in the field as (F₆) and bulked. Modified bulk pedigree method was implemented for generations development during 2009-13 (Table 1). A stable line was selected from F₆ on the basis of early maturing, medium leaf size, tolerant to saline soil & CLCuV, excellent boll weight, desirable fiber length, strength and heat tolerant having Cry1AC gene. RH-662 is highly tolerant to saline soils and drought conditions with CLCuV resistance having yellow pollen, big boll size, medium leaf size, high GOT% and desirable fiber length and fineness with acceptable level. The current line, RH-662 was of the selected progeny from F₅ generation and was subjected for testing in PCCT, AYT, PYT, CVT, PSC, DUS, NCBT, NCVT, 1.25 acre Bt. Cotton trial and agronomic studies during 2014 to 2018. Recommended and uniform agronomic practices were implemented during selection and testing procedures in all trials. Replicated design was used in each yield trial with standard and check varieties. Specifically the data on morphological and quality traits were recorded and statistically analyzed by using statistical tools like MS Stat-C software program for the comparison with standards checks.

Table 1: Developmental history of Cotton variety RH-662

Chronological Development of RH-662	
2006-2007	319/08 x FH-113 cross were attempted in the field
2007-2008	F ₁ was grown in greenhouse in 9x 12" clay pots for generation advancement as (F ₂)
2008-2009	F ₂ generation was grown in the field and superior plant selected
2009-2010	F ₃ was grown in Field and superior plants were selected
2010-2011	F ₄ single plant progeny rows were studied in the field and superior single progeny rows were selected
2011-2012	(F ₅) generation was Normal sown in field
2012-2013	Several superior progeny rows in F ₅ generation were studied in the field as (F ₆) and bulked
2013-2014	Preliminary Yield Trial
2014-2015	Preliminary Yield Trial
2015-2016	Advanced Yield Trial
2016-2017	Advanced Yield Trial
2016-2017	PCCT, NCVT, DUS, Biosafety Trial, Agronomy Trial
2017-2018	PCCT, NCVT, DUS, Biosafety Trial, Agronomy Trial

Results and Discussion

Development procedure of RH-662 was started in 2006-07 in which 319/08 x FH-113 cross were attempted in the field as parents having good quality traits. After that in 2007-08 F₁ was grown in greenhouse in 9x12" clay pots for generation advancement as (F₂) that respond excellently. Next year (2008-09) F₂ generation was grown in the field and superior plants with maximum desired traits were selected. In 2009-10 F₃ was grown in Field and superior plants were selected. For F₄ single plant progeny rows were studied in the field and superior single progeny rows were selected in 2010-11. During 2011-12 generations (F₅) was

Normal sown in field. While in 2012-13 several superior progeny rows in F₅ generation were studied in the field as (F₆) and bulked. Onward to 2013 different Preliminary, Advanced, PCCT, NCVT, DUS, Biosafety and Agronomy Yield Trials were made.

Yield Trials

The new strain RH-662(Bt.) was tested in Preliminary Yield Trial at Cotton Research Institute, Khanpur during 2013-15. RH-662 showed top performance increase in yield over standard variety MNH-886 as given in table 1.

Table 1: of RH-662 in Preliminary Yield (Yield Kg/ha) Trials in Khanpur (RYN) during 2013-15

Performance of RH-662					
Name of the Trial	Year	No. Entries tested	Variety	Seed Cotton Yield (kg/ha)	(%) increase over Check
PYT-3	2013-14	12	RH-662	2832	10.7%
			MNH-886 (Std.)	2558	
PYT-1	2014-15	12	RH-662	709	6.5%
			MNH-886 (Std.)	666	

During 2015-17 it was tested in Advanced Yield Trials named AYT-5 and AYT-1 at Cotton Research Institute, Khanpur along with FH-142 (Std.). In 2015-16 twelve entries were tested in RH-662 produced 33.4% high cotton

yield. While in 2017-18 it was again best in trail as produced 5.6% more yield as compared to FH-142 as given in table 2.

Table 2: Yield Performance of RH-662 in AYT

Name of the Trial	Year	No. Entries tested	Variety	Seed Cotton Yield (kg/ha)	(%) increase over Check
AYT-5	2015-16	12	RH-662	3872	33.4%
			FH-142 (Std.)	2903	
AYT-1	2016-17	12	RH-662	2769	5.6%
			FH-142 (Std.)	2621	

Provincial Coordinated Cotton Trials (PCCCT)

Excellent results were observed in PCCT trails conducted from 2016-18. Average yield in KG per hectare was calculated in comparison with FH-142. Experiment conducted in 2016-17 at 16 different locations in which

yield of RH-662 was 2173 and FH-142 was 1858 that was 17% more than standard. PCCT trail conducted in 2017-18 was at 13 different locations in which yield of RH-662 was 2173 and FH-142 was 1858 that was 16% more than standard as given in the table 3.

Table 3: Summary of Yield (Yield Kg/ha) Performance of RH-662 in PCCT in Punjab

Name of Trial	Trial Year	No. of Locations	RH-662	Standard	
				FH-142	% Increase
PCCT	2017-18	13	2860	2465	16.0
	2016-17	16	2173	1858	17.0

National Coordinated Varietal Trials (NCVT)

RH-662 was checked in NCVT trails conducted during 2016-17 and 2017-18 along with two standards (CIM-602 & FH-142). During 2016-17 RH-662 production was 2669 which was 7.7% high than CIM-602 and 8.4% high than

FH142 variety. Similarly, cotton yield in 2017-18 was 3216 which was 29.2% high from CIM-602 and 22% high from FH142 variety as shown in table 4. It has been proved that RH-662 remained on the top in yield performance in Punjab in NCVT 2017-18.

Table 4: Summary of Yield (Yield Kg/ha) Performance of RH-662 in NCVT in Punjab

Name of Trial	Trial Year	No. Locations	RH-662	Standard 1		Standard 2	
				CIM-602	% Increase	FH-142	% Increase
NCVT	2017-18	15	3216	2489	29.2	2635	22.0
	2016-17	12	2669	2479	7.7	2463	8.4

CLCuV Infestation

In case of CLCuV data of RH-662 was recorded in PCCT at CRI, Khanpur during 2017-18 along with other regular cultivars presently existing in the field. In this trail RH-662 performed excellent and showed slight susceptibility with respect to other cotton varieties. The intensity of CLCuV in

FH-142 was 5.90%, in VH-Gulzar 10% and NIAB-898 was 16% etc as given in the table 5. In RH-662 variety the CLCuV intensity was 4.36% only. Under field trails it was observed that RH-662 has brilliant performance against CLCuV infestation and produced maximum yield in different local trails as discussed below.

Table 5: The CLCuV intensity data of RH-662 in PCCT with 40 different varieties at CRI, Khanpur (RYN), 2017-18

CLCuV data of PCCT-1 Trial conducted at CRI, Khanpur 2017-18						
SR./Code	Varieties	R-1	R-2	R-3	Mean	CLCuV %
1	SLH-6	6	15	8	10	8.58
2	SLH-19	8	5	8	7	6.14
3	SITARA-15	13	15	15	14	12.99
4	SITARA-16	7	5	8	7	5.71
5	THAKAR-808	5	7	9	7	6.16
6	NS-181	13	15	21	16	14.45
7	NS-191	10	12	8	10	8.47
8	NIAB-545	6	6	8	7	5.76
9	NIAB-1011/48	8	6	9	8	6.50
10	NIAB-898	27	13	18	19	16.38
11	AA-933	11	9	8	9	8.24
12	FH-152	16	18	10	15	12.36
13	FH-444	4	3	3	3	2.84
14	FH-490	8	8	7	8	6.48
15	RH-662	6	5	4	5	4.34
16	RH-668	2	5	7	5	3.94
17	RH-Afnan	6	6	5	6	4.72
18	RH-Manthar	4	8	15	9	7.71

19	AGC-Nazeer-1	8	8	4	7	5.70
20	WEAL-AG-1606	12	21	8	14	11.75
21	WEAL-AG-5	4	4	7	5	4.19
22	WEAL-AG-6	2	3	4	3	2.56
23	SAHARA-2020	10	8	12	10	8.47
24	NU-21	9	7	13	10	8.06
25	BH-201	10	5	10	8	7.12
26	BH-221	6	6	5	6	4.80
27	VH-189	4	6	6	5	4.60
28	VH-383	5	5	11	7	6.19
29	VH-Gulzar	15	9	8	11	10.00
30	BAHAR-07	12	8	6	9	7.37
31	BAHAR-2017	8	6	11	8	7.44
32	FH-142*	7	6	7	7	5.90
33	IR-NIBGE-9	2	2	3	2	2.01
34	IR-NIBGE-10	5	10	8	8	6.65
35	CYTO-313	4	7	7	6	5.14
36	CIM-632	7	2	7	5	4.75
37	TIPU-1	3	5	8	5	4.48
38	TIPU-9	4	5	16	8	7.04
39	SHAHEEN-1	2	4	7	4	3.77
40	FH-342	13	20	10	14	12.43

Bt. Gene Quantification & Expression

Bt. Gene Quantification in leaf (80 days after sowing $\mu\text{g}/\text{gram}$ of fresh leaf) of RH-662 through ELISA Year 2017-18 was tested in four different laboratories with two parallel standards CIM-602 (Std. 1) and FH-142 (Std. 2) respectively. High purity percentage was observed in RH-662. Protein concentration was high 1.6 ($\mu\text{g}/\text{gram}$) as compared to FH142 (1.4) as shown in the table 6.

Furthermore, trait Confirmation (+/-) PCR, trait purity% and Quantification (Elisa/ $\mu\text{g}/\text{gram}$ expression of Bt gene Cry1Ac, Event Mon-531 was tested from two different laboratories. All these properties were tested compared with standard (FH-142). Both trait confirmation and quantification (Elisa / $\mu\text{g}/\text{gram}$) were very high in RH-662 with 100 percent purity as given in table 7.

Table 6: Bt. Gene Quantification in leaf (80 days after sowing $\mu\text{g}/\text{gram}$ of fresh leaf) of RH-662 through ELISA Year 2017-18

	RH-662					CIM-602 (Std. 1)					FH-142 (Std. 2)				
	ABRI	NIGAB	CEMB	NIBGE	Avg.	ABRI	NIGAB	CEMB	NIBGE	Avg.	ABRI	NI-GAB	CEMB	NIBGE	Avg.
Event	Mon-531	Mon-531	Mon-531	Mon-531		Mon-531	Mon-531	Mon-531	Mon-531		Mon-531	Mon-531	Mon-531	Mon-531	
Gene	Cry 1Ac	Cry 1Ac	Cry 1Ac	Cry 1Ac		Cry 1Ac	Cry 1Ac	Cry 1Ac	Cry 1Ac		Cry 1Ac	Cry 1Ac	Cry 1Ac	Cry 1Ac	
Purity (%)	100	75	70	-	82	100	75	80	-	85	100	75	80	-	85
Protein concentration ($\mu\text{g}/\text{gram}$)	1.18	0.94	2.73	-	1.6	1.15	0.78	2.99	-	1.6	1.12	0.84	2.19	-	1.4

Table 7: Confirmation, Purity and Quantitative expression of Bt gene Cry1Ac, Event Mon-531 Year 2016-17

	RH-662			FH-142 (Std)		
	ABRI	NIGAB	Avg.	ABRI	NIGAB	Avg.
Trait confirmation (+/-) PCR	+	+	+	+	+	+
Trait purity %	100	100	100	100	100	100
Quantification (Elisa / $\mu\text{g}/\text{gram}$)	2.78	3.39	3.085	2.34	1.48	1.91

Spot Examination & Fiber Quality Traits

Spot examination trail was conducted at Punjab seed corporation Kanpur in 16th October 2017 where it was sown with varietal code A-8. Fiber quality traits like Staple Length (mm), Mic. ($\mu\text{g}/\text{inch}$), Strength (tppsi /g/tex), Strength (g/tex), Uniformity Index (%) and U.R (%) were analyzed by four different laboratories named as NIBGE Faisalabad, CCRI Multan, CRS Faisalabad and APTMA

Lahore as given in table 8. Good fiber quality was recorded in which staple length was 29.07 mm, GOT 39.9%, UI 85.57% and U.R 48.14% as given in the table 9 RH-662 with good varietal characteristics Performed excellent in PSC, trail conducted in the cotton research institute Khanewal on 1.25 Acre Plot during 2017-18 where its production was 31.61 mounds per acre table 10.

Table 8: Fiber Quality Traits of RH-662 (Bt) Spot Examination (October-2017)

Code	Variety Name	GOT (%)	Mic.	Staple Length (mm)	Strength		U.I (%)	U.R (%)
					(tppsi)	(g/tex)		
A-8	RH-662	39.9	4.3	29.07	-	35.1	85.57	48.14

Table 9: Fiber Quality Traits of RH-662 (Bt) of Different Laboratories of Spot Examination during October-2017

Variety RH-662 Code A-8	NIBGE, FSD	CCRI, MTN	CRS, FSD	APTMA, LHR	Average
Staple Length (mm)	29.88	29.3	29.6	26.99	29.07
Mic. (ug/inch)	4.55	5.0	3.7	4.08	4.3
Strength (tpsi /g/tex)	-	99.3	-	84.39	91.85
Strength (g/tex)	36.07	29.3	40.9	-	35.1
Uniformity Index (%)	85.57	-	-	-	85.57
U.R (%)	-	-	-	48.14	48.14

Table 10: Performance (Yield mounds/acre) IN PSC, Punjab Seed Corporation Khanewal 1.25 Acre Plot

Name of Trial	Trial Year	Locations	RH-662
1.25 ACRE	2017-18	PSC, Khanewal	31.61

Agronomic Studies

For agronomic trails, sowing date trial, RH-662 gave yield of 4704 kg/ha when it was sown on 16th April during 2016-17. Similarly, RH-662 gave the better yield of 6068 kg/ha when it was sown on 1st May, 2017-18 at CRI, Khanpur as elaborated in table 11. In comparison with standard variety (FH-142) the RH-662 was sown at four different plant to

plant distances (22.5, 30, 37 and 45cm) during 2016-17 and 2017-18. At all treatments RH-662 performance was best with respect to standard variety. It was observed that RH-662 performed very well at P x P distance of (S2=30.0 cm) and gave seed cotton yield of 4620 kg/ha during, 2016-17 and 4208 kg/ha during 2017-18, respectively as given in table 12.

Table 11: Agronomic Trials for Performance of RH-662 in Sowing Date Trials 2016-2018

Performance of RH-662 in Sowing Date Trials 2016-2018				
Sowing date	RH-662 (Yield kg/ha)		FH-142(Std) (Yield kg/ha)	
	2016-17	2017-18	2016-17	2017-18
16 th April	4704	5894	3258	5957
1 st May	3545	6068	3692	4325
16 th May	2228	4166	2512	3483
1 st June	2264	3657	2522	3578
16 th June	1127	1760	1191	2021

Table 12: Comparative Effect of Plant Spacing on the productivity of RH-662 with standard

Effect of Plant Spacing on the productivity of RH-662				
Plant Spacing	2016-17		2017-18	
	RH-662 Yield kg/ha	FH-142 (Std) Yield kg/ha	RH-662 Yield k/ha	FH-142 (Std) Yield kg/ha
(S1) 22.5 cm	4450	4020	3485	3218
(S2) 30.0 cm	4620	4190	4208	3842
(S3) 37.0 cm	3970	3430	3194	3050
(S4) 45.0 cm	3418	3150	3161	2876

4. Conclusion

It has been proofed that RH-662 remained on the top in yield performance in Punjab in NCVT 2017-18. It is high yielding Bt. variety with good fiber quality traits and wide adaptation. Plant growth pattern is semi bushy and have big boll size. The RH-662 is friendly to manual pickings due to good fluffy opening. RH-662 is a Bt. variety that endures acceptable resistant to bollworms (American & spotted and good tolerance to plant sucking insect species). RH-662 is highly input responsive and highly tolerant to marginal and saline soil conditions. It is highly tolerance to CLCuV. It has wide adaptation in all cotton growing areas of Punjab with heat and drought tolerance characters. It performs well even under low fertile soil and stress conditions with medium flowering duration. Hence RH-662 is a new high yielding Bt. Variety with good characteristics is highly recommendable in all over the Punjab.

5. References

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