



**International Journal of Biology, Pharmacy  
and Allied Sciences (IJBPAS)**

*'A Bridge Between Laboratory and Reader'*

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**EVALUATION OF RELEASE OF NEW COTTON VARIETY "RH-668"**

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Received 15<sup>th</sup> Aug. 2019; Revised 18<sup>th</sup> Sept. 2019; Accepted 15<sup>th</sup> Sept. 2019; Available online 1<sup>st</sup> Feb. 2020

<https://doi.org/10.31032/IJBPAS/2020/9.2.4969>

**ABSTRACT**

A new cotton variety; RH-668 is high yielding, resistant to environ adversities and governing improved fiber quality traits. This heat & drought tolerant variety that withstands harsh hot & dry climatic conditions was developed by Cotton Research Institute, Khanpur (RYK) for cultivation in the Punjab. RH-668 was established by cross of diversified cultivars VH-259 & RH-620 in 2006-2007 followed by gene pyramiding to retain superior combinations with higher yield potential, CLCuV tolerance and good fiber quality right from F2-F5 generations via. Bulk Pedigree method. This advanced line was then tested under variant agro-climates at local, provincial and then national trials during 2013-2018. In national trials (NCVT) RH-668 outperformed others in yield and fibre related characters against the Checks; CIM-602 & FH-142 and was approved by Punjab seed Council in 2018 for general cultivation. This cultivar was commercialized as superior new cotton variety "RH-668" having 39.4 % GOT, 4.5 Mic., 28.78 mm staple length and yield potential of 30-60 mounds/acre.

**Keywords: *Gossypium hirsutum* L., RH-668, gene pyramiding, pedigree selection, drought resistant, CLCuV tolerance**

**INTRODUCTION**

*Gossypium hirsutum* and *G. arboreum* are called white gold grown more than 50 countries around the world [1-4]. Currently cotton is the most leading fibrous crops that

is grown over thousands acres annually [5-7]. In certain areas of the world including Pakistan, Egypt, India, China, USA, the Middle East and Australia have the most suitable environment like temperature, rainfall and humidity [8]. There are about 50 cotton species in which 4 are cultivated in majority. Among these 4 species *G. hirsutum* and *G. barbadense* are tetraploids cultivated over 80% of the world's cotton sowing area while *G. herbaceum* and *Gossypium arboreum* are the diploids [9-12]. In Middle East and Asian countries diploid cotton is grown but in India almost all varieties are grown at variable strength [13, 14]. Hence, Cotton is an significant cash crop of the world that plays a major role in stabilizing the national and international development, reduction of unemployment and is a huge source of raw material for textile and other industries [6, 15]. From last few years in Pakistan water scarcity and intensive changes in the environment has badly affected the cotton growth and yield [15, 16]. Lack of adequate amount of water for cotton crop and extension of the hot and dry environment in south Punjab of Pakistan is severely effecting [8, 17]. Therefore, cotton sowing area is also reducing because farmer has started to replace it with other short duration crops like maize, sunflower and rice etc [17-20]. For maximization of the

cotton yield the synergistic synchronization of the vegetative and reproductive stress tolerances under a-biotic stressful environment [12, 17, 21]. Each cotton variety responses differently under exposure of the same a biotic environmental condition [22, 23]. The cotton scientists has revealed through comprehensive research work that regulation of plant growth, development and physiology main factors for desirable plant height, boll weight and optimum fruit producing buds of cotton that contributed finally in the enhancement of per unit area seed cotton yield [6, 9]. Calhoun *et al.*, [24] revealed that pedigree information is most comprehensive sources for successful breeding program. Through these findings pedigrees of cultivars were established between 1970 and 2005 and also give some pedigree information on early foundation lines as far back as the 18th century. The derivation of most of the cotton lines was found to be utilized in the mid-20th century and is ranked as a valuable resource [8].

Production of cotton is reduced significantly due to both biotic and abiotic stresses. During last 20 years CLCuV, high temperature and low yield are most important threats to cotton that causes massive losses in cotton production especially in Pakistan. These factors

appeared in outbreak forms that condensed the national cotton yield upto 9.05 m bales during 1992-93 and 8.04 million bales during 1993-94. In the coming years supply of fresh water to agriculture sector is likely to be reduced as a result of competition from environmental, increasing demand from industrial and domestic sectors,. Thus keeping in view the current situation of water shortage it has become essential to breed CLCuV resistance, high yielding and drought tolerant varieties of cotton crop through breeding and selection. Thus molecular techniques and other straight breeding methods are necessary for the development of new cotton varieties through different ways like straight breeding methods and that help in controlling these factors [5, 18]. Development of cotton variety is mostly linked with high yield resistant to disease and pests, early maturity, fiber strength , long staple length, fineness of fiber and with better lint to seed ratio. Hence, the approval of new high yielding, resistant to drought and CLCuV variety with good necessary traits would enhance the sustainable seed cotton production in the country. The breeder generally plays a critical role for cotton crop. Development of new cotton varieties is extremely multiple and hard-working as compared to other crops complication occurs due to

diversity of objectives behind a new variety development. It is expected that new improved variety designate as high yielding with fine and homogeneous lint, high long and strong fibred, high ginning out-turn, resistant to a range of diseases and pests, approachable to higher doses of fertilizer and moisture , adapted to an extensive range of agro-ecological conditions and socioeconomic farming systems. If it contain all of desired and essential features, then it should be suitable for seed multiplication, which means that it should possess standardization, clarity and constancy norms.

It is familiar assessment that the cotton varieties, almost instantaneously after release start losing uniqueness, showing decline in production potential. Reasons behind this include different factors like mixing during farm activities, both cross and self pollination, natural gene mutation, gene frequency changes, and disease epidemics. There is a fundamental need to develop a cultivar (variety) with passage of time which performs well in all types of diseases and stress conditions.

## **MATERIALS AND METHODS**

Elite germplasm was used for selection of various crosses for development of present variety. For development of genetic resistance in cotton genotypes, all available germplasm was exploited during running

breeding program. Development of RH-668 involved a cross between two lines in following fashion i.e., (VH-259× RH-620) during spring season 2006-07 at Cotton Research Institute Khanpur, Rahim Yar Khan, Pakistan. Among parents VH-259 is Bt, late maturing, medium leaf size, good boll weight, CLCuV tolerant, brilliant fiber length, strength and heat tolerant with Cry1AC gene. RH-620 is with medium boll size, yellow pollen, highly tolerant to CLCuV, medium leaf size, high GOT and good fiber length and fineness which is acceptable every stakeholder. The group of superior plants of F1 was raised from single plant progeny in predigree method during 2007-2008. The F2 segregation population was raised with the selection of superior and desirable plant characteristics in predigree selection in 2008-2009. The F3 filial generation was raised from single plant progeny selected in the field and negative selection was applied to remove undesirable plants by roughing. The selected plant progenies were raised in field and desirable single plants characteristics were identified.

During, 2010-2011 single plants were cultivated in field to raise F4 generation and repeated same selection process to endorse for next generation sowing and finally subjected to selection process. Superior plants groups selected from each

single plant progeny were harvested and bulked. Generation of F5 was grown in the field to multiply the seed during year 2011-12. The present line, RH-668 was the selected progeny from F5 generation and was subjected for testing in NCVT, PSC, AYT, PYT, PCCT, 1.25 acre trail, Bt. Cotton trial and agronomic studies during 2013 to 2018. Recommended and uniform agronomic practices were applied during testing and selection procedures. Each yield trial was organized in a replicated design using standard and check varieties. Data on quality and morphological traits was recorded and statistically analyzed by MS Stat-C software application for the comparison with standards varieties.

## RESULTS AND DISCUSSION

Chronological development of RH-668 was laid in 2006-07 with multiple crossing attempts of VH-259 x RH-620 in the field as parental combination possessing genes governing desirable quantitative & qualitative phenotype. Following crossing, for generation advancement in 2007-08 F1 was raised in greenhouse in 9 × 12" clay pots as segregating F2 seed that responds exceptionally. In 2008-09 maximum F2 generation was space planted under normal field conditions and superior single plants with maximum desired traits were harvested after field selection and fibre testing. Then, F3 was raised in 2009-10 in

field and plants with superior phenotype were selected to raise F4. For F4 progeny rows were studied in the field and uniform superior progeny rows and superior plants within those progeny rows were selected in 2010-11. Followed by, F5 generation; raised during 2011-12 under normal field circumstance. While, uniform superior progeny rows selected in F5 generation were then studied in the field as (F6) and bulk plots in 2012-13. Proceeding from 2013 diverse Preliminary, Advanced, Agronomy Yield Trials, PCCT, NCVT, DUS and Biosafety were conducted in sequence (**Table 1**).

### **Yield Trials**

#### **Preliminary Yield Trials (PYTs)**

The new strain RH-668 (Bt.) was tested in Preliminary Yield Trial (PYT) at Cotton Research Institute, Khanpur during 2013-15. RH-668 exhibited topmost increase in yield performance over MNH-886 (Standard) as given in **Table 3**.

#### **Advanced Yield Trials (AYTs)**

RH-668 was then tested in Advanced Yield Trials named AYT-5 and AYT-1 in 2015-17 along with FH-142 (Std.). During 2015-16 twelve entries were tested and RH-668 produced 3.2% cotton yield increase over check variety. While, percentage increase in yield over check variety; FH-142 was boasted to 20.5% as given in **Table 4**.

### **Provincial Coordinated Cotton Trials**

**(PCCT):** Experiment was conducted in 2016-17 at 16 different locations. Commendable Yield Kg/ha results were witnessed in PCCT trial conducted in 2016-17 as 29.0 % increase in yield performance (2397 kg/ha) was obtained for RH-668 in comparison with FH-142; 1858 kg/ha (Std.). Whereas, in 2017-18 PCCT trial was laid at 13 different locations with 6.8 % increase for RH-668 (2633 kg/ha) against FH-142 (2465 kg/ha) as given in the **Table 5**.

### **National Coordinated Varietal Trials (NCVT)**

RH-668 competed in NCVT trails conducted during 2016-17 and 2017-18 having two standards (CIM-602 & FH-142) at 13 different locations in Pakistan. During 2016-17, RH-668 produced 2680 kg/ha that was 8.1% higher than CIM-602 (2479 kg/ha) and 8.8% higher than FH-142 (2463 kg/ha). Correspondingly, RH-668 in 2017-18 produced 2867 which was 15.2% more from CIM-602 (2489 kg/ha) and 8.8% more than FH142 (2635 kg/ha) variety as shown in table 6. Hence, it was established that RH-668 remained on top in yield performance in NCVT, 2017-18 in Punjab.

### **CLCuV Infestation**

CLCuV % data of RH-668 was recorded in PCCT at CRI, Khanpur during 2017-18 along with other regular cultivars. In this trial RH-668 performed well and presented slight

susceptibility as compared to other cotton strains. The intensity of CLCuV in FH-142 was 5.90% followed by VH-Gulzar (10%) and NIAB-898 (16%) etc. as specified in the **Table 6**. While, the RH-668 had 3.94% of CLCuV index in 2017-18. Bt. Gene Quantification for RH-668 and two parallel standards CIM-602 (Std. 1) & FH-142 (Std. 2) were tested in four different laboratories using leaf (80 days after sowing  $\mu\text{g}/\text{gram}$  of fresh leaf) through ELISA during 2017-18. High purity percentage was observed in RH-668. RH-668 had high Protein concentration ( $1.54 \mu\text{g}/\text{gram}$ ) as compared to FH142 ( $1.4 \mu\text{g}/\text{gram}$ ) & CIM-602 ( $1.6 \mu\text{g}/\text{gram}$ ) (**Table 6**). Besides, trait Confirmation (+/-) PCR, trait purity (%) and Quantification of ELISA (Elisa/ $\mu\text{g}/\text{gram}$ ) expression of Bt gene Cry1Ac, event Mon-531 was verified from ABRI & NIGAB's laboratories compared with FH-142 (std.). Both trait confirmation and quantification were considerably high in RH-668 with 100 percent purity based on of cry 1 Ac presence through strip test.

#### Spot Examination & Fiber Quality Traits:

Spot examination was conducted by Punjab Seed Corporation on 16th October, 2017 as

RH-668 had varietal code A-35. Fiber quality traits like Staple Length (mm), Micronaire value ( $\mu\text{g}/\text{inch}$ ), Fibre strength (tppsi /g/tex), strength (g/tex), Uniformity Index (%) and U.R (%) were investigated by CCRI Multan, NIBGE Faisalabad, CRS Faisalabad and APTMA Lahore (**Table 8**). The fiber quality results were impressive too as RH-668 produces GOT of 39.4 %, staple length was 28.78 (mm), Fibre strength was 34.4 (g/tex), UI 84.10% and U.R of 48.18% (**Table 9**). RH-668 with good varietal characteristics performed excellent in PSC, Khanewal 1.25 acre trial during 2017-18 and produced yield of 33.47 in mounds/acre (**Table 11**).

#### Agronomic Studies

In sowing date trial, RH-668 gave yield of 3890 kg/ha when it was shown on 16<sup>th</sup> April during 2016-17. Similarly, it gave the better yield of 6074 kg/ha when it was shown on 16 April, 2017-18 at CRI, Khanpur. Hence, 16<sup>th</sup> April was best sowing date for RH-668 during both years of trials 2016-17 & 2017-18 (**Table 12**).

Chronological Development of RH-668	
2006-2007	VH-259 x RH-620 cross were attempted in the field
2007-2008	(F <sub>1</sub> ) was grown in the field.
2008-2009	(F <sub>2</sub> ) generation was grown in the field and superior plant selected
2009-2010	(F <sub>3</sub> ) was grown in Field and superior plants were selected
2010-2011	(F <sub>4</sub> ) single plant progeny rows were studied in the field and superior single progeny rows were selected
2011-2012	(F <sub>5</sub> ) generation was Normal sown in field

2012-2013	(F6) superior progeny rows were selected in the field) 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

Table 1: Developmental history of Cotton variety; RH-668

Table 2: Yield performance of RH-668 in Preliminary Yield Trials

Name of the Trial	Year	No. Entries tested	Variety	Seed Cotton Yield (kg/ha)	(%) increase over Check
PYT-3	2013-14	12	RH-668	2111	37.3
			MNH-886 (Std.)	1537	
PYT-3	2014-15	12	RH-668	2832	10.7
			MNH-886 (Std.)	2558	

Source: Annual Report (2013-14) &amp; (2014-15)

Table 3: Yield performance of RH-668 in Advance Yield Trial

Yield Performance of RH-668					
Name of the Trial	Year	Entries tested	Variety	Seed Cotton Yield (kg/ha)	(%) increase over Check
AYT-2	2015-16	12	RH-668	4153	3.2
			FH-142(Std.)	4025	
AYT-2	2016-17	12	RH-668	3678	20.5
			FH-142 (Std.)	3053	

Source: Annual Report 2015-16 &amp; 2016-17

Table 4: Yield performance of RH-668 in PCCT trails

Name of Trial	Trial Year	No. of Locations	RH-668	Standard	
				FH-142	% Increase
PCCT	2016-17	16	2397	1858	29.0
	2017-18	13	2633	2465	6.8

Table 5: Yield performance of RH-668 in NCVT Trials

Name of Trial	Trial Year	No. Locations	RH-668	Standard 1		Standard 2	
				CIM-602	% Increase	FH-142	% Increase
NCVT	2016-17	12	2680	2479	8.1	2463	8.8
	2017-18	15	2867	2489	15.2	2635	8.8

Table 6: CLCuV infestation of RH-668 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 &amp; Expression

Table 7: RH-668 is a BT Variety

	RH-668					CIM-602 (Std. 1)					FH-142 (Std. 2)				
	ABRI	NIGA B	CEM B	NIBG E	Avg.	ABRI	NIGA B	CEM B	NIBG E	Avg.	ABRI	NI-GAB	CEM B	NIBG E	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		Cry1Ac	Cry1Ac	Cry 1Ac	Cry 1Ac		Cry 1Ac	Cry 1Ac	Cry 1Ac	Cry 1Ac	
Purity (%)	-	75	80	-	77.5	100	75	80	-	85	100	75	80	-	85
Protein concentration (µg/gram)	1.54	3.54	2.69	-	2.6	1.15	0.78	2.99	-	1.6	1.12	0.84	2.19	-	1.4

Source: NCVT 2017

Table 8: Confirmation, Purity and Quantitative Expression of Bt Gene Cry1ac, Event Mon-531 Year 2016-17

	RH-668			FH-142 (Std)		
	ABRI	NIGAB	Avg.	ABRI	NIGAB	Avg.
Trait confirmation (+/-) PCR	+	+	+	+	+	+
Trait purity %	100	100	100	100	100	100
Quantification (Elisa / µg/gram)	1.38	3.55	2.46	2.34	1.48	1.91

Source: NCVT 2016

Table 9: Fiber Quality Traits of Rh-668(Bt) Spot Examination (16.10.2017)

Code	Variety Name	GOT (%)	Mic.	Staple Length (mm)	Strength		U.I (%)	U.R (%)
					(tppsi)	(g/tex)		
A-35	RH-668	39.4	4.5	28.78	-	34.4	84.10	48.18

Table 10: Fiber Quality Traits OF RH-668 (BT) of Different LABS. of Spot Examination on 16-10-2017

Variety RH-668 Code A-35	NIBGE, FSD	CCRI, MTN	CRS, FSD	APTMA, LHR	Average

Staple Length (mm)	29.42	28.9	29.0	27.8	28.78
Mic. (ug/inch)	4.47	4.8	4.9	3.83	4.5
Strength (tppi /g/tex)	-	104.2	-	86.49	95.4
Strength (g/tex)	34.83	28.3	40.2	-	34.4
Uniformity Index (%)	84.10	-	-	-	84.10
U.R (%)	-	-	-	48.18	48.18

Table 11: Performance IN PSC, Khanewal 1.25 Acre Plot Yieldmounds/ACR

1.25 ACRE	Trial Year	Locations	RH-668
	2017-18	PSC, Khanewal	33.47

Table 12: Performance of RH-668 in Sowing Date Trials 2016-2018

Sowing date	RH-668 (Yield kg/ha)		FH-142(Std) (Yield kg/ha)	
	2016-17	2017-18	2016-17	2017-18
16 <sup>th</sup> April	3890	6074	3258	5957
1 <sup>st</sup> May	3650	5163	3692	4325
16 <sup>th</sup> May	2610	4240	2512	3483
1 <sup>st</sup> June	2600	3481	2522	3578
16 <sup>th</sup> June	1280	1751	1191	2021

Table 13: Effect of Plant Spacing on the productivity of RH-668

Plant Spacing	2016-17		2017-18	
	RH-668 Yield kg/ha	FH-142 (Std) Yield kg/ha	RH-668 Yield k/ha	FH-142 (Std) Yield kg/ha
(S1) 22.5 cm	4381	4020	4010	3218
(S2) 30.0 cm	4214	4190	3480	3842
(S3) 37.0 cm	3695	3430	3250	3050
(S4) 45.0 cm	3291	3150	3040	2876

It is clear from above mentioned data that RH-668 performed very well at P × P distance of (S1=22.5 cm) and gave seed cotton yield of 4381 kg/ha during, 2016-17 and 4010 kg/ha (S1=22.5 cm) during 2017-18, respectively.

### Seed Availability

The seed of RH-668 will be multiplied by Punjab Seed Corporation. RH-668 is recommended for, Rahim Yar Khan, Bahawalpur, Bahawalnagar, Muzaffar Garah, D.G. Khan Khanewal, Lodhran Vahari, and Multan with yield potential 42-70 mound/Acre.

### CONCLUSION

Low germination, poor yield, water deficiency/stress, high CLCuV infestation and less responsiveness of varieties to inputs are major problems in cotton production in Pakistan. RH-668 has been developed to address all the aforementioned glitches. Commercialization of this variety would improve the yield of cotton in Punjab as it can be grown on marginal lands with lesser inputs. Further, RH-668 is high yielding Bt. variety possessing good fiber quality traits and varied adaptability. Plant growth pattern is semi-erect type with single main stem having long sympodial branches. It has

medium boll size with good opening that makes RH-668 pleasant to manual picking.

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