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Procedure for the commercial release of transgenic cotton in Pakistan

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Introduction

Pakistan's reliance on cotton is significant, as it is the raw material of the country's largest manufacturing sector, accounting for the single largest source of foreign exchange earnings (60%) through exports of textile goods. It contributes over 0.6% to GDP and 2.4% to value addition (Anonymous 2021). Punjab and Sindh are the major cotton producing provinces in Pakistan. Over seventy percent of the total cotton is produced from Punjab province, while Sindh accounts for the remaining 30%. The entire cotton crop in Pakistan is irrigated, hand-picked, and raised using indigenous seeds.

Cotton research and development institutions in the public and private sectors develop open-pollinated varieties with desirable traits for biotic and abiotic stress resistance and high yields using conventional breeding techniques. Since cotton in Pakistan faces a pest complex problem, a huge quantity of pesticides had been used, which resulted in the development of resistance in insects, especially in bollworms. The government had to launch a resistance management and monitoring plan and introduced pest scouting-based pest management decision system and IPM, etc. However, farmers with limited knowledge continued to upscale pesticide doses and increased their cost of cultivation substantially.

Background

With new developments in biotechnology and genetic engineering, the government paid special attention and set up a National Commission on Biotechnology in 1985. On the recommendation of the Commission, new biotechnology labs were established in the country, and human resources were trained from the top labs in the world. Local universities started new programmes and produced young scientists in the fields of biotechnology, molecular biology and genetic engineering. The government made a huge investment in this new field of science with the aim of using the knowledge of biotechnology and molecular biology to address medical, industrial, and agricultural issues.

However, scientific infrastructure development and human resource development did not match the legislative and rules formulation until 2005, when the government realized that the introduction of biotech cotton is a need of the day. As per the Cartagena Protocol on Biosafety, commercialization, trade, and research on Genetically Modified Organisms (GMO), including Bt cotton, is subject to the enactment of Biosafety laws and the establishment of Biosafety Centre at Institutional and National levels. Pakistan signed the Cartagena Protocol in 2001, but it was ratified and enforced in 2009. Prior to 2009, regulation of research, import, and commercial cultivation of Bt cotton or GMO were not an international obligation. The country lacked laws on breeder's right, biosafety, and technology patent protection. As stop gape arrangement, under the Pakistan Environmental Protection Act 1997, Biosafety rules were framed, and biosafety guidelines were developed in 2005, as the first legal document to facilitate the introduction of biotech crops, especially GM cotton in Pakistan.

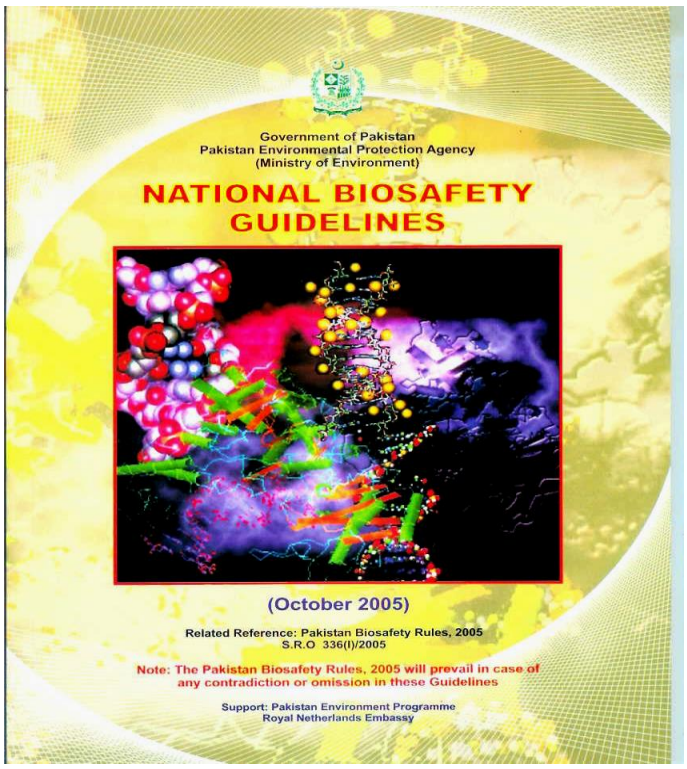


Figure 1. National Biosafety Guidelines 2005

GM cotton in Pakistan

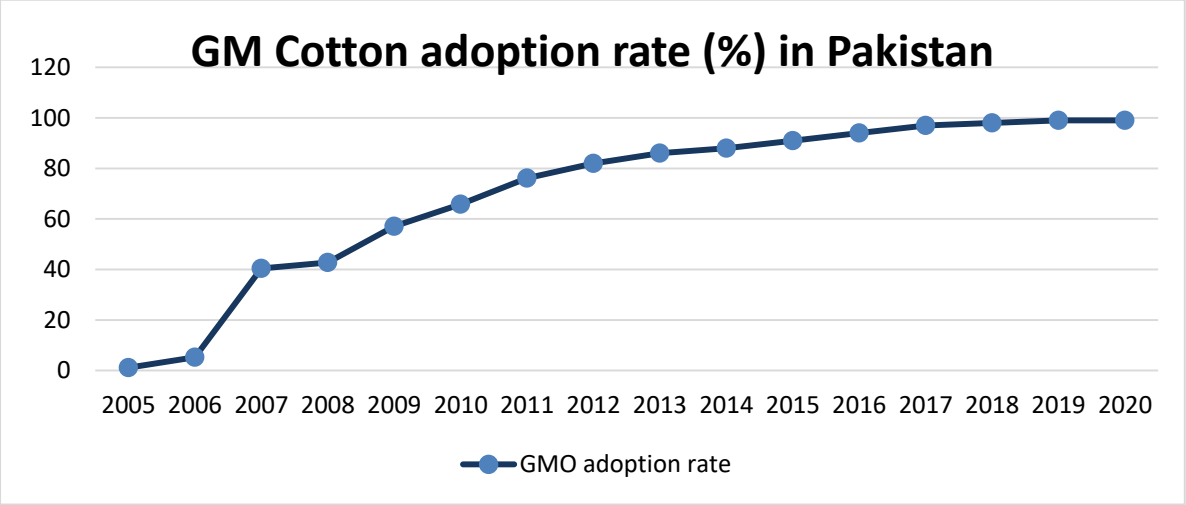
Since India and other countries enacted Biosafety and Plant Breeder's Rights laws earlier than Pakistan and allowed commercial cultivation of Bt cotton, the obvious increase in cotton production in such countries mesmerized Pakistan's cotton growers. Some influential cotton growers and seed industry people

brought in Bt cotton seed from the USA, Australia, and India for direct sowing in the early 2000s, but none of the varieties was successful in Pakistan because of exceptionally high temperatures in cotton areas and the prevalence of a peculiar disease called cotton leaf curl disease (CLCuD).

Meanwhile, scientists at a seed company and some knowledgeable progressive growers successfully backcrossed the Bt trait Mon-531 (Cry1AC) into cultivated commercial varieties and began testing it at farmers' fields under cover, until Monsanto declared that the patent for Mon-531 had expired and they no longer had the right to claim its technology fee in Pakistan. This paved the way for the transformation and testing of Bt cotton varieties with the Mon-531 gene in a legal way by breeders at public and private institutions. The first batch of seven GM cotton varieties was recommended by the Technical Advisory Committee, and the National Biosafety Committee granted a license for commercial cultivation in Pakistan in 2010. These varieties have already passed productivity tests and met fiber characteristic standards.

GMO licensing procedure in Pakistan

Pakistan, being a signatory of the international Cartagena Protocol on Biosafety, has established a Biosafety system in the country to regulate GMOs. The National Biosafety Committee (NBC) and Technical Advisory Committee (TAC) under the Ministry of Climate Change are responsible for evaluating, regulating, and monitoring GMOs for lab or field research and their commercial-scale production or marketing in the country based on data and recommendations from Institutional Biosafety Committees established in each biotech institute. Any concept involving research with GMOs has to be deliberated at the institute level in the Institutional Biosafety Committee (IBC), which is represented by a biotechnology scientist, a social scientist, biosafety officer, and a member of civil society, and makes recommendations to the Technical Advisory Committee (TAC), which is a high-level technical body with representation from provinces, federal ministries, health, industrial, and biotechnological institutes, and is headed by the Director-General of the Pak Environmental Protection Agency (PakEPA). TAC reviews the recommendations of the IBC and, while considering secondary data from other countries, makes its recommendations to the National Biosafety Committee (NBC) for approval of cases or otherwise. NBC is the highest forum for granting approval of GMO-related issues. It is headed by the Secretary of the Ministry of Climate Change and represented by federal ministries, provinces, eminent scientists, and experts in environment, medicine, and agricultural biotechnology.



As of now, cotton is the only transgenic crop allowed for commercial cultivation in Pakistan. Any institution intending to initiate work on GM cotton places its case in IBC, which makes its recommendations to TAC. The Technical Advisory Committee, while considering all risk mitigation and biosafety measures, recommends to NBC for the grant of a license to undertake lab work on GMOs. Scientists are also required to generate biosafety data as per the protocol of National Biosafety guidelines required for the grant of contained facility testing and commercial release licenses. At a semi-advanced stage, when scientists intend to undertake field trials or crossing of GM lines, they will again seek approval for such trials in the contained facility registered with NBC. While applying for a commercial release license, a two-year trial at multiple locations is mandatory, which is usually covered with national yield testing trials at multiple locations required for registration of a variety. TAC considers data from the same trials and makes recommendations to NBC for the grant of a license for commercial release in the country or otherwise. A license to test in a contained facility and for commercial release is required if the variety is being developed utilizing a foreign gene. However, biosafety data from the country of origin is required to be presented for the approval of such a variety.

Sixty-one GM cotton varieties have been developed and approved for general cultivation in Pakistan since 2010. All of these varieties belong to the first-generation or BG-I. In the late 2008, the Government of Pakistan (Ministry of Food and Agriculture) signed an MOU and LOI with Monsanto to facilitate gene technologies in Pakistan, with an agreed protocol for technology fee and a roadmap for the introduction of technologies, including the enactment of the Plant Breeders Right bill, amendments in the existing Seed Act 1976, testing and introduction of cotton hybrids as stopgap arrangements, and effective enforcement of legislation for technology fee recovery, etc. In June 2011, under the 11th constitutional amendments, the Ministry of Food and Agriculture (MinFA) and Ministry of Environment were

devolved to provinces, and subject cotton was entrusted to the Ministry of Textile Industry. This event of devolution created a vacuum, and parties remained unable to meet the committed goals in MOU or LOI.

As Pakistan was using first-generation BG-I (MON 531) seed technology without any resistance management or stewardship programme, it resulted in loss of its efficacy, and insects specially pink bollworm started developing resistance against Cry1AC in 2014-15, hence, it was high time to replace or complement the seed technology. The companies that owned new seed technologies like BG-II, BG-III, or RRF (Roundup Ready Flex) were hesitant to introduce their technologies in Pakistan because legal cover to protect patent violation was not enacted by that time.

Amendments in Seed Act 1977 were made in 2015, and the Plant Breeders Right Act was promulgated in 2016, and its registry was established in 2020. The National Biosafety Committee was again created under the newly established Ministry of Climate Change and became operational, but the technology-providing companies probably lost confidence and interest, did not show any willingness even after a number of attempts by the Government to engage them. The provincial government of Punjab engaged Monsanto one more time in 2017 but was unable to reach a consensus on technology-fee and its collection method.

Development of indigenous GM cotton

The Center of Excellence in Molecular Biology (CEMB), University of the Punjab, Lahore, has developed and patented a parallel technology of Monsanto, i.e., BG-II and RRF, and introduced the technology as “Klean Cotton.” The gene has passed all the regulatory processes of NBC and has been granted a license for commercialization. It can be debated whether the gene has the potential to compete with that of Monsanto, but it is a fact that being a public sector technology, the seed developed with this technology is cost-effective and has a research backup. CEMB entered into a contract with multiple local seed companies, and they are using Klean Cotton technology in their varieties. A leading national seed company pioneering in Biotechnology R&D known as “Four Brothers” has also developed double and triple gene varieties of cotton and got patents. It is worth mentioning here that any seed technology has nothing to do with yield or heat/drought tolerance. These genes only give protection against bollworms and herbicides. In 2021, five double-gene varieties and six triple-gene varieties were approved by the Provincial Seed Council for commercial cultivation in Punjab/Sindh province (Table-1).

Table 1. Transgenic cotton varieties developed using local seed technologies

Variety	Number of genes	Owner	Approved by
Badar-01	2	CEMB Lahore + Four Brothers Lahore	Sindh Seed Council
CA-12	2	CEMB Lahore + Four Brothers Lahore	Punjab Seed Council
CEMB-100	2	CEMB Lahore	Punjab Seed Council
Tahafuz-12	2	CEMB Lahore + Suncrop Multan	Sindh Seed Council
Tahafuz-2020	2	CEMB Lahore + Suncrop Multan	Sindh Seed Council
CEMB-33	3	CEMB Lahore	Punjab Seed Council
CEMB-66	3	CEMB Lahore	Punjab Seed Council
CKC-01	3	CEMB Lahore	Punjab Seed Council
CKC-03	3	CEMB Lahore	Punjab Seed Council
CKC-06	3	CEMB Lahore	Punjab Seed Council
Hataf-03	3	CEMB Lahore + Four Brothers Lahore	Punjab Seed Council

It seems that the priority area in cotton breeding programmes has shifted from core areas such as heat resistance, CLCuD resistance, and high yields to seed technology over the years. The current issues of cotton production require urgent attention to climate resilient genes and their incorporation into high yielding varieties to enable farmers to make a good living from cotton production. Gene editing is a comparatively new area in plant biotechnology, and several products produced through gene editing are available for commercial use. Pakistan is still struggling to formulate policies on how to deal with these products, whether they should be considered as GMOs or not.

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Effect of skips and doubles on the productivity of cotton grown on rainfed Vertic Inceptisols

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Introduction

Cotton production in India is labour intensive. The availability of agricultural labour is declining while the labour wages are increasing, thus reducing farm profits. Human labour accounts 55% of the total operational cost of production (Reddy *et al*. 2018). Cotton picking, weeding and sowing are the main labour intensive field operations. Conventionally long duration, robust cotton hybrids are planted adopting a wide spacing of 3-4 feet between rows and 2-3 feet between plants in a row. However, during the last few years farmers are switching over to new semi-compact, medium duration hybrids and planting them at closer spacing. This is further increasing the cost of seeds required to plant a unit area and additional labour is also required to plant them manually. Therefore, mechanization of sowing is essential to reduce labour cost and ensure timely planting.

Different types of planters and seed drills have been evaluated under Indian conditions for planting cotton (Dixit *et al*. 2011; Sharma *et al*. 2013; Senthilkumar *et al*. 2020). Under irrigated conditions, planting cotton with a tractor drawn inclined plate planter, pneumatic planter and seed drill resulted in a miss index of 0.19, 0.21 and 0.20, respectively and the extent of doubles was lowest in pneumatically planted crop stand (Senthilkumar *et al*. 2020). Among the available planters, the pneumatic planter is most precise in terms of seed singulation and placement (Ramesh *et al*. 2015). Skips (gaps or misses) or doubles through poor singulation are often observed in machine planted crop stands. Both skips and doubles adversely affect yield of widely spaced crops like corn (Nafziger 1996) that has a determinate growth habit. On the contrary, cotton plants with an indeterminate growth habit, have inherent ability to better adapt to missing neighbour plants (skips by altering its branching and fruiting pattern and thereby partially offset the loss in yield. Yield compensation, at low plant density in cotton, occurred primarily by increased boll number per plant and secondarily by increased boll size (Sapkota *et al*. 2022). However, much of the additional yield is produced from outer position bolls developed on monopodial branches (McCarty *et al*. 2017). Although, plenty of literature on the effect of varied uniform plant densities on yield is

available, yet there is no study focussing on the effect of uneven plant stands due to skips and doubles on the yield of cotton crop under Indian conditions. Hence in the present study, we simulated a range of skips and also introduced random doubles and evaluated the plant behaviour and the net effect on the productivity of cotton plants under rainfed conditions.

Material and methods

Field experiments were conducted during the *Kharif* (monsoon) season of 2020-21 and 2021-22 at the research farm of ICAR-Central Institute for Cotton Research, Nagpur, Maharashtra, India under rainfed conditions. The location is a representative of Agro Eco Sub Region 10.2, characterized by hot dry sub-humid bio-climatic condition. The soil of the experimental site was a medium deep black soil (Sub- group: Vertic Haplustepts). It had a pH of 8.1, was low in organic carbon and bicarbonate extractable P but high in ammonium acetate extractable K content. The rainfall received during the crop season was 1359 mm in 2020-21 and 1230 mm in 2021-22 as against the normal seasonal rainfall of 1002 mm.

The experiment was laid out in a randomized block design with three replications. Popular Bollgard II (BG II) hybrid was manually sown with the onset of monsoon, on June 26 during 2020-21 season and June 14 during 2021-22 season at a spacing of 90 cm between rows and 30 cm between plants in a row. The gross plot size was 4.5 m × 6.3 m. Initially, 3 seeds were dibbled 30 cm apart in a row in seven rows. The net plot comprised of the five middle rows and 13 dibbles per row (65 dibbles/plot). On the tenth day after planting 2 plants were retained at each dibble in all the plots by removing the additional plant. On the fifteenth day, 12 treatments were simulated by randomly creating skips by removing both the seedlings per dibble (skip) or randomly retaining both of them (double) in a fixed proportion. In all the other dibbles only 1 seedling was retained. The treatment details are furnished in Table 1.

Table 1. Brief description of the treatments imposed

Code	Treatment	Details of treatment imposed in net plot	Final plants retained/net plot
T1	ALL SINGLE	One plant retained per dibble in all 65 dibbles (no skip)	65
T2	5% S	5% dibbles skipped (gaps), rest one plant/dibble	62
T3	10%S	10% dibbles skipped (gaps), rest one plant/dibble	59
T4	15% S	15% dibbles skipped (gaps), rest one plant/dibble	55
T5	20%S	20% dibbles skipped (gaps), rest one plant/dibble	52
T6	25%S	25% dibbles skipped (gaps), rest one plant/dibble	49
T7	5%S+10%D	5% dibbles skipped (gaps)+ 10% dibbles with 2 plants, rest one plant/dibble	69
T8	10%S+10%D	10% dibbles skipped (gaps) + 10% dibbles with 2	66

Code	Treatment	Details of treatment imposed in net plot	Final plants retained/net plot
		plants, rest one plant/dibble	
T9	15%S+10%D	15% dibbles skipped (gaps) + 10% dibbles with 2 plants, rest one plant/dibble	62
T10	20%S+10%D	20% dibbles skipped (gaps) + 10% dibbles with 2 plants, rest one plant/dibble	59
T11	25%S+10%D	25% dibbles skipped (gaps) + 10% dibbles with 2 plants, rest one plant/dibble	56
T12	ALL DOUBLE	Two plants in all the dibbles	130

The crop was raised following the standard package of practices. All the plots were fertilized with 90 kg N and 20 kg P/ha and 37 kg K/ha. The entire dose of P and K was applied as single super phosphate (6.99% P) and Muriate of Potash (49.8% K) at sowing. N was applied in 3 equal splits at 15 days, 45 days and 70 days after sowing as urea (46% N). At 100 days after sowing the Leaf Area Index was measured using a LICOR LAI 2200 Plant Canopy Analyzer directly from 5 representative spots in each plot and the mean values were recorded.

From the entire experimental plot 50 representative plants belonging to each of the four categories *viz*., normal single, normal double (both with no gaps on either side), skip single (adjacent plant missing on one side in the row) or skip double (double plants adjacent to a skip in the row) were tagged separately. Bolls from these plants were harvested separately to understand the impact of skip or double on the behaviour of the adjacent plant.

All the plots were harvested manually and the entire seed cotton produced was harvested in three pickings done at 20 day interval. Since the treatment effects were similar during both the crop seasons, the data for both years were pooled and statistically analyzed. The pooled data were subjected to ANOVA (Gomez and Gomez 1984) and where ever F test was significant, Student's t test was used to separate difference among treatment means at 5% level of significance.

Results and discussion

Both skips and doubles are commonly observed in machine planted cotton and both add to the spatial variability in crop stand. Our study manually simulated skips and doubles in various proportions to understand their effect on the performance of cotton crop.The data on seed cotton yield as influenced by different proportions of skips and doubles simulated are provided in Table 2. The highest seed cotton yield of 1837 kg/ha was obtained in T1 with evenly spaced (30 cm) plant stand with no gaps (skips). However this yield was statistically at par with the yield realized in the presence of random skips of 5 or 10% of the plants. In the absence of doubles, the seed cotton yield declined in the treatments with 15% or more skips (T4, T5 and T6). The extent of this yield reduction was 16.9% at 20% skips and 24.4 % at 25%

skips. Previous studies on cotton from Texas, USA by Supak and Bowman (1999) indicated that a 25% reduction in cotton stand would result in an yield reduction by 12.8%.

The presence of doubles @ 10% could offset the decline in seed cotton yield occurred due the presence of 15% skips. Therefore the yield in T9, 15% skips + 10% doubles was 1720 kg/ha, that was statistically at par with the yield realized with T1 (uniform plant stand with no skips and doubles). Despite the presence of 10% doubles, the yield with 20% (T10) and 25% (T11) skips was significantly lower than that realized with T1. Retaining 2 plants/dibble and thereby doubling the plant stand (T12) resulted in a significant reduction in yield compared to single plant/dibble (T1) possibly due to competition for resources.

Table 2. Mean (2 year) seed cotton yield (kg/ha) and Leaf Area Index (LAI) recorded in various proportions of skips and doubles

Code	Treatment	Seed cotton yield	LAI at 100 DAS
T1	ALL SINGLE	1837	3.38
T2	5% S	1805	3.34
T3	10%S	1731	3.19
T4	15% S	1648	3.14
T5	20%S	1526	3.11
T6	25%S	1388	2.91
T7	5%S+10%D	1788	3.43
T8	10%S+10%D	1710	3.20
T9	15%S+10%D	1720	3.22
T10	20%S+10%D	1683	2.94
T11	25%S+10%D	1486	2.98
T12	ALL DOUBLE	1683	4.09
	CD (p=0.05)	**129.3**	**0.368**

S = Skips and D = Doubles

At 100 Days after planting, when the plant canopy was at its peak, the Leaf Area Index (LAI) was recorded (Table 1). Compared to uniform stand (T1) the LAI was significantly lower in stand with 25% skips. LAI was the highest, 4.09, when the plant population was doubled by retaining two plants per dibble. Mutual shading due to excessive foliage would have led to drop of fruiting parts and that could have resulted in lower yield in the all double treatment (T12).

Pair-wise comparisons in seed cotton yield due to presence or absence of doubles is graphically depicted in Figure 1. It is evident that the presence of doubles could not positively influence yield at 5% and 10% skips. Nevertheless, at 15%, 20% and 25% skips, the presence of 10% doubles

could result in an increase in yield by 72 kg/ha, 157 kg/ha and 98 kg/ha, respectively. Thus, the presence of occasional doubles in crop stands could partly offset the possible decline in yield due to the reduction in plant stand (15-25% skips). In corn, Mehring (2016) also observed the doubles in corn stand could increase yield but not to the same extent as uniform single spacing.

Compensation at individual plant level in response to skips and doubles was studied by tagging and harvesting single plants separately for seed cotton yield and the results are presented in Table 3. The data indicated that a normal single plant, with no skips on either side in a row, yielded 44.8 g/plant. A cotton plant adjacent to a skip gave 18% higher yield/plant. However, this yield increase did not completely offset the yield loss due to a missing plant (skip). Studies on corn plant stands by Novak and Ransom (2018) concluded that a plant next to a skip had 11% more ear weight than the normally spaced plants, but the resultant increase in yield was not comparable to a missing plant. Combined (two plant) yield of a normal double, with no skips on either side, was 129.4% of that of a normal single plant. Similarly, a double plant next to a skip yielded slightly higher, 142.4%, than a normal double. In corn, Novak and Ransom (2018) reported that the combined weight of ears from double plants weighed 36% more than from a single ear from normally spaced plants. Thus, it is inferred from our study that doubles in cotton crop stand could add to the seed cotton yield but not to the extent that it would have been if they were planted individually at uniform spacing. Further, doubles adjacent to a skip had a slight advantage in contributing to the cotton productivity.

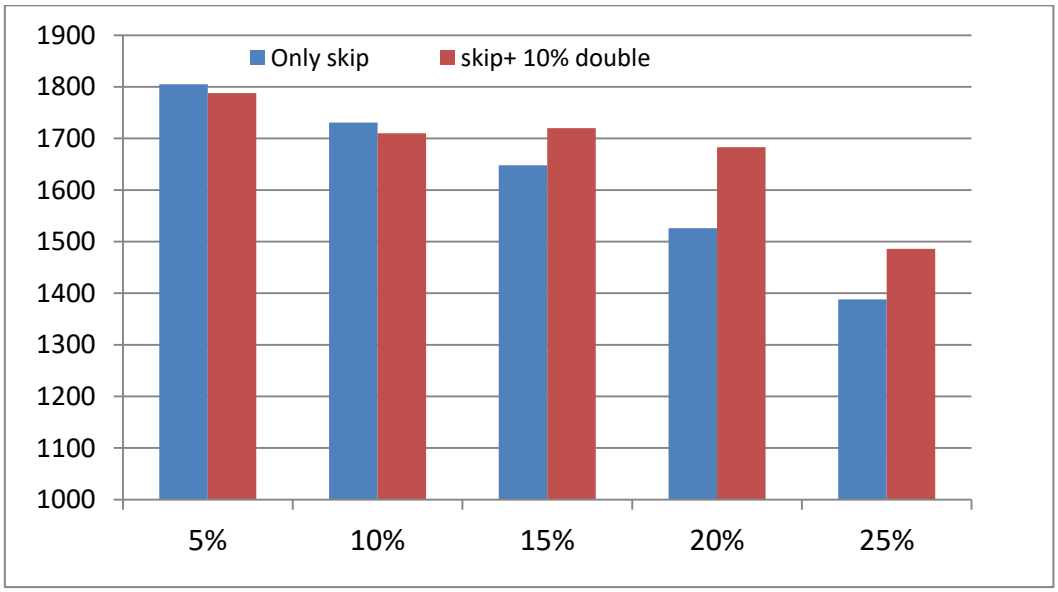

Figure1. Pair-wise comparison in seed cotton yield (kg/ha) due to the presence or absence of doubles

Table 3.Per- plant compensation due to skips and doubles

Situation	Per plant yield (g)	Relative yield (%)
Normal Single	44.8	100
Skip Single	52.9	118.1
Normal Double (each plant)	29.0	64.7
Skip Double (each plant)	32.1	71.7

Conclusion

Both skips and doubles in cotton crop stand contributed to the spatial variability in crop stand but their effect on yield are in opposite directions. Skips beyond 15% could significantly lower seed cotton yield. Nevertheless, the presence of doubles @ 10% could offset the yield loss at 15% skips. A double could add to the seed cotton yield but this is not to the extent that would have been if both the plants were spaced at uniform equidistant spacing.

Acknowledgement

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Challenges in the diagnosis of cotton diseases - A special reference to viruses

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This article is the opinion of Dr. Akhtar Ali who is a Professor of Plant Virology and has rich experience of working on virus diseases of cotton. For example, he worked on the characterization of cotton bunchy top disease in Australia from 2002-2004. Since 2012, Dr. Ali's lab is actively engaged in viruses infecting cotton in southwestern states and have identified viruses of cotton including Cotton leaf roll dwarf virus (CLRDV) in Oklahoma, Kansas and Texas states. Recently, a new virus infection by Tobacco ring spot virus (TRSV) in cotton has been reported in Oklahoma by his lab group.

Introduction

Cotton is one the oldest cultivated crops which is grown for natural fibres in different environments (temperate and tropical) worldwide. It is one of the main commodities globally for millions of people in many different ways such as providing raw materials for clothing industry, jobs and a source of livelihood. In 2021, the top five cotton producing counties in the world were India, China, the United States of America (USA), Brazil and Pakistan that produced 5.9, 5.7, 3.9, 2.76, 0.98 million tons of cotton, respectively (ICAC 2021). Unfortunately, numerous infectious diseases caused by bacteria, fungi, nematodes and viruses infect cotton worldwide and threaten the productivity of cotton. It is very important to reduce the impact of these infectious diseases in order to sustain the cotton industry in the coming future. Although the prevalence and severity of these diseases in cotton might vary from country to country depending on their environmental conditions, cropping patterns, cotton varieties, cultural practices and other management strategies, the most important challenge is the correct diagnosis of these diseases in a timely manner and designing effective control strategies. Although, all the diseases can easily be diagnosed based on their symptoms, there are various challenges to diagnosis of virus diseases of cotton in the field and lab. The main obstacles are the lack of personal expertise. Also working in the lab with cotton leaf tissues that contain phenolic compounds, is a huge impediment to isolate quality nucleic acids of viruses infecting cotton. In this short article, I would briefly describe the standard diagnosis techniques for

various diseases infecting cotton with special focus on the one caused by viruses and suggest the future goals for the diagnosis of virus diseases of cotton.

Bacterial diseases of cotton

Very few bacterial diseases have been reported in cotton. For example, Bacterial blight (also called Angular leaf spot) is caused by *Xanthomonas axonopodis* **pv.** *malvacearum* which is a major disease of cotton in many countries including the USA. The disease affects cotton plant during all the growth stages. Other minor bacterial diseases such as crown gall (*Agrobacterium tumefaciens*) can also infect cotton.

Bacterial diseases in cotton produce various symptoms and the pathogen can easily be diagnosed based on the symptoms and further tests in the lab. The diseased cotton tissues can be cut into small sections and be placed on a specific bacterial media to isolate the causal agent. Bacterial culture of the isolated pathogen can then be used to isolate DNA and subsequent diagnosis by PCR or other staining techniques (gram-positive and gram-negative) as well as microscopy. None of the bacterial DNA is isolated directly from cotton tissues.

Fungal diseases of cotton

Cotton is infected by a large number of fungal diseases worldwide. Some of those fungi cause foliar diseases while other cause soil-borne seedling or root rotting diseases of cotton. A few of the major fungal diseases of cotton are listed below:

Alternaria leaf spot	(*A. macrospora, A. alternata*)
Anthracnose	(*Glomerella gossypii*)
Areolate mildew	(*Ramularia gossypii*)
Ascochyta blight	(*Ascochyta gossypii-Phoma exigua*)
Cercospora leaf spot	(*Cercospora gossypina, Mycosphaerella gossypina*)
Fusarium wilt	(*Fusarium oxysporum* f. sp. *vasinfectum*)
Stemphylium leaf spot	(*Stemphylium solani*)
Verticilum wilt	(*Verticilum spp.*)
Target spot	(*Corynespora cassiicola*)

All of the above fungal diseases cause specific symptoms in leaves, roots, stem and bolls of cotton plants and can easily be differentiated from each other based on the respective symptoms. Even if symptoms of fungal diseases are not helpful, the next step is to perform lab tests. For example, the infected cotton tissues are surface sterilized and cut in small sections which are placed on potato dextrose agar media (PDA) or could be any other fungal media to grow the fungi in a Petri dish. Subsequently the fungal mycelia can be sub-cultured and isolated for morphological studies under the microscope and pathogenicity test on healthy cotton seedlings. In addition, fungal mycelia are routinely used to isolate the fungal DNA

for further application in the nucleic acid-based detection techniques and final identification of the fungus. None of the fungal DNA are directly isolated from cotton tissues for nucleic acid extraction.

Nematode diseases of cotton

Several nematodes infect and feed on cotton roots and cause various diseases in cotton. For example, root knot nematodes (*Meloidogyne spp.*), reniform nematodes and lance nematodes. Most of these nematodes cause obvious symptoms in cotton plants and affect their growth by damaging their roots. In most cases, soil samples around the roots of infected plants or sometime root tissues of the infected cotton plants are collected and processed in the lab to isolates nematodes. These nematodes are used to extract their DNA for subsequent application in the nucleic acid based detection technique for identification. There is nothing to isolate from the leaves of infected cotton plants in disease caused by nematodes.

Among the cotton diseases described above, none of the disease agents are directly isolated from cotton leaves tissues but indirectly on an artificial media in case of bacteria and fungi. Similarly, nematodes can be isolated from soil or indirectly from cotton roots in a medium. Once bacteria, fungi and nematodes are isolated, then the culture is used to isolate DNA from respective pathogens which is not a big challenge. The DNA is used further for nucleic acid based detection tests such as polymerase chain reaction (PCR) to identify the specific pathogen.

Viral diseases of cotton

Although limited virus diseases of cotton have been reported in the world but they pose a big challenge to cotton industry. Both DNA and RNA viruses infect cotton plants worldwide. For example, single-stranded DNA virus which causes cotton leaf curl disease (CLCuD) in Pakistan and India have caused many epidemics in cotton crops for more than 30 years and produced millions of dollar losses in the local economy. Similarly, RNA viruses cause cotton leaf roll dwarf disease (CLRDD) in Argentina, Brazil and the USA while cotton bunchy top (CBTD) in Australia have been reported infecting cotton.

Most of these virus diseases can be identified in the field based on the leaf symptoms of infected cotton plants when the virus disease occurs in the epidemic form and affects a large number of cotton plants in the field. However, if the disease prevalence is less than 1%, it is very hard to identify virus-infected plant(s) located in a 100 acres cotton field. In this situation, a trained virologist is needed to thoroughly survey the field and correctly identify the virus-infected plants.

One of the common challenges in cotton field is to differentiate the symptoms caused by virus from the one due to herbicide applications, drifted 2-4D, and other nutritional issues. Although this can be a challenge for growers, County extension agents and general plant pathologist who has limited knowledge about the nature and symptoms of virus diseases in cotton, a plant virologist should

have the expertise to determine virus-infected cotton plants.

In the lab, symptomatic cotton leaves are the only source to be used for virus identification which is the biggest challenge to isolate intact virus nucleic acids from infected cotton leaves. Since plant viruses cannot be grown on media in the lab, the nucleic acid of viruses shall be isolated directly from infected cotton leaves. Cotton leaves have a lot of phenolic compounds that interfere with the isolation of nucleic acids and it is not easy to isolate a good quality intact RNA or DNA for further identification of the virus.

Although a few nucleic acids isolation kits for plants are available but they are expensive and scientists in the developing countries might not have easy access to it. In such situation, total RNA or DNA of cotton tissues along with viral RNA or DNA shall be extracted together and used in the nucleic acid based detection techniques. This is one of the limitations to work with virus diseases of cotton.

Since the development of high throughput sequencing (HTS), virus detection in cotton became somehow easier. However, HTS also depends on the good quality RNA or DNA from cotton leaves. If any preparations of total DNA or RNA from cotton tissues are contaminated with phenolic compounds during the extraction procedure, HTS might not be able to produce meaningful results from the virus-infected sample.

Serological assays have not been used for virus diseases of cotton due to the difficulty to directly isolate virus-like particles from cotton tissues that could be used for antibody production. Therefore, nucleic acid based detection assays have commonly been used for the virus detection in cotton.

Conclusions

In summary, future challenges for a plant virologist and molecular biologist are to focus on how to isolate a good quality total nucleic acids from cotton tissues. This will require efforts to develop a working lab procedure which shall be robust, reproducible, economical, applicable in developing countries with limited resources and less time consuming for isolating nucleic acids from cotton leaves. With the availability of such an efficient nucleic acid isolating procedure, identifying virus diseases in cotton will become much easier and many more viruses could be identified in future which are continuously threatening cotton productivity and sustainability worldwide.

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It's beyond transmission: crosstalk between Cotton Leaf Curl Virus, whitefly and its harboured endosymbionts

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Introduction

Bemisia tabaci, commonly known as the silverleaf whitefly, is a notorious insect pest that poses a significant threat to agricultural crops worldwide. Besides direct damage as a sap feeder it is more serious pest due to its ability to transmit various plant viruses, including the cotton leaf curl virus (CLCuV). CLCuV belonging to genera Begomoviruses is highly destructive and poses a major threat to cotton crop in north-Indian cotton-growing states. *B. tabaci* mediates the transmission of Begomoviruses in a persistent-circulative manner during which the virus breaches barriers in the digestive, hemolymph, and salivary systems, and interacts with insect proteins along the transmission pathway. Specific insect proteins-virus interactions determine the efficiency and specificity of the transmission. Previous studies have shown that persistent transmission of Begomoviruses by *B. tabaci* vector involves the interaction of viral coat protein with insect-specific genes *Hsp70, Hsp40, Cyclophilin*, and *Knottin*. On the other hand, like other phloem-feeders, *B. tabaci* maintains a close association with bacterial endosymbionts classified as primary and secondary endosymbionts and that can significantly influence their biology. The primary symbiont, *Candidatus Portiera aleyrodidarum* resides in the bacteriocytes transmitted vertically in whitefly. Besides, primary endosymboint, the cryptic species of *B. tabaci* may harbor secondary symbionts namely *Cardinium hertigii, Fritschea bemisiae, Hamilton elladefensa, Arsenophonus spp., Wolbachia spp., Rickettsia spp*. (Gottlieb *et al.* 2006). These endosymbionts are known to play key roles in insecticide resistance, virus transmission, reproduction and development. Based on previous investigations, it is clear that there are two phases of interaction of CLCUV with its vector, first is the interaction of whitefly proteins with CLCuV and second is the crosstalk of vector, virus and endosymbionts. The studies have been undertaken on these lines to understand the underlying mechanism of sex biased transmission of whitefly vectored CLCuV.

Materials and methods

Culture of Asia-II-1 *B. tabaci* is being maintained on *Gossypium hirsutum* cultivar RST9 and that of non-viruliferous whitefly on virus-free cotton plants in walk-in environmental chambers under insect-proof cages at 26 ± 1 °C, 60% RH, and 14h light/10h darkness. The morphological identification of gender was done based on shape and size of abdomen. The initial step was to check the endosymbiotc composition in Asia-II-1 genetic group of *B. tabaci*, which was determined by the diagnostic PCR using total DNA and 16S rRNA and 23S rRNA gene specific primers of *Portiera, Wolbachia, Rickettsia, Arsenophonus, Cardinium,* and *Hamiltonella.* The amplified products were cloned and sequenced; and their identity was confirmed through blast analysis. Once the endosymbiotic composition was established, the antibiotic mediated elimination was carried out using serial concentrations of tetracycline, ampicillin, rifampicin, kanamycin and chloramphenicol administered in artificial diet comprising of 30% sucrose and 5% yeast. For selective elimination of secondary endosymbiont *Cardinium* higher concentrations of tetracycline and rifampicin (150µg/ml) as well as serial concentrations of two additional antibiotics such as streptomycin sulfate and neomycin sulfate were administered to adult flies. The selective elimination of endosymbiont with a particular dose of respective antibiotic was confirmed through PCR and RT-PCR using gene specific primers for16S rRNA and 23S rRNA. In our studies the complete elimination was achieved for *Arsenophonus* with a few antibiotics at a particular dose. The impact of elimination of specific endoosymbiont on CLCuV was analyzed in terms of influence on CLCuV titer in whitefly estimated indirectly through the expression of *coat protein (cp)* gene using RT-PCR. Further, the impact of *Arsenophonus* elimination of transmission efficiency of whitefly was assessed through the release of *Arsenophonus*$^{-ve}$ viruliferous whitefly on virus transmission on healthy plants.

The relationship of whitefly sex with virus transmission was the other objective to understand the three way interaction between whitefly-CLCuV and endosymbionts. To start with this virus load in the whitefly male and female post acquisition of the CLCuV was estimated from the total RNA in terms of the expression of the *cp* gene using RT-qPCR. The relative virus titer was inferred on the basis of relative expression level of the viral *cp* gene calculated using the $2^{-\Delta\Delta Ct}$ method. The expression level of different genes also calculated using ΔCT method and presented in terms of means ± SEM. The expression data were analyzed using Student t- test at $p < 0.05$. The virus coat protein-specific primers qCLCuV_Forward-5'CGTCGACCTGTTGATAAACCTC3' and qCLCuV_Reverse-5'GCATATTGACCA CCGGTAACAG3' were used in the study for quantifying viral copies. Further the sex-biased transmission of CLCuV and viral load in host plant was quantified by releasing equal number of male and female flies on virus free cotton plants at 3 or 5 true-leaf stages for 48h in leaf clip cages. Post inoculation, the plants were regularly observed for the appearance of symptoms. At the appearance of early symptoms such as green islands the virus titer was quantified in the plants from cDNA synthesized from total RNA in terms of the expression of *cp* gene using RTq-PCR.

To elucidate some of the underlying factors that affect sex biased transmission, the relative expression of midgut protein genes (stated to play key role directly or indirectly in *B. tabaci* vectored virus transmission) as well as the infection status of *Arsenophonus spp*. was estimated in male and female flies. The relative expression of *Cyclophilin, Hsp70, Hsp40,* and *Knottin* genes was quantified in viruliferous and non-viruliferous male and females, cDNA synthesized from total RNA through RTq-PCR and gene-specific primers. Similarly, the density of *Arsenophonus* was estimated in both the sexes in terms of the expression of 23S rRNA gene of the bacterium. Functional validation of these studies was performed through knockdown of midgut genes (*Hsp70, Hsp40, Knottin*, and *Cyclophilin*) using gene specific dsRNA (400 ng/ ul of diet) and its impact on viral titer in male and female whiteflies. Further impact of *Arsenophonus* (tetracycline 90 µg/ml) elimination was also estimated in terms of the viral load in male and female flies.

Result and discussion

Based on 16S/23S rRNA sequences it was revealed that the *B. tabaci* Asia II-1 harboured primary endosymbiont *Portiera and* secondary endosymbionts *Arsenophonus* and *Cardinium* and *Rickettsia*. Further, the presence of *Rickettsia* was not uniform. *B. tabaci* is cryptic species complexes which varies in endosymbiotic composition and are capable of transmitting numerous geminiviruses with variable efficiency (Gupta *et al.* 2010; Singh *et al.* 2013). Earlier studies also suggest co-existence of *Arsenophonus* and *Cardinium* in Asia II-1 species of *B. tabaci* (Tang *et al.* 2018). Furthermore, it has been reported that host plants and geographic location play a vital role in shaping bacterial communities associated with whiteflies (Goretty *et al.* 2019).

The complete elimination of *Arsenophonus* quantified in terms of the amplification of 600bp amplicon of 23S rRNA gene through end point PCR was achieved at 90µg/ml of tetracycline and rifampicin administered to the whitefly through sucrose diet. The relative expression of this gene quantified through RTq-PCR also confirmed >95% decrease in expression of this gene in 90µg/ml of rifampicin and tetracycline compared to control. An inverse dose-mortality relationship was observed between the *Arsenophonus* levels and the antibiotic concentrations. However, selective elimination of *Cranidium* could not be achieved with any of the antibiotic and even with the higher concentrations compared to those effective against *Arsenophonus*. In terms of elimination, only 30-35% reduction in expression of 16S rRNA gene of *Cardinium* was observed when flies were fed with 150µg/ml of diet of tetracycline and rifampicin, however significant deleterious effect in terms of expression of primary endosymbiont *Portiera aleyrodidae* gene with different antibiotic concentrations was not observed. Even higher concentrations (150µg/ml) of tetracycline and rifampicin resulted in only 87 to 97.5% reduction in *Porteira* density compared to control flies. In terms of the impact of *Arsenophonus* elimination on CLCuV virus titer in the antibiotic treated flies, it was prominent that the flies fed with 90µg/ml of rifampicin and tetracycline had 80-85% reduction in virus titer compared to control flies. This effect was also was apparent when the treated flies were inoculated on virus free cotton plants showing ~70 % reduction in

the virus titer on 14 DPI (Days Post-Inoculation) compared to control plants. The intensity of CLCuV symptoms in terms of Percent Disease Index (PDI) at 30 DPI in plants inoculated with *Arsenophonus*$^{+ ve}$ whiteflies was ~79 % with severe vein thickening, prominent curling of leaf, leafy enations, minor deformity of internodes and reduction in leaf size in some plants compared to 20-25 PDI and low CLCuD intensity ranging between grade I and II on plants inoculated with *Arsenophonus*$^{-ve}$ whiteflies (rifampicin and tetracycline treated).

The female whiteflies showed higher retention of CLCuV with ~80% higher load compared to males quantified in terms of the *cp* gene expression (Figure 1). This was further prominent in comparative viral transmission mediated by the release of viruliferous male and female whiteflies. The results showed that female inoculated plants recorded 74.3% higher viral inoculum and prominent cotton leaf curl disease (CLCuD) symptoms such as vein thickening and downward curling in comparison to male inoculated plants after 14 DPI (Days Post Inoculation). Due to significantly higher retention and transmission of CLCuV, female whiteflies are more efficient vectors than that of males. The expression of gut protein genes such as *Cyclophilin*, *Knottin*, *Hsp40*, and *Hsp70* was variable among the two sexes. The expression of *Cyclophilin* was about two fold higher in females compared to males and the knockdown of this gene resulted in ~ 48 % reduction in virus titer in the female whiteflies. *Cyclophilin* plays a key role in virus replication, protein refolding, maturation, gene transcription, and cell signaling. Earlier studies with TYLCV have demonstrated that *Cyclophilin* has an active role in virus transmission and knockdown of this gene hampers the virus transmission. Expression of the *Knottin, Hsp40,* and *Hsp70* was significantly low in the viruliferous females compared to the viruliferous males. Earlier studies have also reported an increase in virus ingestion by the whitefly or transmission efficiency by several-fold through the silencing of *Knottin* in whitefly by feeding them on dsRNA. We found that feeding of dsRNA against *Knottin* resulted in 80 to 85% decrease in mRNA levels of *Knottin* in both sexes as comparedto respective dsGFP fed control flies. Previous studies with cotton suggest that dsRNA-mediated knockdown of *Knottin* in whiteflies resulted in 1.9 fold increase in virus titer compared to control and ultimately resulted in 2.0 fold higher transmission efficiency of virus in cotton plants. Similarly, higher expression of *Hsp's* (*Hsp70*and*Hsp40*) (~1.5 fold) was observed in viruliferous males compared to viruliferous females and the knockdown of these genes resulted in significant increase in viral load in females compared to males. Studies suggest that *Hsp*'s may be playing a key role in minimizing the long term effects of virus on the whitefly. Earlier reports also suggest that silencing of *Hsp40* and *Hsp70* in whitefly resulted in increased titer in the whitefly, thereby increasing the transmission efficiency of CLCuV. It is thus inferred from these studies that higher expression of *Hsp40* and 70 might be contributing to poor transmission efficiency of CLCuV by males compared to female whiteflies. Thus, sex-biased expression of gut proteins in males and females may be one of the factors for variable transmission efficiency of CLCuV in both the sexes. The higher density (55 to 75%) of *Arsenophonus* in females might be a factor contributing to efficient female vectoring capacity. Furthermore, the elimination of

Arsenophonus from whitefly using tetracycline showed a 74.9% reduction in viral load in females compared to control female whiteflies. The high viral retention and efficiency of female whiteflies to transmit CLCuV could be attributed to higher density of endosymbiont *Arsenophonus*. The higher density of the *Arsenophonus* might be associated with higher expression of its GroEL protein gene, which is known to safeguard the virion particles (Kaur *et al.* 2020). Our results also suggest higher expression of GroEL protein gene in females compared to males. The studies have suggested higher expression of *Cyclophilin* gene in females as compared to males supplemented by higher expression of *Knottin, Hsp40*, and *Hsp70* genes in females compared to males and thus collectively all these factors might be playing a key role for higher vectoring efficiency of females in whitefly (Singh *et al.* 2021; Kaur *et al.* 2022). Previous studies demonstrate that complex interaction of whitely-virus and endosymbiont interactions cause differential regulation of certain insect genes thus contributing to the transmission efficiency of virus (Gotz *et al.* 2012; Rana *et al.* 2012; Wei *et al.* 2017; Kanakala*et al.* 2019).

The current findings are not sufficient to answers all the key questions arising on the crosstalk between endosymbiont- virus and whitefly and need further in-depth systematic investigations. The reasons for interaction of the symbiotic bacteria with the virus transmitted by their host are not clear, however, there are possibilities that viruses could have evolved to protect themselves in the host by interacting with these GroEL like proteins produced by endosymbionts. It may be other way round that the endosymbiont might be performing their duty of symbiotic relationship to protect the host whitefly from virus by trapping the protein produced by them. The sex-biased expression of the various gut proteins in male and females also need detailed investigations. The complete understanding of these complex cross chemistries of sex- biased expression of various gut genes as well as the density of endosymbionts related to host and its vectored virus may be helpful in devising future strategies for managing the pest or vector through RNAi based technologies or targeting the insect endosymbionts.

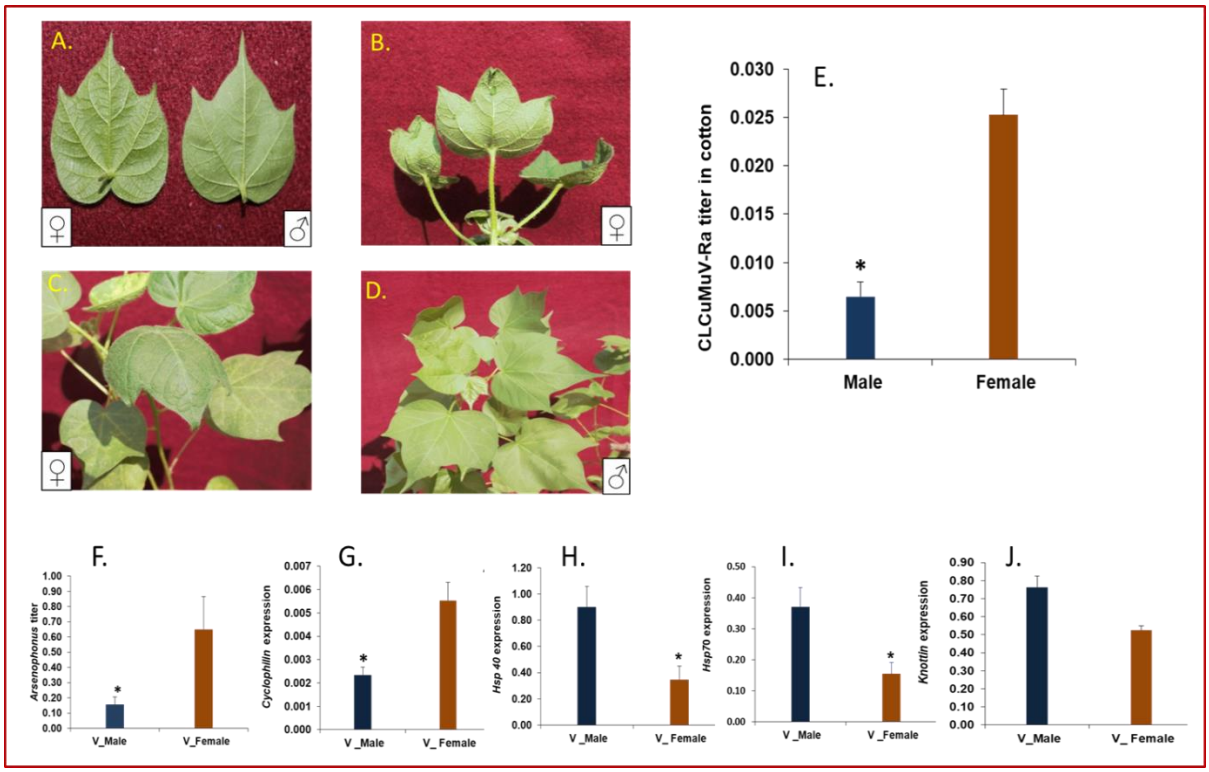

Figure 1. Early vein thickening in cotton plant after inoculation with viruliferous male and female whiteflies; B. Typical curling and enation in cotton after infection with viruliferous females; C. Downward curling in cotton plant after inoculating with viruliferous females D. Symptomless or delayed symptoms in cotton plants after infection with viruliferous male. E. Relative amount of cotton leaf curl virus in *Gossypium hirsutum*. Relative amount of *Arsenophonus* (F), *Cyclophillin* (G), *Hsp 40* (H), *Hsp 70* (I) and *Knottin* (J) in viruliferous females and males.

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Demonstration of balanced crop nutrient delivery system on small holder cotton fields in Vidarbha region of India

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Introduction

The gains obtained in enhancement of cotton production and productivity after the introduction of transgenic Bt cotton in India in 2002 appear to be waning in recent years. Currently, cotton yieldis either stagnant (around 450 kg lint per ha) or declining since 2013-14 when the best national average yield (565.72 kg lint per ha) was obtained (Mayee and Chaudhary 2019). Within the country, the state of Maharashtra boasts of having the highest area under cotton (4.0 to 4.3 m/ha); nearly 1/3rd of the total cotton area in the country but the State has the lowest productivity (320-330 kg lint /ha). Being one of the major cash crops, low cotton productivity has been a matter of great concern for farmers as average yield is hardly 8-10 q of seed cotton per ha or 1.8-2.0 bales of 170 kg lint per ha. There are several reasons for low productivity of cotton in Maharashtra. Among them, the declining reserve of soil nutrients and improper use of fertilizer skewed towards macronutrients are important factors. Improper management of soil nutrition has contributed to depleting soil organic matter, emerging multi-nutrient deficiencies particularly of secondary and micronutrients, declining nutrient use efficiency, negative crop response ratio and negative soil nutrient balance. Nutritional deficiencies; especially those of micronutrients are widespread in the cotton growing areas of Maharashtra and hence efficient nutrient management is essential for realizing high yields and better-quality fibre (Blaise *et al*. 2016)

Integrated Plant Nutrient Supply (IPNS) is promoted as a solution for improving both soil health and cotton productivity (Blaise and Prasad 2005). However, it requires precise monitoring of key soil health parameters and adoption of the integrated nutrient management (INM) practices that improve soil health and ensure that nutrients are managed in a balanced and efficient manner. Government of India launched mega programme to assess soil health, enumerate farmers land and apply nutrients based on soil heath cards. In addition, the Government has also modified Fertilizer Control Order (FCO) 1985, which has created an environment to motivate the fertilizer industry towards development of new and innovative

products based on nutrient requirements of the soil and crop. The modified fertilizer policy triggered the launching of innovative products including specialty fertilizers like fortified fertilizers with appropriate grades of secondary and micronutrients. The approval of innovative complex grade fertilizers such as NPK 8:21:21 and NPK 9:24:24 fortified with S, Mg, Zn, Fe and B is a step in the right direction to replenish the micronutrients along with NPK. In this context, Smartchem Technologies Ltd, a 100% sub-subsidiary of Deepak Fertilizer and Petrochemical Corporation Limited. (DFPCL), Pune introduced a new complex fertilizer called MAHADAN CROPTEK Cotton Solution, FCO registered grade fertilizer specific to cotton crop. A single granule of fertilizer contains all the essential macro, secondary and micronutrients in equal amounts of all the nutrients and is based on Nutrient Unlock Technology (NUT), which increases fertilizer efficiency by increasing nutrient utilization efficiency. CROPTEK Cotton Nutrition Solution is a highly differentiated functional complex fertilizer that meets the crop nutritional requirements of macro, secondary and micronutrients through split application according to crop stage requirement.

To promote and popularize this useful technology, Agrovision Foundation, Nagpur, a non-governmental organization (NGO) decided to launch a project called 'PROJECT POSHAN' with the objective to educate small holder cotton farmers about cotton nutrition and also conduct field demonstrations for farmers to see the benefit of the new nutrition technology.

Materials and methods

To promote the new technology of CROPTEK-SOLUTION among cotton farmers, the strategy adopted was conceptualization, development of framework, implementation and stewardship of outreach programme across cotton growing regions of Vidarbha, Maharashtra and field demonstrations of the technology.Croptek is one of its kind of complex fertilizers that supplies a total of eight plant essential nutrients (Nitrogen, Phosphorus, Potassium, Magnesium, Sulphur, Boron, Zinc and Iron) in balanced manner.

Cotton nutrition has been conceptualized as one of the critical inputs for better cotton growth as it was realized that farmers rarely used balanced nutrition especially the micro and secondary elements. Once soil nutrition is identified as one of the key issues, a framework for implementation was devised. A series of outreach extension activities were executed like; virtual farmer interaction using social media like WhatsApp, YouTube, educational information through pamphlets, leaf lets, hoardings etc. During the peak cotton season, a float was allowed to go through villages for educating the farmers.

Four demonstrations of CROPTEK solution were organized in varied cotton growing situations in Nagpur and Amravati districts of Maharashtra, India. Two demonstrations were planned at farmers' field; village Lakhori and village Metpanjara in Katol Tehsil, Nagpur District. One demonstration was conducted in the best possible field of Ankur Seeds Company at village Kinhi, Nagpur District and one at Shri Shivaji Agriculture College,

Amravati district (Table 1). Each demonstration consisted of half-acre (2000 m^2) plot for treatment and another half-acre plot as control (Farmer Fertilizers Practice). The Croptek fertilizer doses were applied in three splits at 30-32, 48-50 and 65-70 days after sowing depending on rain and soil moisture situation in the treatment plots while recommended schedule of fertilizer application (Table 1) was used in control plots. All other practises recommended by Dr PDKV (an agricultural university); Akola (Anonymous 2022) were followed. Timely observations on number of sympodial branches and green bolls per plant as well as the boll weight were recorded by tagging 10 representative plants in the treated as well as control plots. Three pickings were taken as combined plot yield.

Results and discussion

The cotton season, 2022-23 was an unusually wet season in Vidarbha as the rainfall exceeded by 70 % over the normal of the region and nearly 45% above what was in the last cotton season, 2021-22. High rainfall is considered not suitable for good growth as the vegetative growth gets extended during the main months i.e., July-August-Sept.and delays the onset of fruiting. Despite adverse climatic conditions the Croptek technology appeared to very promising in improving the cotton growth and productivity.

From the data presented in Table 2, it is clear that CROPTEK fertilizer improved cotton growth significantly at all the locations. The best sympodial branch growth was seen at Amravati as also the total number of bolls per plant and average boll weight were the highest at this location. Except the boll number per sympodial branch, all other parameters were found substantially improved in CROPTEK treated plots. Moreover, the seed cotton yield obtained in treated plots was much higher than the control plots. Highest yield of 1359 kg seed cotton per ha was seen in demonstration plot at Kinhi followed by Amravati (1087 kg), Lakhori (1038 kg) and Methapanjara (1112 kg). Based on the means of all the four demonstrations, it was observed that the CROPTEK treatment improved sympodial branches by 31%, number of bolls per branch by 14%, and per plant by 45%. The average boll weight increased by 11% and the overall yield improvement occurred in the range of 18 to 42% (Table 3).

Effective nutrient management in cotton is essential for realizing high yields with desirable fibre quality. Scientific nutrient management is required to save fertilizers, improve nutrient efficiency and avoid nutrient losses (Jingxiu and Xinhua 2019). Recommendations available for fertilizer applications in cotton including those of micro and supplementary nutrients (Singh and Blaise 2000; Anonymous, 2022) are available. However, very rarely farmers apply them due to poor awareness about recommended doses of fertilizers, application of right dose to crop at right stage, access/cost consideration and also lack of knowledge. Repeat application of individual micronutrient involves additional labour cost and farmers avoid their use. Croptek-solution specific for cotton developed by Mahadhan and approved by Government of India as a

complex grade fertilizer, therefore, overcomes these limitations associated with individual application and provides a one-stop solution. The Croptek application to cotton has clearly given an advantage in improving productivity probably due to balanced dosage and synchronized nutrient delivery with crop demand.

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Table 1. Experimental details.

Control (Farmer's Practice)							
Sr. No.	Name of farmer	Upland cotton hybrid	Date of sowing	1st Dose	2nd Dose	3rd Dose	Spacing
1	Mr NarendraBoratkar Village: Lakhori, District: Nagpur	Rasi 659	June 10, 2022	20:20:0:13 - 25 Kg Bensulf- 5 Kg Urea -20 Kg	20:20:0:13 -25 Kg 10:26:26-25 Kg Urea -30 Kg	Urea -25 Kg 20:20:0:13 -25 Kg	150 × 40 cm
2	Mrs. SushamaGokhale Village: Methpanjara, District: Nagpur	Rasi 659	June 11, 2022	20:20:0:13-25 Kg Bensulf -5 Kg Urea -20 Kg	20:20:0:13 -25 Kg 10:26:26-25 Kg Urea -30 Kg	Urea -25 Kg 20:20:0:13 -25 Kg	150 × 40 cm
3	Ankur Seeds Research Farm Village: Kinhi, District: Nagpur	Ankur Kirti BGII	June 23, 2022	10:26:26-25 Kg Urea -10 Kg	10:26:26 -25 Kg Urea -10 Kg	0	90 × 45 cm (HDPS)
4	Shri Shivaji Agriculture College Village: Amravati District: Amravati	Jungee -Mahyco	June 7, 2022	10:26:26 -25 Kg DAP-25 Kg Urea -15 Kg	DAP -20 Kg 10:26:26 -25 Kg Urea -15 Kg	Urea -20 Kg	60 × 45 cm
Demonstration (Croptek solution application)							
1	Mr NarendraBoratkar Village: Lakhori, District: Nagpur	Rasi 659	June 10, 2022	CROPTEK -40 Kg Bensulf -5 Kg Urea -15 Kg	CROPTEK -40 Kg Urea -15 Kg	Urea -15 Kg	150 × 40 cm
2	Mrs. SushamaGokhale Village: Methpanjara, District: Nagpur	Rasi 659	June 11, 2022	CROPTEK -40 Kg Bensulf -5 Kg Urea -15 Kg	CROPTEK -40 Kg Urea -15 Kg	Urea -15 Kg	150 × 40 cm
3	Ankur Seeds Research Farm Village: Kinhi, District: Nagpur	Ankur Kirti BGII	June 23, 2022	CROPTEK -40 Kg Bensulf -5 Kg Urea -15 Kg	CROPTEK -40 Kg Urea -15 Kg	Urea -15 Kg	90 × 45 cm (HDPS)
4	Shri Shivaji Agriculture College Village: Amravati District: Amravati	Jungee -Mahyco	June 7, 2022	CROPTEK -40 Kg Bensulf -5 Kg Urea -15 Kg	CROPTEK -40 Kg Urea -15 Kg	Urea -15 Kg	60 × 45 cm

Table 2. Influence of Croptek solution on comparative growth and yield of cotton in control and treatment plots

Village	Lakhori		Methapanjara		Kinhi		Amravati		Average Cluster	
Growth Parameter	Treated plot	Control plot	Treated plot	Control plot	Treated plot	Control plot	Treated plot	Control plot	Treated plot	Control plot
No of sympods per plant	15	13	16	12	15	12	16	12	15.5	12.25
Boll per branch	4	3	4	4	4	4	4	3	4	3.5
Total Bolls / plant	60	39	64	48	60	48	64	36	62	42.75
Average boll weight (g)	4.5	4.2	4.8	4.4	4.5	4	4.3	3.7	4.53	4
Yield (kg/ ½ acre)	210	152	225	190	275	208	220	155	232.5	176.25
Yield (kg/ha)	1038	751	1112	939	1359	1028	1087	766	1149	871

Table 3. Per cent increase in growth and yield of cotton due to application of Croptek solution.

Treatment	Number of sympods (65 DAP)	Number of bolls per branch (90 DAP)	Number of bolls per plant (120 DAP)	Average boll weight (g) (120 DAP)	Seed cotton yield (kg/ha)
Farm practices	12.25	3.5	42.75	4.00	871
Croptek solution	15.50	4.0	62.00	4.53	1149
% increase over farm practices	31.62	14.3	45.03	11.04	31.92